The Shaping of Tissues in Embryos

Computer simulations of development make it possible to perform experiments that cannot be done in the biological laboratory. They help to reveal some of the basic forces that sculpture the embryo

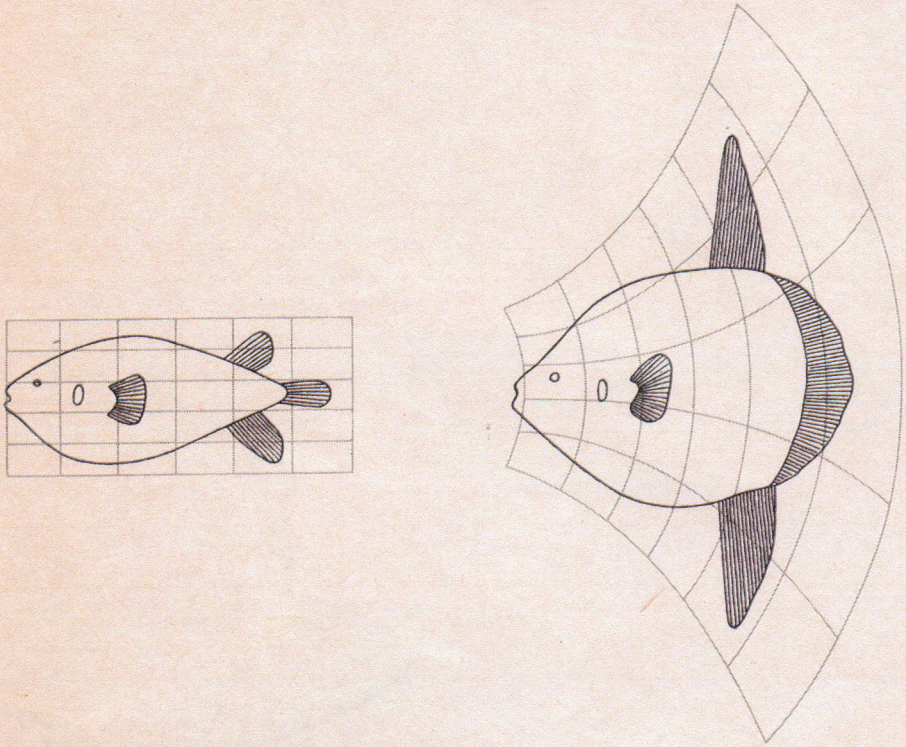
by Richard Gordon and Antone G. Jacobson

After the egg of a higher organism is fertilized it divides into many thousands of cells that arrange themselves into the tissue layers of the embryo. Understanding this elaborate process of embryonic development is one of the outstanding and most difficult problems challenging biologists today. The problem is so complex that it must be broken down into smaller problems before investigation can yield instructive solutions. Therefore in our own work we set out to address the general question of how tissues are shaped in the embryo by analyzing the formation of a single embryonic tissue: the neural plate, the earliest precursor of the central nervous system. The organism we chose for this purpose is the California newt, *Taricha torosa*, which is indigenous to the ponds and streams around San Francisco Bay.

In the first stages of development the successive divisions of the fertilized newt egg give rise to the hollow, single-layered ball of cells called the blastula. Half of the tissue then tucks itself through a slit in the blastula, forming in the spherical embryo two layers: the endoderm and mesoderm. The embryo is now called the gastrula. It is one hemisphere of the outer layer of the gastrula that is destined to become the neural plate. In the course of about 30 hours this hemisphere flattens into a disk and then assumes the shape of a keyhole. The wide part of the keyhole eventually gives rise to the brain and the narrow part to the spinal cord.

