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Science as a Way of Knowing—Developmental Biology[1]

JOHN A. MOORE

Department of Biology, University of California, Riverside, California 92521

SYNOPSIS. This essay is part of the fourth yearly presentation of an educational project of the American Society of Zoologists. The purpose is to offer suggestions for improving the first-year biology courses in colleges and universities. We emphasize the conceptual framework of the biological sciences, show how scientific information is obtained and validated, and relate science to human concerns. The topic for consideration this year is *Developmental Biology*. This essay gives some of the background information—mainly classical experimental embryology. The speakers in the symposium will deal with more recent discoveries and insights.

CONTENTS

[1] From the Symposium on *Science as a Way of Knowing—Developmental Biology* presented at the Annual Meeting of the *American Society of Zoologists*, 27–30 December 1986, at Nashville, Tennessee.

INTRODUCTION

Andrew Bard Schmookler (1984, p. 5), although writing about the study of civilization, describes a general problem in the study of biological science:

> In an age of specialized analysis, there is a prejudice against general questions and general answers: the study of the forest is considered best pursued as the study of particular trees. Even as pictures from satellites open our eyes to sweeping vistas, our world view tends to be myopically mired in the magnifying-glass stage. The parts are delineated in excruciating detail, whereas the whole is left for some invisible hand to assemble or is regarded as no more than the sum of its parts.

A common defect in biology courses taught in the colleges and universities is that students are not provided with a satisfying and useful vista of that forest. We tend to forget that learning is most effective when the student starts with the personal world of things and processes experienced and then confronts the unknown world of concept and abstraction.

But that world of concept and abstraction may not be easy for a student to enter. For many of them schooling has consisted of learning facts—true and eternal facts. Those who study cognitive development suggest that the ability to relate phenomena, to explain them symbolically, and to find joy in seeking answers to unsolved problems develops slowly and for many human beings remains always in a stage of incomplete development.

COGNITIVE DEVELOPMENT

There is increasing evidence that the period including high school and the first two years of college sees important changes in cognitive development. Jean Piaget, the Swiss psychologist, suggests that cognitive development is part of biological development (of course neither takes place in the absence of an environment) and that rather definite stages can be recognized. During the elementary school years there is a concrete-operational (or empirico-inductive) mode of problem analysis. The emphasis is on concrete things and empirical evidence. Flavell (1985, p. 98) describes this stage:

> His is an earthbound, concrete, practical-minded sort of problem solving approach, one that persistently fixates on the perceptible and inferable reality right there in front of him. His conceptual approach . . . does, however, hug the ground of detected empirical reality rather closely, and speculations about other possibilities—that is, about other potential, as yet undetected realities—occur only with difficulty and as a last resort. A theorist the elementary school child is not . . . . The realm of abstract possibility is seen as an uncertain and only occasional extension of the safer and surer realm of palpable reality.

During adolescence (11–15 years) a shift is made to a formal-operational (or hypothetico-deductive) level of problem analysis. Here the thinker

> inspects the problem data, *hypothesizes* that such and such a theory or explanation might be the correct one, *deduces* from it that so and so empirical phenomena ought logically to occur or not occur in reality, and then tests her theory by seeing if these predicted phenomena do in fact occur . . . . If you think you have just heard a description of textbook scientific reasoning, you are absolutely right (Flavell, 1985, pp. 98–99).

A college or university course in biology must be based on the students' ability to employ formal-operational, or hypothetico-deductive, reasoning. There are some disturbing indications, however, that students may not have reached this stage by the time they are ready to enter the colleges and universities. Thus Renner *et al.* (1976, p. 96) found that 66 percent of 12th grade students were still in the concrete-operational stage, 15 percent were transitional, and only 19 percent were formal-operational.

Cognitive development must be due in part to the growth of the brain itself and must be influenced strongly by home, friends, activities, and opportunities but

there is a strong suspicion that some is the result of modes of instruction in the schools. Rote learning tested by objective examinations does little to stimulate an inquisitive mind. One suspects that much of the inquisitiveness and creativity of the child is dampened by such schooling and by television, both of which are associated with passivity and conformity. There are ample data to show that when very young students are put in situations where they are given encouragement and opportunity to explore and reason, they quickly reach significant levels of achievement in hypothetico-deductive reasoning.

References to cognitive development

Brainerd (1978), *Flavell (1985), Hamilton and Vernon (1976), Inhelder and Piaget (1958, 1964), Piaget (1954, 1977), Piaget and Inhelder (1969), Renner *et al.* (1976), Rosen (1977), and Smith (1982).

In the references just given the * indicates a title that provides an excellent introduction to the topic being considered. This plan will be followed throughout this essay. The references given are usually those available to me in our campus library but in some cases I have included titles not seen but suspected to be useful.

A Suggested Approach to Teaching

In any event, many of the students who come to the colleges and universities have had little or no experience with mature conceptual thought and the inquiry approach to learning. Thus it becomes all the more difficult to teach biology, or any science, in the way we suggest. The difficulty in doing so, however, is a measure of prior inadequacies that we must seek to remedy.

The previous volumes of the *Science as a Way of Knowing* series have discussed in some detail our goals and procedures (for example see *Science as a Way of Knowing—I. Evolutionary biology* 1984, pp. 469–476, 524–525; *Science as a Way of Knowing—II. Human ecology* 1985, pp. 486–489; *Science as a Way of Knowing—III. Genetics* 1986, pp. 4–7, 153–154). *Hereafter when reference is made to these earlier publications of this project they will be indicated only by number and page, i.e.,* II, pp. 379–381, etc.

There are some things about the biological sciences that every educated person should know, *i.e.,* organizing ideas that help us to understand and enjoy the natural world. For example, one should be able to look at the vast diversity of life and understand its origin through evolution over vast stretches of time. Knowledge of the structure and function of the human body is not only important in itself but also in maintaining health. Today it is essential to understand the interrelations of all living organisms and the cyclic changes of substance and energy that occur in the environment. Green plants and other living creatures, and their activities, are our life support system, yet in many ways and in many places we are using and abusing the environment beyond its ability to sustain itself—and hence, us. In the eternal quest for resources, all organisms including human beings and their food crops, may become the prey of other organisms and our efforts at control may introduce second-order problems.

As science improves its ability to predict and control natural events, it becomes ever more important that people understand the nature of the scientific process, its strengths and its limitations, and that the importance of science lies in its ability to help us understand and control natural processes. That understanding will provide us with information necessary to reach humane decisions but not to specify what the decisions should be. It is here that science should join ethics and morals in making a better world.

The need for biological knowledge does not end with graduation from the university, and for that reason students must acquire a conceptual framework that will allow the facts of biology to be seen as part of an organized whole. Such a framework becomes a powerful mnemonic device and will provide understanding of new biological facts as they are encountered throughout life.

The goal toward which the *Science as a Way of Knowing* project strives is

> primarily concerned [not] with the history of discovery, but rather with those fundamental conceptions which are ageless and persist, however much they may be altered, extended, or transformed by the discovery of new facts (E. S. Russell, 1930, p. 25).

It must be emphasized, however, that a conceptual framework is something that is worked toward, not started with. Learning is made easier if a variety of biological phenomena are first selected, then studied, and finally united in conceptual schemes that can be tested by the usual procedures of logic and science. That is, a question about some natural phenomenon is asked, a possible answer is framed as a hypothesis, deductions are made and these are tested by observation and experiment. Thus one may reach a level of understanding that, for the time, can be said to be true beyond all doubt. Once a concept has been established as true beyond all reasonable doubt, it serves to organize observations and information acquired subsequently.

This essay will attempt to provide such a conceptual framework for developmental biology. It will be concerned mainly with the sorts of questions that have been asked about development and the scientific procedures employed to answer them. The emphasis will be not on what scientists are working on today but what they have found out. This seems appropriate if the goal is to establish the conceptual framework of the field. When dealing with the past, one knows not only the questions but the procedures for answering them and the answers themselves. The emphasis will be on science as a way of knowing.

