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Early vertebrate development: Neurulation and ectoderm

For the real amazement, if you wish to be amazed, is this process. You start out as a single cell derived from the coupling of a sperm and an egg; this divides in two, then four, then eight, and so on, and at a certain stage there emerges a single cell which has as all its progeny the human brain. The mere existence of such a cell should be one of the great astonishments of the earth. People ought to be walking around all day, all through their waking hours calling to each other in endless wonderment, talking of nothing except that cell.

LEWIS THOMAS (1979)

The phenomenon of life itself negates the boundaries that customarily divide our disciplines and fields.

HANS JONAS (1966)

The vertebrate pattern of development

In 1828, Karl Ernst von Baer, the foremost embryologist of his day,* exclaimed, "I have two small embryos preserved in alcohol, that I forgot to label. At present I am unable to determine the genus to which they belong. They may be lizards, small birds, or even mammals." Figure 1 allows us to appreciate his quandry, and it illustrates von Baer's four general laws of embryology. From his detailed study of chick development and his comparison of those embryos to embryos of other vertebrates, von Baer derived four generalizations, illustrated here with some vertebrate examples.

1. *The general features of a large group of animals appear earlier in the embryo than do the specialized features.* All developing vertebrates (fishes, reptiles, amphibians, birds, and mammals) appear very similar shortly after gastrulation. It is only later in development that the special features of class, order, and finally species emerge (Figure 1). All vertebrate

*K. E. von Baer discovered the notochord, the mammalian egg, and the human egg, as well as making the conceptual advances described here.

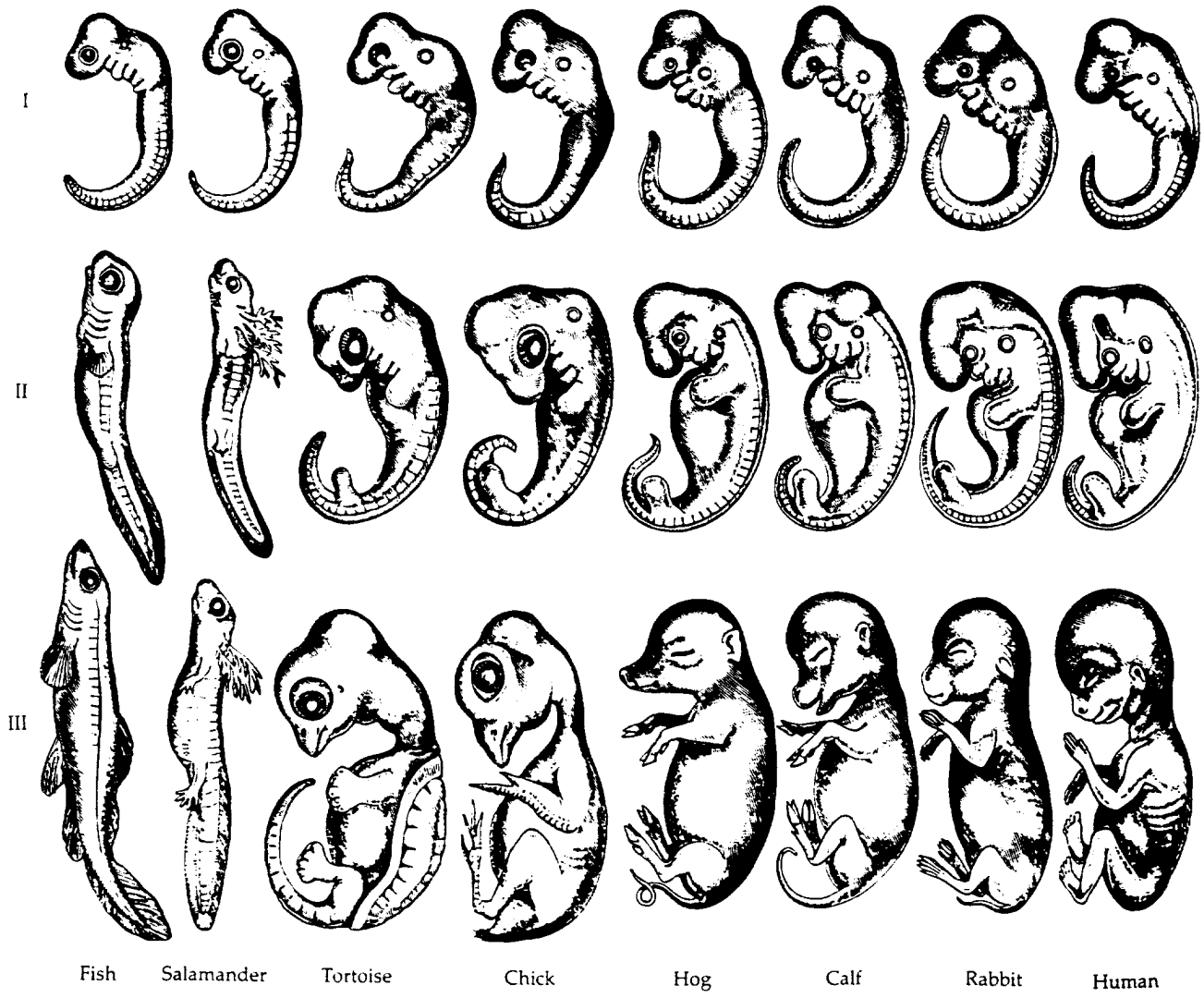


FIGURE 1
Illustration of von Baer's law. Early vertebrate embryos exhibit features common to the entire subphylum. As development progresses, embryos become recognizable as members of their class, their order, their family, and finally their species. (From Romanes, 1901.)

embryos have gill arches, notochords, spinal cords, and pronephric kidneys.

2. *Less general characters are developed from the more general, until finally the most specialized appear.* Certain fishes use a pronephric type of kidney as adults. This type of kidney can be found in the embryos of all other types of vertebrates. However, in these more complex species, it never becomes functional but contributes to the subsequent development of more specialized types of kidneys. All vertebrates initially have the same type of skin. Only later does skin develop fish scales, reptilian scales, bird feathers, or the hair, claws, and nails of mammals. Similarly, the early development of the limb is essentially the same in all vertebrates. Only later do the differences between fins, legs, wings, and arms become apparent.

3. *Each embryo of a given species, instead of passing through the adult stages of other animals, departs more and more from them.* The visceral clefts of embryonic birds and mammals do not resemble the gill slits of adult fishes in detail. Rather, they resemble those visceral clefts of *embryonic* fishes and other *embryonic* vertebrates. Whereas fishes preserve and elaborate these clefts into true gill slits, mammals convert them into structures such as the eustachian tubes (between the ear and mouth).

4. *Therefore, the early embryo of a higher animal is never like (the adult of) a lower animal, but only like its early embryo.*

Von Baer saw that different groups of animals shared certain common features during early embryonic development and that these features became more and more characteristic of the species as development proceeded. Human embryos never pass through a stage equivalent to an adult fish or bird; rather, human embryos initially share characteristics in common with fish and avian embryos. Later, the mammalian and other embryos diverge, none of them passing through the stages of the other.*

These conclusions were extremely important for Charles Darwin because evolutionary classification depended upon finding those similarities that demonstrated that certain diverse animals arose from a common ancestor. "Community of embryonic structure," wrote Darwin in *Origin of Species* (1859), "reveals community of descent." In fact, Darwin believed that embryology was the strongest foundation for his evolutionary theory. Von Baer, of course, was not an evolutionist when he wrote his four laws in 1828. In fact, he never became one. Von Baer believed that he had found the divine plan upon which all the organisms in a group such as the vertebrates developed. Because earlier evolutionary theories had envisioned a single, nonbranching series of transformations, von Baer's observations were often used against evolution. Darwin, however, recognized that von Baer's work supported an evolutionary hypothesis in which a common ancestor could radiate into several different types of organisms by heritable modifications of embryonic development. The reason human embryos, fish embryos, and chick embryos all had visceral clefts was because they had a common ancestor whose embryo had such visceral clefts. Moreover, the reason visceral clefts gave rise to different structures in different groups of

*This work was largely ignored after Ernst Haeckel made a synthesis of German romanticism and Darwinism wherein "ontogeny [the development of an individual] recapitulated phylogeny [the evolutionary history of the species]." Here, the various stages of the human embryo were seen to correspond with the adult stages of "lower" organisms. Even though this view was scientifically discredited before it was even proposed, Haeckel had a gift for showmanship and his theory "explained" human progress. It caught on like wildfire throughout biology and the social sciences before it was shown to be based on false premises (see Gould, 1977).

organisms (gills in fishes, eustachian tubes in mammals) was that the ancestral plan had been modified and selected by different environments.

Von Baer also recognized that there was a common pattern to vertebrate development. The three germ layers gave rise to different organs, and their derivation was constant whether the organism was a fish, a frog, or a chick. ECTODERM formed skin and nerves; ENDODERM formed respiratory and digestive tubes; and MESODERM formed connective tissue, blood cells, heart, the urogenital system, and parts of most of the internal organs. In this chapter we shall follow the early development of ectoderm, focusing on formation of the nervous system. In the next chapter we shall follow the early development of endodermal and mesodermal organs.

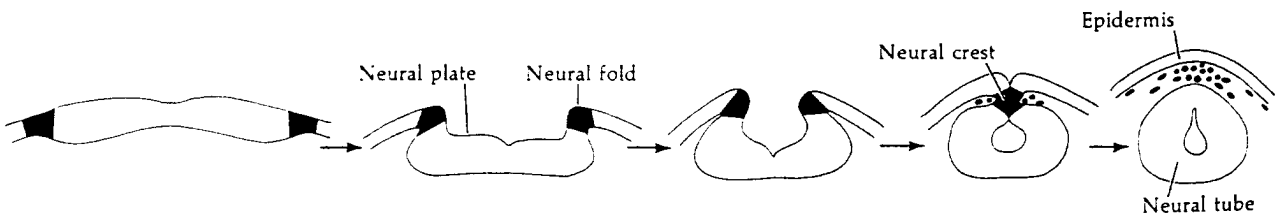
Neurulation

In vertebrates, gastrulation creates an embryo having an internal endodermal layer, an intermediate mesodermal layer, and an external ectoderm. In addition, a cord of mesodermal cells, the notochord, lies directly beneath the most dorsal portion of the ectoderm. The interaction between the notochord and its overlying ectoderm is one of the most important interactions of all development, for the notochord directs the ectoderm to form the hollow NEURAL TUBE, which will differentiate into the brain and spinal cord. Thus, we begin a new phase of development— ORGANOGENESIS, the creation of specific tissues and organs. The action by which the notochord instructs the ectoderm to become neural tube is called PRIMARY EMBRYONIC INDUCTION, and the cellular response by which the flat layer of ectodermal cells is transformed into a hollow tube is called NEURULATION. The events of neurulation are diagrammed in Figure 2. Here, the original ectoderm is divided into three sets of cells: (1) the internally positioned neural tube, (2) the epidermis of the skin, and (3) the neural crest cells, which migrate from the region that had connected the neural tube and epidermal tissues. An embryo undergoing such changes is called a NEURULA. The phenomenon of primary embryonic induction will be detailed in Chapter 8. In this chapter, we are concerned with the response by the various ectodermal tissues.

The process of neurulation in frog embryos is depicted in Figure 3. The mechanisms of neural tube formation appear to be similar in amphibians, reptiles, birds, and mammals (Gallera, 1971), so we will be considering various groups as we proceed through our survey.* The first indication that a region of ectoderm is destined to become neural

*Among the vertebrates, fishes generate their neural tube in a different manner. Fish neural tubes do not form from an infolding of the overlying ectoderm, but rather from a solid cord of cells, which is induced to sink into the embryo. This cord subsequently hollows out (cavitates) to form the neural tube. In mammals, only the most posterior portion of the neural tube forms by cavitation.

FIGURE 2
Diagrammatic representation of neural tube formation in amphibians and amniotes. The ectodermal cells are represented either as precursors of the neural crest (black), the neural tube (white), or the epidermis (gray). The ectoderm folds in at the most dorsal point, forming an outer epidermis and an inner neural tube connected by neural crest cells. (After Balinsky, 1975.)



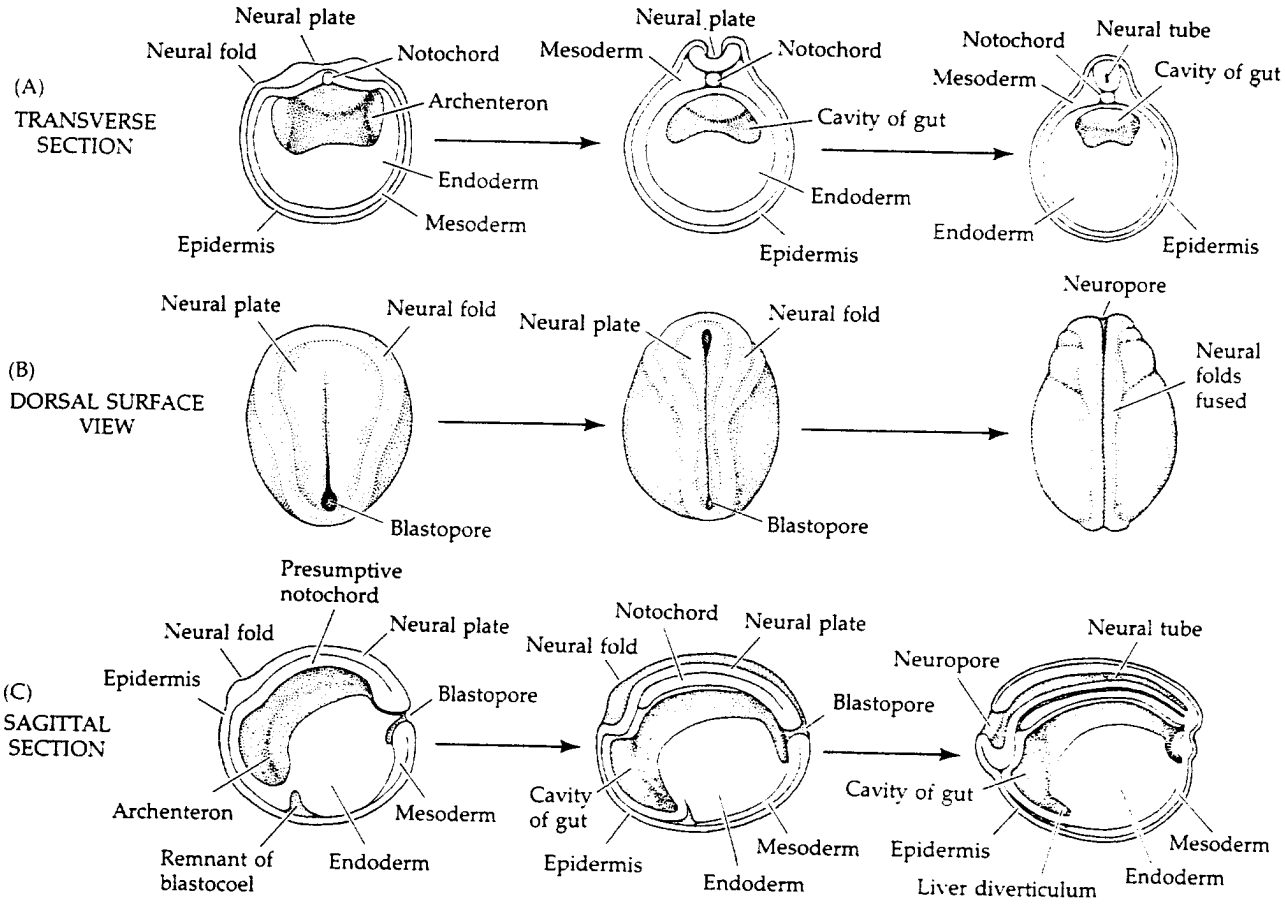


FIGURE 3
Three views of neurulation in a frog embryo, showing early (left), middle (center), and late (right) neurulae in each case. (A) Transverse section through the center of the embryo. (B) The same sequence looking down on the dorsal surface of the whole embryo. (C) Sagittal section through the median plane of the embryo. (After Balinsky, 1975.)

tissue is a change in cell shape (Figure 4). Midline ectodermal cells become elongated, whereas those cells destined to form the epidermis become more flattened. The elongation of dorsal ectodermal cells causes these prospective neural regions to rise above the surrounding ectoderm, thus creating the NEURAL PLATE. As much as 50 percent of the ectoderm is included in this plate. Shortly thereafter, the edges of the neural plate thicken and move upward to form the NEURAL FOLDS, while a U-shaped NEURAL GROOVE appears in the center of the plate, dividing the future right and left sides of the embryo (Figures 3 and 5). The neural folds migrate toward the midline of the embryo, eventually fusing to form the neural tube beneath the overlying ectoderm. The cells at the junction between the outer ectoderm and the neural tube become the NEURAL CREST cells. These neural crest cells will migrate through the embryo and will give rise to several cell populations, including pigment cells and the cells of the peripheral nervous system.

The neural tube and the origins of the central nervous system

The formation of the neural tube does not occur simultaneously throughout the ectoderm. This is best seen in those vertebrates (such as birds and mammals) whose body axis is elongated prior to neurulation. Figure 6 depicts neurulation in a 24-hr chick embryo. Neurulation in the cephalic (head) region is well advanced while the caudal (tail)

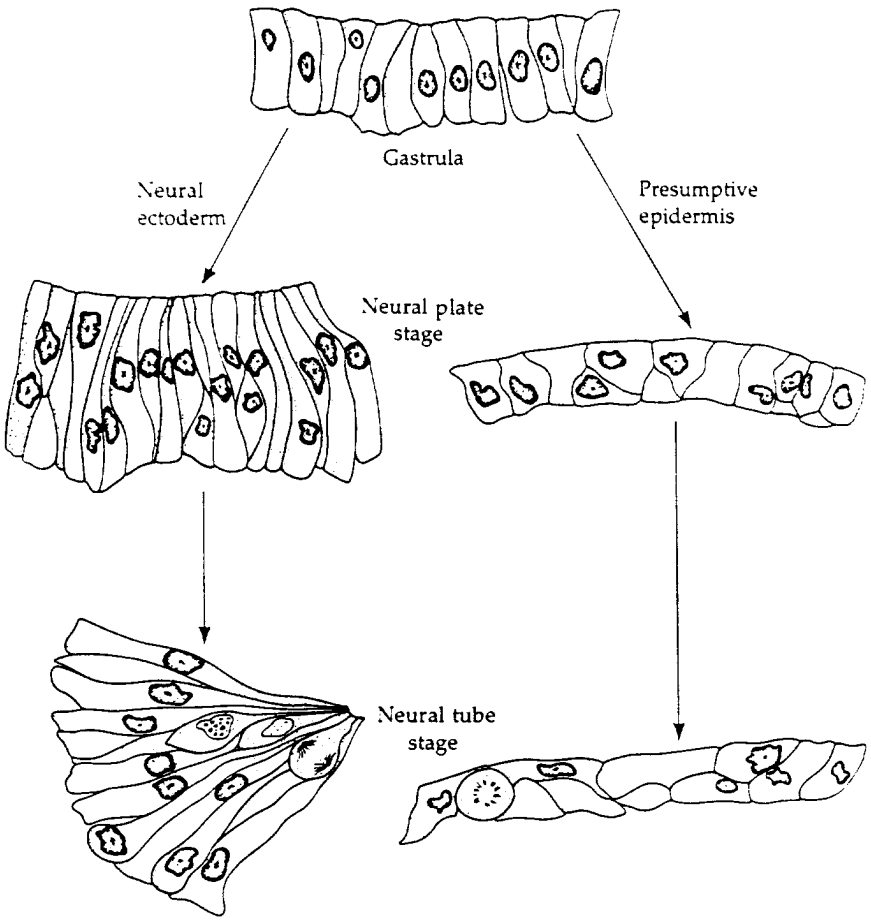


FIGURE 4
Schematic diagram of the shape changes during neurulation in the salamander. At the end of gastrulation, the cells of the ectoderm form a uniform epithelium. During neurulation, neural epithelial cells elongate to form the neural plate and then constrict at their apices to form the neural tube. Presumptive epidermal cells flatten throughout neurulation. (After Burnside, 1971.)

FIGURE 5
Scanning electron micrograph of neural tube formation in the chick embryo. Elongated neural epithelial cells form a tube as the flattened epidermal cells are brought to the midline of the embryo. (Photograph courtesy of K. W. Tosney.)

