NEURAL TUBE DEFECTS
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Introduction

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Each of us comes to this meeting with a particular interest and particular insights and each of us will therefore have the opportunity to catch up with the latest developments in a variety of areas. Each of us is aware of the impact that neural tube defects have had on humans. Some of us have more direct experience with humans, but we can all appreciate that part of the reason for this conference is the potential right now for relieving some of the effects neural tube defects have on humans. Many of us know families or even individuals who have been affected with neural tube defects. The suffering that occurs when such a person is not able to reach their full potential impacts each of us and our societies. So there is a very practical aspect of this meeting—the hope that something can be done to prevent these tragedies.

Neural tube defects are also a model for birth defects in general. Because there are very many unanswered scientific questions, there are various challenges posed by a meeting like this. One aim of this meeting is to provide a synthesis of recent work. We can also consider some of the implications of that work both for new studies and potentially even for formulating some recommendations.

I come as a representative of clinical genetics. We clinical geneticists have a particular view about the world. We believe that the patients with genetic disorders whom we see in the clinic provide an opportunity to learn about mechanisms of disease at the same time that care is being provided. We think of the clinic as a laboratory—as a place where we can see whether or not the things that are being found in basic science make sense in real families. Our job is, of course, to treat, to prevent complications and to help families and patients deal with their situation in the most effective way. So we end up synthesizing basic research with what is happening clinically.

One of the things we also try to do is to figure out whether or not a specific patient 'breaks the rules'—whether a particular family is an exception. So part of what happens in the clinic is that we identify those affected individuals who are unusual and view them as potentially helping to teach us about the normal situation and why things can go wrong.

One of the things therefore that comes out of the clinic is an incredible sense of heterogeneity—lots and lots of what we see does not fit with our current ideas.
We do have the traditional ways of thinking about genetic problems: as chromosomal problems, single gene problems or multifactorial ones. Clearly, these traditional explanations for genetic disease fit many cases of neural tube defects. We know there are chromosomal problems with neural tube defects; we know there are many single gene problems in humans with neural tube defects. But the vast majority of individuals with neural tube defects have what we call multifactorial genetic disorders. During this conference we will hear about known environmental influences; I suspect there are still a number of others that have not been recognized. The common category of human neural tube defects is multifactorial and we should be thinking about the multiple factors, trying to sort out the heterogeneity. That is part of what a conference like this can do; it can help us to identify the many factors suspected to be involved.

In the last five to ten years, clinical geneticists have become increasingly aware of what we call non-traditional types of genetics. Much of our understanding of mechanisms other than single gene defects stems from the work in developmental genetics. Studies on early development in animals are affecting the understanding of the human situation. Cytoplasmic inheritance, maternal effects, mosaicism, both programmed mosaicism and spontaneously arising mosaicism, parent-of-origin effects, growth factors—all these are becoming better understood because of the advances in molecular and developmental genetics. This work will have a large impact on our understanding of birth defects such as neural tube defects in humans.

I would like to take the prerogative of the chair to describe briefly some of our work in British Columbia. We have a very useful situation because all patients with neural tube defects come to one of two health care centres. We also have in British Columbia a registry of all birth defects and genetic diseases that allows us to recognize changing frequencies and, for practical purposes, have total ascertainment. We now have detailed information on over 700 families affected by neural tube defects. These have been well defined as to the level of lesion, other problems and recurrences within the family. British Columbia is a low incidence area. It is known that there are low and high incidence areas around the world; there are also ethnic differences with regard to occurrence and recurrence of neural tube defects. For the population in British Columbia we were able to separate those with high lesions from those with low lesions and to distinguish those families who have additional anomalies from those who do not (Hall et al 1988). We also have a Sikh population which came from India and they seem to have a unique type of anomaly. We suspect that there may be unique susceptibility to neural tube defects in the populations from many other areas of the world. Much of what we know now about neural tube defects involves a Northern European strain of humans. It is likely that there will be specific susceptibility of other strains of humans that we will come to understand. So those physicians who take care of humans need to be careful
about identifying the background of a particular individual (which most researchers working with animals do, of course, very carefully).