The intent is not to move back to some Golden Age of Embryology but to better understand the major concepts of developmental biology. These are relatively secure and they will provide a framework for which current research is attempting to provide a molecular basis.

But, of course, there must be a balance. Research in progress portrays science as a way of trying to find out. This can be a stimulating approach to some while it is threatening to others—because of the student's insecurity of not knowing *the* answer when it is yet unknown but the student assumes it to be necessary by exam time. Thus much of the old and a little of the new may be a balance well suited for first-year students.

This essay is not a history of science. Its intent is to marshal the data that have led to our understanding of developmental phenomena. Since the first questions tend to have been asked at an earlier time than later, the essay will reflect history rather than be it. The discussion of ideas and data is not in strict historical sequence but when it has deviated it is to present a better analysis of a problem. For example, the Spemann-Mangold dorsal lip experiment, although first in time, comes as the climax of work leading to the organizer.

I have selected some key individuals and their discoveries and neglected even more key persons and their accomplishments. Some readers may object that E. B. Wilson will come in for more attention than he may deserve but he was both so outstanding and so quotable that I do not apologize for telling so much about what he did. In many instances I have included long quotations in the belief that original statements will be more valuable than my interpretations.

It is important, I believe, that ideas be associated with individuals. For many students ideas in science come across as a rhetoric of conclusions with no notion of person, place, or time. This is not only regrettable but it makes it ever so much more difficult for a student to imagine what role he or she might play in science. After all, science is a human enterprise—so shouldn't we teach it as such?

PROBLEMS AND PROMISES

The field of developmental biology has long had an unsatisfying element—a lack of conceptual coherence. Its problems are central to biology—how the new individual is deciphered from the universal code—yet their conceptualization remains elusive. Horder (in Horder *et al.*, 1986, p. ix) refers to

> a sense of puzzlement concerning the present state of the discipline of embryology, where, despite all our massive knowledge about embryos at the descriptive level and their basis in molecular and cell biology, the nature of embryological events is generally viewed as mysterious and unsolved . . . . Embryology represents a distinct and significant domain among the biological phenomena, as open to satisfying explanation as any other, and . . . it is a subject which, since embryogenesis has been a precondition for the very existence of living forms throughout evolution, [it] ought to occupy a key position in biology, many areas of which stand to benefit if it were better understood.

In spite of all the dashed hopes, developmental biology may be about to come into its own.

First Questions—First Principles

In contrast with so many other modes of inquiry, science advances by studying what is not known rather than by studying what is already known or assumed to be known. For the working scientist, answers may be interesting and satisfying but their true importance is as a basis for asking new questions—science is process, not position.

Thus the beginning of an inquiry in science is the posing of a question about some puzzling phenomenon of nature. This is not a trivial exercise. Important answers will be obtained only if the question relates to some fundamental aspect of the natural phenomenon, and only if there exist practical means of searching for an answer. Many important questions about nature remained unanswered for millennia and many remain so today mainly because there were or are no methods for initiating the inquiry. Some of the early questions about disease, for example, could not be answered until microscopes had been invented and the previously invisible pathogens could be observed. In fact, satisfying answers to many basic biological questions were unobtainable until the invisible world of life could be entered with the techniques of both microscopy and biochemistry.

And so it has been with developmental biology or, as it was better known over its long history, embryology. It may come as a surprise, therefore, to find that Aristotle (384–322 B.C.), that Greek of universal intelligence, not only established the discipline of embryology but posed the major questions that have lasted to today. But then, according to E. S. Russell (1930, p. 2) this may not be surprising at all.

> In spite of the vast accumulation of detailed knowledge, which is, in some quarters, supposed by itself to constitute science, there is much less difference than one would expect between the fundamental hypotheses or modes of explanation adopted, say, by the Greeks and those in vogue at the present day. This is because there are—apparently—only one or two possible ways of interpreting development open to the human intelligence, and these few alternative methods tend to recur again and again throughout the whole history of biological science. One is accordingly forced to the conclusion that on its constructive or theoretical side biology (and perhaps the other sciences as well) is by no means a simple transcript of fact, but in large measure a construction of the mind, a conceptual edifice, the lines and plans of which may vary according to the type of mind of its architect.

These basic questions may not seem difficult to students since we usually tell them what they are. Their approach to embryology, however, will be more instructive if *they* are asked to suggest what the questions might be and how answers might be sought. This can prove a valuable exercise, especially if those members of the class who have never studied biology are asked first "What are the questions one would like to know about development?" Possibly your students will recapitulate Aristotle and, as Russell suggests, ask the same questions.

This is the problem of development as Russell (p. 1) saw it in 1930:

> The general problem of development is without question one of the most difficult and intriguing in the whole field of

knowledge. That from a minute germ of relatively simple structure there should be gradually built up, by a series of processes beautifully co-ordinated in space and time, the complex organization of the adult is a fact that has never ceased to excite the wonder of mankind. It has provided a constant challenge to the intellect of man, and many and various have been the theories invented to explain it. It ranks as one of the major problems of biology.

But what are the questions that are formulated in such a manner that they can be answered? It may be difficult for your students to suggest good questions but the very fact that they try is most important. Students are rarely asked about matters so basic as this but, surely, a science course can be expected to stimulate their latent heuristic minds. So after listing some questions posed by naive persons today it will be interesting to see how a person, initially even more naive and living two and a half millennia ago, tackled the problems.

THE PERIPATETIC STAGIRITE

The extant biological works of Aristotle consist of *Historia Animalium,* which is a general biology of animals; *De Partibus Animalium,* a comparative physiology and anatomy of animals (Sarton, 1952, p. 532, call this the first animal physiology in any language); *De Motu Animalium,* dealing with movement and some aspects of psychology and metaphysics; *De Incessu Animalium,* also concerned with locomotion; *De Anima,* considering the vital principle of living things; *Parva Naturalia,* mainly psychology; and *De Generatione Animalium,* Aristotle's treatment of developmental biology.

Scholars are reasonably sure that existing forms of these works are relatively accurate. During the Renaissance, when Aristotle's works became known in Western Europe from Arabic editions, it was suspected that translations from Greek to Arabic to Latin might have introduced errors. Subsequently manuscripts in Greek were discovered and these are assumed to be closer to the originals. To be sure some are suspected of containing not only errors made when the manuscripts were copied but also there is sometimes evidence of attempted independent creativity on the copyist's part. When several different manuscripts of the same work are available, however, these errors and insertions can usually be detected and expunged. None of the extant manuscripts are very old. For example, the oldest of the nine most important Greek manuscripts of *Historia Animalium* dates from the 12th or 13th century and the rest are from the 13th to the 16th century. To keep this in perspective: the interval from Aristotle to the 12th or 13th century is roughly the same as the interval from the end of the Roman Empire in the West (476 A.D.) to the present.

HISTORIA ANIMALIUM

Historia Animalium is the earliest known animal biology text and its scope and originality are astonishing. Aristotle knew a very great deal about a very large number of organisms. He was interested in their structure, breeding habits, reproduction, behavior, ecology, distribution, and relationships.

So far as developmental biology is concerned, *Historia Animalium* contains a large amount of factual material that is used for the more theoretical considerations of *De Generatione Animalium.* The "basic facts of life" were known, of course, in Aristotle's time and he described what was believed and suspected of reproduction in human beings and many other animals. Reproduction and development were so basic to understanding the biology of organisms that Aristotle used viviparity and oviparity as important characteristics in classifying organisms (*HA* 489^a, 35ff. It is customary to identify the sections and sentences in Aristotle's works in this manner, which refers to the standard edition of Bekker (1831–1871); *HA* is *Historia Animalium*).

