

PRINCIPLES OF NEURAL DEVELOPMENT

Dale Purves and Jeff W. Lichtman

Contents

EARLY EVENTS IN NEURAL DEVELOPMENT 3

1

Introduction	3
The Rise of Experimental Embryology	3
Box A: Ontogeny and Phylogeny	6
Some Major Events in Early Embryonic Development	8
RESPECTIVE ROLES OF NUCLEUS AND CYTOPLASM IN THE EARLIEST STAGES OF DEVELOPMENT	8
CELL CLEAVAGE AND FORMATION OF THE BLASTULA	9
FORMATION OF THE GASTRULA	11
Initial Formation of Neural Structures in Vertebrates	13
NEURULATION	13
EMERGENCE OF THE VERTEBRATE BRAIN	17
Initial Formation of Neural Structures in Invertebrates	20
Box B: Metamorphosis	22
Conclusions	22

NEURONAL DIFFERENTIATION 25

2

Introduction	25
Influences on Cell Differentiation	25
INFLUENCE OF THE NUCLEUS	25
Box A: What Is the Relation of Genes to Development?	28
INFLUENCE OF THE CYTOPLASM	28
INFLUENCES ARISING FROM OTHER CELLS	29
Fate Mapping and Cell Determination	30
Differentiation of Nerve Cells	32
EARLY ATTEMPTS TO DEFINE THE ORIGIN OF NERVE AND GLIAL CELLS	32
MODERN STUDIES OF NERVE CELL LINEAGE	33
Box B: Molecular Biologists Captivated by Neurobiology	34
ACQUISITION OF TRANSMITTER PROPERTIES	42

DIFFERENTIATION OF ELECTRICAL EXCITABILITY	48	
DIFFERENTIATION OF NEURONAL FORM	49	
Conclusions		51

3

PATTERN AND POSITIONAL INFORMATION 53

Introduction		53
Quest for an Organizing Principle in Embryonic Development		53
Box A: Hans Spemann (1869-1941)		54
Compensatory Phenomena in the Genesis of Embryonic Form:		
Regulation and Morphogenetic Fields		57
Positional Information		59
EVIDENCE FOR POSITIONAL INFORMATION IN A ONE-DIMENSIONAL SYSTEM	59	
EVIDENCE FOR POSITIONAL INFORMATION IN TWO-DIMENSIONAL SYSTEMS	60	
EVIDENCE FOR POSITIONAL INFORMATION IN A SIMPLE THREE-DIMENSIONAL SYSTEM	61	
EVIDENCE FOR POSITIONAL INFORMATION IN A MORE COMPLEX THREE-DIMENSIONAL SYSTEM: MORPHOGENESIS OF THE VERTEBRATE LIMB	63	
How Positional Information Might Operate		65
GRADIENTS	65	
Box B: Propriety of Positional Information in Limb Morphogenesis		68
FRENCH FLAG MODEL	69	
POLAR COORDINATE MODEL	71	
COMPARTMENTS	72	
Conclusions		77
Box C: Segmentation		78

4

MOVEMENT AND MIGRATION OF NEURONS 81

Introduction		81
Mechanisms of Cell Movement		81
Cues for the Initiation, Direction and Cessation of Cell Movement		83
INHERENT DIRECTIONAL PREFERENCES	83	
CHEMOTAXIS	85	
DIFFERENTIAL ADHESION	86	
Box A: Johannes Friedrich Karl Holtfreter (b. 1901)		88
Neuronal Migration		90
MIGRATION OF NEURONS IN THE PERIPHERAL NERVOUS SYSTEM	90	
MIGRATION OF NEURONAL PRECURSORS IN THE DEVELOPING CENTRAL NERVOUS SYSTEM	91	

Nerve Cell Growth Cones and their Mechanism of Movement	94
STRUCTURE OF GROWTH CONES	94
Box B: Ross Granville Harrison (1870–1959)	98
MECHANISMS OF NEURITE GROWTH	98
Conclusions	103