I also want to tell you about our most recent venture using the results from studies on mice to understand the abnormalities seen in humans. For the mouse there is a multisite closure model that explains certain types of abnormalities that occur among neural tube defects in mice (Golden & Chernoff 1993, Juriloff et al 1993). We are finding that the same model seems to explain all of the neural tube defects among the children seen in our clinic, in terms of the specific site of the defect (Van Allen et al 1993). If we use the numbers of particular closure sites which were described by Golden & Chernoff (1993) to define the multiple sites of initiation of closure of the neural tube in the mouse, we can explain the defects observed in humans by either single-site closure anomalies or multiple-site anomalies. This model also explains the sites of encephaloceles. In humans, commonly encephaloceles are either in the nasal forehead area or in the occipital area; these appear to be areas of closure sites in humans. We think that each of these closure sites in humans has different genetic control and different environmental susceptibilities. This concept creates a framework for thinking about the results of animal studies and the effects of environmental factors on human development that we will hear about during this symposium. In this context closure 2 and closure 1 are the sites that seem to be susceptible to folic acid. We have proposed the hypothesis that these closure sites in humans can be identified, that the specific genes which regulate closure can be determined, and specific environmental components can be identified which influence each closure site.

Finally, this approach emphasizes the importance of integrating observations from many fields. Early developmental embryology appears to be very similar in many animal species, as do the genes that control the different processes. These observations support the importance of basic work being done in mice because of its applicability to the human situation. The hope, of course, of this symposium is to bring together workers from many different fields for cross fertilization, for consensus and status taking, and, if at all possible, to develop practical recommendations regarding neural tube defects in humans.

References

DISCUSSION

Opitz: Judy, in the Sikh population in British Columbia, you see an increased incidence of neural tube defects. What kind are these?

Hull: There is both an increased occurrence rate among the BC Sikh population and there is a subgroup which has a different kind of neural tube defect. They have high thoracic defects, with which the affected Sikh individuals cope extremely well. Somebody of Northern European ancestry who has a high thoracic neural tube defect is usually very incapacitated, whereas the BC Sikh affected individuals can walk, are of normal intelligence and generally do very well.

Opitz: Where are the areas of greatest variability in vertebral structure in Sikhs? This relates to the old hypothesis that neural tube defects occur in those sites in the neuraxis where there’s the greatest normal developmental variability—the lumbosacral area and the occipital/cervical area. This is based on data from the 1920s and 1930s when people used to count these things and analyse them in great detail. Are the Sikhs, on the basis of their normal vertebral structure, different from other Indians or European populations?

Hull: Yes, they are. There is a very high incidence of spina bifida occulta among Sikhs. We went to Sikh families in which there had been recurrence of neural tube defects and X-rayed the parents. All of them had spina bifida occulta in the lumbar area. So we thought we would be able to screen and tell who is at increased risk among the Sikhs. We studied X-rays of this population that had been taken for some other reason and found an incidence of about 50% spina bifida occulta among the Sikhs. In Northern Europeans this incidence is more like 10%. So the background incidence among Sikhs of lumbar spina bifida occulta is high, but there does not appear to be an increased incidence of thoracic segmentation problems.

Opitz: The hypothesis that there is a direct correlation between the incidence of neural tube defects in humans and the normal variability of their spine should be tested. The hypothesis is that these malformations occur at sites of ongoing evolutionary modification of structure. According to the developmental field model, these sites would be less buffered or less well canalized. So one wonders if the lumbosacral spina bifida occulta is an indicator of an increased sensitivity due to some epigenetic dysregulation or susceptibility to spina bifida in the mid-thoracic area.

Hull: I don’t think so. I think it is due to a specific recessive gene. In those specific families, there is a high incidence of stillbirth and also a high incidence of hydrocephalus.

Opitz: Is the hydrocephalus Arnold Chiari?

Hull: No, it’s not and the siblings with hydrocephaly do not have spina bifida. When you add up all the recurrences, there is almost a one in four chance of some type of problem. So I think the predisposition to neural tube defects among the Sikhs in British Columbia is due to a different gene.
Opitz: It could be due to an autosomal recessive gene. On the other hand, it may be that inbreeding within this population has made them homozygous for many genes, thereby reducing buffering which normally keeps neural tube development proceeding correctly. One way to answer that question is to study the inbred and outbred populations. Given that the population in British Columbia is now outbreeding, is the incidence of neural tube defects decreasing?

Hall: We think that it's like the Irish when they moved to Massachusetts: the incidence of neural tube defects decreased, but it didn't go away altogether. Socially, it's unacceptable for East Indians to have any kind of congenital anomaly in their family, so these are 'family secrets' that are often not shared with the medical profession. It is very difficult to get good family histories from before they emigrated to North America. We know there is a high incidence of neural tube defects in the area of India from which the Sikhs in British Columbia come. Also, in British Columbia there is still a very strong influence to have arranged marriages, so the inbreeding may persist.