It was generally accepted that development began after "something," assumed to be secretions, from the male parent and the female parent became associated. In the case of human beings the male secretion was semen and the female's contri-

bution was assumed to be something like menstrual blood. No one living at that time, or for the subsequent two millennia, had any accurate notion of sperm or ova. Aristotle knew that many animals produced visible eggs and that from these the young slowly developed. But since other species did not seem to have eggs, there must be "something" more basic.

> What we term an egg is a certain product of conception from which the animal will develop . . . the developing embryo comes from only part of the egg and the rest serves as its food (*HA* 489^{b}, 6).

Aristotle was familiar with the early embryos of numerous animals, and his most complete description is of the developing chick (*HA* 561^{a}, 3–562^{a}, 21). When this is read by a biologist today, it sounds so familiar that one tends to forget the tremendous intellectual steps that Aristotle took when he wrote: "Development from the egg proceeds in an identical manner in all birds." This implies that Aristotle was familiar with development in at least a few other species and, assuming a basic uniformity of natural phenomena, felt secure in extending the conclusions based on a few species to all species of birds.

His belief that at a fundamental level nature is not capricious is a necessary working premise for all scientists. Today we feel confident that, for all intents and purposes, the genetic code is universal, yet that confident feeling is based on acceptable data for no more than a trivial fraction of one percent of all species.

Of great importance was Aristotle's use of data from as many different species as he could obtain. This is so basic to biology today that we accept it as the obvious thing to do. When the comparative method is used, one sees variations in the phenomenon being studied—with some species giving a glimpse into one part of the process and another species giving a different glimpse. Each species, in a sense, is an experiment and, when all of the observations have been made, there is a better chance of understanding the fundamentals of the phenomenon. In the early days of cytology and genetics this procedure was basic for establishing the concepts of those fields (for example, III, pp. 669–670). Observations on the kidney of the goosefish were basic for the discoveries of how the mammalian kidney functions. Time and time again it has been found that if you cannot obtain an answer from one species, try another and you may succeed.

We must note that Aristotle was busying himself with an essentially "useless" task. His biology was not making people either richer, or better, or producing better crops, or helping to fight disease. Aristotle was, instead, providing materials for the inquisitive mind. This was "pure science," that is, science for its own sake. For many centuries attempts such as his to understand the natural world were followed by only a few, often lonely, individuals; and for the most part there were to be few practical fruits of their studies until the Renaissance.

And in contrast with the way many people reasoned then and now, Aristotle tried to base his search for the "hows" and "whys" on the "whats."

Historia Animalium has a very large amount of data on the development of the chick. Aristotle reported that the first visible indications of the embryo came after three days but earlier in small species of birds and later in large species. At this time the heart appears as a tiny red spot, it pulsates, and what we now call the vitelline veins are seen to be carrying blood. A little later the body differentiates and the head, with very large eyes, can be made out.

All these observations were made without a microscope, so he was working at the limits of the unaided eye. His description of the much larger embryo at 10 days is far more complete. The head and the eyes are relatively large, and the main internal organs are visible. He provides a fairly accurate description of the embryonic membranes—those structures so baffling to our students in embryology courses today. He even dissected the eye of the 10-day chick.

Some later observers belabored Aristotle for saying that the heart is the first structure to develop. He did not quite say that but said "Blood is developed first of all in the heart of animals before the body is dif-

ferentiated as a whole." That is a perfectly reasonable statement if made when magnification was impossible and Lillie's *The Development of the Chick* unavailable for reference. One sees blood because it is red while the rest of the embryo is not only tiny but also mostly colorless. One must admit that Aristotle did not know all we know today but one might hope that he would be celebrated for his enormous accomplishments in bridging the gap from no science to science. Some detractors would not be satisfied, I suspect, unless Aristotle had come down from the heavens, landed on the Acropolis and said:

$$\Delta\nu\alpha \rightarrow \rho\nu\alpha \rightarrow \pi\rho o\tau\epsilon\iota\nu$$

Aristotle devoted Chapter VII of *Historia Animalium* mainly to human reproduction and development and he describes a human embryo of forty days, when it was as big as one of the larger ants. His observations appear to have been made on an aborted embryo.

There are many other observations in *Historia Animalium* dealing with embryology. He notes, for example, that in a general way development in birds and fishes is the same (*HA* 564^b, 30) and that development is the same in fishes that are oviparous internally and oviparous externally (*HA* 567^b, 27). Aristotle was discovering the natural order behind the putative chaos.

It is clear that Aristotle added the observations of others to his own (Preus, 1975, pp. 21–47). In so doing he suffered the fate of Darwin, who in *The Variation of Animals and Plants under Domestication* included erroneous observations, such as that on Lord Morton's mare, that rendered accurate conclusions impossible (III, pp. 602, 603). For example Aristotle quoted reports that the sorts of water drunk by rams could determine the hair color of the lambs they sired. If the water was from Assyritis the lambs were black; they were also black if the water was from one river in Antandria, but white if from another; consumption of water from the Scamander River caused the lambs to be yellow (519^a, 10–20).

If one accepts these observations, which Aristotle probably obtained from others, one must conclude that inheritance and development are extremely labile and easily influenced by external conditions. Like need not beget like all of the time.

De Generatione Animalium

There are similar problems in *De Generatione Animalium* of basing a theory on incorrect observations. Although many animals, especially those with blood, produce young as a result of copulation, the young of some develop from decaying matter or feces (*GA* 715^a, 25; 715^b, 5). Thus, genetic continuity cannot be true for all species.

De Generatione Animalium contains many observations, some repeated from *Historia Animalium*, about the nature of semen and how the embryo is formed from it. Observations on very many species are offered and it is clear that Aristotle had more firsthand experience with a variety of developmental patterns than most embryologists today. He organized the data to answer specific questions and to develop general principles.

First he sought to establish what it is that parents transmit to offspring. He accepted that it must be substance and can be called "semen" in both fathers and mothers. The first question considered, which was not posed initially by Aristotle, was the relation of the structure of the body to what was in the semen (721^b). One prevailing view was that every part of the adult body contributes some specific material to the semen—a notion that, millennia later, was to be known as the Theory of Pangenesis. Some of the observations and arguments in support of pangenesis are given but Aristotle thought them not convincing. One of his arguments, paraphrased, is as follows (722^a, 35ff.): If flesh and bones are constructed out of fire and similar substances, the semen would have to be drawn from the element fire in flesh and bones of all sorts. Reduced to this elemental state, the elements in semen would not be one sort of fire from flesh or another sort of fire from bones. Therefore, "Blood is formed out of something that is not blood" (723^a, 5).

Aristotle is proposing that something more fundamental than a specific structure

must be transmitted in semen. This is inevitable since there are very few elements, probably just four of which fire is one, and how would the elements "know" they were to form specific sorts of flesh and bones and not something else composed mainly of the element fire?

Another of his arguments against pangenesis is that if all parts of the body of the male parent and all parts of the female parent produce something that is transmitted, then the result should be two embryonic bodies, not just one. Or in those cases where there are many offspring how can it be that the specific determinants for all of the body structures are packaged so that every offspring gets that entire package (729^a, 5ff.)?

Aristotle concludes that either the semen does not come from all parts of the body or, if it does, some additional mechanism must be responsible such as one attributed to Empedocles: each parent contributes only part of what is required to form a complete body and sexual intercourse is needed so their semens can join to form the entire offspring (722^b, 10ff.).

Thus one can read into Aristotle the notion that parents transmit not structures to their offspring but "information" to construct those structures in the course of development.

