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A review of the theories of vertebrate neurulation and their relationship to the mechanics of neural tube birth defects*

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SUMMARY

All of the published theories of neurulation, (some of them forgotten but never disproved), are reviewed for the purpose of assessing just where we are in coming to a satisfactory explanation of this critical step in the formation of the brain and spinal cord, whose occasional failure leads to neural tube birth defects. A new approach to evaluating these theories is introduced, namely finite element analysis, along with a discussion of its promise and present limitations.

'*To think that heredity will build organic beings without mechanical means is a piece of unscientific mysticism*' (His, 1888).

INTRODUCTION

Neurulation is an unsolved process whose unravelling has occupied embryologists since the time of His (1874) and Roux (1888). Numerous hypotheses have been proposed over this time to explain the mechanism of neurulation and its cellular basis. Most of these have been expressed qualitatively and have not been evaluated for their ability to quantitatively predict neurulation, nor have they been eliminated as reasonable explanations. In what follows I will review the theories of neurulation and their bearing on errors in neural tube closure that produce congenital malformations of the brain and spinal cord (anencephaly and spina bifida, *cf.* Recklinghausen, 1886; Källen, 1968). (I include the material covered by Karfunkel (1974), Jacobson & Gordon (1976*a*) (*cf.* Jacobson & Gordon, 1976*b, c*) and Gordon & Jacobson (1978).) I will also present a new approach to testing models, based on finite element analysis, that holds promise for explaining the origin of the neural tube defects.

Trinkaus summarized the state of knowledge regarding formation of the neural plate and neural tube in 1969 as follows:

> '... the descriptive aspects of the folding movements of the neural plate during formation of the neural tube ... have been studied so exhaustively with vital dyes and transplantation that from a descriptive point of view neurulation is one of the best understood aspects of development. It is therefore particularly frustrating that the mechanism whereby this

*Dedicated to Leslie J. Biberman.

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> deceptively simple process occurs has until now resisted all analysis. Valiant attempts to discover the key have been made several times during the past century, beginning with Wilhelm His (1874). But all proposals have either been since disproved or rest unproved for lack of sufficient evidence.'

The wording changed little in the second edition (Trinkaus, 1984).

The human impact of solving this problem could be considerable. Congenital defects represent a rising fraction of early childhood disorders (Saxen & Rapola, 1969). In *anencephaly* the brain is severely reduced or missing, a condition which often comes to term but is always fatal (Hanaway & Welch, 1970). It is observed in

> '...0·1 to 6·7 per thousand births, making it the most common central nervous system malformation incompatible with life'

(Shulman, 1974). *Spina bifida*, on the other hand, involves incomplete formation of the spinal cord and occurs in the non-occult form about half as often as anencephaly (Shulman, 1974; Mann, 1977). The result is most often paralysis of the extremities, but otherwise the condition is compatible with life (Althouse & Wald, 1980), especially if surgery is carried out promptly to cover up exposed neural tissues:

> 'The survival of an increasing number of affected infants poses serious social and economic problems'

(Mann, 1977). No etiologic agent has been found for these malformations (Nakano, 1973; Roberts & Powell, 1975). Flat statements such as

> 'the causation of neural tube defects is unknown'

(Brock, 1982) are rife.

Theories of neurulation

Lateral compression by the epidermis

It has sometimes been assumed (His, 1874) that the epidermis lateral to the neural plate expands actively and in doing so compresses the neural plate, making it change shape and buckle. His (1894) conducted an extensive series of experiments on the manner of bending of sheets of many materials, including metal, clay, paper, cardboard and complex laminates, in an effort to understand how lateral compression leads to neural tube rolling and closure. Schroeder (1970) suggested that epidermis

> '...forcibly aids closure of the neural tube by pushing the neural folds together...',

as have Bragg (1938), Selman (1955) and Jacobson & Jacobson (1973). However, the notion of lateral compression had been dismissed already by Roux (1888) (*cf.* Giersberg, 1924; Weiss, 1955; and Karfunkel, 1974):

> '...I was able to demonstrate, by separation of their primordia from the parts lateral to them, that... in spite of their isolation the development of the primordia was completed, and even faster than normally. According to this we should look to the formative causes effective in the development of these tubes in the parts which compose the tube itself, while the neighboring regions even offer a resistance to the development of the tubes, which must

> gradually be overcome . . . the elevation of the neural folds on the material of the neural plate does not occur passively from the pressure of the lateral parts . . .'

Picken (1956) suggests that His changed his view to encompass that of Roux. Karfunkel (1974) agrees with Roux that the epidermis

> '. . . restrains the neural folds from approaching each other.'

This contrary effect may be due to epidermal tensions. Lewis (1947) found that a slit between the neural plate and the epidermis gapes wide open:

> 'This indicates that the general ectoderm exerts contractile tension in all directions and opposes the invagination of the neural plate.'

Burnside (1971) noted that microfilaments of the epidermal cells

> 'are usually straight as though under tension.'

Lewis' observations were repeated by Jacobson & Gordon (1976*a*), who found that:

> 'Wherever and whenever the epidermis is slit, the gape is large. The direction of slit makes no difference in the epidermis. The result is always a large round gape. One can conclude that . . . the epidermis is under a considerable tension which is uniform in every direction. These experiments suggest that the epidermis cannot possibly be 'pushing' on the neural folds to augment neurulation movements . . . Since isolated early neural plates will complete neurulation, including forming a neural tube, in the absence of the epidermis, the tension provided normally by the epidermis appears not to be an essential force in neurulation.'

Could some failures of neural tube closure be due to excess epidermal tension? Rough measurements of the force needed to stop closure were performed by Waddington (1939, *cf.* Waddington, 1942; Waddington, 1956) using a small metal sphere pulled by a magnet. Since this was done on an intact embryo, the tension from the epidermis was also present, resulting in an underestimate. (*cf.* Holtfreter's, 1943, criticism and Jacobson's, 1978, discussion of Selman's, 1958, further experiments with magnets.) Exactly how was the load, stress and strain distributed over the tissue, an important, but overlooked detail if one is to view the process from the perspective of mechanical or structural engineering? (*cf.* Hertel, 1966.) What is the relationship between single measurements, as with magnets, and the forces exerted by individual cells? These questions are approachable by the finite element methods below.