Mills: The Sikhs tend to be vegetarians. They also cook food very heavily, which raises the possibility of both folic acid and vitamin $B_{12}$ deficiency in relation to their high incidence of neural tube defects. That would make them a potential risk group both in terms of genetics and in terms of diet. In the population of Sikhs in British Columbia, it would be interesting to look at those Sikhs who don't eat a traditional vegetarian diet.

Hall: We are interested in the diet and would like to look at this.

The buffering that John Opitz mentioned suggests that mammals have evolved with several points of closure of the neural tube, which provide back-up mechanisms in case one closure site fails. It does look as if there is more than one site of closure in humans. In particular individuals, there may be a predisposition to a closure not working properly. One thing in which we are interested is a possible interaction between diet and different closure sites. In amphibia and birds is there a similar back-up mechanism or is there really one closure process that 'zips' all the way up?

Jacobson: In the amphibia it appears as though it is one process. There is one site at which closure starts and the animal ends up with two neuropores.

Schoenwolf: In birds, macroscopically there are about three different sites of closure—anterior neuropore, posterior neuropore and something in between at the level of the auditory placodes. Henny van Straaten has looked very carefully at posterior neuropore closure with a high-definition microscope. Even within an area that macroscopically looks as if it's closing all at one time, there is pulsatile activity and there are localized sites of closure within that area. Closure is not as smooth as we initially thought.
Normal neurulation in amphibians

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Abstract. How does cell behaviour accomplish neurulation in amphibian embryos? During neurulation, the neural plate (while preserving the same volume) doubles its length, triples its thickness, narrows 10-fold, greatly decreases its surface and rolls into a tube. Cells that compose the neural plate produce these changes in three ways. They change shape, change neighbours and attempt to crawl beneath the contiguous epidermis. Plate width, length and area are decreased and the plate thickens when apical surfaces of plate cells contract radially, but plate length increases and width is further decreased when cells reposition themselves and collect along plate boundaries. Contraction of the apical surfaces of plate cells also helps roll the plate into a tube. Poisson buckling resulting from elongation of plate borders may contribute bending forces that help tube formation. The main folding force in tube formation is a rolling moment toward the midline produced by neural plate cells attempting to crawl beneath the contiguous epidermis. Experiments, observations and computer simulations support these assertions, reveal the organization of cell behaviour and implicate contraction of actin filaments as the main source of the necessary forces.

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The study of neurulation requires analysis of the behaviour of the cells of the neural plate. Amphibian embryos offer several advantages for the study of the mechanics of neurulation. Their embryos are accessible and have large cells. Behaviour patterns of single cells are easy to observe. Variegation in egg pigmentation provides built-in markers for the tracking of individual cells. Perhaps the greatest advantage of most amphibian embryos is that there is no growth until well after neurulation has been completed. There is some cell division during neurulation, but the daughter cells do not enlarge, so there are no immediate morphogenetic consequences. Measurements of the volume of the neural plate of a newt (Jacobson & Gordon 1976) reveal that there is no increase in size during neurulation. In contrast, growth during neurulation in amniote embryos may be a confusing factor.

The neural plate is an epithelium that is one cell thick in most amphibians. Neural plate formation begins in newt embryos when a hemisphere of neural ectoderm converts to a disc of the same diameter. The cells of the plate double
Neurulation in amphibians

in height during this conversion (Jacobson & Gordon 1976). In the disc-shaped plate, the cells are taller than the cells of the contiguous epidermis. During a relatively long period, the neural disc reshapes itself into the form of a keyhole, remaining flat as it becomes longer, narrower and thicker. Neural folds begin forming at the plate edges. Then, in a relatively short period, the neural folds rise and roll toward the midline, closing the plate into a tube, while the plate abruptly elongates, narrows and thickens (Fig. 1). The rate of elongation of the midline of the neural plate is 10 times faster during the period when the plate is rolling into a tube than in the periods before or after tube formation (Jacobson & Gordon 1976). When the elastic neural plate is stretched along its midline or along its edge, as it is late in neurulation, the resulting Poisson buckling may help fold the plate toward the midline (Jacobson 1978).

Many studies of neurulation are based upon transverse sections and ignore other dimensions of the neural plate. There has been a general belief that the neural plate narrows and rolls into a tube because the apical surfaces of the cells of the plate contract radially. Such a contraction would also shorten the midline and fold the anterior and posterior ends of the neural plate upward. Instead, the midline elongates and the anterior and posterior ends of the neural plate bend downward over the spherical surface of the embryo. Different patterns of cell behaviour must be invoked to explain this and other aspects of neurulation. Several types of cell behaviour together are responsible for neurulation.