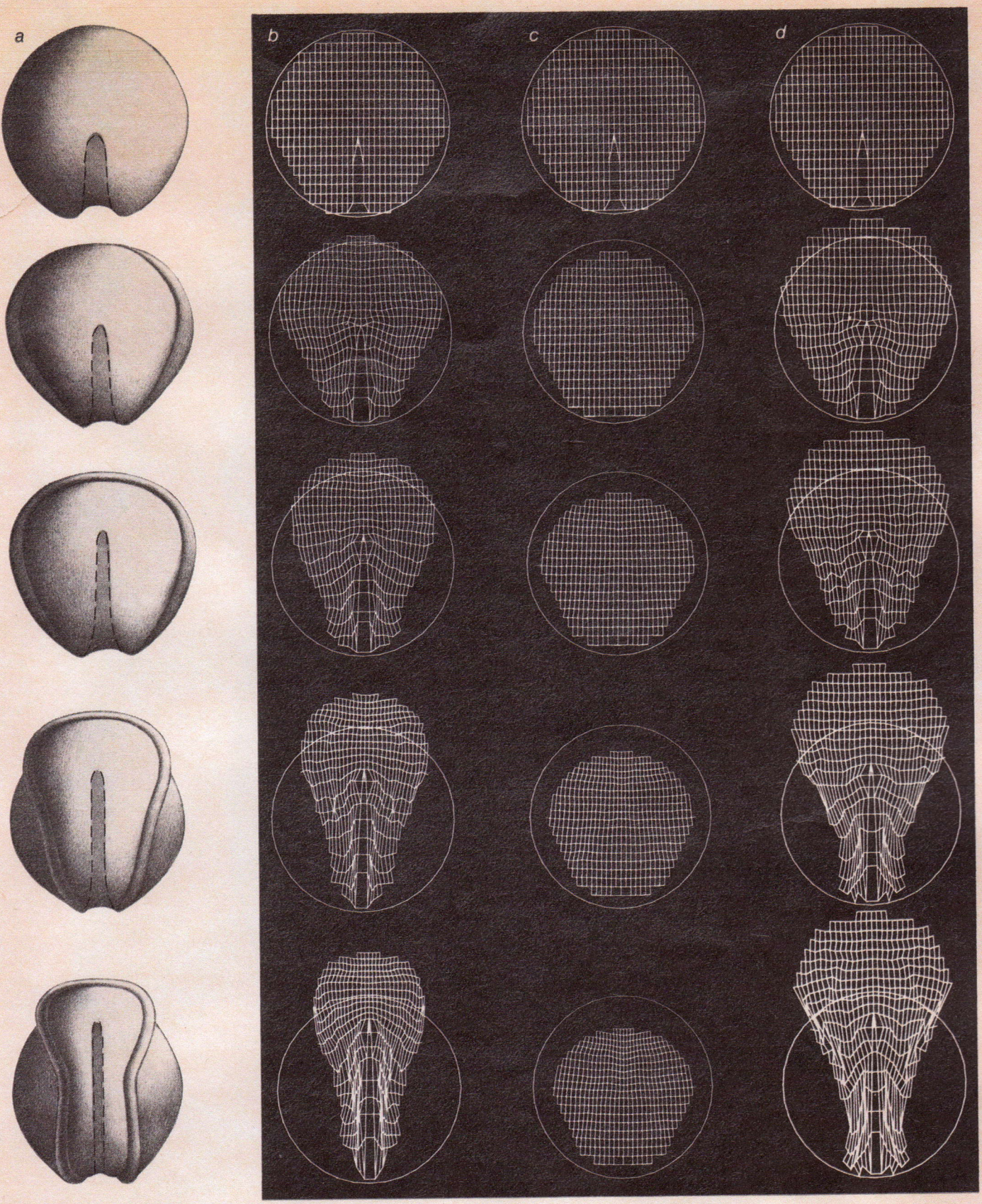
In the course of the neural plate's transformation from the disk stage to the keyhole stage its constituent cells move in a complex spatial and temporal pattern. In undertaking to analyze this pattern we made use of a computer simulation to test the adequacy of different modes of cell behavior to explain the observed changes in the shape of the tissue. Writing the simulation program required that we express the behavior of the cells quantitatively and that we make all our assumptions explicit. These requirements led in turn to more precise observations of the living embryo. Through such a process of simulation alternating with observation we began to discern the forces involved in the shaping of the neural plate (and by analogy in the shaping of many other embryonic tissues as well).

The newt embryo was chosen for a number of reasons. It is hardy and tolerant of experimental surgery, and its neural plate is one cell thick, making observation and analysis relatively easy. The embryonic newt cells are large enough to be examined with a low-magnification dissecting microscope, and they contain varying amounts of dark pigment, so that individual cells can be identified and followed during development without the need for stains or other markers. Moreover, each embryonic cell contains its own supply of stored food (yolk), so that it is possible to culture groups of embryonic cells or even single cells in simple salt solutions.