AXON OUTGROWTH AND THE GENERATION OF STEREOTYPED NERVE PATTERNS 105

Introduction	105
Studies of Axon Outgrowth in Relatively Simple Systems	105
Studies of Axon Outgrowth in More Complex Systems	108
ESTABLISHMENT OF STEREOTYPED PROJECTION PATTERNS IN VERTEBRATES	110
Box A: Cell Marking with Horseradish Peroxidase	114
SIGNIFICANCE OF STEREOTYPED NERVE BRANCHING	117
EVIDENCE FOR AXONAL PATHFINDING	117
Mechanisms of Directed Axon Outgrowth	119
STEREOTROPISM	120
Box B: Axon Outgrowth during Regeneration	122
TROPISM BASED ON DIFFERENTIAL ADHESIVENESS	123
GALVANOTROPISM	124
CHEMOTROPISM	128
Conclusions	129

NEURONAL DEATH DURING DEVELOPMENT 131

Introduction	131
Box A: Counting Cells	132
Programmed Cell Death	132
Target-Dependent Neuronal Death	135
RELATION BETWEEN THE NUMBER OF CELLS IN NERVE CENTERS AND THE SIZE OF THEIR TARGETS	135
Box B: Viktor Hamburger (b. 1900)	140
PROXIMATE CAUSE OF TARGET-DEPENDENT NEURONAL DEATH	140
Box C: Cell Death Induced by Hormones	143
EVIDENCE FOR COMPETITION IN NEURONAL DEATH	144
THE OBJECT OF COMPETITION DURING NEURONAL DEATH	146
ROLE OF SYNAPTIC CONNECTIONS IN NEURONAL DEATH OR SURVIVAL	149
Innervation-Dependent Neuronal Death	151
Box D: Cell Death and Neuronal Proliferation in Maturity	152
Conclusions	153

TROPHIC EFFECTS OF TARGETS ON NEURONS 155

Introduction	155
Nerve Growth Factor: The Preeminent Example of a Trophic Agent	155
DISCOVERY OF NERVE GROWTH FACTOR	155
Box A: Rita Levi-Montalcini (b. 1909)	158
CHARACTERIZATION OF THE NERVE GROWTH FACTOR	
MOLECULE	159
Box B: Why Does a Mouse Sarcoma Secrete NGF?	160
Biological Role of Nerve Growth Factor	162
SPECIFICITY OF NERVE GROWTH FACTOR EFFECTS	163
BIOLOGICAL SOURCES OF NERVE GROWTH FACTOR	165
EFFECTS OF NERVE GROWTH FACTOR ON NEURON SURVIVAL	166
TROPIC EFFECT OF NERVE GROWTH FACTOR	168
LOCAL MAINTENANCE OF TERMINAL ARBORIZATIONS BY NERVE	
GROWTH FACTOR	168
How Does Nerve Growth Factor Achieve its Effects?	170
Evidence for Agents Analogous in Function to Nerve Growth Factor	
in Other Parts of the Nervous System	174
Box C: Familial Dysautonomia:	
A Disorder of Trophic Function?	176
Conclusions	177

LONG-TERM EFFECTS OF NEURONS ON THEIR TARGETS 179

Introduction	179
Long-Term Effects of Neurons on Muscle	179
EFFECTS OF DENERVATION ON MUSCLE FIBERS	179
EVIDENCE FOR A TROPHIC FACTOR IN THE REGULATION OF MUSCLE	
PROPERTIES	181
EVIDENCE FOR ACTIVITY IN THE REGULATION OF MUSCLE	
PROPERTIES	182
Box A: "Sciaticin:" Demonstration of a Neurotrophic Factor	
or a Cautionary Tale?	183
MUSCLE FIBER PROPERTIES THAT DEPEND UPON MOTOR NEURON	
TYPE	187
EFFECTS OF NEURONS ON MUSCLE SURVIVAL DURING LIMB	
REGENERATION	189
Long-Term Effects of Neurons on Sensory Receptors	191
Box B: Effects of Innervation	
on the Morphology of Crustacean Claws	192
Long-Term Effects of Neurons on Other Nerve Cells	195
EFFECTS OF INNERVATION ON PERIPHERAL NERVE CELLS	195