In a newt embryo, from the inception of the neural plate to completion of tube formation, the thickness of the plate almost triples, the length doubles, the width narrows at least 10-fold, the surface is reduced and the plate rolls into a tube.

![Diagram of neurulation](image)

FIG. 1. Drawings, to the same scale, of dorsal views of the neural plate and tube of a newt, *Taricha torosa*. The plates were excised from the embryo and laid flat on an agar bed, then photographed immediately. This method avoids any optical foreshortening. The times between stages, at 17 °C, are from a time-lapse film of neurulation. Modified from Jacobson (1980).
Evidence given below suggests that cells of the amphibian neural plate themselves accomplish the deformations of neurulation. Some adjacent and underlying tissues are necessary to affect the behaviour of the plate cells, but they do not exert forces on the plate that are necessary for neurulation.

Cells have limited ways to apply the forces that accomplish the work involved in the tissue deformations of neurulation. In all cases, the forces provided by the constituent cells must be in the plane of the tissue.
The necessity for maps of cell positions during neurulation

Any analysis of cell behaviour in a field of shifting cells requires that the cell positions be mapped through time. One cannot quantitatively describe changes of cell shape during neurulation without a map of the neural plate and a knowledge of trajectories of the individual cells. More than one investigator has described changes of cell shape by measuring sample cells in typical transverse sections at different times without regard for the changes in the locations of the cells. There is a general flow of cells during neurulation toward the midline and toward the anterior (C.-O. Jacobson 1962, Burnside & Jacobson 1968, Keller et al 1992). Keller has called this set of cell movements ‘convergent extension’. During neurulation, cells flow through cross-sections, so it is necessary to measure cells in a section at an early stage, then deduce from cell trajectories the correct section in which to measure them at a later stage.

Maps of cell trajectories in the neural plate from gastrula to mid-neurula stages are available for a newt and a frog (Fig. 2B, E). A fate map of the mid-neurula axolotl neural plate was made by C.-O. Jacobson (1962). This map was projected onto a mid-neurula stage embryo of Taricha torosa, a newt, and re-mapped to late gastrula stage 13 by running backwards a time-lapse movie of neurulation and following many of the cells (Fig. 2C, A; Jacobson & Gordon 1976). Burnside & Jacobson (1968) mapped cell trajectories during early neurulation in the same newt (Fig. 2B). Eagleson & Harris (1990) made a fate map of the mid-neurula (stage 15) neural plate of the frog Xenopus laevis (Fig. 2F). Keller et al (1992) used a video tape of Xenopus neurulation to map this fate map back to mid-gastrula stage 11.5 by following cells at the intersections of the grid on the stage 15 embryo back to the earlier stage (Fig. 2F, D). In maps of both species at gastrula stages, the brain occupies most of the prospective neural half of the animal hemisphere. The spinal cord and the hindbrain are narrow strips that lie perpendicular to the long axis near the blastopore. They occupy the region called by Keller the ‘non-involuting marginal zone’ (NIMZ). Convergent extension movements greatly lengthen the spinal cord and hindbrain along the axis during neurulation and the movements are largely confined to these regions.

FIG. 2. The fate map of the open neural plate stage of an axolotl (C.-O. Jacobson 1962) was projected onto the same stage in a time-lapse film of neurulation of the newt, Taricha torosa (C). The film was run backwards and cells at the numbered points were followed to a beginning neural plate stage (A) (from Jacobson & Gordon 1976). Cell trajectories between the two stages, as described by Burnside & Jacobson (1968), are shown in B. The beginning of each arrow is the cell position at the earlier stage; the arrow head marks the position at the later stage. D, E, F is a similar study from a video recording of neurulation of a frog, Xenopus laevis, by Keller et al (1992). Cells at the intersections of the grid lines were followed. The fate map of the stage 15 Xenopus embryo is from Eagleson & Harris (1990).
Cell behaviour during neurulation

In *Xenopus*, the movements of convergence and extension begin in the prospective posterior neural plate by stage 11.5 and continue through neurulation. Convergent extension is driven mostly by intercalations among cells in the mediolateral direction (Keller et al 1992).

Keller et al (1985) explanted large pieces of tissue from above the dorsal lip of the blastopore of *Xenopus* embryos. These explants contained both the prospective chordamesoderm and much of the area that later would be neural plate. Two explants were positioned with the basal ends of chordamesodermal cells in contact and with the basal ends of prospective neural plate cells together. These ‘Keller sandwiches’ were held flat in culture while convergence and extension movements of cells started in the chordamesoderm, followed by similar movements in the posterior regions of the prospective neural plate. The two regions extended themselves in opposite directions. If the sandwiches were cut along the line between chordamesoderm and prospective posterior neural plate before culturing, each separated part made its own movements of convergence and extension.