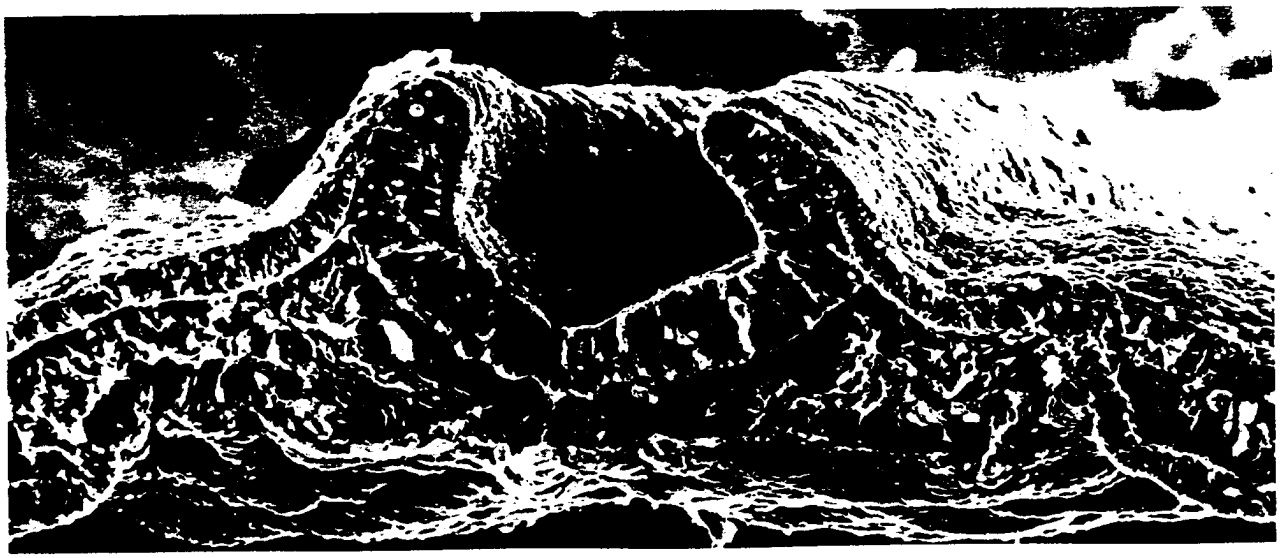
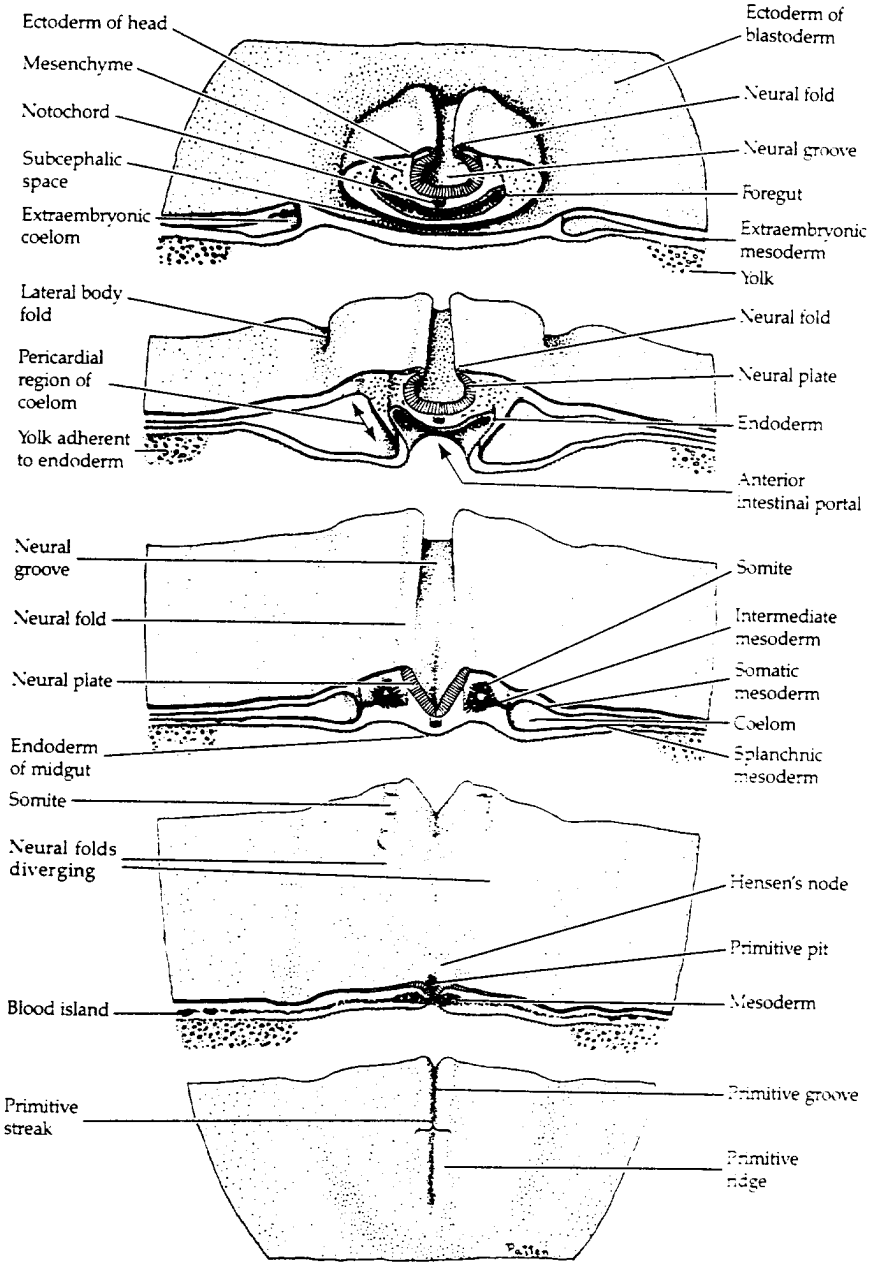


FIGURE 6
Stereogram of a 24-hour chick embryo. Cephalic portions are finishing neurulation while the caudal portions are still undergoing gastrulation. (From Patten, 1971; after Huettner, 1949.)



region of the embryo is still undergoing gastrulation. Regionalization of the neural tube also occurs as a result of changes in the shape of the tube. In the cephalic end (where the brain will form), the wall of the tube is broad and thick. Here, a series of swellings and constrictions define the various brain compartments. Caudal to the head region, however, the neural tube remains a simple tube that tapers off toward the tail. The two open ends of the neural tube are called the ANTERIOR NEUROPORE and the POSTERIOR NEUROPORE. In mammals, these openings allow amniotic fluid to flow through the neural tube for a time (Figure 7). Failure to close the human posterior neuropore at day 27 (or its subsequent rupture shortly thereafter) results in SPINA BIFIDA, the severity of which depends upon how much of the spinal cord remains open. However, failure to close the anterior neuropore results in a lethal condition, ANENCEPHALY. Here, the forebrain remains in contact with the amniotic fluid and subsequently degenerates. Fetal forebrain devel-

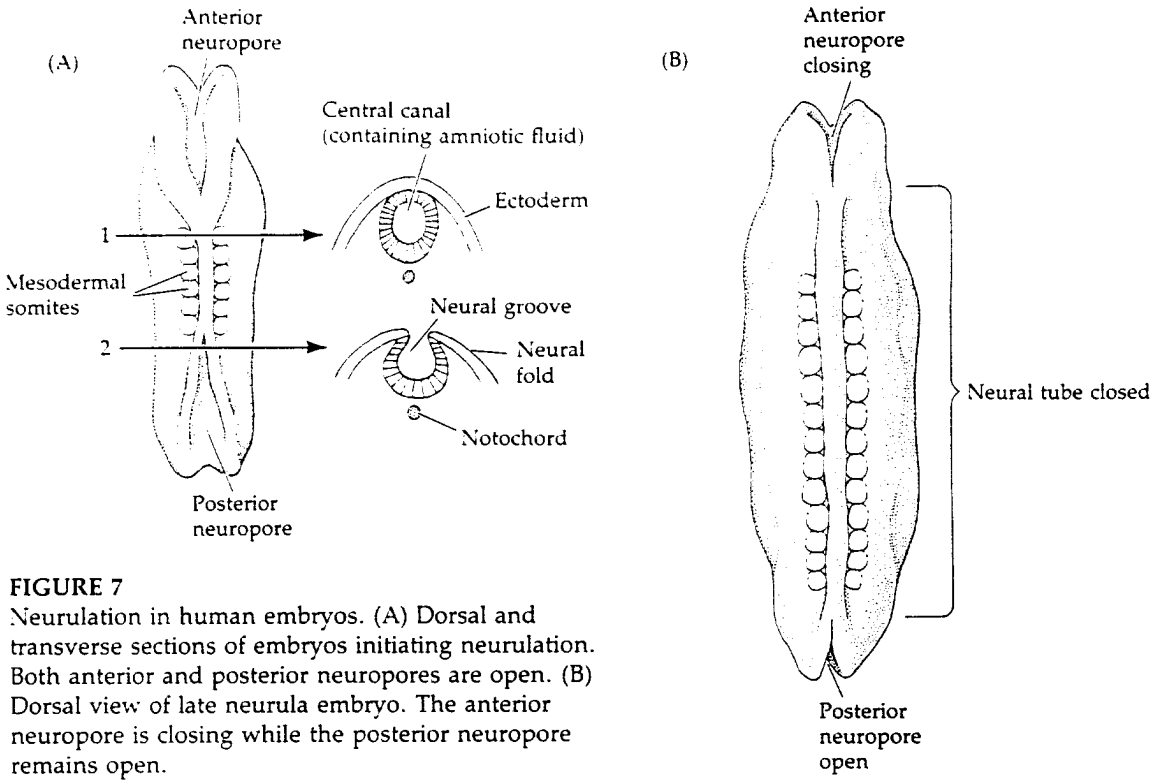


FIGURE 7
Neurulation in human embryos. (A) Dorsal and transverse sections of embryos initiating neurulation. Both anterior and posterior neuropores are open. (B) Dorsal view of late neurula embryo. The anterior neuropore is closing while the posterior neuropore remains open.

opment ceases, and the vault of the skull fails to form. This abnormality is not that rare in humans, occurring in about 0.1 percent of all pregnancies. Neural tube closure defects can now be detected during pregnancy by various physical and chemical tests.

Mechanism of neural tube formation

Neural tube formation is intimately linked to changes in cell shape, and microtubules and microfilaments are both involved in these changes. Ectodermal cells elongate as the randomly arranged microtubules of these cells align themselves parallel to the lengthening axis (Figure 8). This stage of neural tube formation can be blocked by colchicine, an inhibitor of microtubule polymerization (Burnside, 1973). A second change in cell shape involves the apical constriction of cells to form a cylinder; this change is coordinated by a ring of actin microfilaments encircling the apical margins of the cells. The contraction of these microfilaments produces a "purse-string" effect, constricting the apical end of each cell. Burnside (1971) and Karfunkel (1972) have shown that when embryos are cultured in the presence of cytochalasin B, the neuroectodermal cells can elongate but cannot constrict to form the neural folds. The actin is linked to myosin from which it is able to get the energy for contraction (Nagele and Lee, 1980), and it is also linked to the apical cell membrane. This linkage is effected through SPECTRIN, which is an integral protein of the plasma membrane that can bind to microfilaments inside the cell (Sadler et al., 1986). The patterns of spectrin localization follow that of the microfilaments, suggesting that the microfilaments are actively involved in changing cell shape.

An interdisciplinary effort by biologists and mathematicians has succeeded in showing that changes in elongation and constriction may be all that are needed to change a flat sheet into a hollow tube within

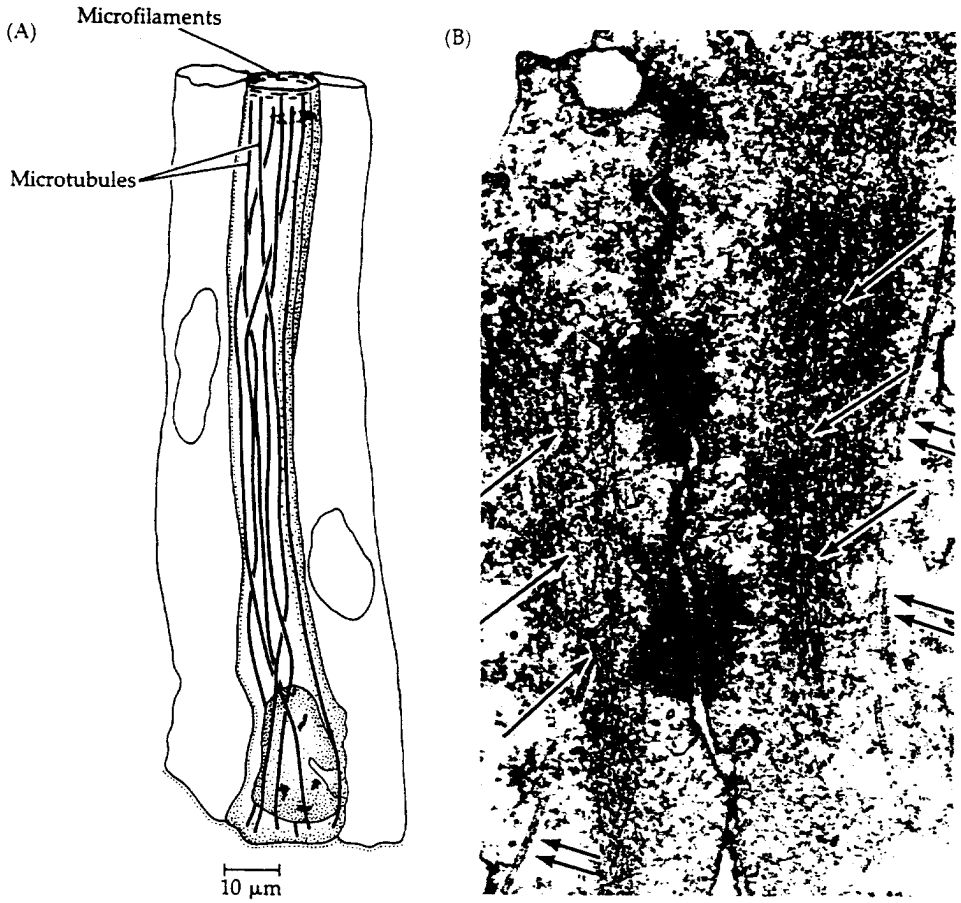
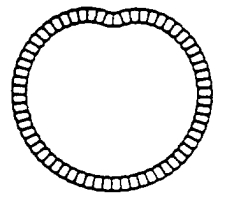
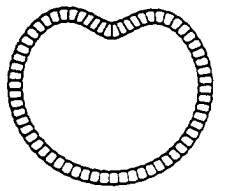
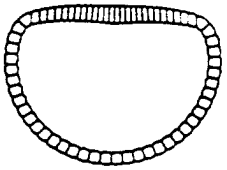


FIGURE 8
(A) Microtubule and microfilament orientation in neural plate cells. Microtubules align parallel to the long axis of the cell; microfilaments encircle the apex. The microfilaments are often seen to attach to desmosomes, specific structures on the cell membrane where two cells come into contact. (B) Apical microfilament bundles (long single arrows) seen near desmosomes on both sides of the cell–cell contacts. Microtubules (double arrows) are also seen. (From Burnside, 1971; photograph courtesy of B. Burnside.)

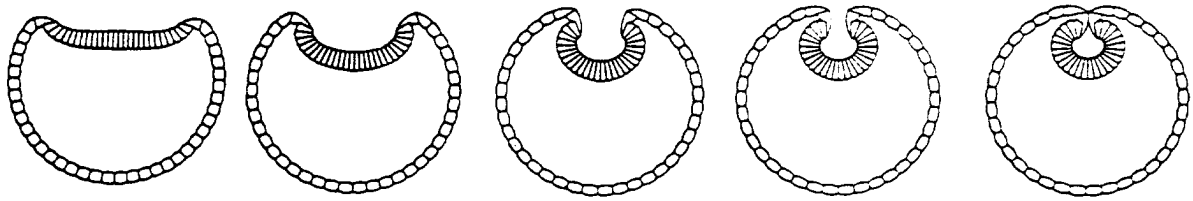






an embryo (Odell et al., 1981). In a computer simulation of neurulation, the original ectoderm layer was made to consist of cuboidal cells aligned side-by-side but joined together tightly at their apical ends (Figure 9). At these apices were the bands of contractile microfilaments. The major unproven assumption of this model is the limitation on the ability of the "purse-string" to be stretched; above this elastic threshold, contrac-

FIGURE 9
Computer-generated model of neural tube formation in amphibians. (After Odell et al., 1981.)



tion is initiated and draws the ring to a new circumference shorter than its original one. The remainder of the cell is presumed to be an elastic body of constant volume, and the apical end of each cell is presumed to be tightly joined to its neighbors. Thus, contraction in one cell would stretch the apical circumference of any adjoining cells and produce a wave of contraction as sequential cells are stretched past their elastic threshold and contract. Thus, a wave of contraction is generated, with all the cells being linked tightly at their apical ends. When these constraints are programmed into a computer, the computer is able to predict that neurulation should occur in a manner consistent with the embryological observations.

Differentiation of the neural tube

Formation of brain regions. The differentiation of the neural tube into the various regions of the central nervous system occurs simultaneously in three different ways. On the gross anatomical level, the neural tube and its lumen bulge and constrict to form the chambers of the brain and the spinal cord. At the tissue level, the cell populations within the wall of the neural tube rearrange themselves in various ways to form the different functional regions of the brain and the spinal cord. Finally, on the cellular level, the neuroepithelial cells themselves differentiate into the numerous types of neurons and supportive (glial) cells present in the body. The early development of most vertebrate brains is similar, but because the human brain is probably the most interesting organ in the animal kingdom, we shall concentrate on the development that is supposed to make *Homo* sapient.

The early mammalian neural tube is a straight structure. However, even before the posterior portion of the tube has formed, the most anterior portion of the tube is undergoing drastic changes. In this anterior region, the neural tube balloons into three primary vesicles (Figure 10): forebrain (PROSENCEPHALON), midbrain (MESENCEPHALON), and hindbrain (RHOMBENCEPHALON). By the time the posterior end of the neural tube closes, secondary bulges—the OPTIC VESICLES—have extended laterally from each side of the developing forebrain. Moreover, the future brain has bent so that the creases demark the boundaries of the brain cavities. The major creases are the CEPHALIC FLEXURE and the CERVICAL FLEXURE.

The forebrain becomes subdivided into the anterior TELENCEPHALON and the more caudal DIENCEPHALON (Figure 11). The telencephalon will

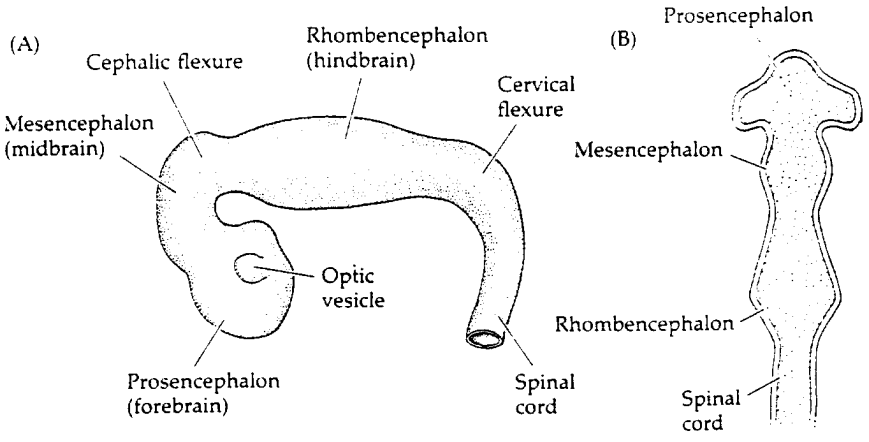
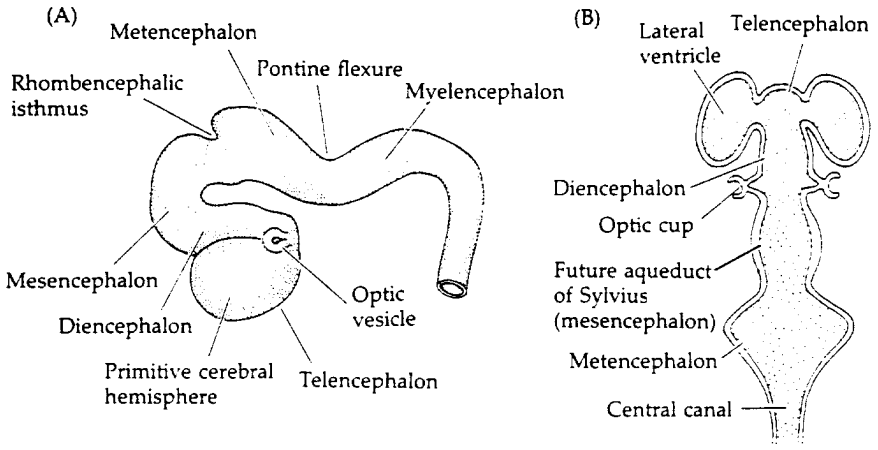


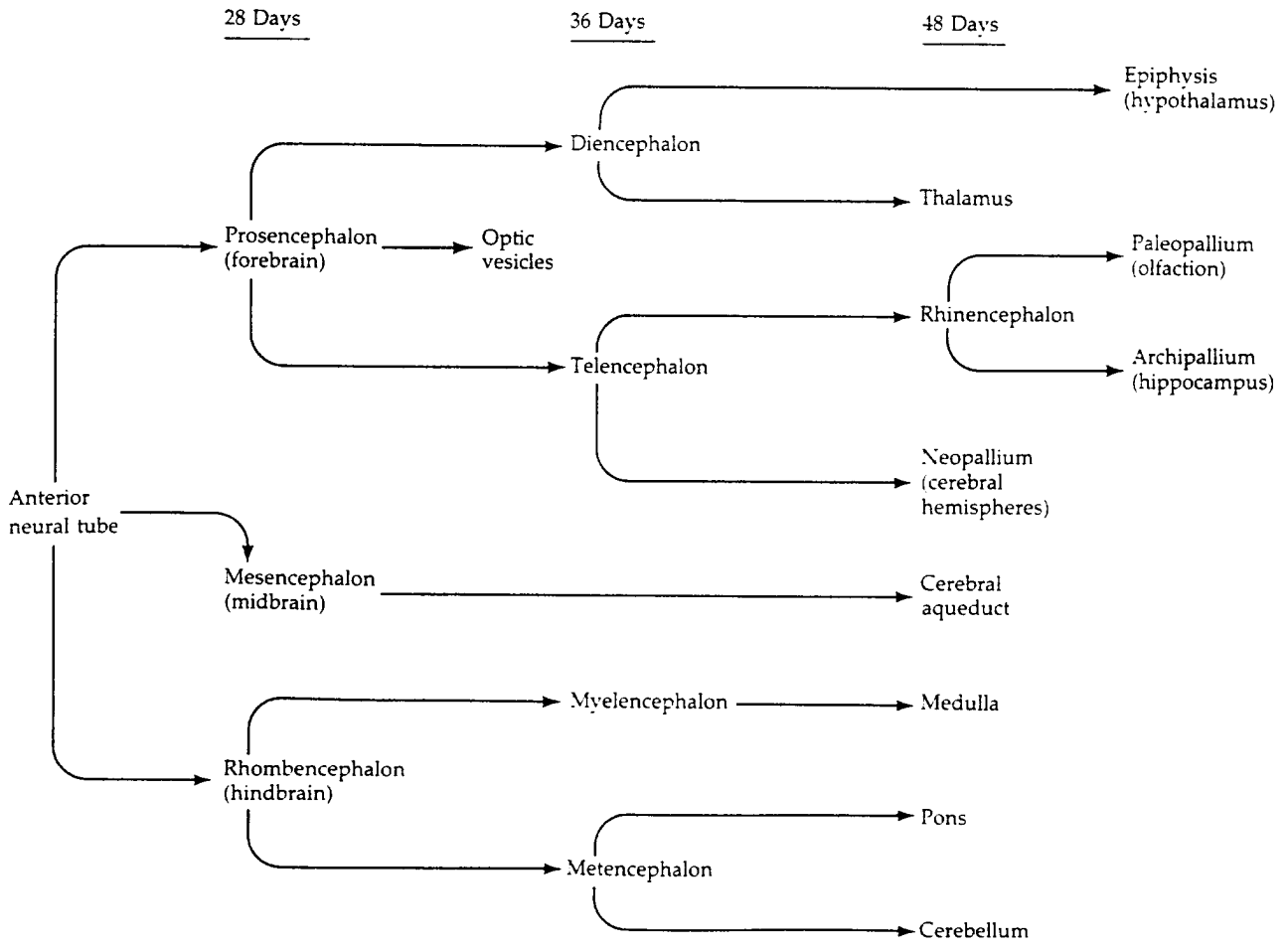
FIGURE 10
Early brain development in a 4-week human embryo. (A) Lateral view. (B) Extended diagram to illustrate the bulges of the neural tube. (After Langman, 1969.)