Migration of the neural folds

The issue of the role of the epidermis has been somewhat confused by the observation of Jacobson (1962, *cf.* Jacobson, 1970) that an embryo whose neural plate has been removed, leaving the epidermis and attached neural folds, appears to undergo normal neurulation. He attributed this to active migration of the neural folds. Karfunkel (1974) has tested this notion by more detailed surgical experiments and concludes

> '. . . that the mediad migration of the neural folds observed after the neural plate is removed merely constitutes wound healing.'

Jacobson & Gordon (1976*a*) also observed wound healing of the gaping cuts made to analyse unresolved tensions. This embryonic wound healing, prior to significant cellular differentiation, working against the epidermal tension, may involve microtubules (Stanisstreet & Panayi, 1980; cf. Smedley & Stanisstreet, 1984).

Forces generated by the mesoderm

The mesoderm in the newt is also a monolayer epithelium of similar size, shape, and movements to the neural plate (Jacobson & Löfberg, 1969). These movements of this underlying tissue have frequently been implicated in neurulation. Boerema (1929) transplanted newt neural plates to the belly attempting to discount the role of the mesoderm. His result was inconclusive, however, because he

> '... did not consider the possibility that such a transplanted neural plate might induce the host mesoderm under it to proliferate and thus act to cause elevation of the donor neural folds...'

(Karfunkel, 1974). Later

> 'Schroeder (1970) ... noted that the elevation of the neural folds seems to be at least enhanced by the thickening of the mesoderm found to lie immediately beneath these folds, correlated with the formation of somites by that mesoderm. This seems to be the case for *Xenopus* but not for either urodeles or chick embryos'

(Karfunkel, 1974). Youn, Keller & Malacinski (1980) point out that:

> 'there exists an elevated ridge at each side of the lateral mesoderm, which appears to resemble the contours of the neural folds. The elevated ridges are most sharply defined in *Ambystoma*, less so in *Rana* and *Pleurodeles*, and not easily detectable in *Xenopus*. With further development those mesodermal contours migrate mediad as the neural folds do... However, it is not clear at this point whether such elevation and migration of the mesoderm are 'results' or 'causes' of the neural-fold formation.'

Indeed, the role of the mesoderm has been completely discounted in neurulation by Karfunkel (1974). Theodosis & Fraser (1978), discussing failure of neural tube closure in mouse caused by excessive vitamin A, dismissed

> 'one theory [that] proposes that any stimulus which adversely affects mesodermal proliferation can secondarily affect closure by producing a lack of supporting mesenchyme for the rising neural folds (Marin-Padilla, 1966; Geelen, 1973; Morriss, 1973).'

(They found, instead, damage to the nuclear and cytoplasmic membranes of the neuroepithelial cells.)

On the other side of the coin, however, when we tried to explain neural plate formation without invoking the mesoderm (Jacobson & Gordon 1976*a*), we failed: our computer simulations and mathematical analysis suggested the inability of the isolated neural plate to form a keyhole. This was confirmed when experimental removal of all mesoderm tissue lead to shrinkage of the excised neural plate, but the tissue did not form the proper keyhole shape (Jacobson & Gordon, 1976*a*, their fig. 3). The following eight experiments and observations led to the conclusion that, despite the long history of suggestions to the contrary, part of the mesoderm,

namely the *notochord* (Jacobson & Gordon, 1976*a*, their figs 1 and 7), seemed essential for obtaining the keyhole shape:

(a) Vital dye experiments by Jacobson & Löfberg (1969) (*cf.* Keller, 1976) indicated that while mesoderm movements during neurulation were similar to those of the neural plate, they were identical in the region of the notochord (fig. 5 in Jacobson & Löfberg, 1969).
(b) Time-lapse movies clearly showed that the notochord and the translucent supranotochordal region of the neural plate (the 'notoplate') moved together (Jacobson & Gordon, 1976*a*, their fig. 1). (I coined the word *notoplate* to designate that part of the neural plate which normally lies over the notochord.)
(c) In histological sections the notochord appears more firmly attached to the neural plate than the rest of the mesoderm (Jacobson & Gordon, 1976*a*, their fig. 7).
(d) When the neural plate is excised the plate is readily separated from all mesoderm except the notochord, indicating firm mechanical attachment of the notochord to the notoplate.
(e) As mentioned above, the neural plate without notochord did not form a keyhole (Jacobson & Gordon, 1976*a*, their fig. 3), contrary to the observations of Roux (1888).
(f) On the other hand, neural plate isolated with only the notochord attached does give a proper keyhole shape (Jacobson & Gordon, 1976*a*, their fig. 8).
(g) Embryos whose neural plate and mesoderm were left intact, except for the removal of the notochord, did not yield keyhole shapes (Jacobson & Gordon, 1976*a*, their fig. 9).
(h) A piece of tissue consisting of the notochord and the notoplate elongated at a normal rate (Jacobson & Gordon, 1976*a*, their fig. 10).

Neither the neural plate nor the notochord isolated alone would elongate. Their continued association appeared to be required, and we could not conclude whether the notochord, the notoplate, or both, are actually generating the force for elongation. Our work has often been misinterpreted as suggesting that the notochord must be the motive force for anterior/posterior elongation of the neural plate. In retrospect, major damage to both early notochord and notoplate may have been done when they were mechanically separated, as in experiments (e) and (g) above (*cf.* fig. 9 in Jacobson & Gordon, 1976*a*).

Later on, at the stage of neural tube closure, in newts, the notochord and notoplate may be separated from one another with no consequence to further elongation of the neural plate (Kitchin, 1949). Clearly, at this time, both tissues may be regarded as autonomous and self-elongating. A sheath begins to form around the notochord (Bancroft & Bellairs, 1976; Waterman, 1979), so the tissues may be physiologically separated already (*cf.* Mann & Persaud, 1979).

Youn & Malacinski (1981) and Malacinski & Youn (1983) found that

notochordless embryos of *Xenopus* could be produced by ultraviolet irradiation of the eggs, undergoing apparently normal neurulation. The method unfortunately did not work on urodele embryos (Malacinski, personal communication). Nevertheless, these results, put together with the above, suggest that the notoplate itself is capable of autonomous elongation from the beginning of neurulation. The previous notions could even be reversed, by considering the possibility that the notoplate carries the underlying notochord along. In this case, we come to the startling conjecture that the neurulation-like movements of the mesoderm observed by Jacobson & Löfberg (1969) may be passive movements driven by neurulation.

The role of the mesoderm is, then, uncertain, but in light of the observation that neural tube closure will procede without it, we may assume that that role is secondary.