Aristotle suspected that the contributions of male and female parent are quite different. The semen of the female was thought to be menstrual fluid (729^a, 26) and it differed in a fundamental way from male semen in supplying the substance (727^b, 32) for the embryo whereas male semen (728^a, 30) supplies the form and principle of movement (which can probably best be thought of as meaning "animal life"). The action of male semen on the female secretion was thought to be analogous to the action of rennet upon milk. Rennet "sets" the homogeneous milk just as male semen "sets" the menstrual fluid (739^b, 20).

When it comes to the formation of the embryo itself, the analytical mind of Aristotle reasoned that it must be formed out of something, by something, into something (733^b, 25). The "out of something" is the life-giving material in the semen of the male plus the material substance supplied by the female. The "by something" is assumed to be carried in the semens of both parents. When it turns "into something," *i.e.*, develops, Aristotle considers two possibilities. Some philosophers held that all parts of the embryo's body form at the same time. Aristotle refuted this hypothesis by observation—the heart in the chick embryo appears before the lungs. One cannot deny the validity of this observation, says Aristotle, by suggesting that the lungs are too small to see because, in fact, they are larger than the heart and hence should be visible first. New things appear in the course of development.

Thus, in this longest of any debate in embryology, preformation *vs.* epigenesis, Aristotle comes down on the side of epigenesis.

He makes clear his belief that the semen transmits only the potential for the embryo's structures, not the actual structures themselves (737^a, 20). The potential is in the female's contribution to which the male provides the mechanism for potential to become actual (740^b, 20ff.). E. S. Russell (1930, p. 17) recognizes this as a basic point and says,

> [Aristotle's] fundamental idea, that development is the functional actualization of a functional potentiality, is a profound one and gets down to the root of the matter.

Aristotle has so much to say about so many things that it is easy to read many modern ideas into his statements. One might be tempted, for example, to see in his remarks about some structures being formed first that are necessary for later developments (742^a and 742^b) an anticipation of organizer theory. There are many similar statements.

But Aristotle obviously was not always correct in his biology. He was convinced, for example, that spontaneous generation was the rule for some creatures (762^a, 763^a). This belief was based on many observations of the apparent generation of some insects from decaying matter and the appearance of marine invertebrates on pots

and other objects placed in the sea. It took a large amount of careful observation and experimentation by many naturalists, from Redi (1626–1698?) to Pasteur (1822–1895), to bell that cat.

ARISTOTLE'S ACCOMPLISHMENTS

Joseph Needham, the famous embryologist and even more famous historian of Chinese science and technology, credits Aristotle with extraordinary accomplishments (1959, p. 42).

> [Aristotle] stood at the very entrance into an entirely unworked field of knowledge; he had only to examine, as it were, every animal that he could find, and set down the results of his work, for nobody had ever done it before .... The extraordinary thing is that building on nothing but the scraps of speculation that had been made by the Ionian philosophers, and on the exiguous data of the Hippocratic school, Aristotle should have produced, apparently without effort, a text-book of embryology of essentially the same type as Graham Kerr's or Balfour's .... The depth of Aristotle's insight into the generation of animals has not been surpassed by any subsequent embryologist, and, considering the width of his other interests, cannot have been equalled.

Aristotle sought to understand by first observing; realizing that general concepts might emerge from the study of the same phenomenon in a variety of species; recognizing the fundamental similarity of development in fish, bird, and mammal; arguing for a physical basis of inheritance; providing argument and observation to support epigenesis; and in suspecting that problems of development and regeneration are similar.

And there are a host of minor observations of great interest. For example, one reads with incredulity his realization that nails, hair, and horns all form from the skin (745^a, 20). But as Dante was to say in the *Divine Comedy* (*Inferno*, Canto IV), Aristotle was the "Master of them that know."

One of the most basic contributions that Aristotle made to the field of developmental biology was that he got it started. He collected all the data he could, in a true Baconian fashion, and tried to bring order to the seemingly random phenomena. Considering the time and the newness of such concerns, he did remarkably well. His scientific methodology was deficient only in lacking the widespread use of controlled experimentation.

D'Arcy Thompson (1922, p. 144) praised him for yet another accomplishment:

> He was the first of Greek philosophers and gentlemen to see that all these things were good to know and worthy to be told. This was a great discovery.

Possibly his greatest overall contribution to biology was his firm belief in naturalistic interpretations. This comes through clearly in his attempts to understand the generation of bees. He concluded (760^b, 29ff.):

> This, then appears to be what can be said about the generation of bees—at least as far as theory and what appear to be the facts can take us. But the facts have not been firmly established. If at any future time they are ascertained, one must rely on observations rather than theories—and on theories only if they agree with the facts.

That's a splendid statement. Unfortunately that sound advice was rarely heeded by Aristotle's followers. Arthur Platt, in his translation of *De Generatione Animalium* (footnote 760^b), comments as follows:

> It should have been kept in mind by those bastard Aristotelians who at the revival of learning refused to accept observed facts because they were supposed to contradict Aristotle's statements.

References to Aristotle

The primary sources are Aristotle's *Historia Animalium* and *De Generatione Animalium*. The Loeb Classical Library and Oxford University Press editions, and Balme have useful comments by the translators.

Other sources are Adelmann (1942,

*1966), Balss (1936), Cole (1930), Downey (1962), Düring (1966), Farrington (1949), Grene (1963), Jaeger (1948), Locy (1925), Lones (1912), Magner (1979), Morsink (1982), *Needham (1959), Oppenheimer (1955, 1971*a*), Owen *et al.* (1970), Peters (1968), Preus (1970, 1975, 1977), Randall (1960), Ross (1930), E. S. Russell (1930), Sarton (1952), Singer (1922, 1960), D'Arcy Thompson (1922, 1940), and Woodbridge (1965).

THE DAWN OF NATURALISTIC THOUGHT

One of the most astonishing events in intellectual history is the sudden appearance, seemingly *de novo,* of naturalistic thought—so dominant in the science of Aristotle. This is the procedure of basing explanations of natural phenomenon on the things and processes of nature. For example, when ascertainable and specific meteorological conditions prevail, liquid water is precipitated from clouds as rain. This is in marked contrast to supernatural or mythical explanations, which assume that some god or intangible force is the cause, such as rain is the tears of weeping gods. W. K. C. Guthrie (1962, p. 40) credits Aristotle with contrasting these polar modes of thought:

> It is to Aristotle in the first place that we owe the distinction between those who described the world in terms of myth and the supernatural, and those who first attempted to account for it by natural causes. The former he called *theologi,* the latter *physici* or *physiologi,* and he ascribes the beginning of the new, 'physical' outlook to Thales and his successors at Miletus, hailing Thales himself as 'first founder of this kind of philosophy'.

"This kind of philosophy" has been fundamental to the advance of science.

Miletus, a seaport on the Ionian coast (now Turkey), was settled by Greeks about 1000 B.C. It was the home of three philosophers who, in the absence of earlier evidence, are the first we know who systematically used naturalistic thought to explain natural phenomena. Thales (*ca.* 625–547 B.C.), the first, was followed by his pupil Anaximander (*ca.* 611–547 B.C.) and later by Anaximenes (*ca.* 585–528 B.C.). Among other problems these Milesians were concerned with the basic materials of which all physical objects are composed. Thales thought the elemental substance was water, Anaximenes thought it was air, and Anaximander assumed some unknown and even more basic substance.

Aristotle (*Metaphysics,* 983^{b}, 20ff.) offers the following suggestion for the origin of Thales' view:

> Thales . . . says the principle is water (for which reason he declared that the earth rests on water), getting the notion perhaps from seeing that the nutriment of all things is moist, and that heat itself is generated from the moist and kept alive by it (and that-from-which-they-come-to-be is the principle of all things). He got his notion from this fact, and from the fact that the seeds of all things have a moist nature, and that water is the origin of the nature of moist things.