FIGURE 11
Further human brain development. Lateral view (A) and midline extended diagram (B) of a 6-week human brain showing secondary bulges of the neural tube. (After Langman, 1969.)



eventually form the CEREBRAL HEMISPHERES, and the diencephalon will form the thalamic and hypothalamic brain regions as well as the region that receives neural input from the eyes. The mesencephalon (midbrain) does not become subdivided, and its lumen eventually becomes the cerebral aqueduct. The rhombencephalon becomes subdivided into a posterior MYELENCEPHALON and a more anterior METENCEPHALON. The myelencephalon eventually becomes the MEDULLA OBLONGATA, whose neurons generate the nerves that regulate respiratory, gastrointestinal, and cardiovascular movements. The metencephalon gives rise to the CEREBELLUM, the part of the brain responsible for coordinating move-

FIGURE 12
Regional specialization during early human brain development.

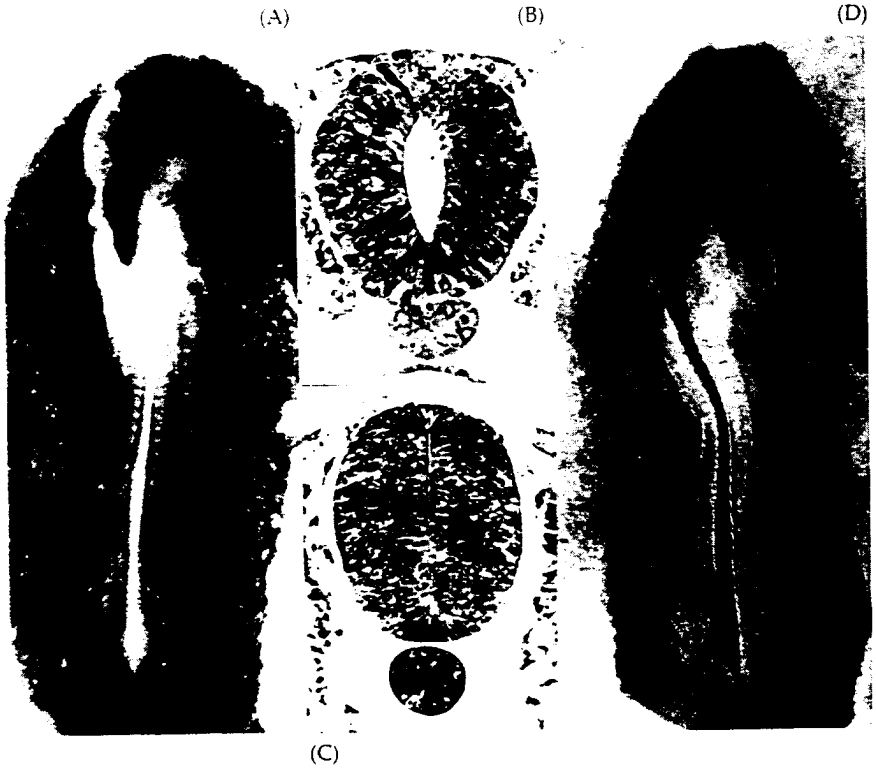


ments, posture, and balance. The development of specialized subdivisions in the brain is summarized in Figure 12.

The ballooning of the early embryonic brain is remarkable in its rate, its extent, and in its being the result primarily of an increase in cavity size, not tissue growth. In chick embryos, the brain volume expands 30-fold between days three and five of development. This rapid expansion is thought to be caused by positive fluid pressure pressing against the walls of the neural tube by the fluid within it. It might be expected that this fluid pressure might have been dissipated by the spinal cord, but this does not appear to be so. Schoenwolf and Desmond (1984; Desmond and Schoenwolf, 1986) have demonstrated that prior to the closing of the posterior neuropore, a constriction forms in the chick neural tube at the base of the brain (Figure 13). This effectively separates the presumptive brain region from the future spinal cord. (Such an occlusion also occurs in the human embryo; Desmond, 1982.) If one removes the fluid pressure in the anterior portion of such an occluded neural tube, the chick brain enlarges at a much slower rate and contains many fewer cells than are found in normal, control embryos. The occluded region of the neural tube reopens after the initial rapid enlargement of the brain ventricles.

Tissue architecture of the central nervous system. The original neural tube is composed of a germinal neuroepithelium, one cell layer thick. This is a rapidly dividing cell population. Sauer (1935) and others have

FIGURE 13
Occlusion of the neural tube to allow expansion of the future brain region. (A) Dye injected into the anterior portion of a 3-day chick neural tube will fill the brain region but does not pass into the spinal region. (B, C) Section of the chick neural tube at the base of the brain (B) before occlusion and (C) during occlusion. (D) Reopening of occlusion after initial brain enlargement allows dye to pass from brain region into spinal cord region. (Photographs courtesy of M. Desmond.)



shown that all of these cells are continuous from the luminal edge of the neural tube to the outside edge but that the nuclei of these cells are at different heights, thereby giving the superficial impression that the wall of the neural tube has numerous cell layers. The position of the nucleus between the luminal edge and the outer edge of the tube depends on the stage of the cell's cycle (Figure 14). DNA synthesis (S phase) occurs while the nucleus is at the outside edge of the tube, and the nucleus migrates luminally as mitosis proceeds. Mitosis occurs on the luminal side of the cell layer. During early development, 100 percent of the neural tube cells will incorporate radioactive thymidine into DNA (Fujita, 1964). Shortly thereafter, certain cells stop incorporating these DNA precursors, thereby indicating that they are no longer participating in DNA synthesis and mitosis. These are the young neuronal and glial (supporting) cells that migrate to the periphery of the neural tube to differentiate (Fujita, 1966; Jacobson, 1968). Subsequent neural differentiation is dependent upon the position these NEUROBLASTS occupy once outside the region of dividing cells (Jacobson, 1978; Letourneau, 1977).

As the cells adjacent to the lumen continue to divide, the migrating cells form a second layer around the original neural tube. This layer becomes progressively thicker as more cells are added to it from the germinal neuroepithelium. This new layer is called the MANTLE ZONE (Figure 15A), and the germinal epithelium is now called the EPENDYMA. The mantle zone cells differentiate into both neurons and glia. The neurons make connections among themselves and send forth axons away from the lumen, thereby creating a cell-poor MARGINAL ZONE. Eventually, glial cells cover many of these marginal zone axons in myelin sheaths, thereby giving them a whitish appearance. Hence, the mantle zone, containing the cell bodies, is often referred to as the GRAY MATTER; and the axonal, marginal layer is often called the WHITE MATTER.

In the spinal cord and medulla, this basic three-zone pattern of ependymal, mantle, and marginal layers is retained throughout development. The gray matter (mantle) gradually becomes a butterfly-shaped

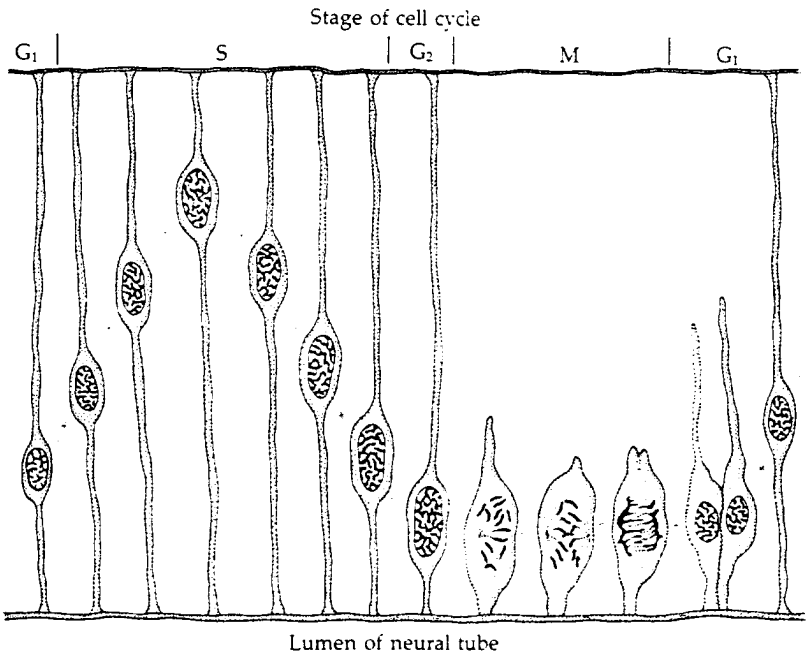


FIGURE 14
Neuroepithelial cell of the neural tube of a chick embryo. Schematic section showing the position of the nucleus in a neuroepithelial cell as a function of the cell cycle. Mitotic cells are found near the center of the neural tube, adjacent to the lumen. (After Sauer, 1935.)

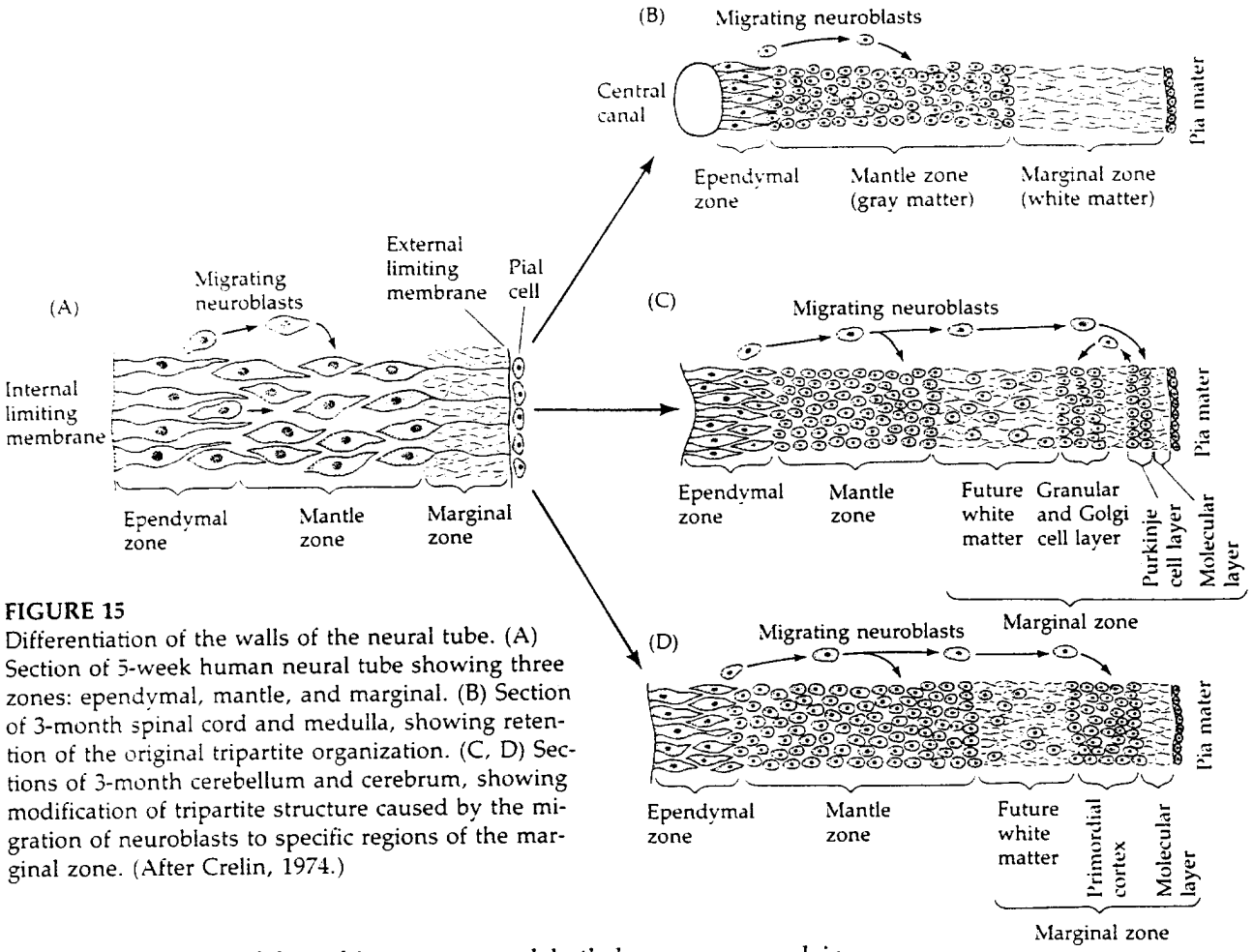


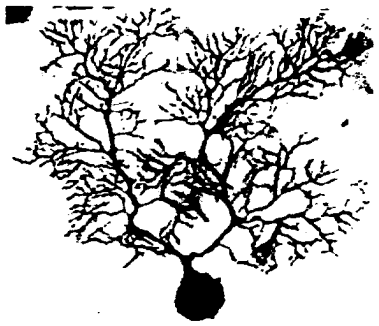
FIGURE 15
Differentiation of the walls of the neural tube. (A) Section of 5-week human neural tube showing three zones: ependymal, mantle, and marginal. (B) Section of 3-month spinal cord and medulla, showing retention of the original tripartite organization. (C, D) Sections of 3-month cerebellum and cerebrum, showing modification of tripartite structure caused by the migration of neuroblasts to specific regions of the marginal zone. (After Crelin, 1974.)

structure surrounded by white matter; and both become encased in connective tissue. As the neural tube matures, a longitudinal groove—the SULCUS LIMITANS—appears to divide it into dorsal and ventral halves. The dorsal portion receives inputs from sensory neurons whereas the ventral portion is involved in effecting various motor functions.

In the brain, however, cell migration, differential growth, and selective cell death produce modifications of the three-zone pattern, especially in the cerebellum and cerebrum (Figure 15B,C,D). Within the deeper layers of the cerebellum, near the lumen, the neuroblasts form clusters of neurons called NUCLEI. Each nucleus works as a functional unit, serving as a relay station between the outer layers of the cerebellum and other parts of the brain. Other gray matter neuroblasts migrate over the outer surface of the developing cerebellum, forming a new germinal zone near the outer boundary of the neural tube. The neuroblasts formed by these germinal cells migrate back into the developing cerebellar white matter to produce a region of GRANULAR NEURONS. The original ependymal layer of the cerebellar cortex generates a wide variety of neurons and glial (support) cells, including the distinctive and large PURKINJE NEURONS. Each Purkinje neuron has an enormous DENDRITIC APPARATUS, which spreads like a fan above a bulblike cell body (Figure 16A). A typical Purkinje cell may form as many as 100,000 synapses with other neurons, more than any other neuron studied. Each Purkinje neuron also emits a slender AXON, which connects to other cells in the deep cerebellar nuclei.

The development of spatial organization, then, is critical for the

(A)



(B)

FIGURE 16
Purkinje neurons. (A) Normal mouse Purkinje neuron. (B) Stunted Purkinje neuron caused by the *staggerer* mutation of the mouse. This mutant has impaired cerebellar function, leading to its characteristic walk. (From Berry et al., 1980; photographs courtesy of M. Berry.)

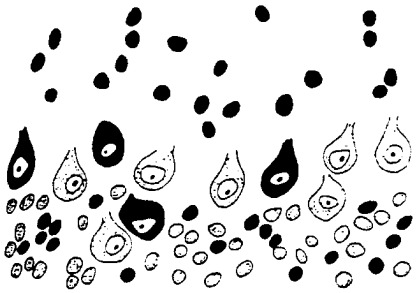
proper functioning of the cerebellum. All impulses eventually regulate the activity of the Purkinje cells, which are the only output neurons of the cerebellar cortex. For this to happen, the proper cells must differentiate at the appropriate place and time.

Some insight into the mechanism of spatial ordering has come from the analysis of neurological mutations in mice. Over 30 mutations are known to affect the arrangement of cerebellar neurons. The defect in *staggerer* mice appears to reside in the Purkinje cells. These cells are smaller than normal and have stunted dendrites with very few spines (Figure 16B). The granule cells originate on schedule, migrate normally, but then die. Sidman (1974) and Sotelo and Changeaux (1974) have suggested that the granule cells die because they are dependent upon connections with the Purkinje dendrites. The resulting mice are small, have tremors, and exhibit a characteristic staggering walk.

Many of the numerous cerebellar mutants have been found because the phenotype of such conditions can be easily recognized. The mice have problems with balance and walking. The *weaver* mutation gives a similar phenotype to *staggerer*, and these mice also have aberrant Purkinje neurons. But in *weaver* mice, the stunting of Purkinje growth appears to be secondary to a genetic defect in the granule cells. This was shown by constructing chimeric mice from wild-type and *weaver* embryos (Goldowitz and Mullen, 1982). The wild-type cells could be recognized by their lower level of β-glucuronidase (which can be stained histochemically) and their different nuclear shape. In the *weaver* cerebellum, there were fewer Purkinje cells and those that were present were not in their correct location in the cortex. In the chimera made with *weaver* and wild-type embryos, some of the Purkinje cells were also in the wrong region. These ectopic cells included neurons of both

FIGURE 17
Failure of granule cell migration in the *weaver* mutant cerebellum. (A) Schematic diagram of the neurons in a chimeric mouse created by fusing a wild-type and a heterozygous *weaver* mouse embryo. The wild-type cells are white, while the cells from the weaver embryo are shown in black. Nearly all the granule cells in abnormal positions are from the *weaver* embryo. (B) Combinations of cells cultured together from wild-type and *weaver* embryos. The wild-type granule cells form close appositions to either wild-type of *weaver* glial cells, but the *weaver* granule cells do not form tight associations with either type of glial cell. (A after Goldowitz and Mullen, 1982; B after Hatten et al., 1986.)