Differential proliferation in the neural plate

A commonly expressed opinion on the morphogenesis of tissue sheets is that 'mitotic pressure' due to the proliferation of cells can generate the shape changes (*cf.* the model for chick lens in Hendrix, 1972; Hendrix & Zwaan, 1974). Gillette (1944), while counting the numbers of cells in various regions of the newt epidermis and neural plate, concluded that the neural plate changes shape with no increase in volume (*cf.* Hutchinson, 1940; Hutchinson, 1944). Jacobson & Gordon (1976*a*) measured the volumes of serially sectioned neural plates and also found them to be constant. A consequence of this is that on the average, and probably in every case, daughter cells must be of smaller volume than their progenitor, and do not grow. Thus it is unlikely that they could exert a pressure that would move the rest of the tissue. Piatt (1948), reviewing this question, notes additionally that

> 'Hutchinson (1940, *cf.* Hutchinson, 1944) found a slight decrease in number of cells per unit volume of neural plate between stages 13 and 16 of *Amblystoma*, and Burt (1943), using the same species, concluded that neurulation is accompanied by mitosis but that the mitotic rate rises only after closure of the neural folds.'

We can conclude that, for the newt, cell proliferation does not contribute to the forces of neurulation.

Jelínek & Friebová (1966) found that the surface area of the chick neural plate increased due to posterior/anterior lengthening while the lateral width remained constant. They concluded that

> 'the proliferation activity may be considered as the main dynamic factor of neurulation movements, the static factor being the firm anchoring of the neural plate cells in the developing internal limiting membrane system [at their apical ends].'

(*cf.* Jacobson & Tam (1982).) In contrast to this work, Piatt (1948) observes that:

> 'Important confirmation of the dissociability between neural tube formation and alterations in cell number and volume comes from irradiation experiments on early chick embryos. Hinrichs (1927) found that ultra-violet radiation did not affect the ability of neural plate cells to divide and proliferate but that in many cases it did inhibit the

> closure of the medullary plate. Davis (1944) observed that ultra-violet radiation of wavelengths 2483–3130Å inhibited the folding process of neural tube formation while cell division and volume changes continued undisturbed.'

Jacobson (1984) added

> 'the finding that the UV effects on brain closure and elongation are photoreversible [which] suggests that nucleic acids may be the UV targets, and therefore have some crucial role in elongation and closure of the brain'.

(The target of the UV irradiation may be the notoplate.)

An unusual effect, probably related to differential proliferation, has been found in the curly-tail mouse, a mutant that arose spontaneously in 1950 (Grüneberg, 1954). About 60 % of the mice have either exencephaly, spina bifida aperta or a curly tail (Embury, Seller, Adinolfi & Polani, 1979; Copp, Seller & Polani, 1982). When various doses of vitamin A were given to the mother on day 8 of gestation, the frequency of neural tube defects increased from a baseline of 60 % (*cf.* Morriss, 1973), while a reduction in penetrance was found when vitamin A was given on day 9 (Seller, Embury, Polani & Adinolfi, 1979). Seller (1983) and Seller & Perkins (1983) have produced a similar effect by administering metabolites which block or hinder DNA synthesis. (The many effects of vitamin A on the rodent embryo include reduction of DNA synthesis, Kochhar, 1968, and prolongation of the cell cycle, Langman & Welch, 1967. Insulin similarly causes

> 'a reduction in neuroectoderm cell proliferation . . . in exencephalic embryos',

Cole & Trasler, 1980.) These observations led Seller (1983) to suggest that changes in DNA synthesis and altered cell proliferation could be involved in the disturbance in morphogenesis of the neural tube in the curly-tail mouse. The regions of the neural plate that are primarily affected may be those identified by Jacobson & Tam (1982) where most cell proliferation is occurring (*cf.* Morriss & New, 1979; Tuckett & Morriss-Kay, 1985). These regions consist of two broad areas lateral to the dorsoventral midline. Jacobson & Tam (1982) suggested that these regions are significant contributors to neural plate closure, providing an additional or alternative mechanism of neurulation compared to urodele amphibians (Jacobson & Gordon, 1976*a*; Gordon & Jacobson, 1978; Gordon, 1983).

On day 8 in the mouse, the neural plate is still relatively flat and open at the posterior and anterior ends, with a deep groove in the centre. The curvature is actually the opposite of that expected of a tissue rolling into a tube. Lateral cell proliferation may be essential at this stage to help buckle the tissue towards closure. Vitamin A administered on day 8 would slow cellular division and growth of the lateral parts of the neural plate. Thus failure of closure would be more probable. On day 9 in the mouse, the neural plate is well on its way towards being closed at both ends. Growth of the lateral flanks, were it to continue, could actually counteract the closure at this stage, since the perimeter of the remaining opening must decrease for closure to go to completion. Continuation of such growth may be the primary defect in curly-tail mice. If so, administration of vitamin A would slow

down the excess growth, permitting closure to go to completion more often. This hypothesis also suggests, then, that there are two kinds of neural tube defects: failure to lift and failure of final closure.

Perhaps the success of vitamin A supplement, in decreasing the chances of a second occurrence of a neural tube defective birth in women who have had one such birth (Rose, Cooke, Polani & Wald, 1983; Schorah *et al.* 1983; Smithells *et al.* 1983), lies in the plausible hypothesis that embryos with fully open neural tubes have a higher spontaneous abortion rate than those that are nearly closed. While vitamin supplements for the general populace may reduce the overall rate of neural tube defects, they may accomplish this by shifting the burden of those defects from one group of people to another.

Formation of bottle-shaped cells by non-uniform swelling

Glaser (1914) proposed that the rolling up of the neural plate into a neural tube was due to greater uptake of water by the basal ends of the neural plate cells than at their apical ends. The resultant pressure and swelling would produce bottle-shaped cells of increased volume. (*cf.* Moore, 1930; Moore, 1941; Moore & Burt, 1939). Sjodin (1957) found that swelling of the neural epithelium occurred under low oxygen tension and resulted in multiple folding, without cell division. Moore (1945) points out that Bütschli (1915) and Spek (1920)

> 'postulated, as the result of the supposed arrangement of lyophilic and lyophobic colloids in the endodermal plate, a differential swelling between the inside and outside of the endodermal layer'

during gastrulation. The hypothesis for neural plate was discounted by Glaser (1916) himself and later by Brown, Hamburger & Schmitt (1941) on the basis of constant mass density of the cells throughout neurulation. The doubling of the volume of neural plate cells during neurulation observed by Glaser (1914) in the newt *Cryptobranchus* was also not confirmed for *Ambystoma* by Gillette (1944). Trinkaus (1969) retains some doubt that this hypothesis has been adequately eliminated:

> 'But others have inhibited folding of the neural plate with hypertonic sugar solutions and caused rolled-up plates to unroll when treated with solutions of glycerine. The evidence is therefore contradictory ... none of these tests examined possible differences in water uptake between the basal and apical parts of the cells, which is the really crucial part of the hypothesis.'

