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0014-4754/88/010058-03$1.50 + 0.20/0

Intrinsic forces alone are sufficient to cause closure of the neural tube in the chick [1]

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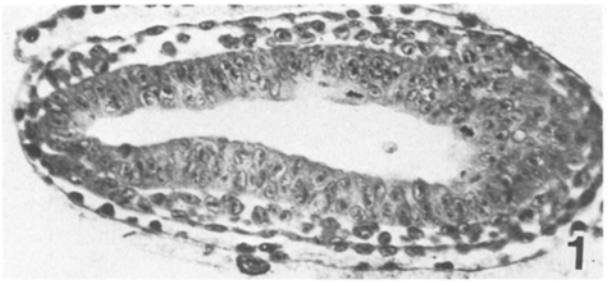
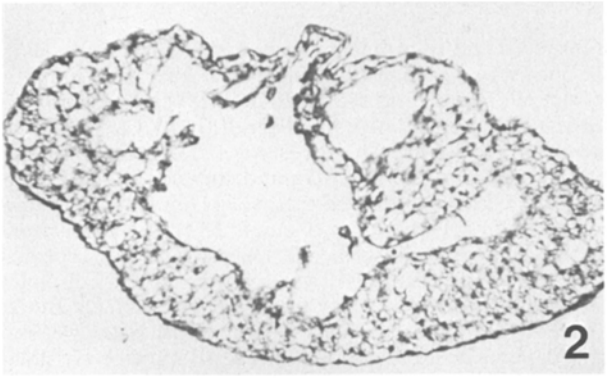
Summary. An isolated neural plate or a postnodal piece of early chick embryos, when cultured under appropriate experimental conditions, can undergo morphogenetic movements and form tubular structures closely resembling neural tubes of early chick embryos.
Key words. Chick embryo; neurulation; intrinsic forces.

Neurulation represents a situation whereby a flat neuroepithelial sheet (the neural plate) progressively changes its shape to become a hollow cylinder (the neural tube). This shape change is caused by forces originating from within neuroepithelial cells (intrinsic forces) and/or from outside the neuroepithelium (extrinsic factors)[2]. Waddington and Perry[3], working on the developing amphibian neuroepithelium, originally reported that an apicobasal alignment of microtubules coincide with a period of elongation of neuroepithelial cells. This and similar observations on other vertebrates such as birds and mammals[4, 5] have formed the basis for the hypothesis that microtubules are involved in the formation and maintenance of the elongated shape of neuroepithelial cells. Baker and Schroeder[6] proposed that immediately following the elongation of neuroepithelial cells, apical microfilaments constrict in a 'purse-string-like' fashion to generate a wedge- (or bottle-) shaped appearance. In support of this idea, numerous subsequent studies[4, 5, 7] have shown that chemical agents (e.g., cytochalasins, local anesthetics, calcium agonists and antagonists, and calmodulin inhibitors), which interfere with microfilament-dependent cellular processes in many other developing systems, inhibit apical constriction of neuroepithelial cells and cause a variety of neural tube closure defects in vertebrates. This finding strongly suggests that the major driving forces for closure of the neural tube originate from microfilament-mediated changes in the shape of neuroepithelial cells (especially apical ends). However, some investigators still believe that extrinsic forces (e.g., tension generated by pulling of the elongating notochord and mediad pushing forces exerted by the somites, perineural extracellular matrix and expanding surface ectoderm) play a key role in neural tube closure[5, 8]. To clarify this uncertainty, we present several lines of evidence to demonstrate that intrinsic forces (e.g., cytoskeleton-mediated cell shape changes, cell rearrangement and programmed cell division) alone are sufficient to bring about closure of the neural tube (especially the cephalic region) in the chick.

Materials and methods. Fertile White Leghorn eggs (Shamrock Poultry & Breeding Farm, North Brunswick, New Jersey) were incubated at 37.5 °C to obtain embryos at stages 4–6 of development[9]. Some embryos were explanted either dorsal side up or down using New's[10] technique and grown for about 15 h in nutrient medium (thin albumen). Others were removed from the vitelline membrane and used in the following two experimental series: In the first series, a piece of the neural plate ($0.5 \times 0.5\ mm^2$) was removed from the region just anterior to the primitive streak at each stage 5 or 6 embryo and grown in Medium 199 (Grand Island) at 37.5 °C in an atmosphere of 5% CO_2 in air. After 24–36 h of incubation, explants were fixed and processed for microscopy[11, 12]. In the second series, the area opaca was trimmed off and the remaining area pellucida was transected 0.6 mm posterior to Hensen's node as previously described[11]. The posterior portion, which is now referred to as the postnodal piece (PNP), was grown in nutrient medium (avian Ringer's solution-whole egg extract) with or without nerve growth factor (NGF)[12]. After 4 days of incubation, PNPs were fixed, serially sectioned at 4 µm, and examined microscopically for identifying recognizable (differentiated) structures.