(A)



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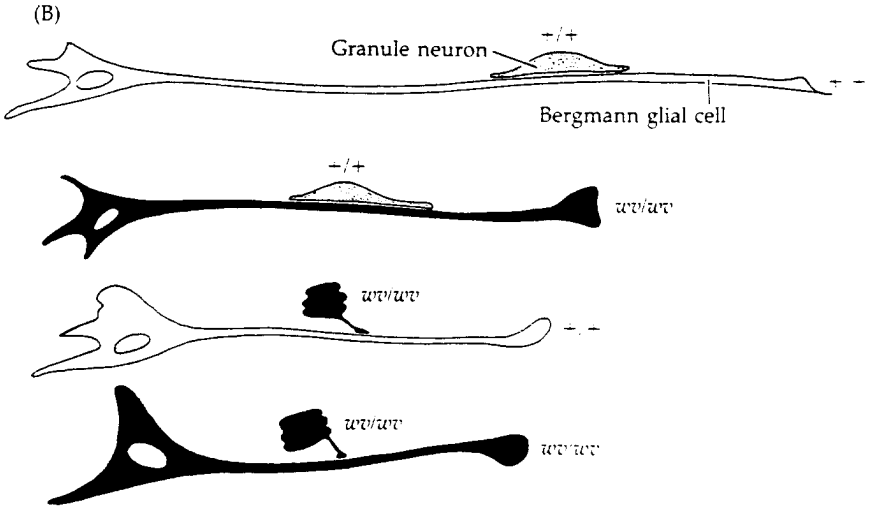


genotypes. However, all the wild-type granule cells were in their correct positions, while the granule cells of the *weaver* embryo were randomly scattered across the cerebellar cortex (Figure 17A). The defect in the *weaver* mice thus appears to involve the inability of the granule cells to migrate from the outer germinal layer to their proper cortical site.

The granule cells usually migrate by traveling upon the extended processes of Bergmann glial cells (Rakić and Sidman, 1973). But Hatten and her colleagues (1986) have demonstrated that the granule cells of *weaver* mice fail to recognize the Bergmann glia as substrates on which to migrate. They cultured different combinations of wild-type and *weaver* granule cells and glial cells (Figure 17B), and found that wild-type granule cells would adhere tightly to either wild-type or *weaver* glial cells (as they would when migrating). However, granule cells from *weaver* embryos would not form close appositions to either *weaver* or wild-type glia. These data strongly suggest that the genetic defect of *weaver* involves the inability of the granule cells to recognize and migrate down the surface of the Bergmann glial cells.

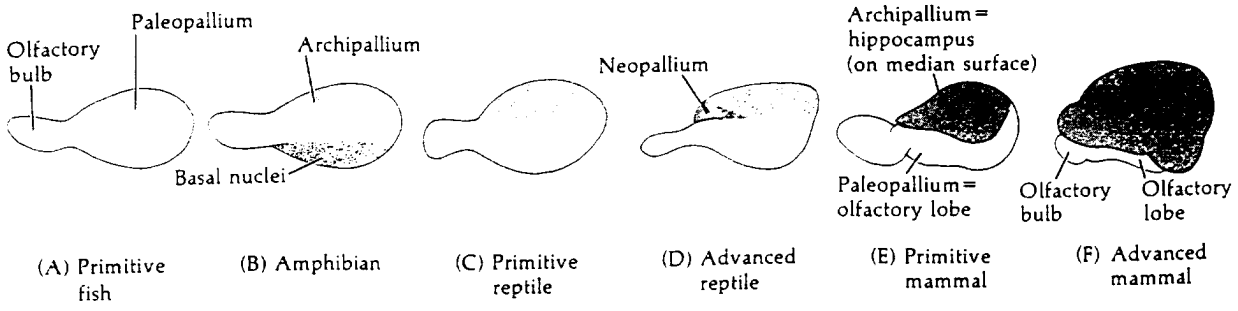
In the cerebrum, the three-zone arrangement is also modified. Certain neuroblasts from the mantle zone migrate through the white matter to generate a second zone of neurons. This new mantle zone is called the NEOPALLIAL CORTEX. This cortex eventually stratifies into six layers of cell bodies, and the adult forms of these neopallial neurons is not completed until the middle of childhood.

SIDELIGHTS & SPECULATIONS

The evolution of cerebral development

The evolution of the cerebral cortex is among the most spectacular stories of vertebrate anatomy. The cerebral lobes originated as paired outgrowths of the forebrain. In fishes, for example, the cerebral lobes function primarily for olfactory perception. Amphibians and reptiles overlaid this primitive olfactory-oriented ARCHIPALLIUM cortex with the PALEOPALLIUM cortex and the CORPUS STRIATUM (Figure 18). The archipallium appears to be associated with "emotional" types of behavior, whereas the paleopallium and corpus striatum are concerned with automatic "instinctual" responses. In birds, the corpus striatum is greatly developed. In advanced reptiles, the first neurons associated with the NEOPALLIAL CORTEX develop. It is the tremendous growth and expansion of the neopallium that characterizes mammalian and especially human evolution. Here, the archipallium has been pushed internally to form the hippocampus (which is involved in sexual

FIGURE 18
Evolution of the vertebrate brain, showing the progressive differentiation of the cerebral cortex. (A) In its most primitive form, the cerebrum is solely for olfaction (smell). (B) In amphibians, the dorsal and ventral portions of the cerebrum become differentiated into the archipallium (hippocampus) and the basal nuclei (corpus striatum). (C) In reptiles, the basal nuclei are found deeper within the cerebrum, and a new group of neurons, the neopallium, is seen on the cortex of advanced reptiles (D). With the advent of mammals (E, F), the expanding neopallial neurons displace the archipallium, eventually dominating the cerebral cortex. (After Romer, 1976.)



and aggressive behaviors), and the corpus callosum has become a relay center for certain involuntary reactions. The neopallium has become the seat of learning, memory, and intelligence. Birds lack a neopallium, and those species capable of learning have developed a HYPERSTRIATUM tissue, where these abilities reside (Romer, 1976).

The development of the human neopallium continues for a remarkably long time. In fact, the human brain continues to develop at fetal rates even after birth (Holt et al., 1975). Portmann (1941, 1945) has suggested that, compared to other primates, human gestation is very much too short. By comparing human development at birth with that of other primates, Portmann determined that human gestation "should" be 21 months long. Moreover, he and Gould (1977) have speculated that we actually do have such a long gestation. During our first year of life, we are essentially extrauterine fetuses.

The reason for this state is that our brain keeps growing at its enormous fetal rate. No woman could deliver a 21-month-old infant because the head would not pass through the birth canal. Birth must occur while the head is still small enough to fit through the mother's pelvis. Thus, humans give birth at the end of 9 months rather than at the end of 21 months. It is during this time, moreover, that we are first exposed to the world. Our developing nervous system takes advantage of the incredible stimulation given it during its first year.

Neuronal types. The human brain consists of over 100 billion nerve cells (neurons) associated with over a trillion supporting glial cells. Those cells that remain as integral components of the neural tube lining become EPENDYMAL CELLS. These cells can give rise to the precursors of neurons and glial cells (Figure 19). It is thought that the differentiation of these precursor cells is largely determined by the environment that they enter (Rakič and Goldman, 1982), and that, at least in some cases, a given precursor cell can form both neurons and glial cells (Turner and Cepko, 1987). There is a wide variety of neuronal and glial types (as is evident from a comparison of the relatively small granule cell with the enormous Purkinje neuron). Some neurons develop only a few cytoplasmic regions where other cells can relay electrical impulses, whereas other neurons develop extensive areas for cellular interaction. The fine extensions of the cell that are used to pick up electrical impulses are called DENDRITES (Figure 20). Very few dendrites can be found on cortical neurons at birth, but one of the amazing things about the first year of life is the increase in the number of such receptive regions in the cortical neurons. During this year, each cortical neuron develops enough dendritic surface to accommodate as many as 100,000 connections with other neurons. The average cortical neuron connects with 10,000 other neural cells. This pattern of neural connections enables the human cortex to function as the center for learning, reasoning, and memory, to develop the capacity for symbolic expression, and to produce voluntary responses to interpreted stimuli.

Another important feature of a developing neuron is its AXON. Whereas the dendrites are often numerous and do not extend far from the nerve cell body (or SOMA), axons may extend for several feet. Thus, the rhythms of the heart are controlled by nerves whose cell bodies are located as far away as the medulla oblongata. The pain receptors on one's big toe must transmit their message a long way, to the spinal cord. One of the fundamental concepts of neurobiology is that the axon is a continuous extension of the nerve cell body. At the turn of the last century, there were still numerous competing theories of axon formation. Schwann, one of the founders of the cell theory, believed that numerous neural cells linked themselves together in a chain to form an axon. Hensen (the discoverer of the embryonic node) thought that the

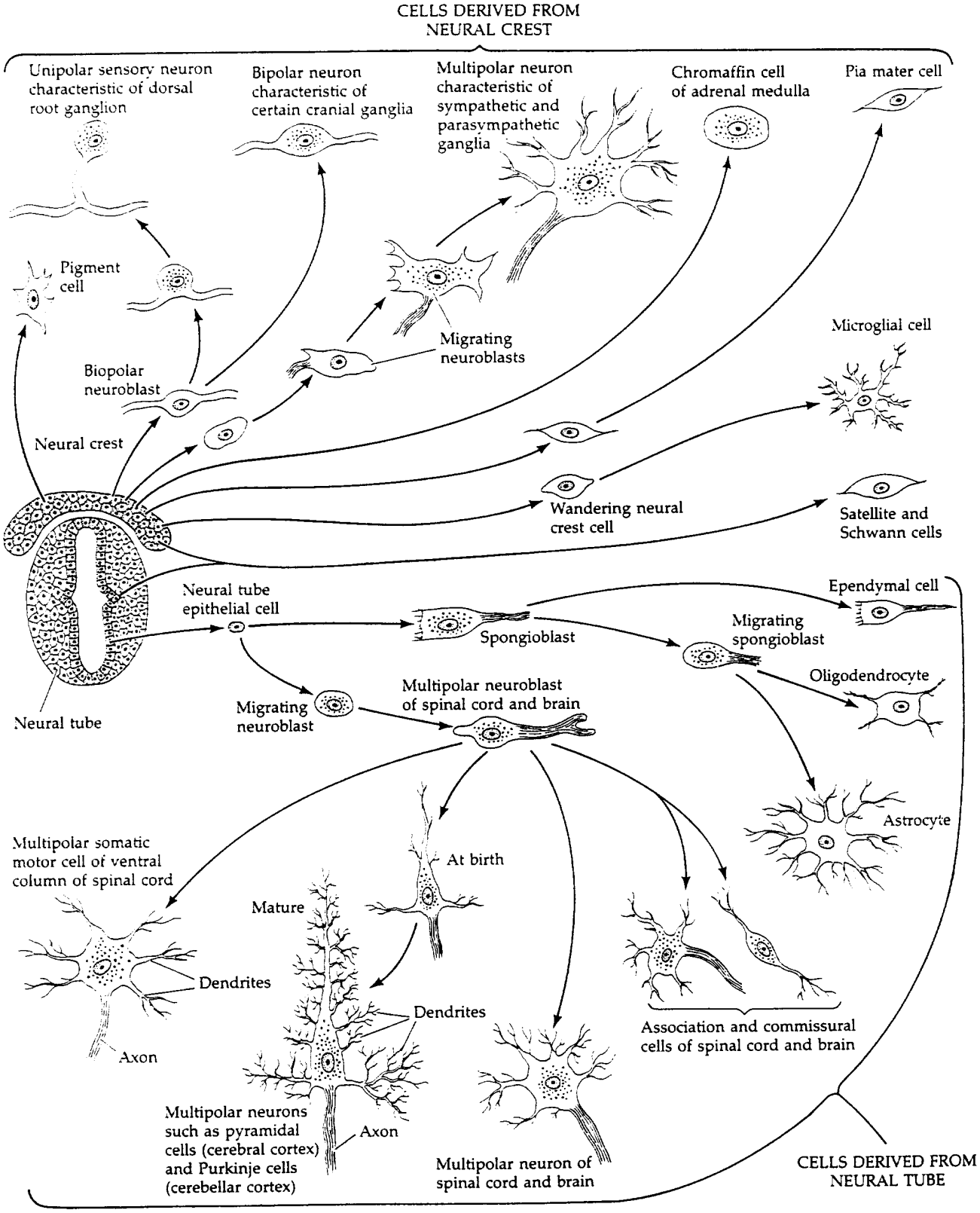


FIGURE 19
Summary of some of the differentiated cell types of the nervous system and their derivation. (After Crelin, 1974.)

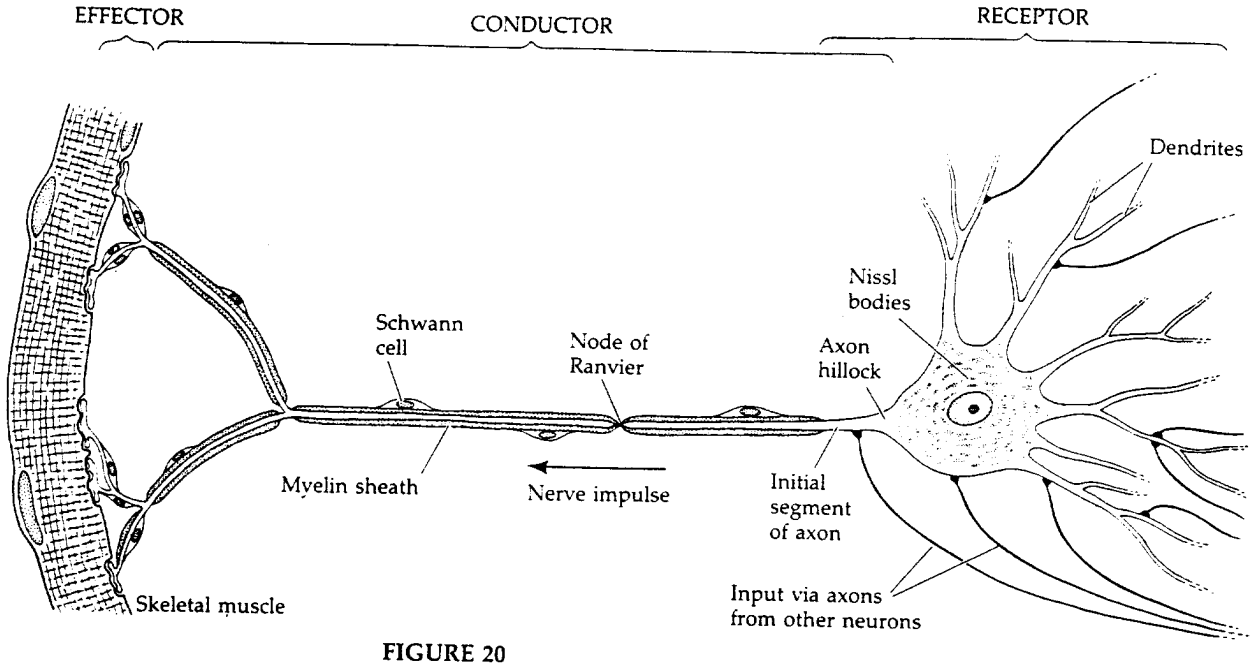


FIGURE 20
Diagrammatic representation of a motor neuron. Impulses received by the dendrites and the stimulated neuron can transmit electrical impulses through the axon (which may be 2–3 feet long) to its target tissue. The myelin sheath, which provides insulation for the axon, is formed by adjacent Schwann cells. (After Bloom and Fawcett, 1975.)

axon formed around preexisting cytoplasmic threads between the cells. Wilhelm His (1886) and Santiago Ramón y Cajal (1890) postulated that the axon was indeed an outgrowth (albeit an extremely large one) of the nerve soma.

In 1907 Ross Harrison demonstrated the validity of the outgrowth theory in an elegant experiment that founded both the science of developmental neurobiology and the technique of tissue culture. Harrison isolated a portion of neural tube from a 3-mm frog tadpole. At this stage, shortly after the closure of the neural tube, there is no visible differentiation of axons. He placed these neuroblasts in a drop of frog lymph on a coverslip and inverted the coverslip over a depression slide so he could watch what was happening within this "hanging drop." What Harrison saw (Figure 21) was the emergence of the axons as outgrowths from the neuroblasts, elongating at about 56 μm/hr.

Such nerve outgrowth is led by the tip of the axon, which is called

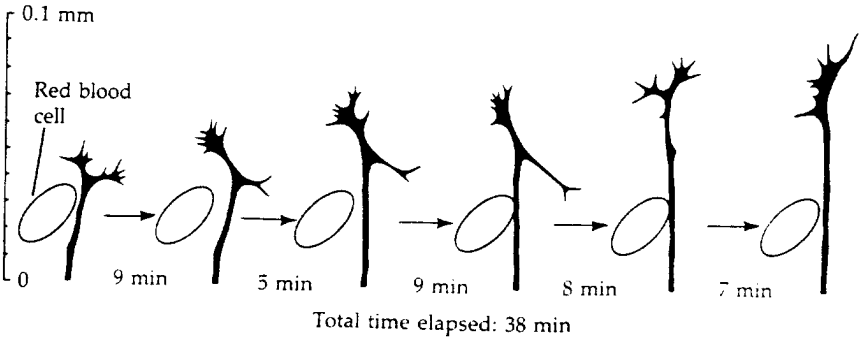


FIGURE 21
Nerve fiber growth in culture. Sketches of a growing tadpole neuron were made as it passed a red blood cell. (From Harrison, 1910.)

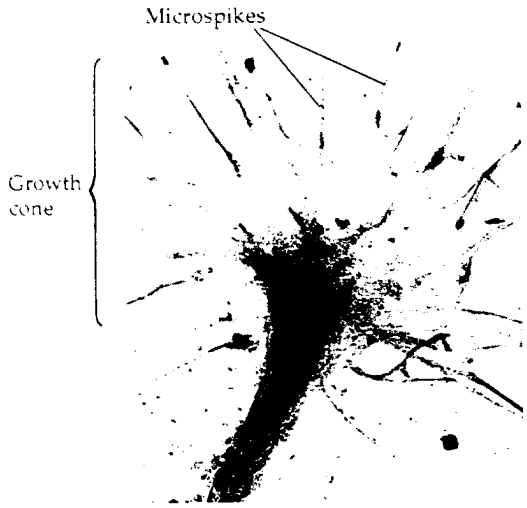


FIGURE 22
Growth cone of the axon. Transmission electron micrograph of the growth cone region showing microspikes. (From Letourneau, 1979; photograph courtesy of P. C. Letourneau.)

the GROWTH CONE (Figure 22). This cone does not proceed in a straight line but rather "feels" its way along the substrate. The growth cones move by the elongation and contraction of pointed filopodia called MICROSPIKES. These microspike filopodia contain microfilaments, which are oriented in an array parallel to the long axis of the axon. (Microfilaments have been associated with the migratory processes of several cell types and are often included in contractile activities.) Treating neurons with cytochalasin B will inhibit their further advance (Yamada et al., 1971). Within the axon itself, structural support is provided by microtubules, and the axon will retract if placed in a solution of colchicine (Figure 23). Thus, the developing neuron retains the same features that we have already noted in neural tube formation, namely, elongation

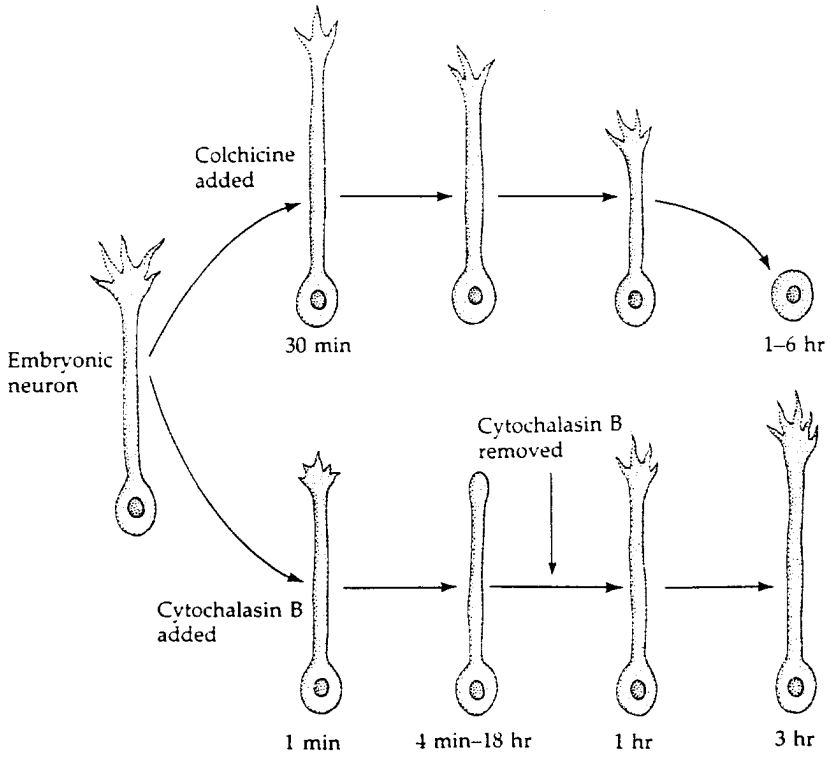


FIGURE 23
Effects of cytochalasin B and colchicine on axon elongation in cultured embryonic neurons. Colchicine causes the collapse of the axon, an event that correlates with the disruption of the microtubules. Cytochalasin B causes the reversible depolymerization of microfilaments, thereby leading to the disappearance of the microspikes. When cytochalasin B is removed from the medium, the growth cone reappears.

by microtubules and apical shape changes by microfilaments. In most migrating cells, the exploratory filopodia will attach to the substrate and pull the rest of the cell over to it. This, of course, would be difficult given the length of the axon. Thus, the contractile apparatus in the axonal filopodium appears to be solely exploratory; and elongation is accomplished by cytoplasmic movement through the axon.