Cell elongation by increased intercellular adhesion

The inverse relationship between increasing height and decreasing area of the neural plate cells is implicit in Gillette's (1944) observation that neurulation proceeds at constant volume of the tissue. The correlation was noted by Jacobson (1962) and measured quantitatively by Burnside & Jacobson (1968). One possible explanation for this behaviour is increased lateral adhesion between the neural plate cells, which presumably would increase the area of the lateral membranes of

the cells while decreasing their apical and basal areas. Such an hypothesis was proposed by Brown *et al.* (1941) and discussed for cell sheets in general by Gustafson & Wolpert (1967). This

> '... mechanism is based on the concept that the degree of contact between cells is determined by the adhesive force between them and the resistance of the cells to deformation [due to, say, a common basement membrane]. Applied to neurulation, one can easily see how increased surface adhesiveness coupled with resistance of the basal part of the cell to deformation could cause cells of the neural plate to assume an elongated wedge shape' (Trinkaus, 1969).

Disruption of the basement membrane does lead to abnormalities (Bernfield, Cohn & Banerjee, 1973). However, preliminary experiments using disaggregation kinetics (which are not a reliable indicator of adhesive strengths)

> '... do not support the conclusion that the curling of the neural plate is correlated with an increased adhesiveness of the cells of the neural tissue of *Xenopus* embryos ...'

(Karfunkel, 1974; *cf.* Karfunkel, Hoffman, Phillips & Black, 1978). We need a set of experiments based on equilibrium measurements to test differential adhesion properly (Mostow, 1975). (The methods of Gordon, Goel, Steinberg & Wiseman (1972, 1975) allow changing adhesive strengths to be measured.) Thus, while this hypothesis tends to be disregarded now with the discoveries of microtubules and microfilaments in neural plate cells, it is by no means disproved. Nardi (1981) argues that the inhibitors of microtubules and microfilaments

> '... do not exert their effects solely on the cytoskeleton. They also affect cell surface properties ... Emphasis on the role of cytoskeletal elements in governing the folding of epithelia should be tempered by an appreciation of the importance of cell adhesivity. The observed accumulation of cell surface molecules at sites destined to invaginate may contribute to increased adhesivity of the invaginating population of cells, and oriented microtubules and microfilaments may simply stabilize the cellular configurations assumed as cell surface properties drive changes in cellular form.'

Revel (1974) suggests that since

> 'the desmosomes display properties which change with the age of the embryo, ... they could therefore form the basis of some of the changes in intercellular adhesivity which are such prominent features of development.'

Lewis (1947) noted that

> 'Dissections of neural plates show that its cells are firmly adherent to one another throughout the thickness of the plate.'

If only the microfilament purse-string held them together (see below), we wouldn't expect this.

Moore (1945) dismissed a suggestion by Assheton (1916)

> 'that an attraction between the cells, the greatest force being exerted along the line passing through the nuclei, could account for the inpocketing...'

of the endodermal plate during gastrulation. He then proposed a most attractive hypothesis, also applicable to the neural plate, which hasn't been considered since:

> 'The possibility does remain that [Assheton's] basic idea is sound, and that instead of the nuclei exerting an attraction there is a differential cohesion between the interior and the exterior halves of the cells of the endoderm.'

Today we ordinarily regard every cell–cell interface as being characterized by a single specific adhesion (*cf.* Gordon *et al.* 1972, 1975). However, given the highly structured nature of cell–cell contacts in neuroepithelium, it is quite reasonable to postulate a gradient in specific adhesion from the basal to the apical end of each cell. (This is quite distinct from the adhesion of anisotropic cells that have uniform specific adhesions on each 'face' (Goel & Leith, 1975; *cf.* Elsdale & Bard, 1974).)

Löfberg (1974) suggested that adhesive bonds between apical projections of the neural plate cells may be responsible for increasing intercellular adhesiveness. Support for this idea comes from Wiley's (1980) observation that *in vivo* in the hamster,

> 'The apices of the neuroectoderm cells in the embryos of females exposed to cytochalasins B and D were found to have lost much of their complex surface architecture and to bulge into the neural groove.'

(These embryos, which failed to undergo neural tube closure, showed no alterations of their microfilaments. He suggests that *in vitro* studies, such as those of Karfunkel (1972) and Webster & Langman (1978) involved higher concentrations of the teratogen than necessary *in vivo*.)

Other connections between the apical surfaces of cells are seen by scanning electron microscopy (SEM):

> '... over the ectoderm at early stages are long cellular extensions with prominent dilatations along their lengths. They connect ectodermal cells and may extend over five or six intervening cells ... they are associated with approximately 5–15 % of the cells at early stages (Bancroft & Bellairs, 1975), and remain prominent throughout the process of neurulation (Bancroft & Bellairs, 1975; Jacob, Christ, Jacob & Bijvank, 1974). They are seen over all regions of the ectoderm at early stages (Backhouse, 1974; Harri, 1974) but appear more prevalent in association with the neural regions as neurulation proceeds (Backhouse, 1974; Bancroft & Bellairs, 1975) ... They ... most probably represent cytoplasmic bridges between daughter cells, the prominent dilatations along their length being midbodies (Bancroft & Bellairs, 1975; Bellairs & Bancroft, 1975)'

(Waterman, 1979).

These bridges have yet to be related to those seen by Löfberg (1974). Whether or not they play a role in cell–cell adhesion, they are at least direct indicators of mitotic activity and cell neighbour changes.

