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Delamination of neuroepithelium and nonneural ectoderm and its relation to the convergence step in chick neurulation

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ABSTRACT

We have analysed the characteristics of the neuroectoderm-nonneural ectoderm meeting point at several axial levels in relation to the mechanics of neurulation in each level. The results show wide differences at cephalic and somitic levels. At cephalic levels, where convergence plays an important role, the delamination process appears at the beginning of the convergence step. This phenomenon produces a major isolation of the basal lamina, forming a space between this structure and the epithelial sheet in whose basal surface a new basal lamina begins to form. This cavity contains abundant extracellular matrix stained with ruthenium red (RR) and tannic acid (TA), and its increase in volume correlates with the progressive convergence of neural folds. At somitic levels, where the convergence is not important, delamination involves the progressive formation of a half-moon-shaped cavity. This structure appears between a dorsal attachment point, in the tip of neuroectodermal wall, and a ventral attachment point which coincides with the point of bending that determines the bilateral furrow, if it exists. In this small cavity, delamination is not related to an isolation of basal lamina. The RR-staining of the extracellular matrix in this cavity is scarce and the volume increase is smaller than in the cephalic region. These results are discussed in terms of neural fold convergence and neural tube closure.

INTRODUCTION

The formation of the neural tube in chick embryos occurs through a bending of the neural plate (in 2 phases: elevation and convergence of the neural folds) and the fusion and separation of neuroepithelium from the epidermal ectoderm (Schoenwolf, 1982). Nevertheless, morphogenetically the formation of the neural tube is not uniform along the longitudinal axis. Thus at cephalic and caudal levels convergence is more important than at somitic levels. Elevation and convergence involve the delimitation of 2 different hinge points in the neuroepithelium that determine a medial furrow or 2 bilateral furrows respectively.

There is still controversy about the contribution of intrinsic and extrinsic forces to morphogenetic mechanisms involved in neural fold elevation and convergence, and neural tube closure (reviewed by Karfunkel, 1974; Schoenwolf, 1982; Gordon, 1985; Schoenwolf & Smith, 1990).

On the one hand very suggestive results have been obtained by studying cell shape changes, particularly in relation to cytoskeletal elements and interkinetic nuclear migration (Nagele & Lee, 1979, 1980, 1987; Schoenwolf & Franks, 1984; Schoenwolf, 1985; Lee & Nagele, 1985; Nagele et al. 1987; Fernández et al. 1987; Schoenwolf et al. 1988; Smith & Schoenwolf, 1988). In this sense, Lee & Nagele (1988) concluded that intrinsic forces alone are sufficient to cause closure of the neural tube in the chick embryo. However, Schoenwolf et al. (1988) reported that microfilament-mediated constriction of neuroepithelial cell apices is not the major force for medial neuroepithelial cell wedging and elevation of the chick neural plate. Microfilaments may, therefore, only be necessary towards the end of neurulation. These last results show several neurulation events that occur independently of microfilaments, limiting the role proposed for microfilaments as a fundamental intrinsic driving force in neurulation and suggesting the existence of other motor(s) for these processes.

On the other hand, hyaluronidase digestion of the

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extracellular matrix originates convergence and fusion defects in chick embryos (Schoenwolf & Fisher, 1983). Moreover, the role of basal lamina is fundamental in neural crest cell migration (Martins-Green & Erickson, 1986, 1987; Duband & Thiery, 1987; Bronner-Fraser & Lallier, 1988), and may be important in the last phases of neurulation (Martins-Green, 1988). Studies of the basal lamina have in the main been directed in neural crest cell migration; only Martins-Green's (1988) work applied this directly to neurulation mechanisms, giving a new general view of this problem. However, this work was done only in the caudal trunk region. At this level convergence starts before elevation has finished. This fact obscures the interpretation of the mechanisms of both neurulation phases. Furthermore, the neurulation process may present some differences in this region when neural tube closure has already finished over most of its length.

In this work we report new observations in the neuroectoderm-nonneural ectoderm meeting point. These observations were carried out at all axial levels, especially in cephalic and somitic regions of the chick embryos at stages 6, 8, 10 and 12 (Hamburger & Hamilton, 1951). The results may complete the general view enunciated by Martins-Green (1988).

MATERIALS AND METHODS

Fertile Hubbard hen eggs were incubated at 38 °C to obtain embryos at stages 6, 8, 10 and 12 (Hamburger & Hamilton, 1951). Embryos were processed for transmission and scanning electron microscopy.

Transmission electron microscopy (TEM)

Ruthenium red (RR) processing was accomplished according to Luft (1971), and tannic acid (TA) was applied according to Vanroelen & Vakaet (1981). Briefly, 0.1 % RR was added to 2 % glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) and to 1 % osmium tetroxide in the same buffer. TA was added to 1 % glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4); postfixation was carried out using 1 % osmium tetroxide in the same buffer. Fixed embryos were washed 3 times in cacodylate buffer and cut into small pieces (Fig. 1): at stages 6 and 8 the entire area of the neural plate situated cranially at the level of Hensen's node was taken; at stages 10 and 12 the regions corresponding to the 3 encephalic vesicles, somitic region and segmental plate region were taken individually. Further processing was accomplished as for routine TEM, and specimens were embedded in Epon 812 (Merck). Longitudinally well oriented specimens were sectioned transversely (1–2 μm) and stained in 0.5 % toluidine blue in a 1 % aqueous solution of sodium borate. Thin sections from selected areas (Fig. 1) were stained with uranyl acetate and lead citrate. The grids were observed in a JEOL 100CX transmission electron microscope operating at 60 kV.