Neurons transmit electrical impulses from one region to another. These impulses usually go from the dendrites into the nerve soma, where they are focused into the axon. To prevent dispersion of the electrical signal and to facilitate its conduction, the axon in the central nervous system is insulated at intervals by processes that originate from a type of glial cells called OLIGODENDROCYTES. An oligodendrocyte wraps itself around the developing axon. It then produces a specialized cell membrane that is rich in myelin basic protein and that spirals around the central axon (Figure 24). This specialized membrane is called a MYELIN SHEATH. (In the peripheral nervous system, Schwann glial cells accomplish this myelination.) The myelin sheath is essential for proper neural function, and demyelination of nerve fibers is associated with several severely debilitating or lethal diseases.

The axon must also be specialized for secreting a specific neurotransmitter across the small gaps (synaptic clefts) that separate the axon of one cell from the surface of its target cell (the soma, dendrites, or axon of a receiving neuron or a receptor site on a peripheral organ). Thus, the developing neuron must also develop the specific enzymes

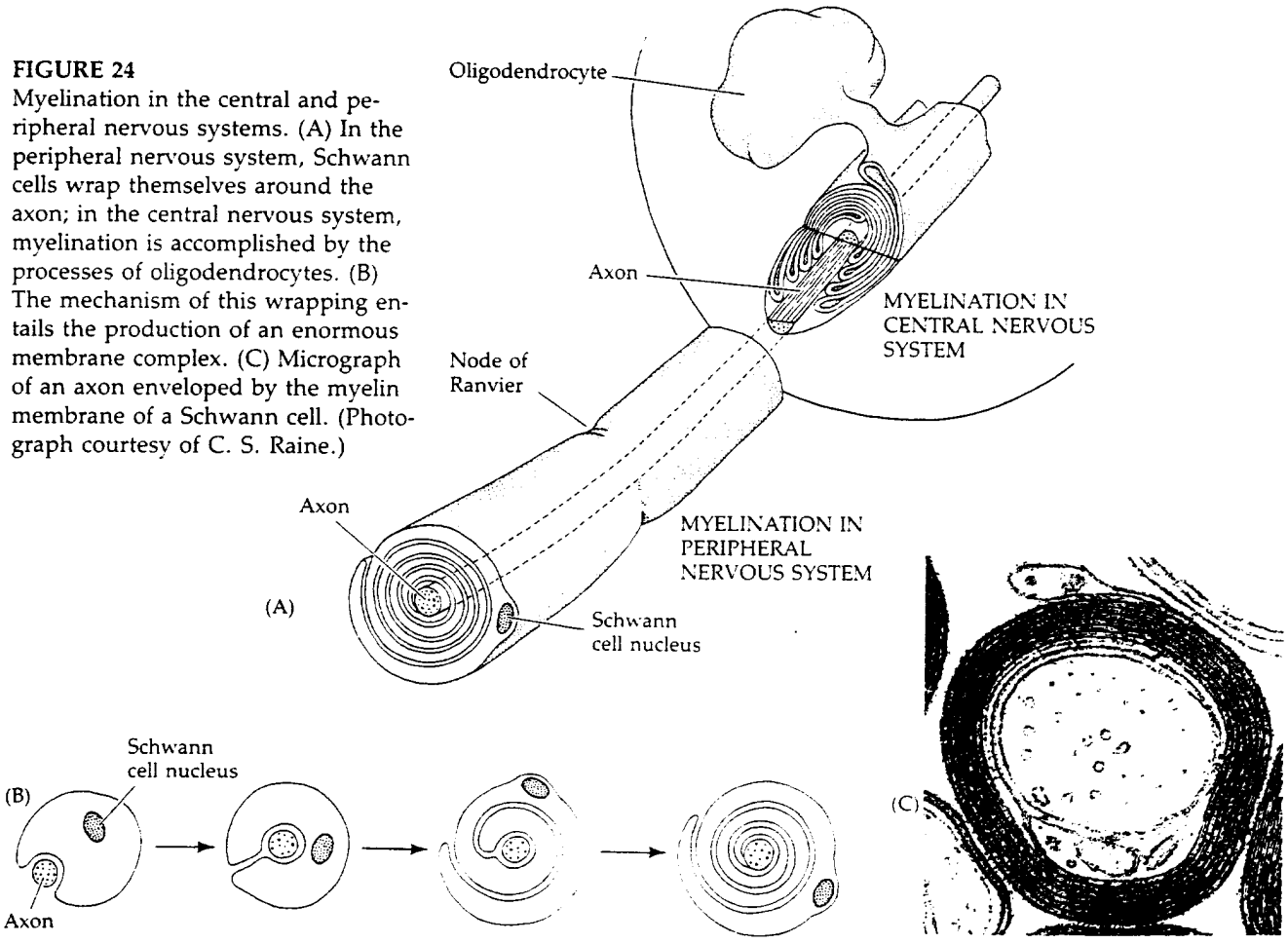


FIGURE 24
Myelination in the central and peripheral nervous systems. (A) In the peripheral nervous system, Schwann cells wrap themselves around the axon; in the central nervous system, myelination is accomplished by the processes of oligodendrocytes. (B) The mechanism of this wrapping entails the production of an enormous membrane complex. (C) Micrograph of an axon enveloped by the myelin membrane of a Schwann cell. (Photograph courtesy of C. S. Raine.)

required for the production and degradation of its neurotransmitter substance. Neuronal development involves both structural and molecular differentiation.

Development of the eye

Dynamics of optic development. An individual gains knowledge of its environment through its sensory organs. In this section, we will focus on the eye, because this organ, perhaps more than any other organ in the body, must develop with precision and perfect coordination of all its components.

The story of optic development begins at the wall of the diencephalon. In humans, optic development begins as the wall of the 22-day embryonic diencephalon bulges out laterally from the neural tube. This differential growth produces the OPTIC VESICLES, which are connected to the diencephalon by the OPTIC STALK. Subsequently, these vesicles contact the surface ectoderm and induce this ectoderm to form the LENS PLACODES (Figure 25).* This induction is specific, since isolated optic vesicles implanted adjacent to any region of head ectoderm will induce that ectoderm to form lens tissue instead of epidermal cells. In the absence of optic vesicle contact, no lens forms. The necessity for close contact between the optic vesicles and the surface ectoderm is also seen

*The inductions forming the eye will be detailed in Chapters 8 and 16.

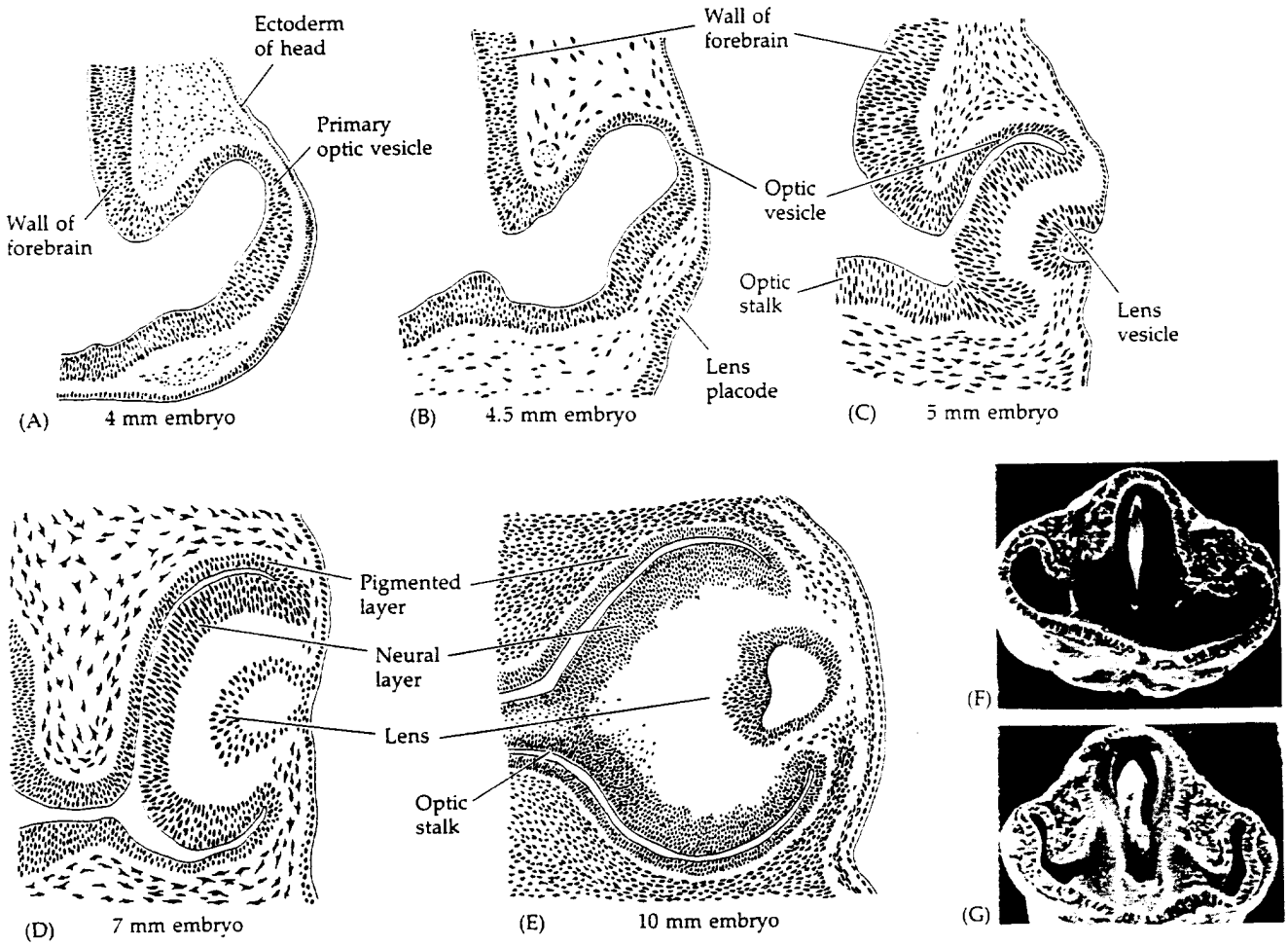


FIGURE 25
Development of the eye. (A) Optic vesicle evaginates from the brain and contacts the overlying ectoderm. (B, C) Overlying ectoderm differentiates into lens cells as the optic vesicle folds in on itself. (D) Optic vesicle becomes the neural and pigmented retina as the lens is internalized. (E) The lens induces the overlying ectoderm to become the cornea as the optic stalk develops to carry impulses from the eye to the brain. (F) and (G) are scanning electron micrographs corresponding to (A) and (B). (A–E from Mann, 1964; F–G from Hilfer and Yang, 1980. Photographs courtesy of S. R. Hilfer.)

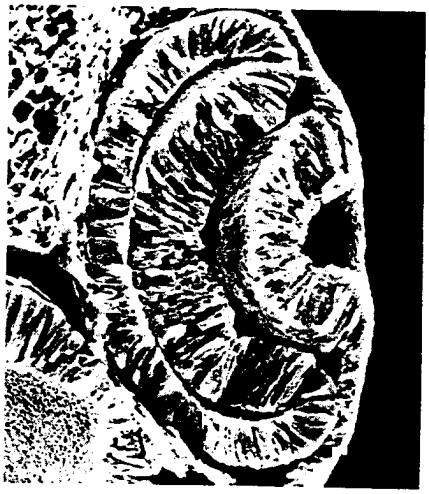


FIGURE 26
Scanning electron micrograph of the formation of the optic cup and lens placode in a chick. (From Hilfer and Yang, 1980; courtesy of S. R. Hilfer.)

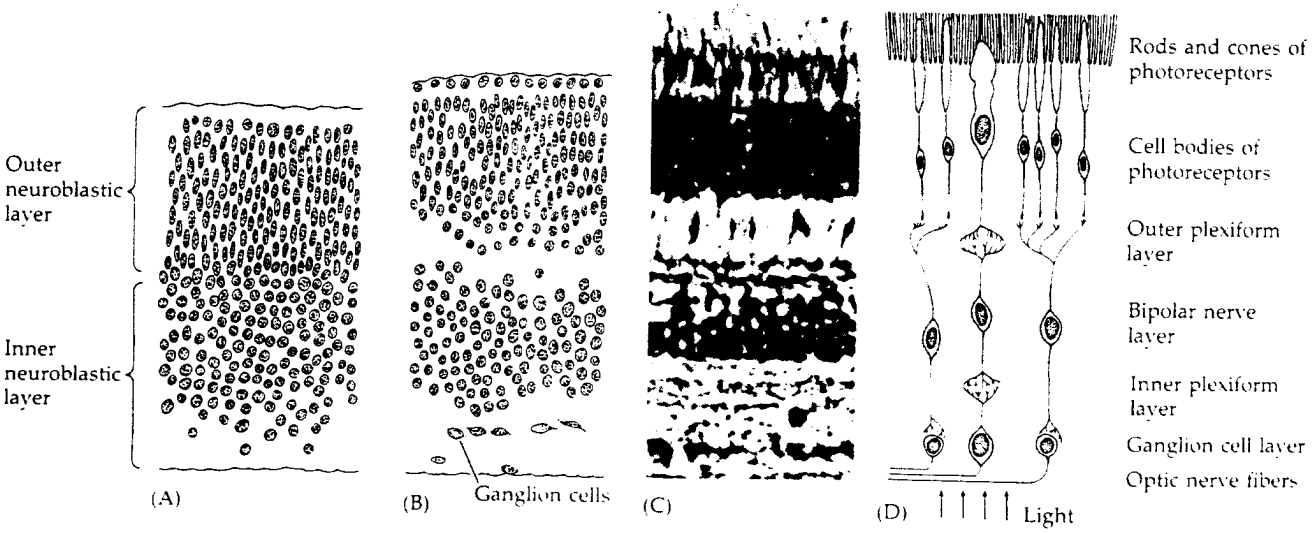
in both experimental cases and in certain mutants. For example, in the mouse mutant *eyeless*, the optic vesicles fail to contact the surface and eye formation ceases (Webster et al., 1984).

Once formed, the lens placode reciprocates and causes changes in the optic vesicle. The vesicle invaginates to form a double-walled OPTIC CUP (Figure 26). As the invagination continues, the connection between the optic cup and the brain is reduced to a narrow slit. At the same time, the two layers of the optic cup begin to differentiate in different directions. The cells of the outer layer produce pigment and ultimately become the PIGMENTED RETINA. The cells of the inner layer proliferate rapidly and generate a variety of light-sensitive photoreceptor neurons, glia, interneurons, and ganglion cells. Collectively, these cells constitute the NEURAL RETINA. The axons from the ganglion cells of the neural retina meet at the base of the eye and travel down the OPTIC STALK. This stalk is then called the OPTIC NERVE.

Neural retina differentiation. Like the cerebral and cerebellar cortices, the neural retina develops into a layered array of different neuronal types (Figure 27). These layers include the light- and color-sensitive photoreceptor cells, the cell bodies of the ganglion cells, and the bipolar interneurons that transmit the electrical stimulus from the rods and cones to the ganglion cells. In addition, there are numerous glial cells that serve to maintain the integrity of the retina, as well as amacrine and horizontal neurons that transmit electrical impulses horizontally.

In the early stages of retinal development, cell division from a germinal layer and the migration and differential death of the resulting cells forms the striated pattern of the neural retina. The formation of this highly structured tissue is one of the most intensely studied prob-

FIGURE 27
Development of the human retina. Retinal neurons sort out into functional layers during development. (A,B) Initial separation of neuroblasts within the retina. (C) The three layers of neurons in an adult retina, and the synaptic (plexiform) layers between them. (D) A functional depiction of the major neural pathway in the retina. Light traverses the layers until it is monitored by the photoreceptors. The axons of the photoreceptors synapse with bipolar neurons that transmit the depolarization to the ganglion neurons. The axons of the ganglion cells join to form the topic nerve that enters the brain. (A and B after Mann, 1964. Photograph courtesy of G. Grunwald.)



lems of developmental neurobiology. It has recently been shown (Turner and Cepko, 1987) that a single retinal neuroblast precursor cell can give rise to at least three types of neurons or to two types of neurons and a glial cell. This analysis was performed using a most ingenious technique to label the cells generated by one particular precursor cell. Newborn rats (whose retinas are still developing) were injected into the back of their eyes with a virus that can integrate into their DNA. This virus contained a β-galactosidase gene (not present in rat retina) which would be expressed in the infected cells. A month after infecting the rat eyes, the retinas were removed and stained for the presence of β-galactosidase. Only the progeny of the infected cells should stain blue. Figure 28 shows one of the stripes of cells derived from an infected precursor cell. The stain can be seen in five rods, a bipolar neuron, and a Müller glial cell.

Of the three major neuronal types in the retina (ganglion, bipolar, and photoreceptor), the photoreceptive rods and cones are probably the last of the neurons to complete their differentiation. As they develop, the cell bodies of these outer neurons produce a bud of cytoplasm that contains several specialized organelles. These organelles elongate the bud and adjust the size and shape of the photoreactive regions (Detwiler, 1932). The cell membrane of these cells folds back upon itself to form sacs upon which the photoreceptive pigments are placed. Light induces these pigments to undergo chemical changes that ultimately result in a change of membrane potential. This change in membrane potential effects the release of neurotransmitters to a group of bipolar neurons that relay the electrical signal to the ganglion cells. The ganglion cells, whose axons bundle together to form the optic nerve, relay this information to the brain (Fesenko et al., 1985; Stryer, 1986).

FIGURE 28
Determination of the lineage of a precursor cell in the rat retina. (A) Technique whereby a virus containing a functional β-galactosidase gene is injected into the back of the eye to infect some of the retinal precursor cells. After a month to 6 weeks, the eye is removed and the retina is stained for the presence of β-galactosidase. (B) Stained cells forming a strip across the neural retina, including five rods (r), a bipolar neuron (bp), a rod terminal (t), and a Müller glial cell. The identities of these cells were confirmed by Nomarski phase contrast microscopy. Scale bar, 20 μm. (From Turner and Cepko, 1987; photograph courtesy of D. Turner).

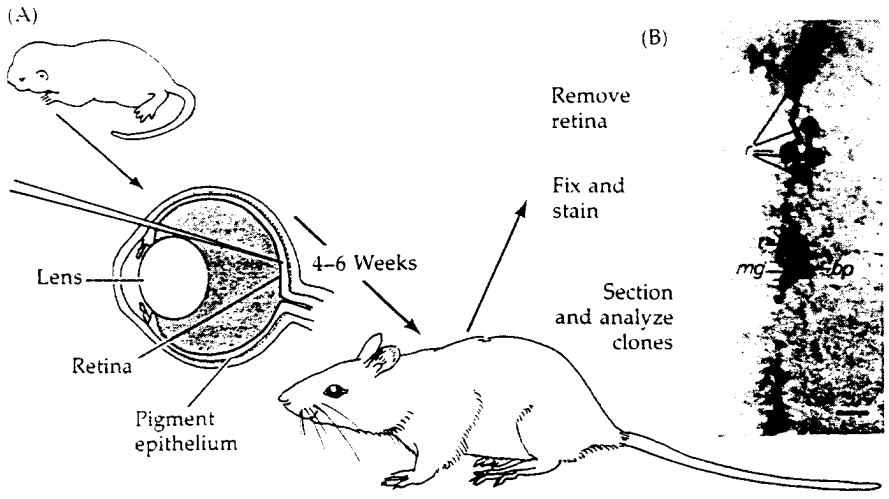


FIGURE 29
Scanning electron micrograph of the rounded and internalized lens placode in chick embryo. (Courtesy of K. W. Tosney.)

Lens and cornea differentiation. During its continued development into a lens, the lens placode rounds up and contacts the new overlying ectoderm (Figure 29). The lens placode then induces the ectoderm to form the transparent CORNEA. Here, physical parameters play an important role in the development of the eye. Intraocular fluid pressure is necessary for the correct curvature of the cornea so that light can be focused upon the retina. The importance of such ocular pressure can be demonstrated experimentally; the cornea will not develop its characteristic curve when a small glass tube is inserted through the wall of a developing chick eye to drain away intraocular fluids (Coulombre, 1956, 1965). Intraocular pressure is sustained by a ring of scleral bones (probably derived from the neural crest), which act as inelastic restraints. Without these bones, the intraocular pressure could not be maintained, and the cornea would not be properly formed.

The differentiation of the lens tissue into a transparent membrane capable of directing light onto the retina involves changes in cell structure and shape as well as synthesis of lens-specific proteins called CRYSTALLINS. These crystallins are synthesized as cell shape changes occur, thereby causing the lens vesicle to become the definitive lens. The cells at the inner portion of the lens vesicle elongate and, under the influence of the neural retina, produce the lens fibers (Piatigorsky, 1981). As these fibers continue to grow, they synthesize crystallins, which eventually fill up the cell and cause the extrusion of the nucleus. The crystallin-synthesizing fibers continue to grow and eventually fill the space between the two layers of the lens vesicle. The anterior cells of the lens vesicle constitute a germinal epithelium, which keeps dividing. These dividing cells move toward the equator of the vesicle, and as they pass through the equatorial region, they too begin to elongate (Figure 30). Thus, the lens contains three regions: an anterior zone of dividing epithelial cells, an equatorial zone of cellular elongation, and a posterior and central zone of crystallin-containing fiber cells. This arrangement persists throughout the lifetime of the animal as fibers are continuously being laid down. In the adult chicken, the differentiation from an epithelial cell to a lens fiber takes 2 years (Papaconstantinou, 1967). The details of lens and cornea formation are discussed in Chapter 16.