The model of increasing intercellular adhesion presupposes that apical and basal membrane can be exchanged for lateral membranes. No one has yet tried to observe such an exchange, say by the fluorescence labelling technique of Edidin (1974) that originally showed the fluid nature of the plasma membrane in cells. (Exchange of membrane units through the cytoplasm is another plausible route.) However, let us calculate the total membrane surface area of a cylinder (ignoring foldings, microvilli, projections, etc.): $S = 2\pi r^2 + 2\pi rh$, where r and h are the radius and height, respectively. If the volume $V = \pi r^2 h$ is constant, then $S = 2\pi r^2 + 2V/r$. As

the apical radius decreases, the total membrane decreases to a minimum at $r_{min} = \sqrt[3]{V/(2\pi)}$ and $h_{min} = \sqrt[3]{4V/\pi}$ and then increases approximately hyperbolically with decreasing r. Since the minimum area is reached at $h_{min}/(2r_{min}) = \sqrt[3]{2} \cong 1{\cdot}26$, it is clear that the columnar cells of the neural plate must be increasing their surface areas.

Most models for cell–cell adhesion assume constant membrane area of the interacting cells. Thus we will have to sort out the effects of changing specific adhesions between membranes and the increased area over which these adhesions may occur. The hypothesis here is that the latter are caused by the former. The dynamic involvement of cell membranes may be seen in Löfberg's (1974) proposal that the projections seen on the apical surfaces of neural plate cells (his fig. 30) may be the driving force for their apical constriction:

> 'By fusing, some projections enclose an endocytotic vesicle, which is then pinched off and translocated basally. If the influx of membrane materials from the surface is greater than outflow from the cytoplasm the apical surface successively shrinks, being transformed into intracytoplasmic vesicles, and the cell narrows . . . In a constantly turbulent cell surface that is thrown into new folds and slender processes, certain projections at the cell peripheries come into contact with similar features of adjoining cells and establish new focal attachments . . . Some cells may become so narrow that surrounding cells can bridge over them and force them below the surface . . . The radius of curvature at the tips of the projections [is low enough] . . . to penetrate the potential barrier of an adjacent cell.'

Active cell elongation perpendicular to the neural plate

Brown *et al.* (1941) suggested that neural plate cells could change shape via

> 'changes in protoplasmic structures such as oriented cytoskeleton.'

The idea was widespread at the time:

> 'several authors (e.g. Harrison, 1936; Needham, 1936; Waddington, 1940) have drawn attention to the possibility that the facts could be explained if we could postulate the formation of submicroscopic fibrils (an oriented cyto-skeleton) within the cytoplasm'

(Waddington, 1942). Active elongation of isolated neural plate cells was observed by Holtfreter (1943, 1946, 1947) and confirmed by Burnside (1973*b*). Microtubules were first observed in newt neural plate by Waddington & Perry (1962) and were subsequently confirmed in chick (Messier, 1969; Karfunkel, 1972) and *Xenopus* (Schroeder, 1970; Karfunkel, 1971). In each case their orientation is perpendicular to the sheet and it is often presumed that they are actively responsible for the elongation of the cells in the apical/basal direction. Vinblastine sulphate, which disrupts both microtubules and microfilaments, and colchicine, which disrupts only microtubules, both stop neurulation (Karfunkel, 1971; Burnside, 1972, 1973*a*). Karfunkel (1974) was unable to get colchicine to penetrate the *Xenopus* embryo, but succeeded in using it to stop chick neurulation (Karfunkel, 1972). Burnside (1971) found that:

(1) the number of *paraxial microtubules* (along the apical/basal cell length) in a newt neural plate cell decreases as it lengthens;

(2) the total length of microtubules is constant;
(3) the number of microtubules is constant along the length of each cell;
(4) there is no difference in the number of paraxial microtubules in a cell from one region compared with another;
(5) the rate of elongation of a cell is not correlated with the number of paraxial microtubules it contains;
(6) the number of paraxial microtubules is not correlated with the cross-sectional area of the cell.

There is an apparent contradiction between observations (1) and (4). If all cells start out with the same height and the same complement of microtubules, then at a later stage the count ought to be less in taller cells. Instead it is found to be the same (at newt stages 13 and 15). Clearly one of these conclusions is erroneous. The large standard deviation in microtubule counts (Burnside, 1971) may be the source of the difficulty.

Since neural plate cells with disrupted microtubules round up, we can at least conclude that microtubules are necessary to support the elongate, columnar shape of the cells. Whether microtubules, via changes in their number, length, protoplasmic transport, or by other mechanisms actively change the shape of the cells cannot be ascertained from the available evidence. Burnside (1971) eliminates a sliding filament mechanism by observation (3) above. She suggests that either the polymerization process itself pushes the cell to elongate, or that the microtubules transport cytoplasm in the apical/basal direction(s), including their own recycled subunits. She favours the latter hypothesis. A version of such a model asymmetric in the apical/basal direction could lead to bottle-shaped cells, with no change in cell volume. A non-uniform distribution of the membrane attachment sites of the microtubules could be the basis for such an asymmetry.

It seems that a proper statistical mechanical study of the polymerization of microtubules could settle the question of whether they are capable of forcing a cell to elongate by their very polymerization, or are merely capable of retaining its current shape. (*cf.* force generation by the polymerization model for the spindle apparatus, Salmon, 1975.) Monte Carlo computations may be useful (*cf.* Gordon, 1980), possibly revealing the reasons that some microtubules elongate while others dissolve. Direct observation of the microtubules growing and dissolving, by video contrast enhancement microscopy, may now be feasible (Inoue, 1981; Miller, 1981), especially in isolated neural plate cells in newts (Burnside, 1973*b*).

> 'Hobson (*cit.* Schmitt, 1941) ... found that the walls of the [chick] neural tube show birefringence which is positive with respect to the long axis of the cells...'

(Holtfreter, 1943).

Active apical constriction of neural plate cells

Models for neural tube formation by active apical constriction go back at least to Rhumbler (1902). (*cf.* Moore, 1945.) This model was favoured in the review of

Weiss (1955), based on proposals by Lewis (1947) and Holtfreter (1943). Cloney (1966) and Baker & Schroeder (1967) suggested that circular bands of microfilaments just beneath the apical surface of neural plate cells in anuran neurulation act as contractile purse strings. Other reports, in chick, include Ruggeri (1967), Handel & Roth (1971), Camatini & Ranzi (1972), Portsch & Barson (1974) and Bancroft & Bellairs (1975). (Jelínek & Friebová (1966) were apparently unable to see them.) Disruption of microfilaments (Karfunkel, 1971; Karfunkel, 1972; Linville & Shepard, 1972), especially with cytochalasin B, which was presumed to be specific for them, led to flattening of the neural plate with retention of the columnar shapes of the cells. Similar work on morphogenesis of salivary gland epithelium led Wessells *et al.* (1971) to

> '... the general conclusion ... that microfilaments are probably the active agents, in these sorts of morphogenesis. Microtubules are not causal agents, except ... in the maintenance of a columnar cell shape (Byers & Porter, 1964; Tilney, 1968).'