Scanning electron microscopy (SEM)

Two methods of processing for SEM were employed. Embryos destined for routine SEM were fixed for 2 h with 2 % glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4). After this primary fixation, they were cut transversely at several axial levels with razor blades. Fragments were postfixed with 1 % osmium tetroxide for 1 h, dehydrated in ethanol and critical-point dried in liquid carbon dioxide. Specimens mounted onto aluminium stubs were coated with gold-palladium and examined with a JEOL 35C scanning electron microscope operating at 15 kV.

On other occasions, glutaraldehyde-fixed embryos were embedded in paraffin (Armstrong, 1971; Armstrong & Parenti, 1973), paraplast or polyethylene glycol (Nagele et al. 1984). Serial 10–20 μm sections were mounted in order of cutting on gelatin-coated plastic coverslips of cellular culture. In this way the entire length of the embryos was analysed by means of a series of transverse cuts. Plastic coverslips for this serial study were used because of the ease with which they can be cut with scissors. In this way they can be cut into short lengths which can later be introduced into the microscope without the loss of sections, and several cuts may be analysed at the same time. Sections were disembedded by plunging into toluene (paraffin, paraplast) or water (polyethylene glycol). Then they were rinsed 3 times (15 min each) in 100 % ethanol, critical-point dried and mounted as above. In this way, the entire axial length of embryos at stages 6, 8, 10 and 12 was analysed by 10–20 μm serial transverse sections.

RESULTS

Stage 6

At this stage neuroepithelium forms an incipient neural groove of which neural folds exist at the beginning of the elevation phase. The study of the embryo at the level indicated in Figure 1 and proximate levels shows that both neuroepithelium and nonneural ectoderm rest on an unbroken basal lamina (Fig. 2).

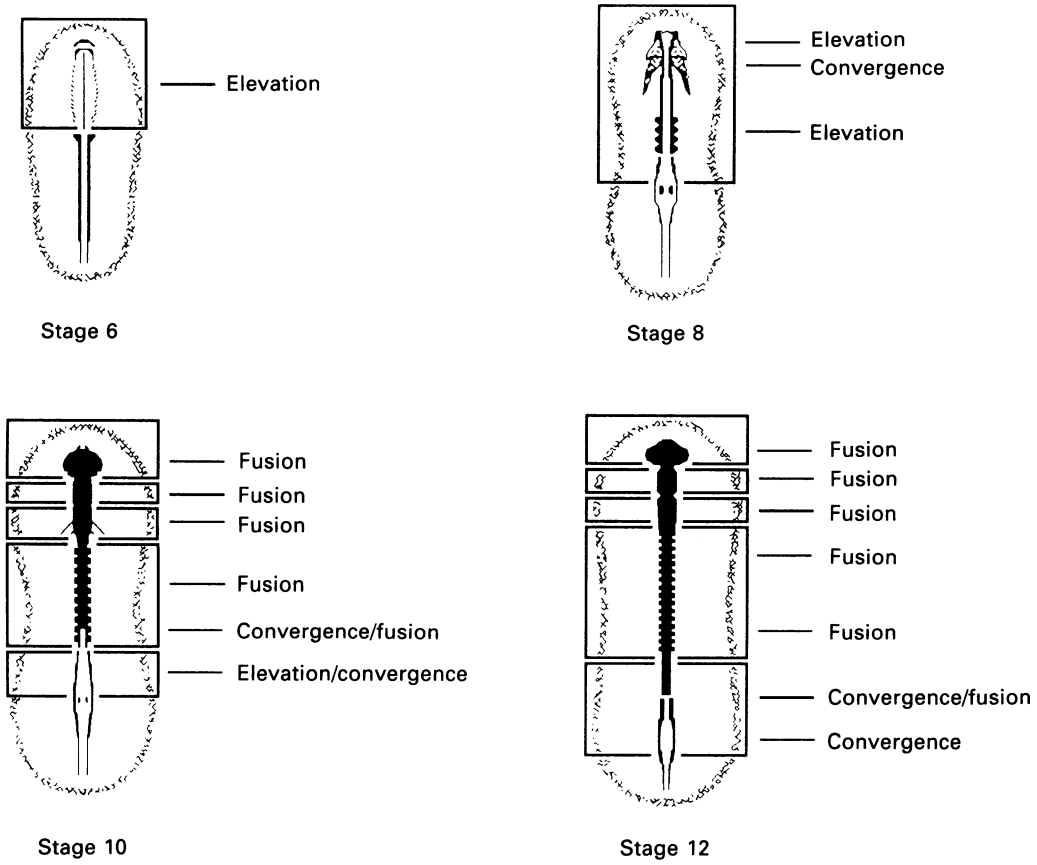

Fig. 1. Regions selected in the different stages of development for individual study. The ultrastructural study was made initially at the levels indicated on the right-hand side of each stage.

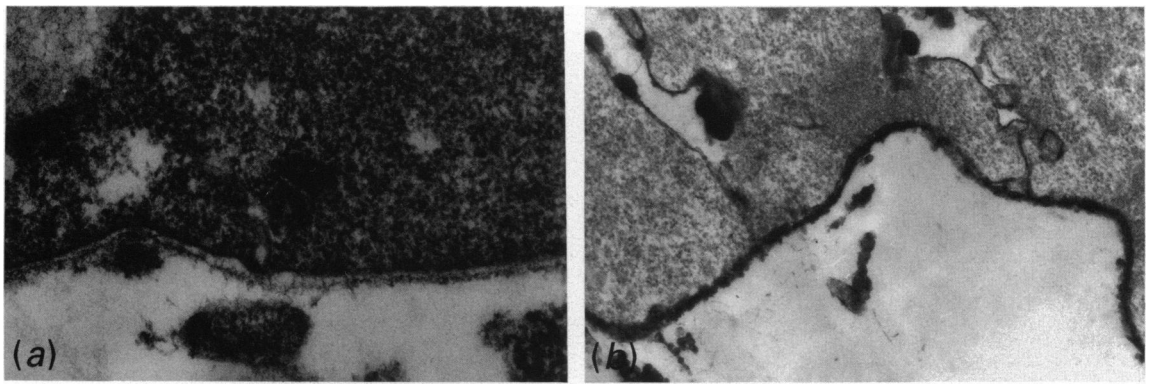

Fig. 2. Transmission electron micrographs of nonneural ectoderm basal lamina TA-stained (*a*) and neuroectodermal basal lamina RR-stained (*b*) from a stage-6 chick embryo carried out at the level indicated in Figure 1. (*a*) Nonneural ectoderm in the area of the neuroectoderm-nonneural ectoderm meeting point. (*b*) Middle region between the medial point of bending and the nonneural ectoderm. *a* × 54000; *b* × 21000.

