Some Aspects of Developmental Neurology: A Review*

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The increasing interest in the ontogenetic and genetic aspects of abnormal development and neoplasia in the nervous system makes a presentation of some aspects of neuroembryology timely. Seemingly unrelated medical problems of growth are coming more and more to have a common meeting ground in basic fields of developmental biology. In medical practice, for example, a newborn anencephalic monster, an idiot child who excretes phenylpyruvic acid in its urine, a young adult with a retinoblastoma, and a victim of an hereditary ataxia might seem to have nothing in common but simply to be the result of "environmental accidents." Actually, these are no accidents but represent specific problems in development, with a promise of real answers. These particular abnormalities have a certain inevitability now, because their origin can be traced back to the primary genetic material in the germ cells; but progress is being made toward a better understanding of the steps that lie between a peculiar chromosome configuration and such deviations from normal ontogeny. When those steps are understood, prevention may be possible.

The study of how the nervous system may develop abnormally must be based on a knowledge of its normal embryology, for what we call abnormalities are really variations or deviations from normal or average pathways of development. A primary goal in any such study is to learn how the nervous system functions and what things disturb it. The foundation for this is how it was put together in the first place, since morphogenesis determines the structural basis of function. The morphogenesis of the nervous system is complicated, and it cannot, unfortunately, be reduced to only a few simple laws based on physical chemistry or enzyme action. Actually, there is a great deal more to the problem. Weiss (1950) has emphasized this by saying that "The complex engineering performances of technology are a much more pertinent model of the nature of morphogenesis than are the more elementary phenomena dealt with in basic physics and chemistry. . . . As we now view it, development is an assembly-line process in which countless component events are brought together in orderly patterns in regular succession and are interwoven with one another by innumerable specific interactions."

What are some of these engineering performances, and what lies behind them? In the present state of our knowledge, with its numerous gaps, we are still at the stage of studying the component events and describing the parts that go into what we see as morphogenesis. We are a long way from Weiss' ideal of understanding the assembly process as a whole, but a number of areas have been of especial interest to experimental embryologists, biochemists, anatomists, and geneticists. Some of these may be enumerated. One concerns the problem of how the nervous system makes its first appearance—the so-called induction of the nervous system. The means by which one group of cells "induces" another group to differentiate is a major problem, not only in the inception of the neural plate but as a basic phenomenon throughout embryogenesis. Another area is the study of the mechanics by which cells that make up the nervous system originate, differentiate, migrate, aggregate, and interconnect to form the complex functioning organization that we know as the nervous system. During ontogeny each neuron acquires a biochemical submicroscopic specificity that makes almost every nerve cell different from every other, and the relation of this to integrated behavior has been much studied. How the genic material at the beginning of development translates itself into the specific form and function of a mature organism is receiving increased attention from multiple disciplines. The impact of embryology has been more superficial in oncology, but some interrelations have attracted interest.

This review will tell about some of the work going on in the areas just enumerated, and emphasis will be on early morphogenesis. This can perhaps be done best by first refreshing the reader's mind about how animals with backbones develop, then going on into the subjects of neural induction, how the components of the nervous system are put together, the early development of functional specific-

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Verterbrate Development

The earliest vertebrates perhaps had simply an elementary receptor-effector neural axis. During evolution there was added a variety of increasingly complex associative and information-storing devices that integrated and modified neural function in adaptation to surroundings. Despite the great array and different complexities of present-day vertebrate nervous systems, there are common features in their development (9, 12, 18, 48, 49, 54, 55, 65, 66, 72).

Following fertilization (or parthenogenesis) and various patterns of cleavage, the vertebrate cell mass comes to a stage called a blastula in which the beginnings of what we arbitrarily call an embryo first appear (63, 67, 84, 95, 104, 115). A process called gastrulation (“forming a little stomach”) occurs in which relatively undifferentiated cells actively migrate and stream into certain relative positions that give rise to a “layered” early embryo. The amphibian blastula is a hollow sphere of cells with a thin roof and a thick yolk-cell floor. An indentation on the upper surface appears, invaginates, and outer cells stream toward and into this opening (blasto pore) to underlie cells on the outside and form by their relative positions the so-called germ layers. The relations are so rapidly changing that in point of time ectoderm, endoderm, and mesoderm are very arbitrary terms applied to the layers. In the bird the blastula forms a disc, instead of a vesicle, on a sea of yolk, and during gastrulation cells stream toward and into a groove in the primitive streak, a central concentration of cells in the disc. The rat and man, because of placentation, are somewhat more complex. The hollow blastula of the rat develops a cell mass at one pole, the outer part of which becomes ectoplacenta. The inner part of the cell mass forms a hollow cylinder with an inner ectoderm and an outer entoderm. The embryo proper then develops as a focal thickening of the ectoderm called a primitive streak. Between the entoderm and the thin blastula vesicle wall is a space, the yolk sac, sometimes containing maternal erythrocytes. It is assumed that the layered embryo that follows results from gastrulation analogous to that in the simpler vertebrates. Man develops through these stages somewhat similarly (115), the inner cell mass becoming an embryonic disc with an epiblastic and a hypoblastic layer. Above this is the amniotic sac, below the yolk sac. Mesoderm forms at the disc rim and gradually forms a middle “germ layer” by the time that a primitive streak forms. A mesodermal node forms in the streak and apparently by gastrulation forms the prechordal and chordal mesoderm whose importance will be discussed. The lateral mesoderm is not believed to flow into the streak to become redistributed by gastrulation.

These vertebrates have arrived by different paths at a stage where mesoderm underlies ectoderm. The mesoderm is essential to the transformation, or induction, of the ectoderm into neurectoderm, which forms the nervous system. With mammals used now as examples of further development, the transformed central ectoderm, now a neural plate, broadens anteriorly to be shaped like a long blunt triangle. Caudally the plate lengthens but is narrower. The anterior brain-to-be grows laterally, the edges curl up and inward, and the caudal spinal cord-to-be does the same on a smaller scale. Both close medially to form the neural tube, enlarged anteriorly. By the time the lateral folds have become well developed the anterior end is bent ventrally over the early heart and gut structures, and the optic pits are recognizable ventrolaterally. At about the 20-somite stage in the rat and at about the same in man, cerebral vesicles are becoming evident as bulges of the anterior neural tube, and the neuraxis may be arbitrarily divided into forebrain, in-between-brain (diencephalon), midbrain, hindbrain, and spinal cord. Subsequent growth involves a complex sequence of gross flexures and disproportionate growths. The cerebral vesicles with their ventrolateral striatal masses bulging into the ventricles increase greatly in size. The thalamic structures develop medially in the diencephalic region, and the cerebellum arises from the anterior part of the rhomboid flexure of the medulla still later on (see Chart 1).