Directly in front of the lens is a muscular tissue called the IRIS. These muscles control the size of the pupil (and give an individual his or her characteristic eye color). Unlike the other muscles of the body (which are derived from the mesoderm), the iris is derived from the ectodermal layer. Specifically, the iris develops from a portion of the optic cup that is continuous with the neural retina but does not make photoreceptors.

The neural crest and its derivatives

Although derived from the ectoderm, the neural crest has sometimes been called the fourth germ layer because of its importance. The neural crest cells migrate extensively and give rise to a bewildering number of differentiated cell types including (1) the neurons and supporting glial cells of the sensory, sympathetic, and parasympathetic nervous systems, (2) the epinephrine-producing cells of the adrenal gland, (3) the pigment-containing cells of the epidermis, and (4) skeletal and connective tissue components of the head (Table 1). The fate of the neural crest cells depends upon where the cells migrate and settle.

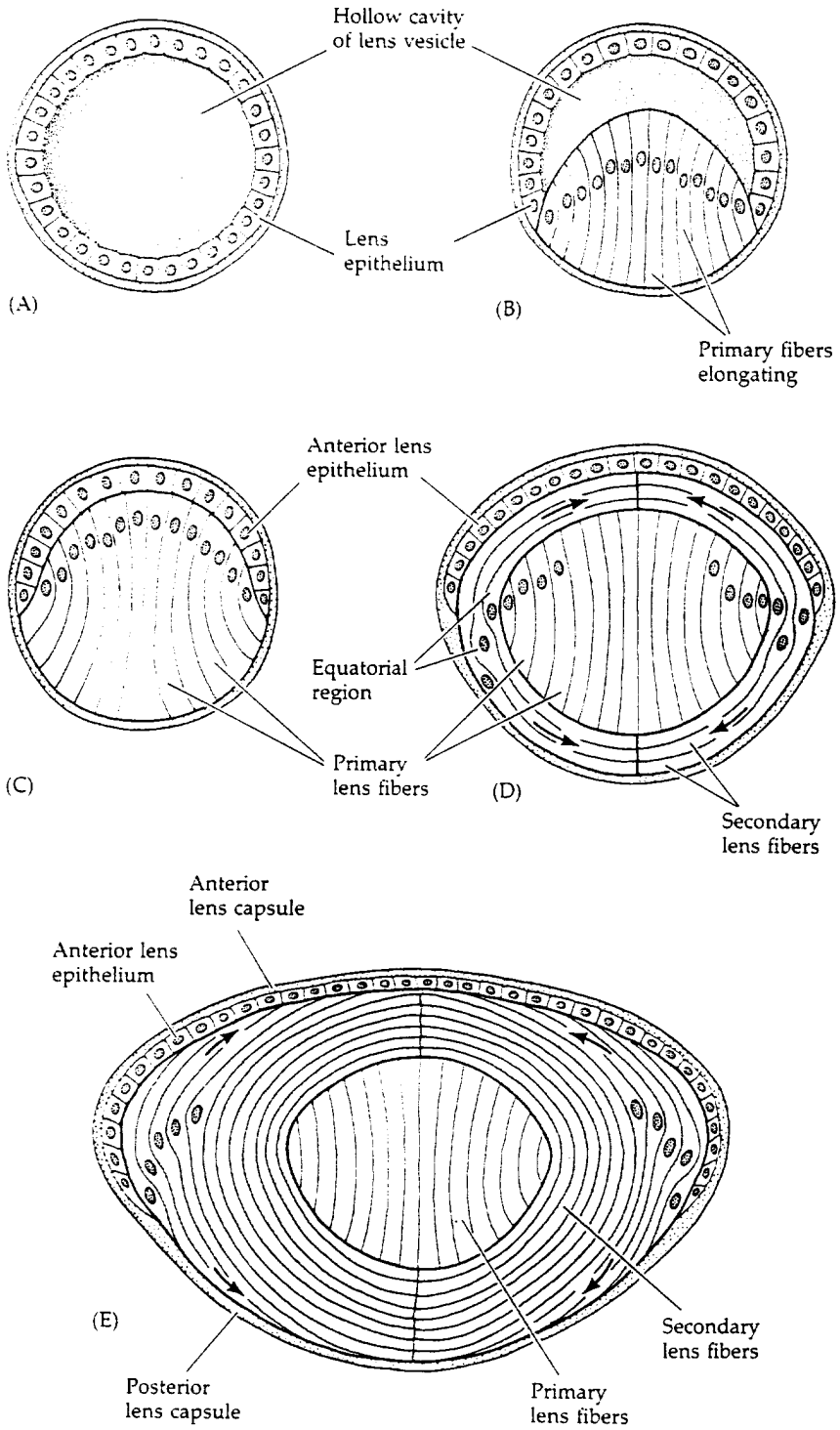


FIGURE 30
Differentiation of the lens cells.(A) Lens vesicle as shown in Figure 29. (B) Elongation of the interior cells, producing lens fibers. (C) Lens filled with crystallin-synthesizing cells. (D) New lens cells derived from anterior lens epithelium. (E) As the lens grows, new fibers differentiate. (After Paton and Craig, 1974.)

Migration pathways of neural crest cells

As shown in Figure 2, the neural crest is a transient structure, its cells dispersing soon after the neural tube closes. By grafting a portion of the chick neural tube and its associated crest from radioactively or genetically marked embryos into other embryos (Weston, 1963; Thiery et al., 1982), investigators have been able to trace the routes of neural crest cell migration (Figure 31). More recent studies have followed neural crest cell migration with fluorescent antibodies that bind almost

TABLE 1
Major neural crest derivatives

Pigment cells	Sensory nervous system	Autonomic nervous system	Skeletal and connective tissue	Endocrine
TRUNK CREST (INCLUDING CERVICAL CREST)				
Melanocytes Xanthophores (erythrophores) Iridophores (guanophores) in dermis, epidermis, and epidermal derivatives	Spinal ganglia Some contributions to vagal (X) root ganglia	Sympathetic Superior cervical ganglion Prevertebral ganglia Paravertebral ganglia Adrenal medulla Parasympathetic Remak's ganglion Pelvic plexus Visceral and enteric ganglia	Mesenchyme of dorsal fin in amphibia Walls of aortic arches Connective tissue of parathyroid	Adrenal medulla Calcitonin-producing cells Type I cells of carotid body Parafollicle cells of thyroid
		Some supportive cells Glia (oligodendrocytes) Schwann sheath cells Some contribution to meninges		
CRANIAL CREST				
Small, belated contribution	Trigeminal (V) Facial (VII) root Glossopharyngeal (IX) root (superior ganglia) Vagal (X) root (jugular ganglia)	Parasympathetic ganglia Ciliary Ethmoid Sphenopalatine Submandibular Intrinsic ganglia of viscera	Most visceral cartilages Trabeculae craniae (ant.) Contributes cells to posterior trabeculae, basal plate, parachordal cartilages Odontoblasts Head mesenchyme (membrane bones)	
		Supportive cells		

Sources: Weston (1970); Bockman and Kirby (1984).

exclusively to these migrating cells. This procedure allows the identification of individual cells within large areas. Using such antibodies, investigators in several laboratories have identified three major routes on which neural crest cells migrate in the trunk region (Rickmann et al., 1985; Bronner-Fraser, 1986a; Teillet et al., 1987; Loring and Erickson, 1987; Figure 32). The first pathway extends ventrally *through* the *anterior* portion of the somite. As can be seen in Figure 33 and the color endplate, migrating neural crest cells are able to enter the anterior section of these structures but not the posterior section. Some of these cells reach the dorsal aorta, where they give rise to the SYMPATHETIC GANGLIA. These ganglia are clusters of neurons that transmit impulses to target cells when stimulated by spinal cord neurons.* At specific regions of the trunk, crest cells migrating along the same pathway aggregate and form the epinephrine-secreting cells of the ADRENAL MEDULLA.

*The parasympathetic division of the peripheral nervous system is also formed by neural crest cells migrating by this pathway, but only in the cranial and cervical regions of the embryo.

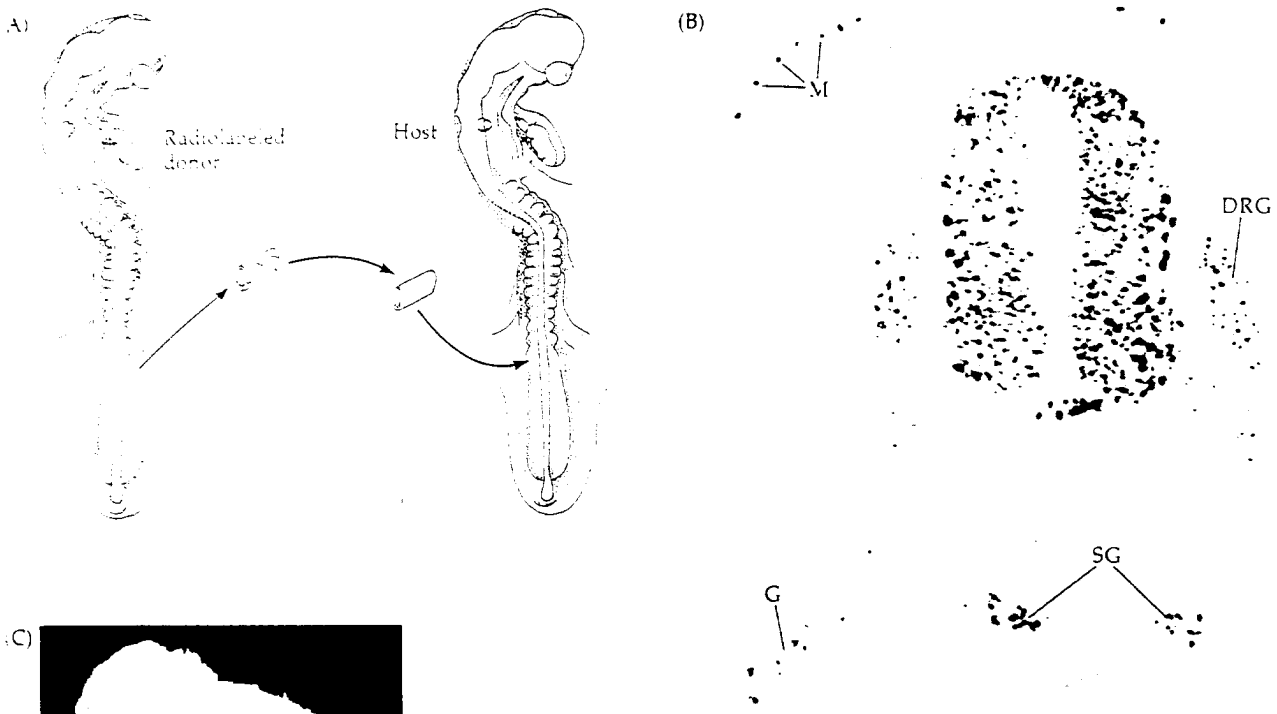


FIGURE 31
Neural crest cell migration. (A) Grafting technique for mapping neural crest cells. A piece of the dorsal axis is excised from a donor embryo; the neural tube and its associated crest are isolated and implanted into a host embryo whose neural tube and crest have been excised. When the donor crest cells are radiolabeled (with tritiated thymidine) or genetically labeled (from a different species or strain), their descendants can be traced in the host embryo as development proceeds. (B) Autoradiograph showing locations of neural crest cells that have migrated from transplanted radioactive donor neural crest to form melanoblasts (M), sympathetic ganglia (SG), dorsal root ganglia (DRG), and glial cells (G). (C) Chick resulting from the transplantation of a neural crest region from a pigmented strain of chicken into the crest region of an unpigmented strain. The crest cells that gave rise to pigment were able to migrate into the wing skin. (After Weston, 1963; photographs courtesy of J. Weston.)

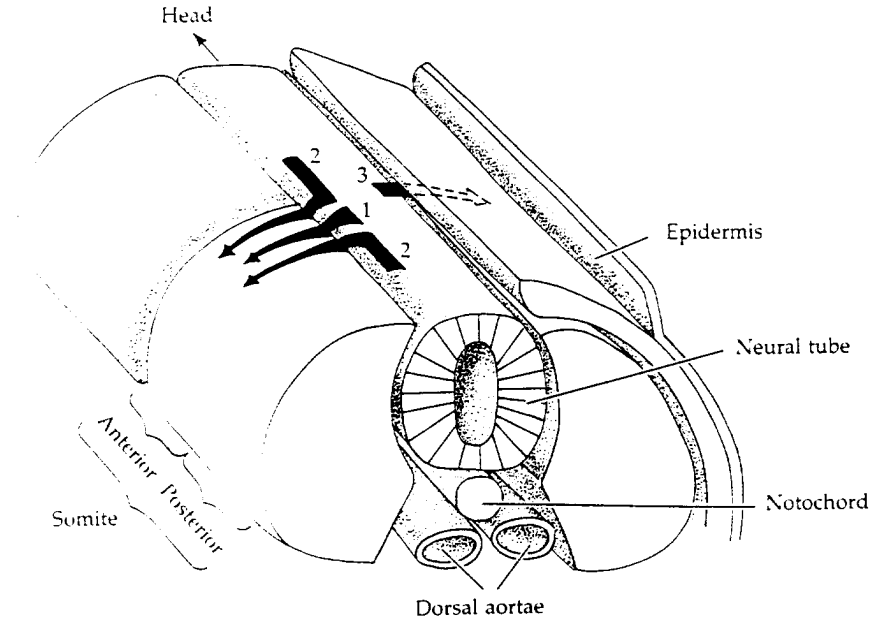


FIGURE 32
Neural crest cell migration in the trunk of the chick embryo. Path 1: Cells travel ventrally through the anterior of the somite. These cells probably contribute to the sympathetic and parasympathetic ganglia as well as to the adrenal medullary cells and dorsal root ganglia. Path 2: Cells adjacent to the posterior portions of the somites migrate between the neural tube and somite to enter an adjacent anterior somite. These cells have been found in the dorsal root ganglia. Path 3: Cells take a dorsolateral route beneath the ectoderm. These cells become pigment-producing melanocytes.

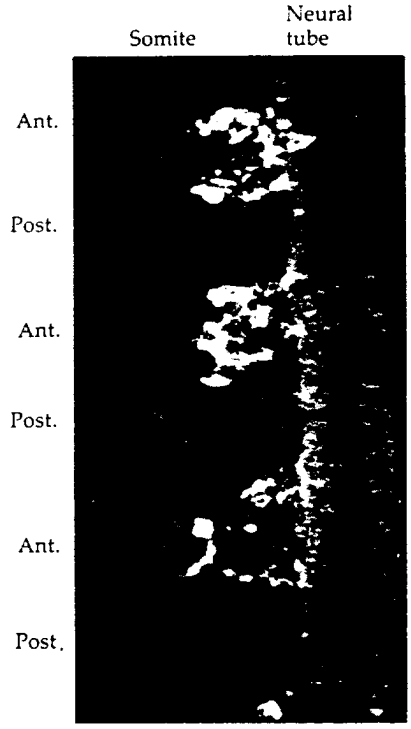


FIGURE 33
Neural crest cell migration. These fluorescence photomicrographs of longitudinal sections of a 2-day chick embryo are stained with antibody HNK-1, which selectively recognizes neural crest cells. Extensive staining is seen in the anterior, but not in the posterior, half of each somite. (From Bronner-Fraser, 1986a; photograph courtesy of M. Bronner-Fraser.)

But what happens to those neural crest cells that are adjacent to a *posterior* region of the somite? The antibody can provide information about space, but it cannot provide information about the direction in which a specific group of cells is traveling. Teillet and co-workers (1987) combined the antibody approach with transplantation of genetically marked quail neural crest cells into chick embryos. The antibody marker recognizes and labels cells of both species as neural crest cells; the genetic marker enables the investigator to distinguish between the quail and chick cells. These studies identified a second pathway of neural crest cell migration by which neural crest cells opposite the posterior regions of the somites migrate anteriorly or posteriorly along the neural tube and enter the anterior regions of their own or the adjacent somites. These cells combined with some of the cells that were initially opposite the anterior portion of the somite to form the DORSAL ROOT GANGLIA. These are the clusters of neurons that relay sensory information to the spinal cord. Thus, each dorsal root ganglion is composed of three neural crest populations: one from the neural crest opposite the anterior portion of the somite, and one from each of the adjacent neural crest regions opposite the posterior portions of the somite.

The third major pathway of neural crest cell migration follows a dorsolateral route beneath the embryonic ectoderm. Some of the cells of this pathway differentiate into pigment cells (in mammals, the MELANOCYTES). These cells travel along the ventral surface of the ectodermal layer from the central dorsal region to the most ventral region of the skin, eventually terminating in the belly skin of the organism. In several mutations that affect neural crest migration, there is a white "belly spot," indicating that pigment cells did not arrive there (Figure 34).

Thus, neural crest cells do not migrate randomly through the body; rather, they follow precise pathways. Although the nature of these pathways is still a major question, the migration of neural crest cells appears to be controlled by the substratum over which they travel. In 1941, Paul Weiss speculated that temporary linkages would form between specific molecules of the cell surface and complementary mole-

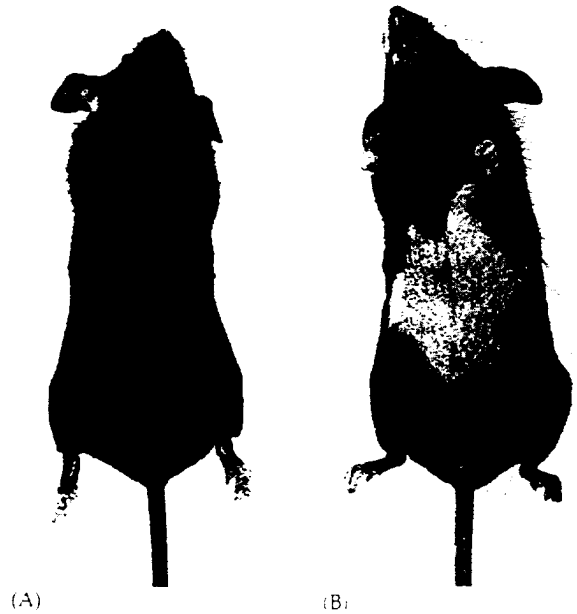
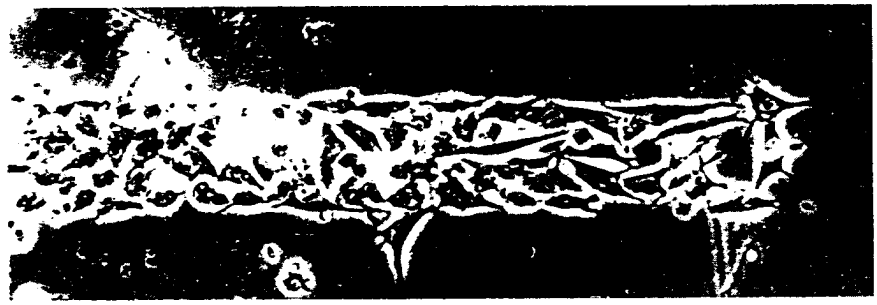


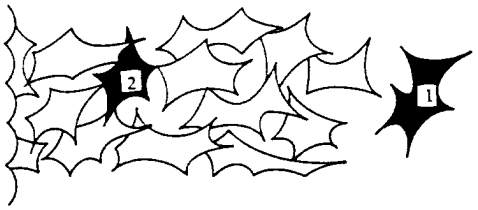
FIGURE 34
Pigment cell migration. The dorsal (A) and ventral (B) sides of a mouse heterozygous for the mutation *White*. The melanoblasts have not migrated completely around the mouse; a white spot (lacking pigment cells) remains in the center of the belly. In mice homozygous for this mutation (not shown), the whole body is white, but the melanin pigment that forms in the retina can be seen. (Photographs courtesy of The Jackson Laboratory.)

cules on the substratum. More recent evidence suggests that this is probably the case for neural crest cells. First, when the neural tube and its associated crest are inverted, the crest cells continue to stream out. However, they now move dorsally instead of ventrally, thereby indicating that they keep their original orientation with regard to the neural tube. Moreover, when neural crest cells or their derivatives are placed (either by transplantation or by injection) on a normal neural crest pathway in a host, they will migrate along these pathways. Other embryonic cells will not orient themselves in this manner and will stay where they are placed (Erickson et al., 1980; Bronner-Fraser and Cohen, 1980). Therefore, the neural crest cells are able to recognize certain pathways in the embryo and migrate along them.