Stage 8

In the cephalic region, the neural plate forms a deep neural groove. Convergence is wide and starts when elevation finishes, so the cephalic region of a stage 8 chick embryo provides a very suitable model for studying these phases separately (Schoenwolf, 1983).

The morphological characteristics observed in the neuroectoderm-nonneural ectoderm meeting point are morphogenetically important in relation to the different steps of cephalic neurulation. During the elevation phase (V-shaped neural groove), this region remains compact and limited by a basement membrane. At the end of the elevation process, cavitation develops in the confluence of these 2 ectodermal derivatives (Fig. 3*a*). This cavity, initially formed by a

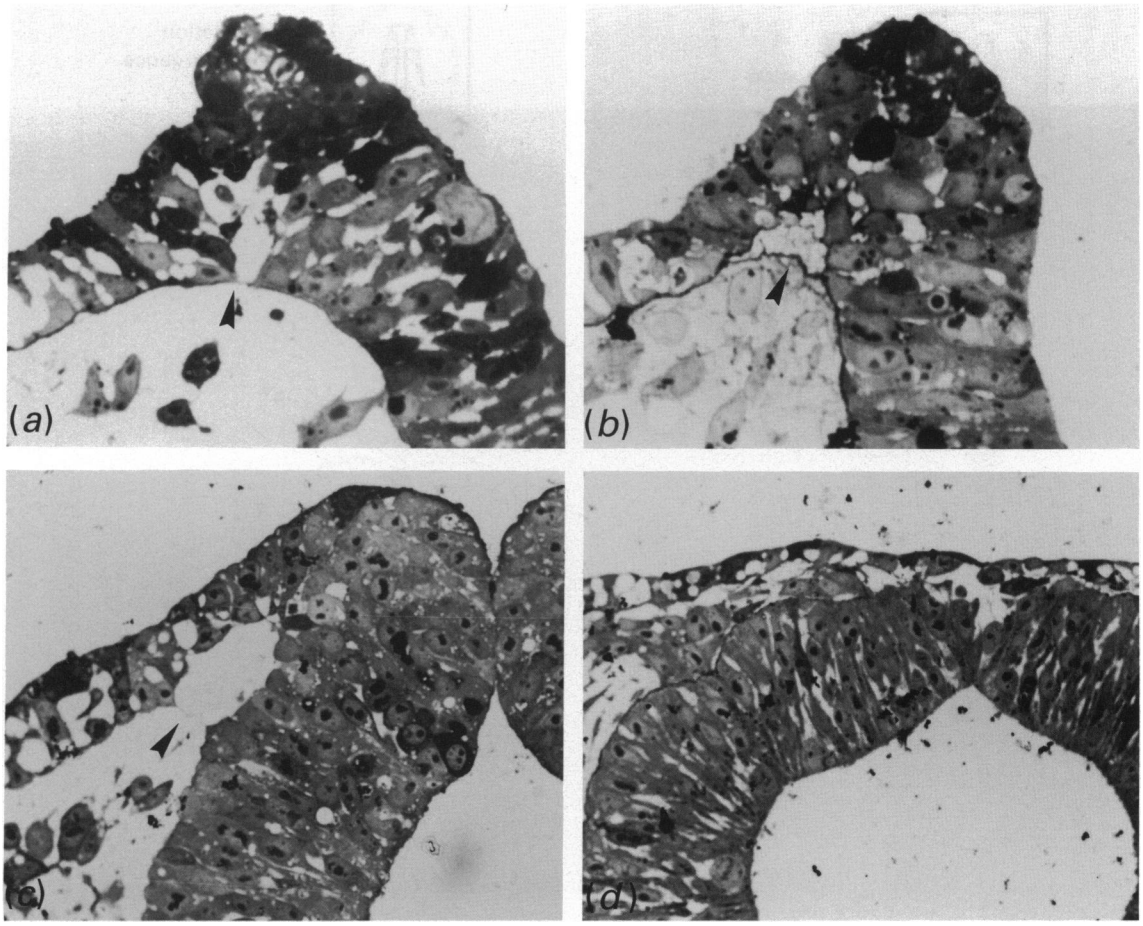

Fig. 3. Delamination process at cephalic level observed with light microscopy of stage 8 (*a,b*) and stage 10 (*c,d*) chick embryos. (*a*) Cavity formation in the elevation phase (arrowhead). (*b*) Basement membrane isolation in the convergence phase (arrowhead); note the presence of a bilateral furrow. (*c*) Neural folds apposition; the isolated basal lamina is much less evident (arrowhead). (*d*) Neural crest cell migration phase; the isolated basal lamina is not a barrier for migration. *a,b,c* × 500; *d* × 350.

progressive cellular de-adhesion, constitutes a wide irregularly shaped extracellular space that broadens until it reaches the basement membrane. Therefore the basement membrane borders a cell-free space at the neuroectoderm-nonneural ectoderm meeting point (Fig. 3*b*). The appearance of this subepithelial space is temporally correlated with the start of convergence. This process implies the formation of a bilateral furrow in each one of the neuroepithelial walls that form the neural groove. It is interesting that the isolated basement membrane should enter into contact with the neuroectoderm at bilateral furrow level; at more ventral levels the basement membrane does not detach from the neuroepithelial cells.