At the cellular level, the early nervous system derives from the primitive neural plate, a layer of proliferating cells. Similar cells later line the neural tube and are called neur ectoderm, germinal layer, or ependymal layer. Their derivatives are the precursors of neurons and glia, and these accumulate at first just outside the lining to form a “mantle.” By varying processes of proliferation, migration, and grouping, these germinal and differentiating cells form the future nuclei, cell layers, and zones of the gray matter. The neurites of the nerve cells form the interconnections, the future fiber systems and neuropil within the central nervous system, and the nerves that communicate between the
central nervous system and peripheral tissues. The vascular system develops according to precise patterns related more to the apparent local needs of each region than any over-all gradient pattern (30, 35). It is the sum of these processes that delineates the developmental anatomy of the vertebrate nervous system. Some details of them will be given later.

Two other sources of cells contribute to the peripheral nervous system and related structures. The neural crest (62) appears as a transformation of the ectoderm along the lateral margin of the neural plate at its junction with the prospective epidermis and lies as a strip of cells between the central nervous system and the epidermis when the neural tube has formed. From it are derived spinal and autonomic ganglion neurons, pigment cells (melanocytes), nerve sheath cells, leptomeninges, much of the connective tissue of the head and some of the trunk, and certain peripheral neural organs such as the adrenal medulla and chromaffin system. In some lower vertebrates it forms cartilage. Still other focal transformations, called placodes, occur as thickenings in the lateral ectoderm regionally associated with the cranial part of the neuraxis. They are very much like focal “neural crests,” and contribute to parts of sense organs (olfactory epithelium, ear, and lens) and, with neural crest cells, to ganglia and possibly connective tissue.

**Induction of the Nervous System**

It is generally agreed that in normal amphibian development the mesoderm forming the roof of the archenteron (primitive gut) of the gastrula is responsible for the transformation of the overlying ectoderm into the neural plate. (Reviews: Holtfreter and Hamburger [59], Spemann [90], and references below). A similar mechanism is probably at work in other vertebrates, including birds and mammals (38, 39, 41, 53, 67, 84, 85, 115). Precisely how the mesoderm (the “organizer”) does this is not known, but the problem has stimulated a tremendous number and variety of experiments, including extirpations, biochemical and immunological studies, transplantations and culturing of various parts of the embryo. Some of these illustrate the problem. It may be said summarily at the outset that the anterior or prechordal part of the mesoderm, the roof of the archenteron or primitive gut in amphibia, “induces” anterior parts of the brain, while caudal parts of the mesoderm (future notochord) induce brain stem and spinal cord. The mesoderm is also apparently responsible for the differentiation of the neural crest and placodes. Whether this early induction simply involves the laying down of two general regions of the neuraxis or whether it is responsible for a very complex mosaic of the future nervous system will be considered later.

Speculations concerning the transformation of the ectoderm into neurectoderm have ranged from considerations of cell radiations to specific chemical inductors. Experimenters have tried almost everything under the sun from organic substances to extracts of embryonic or adult tissues. It is true that most of these will induce prospective neural plate to become some kind of neural structures, but it is becoming increasingly evident that this is different from producing a complete “individuated” nervous system (13, 104). Holtfreter (56, 58) concluded that a common denominator in all these experiments with artificial inductors was damage to cell surfaces and that breakdown products of this cellular disintegration acted as artificial inductors.

Induction is a trigger reaction in which cells having differentiated as far as ectoderm are steered into one of the remaining paths of further differentiation still possible for them. Experimentally, it can be shown that just before neuralization the ectoderm has the “competence” to become epidermis, neurectoderm, or mesodermal tissue, although its normal “prospective” path is toward neurectoderm. It may be considered to have a limited number of chromosome-controlled metabolic pathways open to it, and induction is the process by which the mesoderm activates or inhibits these potentials (Waddington [104]). Once the cell has differentiated to neurectoderm, it is irrevocably limited to the pathways of differentiation open to neurectoderm.

**Molecular and biochemical aspects.**—Weiss (110)
has put forward a hypothesis involving cellular molecular ecology that is helpful in thinking about transformations in cells. It applies not only to early neural induction but to thinking about how almost any cell influences the future differentiation or transformation of another cell. He says: “Let us suppose that the surface of the ‘inducing’ substratum is saturated with a certain species A of polarized molecules of such configuration as to match precisely one single component of the molecular populations a, b, c, d, etc., of the overlying ectoderm cells. Due to their complementary shapes, these two types, A and a, would form strong unions. Thus, given a certain degree of mobility of the cell content, all of the a units will gradually be trapped along the surface exposed to A, just as a film of antigen traps antibody molecules. Faced with a different substratum, containing key molecules B complementary to b, the same ectoderm cell would become covered with a b layer, furnished again from its own stock, and thus become turned into a wholly different course of differentiation.

“Progressive determination would occur through a succession of such steps. A given contact situation would bring a certain key species to the surface. Its residence there would affect the chemical processes in the interior, entailing presumably further regrouping along internal interfaces, setting off a chain of effects which will reach the nucleus and chromosomes, whose reactions, in turn, will rebound on the chemical composition of the cytoplasmic population. As a result, new compounds will arise, and when the cell is later faced with a new contact substratum this may attract some of these new species, initiating the next phase of differentiation, and so forth. At any one stage, the cell will thus have only a limited assortment of specific key species, and its reaction to ‘inductive’ surface contact will therefore vary with time. This is the molecular version of what is usually referred to as the development of responsiveness, or ‘competence,’ in embryonic cells.”