(Spooner (1974) restored microtubules to a possible role in salivary gland epithelium.) Burnside (1971) concludes:

> 'In summary, it is strongly suggested that apical filaments interact by sliding to play a contractile role in the constriction of the cell apex. They are morphologically similar to other cytoplasmic filaments considered 'contractile'; their purse-string arrangement would enable them to cause constriction of the cell apex; they retain a parallel alignment within the bundles as the cell narrows; and the increase in thickness of the bundle of filaments is inversely related to the decrease in circumference of the purse-string bundle in such a way that increased thickness of the bundle may be accounted for by increased overlap.'

(The presence of a few microtubules parallel to the apical surface and intertwined with the apical microfilaments (Burnside, 1971) should not be ignored.) Thus apical constriction by microfilaments seems to have a definite role in changing the cell shapes and possibly a role in the rolling up of the neural plate into a neural tube.

Odell, Oster, Burnside & Alberch (1980) have suggested that the apical

> 'microfilament band behaves like smooth muscle fibres ... small extensions can trigger major contractions.'

Time-lapse movies of newt neurulation often show an irregular 'puckering' phenomenon, in which a wave of contraction starts at a point and spreads out over a considerable portion of the embryo (A. G. Jacobson, personal communication). While this puckering is apparently not a necessary component of the neurulation movements, it may represent an overshoot phenomenon reflecting the underlying mechanism of cell shape change. It may, then, support the notion of actin/myosin-like involvement of microfilaments proposed by Odell *et al.* (1980), which permits hysteresis in the stress/strain relationship of the cell, and supposes propagation of contraction from cell to cell. A similar phenomenon of irregular, step-like closure of newt neural tubes has been found by Selman (1958) and attributed to hysteresis by Jacobson (1978). (*cf.* Bancroft & Bellairs, 1975.)

Nagele & Lee (1980), on the other hand, have some reservations about the directing role of microfilaments:

> 'The finding of 'microfilament-deficient' regions in the cell apex is inconsistent with the depiction of apical microfilaments as forming a single and continuous circumferential bundle . . . The fact that some areas of the cell apex appear more 'pulled in' than others suggests that the extent of contraction of filament bundles may vary from one cell to another or even within the same cell. Thus, movement of the neuroepithelium may be achieved by regulating the magnitude and direction of the contractions of filament bundles.'

How the latter would be established by the embryo is not discussed.

Morris & New (1979), investigating rat embryos that failed in neural tube closure in the presence of high oxygen concentrations, yet had the normal complement of microfilaments,

> 'suggest that their presence may be essential for control of shape (e.g. surface area) and maintenance of cohesion of the neural epithelium, but that they do not actually bring about neural tube formation.'

Tensions generated by the cells of the neural plate

Beloussov (1980) has suggested

> 'that the normal morphogenesis of the axial rudiments is oriented and ordered by a non-specific anisotropic tensile field of tissue stretching'

(*cf.* Holtfreter, 1943). He found that pieces of tissue of frog embryos, transplanted at late gastrulation, after determination was presumably complete, took on the orientation of surrounding tissues. Beloussov, Dorfman & Cherdantzev (1975) distinguish passive and active stretching, the latter being a much slower process sensitive to cold, cytochalasin B and cyanide.

Beloussov's (1980) suggestion may apply to the effect of the tongue of neural plate cells anterior to the notoplate (Fig. 15B in Jacobson & Gordon, 1976*a*). These cells, which exhibit maximal apical contraction, may produce an orienting force on the notoplate cells, as discussed in Gordon (1983).

In mouse neurulation (Jacobson & Tam, 1982), one fundamental parameter requiring explanation is the anterior/posterior orientation of pairs of daughter cells. It could be due to tensions in the neural plate that mechanically align the spindles. (*cf.* the shaping of Sinnott's, 1944, 1960, gourds and Furuya, 1984.) The numerical values of these tensions are available in the course of a finite element analysis, and could be used to simulate dynamic alteration of the degree of spindle orientation.

Coordinated cell elongation and apical constriction

I would now like to consider the consequences of both cell elongation and apical constriction being active in the cells, as concluded by Burnside (1971). An interaction between the two mechanisms has been proposed:

> 'In *Xenopus*, it seems possible that the apical constriction of the cells at the midline of the neural plate produces lines of stress within the tissue, and that the microtubules line up along these lines. Thus, prior to cell elongation, microtubules that were randomly oriented at . . . end of gastrulation become aligned parallel to one another . . . when the neural folds have just formed' (Karfunkel, 1974).

Considering that apical stretching occurs in directions perpendicular to the paraxial microtubules (Jacobson & Gordon, 1976*a*), which are in the majority (Burnside, 1971), this interaction would seem unlikely. On the other hand, in plant cell walls the alignment of microtubules is perpendicular to the direction of cellular elongation (Green & Lang, 1981; Gunning & Robards, 1976), as we require.

If both cell elongation and apical constriction are accomplished by active mechanisms, then they must be coordinated during neural plate formation. If they were not, the cells would become bottle shaped during the stages in which the neural plate is essentially flat; i.e., apical shrinkage and height increase of the cells must be strictly inversely related to retain a columnar shape during formation of the neural plate. (Apical shrinkage thus occurs both during neural plate and neural tube formation, not just the latter, as suggested by Burnside (1971).) Webster & Langman (1978), studying the vertical (basal/apical) *interkinetic migration* of nuclei of the cells in the neural plate, suggest

> 'that the microtubules and microfilaments are in balance to maintain the neuroepithelium.'

When neural tube formation begins, cell elongation and apical constriction could and must deviate from this simple inverse relationship:

> 'In the neural plate the microtubules and microfilaments would have to act in concert, so that the shape transformation occurs in a plane. However, they must then deliberately go out of register to change each cell from a columnar to a bottle shape. Any lack of coordination could lead to too much curling or curling the wrong way (and could thus be the basis for neural tube defects). Such curling could constitute the bending movements discussed by Gierer (1977)'

(Gordon, 1983).