The initial cavitation process is simultaneous with the beginning of convergence, while the appearance of the isolated lamina always implies the existence of a wide convergence. In neither case was one process observed in the absence of the other (Fig. 4).

In TEM observations more conclusive images were found in RR-fixed specimens. Detached basal lamina appears as a thick line widely stained, similar to neuroectoderm and nonneural ectoderm basal laminae, although it is often discontinuous (Fig. 6). This structure borders an extracellular space which contains an extracellular matrix stained as thin fibrils and associated small granules. Morphologically this matrix is more concentrated and homogeneous than the one situated on the other side of the isolated basal lamina. RR-staining differences between them are due to the fibrils in the first being shorter, which determines a fine network, and to a smaller size of granules, giving a more regular and homogeneous image.

At somitic levels, neural groove closure is delayed in relation to the cephalic region and a clear convergence phase does not exist. In stage 8, neuroepithelium forms a neural groove with less elevated folds, and usually with straight walls (Fig. 5).

The described morphological features of the tip of the neural folds at cephalic levels do not appear in the somitic region in this stage. In some sections only,

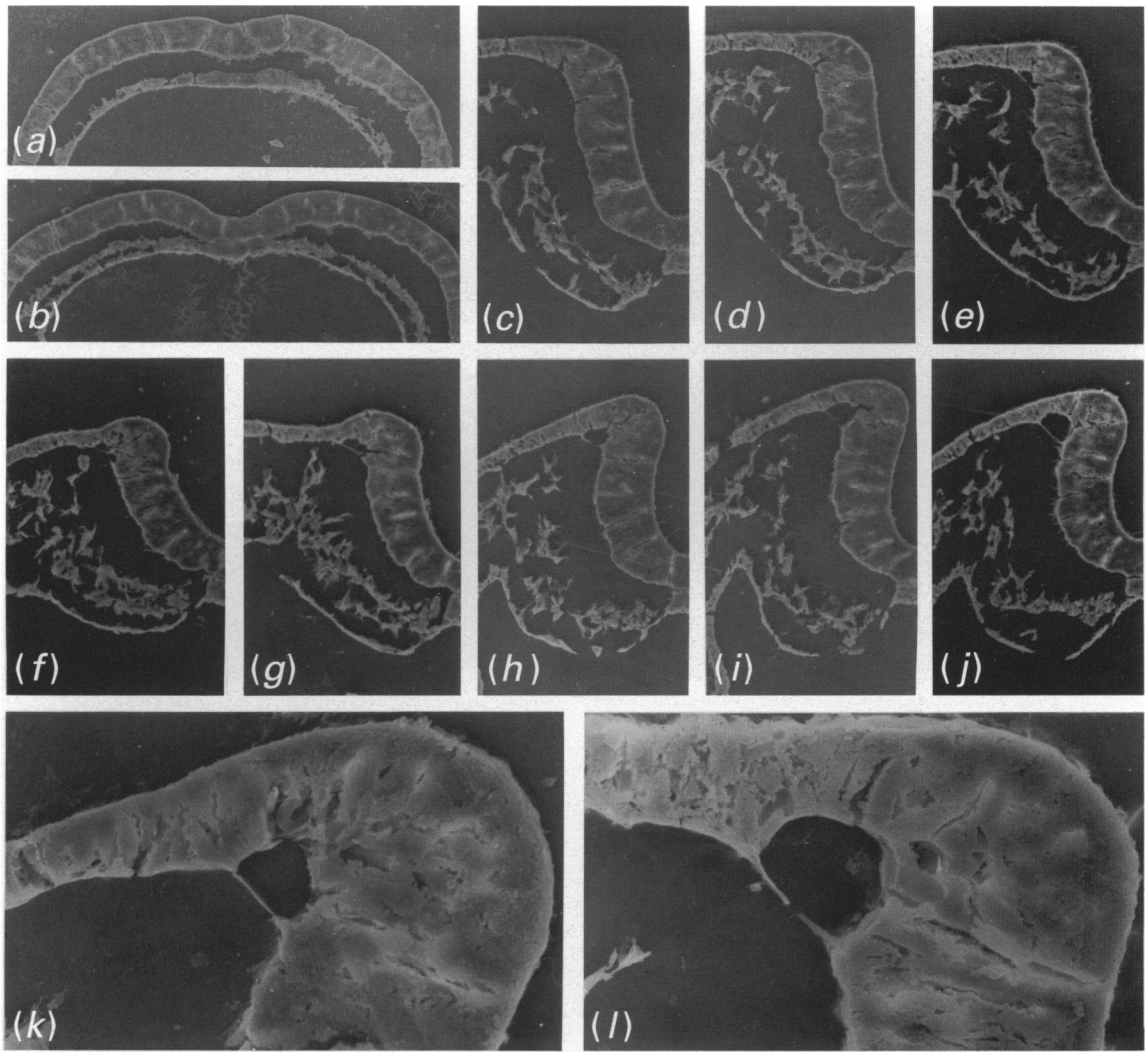

Fig. 4. Scanning electron micrographs of transverse sections through the cephalic region of chick embryos (*a*, *b*, stage 6; *c–l*, stage 8) showing the progressive cavitation and basement membrane isolation and its relation to the convergence phase. *a*, *b* × 120; *c–j* × 170; *k*, *l* × 700.

cavitation is observed in the cranial somitic levels, similar to that previously described, coinciding with a small degree of convergence (Fig. 5*a*, *b*). In these sections, the hinge point is situated in a very dorsal position near the terminal end of the groove walls. The result is a slight degree of convergence. Detachments of basement membrane from the cells at these levels were never observed. Moreover, in TEM observations no extracellular RR-stained material appears in the cavities occasionally formed at the neuroectoderm-nonneural ectoderm meeting point, at least not in considerable quantity.