Waddington (104), on the other hand, conceives of the passage of evocating or inducing substances into the cells and their more direct action on the surfaces of microsomes rather than on the outer cell surface as in Weiss’ model. In support of this idea is the increase in number of microsomes during induction in cells concerned, and the fact that microsomes are nucleoprotein in nature links them to protein synthesis, a characteristic aspect of differentiation (Brachet [13]).

Details about measured biochemical processes involved in neural induction are relatively scant, despite careful investigation. A large array of chemical measurements of embryos as a whole and of their parts, however, is available. (Reviews: [11, 13, 80, 97, 103]). In amphibians, the mesodermal and ectodermal cells involved in the inductive process rapidly acquire considerable histochemically measurable cytoplasmic glycogen, and intense glycolysis that can proceed anaerobically is demonstrable in the mesodermal region. The presumptive nervous system requires considerable oxygen, in contrast. Between the presumptive neural plate and the mesoderm there is intensification of sulfhydryl ribonucleoproteins, and these substances then increase in both the neural plate and inducing mesoderm.

These studies indicate some possible chemical mechanics that drive and implement protein synthesis during inductive transformation, but much remains to be learned about how the proteins are made. Indeed, there is some question whether early embryos make up many of their proteins from simple building blocks at all. Schectman (87, 88) advances the concept, factually supported, that many of the embryo’s proteins (measured as antigens) are originally maternal in origin. Macromolecules of a wide variety are constantly obtained by the early embryo from the original maternal source, whether it is from yolk cells or through the placenta. That the embryo is dependent on maternal sources for normal development is suggested by the failure of early mammalian embryos to survive and grow in artificial surroundings unless they include adult tissues and serum. Among other functions, the maternal molecules might act as (environmental) templates in conjunction with the embryo’s own genetic machinery to manufacture like or complementary substances, or partly dismembered molecules might also act as substrates to stimulate enzyme formation.

There are many changing patterns of antigen make-up during early embryogenesis that are temporarily linked to morphologic differentiations. A well studied example, though it does not directly concern neurogenesis, has been presented by Ebert (28–30), who showed by immunologic techniques that myosin appears in the chick just after invagination of the mesoderm into the primitive streak. It becomes widespread in the presumptive mesoderm, then localized more and more into the anlagen of the heart as that organ shows signs of developing. The mechanism of the relative disappearance of the myosin has not been explained yet, but a number of antigens may come and go in early embryos, some related and some not seemingly related to adult ones. Early embryonic brain contains some antigens common to later stages but not to adult brain. As Schectman points out, it is
presently hard to know which proteins are the products of differentiation and which ones are involved in the differentiation process.

**Morphology.**—The actual morphologic aspects of induction of the nervous system have been much studied. Dalcq (21) cut amphibian embryos "in half" at gastrula stages, rotated them 180°, and reapposed the halves, whereupon two nervous systems formed. By this procedure he was able to deduce what part, how much, and how long the mesoderm was able to influence the ectoderm in each half. The strongest evocator of neural tissue was the presumptive prechordal material, and its strength fell off caudally and laterally from its antero-medial position. Normally this gradient produced the forebrain anteriorly, the brainstem and cord caudally, and the neural crest and placodes laterally. Experimentally, maximal induction produced forebrain-like structures, the weakest effect resulted in melanocytes (neural crest derivative). Dalcq held that one inductor was responsible.

Nieuwkoop (81) showed that the time and intensity of the inductive influence were normally a result of the gastrulation movements of invagination at the blastopore. Prechordal mesodermal cells invaginated first, passing forward from behind under the whole prospective neural area, exerting a maximum neuralizing effect. However, he concluded that the chorda-mesoderm cells that followed inhibited the process, forming a gradient of neuralization strongest anteriorly and falling off laterally and caudally. Eyal-Giladi (32) transplanted prechordal mesodermal tissues to other parts of the embryo, and from what did and did not develop in the transplant interpreted what influence the underlying tissue had had on it. The duration of contact during morphogenetic movements seemed to be a prime factor, as in Nieuwkoop's work, but the conclusion was that two inductors acted. Holtfreter (58) believed that capacities in the anterior inductive region to produce both neural and mesodermal tissues were based on qualitatively different substances. Toivonen (102) and Waddington (104) proposed that the inductors are the result of distinct permeating substances and that one may be a pure primary neural inductor and the other may induce both spinal cord and associated mesodermal structures.

**Implications.**—Why is this problem of the induction of the nervous system important? It is the time when the basic plan of the whole future nervous system is first laid out, and the minutest deviations in the process can be magnified into the grossest malformations. The bilaterality of the brain and the eyes (2), the gradient from forebrain to spinal cord, the relative proportions and relations of the major divisions of the brain, and the formation of the anlagen of components of the peripheral nervous system are determined here. Severe deformities involving the primordia of the neuraxis that lead to death of the embryo are relatively common in teratology, and in at least a few instances the earliest neural inductive processes for various reasons seem to be abnormal (38, 39).

**How Does the Nervous System Evolve?**

With the establishment of the neural plate, the next problem is: how does a whole, complicated nervous system arise from it? Virtually all the work on the induction of the nervous system assumes that the central neuraxis is divided into a front, middle, and back part, the archencephalic and deuterencephalic divisions tapering to the spinal cord. Like the germ layers, these parts describe the embryo at a certain time and reveal little about the adult. The popular division of the neuraxis into several vesicles in the early neural tube stages is similarly descriptive.1 To look deeper into this, a good deal of work has centered on the study of the proliferation and migration centers in the embryo neural tube that form a mosaic of the future brain and spinal cord, the mechanics of these morphogenetic processes, and factors that influence the development and organization of nerve cell fibers centrally and peripherally. Some of these may be presented.