The notion that each cell actively forms itself into a preprogrammed bottle shape (*cf.* Holtfreter, 1944; Lewis, 1947) should be directly testable by repeating Holtfreter's (1946; *cf.* Holtfreter, 1947) and Burnside's (1973*b*) isolations of individual neural plate cells and measuring their changing shapes. To explain a positive observation we would need to identify two *mathematically independent* parameters such as length and apical radius and attribute them to, say, microtubules and microfilaments (Gordon, 1983). (A model such as that of Odell *et al.* (1980) implicitly assumes a particular relationship between the height and apical programs for a cell, reducing the number of independently controllable parameters to one.)

Cell rearrangement due to gradients of intercellular adhesion

In explanations of embryonic cell sorting by differential adhesion, it is not cell shape, discussed above, but rather cell motion or rearrangement that is generally addressed (Mostow, 1975; *cf.* Mittenthal, 1981). I have attempted to simulate notoplate shaping in a manner that incorporates both cell shape changes and cell rearrangement, both driven by the same cell–cell adhesivities, using a bilateral gradient of adhesion, in which cells adhere to one another with an interfacial

tension that increases with their *initial* lateral distance apart. (Perhaps a molecular model, based on multiple states of cell adhesion molecules (CAM), could be formulated to produce such a gradient (*cf.* Cummings, 1985).) The polygonal representation of Gordon (1983) was used, in which the vertices where three cells meet are moved in directions and speeds dependent on maximizing the local interfacial energy. These simulations failed to produce notoplate elongation. Further investigation showed that they also fail to simulate sorting out of a random cell mixture and even fail to simulate rounding up of an isolated embryonic tissue. Since the computer program accurately reflects the presumed physics of cell sorting, I am led to the conclusion that the cells must provide the energy necessary to get over the local energy barriers that keep the simulation trapped in metastable states (*cf.* Stillinger & Weber, 1984). Brownian motion of the cell membranes relative to one another (Gordon *et al.* 1972, 1975) is clearly inadequate. Keller's work (this volume) shows that four mechanisms may be involved in providing this energy and permitting cells that are not touching to make the initial contact that lets them draw closer together: rounding up with rotary blebbing or cyclosis (*cf.* Holtfreter, 1943), extension of processes, intercolation of cells from lower layers (applicable only to embryos, such as *Xenopus*, whose neural plate is not a cellular monolayer), and the formation of gaps. In intact embryos, the latter could be caused or accentuated by the tension under which we find the whole epidemis and neural plate (Jacobson & Gordon, 1976*a*).

If a differential adhesion model for shaping of the notoplate is correct, it may be extendable to the whole neural plate (Gordon, 1983; *cf.* Phillips & Davis, 1978). The idea that bilateral symmetry itself is a product of differential adhesion has been put forward by Jehle (1970).

Stein & Gordon (1982) give a method for estimating gradients of differential adhesion directly from the shapes of cell–cell boundaries in monolayer epithelia, such as the neural plates of urodeles, birds and mammals.

Neural tube formation by Eulerian buckling of an elastic sheet

Elongation of the notochord and/or notoplate (uniform along the length of the notochord in urodeles, Jacobson & Gordon, 1976*a*), might in itself explain formation of the neural tube. If a sheet of rubber is stretched along a line, it buckles and the part near the line of stretching rolls up into a tube (Jacobson, 1978; Gordon & Jacobson, 1978). Since the neural plate is an elastic material, then we may presume that it ought to act the same way.

Consider a long, narrow dowel which is compressed from its ends. It will tend to bow out. Actually, there are many modes of bowing, leading to bends along the dowel of certain wavelengths. (There are some embryos which show multiple buckling: Solurish, 1978). This phenomenon of bending, rather than compression of the dowel is called *Eulerian buckling* (Wainwright, Biggs, Currey & Gosline, 1976). As is typical for instability phenomena (*cf.* Gordon *et al.* 1972, 1975), the buckling can be described analytically in terms of sine waves for small amplitude perturbations.

It is especially likely to occur in a thin, wide material (Southwell, 1941). (Ghiradella (1974) has shown that ridges on butterfly scales can be explained using the theory of buckling of thin films (Timoshenko & Gere, 1961). The driving force may be contractile microfilament bundles.)

Stretching an elastic sheet along a line leads to compressive forces with components perpendicular to the line, and thus buckling. When the bends become large in amplitude, the analysis of Eulerian buckling becomes non-linear and mathematically intractable, requiring advanced forms of finite element analysis (*cf.* Brodland, 1985).

This notion that lateral compression is caused by anterior/posterior elongation of the neural plate reconciles the contradiction between the original theory of His (1874) and the observations of Roux (1888), discussed above. No external force is necessary to achieve lateral compression, and thus an isolated neural plate (albeit with a functioning, intact notoplate, which Roux must have inadvertantly retained) can undergo neural tube closure.

One observation pointing towards the buckling model is the sudden spurt in the elongation rate of the notochordal region during neural tube closure (Jacobson & Gordon, 1976*a*, their fig. 38). This spurt consists of a temporary *tenfold* increase in the rate of elongation:

> "Since the neural plate is a viscoelastic system, Gordon and I suspect that the increased rate of elongation during tube formation may be necessary to emphasize the solid or elastic properties of the plate that would lead to buckling out of the plane . . . . There may be just one chance, during rapid elongation, when the plate can roll into a tube [a 'critical' event]. This might account for some of the persistent defects in tube closure which are of common occurrence' (Jacobson, 1980).

In chick, Jacobson (1980) has demonstrated that a propagating spurt of elongation (29 %) accompanies the propagating position at which the neural tube closure is occurring. Jacobson (1984) has shown that neural tube closure in the chick may be photoreversibly stopped with ultraviolet radiation. However, he did not discriminate between elongation of the notoplate and elongation of the lateral neural plate.

The mechanisms of rounding up and gap formation (Keller, this volume) suggest the self-dissolution of chemical links between cells. The beginnings of this process may be apparent from the work of Opas, Turksen & Kalnins (1985) who observed that vinculin is present everywhere between columnar cells in retinal pigmented epithelium, except at the vertices where three cells meet (*cf.* Crawford, 1980). The transitory speedup in elongation of the notoplate could possibly be explained by postulating that dissolution occurs. (Sheath formation by the notochord, Bancroft & Bellairs, 1975, could provide an alternative source of the spurt in elongation. This could be tested using notochordless embryos (Malacinski & Youn, 1983).)

The dissolution of cell connections during cell rearrangement may be the basis for the shear lines postulated by Jacobson & Gordon (1976*a*) to be responsible for the division of tissues into separate domains (*cf.* Jacobson, 1975).