Thus such elemental stuff as water could be modified as plants, animals, mountains, soil, clouds, etc. Human beings consume water, air, plants, and animals and convert them into human substance and, upon death, all change back to water once again. Hence, it was not too far fetched to suspect that there was a common building block for all matter. This search for elemental particles, of which all substances are composed, has concerned philosophers and later scientists (when the two groups became different after the Middle Ages) until the present. It remained for the English scientist, John Dalton (1766–1844), to provide acceptable evidence for atoms, predictions for which go back to ancient times. In our century, the "indivisible" atoms have dissolved into a hierarchy of subatomic particles.

The specific hypotheses of the Milesians were of little value; it was their approach that was so novel and so important. Many things suggested to Thales

> that *if there is any one thing at the basis of all nature,* that thing must be water. If

> there is any one thing! This supposition, that is to say, the asking as it were of this question, constitutes Thales' claim to immortality. The fact that he made a guess at the answer, and a pretty good guess at that, is of minor importance. If he had championed the cause of treacle as the sole "element" he would still have been rightly honoured as the father of speculative science. True, others before him (such as Homer and Hesoid) had sketched the origin of the world from one substance, but they were not content to deal with *verae causae,* that is with things whose existence can be verified by observation. To attempt to explain the origin and process of the world by having recourse to gods and spirits endowed with special powers, is merely to beg the question, since the existence of such beings can never be proved (nor of course disproved) by the means wherewith we know that world. In a word, it was Thales who first attempted to explain the variety of nature as the modifications of something *in nature* (Wightman, 1951, pp. 10–11).

The Milesians were asking fundamental questions and proposing *naturalistic* hypotheses in contrast to all others who invoked supernatural forces—the earth being formed from the body of the goddess Tiamat, for example.

One might have imagined that such an important philosophical shift would be based on a substantial amount of original written material. In fact, there is none. What is known about Thales is based on brief mention by Aristotle (*Metaphysics* 983^{b}, 20ff.; 984^{a}, 2) and a few other ancient writers. Our evaluation of the Milesians, therefore, is based mainly on the opinion of Aristotle—*he* thought they had made an intellectual breakthrough and there is no reason not to accept his conclusion.

The Frankforts (1977, p. 376) offer this paean:

> The Ionian philosophers gave their attention to the problem of origins; but for them it assumed an entirely new character. The origin . . . which they sought was not understood in the terms of myth. They did not describe an ancestral divinity or a progenitor. They did not even look for an "origin" in the sense of an initial condition which was superseded by subsequent states of being. The Ionians asked for an immanent and *lasting* ground of existence . . . .
>
> This change of viewpoint is breath-taking. It transfers the problem of man in nature from the realm of faith and poetic intuition to the intellectual sphere. A critical appraisal of each theory, and hence a continuous inquiry into the nature of reality, became possible. A cosmogonic myth is beyond discussion. It describes a sequence of sacred events, which one can either accept or reject. But no cosmogony can become part of a progressive and cumulative increase of knowledge . . . . Myth claims recognition by the faithful, not justification before the critical. But a sustaining principle or first cause must be comprehensible, even if it was discovered in a flash of insight. It does not pose the alternative of acceptance or rejection. It may be analyzed, modified, or corrected. In short, it is subject to intellectual judgment.

And for Guthrie (1962, p. 70),

> the perennial fascination exercised by the Milesians lies in just this, that their ideas form a bridge between the two worlds of myth and reason.

Your students might find it interesting to estimate the relative frequencies with which they use various sorts of reasoning in making everyday decisions. They may discover that naturalistic thought may not be the prevailing mode even in this Age of Science.

References to Ionian philosophy

Baldry (1932), Cherniss (1951), Cornford (1952, 1957), Dicks (1959), Farrington (1949), Frankfort *et al.* (1977), *Guthrie (1962), W. T. Jones (1952), Longrigg (1976), Magner (1979), Neugebauer (1957), O'Connor (1964), B. Russell (1945),

Sarton (1952), Taton (1963), Waerden (1961), and Wightman (1951).

GALEN

One might have anticipated that the combination of those "right-thinking" Ionians and that omnivorous observer and speculator about nature, Aristotle, would have begun a vigorous investigation of development, as well as other biological problems. Not at all. A peak was reached with Aristotle and, thereafter, there was a decline of interest and accomplishment for roughly two millennia.

The next major figure who wrote on embryological matters, five centuries after Aristotle, was the Greek physician Galen (*ca.* 130–200 A.D.). He was interested mainly in human anatomy and physiology but he did have a few things to say about development (Galen, 1916, book I, chapters 5–7, book II, chapter 3, and book III, chapter 3; Galen, 1968, books 14 and 15; see also Adelmann, 1942, *1966; Kudlein and Wilson, 1972 and Needham, *1959). He added little to Aristotle's work. The following quotation gives the flavor of his views, which were important since Galen's work was known in Western Europe during the Dark Ages when Aristotle's biology was not:

> Genesis [=embryogeny], however, is not a simple activity of Nature, but is composed of alteration [=histogenesis] and of shaping [=organogenesis]. That is to say, in order that bone, nerve, veins, and all other tissues may come into existence, the *underlying substance* from which the animal springs must be *altered.* In order that the substance so altered may acquire its appropriate shape and position, its cavities, outgrowths, attachments, and so forth, it has to undergo a *shaping* or formative process. One would be justified in calling this substance which undergoes alteration the *material* of the animal, just as wood is the material of a ship, and wax of an image (1916, p. 19).

Looking back, with the knowledge of what was to come, we can say that Galen was defining the fundamental problem of development—differentiation. The formative material must be "altered" since the early embryo, which is to become that adult, lacks the tissues and organs characteristic of the adult. The conversion itself would involve a variety of morphogenetic movements. Thus, novelty would appear in the course of development—Galen was an epigeneticist.

He was also the end for centuries. "The death of Galen in 200 A.D. marks the end of progress in embryological learning for over thirteen centuries" (Adelmann, 1942, p. 45). That length of time is really beyond comprehension. The American Revolution seems remote to most of us, yet those thirteen centuries were more than six times the interval between our national birth and today.

THE MIDDLE AGES

There are many possible reasons for those dark centuries. The political and social stability sustained by the Roman Empire was swept aside by degeneration from within and invasion from without. The rise of Christianity and the establishment of the church as the only effective institution in the West changed the topics for serious thought. The problems of the natural world were replaced by those of the supernatural world. The ability to read and write became rare skills. To be sure there was very little to read apart from theology. What education there was consisted mainly of instructions for those seeking a career of service to the Mother Church. Those with interests in science were rare, as they always had been, and insufficient to form that critical mass which is essential for sustained scientific progress. There were no universities where science was taught, no scientific academies, and few libraries. Essentially no Greek science, except for Galen, was available in the West.

But even if these constraints of the Middle Ages had not existed, *what* was one to do in order to extend Aristotle's and Galen's analysis? The answer is far from obvious. The major questions they had raised were not really approachable until the 19th century when it first became possible to work at the cellular level. One could continue to observe the gross features of

development in any embryos that were available. Basic processes and causal relations could not be studied.

It is most unlikely that fascination with the mystery of development, especially human development, ever ceased.

> We may assume that every thinking man has asked himself some questions with regard to the formation and development of embryos, for such questions are continually forced upon him by life itself (Sarton, 1931, p. 315).

Needham (1959, p. 65) reports that "Cleopatra, the Ptolemaic queen, had investigated the process of development by the dissection of slaves at known intervals of time from conception, following the precepts of Hippocrates with regard to hen's eggs." Kottek (1981) adds the following to this report, quoting ancient sources:

> It happened that Cleopatra, the Queen of Alexandria, presented to the physicians some of her maids who had been condemned to death and they were dissected. It was found that the male embryo is complete after forty-one days and the female embryo after eighty-one days.

Other versions of the account differ in maintaining that there are no differences in the times males and females are "complete."