Stage 10

At this stage, the neural tube is already closed in the cephalic region except for the cranial neuropore.

In the cephalic region the closure of neural tube starts when neural folds enter into contact. At this moment, the neural and nonneural ectoderm are in apposition by their basal surfaces in which 2 discontinuous newly formed basal laminae border a narrow space (Fig. 3*c*, *d*). The isolated basal lamina described in the tip of the neural folds of stage 8 embryos in relation to the convergence step no longer appears at this stage in which the neural folds are in the fusion phase. The disappearance of this isolated basal lamina causes the pre-existing neuroectoderm and nonneural ectoderm basal laminae to be extended at this point by these basement membranes which are formed on the basal surface of the neuroectoderm and the nonneural ectoderm in the region of epithelium which had been left uncovered through the detachment of the basal lamina, as described in stage 8 (Fig. 3*c*, *d*). The morphology of these newly formed

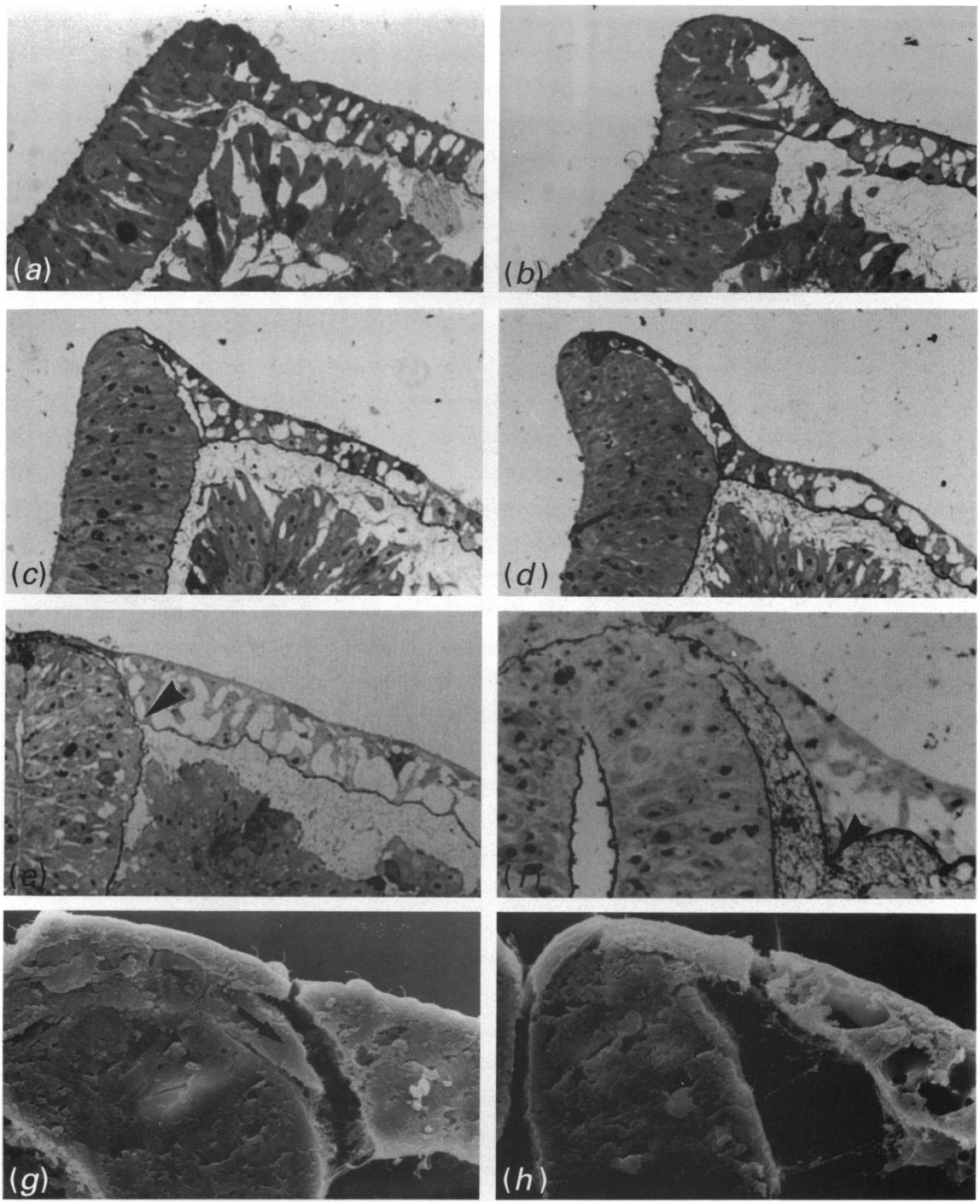

Fig. 5. Delamination process at somitic level observed with light and scanning electron microscopy of stage 8 (*a,b*) and stage 10 (*c–h*) chick embryos. (*a,b*) Formation of a small cavity which coincides with the beginning of the convergence phase. (*c,d*) Formation of a half-moon-shaped cavity; this cavity is present in both elevation (*c*) and convergence (*d*) phases. (*e,f*) Neural folds apposition (*e*) and fusion (*f*); the ventral attachment point of nonneural ectoderm to neuroectoderm (arrowheads) comes off the neuroectoderm and moves from it when the tube closes. (*g,h*) Scanning electron micrographs of ventral attachment point separation and neural crest cell migration (arrow); note the orientation of extracellular fibrils. *a,b* × 400; *c–e* × 300; *f* × 500; *g,h* × 725.

basement membranes has been exhaustively described (Martins-Green & Erickson, 1986, 1987; Duband & Thiery, 1987; Bronner-Fraser & Lallier, 1988). Initial neural crest cell migration takes place across this narrow space; then this space progressively widens, separating the 2 ectodermal derivatives. The discontinuous basement membrane is completely formed only when the migration finishes.