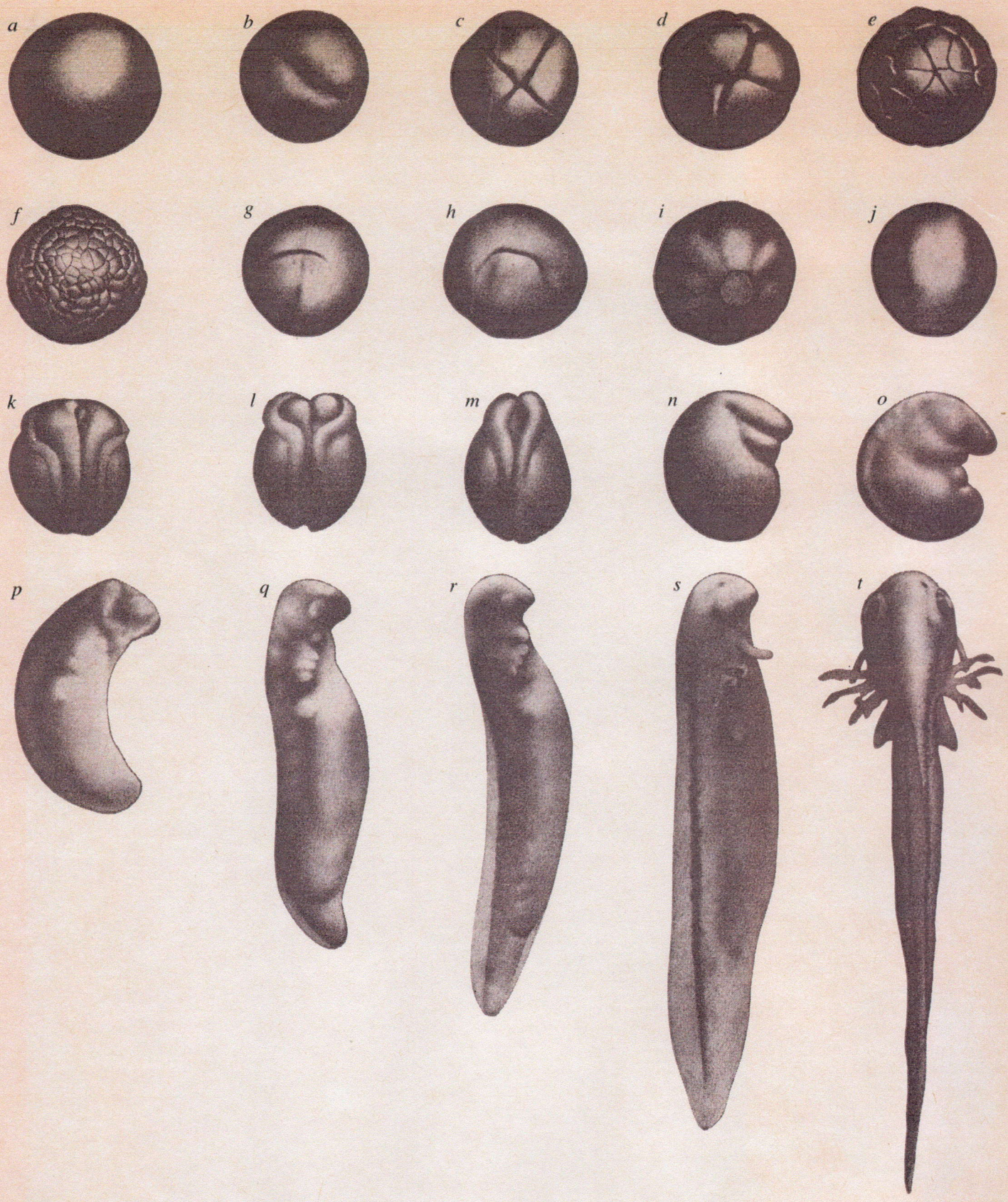


GRID-TRANSFORMATION METHOD was devised by the Scottish biologist D'Arcy Wentworth Thompson, who showed how related organisms could be represented as relatively simple distortions of each other. This illustration, based on one in Thompson's 1917 work *On Growth and Form*, shows the geometrical relations between the porcupine fish *Diodon* (*left*) and the sunfish *Orthagoriscus mola* (*right*). Thompson's grid-transformation method has proved useful in understanding the forces that shape sheets of cells into the tissue layers of the embryo.

The mathematical foundations of our computer simulation go back to the work of D'Arcy Wentworth Thompson of the University of St. Andrews at the turn of the century. In 1917 Thompson published his classic work *On Growth and Form*, in which he proposed that the evolutionary transformation of one species into another is a process involving the entire organism rather than successive minor alterations in the body parts. Thompson represented the transforma-



COMPUTER SIMULATIONS shown here were developed by the authors to model the formation in the embryo of the neural plate, the earliest precursor of the central nervous system. The shaping of the tissue is represented as the distortion of a geometric grid placed over the embryo. Column *a* shows a sequence of schematic diagrams of the developing neural plate, derived from a time-lapse motion picture. Column *b* shows a computer simulation that incorporates two forces: nonuniform shrinkage of the neural-plate cells and elongation of the supranotochordal region along the midline of the neural plate. The resulting shape of the transformed grid is virtually identical with that seen in the embryo. In column *c* supranotochordal elongation has been turned off so that the only force is provided by cell shrinkage; the resulting grid is reduced in size but does not attain the keyhole shape. In column *d* cell shrinkage has been turned off so that the only driving force is provided by supranotochordal elongation; this experiment could not be done with the living embryo. Although the grid does attain the keyhole shape, the top end is overlarge. Thus both cell shrinkage and cell movement appear to shape the neural plate.



DEVELOPMENTAL STAGES of the California newt *Taricha torosa* are shown in this abbreviated version of a sequence prepared by Victor C. Twitty and Dietrich Bodenstein at Stanford University. First the fertilized egg divides many times over (*a–f*), giving rise to a hollow sphere of cells, or blastula. Then a groove appears below the equator of the embryo (*g*) and gradually deepens to form a spacious internal cavity as more and more surface tissue moves into the interior (*h, i*). This process, termed gastrulation, gives rise to two internal tissue layers: the endoderm and the mesoderm. The mesoderm later forms the main body musculature and the notochord (a rod beneath the embryonic nervous system). One hemisphere of the outer layer of the embryo then flattens into a disk (*j*) and forms itself into the keyhole-shaped neural plate (*k*). At the same time the supranotochordal region of the neural plate and the underlying notochord undergo a considerable elongation and narrowing along the midline. In the succeeding stages the neural plate rolls up to form the neural tube (*l–n*), the eyes develop and the embryo elongates into the larval form (*o–t*). Views *a–f* show the top of the embryo, *g–i* show the bottom, *j–m* and *t* show the back and *n–s* show one side. The newt embryo is particularly amenable to analysis because it is hardy and its cells are quite large.

tion as the geometric distortion of a grid placed over the organism, resulting in extensive changes in spatial relations. As a consequence two organisms having a common ancestor will have shapes that are related by an often simple transformation. Thompson's method of geometrical transformation has proved to be extremely useful in mathematically analyzing the shaping of embryonic tissues.

In 1968 Mary Beth Burnside, who was then working at the University of Texas at Austin, analyzed the movement of individual pigmented cells in developing newt embryos by following the cells at the intersections of a superposed D'Arcy Thompson grid. By means of time-lapse motion pictures she determined how the geometry of the grid was distorted during the forming of the neural plate. The transformation was remarkably consistent from one embryo to the next: when she superposed the transformed D'Arcy Thompson grids of three different embryos, they overlapped almost exactly. She also observed that the distortion of the neural plate was correlated with shrinkage of the exposed surface areas of the cells, and that the extent of the shrinkage varied over the surface of the plate. As the plate shrank, the surface area of each cell remained inversely proportional to its height (its dimension perpendicular to the sheet). Moreover, the increase in height occurred without growth, with the volume of the neural plate remaining constant during the transformation.

The mechanism by which the neural-plate cells lengthen, or increase in height, and thereby reduce their exposed surface area is understood to some extent. With the aid of the electron microscope Burnside observed contractile microfilaments arranged like a purse string around the top end of each cell. Contraction of the microfilaments appears to be responsible for the shrinkage of the exposed cell surface. Larger fibers known as microtubules are oriented along the cell's length and are essential for its lengthening.