Preliminary cell marking experiments have shown that cells on the midline of the neural plate in mouse can have descendents that are dispersed along the midline

(Kirstie Lawson, personal communication), suggesting that mammals also have a notoplate.

On distinguishing between models of neurulation

There are a number of questions one may ask in deciding whether or not a given model matches the observed (or observable) facts of neurulation. Only one of the many models has been put through the test of a computer simulation with detailed comparison to cell behaviour, to see if it holds up to the light of quantitative scrutiny, and that only for flat neural plate shaping (Jacobson & Gordon, 1976*a*).

The embryo may not care a whit about our abstract discriminations, using what we would call multiple mechanisms and blurring our distinctions between heirarchical levels (*cf.* Gordon, 1983) in achieving its goals. Such redundancy may, for example, account for the ability of starfish embryos to achieve gastrulation, albeit by different routes, in the face of widely varying sizes of eggs (Berrill, 1961). Each mechanism may simply 'kick in' in proportion to its ability to function in the given circumstances. (Two quite different neurulation mechanisms can indeed operate in the same embryo, as in the fish-like 'secondary neurulation' of chick (Criley, 1969; Jelínek, Seichert & Klika, 1969; Schoenwolf & Delongo, 1980).) Lewis (1947) also recognized that:

> 'Many factors are involved in each invagination. Invagination areas or plates differ in size, shape, thickness, location and environment. Changes occur in them and in their environment during invagination. Their cells differ in number, size, shape, adhesiveness, viscosity, mitotic rate, growth rate, cytoplasmic differentiation, etc. The above factors are involved in every invagination and play a part in modifying and determining its character.'

There are two general approaches we may take in trying to unravel this situation. First, we may consider each model in its extreme form, design 'critical' experiments, and carry them out on the embryo and on a computer embodiment of the model. Without such computer simulations, we are only engaging in handwaving and wishful thinking about the performance of our models. It is for this reason that our pre-computer predecessors, such as His (1894) and Lewis (1947; see fig. 5 in Gordon, 1983), built machines to simulate their conceptions of what causes involutions of sheets of cells. Computer simulations must themselves accurately represent the model: geometrically inappropriate simplifications, such as those based on histological cross sections, can be misleading (Gordon, 1983; *cf.* Odell, Oster, Alberch & Burnside, 1981; Hilfer & Hilfer, 1983).

The second approach is to combine all models that are not physically mutually exclusive into one grand computer simulation. There may then be a large number of undetermined parameters for which we would attempt to obtain a best fit. If we found that many different sets of parameters gave equally good fits, we would have to devise experiments or observations that would narrow the options. This kind of *inverse problem* is fraught with subtle difficulties, including the possibility of total indeterminacy (Gordon, 1979; Smith, Solmon & Wagner, 1977). Fortunately embryos and

their cells are sufficiently large that most underlying parameters could, conceivably, be measured by *tour de force* methods, if necessary. (See Gordon, 1983, for a review of applicable methods of image processing and micromanipulation.)

Synthesis of neural tube closure using the finite element method

The finite element method generally involves the division of a complex object into a number of small, contiguous pieces, with the purpose of making it possible to calculate how the object will deform in response to various external forces (Bathe & Wilson, 1976; Owen & Hinton, 1980; Cook, 1981). It is essentially a numerical formulation of continuum mechanics (Lin & Segel, 1974; *cf.* 'morphodynamics' in Jacobson & Gordon, 1976*a* and the review by Silk, 1984).

Current finite element methods cannot handle three basic features of developing systems: the cells (the fundamental finite elements in an embryo, *cf.* Gordon, 1983) can change positions relative to one another, new elements are continually being created by cell division, and as a result the geometric transformations do not have inverses in the mathematical sense. (This difficulty might be approachable through new methods of 'moving finite elements', Gelinas & Doss, 1981.) Further non-standard features of morphogenetic problems are: (1) the forces are generated internally, by the finite elements (cells) themselves (*cf.* Nowinski, 1981) and the directions change as the cells move ('following' forces); (2) the elements are, in general, growing, with large strains (up to 10 or more); (3) the growth violates the usual assumption of conservation of matter, since cells (and thus embryos) are open systems; (4) the mechanical parameters (constitutive properties) can vary over time and in response to cell–cell interactions; (5) the values of the constitutive properties are unknown, so we are actually faced with an inverse finite element problem. We have tried three-dimensional versions of the 'repacking' algorithm in Jacobson & Gordon (1976*a*), but they have all proven to be numerically unstable. What may be needed is a new form of finite element calculation which combines their numerical stability with an ability of the cells (elements) to change neighbours in an orderly fashion.

By using thermal expansion coefficients that are huge compared to ordinary materials, we can simulate the growth of a finite element. We can let an element represent a group of cells within which cell division, spindle reorientation, and cell rearrangement can be approximated by anisotropic expansion coefficients in the plane of the neural plate. A third, perpendicular expansion coefficient can be used to simulate the local, changing thickness of the neural plate. We are interpolating and extrapolating the measurements of Jacobson & Tam (1982) to attempt such a finite element analysis of mouse neurulation, which will be presented at a later date. If this approach succeeds in synthesizing normal neural tube closure, we will then be able to test if variations in the parameters (such as embryonic size: *cf.* Snow & Tam, 1979; Snow, Tam & McLaren, 1981; Cooke, 1981) may lead to an understanding of the range of values under which normal neural tube closure occurs. If any of these ranges are narrow, we may then have an explanation of the high frequency of neural tube defects and the critical nature of neural tube closure.

Of course, it will eventually be necessary to have detailed fate maps, time-lapse studies, cell trajectories, cell height programs, and neighbour changes over normal and abnormal mammalian embryos to corroborate the theoretical investigations. This will clearly involve some new technology (*cf.* Gordon, 1982).

CONCLUSION

The most promising model of neural tube closure in amphibians is one of Eulerian buckling driven by notoplate elongation. In birds and mammals, oriented cell proliferation may be an essential additional process. However, a wealth of other plausible models have been proposed over the past century. Each has had its proponents and antagonists, but all remain inadequately tested. Testing will require coordination of experiments, observations, and finite element analysis. The design of numerically reliable finite element methods for morphogenetic systems is a significant challenge.

It would seem that we are on the verge of understanding the mechanics of neurulation, and possibly the manner in which neural tube defects come about (but *cf.* Gardner, 1961). If so, the outstanding problems will be those of the cellular and molecular bases of the mechanics, and how these molecular and cellular properties are initiated during primary neural induction (*cf.* Gordon, 1983).

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