Results and discussion. Nearly all (23 out of 24) chick embryos, which were explanted either dorsal side up or down using New's[10] technique, underwent neurulation indistinguishable from one another. This finding suggests that tension generated by the gravity and by the weight of the blastoderm has no effect on neural tube closure. Similarly, all (34) neural plate explants underwent morphogenesis to form tubular structures closely resembling the 'C'-shaped neuroepithelium or the closed neural tube of early chick embryos (fig. 1). This is probably the best evidence that the forces for neural tube closure originate from within individual neuroepithelial cells, because almost all of the outside influences, that could conceivably contribute to bending of the neuroepithelium, have been eliminated. As for PNPs, no axial structures were observed in any of the 18 untreated controls (fig. 2), a finding consistent with that of previous studies[11–13]. In contrast, all (36) of the PNPs grown in the presence of 10 ng/ml NGF were induced to undergo neuralization ranging from a local thickening of the epithelium (resembling a neural plate) to a completely closed neural tube (fig. 3). In addition, the induced neural tissue showed no preferential orientation and dimension, and in many instances, there were two or more regions with varying degrees of neuralization in a single PNP (fig. 3). We also have learned over the years that 1) the cephalic regions of early chick embryos are devoid of organized mesodermal structures (e.g., somites); they can nevertheless elevate their neural folds and 2) a section of the chick neural plate, when isolated, turned over and put back in place, proceeds to form a neural tube in an inverted orientation. These and other related findings strongly suggest that intrinsic forces alone are sufficient to bring about closure of the neural tube. There has been some speculation that mesodermal structures play a primary role in uplifting and apposition of neural folds[5]. Although





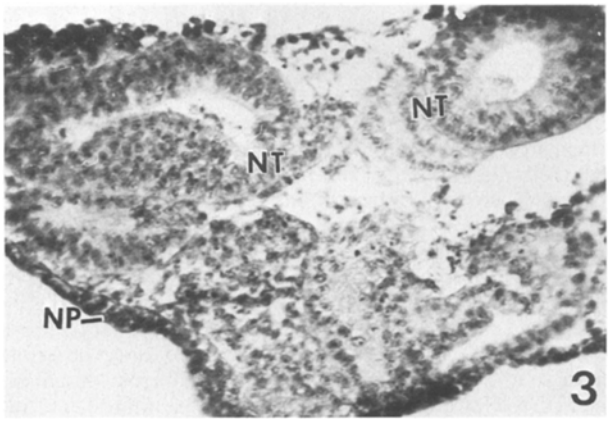


Figure 1. Light micrograph showing a transverse section through a neural plate explant after 28 h of cultivation in Medium 199. This neural tube closely resembles that of an early chick embryo. × 240.

Figure 2. Light micrograph showing a section of a control postnodal piece grown for 4 days in plain nutrient medium. This and other controls showed no identifiable (differentiated) structures. × 30.

Figure 3. Light micrograph showing a section of a postnodal piece grown for 4 days in the presence of 60 ng/ml nerve growth factor. This section shows a neural plate-like structure (NP) and two neural tubes (NT). × 140.

the formation of somites may contribute somewhat to bending of the neuroepithelium in the future spinal cord region, the evidence presented above seems to weigh against this idea.

Langman, Guerrant and Freeman[14] proposed that interkinetic nuclear migration, during which neuroepithelial cells undergo drastic changes in shape, may in some way serve to facilitate neural tube closure mechanically by accumulating a large number of wedge- (or bottle-) shaped cells in regions inquiring bending (e.g., the midline of the V-shaped neuroepithelium and midlateral walls of the 'C'-shaped neuroepithelium). This view was later confirmed by Schoenwolf and Franks[15] who systematically determined the percentages of wedge-shaped and other cell types in various regions of the developing chick neuroepithelium. They concluded that bending of the neuroepithelium is immediately preceded by a localized increase in the percentage of wedge-shaped cells. Our recent morphometric study[16] has shown that the degree of apical constriction and apical surface folding of chick neuroepithelial cells is not uniform throughout the developing neuroepithelium, suggesting that certain regions contribute more to shaping the forming neural tube than others. This finding is in line with those of indirect immunofluorescence studies which have revealed the presence of a high concentration of motility-related proteins (e.g., actin and myosin) in the apical regions of neuroepithelial cells directly involved in bending of the neuroepithelium[17, 18]. Further studies are needed to determine the exact origin of driving forces for bending of the neuroepithelium and how these forces are distributed within the neuroepithelium at different phases of neural tube formation.

1 This work was supported by grants from the NIH (NS 23200 and NS 21730) and the Busch Fund of Rutgers University.

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