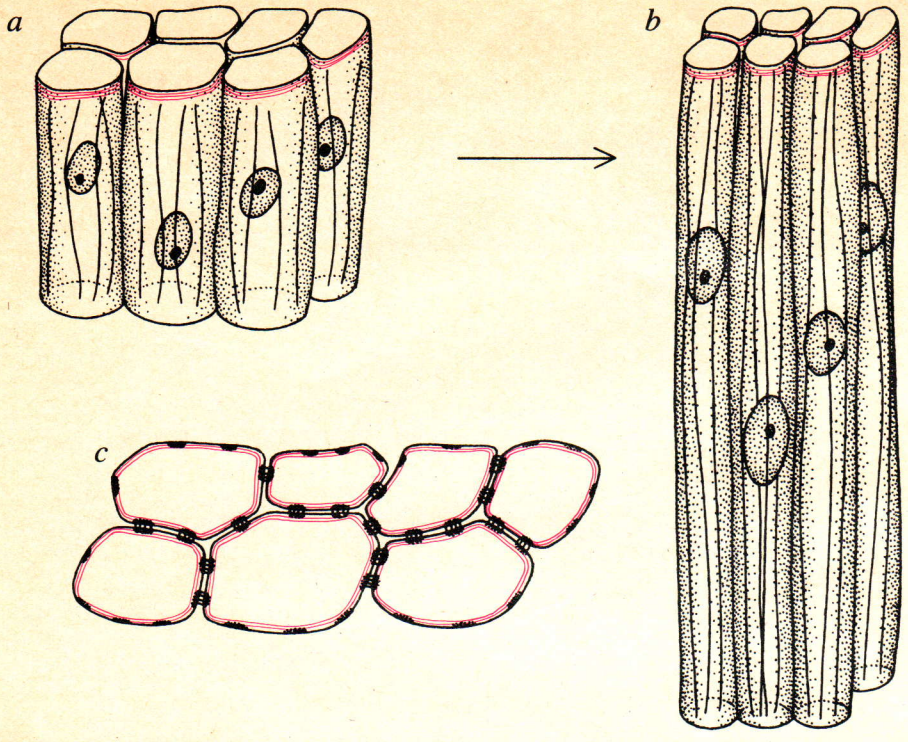
On the basis of Burnside's observations we undertook to devise a mathematical model of the neural plate as a sheet of cells, each cell of which lengthened perpendicular to the sheet while maintaining a constant volume. In formulating a quantitative model of the behavior of a neural-plate cell we had to know whether or not height increase was influenced by the behavior of neighboring cells. We transplanted small groups of neural-plate cells from one area of the neural plate to another, where they lengthened in accordance with their original positions. In 1946 Johannes Holtfreter, working at McGill University, isolated single neural-plate cells and observed that they continued to lengthen when they were cultured in laboratory glassware. His observation has been confirmed by Burnside. Therefore lengthening is an intrinsic property of each cell: the cell will increase its height by a characteristic amount wherever it is. Each neural-plate cell can be viewed as an autonomous entity whose "height program," or prospective course of lengthening, has been determined in advance.

NEWT EMBRYO is magnified 40 times in this photomicrograph (the embryo is 2.4 millimeters in diameter). The keyhole-shaped structure in the center of the embryo is the neural plate. The wide part of the keyhole is destined to become the brain and the narrow part the spinal cord. Because the newt neural plate consists of a single layer of cells, it provides a simple but representative system for examining how tissues are formed in the embryo. Moreover, the amount of brown pigment varies from cell to cell, so that it is possible to follow the movements of individual groups of cells at low magnification without the need for stains or other markers.

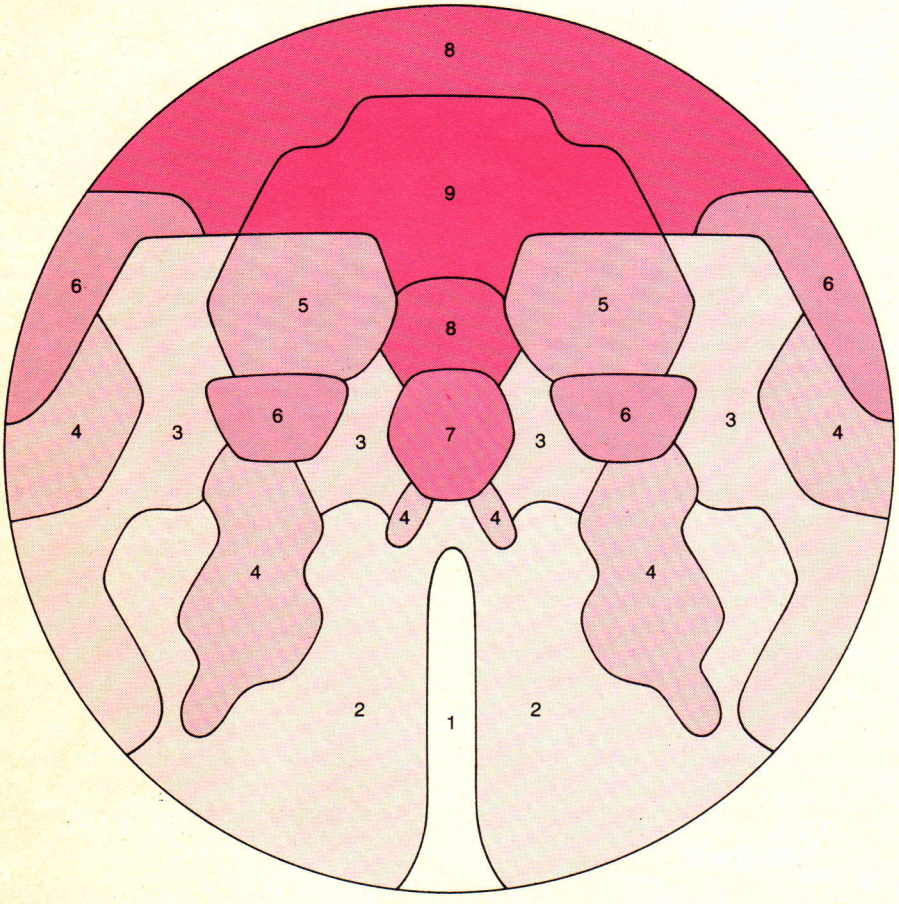
We obtained the height programs of different cells in the following manner. We first measured the heights of the cells in the disk stage in sectioned embryos and then used time-lapse motion pictures to see where the corresponding cells were relocated in the keyhole stage. (Some cells moved as much as .9 millimeter across the 2.4-millimeter embryo!) We then sectioned a keyhole-shaped neural plate and measured the new heights of the relocated cells.