When grown in a culture medium adjusted to resemble blastocoel fluid, ‘open face’ sandwiches of chordamesoderm converge and extend, but the prospective neural plate parts will not extend unless some tissue is apposed to their basal surfaces (Keller et al 1985). In normal embryos the underlying tissue would be the involuted notochord. In *Xenopus* embryos lacking a notochord, Malacinski & Youn (1981) observed normal neurulation. These embryos had somites, rather than notochord, beneath the posterior neural plate. In Keller sandwiches, posterior neural plates face one another and the cells make normal movements. In all these cases with one or another of the various tissues beneath the neural plate, convergent extension movements occur and the movements are intrinsic to the cells of the prospective posterior neural plate.

Jacobson & Gordon (1976) analysed in a newt embryo the details of cell behaviour during the period from stage 13 when the plate is disc shaped to mid-neurula stage 15 when the plate is shaped like a keyhole but has not yet started to roll into a tube (Fig. 1). We called this the ‘shaping period’, it takes about 90% of the time of neurulation. The period of tube formation that follows takes only about 10% of the time.

Burnside (1973) measured the changes in shape of some cells between the disc and keyhole stages in the newt embryo. The lengths of cells were measured from sections and their apical areas were calculated as the ends of cylinders of the measured heights. As the cells elongate during this shaping period they preserve volume and remain cylindrical so the apical areas are inversely proportional to cell heights.

Because height measurements were made from sections, it was necessary to refer to the map of cell trajectories made by Burnside & Jacobson (1968) to find
appropriate sections in which the same cells appeared at both times. Obviously, the measurements were made in different embryos, so some estimate of the consistency of cell trajectories from embryo to embryo was necessary. Burnside & Jacobson (1968) found that the cells at intersections of grids on three different embryos followed the same trajectories, so it is reasonable to make these measurements on different embryos.

Jacobson & Gordon (1976) similarly measured many cell groups over the entire neural plate at the beginning and at the end of the shaping period to map prospective shrinkage of apical areas of cells in the disc-shaped neural plate (Fig. 3B).

After viewing many time-lapse movies of neurulation of newt embryos, we noticed that the cells of the neural plate overlying the notochord behaved differently from the rest of the neural plate cells. At that time we called these cells the ‘supra-notochordal cells’, but later named them the ‘notoplate’ (Jacobson 1981). The notoplate eventually forms the initial floor plate of the neural tube.

The boundary between the notoplate and the rest of the neural plate is visible in time-lapse movies. We suspected that the notoplate has a role in elongating the midline of the neural plate during neurulation. At that time it was not yet clear that the convergent extension movements seen in and around the notoplate were intrinsic to it, rather than being driven by the underlying notochord. The observations from Keller sandwiches noted above make it clear that convergent extension movements may occur independently in the posterior neural plate (including the notoplate) as well as in the chordamesoderm.

**Computer simulations and models of cell behaviour during neurulation**

Jacobson & Gordon (1976) made a computer simulation of the shaping period of newt neurulation. We used the empirical data of the shrinkage map to simulate the shrinkage of the apical surfaces of the constituent cells during the shaping period. Observed changes in position of the border between the notoplate and the rest of the neural plate were used to simulate the convergence and extension that are seen in the posterior neural plate. These two sets of changes were simulated separately and together. Shrinkage alone (a computer experiment) did not produce a keyhole shape (Fig. 3F), whereas midline elongation alone (another computer experiment) did, but not one that matched the normal embryo (Fig. 3G). The computer simulations showed that apical shrinkage plus the midline elongation (which results from changes in the position of the notoplate border) were both necessary and together were sufficient to get the observed changes in shape of the neural plate from early neurula to mid-neurula stages (Fig. 3E).

These simulations raised questions about how cells accomplish the movements of convergence and extension seen in the posterior neural plate. Observations
FIG. 3. Outline drawings from a time-lapse film of neurulation of a newt show the conversion of the plate from a disc shape (A) to a keyhole shape (D). The notoplate is outlined in the midline of the plate. (B) Map of a stage 13 neural plate outline indicating the amounts of shrinkage of cell surfaces (and concomitant elongation perpendicular to the surface of the cells) from stage 13 to 15. Cells whose surfaces shrink the most have higher numbers and are shaded. The rocket-shape in the lower midline represents the notoplate region. Photographs of a computer graphics screen: (C) A starting grid for computer simulations. (E) Distortion of the grid after the shrinkage pattern of B and the changes in position of the notoplate boundary that reflect midline elongation are both simulated. (F) A computer experiment that simulates shrinkage only. (G) A computer experiment simulating midline elongation only. Shrinkage and elongation are both necessary and sufficient to produce the shape change observed in the normal embryo. Figures modified from Jacobson (1980).

indicate that the neuroepithelial cells are changing neighbours in the plane of the tissue, despite being tied to one another by subapical junctional complexes. If cells changed neighbours in the plane of the tissue randomly, there would be no morphogenetic consequences. Some means of organizing cell rearrangements must exist. The neural plate elongates because more cells intercalate mediolaterally than anteroposteriorly. How then can cells forcefully intercalate to produce the necessary compression that extends the length of the posterior neural plate?

