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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

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*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.'