**Mosaic of the future nervous system.**—It has been known for a century and a quarter that the nervous system shows segments or "neuromeres," and studies of the mosaic of the proliferation centers and migrating cells that generate the brain and spinal cord have engaged the attention of many investigators, including Bartelmez (4), Holmgren (54, 55), Adelman (1), Coghill (17), Streeter (99), Herrick (48, 49), Tilney (101), Bergquist (6), and Rudebeck (83). Wenger (113) has demonstrated in the early chick spinal cord the mosaic of its future neural constituents, showing that cell groups have specific origins in the neural tube analogous to those in the brain. Bergquist and Killén (see several joint and single references to Bergquist or Killén) have carried this approach much further on a comparative basis than have other investigators. They have sought a common basic pattern of proliferating areas in the vertebrate embryonic brain that would account for

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1 The corresponding topographical division of the vertebrate central nervous system into forebrain (telencephalon), in-between-brain (diencephalon), midbrain (mesencephalon), hindbrain (medulla or metencephalon), and spinal cord is certainly useful, although arbitrary.
both similarities and differences in the ultimate adult structures of various species. They note that the many vertebrates show a sequence of proliferations in the germinal layer of the early neuraxis that suggests a series of inductions. The first wave of activity produces a series of six slight bulges, or proneuromeres, faintly evident in the whole early neural tube (about 10 somites in a mouse). These are short-lived but are followed by a second wave of more bulges, called neuromeres, which reach a total of 21 at around 20 somites, and a third pattern of proliferation centers, the migration areas, follows on these. The migration areas are visible only in the brain and brain stem and form a complex pattern which gives the appearance in a reconstructed embryo of the early cerebral vesicle stage of a network spread over the outer surface of the neuraxis. The net is represented by very faint fissures, the net spaces by faint bulges. The migration areas give rise to one or more packages of migrating cells that form specific nuclei, layers, and masses of neural cells that later compose the adult brain.

The pattern of the migration areas (Chart 1) in the lining of the neural tube is formulated as a series of transverse bands of cellular proliferation intersected by four longitudinal columns of proliferating cell centers. The longitudinal columns correspond to the classic dorsal somatosensory, dorsolateral viscerosensory, ventrolateral visceromotor, and ventral somatomotor columns. The dorsal column extends only to the isthmus fold, the dorsolateral as far as the forebrain, the ventrolateral to the diencephalon, and the ventral to the midbrain. There are thirteen transverse bands (also called postneuromeres). The intersections of the first seven of these with the dorsolateral and ventrolateral columns, for example, compose the migrating centers for the future forebrain, diencephalon, and midbrain. These are given zonal names usually arbitrarily related to future adult regions. The divisions of the medulla-to-be are numbered in segments corresponding to a transverse band and a longitudinal column. Chart 1 incorporates a free sketch based on some of Källén’s and Bergquist’s reconstructions to give some idea of the network of migration areas.

Homologization on an ontogenetic basis is difficult, especially when biologists disagree on what homology means (deBeer [83], Szarski [100], and Herrick [48]). Källén and Bergquist attempted it by tracing the adult structure back to migration and proliferation centers and especially by tracing the migration centers forward to whatever they are to produce in a given vertebrate. Some examples may be given, even though word pictures without photographs are hard to convey (68, 69). The forebrain in the early cerebral vesicle stage is divided into a roof or dorsal telencephalic migration area, which gives rise to the future pallium or cerebral mantle. From the floor or ventral telencephalic migration zone evolve certain future basal nuclear or subpallial structures. The latter shows three (in some species two) longitudinally disposed columnar migration zones which are fused caudally in the prospective preoptic region. Rostrally they are labeled a, b, and c lateralward. With some slight exceptions they seem on a comparative basis to be homologous among vertebrates and give rise to similar olfactory and striate nuclei. Column c develops two successive migrations (e^I; and e^II) to form a number of subpallial structures, and these masses of migrating cells then divide themselves further into internal and external components. In mammals the first migration of cells, e, moves out from the neurectoderm to form an external and an internal part. The external part (e^I external) forms the nucleus of the lateral olfactory tract anteriorly and the cortical amygdaloid nucleus behind. The internal portion (e^I internal) becomes rostrodorsally the globus pallidus, and caudoventrally the central amygdaloid nucleus and anterior amygdaloid mass. The second migration occurs and gives rise to an outer part, the putamen, and an inner part, the rostral caudate nucleus. How a second migration of cells can overtake an earlier group of cells and come to lie lateral to it can be visualized when it is realized that the forebrain is changing shape during this growth process. For example, the proliferating ventricular surface c was vertical when it gave rise to e, but by the time it gave rise to group e^I it was lying horizontal and had also been pushed forward. Thus, the derivatives of e^I were in part brought forward and relatively lateral to some of the e^I regions which are displaced caudally. Different vertebrates, of course, differ considerably in the degrees and proportions in which the various migration centers develop. Comparing reptiles with mammals in respect to some derivatives of e, the nuclei of the lateral olfactory tract region are approximately homologous; the large-celled part of the reptilian ventrolateral area corresponds to the globus pallidus and ganglion of Meynert, while the nuclear centralis corresponds to the mammalian central and cortical nuclei. Part of the ventrolateral area and intermediolateral areas of the reptilian subpallium are derivatives of e^II, but as composite adult structures they are not regarded as homologs of the caudate nucleus and putamen of the mammal.

Such comparative studies reveal that there is an extensive mosaic of the future brain laid out
very early in embryonic life and that many of the divergent patterns of development of vertebrate brains have a common meeting ground in these beginnings. Here are some of the first visible morphogenetic expressions of the genetic processes that determine whether the brain will fit an alligator, a man, or whether it will function harmoniously at all.

Tilney (101) compared the morphogenesis of the cerebral cortex of the opossum, rat, cat, and man, and noted its relations to simpler vertebrate brains. The neocortex of these mammals with its six laminations derives from three successive migrations from the germinal layer of certain regions of the cerebral vesicles. The parts of the vesicle that form the base of the olfactory bulb (paleocortex) and the lower part of the mesial walls that arch over the thalamus (archicortex) continuous with the olfactory bulb do not have these complex migrations but mimic the ontogeny of simpler vertebrates, like reptiles. The future septum, mesially, shows no layers. The neocortex is begun when the first migration of cells forms a lamina in the middle lateral vesicle wall. Certain tract beds, especially thalamo-cortical radiations, become prominent as a result of the appearance of numerous spindle spongioblasts that outline where the fiber tracts will be. The first migratory cortical lamina thickens meanwhile and becomes tri-layered corresponding to the zonal, external granule and external pyramidal layers of the cortex. A second migration now brings a new fourth layer under these, the future internal granule layer. Then, during the early pouch stage of the opossum, last fetal days of the rat and cat, and in midgestation in man, the third migration moves out to form the internal pyramidal and multiform layers, completing the six. The corpus callosum tract bed cells (absent in the opossum) first grow down mesially under the cortex from the frontal and parietal regions in front and above the foramen Monroi. They then cross in the midline through the thin mesial archicortex inducing the latter to form the induseum griseum above, hippocampus behind, and subcallosal preseptal area in front.