Box C: Stephen William Kuffler (1913–1980)	196
Box D: Differential Interference Contrast	
(Nomarski) Microscopy	199
EFFECTS OF INNERVATION ON NEURONS	
IN THE CENTRAL NERVOUS SYSTEM	201
Conclusions	204

FORMATION OF SYNAPSES 205

Introduction	205
Time of Synapse Formation	205
Box A: What Is a Synapse?	208
Location and Spacing of Initial Synaptic Contacts	210
Structure and Function of Newly Formed Synapses	212
Some General Questions about Synapse Formation	217
ARE POSTSYNAPTIC MEMBRANES REGIONALLY SPECIALIZED BEFORE	
INNERVATION?	217
Box B: Santiago Ramón y Cajal (1852–1934)	218
DO PRESYNAPTIC ELEMENTS INDUCE OTHER	
POSTSYNAPTIC SPECIALIZATIONS?	221
DO POSTSYNAPTIC CELLS INDUCE CHANGES	
IN PRESYNAPTIC NEURONS?	223
Box C: Synapse Formation between Mismatched	
Presynaptic and Postsynaptic Elements	226
Conclusions	227

SELECTIVE SYNAPTIC CONNECTIONS 229

Introduction	229
Selective Synaptic Connections in Skeletal Muscle	229
SELECTIVE INNERVATION OF NONMAMMALIAN MUSCLES	229
SELECTIVE INNERVATION OF MAMMALIAN MUSCLES	232
SELECTIVE INNERVATION OF MUSCLE FIBER TYPES	235
Selective Synaptic Connections in Autonomic Ganglia	235
INNERVATION OF SUPERIOR CERVICAL GANGLION CELLS	236
REINNERVATION OF AUTONOMIC GANGLION CELLS	240
Box A: John Newport Langley (1852–1925)	242
Selective Synaptic Connections in the Spinal Cord	243
Selective Synaptic Connections in	
the Invertebrate Central Nervous System	246
Conclusions	250

THE MOLECULAR BASIS OF NEURONAL RECOGNITION 251

Introduction	251
The Retinotectal System	251
Box A: Paul Alfred Weiss (b. 1898)	254
The Chemoaffinity Hypothesis	254
Tests of Sperry's Hypothesis	257
Box B: Roger Wolcott Sperry (b. 1913)	259
Attempts to Define the Molecular Basis of Chemoaffinity	260
INTERCELLULAR ADHESION AS A MEASURE OF CELL RECOGNITION	260
IMMUNOLOGICAL ATTEMPTS TO IDENTIFY MOLECULES INVOLVED IN INTERCELLULAR RECOGNITION	263
Box C: Monoclonal Antibodies	264
Alternatives to the Chemoaffinity Hypothesis	267
THE RESONANCE HYPOTHESIS	267
THE CONTACT GUIDANCE HYPOTHESIS	268
THE TARGET LABELING HYPOTHESIS	269
OTHER THEORIES OF RETINOTECTAL SPECIFICITY	269
Conclusions	270