At somitic levels, the neuroectoderm forms a tube at cranial levels, whereas at caudal levels it is still a

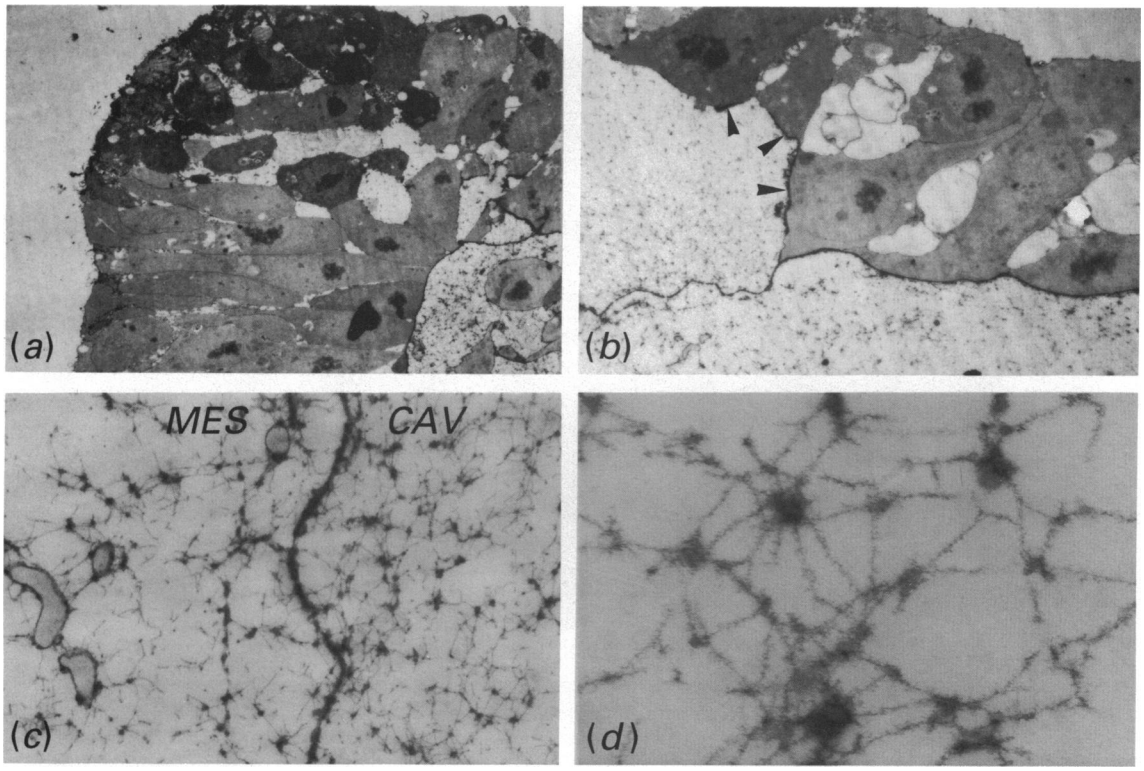

Fig. 6. Delamination process at cephalic level in stage-8 chick embryos, observed with transmission electron microscopy of RR-fixed specimens. (*a*) Cavitation phase. (*b*) Basement membrane isolation and formation of a new basement membrane (arrowheads). (*c*, *d*) Extracellular matrix RR-stained of this region; note the differences between the extracellular matrix present in the cavity (*CAV*) and the general mesodermic extracellular matrix (*MES*). $a \times 825$; $b \times 1600$; $c \times 8725$; $d \times 50000$.

groove. In this last case, the nonneural ectoderm enters into contact with the neuroectoderm at the highest point. Superficial ectoderm cells and neuroepithelial cells are in contact only at 2 points: dorsally in the fold apex, and ventrally in the folding points that form the bilateral furrows (Fig. 5*c*, *d*). There is a narrow gap free of cells between the 2 attachment points. After tube closure, the ventral attachment point detaches itself and gradually moves away from the neuroectoderm (Fig. 5*c*, *h*).

The attachment points are understood as being the only 2 points at which the cells of the nonneural ectoderm make contact with the neuroectoderm at somitic level when the neural groove has not yet closed up (one dorsal and the other ventral) (Fig. 5*c*, *d*); TEM observations show that basal laminae from both epithelia only join in the dorsal attachment point. At the level of the ventral point of attachment which establishes the neuroectodermic and nonneural ectodermic cells in the folding points that form the bilateral furrows (Fig. 5*c*, *d*), the 2 basal laminae do not come into direct contact although they are very close. The short distance between them appears obliterated by very strongly RR-stained amorphous matrix material (Fig. 7).

The delamination process in the somitic region observed by these means agrees with Martins-Green's (1988) observations at more caudal levels. However, our results differ from hers in the nonexistence of continuity in neuroectoderm and nonneural ectoderm basal laminae at the ventral attachment point. Therefore, we think that delamination implies basal lamina isolation in the trunk region.

The cavity described contains some RR-stained matrix elements: thick granules which appear always associated with the basal lamina, thin granules distributed throughout the cavity, and some fibrils. However, this stained matrix is very scarce in relation to the cephalic region.