It was to answer these questions that Jacobson et al (1985, 1986) proposed the ‘cortical tractor model’ of epithelial morphogenesis. The model drew on what was known of mechanisms of cell motility in mesenchymal cells and applied this to epithelial cells. In this model, cell rearrangements in the plane of the epithelial tissue are due to lateral protrusions that spread apically, followed by
displacement of the bulk of the cell to the site of the lateral protrusion (hence a *forceful* intercalation). During protrusion formation and cell rearrangement (cell movement), actin cortical microfilaments depolymerize and repolymerize at the protrusive tips; contraction waves in the actin network follow. Components of the inner cytosol flow to the site of the protrusion and become incorporated into the cortical cytoskeleton and the cell membrane. Cell–cell adhesion molecules are extended through the cell membrane and directly or indirectly attach to the cytoskeleton and to adhesion molecules on adjacent cells. Junctional molecules also insert into the membrane and are carried apically with the cytoskeleton. There is a time-averaged flow of cortical cytoplasm and membrane away from the tip of the protrusion and toward the body of the cell and also more generally toward the apical end of the epithelial cell. This 'cortical tractor' is propelled by waves of contraction in the actin filament network that start at the tips of protrusions. Such peristaltic contraction waves were long ago described in isolated neural plate cells as running from protrusions at the basal end of the isolated cell toward the apical end (Holtfreter 1946). Their effect is to move the cell toward the end of the protrusion.

At or near the apical ends of the cells, there must be a 'sink' or 'sinks' where the membrane, cytoskeleton and associated adhesion and junctional molecules are recycled into the interior cytoplasm. It is quite possible that this tractoring positions the subapical junctional complex. These junctions must be dynamic, constantly turning over, and thus allow cells to change neighbours without disrupting the apical seals. The subapical purse-string of actin microfilaments may also be put in position by the apically directed cortical tractoring; contraction of this bundle may then help reduce the apical surfaces of neural plate cells.

It is clear from direct observation of time-lapse movies that the notoplate border elongates along the axis during neurulation. For a border to elongate, cells must be added next to the border and stay there. We proposed that the boundary between the notoplate and the rest of the neural plate organized the otherwise random intercalations of cells by trapping cells that encounter the border. Cells from either the notoplate side or the neural plate side of the boundary would randomly intercalate among themselves and those that encountered the border would stay there. Cells would not cross the boundary. This might be due to adhesive differences in the two domains of cells or to contact inhibition between the two cell types.

We made observations and computer simulations that confirm important parts of the model (Jacobson et al 1986). We found, as predicted, that there are lateral protrusions in the notoplate that extend two or more cell diameters and that the direction of their extension is random except at the border. We re-examined time-lapse movies of newt neurulation and followed individual cells in both the neural plate and in the notoplate. Cells of each kind move about randomly until they reach the boundary, then they stay at the boundary, as predicted.
We made computer simulations testing the physical properties we proposed. These simulations showed that a rolling moment from the plate edge toward the midline, imposed either by basal tractoring or by apical constriction, can roll the plate into a tube. This was especially realistic if the midline was anchored, which, in fact, it is by the tight adhesion of the notoepithelium cells to the underlying notochord.

The chordamesoderm that underlies the neural plate also makes convergence and extension movements. Keller & Tibbetts (1989) found that small clumps of labelled cells inserted into prospective notochord at the beginning of gastrulation respect the border between notochord and somite as they rearrange during ensuing convergence and extension. They behave as the cells do at the boundary between the notoplate and the neural plate in newt and axolotl embryos.

Keller et al (1992) followed cells during gastrulation and early neurulation of *Xenopus* embryos and did not see any notoplate boundary. Nevertheless, their maps of cell trajectories suggest that no cells cross the lines where one would expect the notoplate boundaries to be.