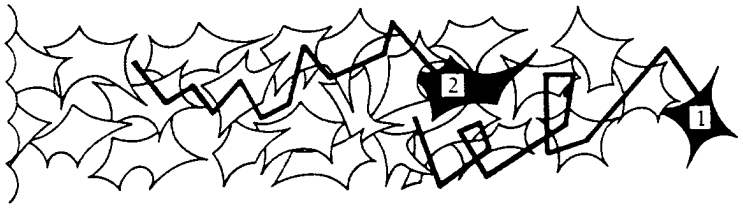
Recognition of the proper road may be due to that road's being marked by the protein fibronectin. Rosavio and his co-workers (1983) have found that migratory neural crest cells attach to and travel upon this molecule. If neural crest cells in a petri dish come into contact with a strip of fibronectin, the crest cells prefer to move only on that strip, and they move away from higher concentrations of cells (Figure 35). This mimics the behavior of neural crest cells in the head where such cells travel on thin fibronectin-rich tracks away from regions of high cell density (Mayer et al., 1981). Neural crest cells also show a preference for traveling on laminin, another protein that is secreted by cells to

FIGURE 35
Neural crest cell migration along a fibronectin substrate. Neural tubes and their associated crests were removed from the embryo and placed in a petri dish on a line of fibronectin. (A) Crest cells migrating from a fixed point (at left) in a nonrandom fashion along the fibronectin line. (B, C) Tracks of representative cells as they traveled on a fibronectin strip. The undulating line at the left represents the neural tube and crest that was the source of the migrating cells. (After Rosavio et al., 1983; photograph courtesy of J.-P. Thiery.)





(B)



(C)

form an extracellular matrix (Newgreen et al., 1986). Bronner-Fraser (1986b) has shown that when antibodies against the neural crest cell receptors for fibronectin and laminin are injected into the heads of early chick embryos, the migration of the neural crest cells is severely disturbed. Thus, recognition of fibronectin and laminin may play some important role in neural crest cell migration.

If the migration of neural crest cells depends upon these extracellular matrix molecules, one might expect the concentrations or localizations of fibronectin or laminin to be different in the places where neural crest cells migrate. Loring and Erickson (1987) have proposed that such differences can be seen between the anterior portion of the somite (into which neural crest cells migrate) and the posterior portion of the somite (into which neural crest cells do not migrate). As we will see in the next chapter, the blocks of somite tissue break up and give rise to the inner sclerotome, which will form the cartilage of the spine, and the dermamyotome, which will generate muscles and the dermis. They found that the neural crest cells in the chick embryo enter the somite as the dermamyotome is forming. This formation occurs in the anterior portion of the somite before it occurs in the posterior portion, and by the time the posterior part of the dermamyotome is made, the neural crest cells have completed their migration through the anterior somite region. Moreover, the migrating cells appeared to adhere to the newly-formed extracellular coating of these cells, an envelope that is rich in fibronectin and laminin (Figure 36).

In this model of neural crest cell migration, the anterior portion of the somite is permitting (and perhaps directing) the migration of neural crest cells by providing them with a suitable substrate upon which to travel. Without this substrate, the cells should not be able to migrate. This conforms with data gathered by embryologists over 50 years ago. Lehmann (1927) demonstrated that the removal of a somite caused the corresponding loss of a sensory ganglion, and Detwiler (1937) showed that the addition of extra somites into an amphibian embryo resulted in the duplication of those ganglia. It is probable, then, that the migration of the neural crest cells is mediated by changes in the extracellular matrix proteins that constitute their embryonic environment.

Other factors besides fibronectin and laminin may also play important roles in determining the pathways of neural crest cell migration. One such factor is hyaluronic acid. Neural crest migration involves the formation of spaces into which the cells can migrate. It is thought that hyaluronic acid causes the formation of cell-free spaces, which can then be invaded by the crest cells (Pratt et al., 1975; Solursh et al., 1979). This model is similar to that proposed for the invasion of the avian blastocoel by mesodermal precursors, and Meier (1981) has found that hyaluronic acid does indeed accumulate in some regions where crest cells migrate.

In addition, the neural crest cells may alter the paths over which they travel, making it more difficult for other neural crest cells to use the same path. Weston and Butler (1966) showed that when older neural crest cells were placed into younger environments, the older cells were able to migrate into all the areas normally colonized by the crest cells. However, when younger crests were placed into older environments, most cells were restricted to the formation of dorsal root ganglia. This observation suggests that some alterations already had occurred in the environment over which these cells were to travel. Either the passage of the earlier neural crest cells modified the pathway, or further development of the somites may have obliterated the pathway.

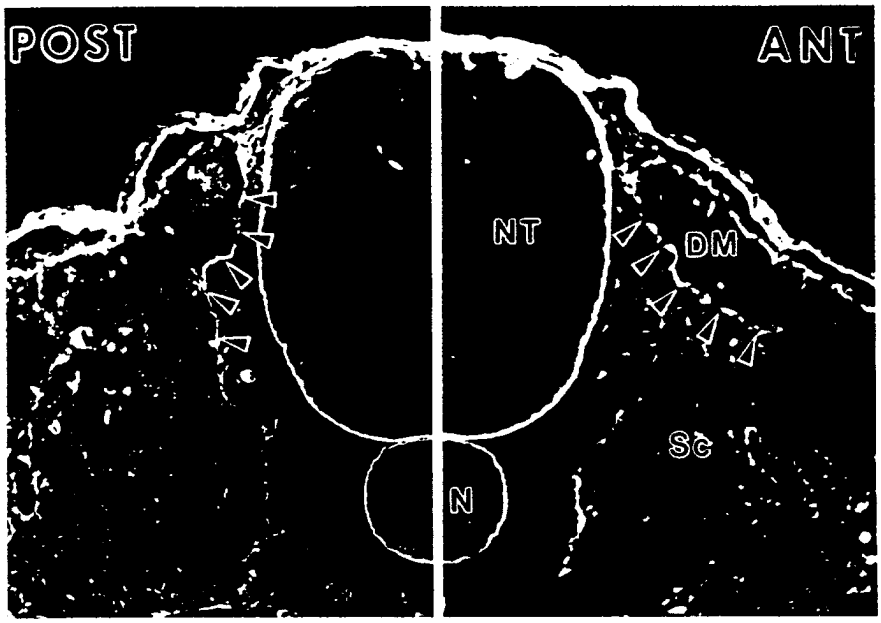


FIGURE 36
Differences between the anterior and posterior portions of the somite. Composite photograph of posterior (left) and anterior (right) regions through the same somite. The sections of the chick somite were made as the somite was splitting into sclerotome and dermamyotome. The microscopic sections were stained with fluorescent antibodies to laminin. In the anterior section, the laminin coating on these cells provides a pathway into the somite. In the posterior section, the split of the dermamyotome from the sclerotome is not complete, and the laminin does not provide a pathway into the somite. NT, neural tube; N, notochord; DM, dermamyotome; Sc, sclerotome. (Photograph courtesy of J. F. Loring.)

Pluripotentiality of neural crest cells

One of the most exciting features of neural crest cells is their PLURIPOTENTIALITY. A single neural crest cell can differentiate into different cell types depending upon its location within the embryo. For example, the parasympathetic neurons formed by the cervical neural crest cells produce acetylcholine as their neurotransmitter. They are therefore CHOLINERGIC neurons. The sympathetic neurons formed by the thoracic neural crest cells produce norepinephrine. They are ADRENERGIC neurons. But when cervical and thoracic neural crests are reciprocally transplanted, the former thoracic crest is found to produce the cholinergic neurons of the parasympathetic ganglia and the former cervical crest forms adrenergic neurons in the sympathetic ganglia (LeDouarin et al., 1975). Thus, the thoracic crest cells are capable of developing into cholinergic neurons when they are placed into the neck, and the cervical crest cells are capable of becoming adrenergic neurons when they are placed in the trunk. Kahn and co-workers (1980) found that premigratory neural crest cells from both the thoracic and the cervical regions had the enzymes for synthesizing both acetylcholine and norepinephrine. The pluripotentiality of some neural crest cells is such that regions of neural crest cells that never produce nerves in normal embryos can be made to do so under certain conditions. Mesencephalic neural crest cells normally migrate into the eye and interact with the pigmented retina to become scleral cartilage cells (Noden, 1978). However, if this

region of the neural crest is transplanted into the trunk region, it can form sensory ganglia neurons, adrenomedullary cells, glia, and Schwann cells (Schweizer et al., 1983).

It is possible that the selection of neurotransmitters occurs at two stages, which correspond to the functional divisions of the peripheral nervous system. The first separation is that of autonomic nervous system precursors (which form the sympathetic and parasympathetic neurons) from the sensory nervous system precursors (which form the spinal and cranial ganglia). LeDouarin (1986) proposed that each migrating neural crest cell has already become committed to develop into clones of either autonomic or sensory neurons. The environment into which these cells migrate selects the appropriate population by stimulating one of these cell types to divide. The sensory ganglia, for instance, develop in contact with the central nervous system, and it is possible that the central nervous system produces short-range factors that allow the selective survival of only sensory neural precursors. Indeed, proteins having such properties have been isolated (LeDouarin, 1986; Barde et al., 1982).

The final differentiation of transmitters is also determined in large part by the environment in which these cells develop; it does not involve cell death (Coulombe and Bronner-Fraser, 1987). Heart cells, for example, can secrete a factor (or factors) that can convert noradrenergic sympathetic neurons into cholinergic neurons without changing their survival or growth (Chun and Patterson, 1977; Fukada, 1980). Identification of these factors has been difficult, because these molecules appear to be present in very small quantities, and the assay takes 2-3 weeks to complete. However, Fukada (1985) has isolated from cultured heart cells a 45,000-d glycoprotein that induces the noradrenergic neurons of rat sympathetic ganglia to synthesize acetylcholine and form cholinergic synapses.

Anderson and Axel (1987) have also found that the hormonal milieu can direct the fate of a neural crest cell. The neural crest cells that migrate into the region destined to become the adrenal medulla can differentiate in two directions. If given nerve growth factor, such cells differentiate into sympathetic neurons. However, if given glucocorticoids like those made by the cortical cells of the adrenal gland, they differentiate into adrenal medullary (chromaffin) cells.

In addition to producing the peripheral neural structures, the neural crest is also responsible for the production of all the melanin-containing cells in the organism (with the exception of certain neural derivatives such as the pigmented retina). In a series of classic experiments, Rawles and others transplanted the neural tube and crest from a pigmented strain of chicken into the neural tube of an albino chick embryo. The result (Figure 31C) was a white chicken with a specific region of colored feathers. It should be noted that the production of norepinephrine and epinephrine (sympathetic neurons and adrenal medulla) and melanin (melanocytes) both involve the hydroxylation of tyrosine and the subsequent oxidation of the product.

From the preceding discussion, it would appear that all neural crest cells are originally identical in their potencies. This, however, is not always the case. For instance, only the cells of the cranial neural crest seem able to produce the cartilage of the head; the thoracic crest cells cannot substitute. Moreover, the cranial neural crest, when transplanted into the trunk region, will participate in forming trunk cartilage that normally does not arise from neural crest components. The study of the neural crest cells provides developmental biologists with a relatively

well defined set of cells with which to study the problems of cell migration and differentiation.

The epidermis and the origin of cutaneous structures

The cells covering the embryo after neurulation form the presumptive epidermis. Originally, this tissue is one cell layer thick, but in most vertebrates this shortly becomes a two-layered structure. The outer layer gives rise to the PERIDERM, a temporary covering that will be shed once the bottom layer differentiates to form a true epidermis. The inner layer, called the BASAL LAYER (STRATUM GERMINATIVUM), gives rise to all the cells of the epidermis (Figure 37). The stratum germinativum first divides to give rise to another, outer population of cells that constitutes the SPINOUS LAYER. These two epidermal layers are referred to as the MALPIGHIAN LAYER. The cells of the Malpighian layer divide to produce the GRANULAR LAYER of the epidermis, so called because the cells are characterized by granules of the protein KERATIN. Unlike the cells re-

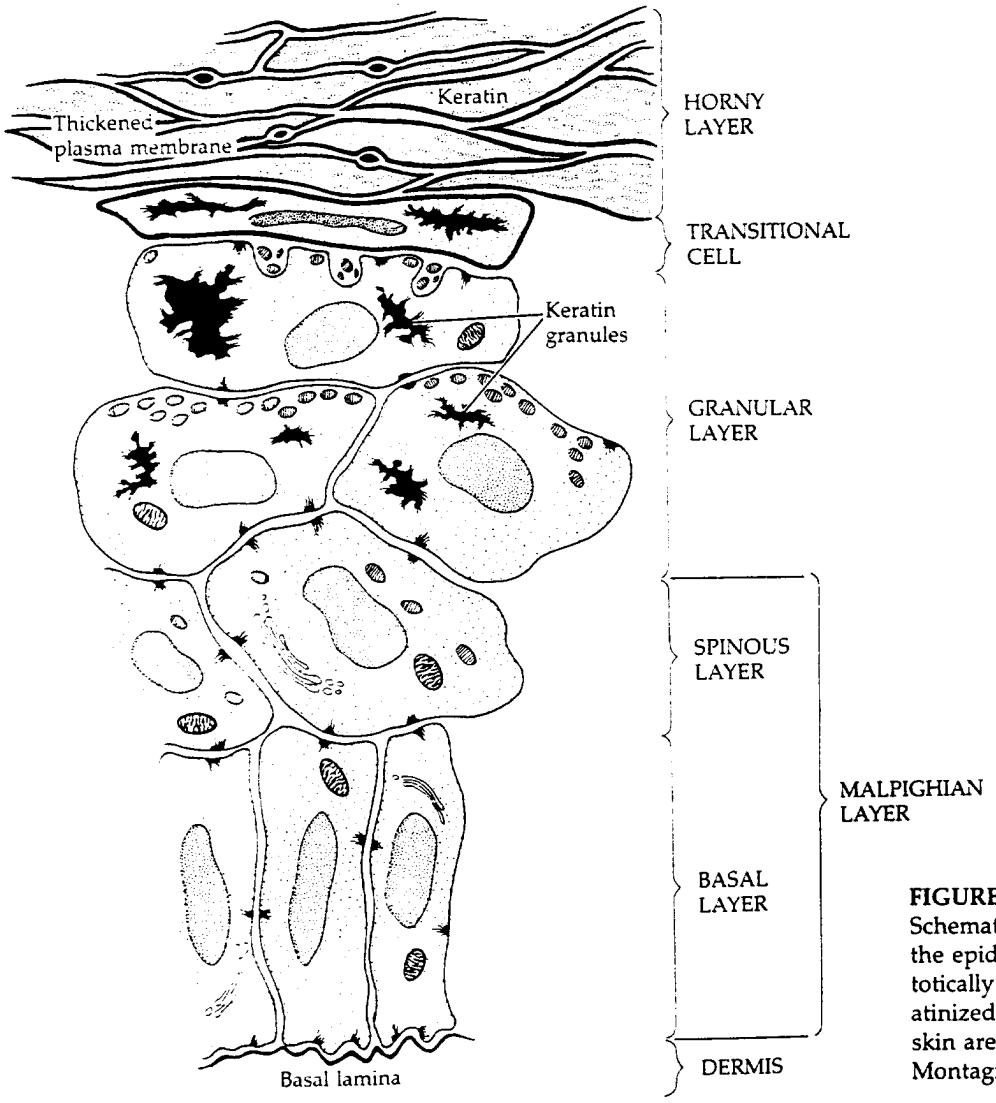


FIGURE 37
Schematic diagram of the layers of the epidermis. The basal cells are mitotically active, whereas the fully keratinized cells characteristic of external skin are dead and shed off. (After Montagna and Parakkal, 1974.)

maining in the Malpighian layer, the cells of the granular layer do not divide. Rather, they begin to differentiate into skin cells (KERATINOCYTES). The keratin granules become more prominent as the cells of the granular layer age and migrate outward. Here, they form the HORNY LAYER (STRATUM CORNEUM), in which the cells have become flattened sacs of keratin protein. The nuclei have been pushed to one edge of the cell. Shortly after birth, the cells of the horny layer are shed and are replaced by new cells coming up from the granular layer. Throughout life, the dead keratinized cells of the horny layer are being shed (we humans lose about 1.5 grams each day*) and are replaced by new cells, the source of which is the mitotic cells of the Malpighian layer. The pigment cells from the neural crest also reside in the Malpighian layer, where they transfer their pigment sacs (melanosomes) to the developing keratinocyte. In adult skin, a cell born in the Malpighian layer takes roughly 2 weeks to reach the stratum corneum. In individuals with psoriasis, a disease characterized by the exfoliation of enormous amounts of epidermal cells, the time required is only 2 days (Weinstein and van Scott, 1965; Halprin, 1972).

The epidermis alone does not make the skin. As we shall see in the next chapter, a region of mesoderm underlies the epidermis and constitutes the DERMIS of the skin. This layer consists of loose connective tissue embedded in an elastic glycoprotein matrix. The dermis and epidermis interact at specific sites to create the cutaneous appendages: hair, scales, or feathers (depending on the species), sweat glands, and apocrine glands.

The first indication that a hair follicle will form at a particular place is an aggregation of cells in the basal layer of the epidermis. This aggregation occurs at different times and different places in the embryo. The basal cells elongate and divide, sinking into the dermis and forming the hair follicle (Figure 38). At this stage, two epithelial swellings begin to grow. The cells of the upper bulge will form the SEBACEOUS GLANDS, which produce an oily secretion, SEBUM. In many mammals, including humans, the sebum mixes with the desquamated peridermal cells to form the whitish VERNIX CASEOSA, which surrounds the fetus at birth. The lower swelling forms the HAIR BUD, which is then split by upwelling mesenchyme. The germinal epidermis directly above this mesenchyme proliferates to form the keratinized shaft of hair.

The first hairs in the human embryo are of a thin, closely spaced type called LANUGO. This type of hair is usually shed before birth and is replaced (at least in part, by new follicles) by the short and silky VELLUS. Vellus remains on many parts of the human body usually considered hairless, such as the forehead and eyelids. In other areas of the body, vellus gives way to the "terminal" hair. During a person's life, some of the follicles that produced vellus can later form terminal hair, and still later revert to vellus production. The armpits of infants, for instance, have follicles producing vellus until adolescence. At that time, terminal shafts are generated. Conversely, in normal masculine "baldness," the scalp follicles revert back to producing unpigmented and very fine vellus (Montagna and Parakkal, 1974). The placement and pattern of hair, feathers, and scales involves the interactions of the dermis and the epidermis, and these will be discussed in more detail in Chapters 16 and 17.

The germinative cells of the epidermis also give rise to the sweat glands and (what are embryologically modified sweat glands) the mam-

*Most of this skin becomes "house dust" atop furniture and floors. Should you doubt this, burn some of the dust. It will smell just like singed skin.

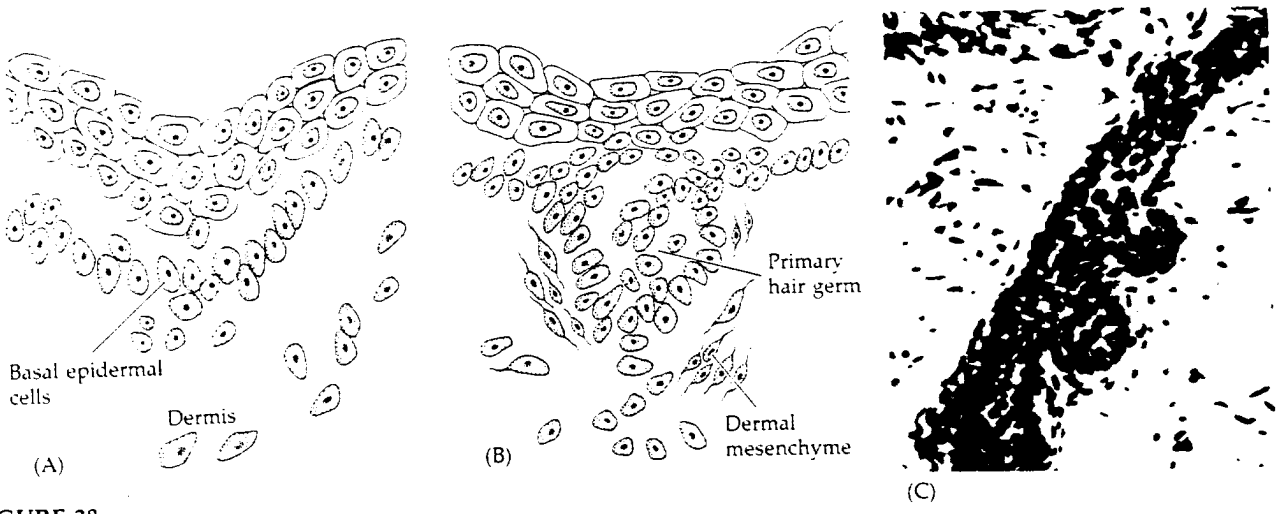


FIGURE 38
Development of the hair follicles in fetal human skin. (A) Basal epidermal cells become columnar and bulge slightly into the dermis. (B) Epidermal cells continue to proliferate, and dermal mesenchyme cells collect at the base of the primary hair germ. (C) Elongated hair germ. The uppermost bulge develops into an apocrine sweat gland, and the central bud forms the sebaceous gland. The lowest bulb differentiates into the hair bud. (Photograph courtesy of W. Montagna.)

mary glands. Like the formation of the hair bulb, these glands are formed by the ingrowth of the germinative basal epidermal cells in the dermis. The epithelial–mesenchymal interactions necessary for mammary gland development will be addressed in Chapter 19.