Stage 12

Stage 12 chick embryos show a closed neural tube at all axial levels except for the caudal neuropore. Neural crest cell migration is a very extensive process along the dorsal surface of the neural tube, particularly in the cephalic region. Disappearance of isolated basal lamina in stage 10 (Fig. 3*c*, *d*) allows the cells to migrate across a very incomplete basal lamina, widening the gap between neuroectoderm and non-

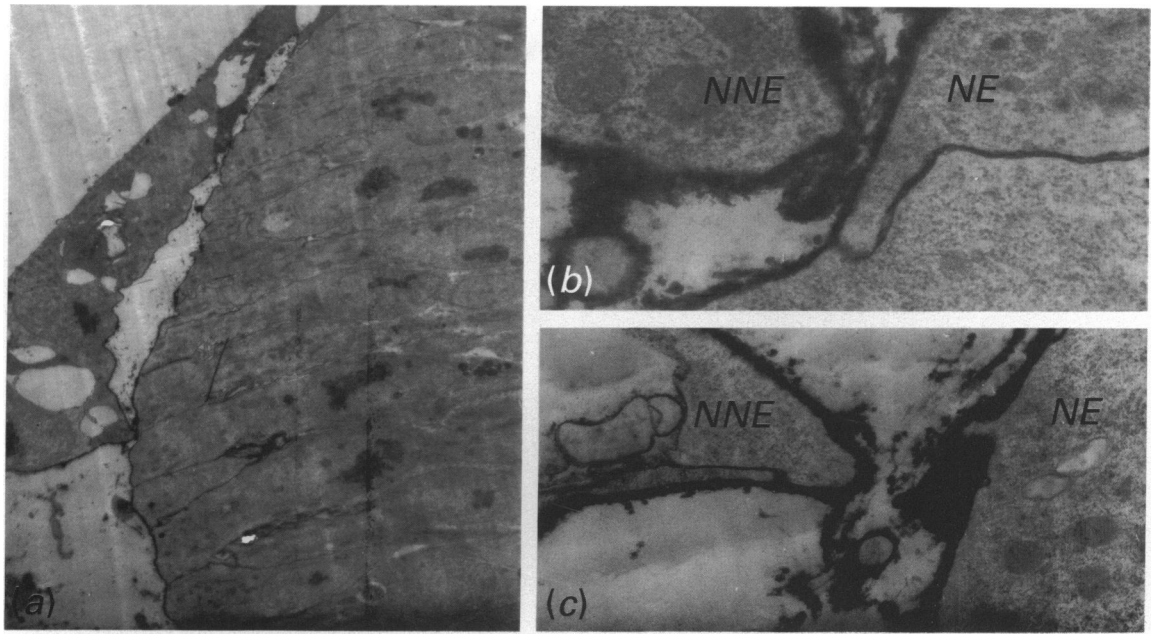

Fig. 7. Delamination process at somitic level in stage 10 chick embryos, observed with transmission electron microscopy of RR-fixed specimens. (*a*) General view of the cavity formed in the neuroectoderm-nonneural ectoderm meeting point; note the absence of basement membrane isolation at this level. (*b*,*c*) Ventral attachment point of nonneural ectoderm (*NNE*) to neuroectoderm (*NE*) showing the lack of continuity at this point between the nonneural ectoderm basement membrane and neuroectoderm basement membrane (*b*) and the beginning of its separation (*c*). *a* × 740; *b* × 34670; *c* × 17350.

neural ectoderm. At somitic levels, the neural tube is also closed and nonneural ectoderm and neuroectoderm cells only are linked in the fold apex (dorsal attachment point). The neural crest cell migration occurs across the nonneural ectoderm and neuroectoderm separation. Therefore, at cephalic as well as somitic levels, the obstacles to the migration of these cells disappear: at cephalic level, the isolated basal lamina disappears and at somitic level, the ventral attachment point which linked the nonneural ectoderm to the neuroectoderm disappears. In both cases the migration takes place across a very incomplete, newly formed basal lamina.

DISCUSSION

Moury & Jacobson (1989) emphasised the central role of the boundary between the neural plate and the epidermis in axolotl neurulation. They concluded that neural folds result from conditions at this boundary, and suggested that local interactions of the cells at this point are sufficient to raise the neural folds.

When Martins-Green (1988) first described a delamination process at the boundary between the neuroepithelium and the nonneural ectoderm of the chick neural groove as a morphogenetically important phenomenon, this description was welcomed as a possible new explanation of neural tube formation. In this work, we analysed this process over the entire length of the chick embryo and in various stages of primary neurulation, since morphogenetic mechanisms of neurulation would not be identical in the different regions and stages. In our results, considerable differences were found between the cephalic and somitic regions. The results establish a constant temporal relation between basal lamina isolation (but not simple delamination) and cavity formation at the tip of neural folds, exclusively with one step of neural morphogenesis: the convergence step.

Cephalic convergence

Neural tube formation in the cephalic region is characterised by an important degree of convergence which initially determines a diamond-shaped neural tube, by bending of the neural walls through 2 deep bilateral furrows. These bilateral furrows show special cellular characteristics consisting of an increase of bottle-shaped cells (Schoenwolf & Franks, 1984), which appear very suggestive but are not conclusive (Schoenwolf & Smith, 1990). In this regard, the intervention of apical bands of microfilaments in the apical constriction that forms the wedge-shaped neuroepithelial cells has been widely accepted. However, cytochalasin-D treatment does not have the same effect on the wedge-shaped cells existing at the 2

points of bending from the cephalic neural plate in the chick embryo: the medial supranotochordal furrow and the bilateral furrows (Schoenwolf et al. 1988). This fact suggests that 2 different mechanisms of bending are implied in elevation and convergence respectively. Smith & Schoenwolf (1989) concluded that cell wedging is not required for bending although it plays a major role in generating the normal morphology of the neural tube.

It is known, moreover, that hyaluronidase treatment likewise causes alterations in convergence, suggesting a role for extracellular polysaccharides in this process (Schoenwolf & Fisher, 1983). Therefore the convergence mechanism can consist in an interrelated and synchronised action of cytoskeletal and extracellular agents. In mouse development, Copp & Bernfield (1988*a*, *b*) pointed out the existence of a correlation between an accumulation of basement membrane-associated hyaluronate and normal neural tube closure, and between a reduction of this accumulation and neural tube defects.