Cuts were made the length of the notoepithelium boundary in the mid-neurula newt embryo to see if a boundary with the notoepithelium must be present for elongation of the neural plate to occur. In the newt, the side of the neural plate lacking a notoepithelium boundary elongated little or not at all; the side of the neural plate having a notoepithelium boundary elongated. If both notoepithelium boundaries were severed, then three pieces were created—right and left neural plate pieces and a central notoepithelium piece—none with a boundary. None of these separated pieces elongated. In control experiments, the notoepithelium was severed down the midline so that right and left neural plate pieces each had some boundary; each side then elongated (Jacobson 1991). Similar experiments with axolotl embryos have given more ambiguous results that suggest that this embryo relies more on the neural plate/epidermal boundary for elongation (A. G. Jacobson & J. D. Moury, unpublished work 1993).

**Events at the epidermal border with the neural plate**

The observations discussed above called attention to the border of the neural plate with the epidermis. We have measured during neurulation the change in length of the border between the neural plate and the epidermis in a newt and in axolotl embryos. We find that the brain border does not elongate, but the border of the prospective spinal cord does (A. G. Jacobson & J. D. Moury, unpublished work 1993). As Keller et al (1992) point out, convergence and extension movements are restricted in *Xenopus* to the spinal cord and hindbrain regions of the neural plate. The measurements we have made on urodeles suggest the same for them.

Elongation of the spinal cord border must involve intercalation of cells into the border from both sides, as seen at the notoepithelium/neural plate border. The brain
FIG. 4. Drawings from transverse sections illustrating how neural plate cells at the epidermal border crawl beneath the epidermis, elongate themselves, raise neural folds and create a rolling moment (arrows) toward the neural plate midline. Figures modified from Jacobson et al (1986).

border, which shortens, could be driven to do so entirely by the large decreases in apical surfaces of the brain plate cells along it. The epidermal cells on the other side of the border would have to make many adjustments, including associating with increasing numbers of brain plate cells; some cells might leave the border. We are trying to quantify the numbers of brain plate cells associated with each epidermal cell at early and at late neurulation.

Jacobson et al (1986) observed that neural plate cells at the plate edge try to crawl beneath the epidermis and thus produce a rolling moment toward the midline that could be a large force in rolling the plate into a tube (Fig. 4). Several complex things thus occur at the neural plate border simultaneously. The brain plate border shortens, the spinal cord border elongates and a neural fold lifts as plate cells attempt to crawl beneath the epidermis. In addition, as the neural folds form, some cells next to the border ingress beneath the epithelia from both the epidermis and the neural plate; these are later part of the neural crest.

Some have proposed that epidermis 'pushes' the neural plate edge, lifting a fold. This idea has been discounted because slits made in the adjacent epidermis gape instantaneously, demonstrating that the epidermis is under tension, not compression (Jacobson & Gordon 1976). To show that association with some contiguous epidermis is necessary for the plate cells at the borders to undergo the complicated behaviours noted, we have isolated neural plates with underlying mesoderm so they will have the proper associations of their basal surfaces, either with or without a strip of contiguous epidermis. These isolated plates never form a tube if there is no epidermis; they also fail to elongate as much as normal. If there is a strip of epidermis included with the isolate, then the explanted neural

Neural plate cells nearest the border with epidermis greatly increase their heights and reduce their apical surfaces to mere points. Continued basally directed crawling concentrates tugging forces at these apical points until the subapical junctions break, which allows the cells to pull themselves out of the epithelium (Jacobson et al. 1986). The ingressed cells most likely become neural crest cells.

**Associations with cells outside the neural plate may help guide the behaviours of cells within the neural plate**

When two domains of different sorts of cells form a common boundary, the cells that abut the boundary on each side behave differently from the rest of the cells in the two domains. As noted above, cells from each side intercalate along the border between the neural plate and the notoplate: they do not cross the border and they remain at the border. Cell behaviour is thus organized along the border so that the border elongates. The epidermal border with prospective spinal cord also elongates, but the border between brain plate and epidermis does not in the amphibians examined. Jacobson & Tam (1982) found that the brain plate of the mouse embryo does elongate along its border with the epidermis. Convergence and extension movements among the cells of the notoplate and the posterior neural plate occur only when some other cells are beneath their basal surfaces. Cells that will satisfy this requirement of basal association include those from notochord (as in a normal embryo), somitic mesoderm (as in notochord-free embryos) or a duplicate of the posterior neural plate (as in Keller sandwiches).

Tube formation depends on lateral contact between neural plate cells and epidermal cells. Neural plate cells attempt to crawl beneath epidermal cells in normal embryos; in the process they elongate as they produce a rolling moment toward the midline that is a principal driving force of tube formation. An explant of neural plate with only a rim of epidermis attached will close into a tube and the sequence of shape changes in neural plate cells that normally starts at the epidermal border occurs in these explants (A. G. Jacobson & J. D. Moury, unpublished work 1993).