Our mathematical description of a neural-plate cell consisted of five quantities: two spatial coordinates for the cell's location, the cell's initial height, its volume and its height program (which was placed in one of nine classes according to the cell's rate of elongation). The shrinkage pattern of the plate could thus be represented as the pattern of different height programs distributed over the disk-shaped plate [*see bottom illustration on next page*]. The pattern turned out to be quite complex.

At first we thought that the shrinkage pattern alone might be sufficient to explain the shaping of the neural plate. We tested this hypothesis by experiment, by mathematical modeling and by com-



LENGTHENING AND SHRINKAGE of the neural-plate cells generate forces that help to shape the tissue. Here the same neural-plate cells are shown immediately after gastrulation (*a*) and after the closure of the neural tube (*b*). Each roughly cylindrical cell lengthens perpendicular to the sheet while maintaining a constant cell volume; as a result its exposed surface area decreases. Because the microfilament bundles (*color*) in each cell are coupled to those of the adjacent cells, the shrinkage of one cell pulls the other cells toward it, thereby reshaping the sheet (*c*).



PATTERN OF CELL LENGTHENING in the neural plate is quite complex, as is suggested by this map. The precise amount by which each cell lengthens (its "height program") varies considerably with the location of the cell in the neural plate. Here the height programs have been placed in nine classes according to the cell's rate of lengthening. The fastest class is *9*.

puter simulation. In our experiments we surgically removed the disk-shaped neural plate from the embryo and grew it in tissue culture. The surface of the isolated neural plate shrank, but the resulting shape was only slightly narrowed at the rear end and looked very little like the expected keyhole shape. We then reasoned that if each cell pulls isotropically (that is, equally in all directions) as it shrinks, the angle between two intersecting lines drawn on the sheet of cells should not change. In mathematical terms such a transformation is called conformal. Yet we knew that large changes of angle did occur in some regions of the D'Arcy Thompson grid during the transformation of the neural plate, indicating that the shape change was nonconformal.

Our computer simulation led us to the same conclusion. We modeled the neural plate as being made up of approximately 300 "shrinkage units," each unit being a cylinder representing a group of approximately 30 cells. When we ran the computer program allowing the shrinkage pattern to deform the D'Arcy Thompson grid, the simulated neural plate did not attain the keyhole shape but merely became smaller, much as had happened with the isolated neural plate grown in tissue culture [*see column c in illustration on page 107*]. It therefore seemed clear that no shrinkage pattern in itself could give rise to the keyhole shape, and we began to look for a second force.

The changes of angle in the D'Arcy Thompson grid of the transformed neural plate implied the existence of a shearing force that was moving some of the cells with respect to one another. In search of what might generate such a force we took a close look at the notochord, which is part of the mesodermal tissue underlying the neural plate. In older embryos the notochord is a rod running most of the length of the embryo under the spinal cord and brain. In the neural-plate stages the notochord is a flat sheet of cells that is in the process of shaping itself into a rod. Carl-Olof Jacobson and Jan Löfberg of the University of Uppsala had shown that although the mesodermal cells followed paths similar to those of the overlying neural-plate cells during the formation of the plate, only the cells in the notochord exactly followed the paths of the overlying cells. Moreover, we found that the cells of the notochord adhere tightly to the overlying neural-plate cells, whereas the rest of the mesoderm does not. This mechanical attachment was evident when we tried to separate the two tissue layers. We have termed the part of the neural plate that lies over the notochord the supranotochordal region.