REARRANGEMENT OF DEVELOPING NEURONAL CONNECTIONS 271

Introduction	271
Synaptic Rearrangement in Different Parts of the Developing Nervous System	271
SYNAPTIC REARRANGEMENT IN SKELETAL MUSCLE	271
SYNAPTIC REARRANGEMENT IN THE AUTONOMIC NERVOUS SYSTEM	275
SYNAPTIC REARRANGEMENT IN THE CENTRAL NERVOUS SYSTEM	280
OTHER REARRANGEMENTS IN THE CENTRAL NERVOUS SYSTEM	286
Box A: Elimination of Some Initial Inputs	
Is Not a Universal Feature of Neural Development	288
Mechanisms Underlying the Rearrangement of Synaptic Connections	289
SYNAPTIC REARRANGEMENT APPEARS TO BE BASED ON COMPETITION	289
Box B: The Relationship of Synaptic Rearrangement to Error Correction	289
THE OBJECT OF COMPETITION DURING SYNAPTIC REARRANGEMENT IS PROBABLY TROPHIC SUPPORT	290
TROPHIC SUPPORT IS SOMEHOW LINKED TO NEURAL ACTIVITY	290
SYNAPTIC REARRANGEMENT AND TARGET CELL SHAPE	296
Conclusions	300

MAINTENANCE AND MODIFIABILITY OF SYNAPSES 301

13

Introduction	301
Modification of Synaptic Connections	301
SPROUTING OF AXON TERMINALS AND THE FORMATION OF NOVEL SYNAPTIC CONNECTIONS	301
RETRACTION OF SYNAPTIC CONNECTIONS IN THE ADULT NERVOUS SYSTEM	308
BALANCE BETWEEN SPROUTING AND RETRACTION	310
NORMAL MODIFICATION OF SYNAPTIC CONNECTIONS	311
MODIFICATION OF SYNAPTIC CONNECTIONS IN OLD AGE	313
Modification of Synaptic Efficacy	315
CHANGES WITH USE AT INDIVIDUAL NERVE-MUSCLE SYNAPSES	315
MODIFICATION OF CENTRAL SYNAPTIC FUNCTION IN INVERTEBRATES	317
Box A: Bernard Katz (b. 1911)	318
Box B: Compensatory Responses to Distorted Perception	324
MODIFICATION OF SYNAPSES FOLLOWING REPETITIVE ACTIVITY IN THE HIPPOCAMPUS	325
Conclusions	327

THE DEVELOPMENT OF BEHAVIOR 329

14

Introduction	329
Innate Quality of Many Behaviors	329
INSTINCTUAL BEHAVIOR	329
EVIDENCE FOR THE DEVELOPMENT OF COMPLEX NEURAL PROGRAMS IN THE ABSENCE OF EXPERIENCE	332
Influence of Experience on the Development of Behavior	333
IMPRINTING	334
EFFECTS OF EARLY EXPERIENCE ON THE SOCIAL BEHAVIOR OF PRIMATES	334
Box A: Konrad Zacharias Lorenz (b. 1903)	336
DEVELOPMENT OF BIRDSONG	338
Refinement of Innate Connections in the Visual System	343
Box B: Neural Correlates of Sexually Dimorphic Behavior in Invertebrates	344
THE CAT VISUAL SYSTEM AT BIRTH	345
Box C: David Hunter Hubel (b. 1926) and Torsten Nils Wiesel (b. 1924)	346
EFFECTS OF EYE CLOSURE	349
RELEVANCE OF DEPRIVATION EFFECTS TO NORMAL DEVELOPMENT	351
Conclusions	354

15	PRINCIPLES OF (AND SOME PREJUDICES ABOUT) NEURAL DEVELOPMENT	357
	ACKNOWLEDGMENTS	364
	GLOSSARY	365
	BIBLIOGRAPHY	377
	INDEX	425

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THE COVER: Halo response of an embryonic chick ganglion after incubation with nerve growth factor; see Chapter 7. (Courtesy of R. Levi-Montalcini.)

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Early Events in Neural Development

INTRODUCTION

The development of the nervous system is difficult to separate, as a topic, from the development of the rest of the animal. The nervous system is ultimately the collection of cells that organizes an animal's behavior. But embryos do not behave, at least in the beginning, and there is really no reason to imagine that neural development is fundamentally different from the generation of other organ systems. Moreover, the nervous system does not, of course, develop in isolation: it both is influenced by and influences the motor and sensory organs that allow an animal to act and to react. Finally, the development of the nervous system depends on a metabolic and hormonal context. Thus, it is no more sensible to talk about neural development without reference to the general course of embryogenesis than it is to discuss the performance of a conductor without reference to the orchestra; it is often done, but not very usefully.