Because the plate edge is parallel to the midline, the rolling moment produced where neural plate cells contact epidermal cells bends the neural plate upward and toward the midline. This bend increases during neurulation until the plate becomes a tube. The bend produces a fold line on the surface of the neural plate comparable to the ‘lateral hinge points’ described by Schoenwolf (1982, 1991) in chick embryos. This fold line begins early in neurulation near the edge of the neural plate, then advances toward the midline. The position of the fold line of the plate coincides exactly with the apical end of the most ventral neural plate cell whose basal surface contacts the epidermis (Fig. 5). We have quantified
FIG. 5. Plastic transverse sections through the neural plates of newt embryos at stage 17 (A) and stage 20 (B). The cell whose apex is at the line of folding in the neural plate (right arrows) is the most ventral cell to have basal contact with the epidermis (left arrows). The amount of epidermis–neural plate contact (between arrow heads) changes with the stage, as do the positions of the fold lines.

the amount of contact between neural plate cells and epidermis along the anteroposterior axis through the course of neurulation in axolotl and newt embryos (A. G. Jacobson, J. D. Moury & Y. Lu, unpublished work 1993). The amount of contact varies with time in all regions. The regions that develop the greatest amount of contact are where the plate is widest. Our findings are consistent with a change in behaviour of neural plate cells that advances as a wave from the plate edge toward the midline. This results in elongation of the plate cells and plate folding as the basal ends of the plate cells sequentially encounter the epidermis.

Because the border between the neural plate and the epidermis seems to organize plate folding, we reasoned that any created border between neural plate and epidermis should raise a fold. Using transplants between different genetic strains (pigmented and albino) of the axolotl embryo, Moury & Jacobson (1989, 1990) demonstrated that artificial confrontation of any portion of the neural plate and epidermis will result in formation of new neural folds and neural crest cells at the created boundary. These results overturn old ideas of neural crest induction and fold formation. Both these processes result from local interactions between neural plate cells and epidermal cells. We also found that neural crest arises from both neural plate and epidermis.
Because the properties of the border between neural plate and epidermis greatly affect neurulation, it is important to know how the initial position of this boundary is established. Zhang & Jacobson (1993) hypothesized that the anterior border of the neural plate is positioned by interactions of the same signals that induce ventral and dorsal mesoderm in \textit{Xenopus laevis}. We suggested that these signals continue through the prospective ectoderm of the animal hemisphere and interact to establish the anterior border of the neural plate near the animal pole.

To increase ventral signals at the animal pole, we transplanted ventral vegetal cells into the animal poles of early blastula (stage 7) embryos. The neural boundary was driven back toward the blastopore, producing embryos with short axes. A mark placed in prospective anterior neural plate ends up in the anterior neural plate of a normal embryo, but in the anterior ventral epidermis in the experimental embryos, suggesting that prospective neural plate has been converted to epidermis. We did the same transplantation at a later stage—just before gastrulation commences (stage 9)—and the embryos developed normally, indicating that the anterior neural plate border is established prior to gastrulation.

To reduce ventral signals that would reach the animal pole, we removed a few ventral vegetal cells at the 64-cell stage. Our prediction that these embryos would have axes that were longer than normal was confirmed. A mark placed in anterior prospective ventral epidermis ends up in the epidermis near the anterior end of the neural plate in a normal embryo, but in the anterior neural plate in these experimental embryos, suggesting that the experiment converts prospective ventral epidermis into anterior neural plate. If dorsal rather than ventral signals are reduced by removing dorsal vegetal cells, then the axes that form are shortened, as predicted.

The experiments above indicate that the same signals that induce ventral mesoderm and dorsal mesoderm during cleavage and blastula stages interact at the animal pole to position the border of the neural plate before gastrulation begins. Once the neural plate boundary is established, and epidermal and neural plate differentiation begin, planar inductions that specify neural crest occur in both directions across the boundary. Other planar interactions between the two cell types raise neural folds. In \textit{Xenopus}, prior to gastrulation, planar induction from contiguous chordamesoderm is responsible for the induction of convergence and extension movements in the posterior neural plate (Keller et al 1992). Planar induction thus plays a major role in setting up the boundaries of the \textit{Xenopus} neural plate and inducing the cellular movements that elongate the neural plate.

\textbf{Conclusions}

The changes in the shape of the neural plate during neurulation may be explained by the behaviour of the neural plate cells themselves, with some important