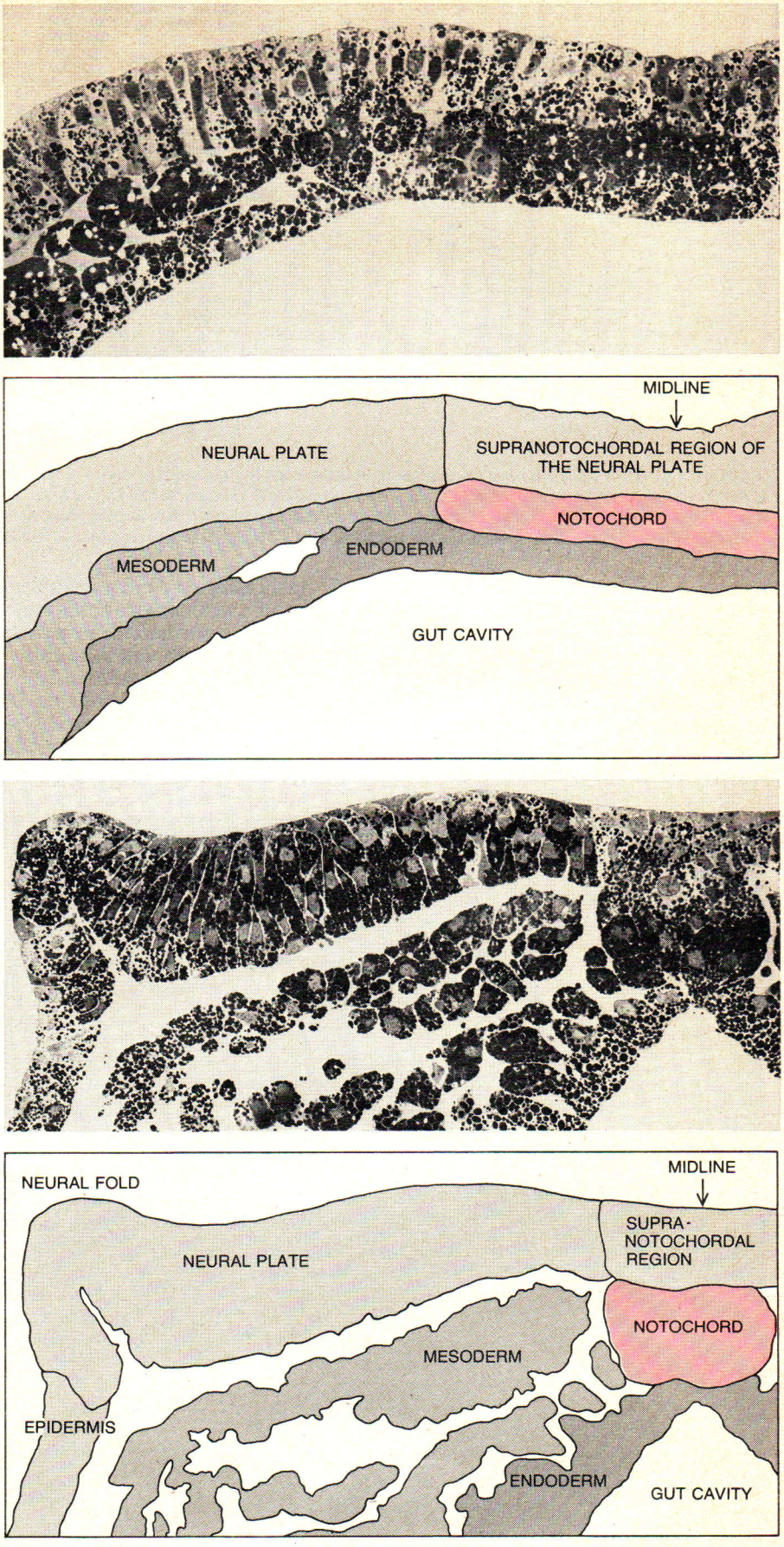
If we removed the neural plate from the embryo along with the notochord

and grew it in isolation, a normal keyhole shape resulted. If, on the other hand, we left the neural plate attached to the embryo and removed the notochord by cutting it away from below, the keyhole shape did not arise. Similarly, if the notochord itself was isolated from the neural plate and grown in culture, the cells did not rearrange themselves into a rod. We concluded that the cells of the notochord and the supranotochordal region of the neural plate move synchronously, but that neither set of cells will execute these movements in isolation. Therefore our next step was to incorporate the elongation of the supranotochordal region into our computer simulation.

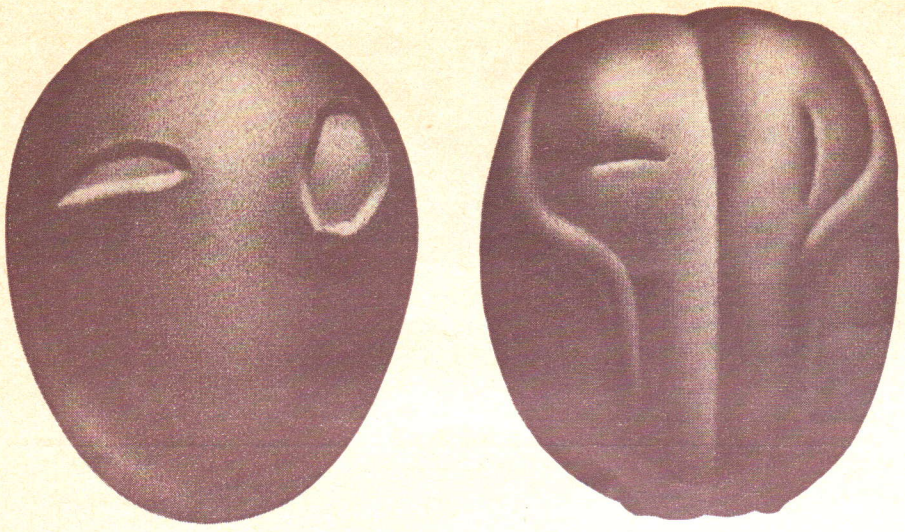
In programming the simulation we had to find a means of holding the shrinkage units together to represent a continuous sheet of cells. The neighboring units were connected by "bonds" that were modeled as if they were rubber bands. Problems soon arose with this approach. As the shrinkage units diminished in size and the supranotochordal region elongated, some of the bonds were stretched to lengths that could not normally be attained by cells in the embryo. Such stretching seemed to point to the possibility of unrelaxed tensions in the neural plate. We looked for signs of these tensions in the embryo by cutting small slits in the neural plate. As we had anticipated, the tensions in the plate during the disk stage caused the slits to gape open. In the keyhole stage, however, when slits were cut, they did not gape, indicating that the tensions in the neural plate had relaxed. Thus the neural plate does not behave simply like an elastic material; it also has a viscous character. Such mixed behavior in a material or a fluid is called viscoelasticity.

In order to model the viscous component of the neural plate we allowed the bonds between the shrinkage units to snap when they got too long; the length at which they snapped was derived from an empirical measurement of the maximum observed stretching of cells in the embryo. The separated shrinkage units were then allowed to join up with new neighboring units. This change of neighbors among the units allows for the viscous component of the flow and accounts for the observed shear.