Throughout this account, then, we have tried to relate the development of the nervous system to development generally, and the views of neurobiologists to the ideas of experimental embryologists.

THE RISE OF EXPERIMENTAL EMBRYOLOGY

Although many historical controversies seem outdated in retrospect, surprisingly few fundamental issues in development have really been settled; they have simply taken on different guises as technical and intellectual styles have changed. "It is easy to sneer at our ancestors," wrote T. H. Huxley, ". . . but it is much more profitable to try to discover why they, who were not really one whit less sensible persons than our excellent selves, should have been led to entertain views which strike us as absurd" (Meyer, 1939).

Perhaps it was Aristotle who began what must surely be one of the most protracted debates in the history of science: Is the development of animals based on preformation, or is it the result of an initial plan operating in conjunction with "external" factors? The idea that the zygote is simply a miniature individual that grows seemed so cogent that many philosophers took this notion of development for granted.

"In the seed," the Roman philosopher Seneca declared about 2000 years ago, "are enclosed all the parts of the body of the man that shall be formed. The infant that is borne in his mother's wombe hath the rootes of the beard and hair that he shall weare one day. In this little masse likewise are all the lineaments of the bodie and all that which Posterity shall discover in him" (quoted from Needham, 1959). Even with the advent of the microscope, the appeal of preformation was so strong that biologists claimed to see a homunculus in the head of a human sperm and a microscopic horse in equine semen. There were, of course, arguments against preformation (Meyer, 1939; Needham, 1959; Oppenheimer, 1967). For example, this view logically requires the inclusion of all humanity in a single ancestral homunculus. In spite of such counter arguments, the thrust of developmental inquiry well into the nineteenth century was not so much to question preformation but to decide whether the key element in this scheme was the egg or the sperm.

By the middle of the nineteenth century, the idea of preformation was in decline, largely as a result of the observations and arguments of C. F. Wolff and K. E. von Baer. In particular, von Baer's *Entwicklungsgeschichte der Thiere* (roughly *Developmental History of the Animals*), published in 1828, convinced many people that embryos simply did not look like miniature replicas of the adults they would become. In fact, embryos, regardless of species, resembled the embryos of other animals much more than they resembled the adults of their own kind (Figure 1). As concern shifted from homunculi to heritability, debates about development took on a decidedly more modern ring; it seemed that gametes might contain information needed to *create* an organism rather than animal rudiments in miniature form (Wilson, 1911; Jacob, 1982). A major proponent of this new view was W. Roux, who is generally credited with founding the discipline of experimental embryology and, indirectly, the more specialized field of neuroembryology (Hamburger, 1981).

Roux, who lived from 1850 to 1924, was the son of the fencing master at the University of Jena, where he became a student of the biologist and philosopher E. Haeckel. Haeckel was the foremost exponent of the biological approach to embryonic development in the late nineteenth century: the key to ontogeny, he argued, lay in phylogeny (see Box A). Haeckel's teachings apparently impressed Roux in two ways. On the one hand, Roux found Haeckel's emphasis on phylogeny unsatisfactory and metaphysical; on the other hand, he was intrigued by Haeckel's interest in the physicochemical basis of development (Gould, 1977). Roux did not accept Haeckel's verdict that phylogeny is a sufficient cause for ontogeny; he realized that proximate causes had to be analyzed. In consequence, he emphasized the importance of discovering a causal scheme of embryogenesis; he called this scheme "Developmental Mechanics" (in an analogy to Newton's laws of mechanics).