Cell mechanics in morphogenesis.—The previous sections dealt with some aspects of organization of cell aggregates. Do we know anything about the individual cells and how they behave normally and under abnormal and experimental circumstances? The cells of the early brain and spinal cord up to the closing of the tube are still rather flexible in their prospective capacities for development. Transplanting one part of the early neuraxis into another region, if done early enough, results in incorporation of the “foreign” part in the region in which it is transplanted while restitution occurs in the defect. Normal development or a good facsimile of it results. The loss of a substantial part, or large numbers of primitive cells, of the early neuraxis in amphibia (26, 27, 47), birds (46), and mammals (50, 53) can in many circumstances be restituted from remaining proliferative sources, provided certain inductive processes are not also interfered with. Thus, the early mosaic we have been speaking of is prospective and has a potential fluidity as to what it can do in abnormal circumstances. As development proceeds the fluidity is gradually lost in a generally head-to-tail sequence. If regeneration or capacity to “remodel” were purely an experimental problem, the matter would have little practical medical importance. The fact that regeneration and restitution can in some circumstances occur to a remarkable degree after injury even in mammals means that destructive injury does not always result in malformation.

The source of neural tube building blocks, the germinal layer, appears to be one or two to a few cells thick, and mitoses are relatively uncommon in the mantle and peripheral differentiating cell populations. With colchicine Watterson has shown in the chick that this layer of potentially mitotic cells is considerably thicker than usually supposed (Watterson [105]; see also Sauer [86]). When Detweiler (27) extirpated half a neural fold of an amphian embryo, he attributed the restitution of the fold to both germinal cells and cells not usually considered to be germinal. Burr (15, 16) in similar experiments concluded contrariwise that regeneration came from the conventional neurectoderm. This seems to be the principal source of regenerating cells as far as has been determined in mammals (53). Notwithstanding, it seems reasonable that primitive cells that would not usually divide again might do so in unusual circumstances.

From the beginning, the nervous system shows tendencies to regionally unequal growth, such as the larger cephalic end, neuromery, various bends, and variations in cell distributions. Many things besides simple proliferation enter into this. In a meticulous study of the patterns of mitotic activity in the chick embryo spinal cord from 2½ to 8½ days, Hamburger (42-44) and Levi-Montalcini (76) and Hamburger and Levi-Montalcini (43) found that the determination of mitotic activity was governed by several interacting factors. How complicated and sometimes unexpected these can be is illustrated by some of their findings. One might have expected greater mitotic activity in the spinal cord at the levels of the developing limbs but would not probably have expected the basal (ventral) plate to differ greatly from the alar...
(dorsal) plate. Actually, the alar plate showed much higher levels of mitotic activity, and it reached a peak at 6 days, whereas the basal plate peaked at 8 days with no special activity at the limb levels. It turned out that uniform ventral gray columns were produced throughout the cord at first, and this occurred earlier than did the dorsal. Two things happened later to account for the ultimate reduction in the size of the motor columns in the cervical, thoracic, and sacral regions, leaving those at the limb levels proportionately larger. One was that many motor neuroblasts degenerated in the cervical region where they were not needed. The other was that "excess" cells in the thoracic and sacral regions moved to the ventromedial part of the cord to become the preganglionic autonomic cell columns. The alar plate growth behavior was less easily explained. Its lag behind the motor columns may in part have been due to the slower development of the posterior horns and related intercolumns. The larger size of the posterior root ganglia supplying limbs was related to the greater number of mitoses in the ganglia of the brachial and lumbosacral levels than elsewhere. Further difference in size resulted from degeneration of partly differentiated neuroblasts in the thoracic and cervical ganglia but not in the limb ganglia.

Influences on development of nerve cells and fibers.

—All sorts of hypotheses have been advanced to explain how the intricate fiber connections of the nervous system are fabricated and how the first neurites of the neuroblast get started. Much current thinking attributes the guidance of the fiber to surface reactions between the fiber and the matrix in which it finds itself (110, 112). The first pseudopodial projection of the primitive neuroblast cytoplasm is apparently exploratory, and in experimental circumstances a fiber aligns itself along oriented surfaces such as fibrin strands, columns of cells, or along other nerve fibers already laid down. Rapidly proliferating centers seem to attract young neurites nonspecifically. Fibers from neuroblasts of the cervical spinal cord may be attracted to the appropriate developing limb bud, but they will also grow toward a transplanted developing eye or nasal placode. Weiss speculates that two proliferation centers, due to local dehydration, will tend to orient the extracellular matrix into lines running between them. Nerve fibers sprouting out from the spinal cord center would be oriented to grow toward the regional proliferating limb bud center. Sharp deflections of fibers in the brain may result (early) if they contact oriented lines in the matrix, or (late) they may simply follow already established fiber bundles. Another orienting factor is the towing effect that a developing limb or other organ exerts on a nerve fiber. The distance between a neuroblast and the target of its fiber is not so great in the beginning as its later relations in the adult suggest, so that after the fiber gets to the limb, subsequent limb growth carries it along "passively."

Gradients, distance chemical influences, neurobiotaxis, and electrical fields are currently less popular as concepts to explain the guidance of nerve fibers than formerly, but the trouble an infant nerve fiber will take to make certain connections is impressive. Mauthner's cell, a single huge neuron in each side of the medulla of the tadpole, sends a long conspicuous fiber to the animal's tail. The cell can be spatially disoriented and blocks placed in the path of its fiber, but the fiber turns and dodges until it finds its way to the tail (review, Stefanelli [96]). The facility with which certain developing nerve fibers, after section and disorientation, can find their way back to the central nervous system and make the right functional connections can be partly explained by assuming specific chemical surface reactions (Weiss [110, 112]), but much remains to be learned. The extracellular spaces and matrix are as much the animal as the cell units, a matter emphasized by Weiss particularly, Holtfreter (57), and Schmitt (89). Opie (82) has shown what an extraordinarily watery milieu the molecular and cellular components of the embryo operate in. The more we understand the matrix perhaps the more we shall know how it makes cells do the things they do.