There were some isolated observations on embryos during the Middle Ages and early Renaissance. The developing hen's egg was the usual object of study. By the time of Albertus Magnus (1193?–1280), the works of Aristotle were becoming available and Albertus was a close student of the Master's works. He described the development of the chick, but seemingly his information came only from the Master, not an opened egg. That was standard procedure for the Middle Ages.

SCHOLASTICISM

The Scholastic Method for arriving at truth has been much maligned by later scholars. A debased variation of it remains an important pattern of thought for many people to this day. The method consists basically of accepting the opinions of others rather than data personally obtained by observation and experiment. Since the opinions of others might differ, a formal way of seeking "truth" became common: proposition, opposition, and resolution. That is, the question was raised and the supporting answers of accepted authorities were listed. Then the opposing answers were listed and, finally, an attempt was made to resolve the differences and reach some acceptable conclusion. This Scholastic Method was eminently suited for those whose disputations were on theological subjects. In fact, it is hard to think of any other way to decide such questions, short of resorting to violence.

Scholasticism was a unification of theology and philosophy with the central goal of proving the existence of God. It would have been of little importance for science had it not been, for centuries, the dominant mode of thought of intellectuals—the group from which those with an interest in science would have been expected to emerge.

Truth existed in the mind of God and it was the task of mortals to fathom what that truth might be. The procedure was logical reasoning based on scripture, church dogma, and the opinions of revered philosophers. Thus, in the last analysis, all data was derived from revelation and right-thinking people. Faith came first, understanding later.

A notable exponent of scholasticism was Peter Abelard (1079–1142) who, however, exposed the fundamental weakness of the approach. In his famous book *Sic et Non* he lined up the "Yes" and "No" opinions about the same question and showed that equally respected sources could hold diametrically opposed points of view. He had real problems with the Church on that score but even more problems of another sort. This is the Abelard who had an affair with the beautiful and loving Heloise. Her father felt strongly about that and had Abelard castrated to cool his ardor. It did.

Scholasticism precluded science. Even those who were interested in science looked to Aristotle for the answers, not to nature herself—as we have already observed for Albertus Magnus. This was a far cry from

those naturalistic Greeks who probed nature with mind to obtain understanding in contrast to those who sought understanding by probing mind with mind.

This point of view may be difficult for us to comprehend today but possibly this appraisal by Brehaut (1912, pp. 67–68) of Isidore of Seville (the author of the most extensively used encyclopedia of the Middle Ages) may help:

> The view held in the dark ages of the natural and the supernatural and of their relative proportions in the outlook on life, was precisely the reverse of that held by intelligent men in modern times. For us the material universe has taken on the aspect of order; within its limits phenomena seem to follow definite modes of behavior, upon the evidence of which a body of scientific knowledge has been built up. Indeed at times in certain branches of science there has been danger of a dogmatism akin to, if the reverse of, that which prevailed in medieval times with reference to the supernatural. On the other hand, the certainty that once existed in regard to the supernatural world has faded away; no means of investigating it that commands confidence has been devised, and any idea held in regard to it is believed to be void of truth if inconsistent with the conclusions reached by science. In all these respects the attitude of Isidore and his time is exactly opposite to ours. To him the supernatural world was the demonstrable and ordered one. Its phenomena, or what were supposed to be such, were accepted as valid, while no importance was attached to evidence offered by the senses as to the material. It may even be said that the supernatural universe bulked far larger in the mind of the medieval thinker than does the natural in that of the modern, and it was fortified by an immeasurably stronger and more uncritical dogmatism.

Isidore of Seville was a man of extraordinary intellectual powers yet he was molded by his time—as we are by ours. Had he been alive today, he could have been a truly first-rate molecular biologist.

The medieval mind remains hale and hardy in many today and it continues to be resented by scholars in and out of the sciences. Bertrand Russell's (1945, p. 463) evaluation of Saint Thomas Aquinas deals with the medieval mind, whatever the period of its existence:

> He does not, like the Platonic Socrates, set out to follow wherever the argument may lead. He is not engaged in an inquiry, the result of which it is impossible to know in advance. Before he began to philosophize, he already knows the truth; it is declared in the Catholic faith. If he can find apparently rational arguments for some parts of the faith, so much the better; if he cannot, he need only fall back on revelation. The finding of arguments for a conclusion given in advance is not philosophy, but special pleading.

References to Medieval thought

Artz (1965, ch. 7), Haskins (1927, ch. 11), Knowles (1962), Taylor (1951, chs. 35–37).

The Rebirth of Naturalistic Thought

Slowly scholasticism revealed its inadequacy as a method of understanding man or nature and the inquisitive turned elsewhere. By the 13th century essentially all of Greek philosophy and science, with their fresh and open-ended procedures, became available to western scholars. Once the awe of the Greek accomplishment was overcome and the bondage to accepted authority had been broken, scholars could imitate what the Greeks did, not parrot what they said. Science became possible once again.

There was renewed interest in embryology. Leonardo da Vinci (1452–1519) both observed human embryos and left us some beautiful drawings of them and there are many other fragments of embryological observations and speculations. It is more realistic, however, to renew the narrative with Fabricius (1533?–1619) who for most of his active life was a crusty professor of medicine at the University of Padua and the teacher of William Harvey.

HIERONYMUS FABRICIUS OF AQUAPENDENTE

There was still the basic problem of trying to formulate questions that could be answered. This proved elusive so the study of development concentrated on what was possible—describing normal development. This is not an unworthy goal. Science seeks to associate and conceptualize the phenomena of nature. That activity, quite obviously, depends on knowing what the phenomena are.

Fabricius's *De Formatione Ovi et Pulli,* mainly about the chick, and *De Formato Foetu,* mainly about mammalian development, date from about 1600. Chicken eggs were opened daily after the beginning of incubation and the embryos were studied and drawn as in Figure 1—the earliest illustrations that have survived. No magnification was used (compound microscopes were just in the process of being invented). No wonder he said that in the four-day chick the body looks like a very tiny flea. By the fifth day, however, Fabricius could make out the head, eyes, heart, arteries, veins, liver, and lungs.

In this description of the developing chick, Fabricius combined the work of Aristotle and Galen with his own. When we remember that these three span nearly two thousand years, the advances made in embryology appear most modest. There is a strong Scholastic streak that makes Fabricius most reluctant to disagree with his illustrious predecessors, especially Aristotle. Nevertheless, he helped to keep alive an interest in the subject, he corrected some of the errors of Aristotle and Galen and adds a few of his own—such as believing that the embryo arose from the chalazae instead of the blastoderm, which he regarded as a scar representing the place where the egg attached to the ovary.

Those who studied embryology of the chick in the 15th–17th centuries were not biologists in the modern sense but physicians who were studying more convenient organisms in order to better understand human development. It is remarkable that they thought this was possible but, ages before, Aristotle and Galen had shown that the vertebrate embryos with which they were familiar all resemble one another to some degree. Therefore it seemed acceptable to study the chick, so easy to obtain, instead of the human embryo that was impossible to obtain in the early stages.

None of these observations led to any practical medical result so, one might ask, why was such work done? To put this question in perspective it must be remembered that very few individuals in the 16th and 17th centuries were so occupied. For those who were, however, attempts to gain understanding were most serious. They studied ancient authorities with care, made what observations they could, and speculated according to the canons of contemporary philosophy. They sought knowledge for its own sake. Progress was well nigh imperceptible—awaiting the tools and technology necessary to collect the necessary data and the addition to observation and speculation of the third element of basic scientific procedures—controlled experimentation.

The embryological treatises of Fabricius have been translated by Adelmann (*1942), who provides a biography and discussion of Ancient and Medieval embryology. The two volumes are a monument to scholarship and publishing. See also Meyer (1939) and Needham (1959).