In the extracellular factor, however, there were no morphological observations that could reliably show the possibility of its action. The present study agrees with Martins-Green's (1988) report, which also provided morphological observations which support the possibility of a determinant action of extracellular matrix in neurulation.

Serial study of the convergence phase at cephalic levels allows us to establish a correlation between the degree of convergence in a particular section and the extracellular matrix characteristics of the neuroectoderm-nonneural ectoderm meeting point.

The detached basal lamina is of the same approximate thickness as the contiguous basal laminae, but its structure changes. Thus it appears in RR-staining to be composed of granules and an amorphous very electron-dense material, with occasional small interruptions. Judging from RR-staining, this lineal succession of granules and amorphous very electron-dense material seem to contain a high relative proportion of proteoglycans. The observation of detached basal lamina in routine sections is very difficult, and previous studies in this region using monoclonal antibodies did not show these structures (Martins-Green & Erickson, 1986, 1987; Duband & Thiery, 1987; Bronner-Fraser & Lallier, 1988); this difficult observation in conventional TEM can result from the extraction of its, hypothetically, main component. RR is a protective component which prevents proteoglycan depletion.

RR-staining of the extracellular matrix present in the subepithelial space is morphologically different from that observed on the other side of detached basal lamina. This morphology in RR-staining suggests that they are proteoglycans (Mayer et al. 1981).

After the appearance of the detached basal lamina, a series of electron-dense patches form on the basal surface of cells that border with this cavity dorsally. This process is very similar to that observed by Csato & Merker (1983) in the formation of basal laminae in mouse embryonic tissues studied by RR-staining.

These facts seem to support a hypothesis on the mechanism of neural fold convergence in the cephalic neurulation of the chick embryo. The local production of a special extracellular matrix at the neuroectoderm-nonneural ectoderm meeting point, bounded by a small detachment of the basal lamina, increases in volume by new synthesis or by hydration, bending the neuroepithelial walls exactly through the point where the detached lamina and neuroectoderm join. This hypothesis would require a certain rigidity in the surface ectoderm so that the hypothetical generated forces were transmitted in medial direction. The nonexistence or incompleteness of the basal lamina in the neuroectodermic basal surface of this region may make this epithelium more sensitive to deformation in this point. This mechanism could be entirely or partly responsible for cephalic neural fold convergence.

Some authors have already suggested the possible role in neurulation of an increase in the volume of the extracellular matrix beneath the neural folds (Morriss & Solursh, 1978*a*, *b*). However, they have not provided morphological facts which support this idea, basing this possible role only on experimental data from treatment, fundamentally with hyaluronidases (Schoenwolf & Fisher, 1983). The present paper is in agreement with Martins-Green's (1988) report for caudal levels and supports this morphological possibility.

Tosney (1982) reported the existence of a longitudinal ridge developed from a zone of close apposition between the neural tube and the nonneural ectoderm. Newgreen (1989) confirmed this fact by light microscopy of transverse slices through the midbrain level from quail embryos. He reported a balloon-like increase in the space between the neural tube and superficial ectoderm limited by the apposition. In Newgreen's work, however, the formation of this space starts in the already closed neural tube (see Fig. 4*a*, *b*). He deduced that the expansion of this space depresses neighbouring portions of the neural tube already formed, causing the apposed region to form a ridge. Consequently, the zone of apposition could be a point of adhesion between the 2 tissues, and later the space increases and the apposed tissues

separate. In the present study we show that the zone of apposition is not a point of adhesion and that it originates before neural tube closure.

Somitic convergence

At somitic level, nonneural ectoderm remains in contact with the neuroepithelium at only 2 points (dorsal and ventral attachment points), so that a half-moon-shaped cavity separates the 2 epithelia. Our observations on the formation of this cavity agree with Martins-Green's (1988) observations. However, RR-staining shows that pre-existing basal lamina does not remain long at the ventral attachment point, and quickly appears as an electron-dense and amorphous material that occludes the short distance between the 2 epithelia. Therefore, in this region detachment and isolation of basal lamina do not occur. The basal lamina is simply replaced at the ventral attachment point by amorphous material that isolates the cavity from the rest of the extracellular matrix. Neuroectodermic and nonneural ectodermic basal laminae are continued by newly formed basal laminae present in the cavity.

The ventral attachment point is situated always at bilateral furrow level (when it exists) and it disappears when neural folds are closed. These facts suggest, as in the cephalic region, a possible role for this cavity in the small degree of convergence that can occur at this level, acting by an increase of volume or pressure that would direct neural folds medially. Proteoglycans seem to be scarce in this region, however, judging from the poor RR-staining that appears here. In this regard, Brauer & Markwald (1988) have reported the existence of differences between cephalic and trunk regions in the distribution of glycoproteins and polyanions in the dorsal apex of the neural tube before the appearance of neural crest cells. These differences could be related to this different RR-staining. In a recent study (Tuckett & Morriss-Kay, 1989) heparitinase treatment of rat embryos has shown that specific degradation of heparan sulphate leads to an inhibition of cranial neurulation, but it does not inhibit neural tube closure in the trunk. Heparan sulphate is a polysaccharide closely associated with basement membranes. These facts provide further evidence that different morphogenetic mechanisms are involved for the 2 regions.

In our results, somitic convergence is more related with the small cavity produced in stage 8 than with the half-moon-shaped cavity present in stage 10. In any case, the absence of basal lamina isolation and scanty presence of RR-staining (proteoglycans) in the cavity formed, is associated in the somitic region with an absence or a very low degree of convergence.

In summary, the differences described between the cephalic and somitic regions can be related to the different convergence mechanics of the 2 regions.

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