The metabolic changes of the cerebral nerve cell itself accompanying maturation and the growth of its fibers prior to myelination and maturity have been little studied except by Flexner and his associates (reviews [36, 37]). In rats, swine, and guinea pigs growth of the nucleus is a predominant feature in the neuroblast, but as the cell becomes a recognizable neuron a shift to increased aerobic metabolic processes occurs. Cytoplasmic maturation is accompanied by formation of protoplasmic fibers, Nissl material, a nucleolus, and synthesis of certain enzymes. Following these the beginning of functional maturation is shown by measurable electrical activity and, in appropriate cases, evidence of motor activity.

Peripheral factors influence the development of central neuroblasts and their fibers in several ways, but the relations are not simple, and some of the functional aspects will be discussed later. As early as 1920 Detweiler transplanted a limb to the trunk in a developing amphibian, and this caused overgrowth of the corresponding innervating spinal ganglia. It had been known earlier that
tracts cross completely, removal of an eye before the fibers contact the optic tectum results in un-

related type of specificity in muscle innervation. Stone and Sperry extended Matthey’s experiments and explored other neural systems.

Specificity in neural organization.—In Weiss’ amphibian experiments a single muscle or a whole limb was transplanted to a position adjacent to a normal limb early in development. The limb developed essentially normally and was innervated by branches from those to the normal limb. The coordinated movements of its muscles functioned in a synchronous pattern identical to that of the normal limb. Each muscle conferred upon the nerve growing into it a mark or a sign, apparently at the molecular level, that conveyed to the neural center precise information as to just which muscle it was connected with. If the transplanted limb was put on backward, the nerves that grew into the muscles were informed exactly what the “name” of the muscle was that each innervated. This information was so precise that the movements of the transplanted limb synchronously mirrored every movement of the adjacent normal limb. This movement persisted automatically despite its inconvenience to the animal.

Clearly, there was more to this than a muscle contracting in response to a nerve that had become specified to it. In normal development a much more complex system of coordination processes was involved. At the same time that the specific muscle was applying its own “name” or “sign” to the motor nerve that had grown into it, proprioceptive afferent fibers were also being marked. The central connections of these afferent nerves were also becoming specifically integrated into the corresponding central motor mechanism that was developing. One of the harmonious results of this process in respect to limbs was the evolution of a coordinated mechanism for walking and running, and this machinery developed in a self-determining manner characteristic for the animal. Weiss exchanged the right and left front limbs of Amblystoma at many stages of development and with adult Triturus in

Specificity in Neural Development

Weiss (106) and Matthey (78) first fixed special attention on the extraordinary specificity of the components of the nervous system. Matthey cut the optic nerve of the newt and discovered that the fibers regenera
ted and found their way back to the right part of the brain so precisely that normal vision was recovered. Weiss at this time and in subsequent extensive studies (107–109) showed a

an absent limb caused undergrowth of the corresponding ganglia. In amphibia where the optic fibers contact the optic tectum results in un-

nervous technical reasons. Still other patterns of this sort can be demonstrated. Extirpation of the otocyst including the eighth ganglion primordium in the 2-day-old chick embryo is followed by normal differentiation of neurons of the secondary vestibular nuclei up to a point. Then after they are mature degeneration occurs, and this is greatest in the nucleus angularis, less in the nucleus magnocellularis, and least in other vestibular centers. Angularis seems to depend solely on afferents from the ear, whereas the other nuclei are “protected” by having additional synapses from other sources (Levi-Montalcini [75]).

Still another interesting influence on central nervous system growth is the stimulating effect that some as yet unknown substance in a mammalian sarcoma has on the development of ganglion neurons in the bird (14, 44, 77). Planted near the leg bud of a 2-day chick embryo, mouse Sarcoma 180 stimulated the regional spinal and sympathetic ganglia to become abnormally large owing to increased numbers of primitive neuroblasts and rapid differentiation. Fibers from these ganglia grew out and invaded the tumor, but there was no trophic effect in the somatomotor nerves. Planted in the extra-embryonic membranes, the tumor stimulated the same kind of hypertrophy of cells. Nerve fibers grew out prematurely and in excessive numbers into the viscera, often passing through blood vessel walls to form fibrous proliferations in their lumens. Considering how nicely balanced peripheral innervation usually is, the tumor substance must be considered to be an extraordinary alterant of development.

Stone (98) and Sperry (92–94) explored specificity in the visual system of amphibia. Stone experimented with the eyes of Amblystoma at many stages of development and with adult Triturus in
which a capacity for an embryonic type of regeneration persists much longer than in most animals. Eyes surgically removed and replaced promptly degenerated except for a ring of peripheral cells of the ciliary margin. From these cells a complete functioning new eye developed in about 2 months, provided the eye was normally oriented when it was replaced. If both eyes were removed and one was rotated 180° and replaced in its own orbit, regeneration occurred, and fibers of that eye connected up with the optic tectal cells precisely as they had previously. (The other eye was discarded.) However, images that used to be received on the lower part of the retina were now received on the upper part and vice versa, and the animal saw everything upside down. It responded accordingly and kept upward at a lure held below eye level, and dived down in its tank toward a lure held above it. If the right eye was transplanted to the left side, but not rotated, objects in front were seen behind and vice versa. This polarization of the retina first showed specificity at about the time the first motor responses occurred in the embryo and was well established by the time the heart was beating prominently. Rotation of the eye after this time resulted in the characteristic dysfunctions just described. Sperry made similar experiments in fish, frogs, and newts and found the same specificity. He also studied histologic sections and found that the nerve fibers were disarranged at the site of regenration but had ultimately found their way back to the proper centers. Altogether, these experiments indicate that “optic fibers arising from different points in the retinal field differ from one another in quality,” just as Weiss showed for the motor nerves in muscle.