A central question that absorbed Roux's interest was the cause of embryonic differentiation: How do cells that develop from a single fertilized egg become so different in form and function? To attack this

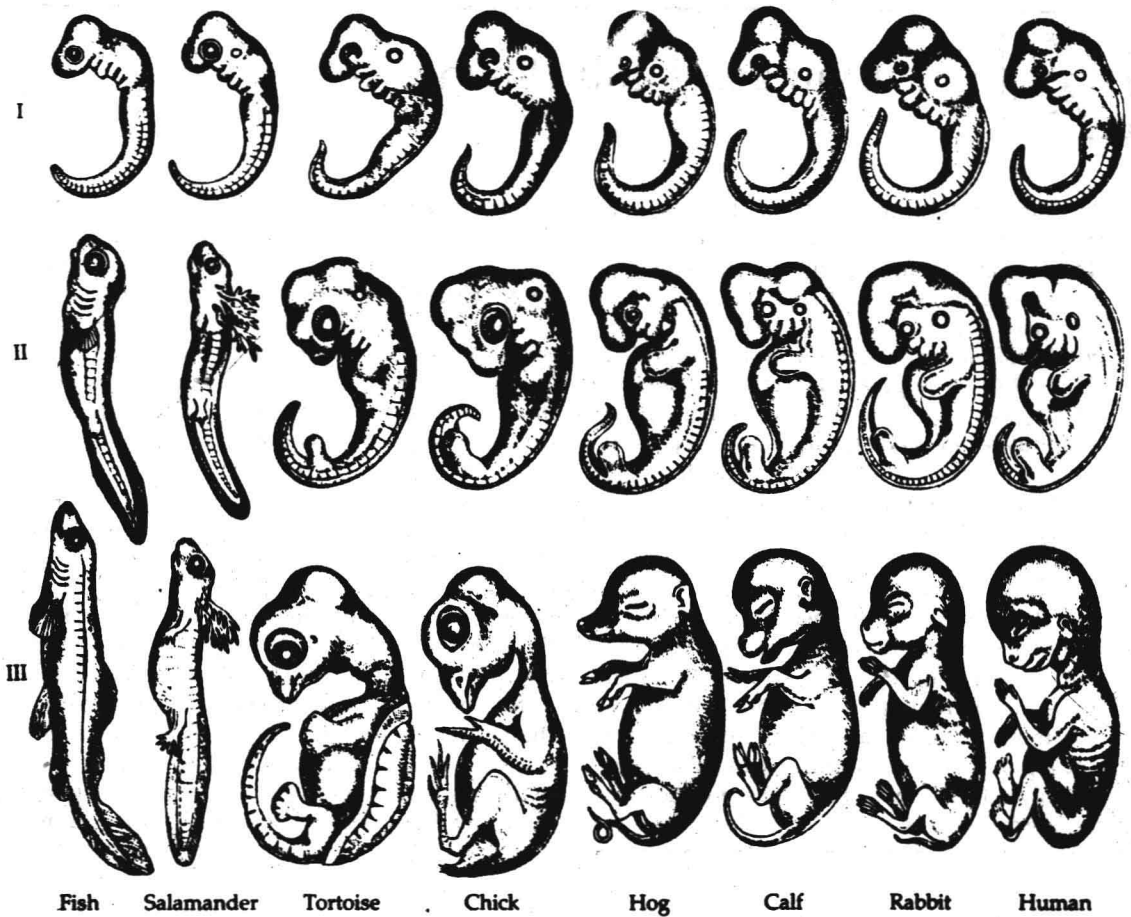


FIGURE 1. *The appearance of vertebrate embryos at various stages of development. The similarity of different embryos during early development is striking; this fun-*

damental observation suggests that these different animals share both a common ancestor and the same basic mechanisms of development. (From Romanes, 1901.)

problem Roux took up an experiment initially performed by Haeckel in 1869. Haeckel had tried to kill one of the first two cells produced by the cleavage of a fertilized frog egg, but he met with little success. Roux realized that the embryo arising from the remaining cell should indicate whether each cell generates a unique part or whether individual cells have a broader potential. In 1888 Roux published the results of experiments in which he succeeded in killing one blastomere at the 2-cell stage (Roux, 1888). The structures that grew from the residual cell appeared to constitute half an embryo, and Roux therefore concluded that each blastomere develops independently. On the basis of this result he proposed a mosaic theory of development that held that the fate of cells is preordained: each cell was regarded as having only the information necessary to create a particular part of the embryo.