We found that by combining the shrinkage pattern and the movements of the supranotochordal region in our simulation we could obtain a transformed D'Arcy Thompson grid that was virtually identical with the keyhole shape observed in the embryo. We wondered, however, if the second force alone might be sufficient to effect the transformation. Unfortunately we could not test this hypothesis by experiment because we knew of no way to prevent the shrinkage of the neural-plate cells without affecting the movement of the su-



CROSS SECTIONS through the newt embryo at two stages of its development illustrate the relations between the neural plate and the underlying tissue layers. The micrograph at the top shows the tissues of an embryo that has just completed gastrulation; the neural plate, the mesoderm and the endoderm are visible. The micrograph at the bottom is of an embryo with a keyhole-shaped neural plate. Note that between the two stages the cells of neural plate have lengthened, whereas those of notochord and supranotochordal region have converged along midline.

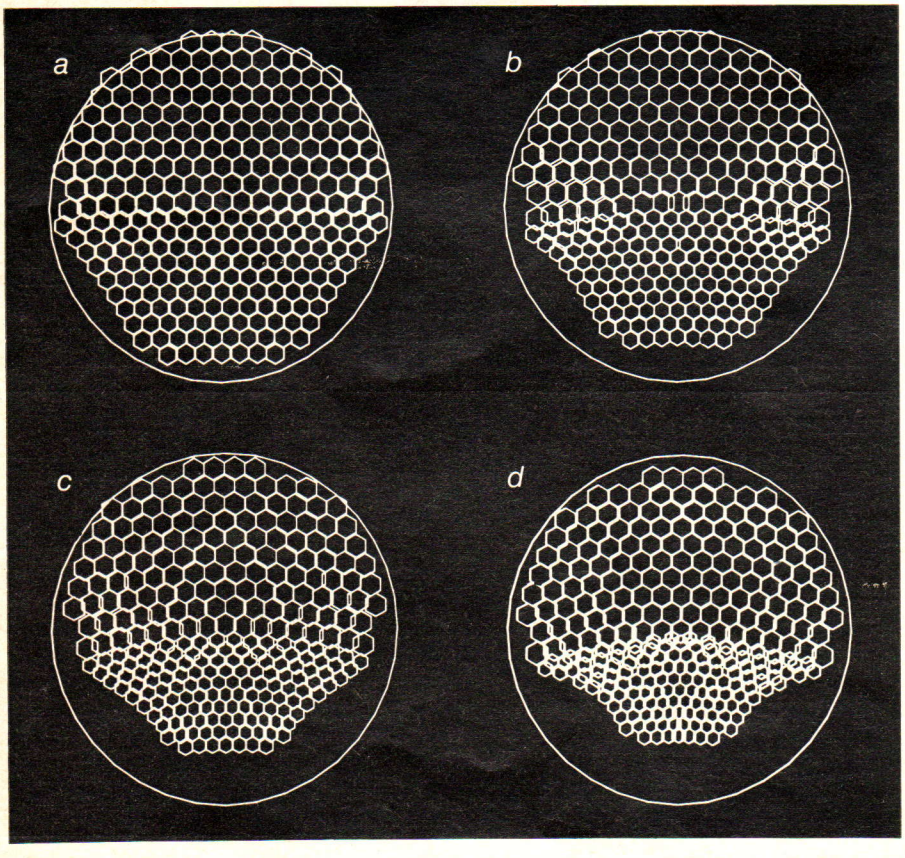


GAPING OF WOUNDS when the neural plate is slit reveals the presence of internal tensions in the disk-shaped stage of the embryo (*left*). The tensions have relaxed by the keyhole-shaped stage (*right*). The presence of these tensions indicates that the neural plate is both elastic and viscous, a property shared by nonbiological materials that flow, such as metals under pressure.

pranotochordal region. It was a simple matter, however, to modify the computer simulation so as to turn off the shrinkage as the supranotochordal region elongated. Although the resulting transformed D'Arcy Thompson grid had a keyhole shape, its front end was oversized [*see column d in illustration on page 107*]. We therefore concluded that both the shrinkage pattern and the elongation of the supranotochordal region are necessary and sufficient to account for the shaping of the neural plate.

In vertebrate animals the main role of the notochord is apparently to participate in the formation of the embryonic nervous system. Once the backbone has appeared the notochord has no further function and largely disintegrates.

Three lines of evidence led us to the conclusion that shear occurs in the neural plate: the nonconformality of the transformation of the D'Arcy Thompson grid, the need to introduce viscoelasticity into our computer model and direct observations of groups of cells changing neighbors. There are at least two regions of the neural plate where shear may be found: in the supranotochordal region and at the boundary between the neural plate and the epidermis (the rest of the outer layer of the embryo). In the latter region the neural-plate cells, which are shrinking at a rapid rate, are juxtaposed with epidermal cells that are actually flattening and increasing their exposed surface area. As a result an abrupt boundary is formed along which shear can develop [*see bottom illustration on this page*]. The massive amount of shear among the neural-plate cells in the supranotochordal region may play a major role in subdividing the neural plate into two bilaterally symmetric halves.



LINES OF SHEAR in the embryo are generated at the interface between a sheet of cells whose surface area is shrinking and an adjacent sheet of cells whose surface area remains the same or actually increases. This computer simulation shows a simplified example of such an interface, with shear (the changing of cell neighbors) occurring at the downturned edges. Shear occurs along midline of the neural plate during the elongation of the supranotochordal region and at the border between neural plate and the epidermis (the rest of the outer layer of the embryo).