Sperry (94) and Miner (79) investigated other central-peripheral connections. If a segment of belly skin in a tadpole was exchanged with one from the back, the frog that grew up had a patch of white belly skin on its back and back skin on its belly. When the displaced belly skin was stimulated the frog wiped its belly. The displaced belly skin had conferred its positional identification on the nerves that grew into it and histologically these were the normal regional nerves. In other experiments the developing afferent nerves of the limb skin were removed, and adjacent trunk nerves were allowed to grow into it. The trunk nerves acquired the “sign” of the limb, and when the tadpole became a frog these nerves mediated limb reflexes, not trunk reflexes. Sperry also reported experiments involving the vestibular system in which the results were analogous to those in the optic experiments. In still other amphibian experiments a posterior sensory spinal nerve root was cut and the distal end experimentally made to regenerate into the central stump of the contralateral root. Regenerating fibers found their way to proper central connections even though the histologic picture of the abnormal root entrance was one of chaos.

Variation in different vertebrates.—Development of specificity of the cells of the nervous system varies in different members of the vertebrate kingdom and in different parts of the nervous system in a given animal. In muscles the capacity to remodel the specificity persisted for a long time, but in the eye it was “crystallized” quite early in development. That the fine structure of the nervous system, at least below the pallium, is apparently irreversibly set by the time of maturity in mammals as well as lower forms is supported by observations in rats and man. Sperry (1945) transposed sensory nerves in the hind legs of adult rats. By this stage of development the nerves were specific for coordinating sensory stimuli with motor responses of the right and left sides. The nerves regenerated anatomically, but a stimulus to one leg induced an inappropriate motor response in the other. The same sort of thing is familiar in man. When a hypoglossal or spinal accessory nerve is anastomosed to the distal part of a cut facial nerve, the hypoglossal nerve is never functionally transformed into “facial” nerve, and it never takes over real facial nerve function (Coleman [19]).

Implications.—These experiments illustrate that much of the basic structural patterning of the nervous system is built in by the developmental processes in a predetermined way and the degree of specificity is extremely refined. This does not deny that a nervous system is what it is, an adaptive or adjusting mechanism. Depending on the number and complexity of the repertory of alternate neural mechanisms available to it, an animal can compensate for malfunctioning parts. The salamander with its legs on backward had neither a repertory of alternate limb mechanisms nor a cerebral cortex to implement them if it did. Sperry’s rats could not “learn” to compensate for the crossed nerves in the hind limbs, but in experiments in which the forelimb nerves were crossed some compensation through available alternative movements was accomplished. In more complex mammals and man there is a considerable capacity for higher centers to dissociate motor coordination mechanisms and substitute other mechanisms when temporary or permanent injury interferes with normal function. Sometimes this leads to a remarkable degree of compensatory function. There is presently no evidence that the actual nerve fibers involved (as in the hypoglossal-facial
anastomosis) are ever “developmentally” altered in the adult.

Most of what has been dealt with has concerned the early organization of the structures upon which future function will be based rather than the chemical or physiological nature of the specificity. Most neurochemical and functional studies relating to behavior are carried out much later in development than the “putting together” stage, but the evolution of mammalian fetal reflexes has been the subject of investigation (8, 60, 114). The neurochemical changes that underlie the extraordinarily refined degrees of specificity of functional development are still beyond biochemical methods presently available.

**GENETIC ASPECTS OF NEURAL DEVELOPMENT**

Genetics has been described as “the study of the origin, development, and distribution of individual differences” (David and Snyder [22], in reference to the nervous system), and it equally concerns the mechanisms by which individual similarities and species are perpetuated. These mechanisms have their beginnings in the genetic material, the chromosomes. Recent biochemical studies of chromosomes and nucleic acids have culminated in the formulation of chemical models that aid in thinking about how this generic material may initiate and determine normal and abnormal development (review of various concepts, DeBusk [24]; popular summary, Horowitz [61]).

The chromosome may be regarded as having a protein core wound with a parallel pair of helical chains of segmented deoxyribonucleic acids (DNA). The arrangement of the sequence of the usual four pyrimidine and purine bases in the chain provides a theoretically almost limitless variety of combinations, since the helixes have millions of turns. Segments of DNA, perhaps corresponding to the gene unit as it is popularly conceived in development, may act as templates, possibly by imprinting a specific code on nuclear protein or by forming RNA templates to be used in cytoplasmic protein synthesis. The remarkable degree of specificity that is implicit in the developmental processes is made palbable by these concepts. By the same token mutations that lead to biologic variation or frank abnormality are viewed as alterations in the coded templates. A mutated template results in a mutated protein in the initial steps of development.

How the genetic material ultimately translates its code into the form and function of an adult organism is a matter of enormous complexity. There is not a group of genes for the brain, another for an eye, and still another for a leg or a nerve. Rather, a given gene or part of a chromosome may be responsible for the development of a number of characteristics diversely represented in separate organs, cells, enzymes, and other attributes. Developmental characteristics, as far as they can be defined, come in a diverse assortment of packages, and it is often hard to define what is meant by one in the adult brain until a mutation reveals it. The seeming diversity of normal and abnormal forms (phenotypes) that is seen may depend more on shades of environmental influence than on innumerable genotypes. Despite the enormous theoretical numbers of possible DNA codes, there may be only a relative few basic combinations that can accomplish successful development (Goldschmidt [40]), and the number of possible or likely chemical configurations may be considerably less than is inferred from the models. Answers to these questions may help to explain why the neuropathologist does not see an unlimited variety of malformations and why certain patterns seem to crop up throughout the vertebrate kingdom.

Examples of mutations that are expressed as biochemical faults are well known, the chromosomal abnormality resulting somehow in a missing enzyme or groups of enzymes. Phenylketonuria, galactosemia, and Wilson’s hepatolenticular degeneration are examples, but it is not possible at present to know whether these solely represent biochemical defects at the end of the developmental line or something more complicated. In the idiocy of phenylketonuria it is not known whether the accumulation of phenylalanine impairs what would otherwise be normal brain function or whether the mutation also impaired the molecular structure of the nerve cells in other ways besides enzymes concerned with phenylalanine. In other words, it is usually impossible to know at just what stage a developmental process first got off the track and consequently what is a “primary” defective process and what is “secondary.”