Three years later, in 1891, H. Driesch tested Roux's conclusion more

Ontogeny and Phylogeny

An important controversy that bears on the modern view of development is the relationship of embryogenesis to the obvious hierarchy of animal species (Gould, 1977). Although the argument can be traced earlier, the modern portion of the story begins with K. E. von Baer (1792–1876). A popular idea in the eighteenth century was that the embryos of higher animals recapitulate the *adult* features of lower forms. Von Baer (who figured in the discovery of the mammalian ovum, put forward the germ-layer theory and discovered the notochord in chick embryos, among other accomplishments) vigorously attacked this notion of recapitulation (von Baer, 1828). He suggested instead that the more general features of animals appear earlier than special features and that developing embryos of different species simply depart more and more from an early form common to all (see Figure 1). This sensible argument was temporarily eclipsed by E. Haeckel's assertion that the normal events of early development are a

recapitulation of biological history (Haeckel, by the way, coined the terms *ontogeny* and *phylogeny*). For Haeckel, development was simply an accelerated version of evolution. However, Haeckel's idea that development proceeds through a series of adult stages of lower forms (a human embryo is first a fish) simply did not fit the facts. Embryonic men are not really like fish at some point; rather, human embryos and fish embryos are at early times very similar.

The similarity of early embryos is relevant to theories of evolution because it implies that more complex forms arose from a common ancestor—evidently the strategy of early development is highly conserved. In accord with this idea is the fact that the genes of closely related species (man and monkey, for instance) differ very little. This presumably means that the profound differences between the two species do not arise from major differences in genetic programs. One view of speciation is that many differences between animals arise from

carefully with sea urchin eggs at the Zoological Station in Naples (Figure 2). Instead of killing one of the first two blastomeres, Driesch separated them so that each cell could develop independently. In this circumstance Driesch found that the isolated blastomeres developed into fully formed, if smaller, larvae (Driesch, 1892). Subsequently, H. Spemann and others confirmed Driesch's work in vertebrates, thus invalidating Roux's major experimental contribution. The reason for Roux's misinterpretation was probably that the damaged cell, which remained in contact with the other blastomere, caused development to proceed abnormally.

In the end, however, it was Driesch who gave up science after a few years to become a professor of philosophy; in this post he argued that the "harmonious equipotential system" that the embryo represents is beyond analysis. He felt that no system of mechanics could explain how a part could be transformed into a whole. Roux, on the other hand, became a leader of German science, lectured widely, and continued to promote experimental embryology. Not the least of his achievements was founding the *Archiv für Entwicklungsmechanik* (Archives of Developmental Mechanics) in 1894 (now *Wilhelm Roux's Archives*). Influenced



A juvenile and an adult chimpanzee. The resemblance of the juvenile chimp to an adult man suggests that differences in the duration of development pro-

duce major differences in form. (From A. Naef, 1926.)

modulations of the regulatory systems that govern quite general aspects of development, such as rate. S. J. Gould and others have argued that humans and primates may differ because of the more protracted development of humans (Gould, 1977). For instance, by the criterion of ossification, a newborn infant (40

weeks) is comparable to an 18-week monkey fetus (macaque), and the bones of a macaque at birth (24 weeks) are similar in their development to a child of several years! Indeed, the physiognomy of an adult human bears a much greater resemblance to a baby chimp than to a full-grown ape (see figure).