The question of why abrupt boundaries arise between cell domains is largely unsolved, but lines of shear between cells may be an important factor. Several investigators, including Edwin J. Furshpan and David D. Potter of the Harvard Medical School and Werner R. Loewenstein of the University of Miami School of Medicine, have found that most cells in the embryo communicate electrically and chemically by means of permeable adhesions between their outer membranes called gap junctions [see "Junctions between Living Cells," by L. Andrew Staehelin and Barbara E. Hull; SCIENTIFIC AMERICAN, May]. Ions and even fairly large molecules can pass freely among the coupled cells, and this cellular communication may play an important role in the formation of cell domains that exhibit coordinated behavior during embryonic development. The cells of the neural plate comprise one such domain; the cells of the epidermis comprise another. Shear may sever or alter the function of the gap junctions between cells at the boundary between the neural plate and the epidermis, leading to their functional separation. This separation could lead in turn to different subsequent development in each of the two isolated cell domains.

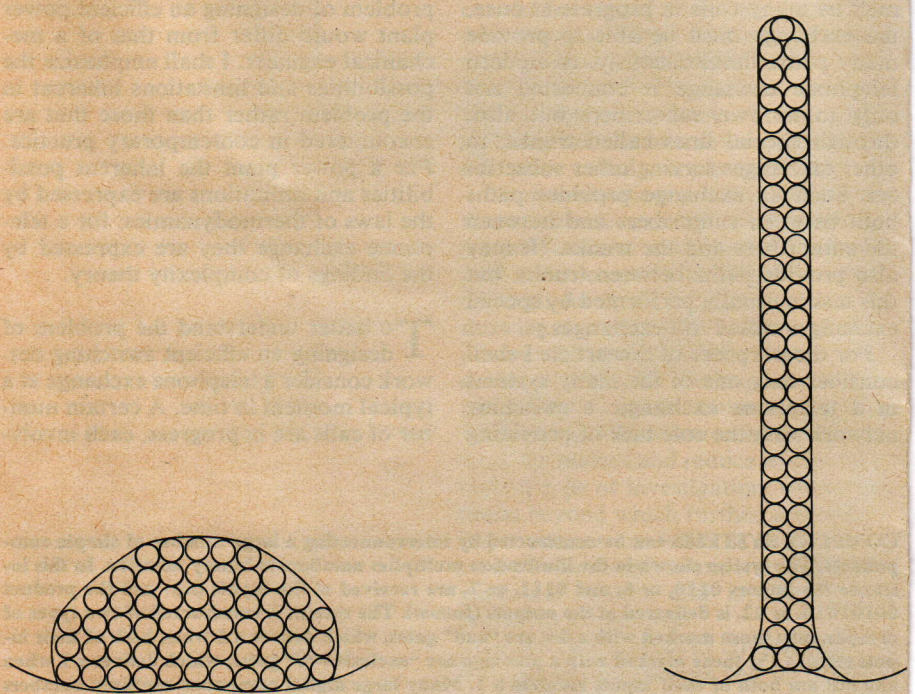
Once the flat neural plate is formed it rolls into a tube. The dominant effect may be the elongation of the supranotochordal region, which at this time increases its rate of elongation tenfold. If one stretches an elastic sheet, such as a thin sheet of rubber or plastic, along a line, it will buckle out of the plane and form a tube. We are now embarking on a new computer simulation to test whether or not this phenomenon will quantitatively explain the formation of the neural tube.

In devising our computer simulation of the neural plate we became aware of the analogy between its formation and the flow of nonbiological materials, such as metals. The branches of physics that deal with the flow of materials are hydrodynamics and the more general discipline of continuum mechanics. Yet there are two major differences between ordinary hydrodynamics and the flow of tissues during development (which we have proposed to name morphodynamics). First, ordinary fluids are passive, responding to the forces applied on them, whereas the forces that drive the flow of tissues are generated by the cells themselves. Second, most fluids are spatially uniform in their intrinsic properties, whereas the fluid properties of a tissue are highly nonuniform, as is evidenced by the complexity of the shrinkage pattern in the neural plate. The added complexity of morphodynamics makes it mathematically intractable and places it at the limits of the descriptive power of hydrodynamics, even with the availability of high-speed computers.

In our work on the neural plate we have shown how the morphogenesis of a sheet of tissue is a consequence of the behavior of its cells. One of these kinds of behavior, namely the carrying out of a height program by each cell, seems in turn to be a consequence of the behavior of the large structural molecules, such as microtubules and microfilaments, within each cell. As we have seen, the height program varies from cell to cell. This observation raises some fundamental questions. What is the mechanism by which the spatial pattern of height programs is established? The answer may well bear on pattern formation in general. Exactly what is it in each cell that varies from cell to cell to give the cells their different height programs? Perhaps the regulation mechanism is like a thermostat whose molecular control may be set differently in each cell. Finally, we need to learn how this control mechanism operates to carry out the height program. The molecular mechanisms seem within grasp for this tissue, so that we may have here an excellent opportunity to investigate these questions.

The formation of the supranotochordal region of the neural plate and the notochord itself remain an enigma. It is not understood how the cells in this part of each sheet can rearrange themselves drastically but in a coordinated fashion to give rise to elongated structures. The answer may lie in surface-tension interactions between the supranotochordal region and the rest of the neural plate that operate by way of the different strengths of adhesions between cells.

The development of an embryo is one of the most profoundly difficult and challenging phenomena to comprehend. After a number of years of study we have succeeded in obtaining a quantitative understanding of how the behavior of cells in a single tissue leads to its change in shape. Although this work has yielded a few important principles of embryogenesis, it has revealed or made explicit a number of other fundamental mechanisms that remain to be grasped.



SUPRANOTOCHORDAL REGION of the neural plate is shown diagrammatically at the disk stage of the neural plate (*left*) and at the keyhole stage (*right*). The circles represent equal numbers of cells. As the supranotochordal region elongates and narrows along the midline of the neural plate, cells from the interior of the flat region intercalate to take positions at the perimeter. The diagram illustrates immense amount of shear that is involved in the shape change.

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