SIDELIGHTS & SPECULATIONS

Teratology

The development of an organism is a complex orchestration of cell divisions, cell migrations, cell interactions, gene regulation, and differentiation. Any agent interfering with these processes can cause malformation in the embryo. In fact, it is estimated that about half of the total number of human conceptions do not survive to be born. Most of these embryos express their abnormality so early that they fail to implant in the uterus. Others implant but fail to establish a successful pregnancy. Thus, most abnormal embryos are spontaneously aborted before the woman even knows she is pregnant (Boué et al., 1985). Hertig and Rock (1949) showed that in 34 embryos between 1 and 17 days old, 13 showed some recognizable anomaly. Nearly 90 percent of a sample of fetuses aborted before one month have developmental anomalies (Mikamo, 1970; Miller and Poland, 1970). Edmonds and coworkers (1982), using a sensitive immunological test that can detect the presence of human chorionic gonadotropin (hCG) 8 or 9 days after fertilization, monitored 112 pregnancies in normal women. Of these hCG-determined pregnancies, 67 showed no further evidence of pregnancy.

It appears, then, that many human embryos are impaired early in development and do not survive long *in utero*. Defects in the lungs, limbs, or mouth, however, would not be deleterious to the fetus (which does not depend on those organs while inside the mother), but can seriously threaten life once the baby is born. About 5 percent of all human births have a recognizable malformation, some of them benign, some very serious (McKeown, 1976).

The study of these congenital ("at birth") abnormalities is called TERATOLOGY, and agents responsible for causing these malformations are called TERATOGENS ("monster-formers"). Teratogens work during certain CRITICAL PERIODS. The most critical time for any organ is when it is growing and forming its particular structures. Different organs have different critical periods, although the time from day 15 to day 60 is critical for many organs. The heart forms primarily during weeks 3 and 4, while the external genitalia are most sensitive during weeks 8 and 9. The brain and skeleton are always sensitive, from the beginning of week 3 to the end of pregnancy and beyond.

There are many types of teratogens. One class are those agents that cause gene mutations. Ionizing radiation and certain drugs can break chromosomes and alter DNA structure. For this reason, pregnant women are told to avoid unnecessary X rays, even though there is no evidence for congenital anomalies resulting from diagnostic radiation (Holmes, 1979). Congenital conditions such as achondroplastic dwarfism (an autosomal dominant leading to shortened limbs and normal torso) or Robert syndrome (an autosomal recessive disease in which the infant has severe limb reduction, cleft palate, and severe mental retardation) are examples of single-gene (Mendelianly inherited) congenital malformations. Conditions such as Down syndrome (an extra copy of chromosome 21 causing mental retardation, heart defects, and the retention of certain fetal muscle patterns) and Klinefelter syndrome (an extra X chromosome in males, causing small testes, sterility, and mild mental retardation) involve extra chromosomes.

Another class of teratogens are viruses. Gregg (1941) first documented the fact that women who had rubella (German measles) during the first third of their pregnancy had a 1 in 6 chance of giving birth to an infant with eye cataracts, heart malformations, and deafness. This was the first evidence that the mother did not fully protect the fetus from the outside environment. The earlier the rubella infection occurred during the pregnancy, the greater the risk that the embryo would be malformed. The first 5 weeks appear to be the most critical, because this is when the heart, eyes, and ears are being formed. The rubella epidemic of 1963–1965 probably resulted in about 20,000 fetal deaths and 30,000 infants with birth defects. Two other viruses, cytomegalovirus and herpes simplex virus, are also teratogenic. Cytomegalovirus infection of early embryos is nearly always fatal, but infection of later embryos can lead to blindness, deafness, cerebral palsy, and mental retardation.

Microorganisms are rarely teratogenic, but two of them can damage human embryos. *Toxoplasma gondii*, a protozoan carried by rabbits and cats (and their feces), can cross the placenta and cause brain and eye defects in the fetus. *Treponema pallidum*, the cause of syphilis, can kill early fetuses and produce congenital deafness in older ones.

The fourth class of teratogens includes drugs and environmental chemicals. Some chemicals that are naturally found in the environment can cause birth defects. Even in the pristine alpine meadows of the Rocky Mountains, teratogens are found. Here grows the skunk cabbage *Veratrum californicum* upon which sheep can feed. If pregnant ewes eat this plant, their fetuses tend to develop severe neurological damage, including cyclopia, the fusion of two eyes in the center of the face (Figure 39). This condition also occurs in humans, pigs, and many other mammals; the affected organism dies shortly after birth (as a result of severe brain defects including the lack of a pituitary gland).

Quinine and alcohol, two substances derived from plants, can also cause congenital malformations. Quinine can cause deafness, and alcohol (greater than 2–3 ounces per day) can cause physical and mental retardation in the infant. Nicotine and caffeine have not been proved to cause congenital anomalies, but women who are heavy smokers (≥ 20 cigarettes a day) are more likely to have infants smaller than those born to women who do not smoke. Smoking also significantly lowers the number and motility of sperm in the semen of males who smoke at least 4 cigarettes a day (Kulikauskas et al., 1985).

FIGURE 39
Head of a cyclopic lamb born of a ewe who had eaten *Veratrum californicum* early in pregnancy. The cerebral hemispheres fused, forming only one eye and no pituitary gland. (From Binns et al., 1964; photograph courtesy of J. F. James and the USDA-ARS Poisonous Plant Laboratories.)

In addition, our industrial society produces hundreds of new artificial compounds that come into general use each year. Pesticides and organic mercury compounds have caused neurological and behavioral abnormalities in infants whose mothers have ingested them during pregnancy. (This was tragically seen in 1965 when a Japanese firm dumped its mercury into a lake, where it was ingested by the fish, which were eaten by pregnant women in the village of Minamata. The congenital brain damage and blindness in the resulting children became known as Minamata disease.)

In the past 5 years, physicians have seen the effects of a new teratogen, 13-CIS-RETINOIC ACID (RA). Retinoic acids are analogues of vitamin A and can mimic the vitamin's effects on epithelial differentiation but are less toxic than high doses of the vitamin itself. These analogues, including 13-*cis*-retinoic acid, have been useful in treating severe cystic acne and have been available (under the name Accutane) since 1982. Because the deleterious effects resulting from the administration of large amounts of vitamin A or its analogues to various species of pregnant animals have been known since the 1950s (Cohlan, 1953; Giroud and Martinet, 1959; Kochhar et al., 1984), the drug contains a label warning that it should not be used by pregnant

women. However, about 160,000 women of childbearing age (15–45 years of age) have taken this drug since it was introduced, and some of them have used it during pregnancy. Lammer and his co-workers (1985) have studied a group of women who had inadvertantly exposed themselves to the retinoic acid and who had elected to remain pregnant. Of the 59 fetuses, 26 were born without any noticeable malformations, 12 aborted spontaneously, and 21 were born with obvious malformations. The malformed infants had a characteristic pattern of anomalies including absent or defective ears, absent or small jaws, cleft palate, aortic arch abnormalities, thymic deficiencies, and abnormalities of the central nervous system.

This pattern of multiple congenital anomalies is similar to that seen in rat and mouse embryos whose pregnant mothers have been given these drugs. Thus, one has ANIMAL MODELS for the human disease. These animals can be experimented on in ways that enable researchers to uncover the mechanism of the teratogen. Goulding and Pratt (1986) have placed day 8 mouse embryos in a solution containing 13-*cis*-retinoic acid at very low concentrations (2×10^{-6} *M*). Even at this concentration, approximately one-third of the embryos developed a very specific pattern of anomalies, including dramatic reduction in the size of the first and second pharyngeal arches (Figure 40). The first arch eventually forms the maxilla and mandible of the jaw and two ossicles of the middle ear, while the second arch forms the third ossicle of the middle ear as well as other facial bones.

The basis for this phenomenon appears to reside in the drug's ability to inhibit neural crest cell migration from the cranial region of the neural tube. In mammalian embryos, the cranial neural crest cells migrate before the neural tube is closed (Tan and Morriss-Kay, 1985) and give rise to the facial mesenchyme cells

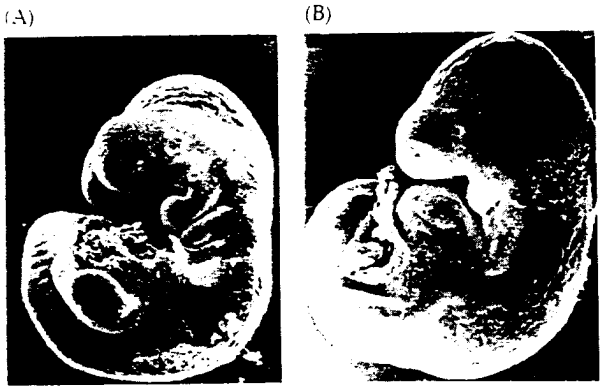


FIGURE 40
Scanning electron micrograph of mouse embryo cultured at day 8 for 48 hours in control medium (A) or in medium containing 2×10^{-6} *M* 13-*cis*-retinoic acid (B). The first pharyngeal arch of the treated embryo has a shortened and flattened appearance and has apparently fused with the second pharyngeal arch. (From Goulding and Pratt, 1986; photographs courtesy of the authors.)

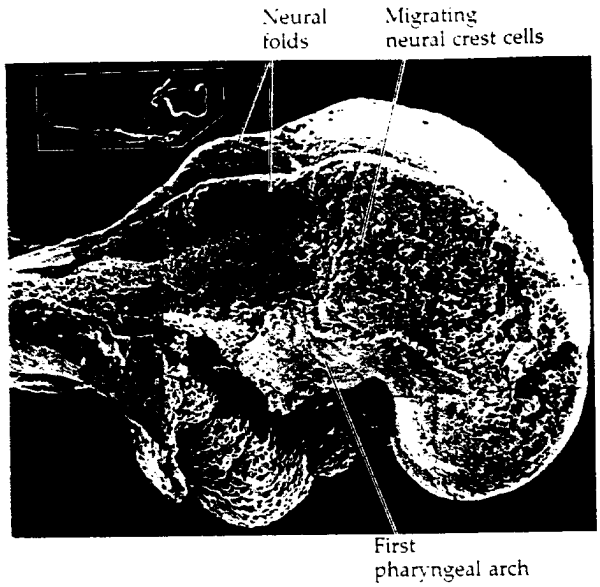


FIGURE 41
Neural crest cell migration in the head of the rat. Scanning electron micrograph of a rat embryo with part of the lateral ectoderm removed from the surface. Neural crest migration can be seen over the midbrain, and the column of neural crest cells migrating into the future first pharyngeal arch is evident. (From Tan and Morriss-Kay, 1985; courtesy of S.-S. Tan.)

(Johnston, 1975), many of which will give rise to cartilage. The crest cells originating in the forebrain and midbrain contribute to the nasal process, palate, and mesenchyme of the first pharyngeal arch (Figure 41). The crest cells originating in the anterior hindbrain region generate the mesenchyme of the second pharyngeal arch, and the cervical crest cells give rise to the mesenchyme of the third, fourth, and sixth pharyngeal arches. Radioactively labeled retinoic acid binds to the cranial neural crest cells and arrests both their proliferation and migration (Johnston et al., 1985; Goulding and Pratt, 1986). The binding seems to be specific to the cranial neural crest-derived cells, and the teratogenic effect of the drug is confined to a specific developmental period (days 8–10 in mice; days 20–35 in humans). The animal models of retinoic acid teratogenesis have been extremely successful at elucidating the mechanisms of teratogenesis at the cellular level. Present efforts are underway to determine the molecular effects of this drug on the cranial neural crest cells.

Before 1961 there was very little evidence for drug-induced malformations in humans. But in that year Lenz and McBride independently accumulated evidence that a mild sedative, THALIDOMIDE, caused the enormous increase in a previously rare syndrome of congenital anomalies. The most noticeable of these anomalies was PHOCOMELIA, a condition in which the long bones of the limbs are absent (amelia) or severely reduced (meromelia), thus causing the resulting ap-

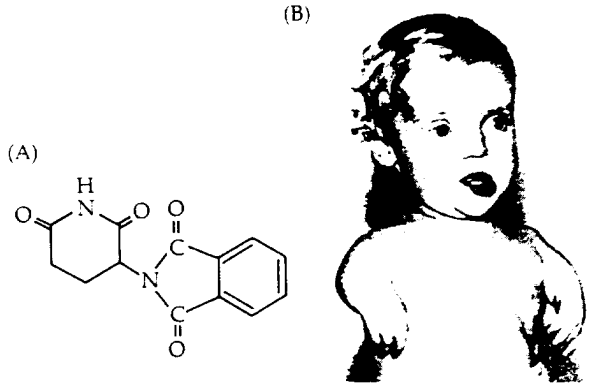


FIGURE 42
Thalidomide structure and effect. (A) Chemical structure of thalidomide. (B) Phocomelia in newborn whose mother had taken thalidomide during the first two months of pregnancy. (Photograph courtesy of W. Scott.)

pendage to resemble a seal flipper (Figure 42). Over 7000 affected infants were born to women who had taken this drug, and a woman need only to have taken one tablet to produce children with all four limbs deformed (Toms, 1962; Lenz, 1966). Other abnormalities induced by the ingestion of thalidomide included heart defects, absence of the external ears, and deformed intestines. The drug was withdrawn from the market in November, 1961.

Nowack (1965) documented the PERIOD OF SUSCEPTIBILITY during which thalidomide caused these abnormalities. The drug was found to be teratogenic only during days 34 to 50 after the last menstruation (about 20 to 36 days postconception). The specificity of thalidomide action is shown in Figure 43. From day 34 to day 38, no limb abnormalities are seen. During this period, thalidomide could cause the reduction or absence of ear components. Malformations of upper extremities were seen before those of the lower limbs since the arms form slightly before the legs during development.

The thalidomide tragedy shows the limits of animal models to test the potential teratogenic effects of drugs. Different species (and strains within species) metabolize thalidomide differently. Pregnant mice and rats, the animals usually used to test such compounds, do not generate malformed pups when given thalidomide. Rabbits produce some malformed offspring,

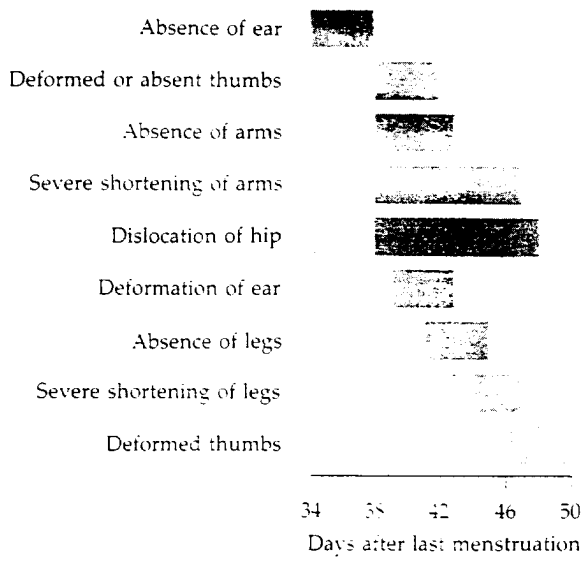
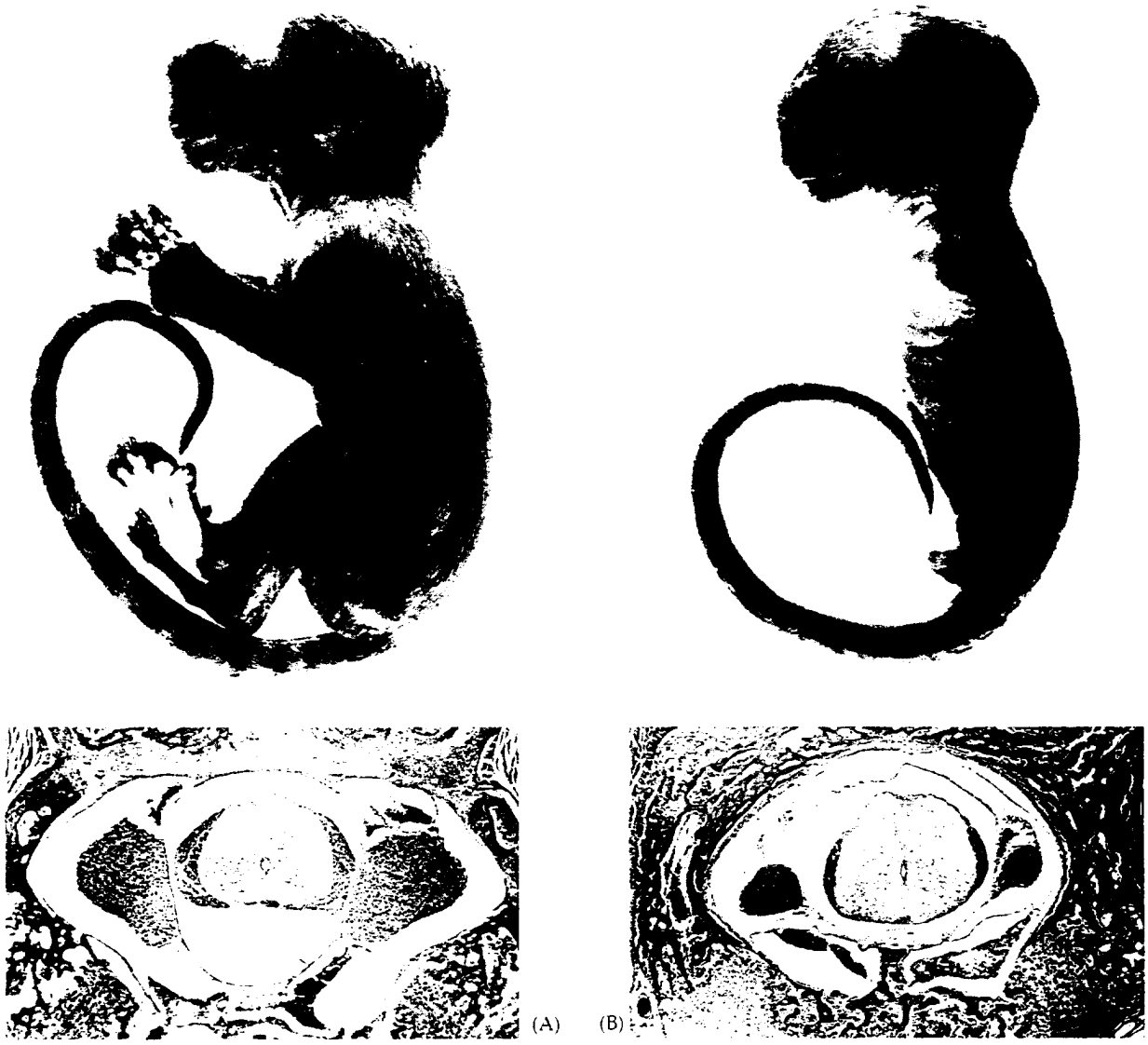


FIGURE 43
Timing of susceptibility to the teratogenic effects of thalidomide. (After Nowack, 1965.)

but the defects are different from those seen in affected human infants. Primates such as the marmoset appear to have a susceptibility similar to that of humans, and affected marmoset fetuses have been studied in an attempt to discover how thalidomide causes these malformations. McBride and Vardy (1983) report that the most noticeable difference seen before limb malformations concerns the size of the dorsal root ganglia and their neurons. The number of neurons in these ganglia are markedly reduced (Figure 44). These authors speculate that the neurons from these ganglia are necessary for maintaining limb development and that thalidomide works by interfering with or destroying these acetylcholine-producing neurons. The molecular mechanism of this selective thalidomide toxicity is still not known, but having an animal model to study should lead to more detailed knowledge.

There are over 50,000 artificial chemicals presently used in our society and about 200 to 500 new materials being made each year (Johnson, 1980). The problem of screening these chemicals is of major importance, and standard protocols are expensive, long, and subject to interspecies differences in metabolism. As yet, there is no consensus on how to test a substance's



teratogenicity for human embryos. Teratogenic compounds have always been with us. Certain metals and plant substances are known to cause severe developmental anomalies. However, as more and more new compounds are being produced and ingested by human populations, the fetus is placed at ever greater risk.

FIGURE 44
Thalidomide effects on fetal marmosets. Top figures show phenotypes of marmoset fetuses late in gestation. Bottom figures show spinal cord cross sections at the level of the forelimbs. (A) Fetus of a control marmoset. (B) Fetus of a marmoset treated with 25 mg kg thalidomide between days 38 and 46 of pregnancy. (From McBride and Vardy, 1983; photographs courtesy of W. G. McBride.)

SUMMARY

In this chapter we have followed the differentiation of the embryonic ectoderm into a wide variety of tissues. We have seen, then, that the ectoderm produces three sets of cells during neurulation: (1) the neural tube, which gives rise to the neurons, glia, and ependymal cells of the central nervous system; (2) the neural crest cells, which give rise to the peripheral nervous system, pigment cells, adrenal medulla, and certain areas of head cartilage; and (3) the epidermis of the skin, which contributes to the formation of cutaneous structures such as hair, feathers, scales, and sweat and sebaceous glands, as well as forming the outer protective covering of our bodies. We also observed how the interactions of epidermal cells are involved in generating the various tissues of the eye.

In later chapters we shall discuss in more detail the induction of the neural tube, the coordinated development of the eye, and the manner in which the neurons are directed to travel to specific sites, thus enabling the development of reflexes and behaviors. Meanwhile, we shall see how the endoderm and the mesoderm layers initiate the formation of their organ systems.

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