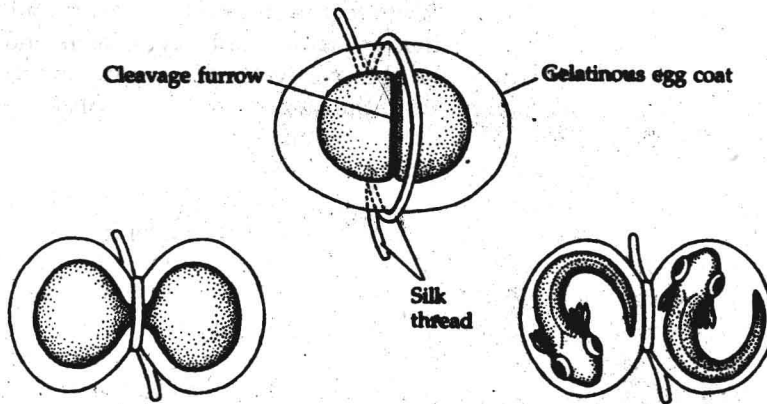


FIGURE 2. *Induced twinning of amphibian eggs. The notion that the earliest embryonic cells in every animal are preordained to give rise to only a part of the embryo had to be discarded when the German zoologist and philosopher H. Driesch showed that each of the first two blastomeres of the sea urchin egg could give rise to a complete larva. This point was confirmed in an experiment carried out by H. Spemann in which a salamander egg was constricted by a fine thread in the plane of the first cleavage furrow, as illustrated here. (After Hamburger, 1963.)*

by Roux's approach, T. H. Morgan, E. G. Conklin, H. Spemann, E. B. Wilson, F. R. Lillie, R. G. Harrison, and others rapidly provided a body of classic experiments in this field (Wilson, 1911; Morgan, 1927; Detwiler, 1936; Waddington, 1936). Before Roux, embryology as a discipline was either a philosophical or an entirely descriptive pursuit; Roux made it an experimental and analytical science. "In zoology," Spemann wrote in 1938, "... the speculations on evolution have, partly, perhaps for accidental ... reasons, outweighed and overpowered every other interest for a number of decades. Here, the initiative of an original thinker was necessary to remind investigators of the fundamental principle [of strict causation]. We owe this achievement to Wilhelm Roux. He will always be honored as the founder of a new discipline in animal embryology" (Spemann, 1938).

In fact, the controversy about whether the fate of early embryonic cells is preordained or determined by interactions with other cells and the environment has never been fully resolved. A variety of observations and experiments indicate that both preordination and flexibility are important in different aspects of development (Chapter 2).

SOME MAJOR EVENTS IN EARLY EMBRYONIC DEVELOPMENT

Respective roles of nucleus and cytoplasm in the earliest stages of development

Development begins with the activation of the egg, usually stimulated by the penetration of a sperm. At the turn of the nineteenth century, the relative importance of the egg nucleus and cytoplasm in development was unclear. In the 1890s, T. Boveri, who later showed that chromosomes are qualitatively different from one another, found that fragments of sea urchin egg that contained only cytoplasm and the genetic material contributed by a sperm developed into an embryo, all parts of which were characteristic of the paternal species (Wilson, 1911). This observation suggested what is now taken for granted: the nuclear material rather than the cytoplasm carries the genetic information. These experiments were extended by I. J. Lorch and J. F. Danielli, who were able to remove (and subsequently reimplant) nuclei from amoebae (Lorch and Danielli, 1950). The enucleated cells failed to survive; they could, however, be rescued by subsequent nuclear implantation.

Other experiments, however, showed that the cytoplasm of the egg also plays a critical role in development. The egg cytoplasm has an uneven distribution of cytoplasmic inclusions such as lipid droplets and yolk granules; such asymmetries are the basis for describing eggs as having an animal pole and a vegetal pole (Figure 3). Different parts of the egg cytoplasm have special functions in development. A striking example is the egg of *Styela* (a sea squirt). Before fertilization the egg has three distinct regions: a peripheral layer that is yellow, a central mass of gray yolk, and a clear germinal vesicle. E. G. Conklin was one of the first embryologists to note that the egg cytoplasm is rearranged within a few minutes of fertilization (Conklin, 1905, 1932). The yellow