But slowly the procedures of modern science were penetrating mind and laboratory. A colleague of Fabricius at Padua, Cesare Cremonini, wrote in 1596 that both teaching and learning must be based on "logic, with the opportune intervention of experience." The requirement for logic is obvious—there must be disciplined reasoning. Experience is necessary "because, though one be instructed by genius [*i.e.,* Aristotle] or by logic, unless he be also experienced in the very thing in which he is to judge, he will there exercise no judgment." But it is not always easy to make that opportune intervention of experience because "in the natural sciences such observation is not so obvious a way of gaining principles, nor is the collection of principles by its employment so easy. There is indeed required a laborious attention, procured from a zealous application to things;

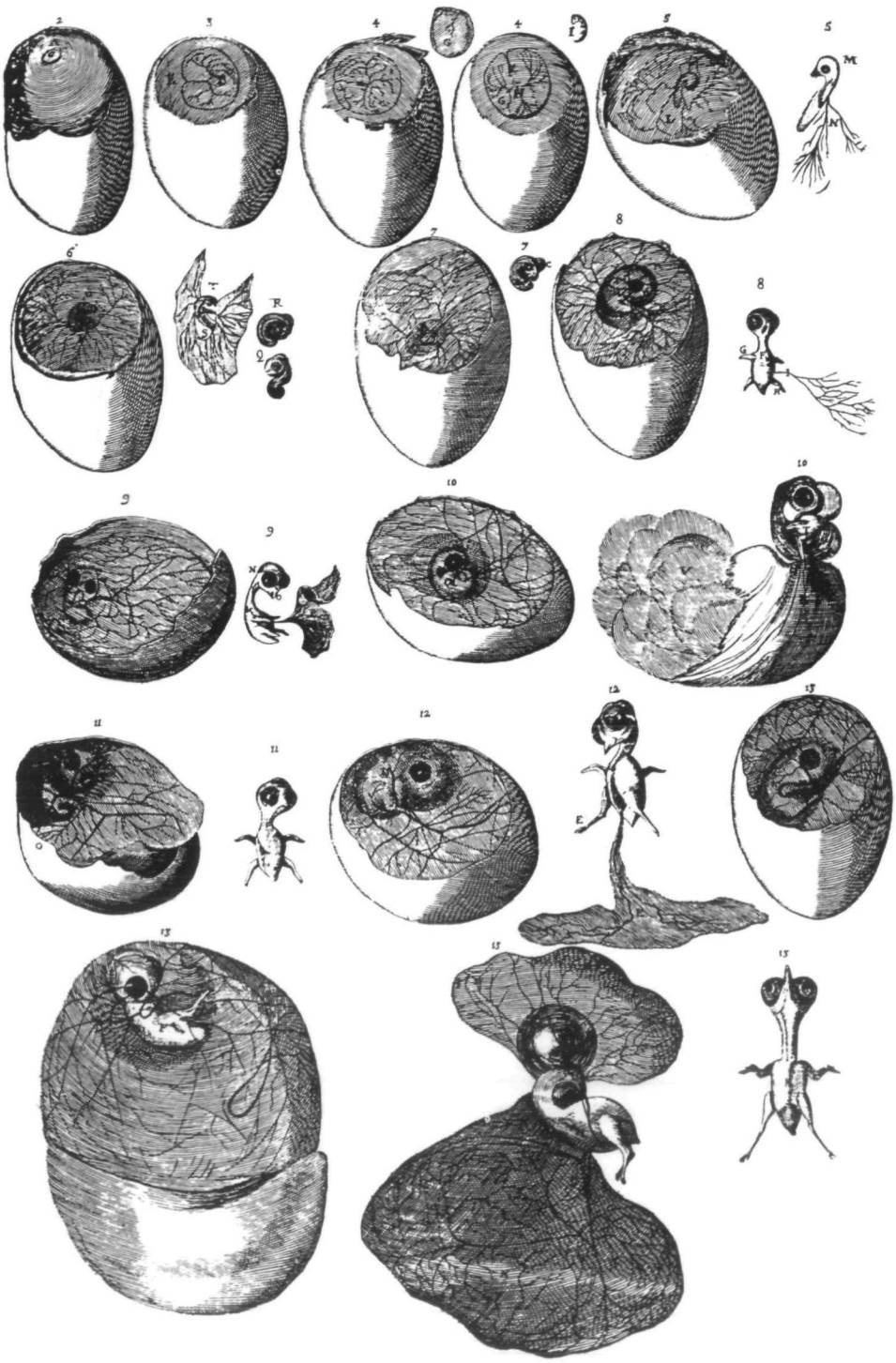


FIG. 1. The formation of the chick as seen by Fabricius in the early 1600s. The numbers above each illustration show the days of incubation. No embryo could be detected on the second day. On the third day there were blood vessels. The tiny figure identified as "I" to the right of the rightmost four-day egg shows the excised embryo. A head and spine could be identified. (From Adelmann, 1942.)

and even with it the principles are arrived at not without keen thought" (these quotations are from Randall, 1940, p. 204). Cremonini felt that progress was simpler in mathematics.

So the "zealous application to things" continued.

WILLIAM HARVEY

With the advent of William Harvey (1578–1657)—yes, the one concerned with the circulation of blood—there began

> the transition from the static to the dynamic conception of embryology, from the study of the embryo as a changing succession of shape, to the study of it as causally governed organization of an initial physical complexity (Needham, 1959, pp. 116–117).

Harvey was a student of Fabricius at Padua from 1598 to 1602 and this is where he first studied embryology. His *Exercitationes de Generatione Animalium* was published in 1651, half a century after his teacher's book on the same subject.

> Since our observations lead us to conclude that many things of great consequence are very different from what they have hitherto been held to be, I shall myself give an account of what goes on in the egg from day to day, and what parts are there transmuted, directing my attention to the first days especially, when all is most obscure and confused, and difficult of observation, and in reference to which writers have more particularly drawn the sword against one another in defence of their several discordant observations, which, in sooth, they accommodate rather to their preconceived opinions respecting the material and efficient cause of animal generation rather than to simple truth (p. 226; all quotes are from the 1965 edition).

Harvey then describes the development of the chick for each day up to the 14th, and in general, thereafter. Fabricius had believed the embryo to arise from the chalazae; Harvey correctly recognizes the origin as the blastoderm (to him the "cicatricula").

> As soon as the egg, under the gentle warmth of the incubating hen, or of warmth derived from another source, begins to pullulate [*i.e.*, to start to become a pullet], this spot forthwith dilates, and expands like the pupil of the eye, and from thence, as the grand centre of the eye, the latent plastic force breaks forth and germinates. This first commencement of the chick, however, so far as I am aware, has not been observed by any one (p. 229).

So, at last, we know where development begins. Harvey used a magnifying glass and that may have been the reason why he was able to make this discovery.

There follows a detailed account of the chick's development in which the observations and opinions of earlier students, mainly Aristotle and Fabricius, are confirmed, extended, and corrected. Thereafter he discusses more general matters where the cogency of the argument replaces direct observation.

For example, he rejects the old Aristotelian view that the female contributes only the substance (menstrual blood) and the male the effective generating stuff (male semen).

> For the egg is to be viewed as a conception proceeding from the male and the female, equally endued with the virtue of either, and constituting a unity from which a single animal is engendered (pp. 270–271).

After watching the daily changes in the developing chick, Harvey accepts epigenesis as the mode.

> The structure of these animals commences from some one part as its nucleus and origin, by the instrumentality of which the rest of the limbs are joined on, and this we say takes place by the method of epigenesis, namely, by degrees, part after part; and this is, in preference to the other mode, generation properly so called (p. 334).

That other mode seems restricted to insects where there is a conversion of a caterpillar into a butterfly

> already of a proper size, which never attains to any larger growth after it is first born; this is called metamorphosis. But the more perfect animals with red blood are made by epigenesis, or the superaddition of parts (pp. 334–335).