Most congenital anomalies are the result of mutations or interference with developmental pathways governed by the normal counterparts of the mutated genes. Because the latter process results in a copy of a mutation the resulting abnormal animal is called a phenocopy (Goldschmidt [40]). The possibility that most phenocopies may be the combined result of mutants that are not ordinarily expressed and unusual environmental factors has been suggested by Landauer (74). A third group of abnormalities results when the embryo or fetus is injured so as to destroy a part of its tissues. The best example of this is the abnormalities that follow the various kinds of extirpative procedures employed by experimental em-
bryologists. Although the response to injury of any organism is primarily determined by its genetic makeup, these responses tend to reflect patterns of growth common to vertebrates, and they can even be designed not to imitate mutants. Ionizing radiation, anoxia, certain traumatic injuries and infections may cause abnormal development of this sort in vertebrates in special circumstances but in man such causes are rarely substantiated (51–58).

**Ploidy and chromosomal changes during development.**—It is at first disconcerting to learn that deviations from the usually accepted number of chromosomes in mammalia and other animal cells is commonplace. Deviations in set multiples are familiar to biologists who work with plants, invertebrates, and lower vertebrates. Animals that are apparently normal may have in their bodies somatic cells with half, double, or triple the usual complement of chromosomes. When this involves all the body’s cells, tissue structure and function may be affected.

Fankhauser and his associates have investigated the role of ploidy in development of the nervous system in salamanders (33, 34). Interference with the second maturation division of the fertilized eggs leads to the development of animals with a triploid number, or three sets, of chromosomes in all their cells. The size of individual cells in such animals is larger than that in the normal diploid animals, but the total size of the animals, and their brains, is the same in both. (Tetraploid and pentaploid animals, which also sometimes occur, have even larger cells roughly proportional to the ploidy.) The triploid animals actually have a smaller total number of neurons than do their diploid brothers in the same brain volume. This is shown by histologic study. In certain “learning” tests to which they were subjected, the triploid animals were significantly less gifted than their diploid brothers. The results of the experiments pointed to the fewer neurons in the brain as being the factor responsible for their poorer performances.

The frequency with which deviations from the normal diploid state in man or other mammals may, by unequal division or as a result of parthenogenesis, have anything to do with the development of the nervous system is presently speculative. The variation in the total number of chromosomes that may be found in mammalian somatic cells is normally very considerable (Beatty [8]). This is compatible with a concept that a precise chromosome number is most essential to cells in early embryonic development and that it may be less essential to the proper behavior of cells of more differentiated tissues (King and Briggs [71]).

**Neoplasia**

The most fruitful applications of embryology to oncology will probably come from an understanding of how chromosomes govern cell differentiation, whether this follows a normal course, a neoplastic course, or some other deviation called abnormal development. The general problem is beyond the scope of this review, and only some particular points as they fit into the problem of neural development have been considered. In clinical and pathologic practice the distinction has to be made between neoplasms and malformations for the purpose of treatment, but the distinction is sometimes really impossible. Some growths involving the nervous system as in von Recklinghausen’s neurofibromatosis, cerebellar “astrocytomas,” vascular malformations, and tuberous sclerosis present features of maldevelopment at one time and neoplasia at another, or both aspects may present themselves. The development of a retinoblastoma, a neuroembryoma of the eye, which usually satisfies the criteria of a malignant tumor, is governed by a dominant gene with high expressivity. In its morphogenesis it possibly passes through a stage of malformation into neoplasia. On the other hand, Zimmerman (116) has demonstrated in animals (having an appropriate genetic makeup) that neoplastic transformation can be induced in one or more of adult glial types directly without resorting to embryonal cells or to what is virtually unsubstantiated in any other form of development, “dedifferentiation” (Ephrussi [81]). The point is that neoplasia in the nervous system is no more an entity or even a single form of abnormal differentiation than it is in any other tissue (Huxley [64]).

One other aspect of neural tumors (and tumors in general) may be touched on. The inference is often drawn that, because two adult tissues originated from the same embryonic germ layer, they will have similar characteristics. Some efforts have been made to dispel this myth (DeBeer [23]), and the recent work of Horstadius (62), Spratt (96), and Witschi (115) among others strengthen them. Considerable discussion, for example, has always surrounded the classification of the nerve sheath cells, the pia and arachnoid cells of the leptomeninges, and the tumors that imitate them. It is often argued whether the leptomeninges and Schwann sheaths are neural tissues or connective tissues, and, because they derive from the neural crest, it is said that they must be neural. Recent work shows that this no longer has any meaning. Pia-arachnoid is pia-arachnoid, and Schwann cells are Schwann cells—and they may, if desired, be designated as neural tissues because of their adult
functions and associations but not at all because of their embryonic origin. The neural crest gives rise to leptomeninges, connective tissue of the head and trunk, Schwann cells, melanocytes, adrenal medulla, and certain neurons—as disparate a group of tissues as could be gathered together.

If a further example is needed to show that so-called embryonic layers are part of the embryo, not the adult, one may recall the adenoacanthoma of the uterus. The keratinizing prickle cell parts of such a tumor are morphologically indistinguishable from epidermoid cancers elsewhere; that its origin can be traced back to the embryonic mesoderm throws little light on its morphologic nature.

CONCLUDING REMARKS

Our knowledge of the early normal and abnormal development of the vertebrate nervous system is still fragmentary, and the fragments have naturally varied more in proportion to the interest they have held for investigators than in their relation to the whole of neurology. Most of the ground work has been laid in experimental neuroembryology of simpler vertebrates, but there is no longer doubt that basic principles of development apply to the vertebrates as a whole and that differences are chiefly a matter of complexity. The development of a normal nervous system is an incredibly complex process to understand, and the deviations that can express themselves in anatomic, functional, biochemical, and neoplastic forms compound the difficulties.

No dynamic scientific study is without controversy, and at least three such studies in developmental neurology seem to hold great promise. One is the comparative approach to the early mosaics and cell mechanics that determine brain anatomy. A second is the study of the extraordinary refinement of specificity that is built into the nervous system during ontogeny. The third is the increasing impact that biochemical and developmental genetics have on elucidating how the phenomena in the first two come about.

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