This hypothesis of epigenesis is strengthened by another belief that all parts of the body are derived from the same basic materials:

> For out of the same material from which the first part of the chick or its smallest particle springs, from the very same is the whole chick born; whence the first little drop of blood, thence also proceeds its whole mass by means of generation in the egg; nor is there any difference between the elements which constitute and form the limbs or organs of the body, and those out of which all their similar parts, to wit, the skin, the flesh, veins, membranes, nerves, cartilages, and bones derive their origin. For the part which was at first soft and fleshy, afterwards, in the course of its growth, and without any change in the matter of nutrition, becomes a nerve, a ligament, a tendon; what was a simple membrane becomes an investing tunic; what had been cartilage is afterwards found to be a spinous process of bone [a remarkable conjecture but based on what Harvey could see], all variously diversified out of the same similar material (p. 339).

A final important hypothesis of Harvey is that all life comes from eggs. The frontispiece (Fig. 2) of the 1651 edition of *De Generatione Animalium* shows Zeus opening an egg from which emerges a bird, human being, katydid, porpoise (?), deer, snake, spider, lizard, and various plants. An inscription appears on the egg: *ex ovo omnia.* This is usually expanded to *omne vivum ex ovo,* but that precise phraseology does not appear. "Exercise the Sixty-Second" carries the title "An egg is the common origin of all animals." It is clear, however, that Harvey is not using "egg" in the customary restricted sense since, quoting Aristotle, he accepts spontaneous generation as a mode of origin for some creatures.

> [These organisms] whether they arise spontaneously, or from others, or in others, or from the parts or excrements of these, have this in common, that they are engendered from some principle adequate to this effect, and from an efficient cause inherent in the same principle. In this way, therefore, the primordium from which and by which they arise is inherent in every animal. Let us entitle this the primordium vegetale or vegetable incipience, understanding by this a certain corporeal something having life in potentia; or a certain something existing *per se,* which is capable of changing into a vegetative form under the agency of an internal principle. Such primordia are the eggs of animals and the seeds of plants; such also are the conceptions of viviparous animals, and the worm, as Aristotle calls it, whence insects proceed: the primordia of different living things consequently differ from one another; and according to their diversities are the modes of generation of animals, which nevertheless all agree in this one respect, that they proceed from the vegetal primordium as from matter endowed with the virtue of an efficient cause, though they differ in respect of the primordium which either bursts forth, as it were, spontaneously and by chance, or shows itself as fruit or seed from something else preceding it. Whence some animals are spoken of as spontaneously produced, others as engendered by parents (p. 457).

Obviously the "efficient cause" is a critical element in Harvey's explanation. Here he is using the Aristotelian terminology, which recognized four causes: final, efficient, formal, and material. The *final cause* is the purpose of the object. The final cause of the chick embryo is to produce a chicken. Somehow the end was thought to influence the process—today we call this teleology and shudder at the notion. The *efficient cause* represents the underlying control. The efficient cause might be developmental mechanisms that control the chick's devel-

FIG. 2. The elegant frontispiece from William Harvey's (1651) *Generatione*.

opment. The *formal cause,* or form, may possibly be thought of as the DNA code that results in the embryo developing as, and becoming, a chicken. The *material cause* is the matter in the egg that is converted to the chick.

Aristotle's four causes have led to much confusion. The main problem is that scholars have failed to employ current words for Aristotle's ideas. Today only the "efficient cause" has even a remotely useful and modern meaning. (See Aristotle's *GA,* 715^{a}, 1–19 and especially the Introduction in Peck's translation, pp. xxxviii–xli.)

Thus when Harvey speaks of the "efficient cause" he means whatever it is that is controlling development. The nature of this efficient cause was an insoluble question for Harvey largely because he could not establish any continuity between material derived from the male and female and the offspring:

> Neither is there anything contained in the uterus immediately after intercourse, which, proceeding from the male, or from the female, or from both, can be regarded as the matter or rudiment of the future foetus (p. 356).

Thus he was demolishing Aristotle's hypothesis of menstrual blood and male semen as the contribution of parents to offspring. But something had to be the basis of genetic continuity, even where there seemed to be none, and Harvey proposed this hypothesis:

> So much is certain, and disputed by no one, that animals, all those at least that proceed from the intercourse of male and female, are the offspring of this intercourse, and that they are procreated as it seems by a kind of contagion, much in the same way as medical men observe contagious diseases, such as leprosy, lues venera [syphilis], plague, phthisis [tuberculosis], to creep through the ranks of mortal men, and by mere extrinsic contact to excite diseases similar to themselves in other bodies; nay, contact is not necessary; a mere halitus [breath] or miasma suffices, and that at a distance and by an inanimate medium, and with nothing sensibly altered: that is to say, where the contagion first touches, there it generates an "univocal" like itself, neither touching nor existing in fact, neither being present nor conjunct, but solely because it formerly touched. Such virtue and efficacy is found in contagions. And the same thing perchance occurs in the generation of animals (p. 358).

This was not a satisfactory solution and Harvey recognized as much. Embryology awaited Leeuwenhoek, von Baer, and Oscar Hertwig to establish the contributions of the parents. Furthermore, the fundamental questions that had concerned all embryologists from Aristotle to Harvey were to remain unanswered until controlled experimentation became possible and practiced. The field had to await George Newport, two centuries in the future. Harvey was a skillful experimenter in other fields, but not in embryology. According to Adelmann (1942, p. 121) Harvey

> in his work on generation did not escape the influences which mar the work of Fabricius; both were, in fact, deeply imbued with the spirit of the times in which they lived. Harvey built upon the foundations laid by Fabricius, and so in some cases approximated more closely the truth as we see the truth today; but Fabricius no less than Harvey contributed to its slow advance. Both struggled with problems far too difficult for their age to solve, but both contributed documents precious in the history of biological thought.

THE SCIENTIFIC REVOLUTION

So, once again, an embryologist provided better observations, corrected more errors, and sharpened speculation, but achieved no paradigm shift. Harvey lived during the early decades of the Scientific Revolution of the 17th century—a period of great intellectual ferment. The umbilicus to Aristotle was being severed, the world of nature was being accepted as fit for inquiry, science was becoming respect-

able, observation and experimentation were replacing sole reliance on authority and deductive speculation, order was being discovered in the physical universe, censorship of Church and state was being challenged, theology was dethroned as the Queen of the Sciences, freedom of person and thought was increasing, theory and practice could be united in the same individual, universities were spreading, the Royal Society for the Improving Natural Knowledge was established (1662), the followers of Gutenberg (*ca.* 1400–1468) were hard at work, and the spread of prosperity increased the number of scholars who could work outside the Church.

There was no sudden springing to the barricades at the onset of the Scientific Revolution. In fact, there was no obvious beginning—only a slow spread of a new way of defining the methods of obtaining understanding of natural phenomena. Some historians date the onset of the Scientific Revolution at about 1660, near the time of the founding of the Royal Society of London (Burns *et al.*, 1986, pp. 861, 863). Such a date, however, seems odd since it excludes Vesalius, Harvey, Bacon, Copernicus, Galileo, Kepler, and Brahe and thus omits most of the intellectual giants who truly gave us science as a way of knowing. They are defined as the anticipators of the Scientific Revolution, and their exclusion leaves the period of the Scientific Revolution rather depopulated—since B. Russell (1945, pp. 525–526) writes, "Four great men—Copernicus, Kepler, Galileo, and Newton—are pre-eminent in the creation of science."

For reasons about to be mentioned my preference is 1543. No matter which birth date is selected we can speak of a "revolution" because some exceptional contributions to natural knowledge were made in a relatively brief time.

The year 1543 saw three key events in the history of science. One was the publication of *De Humani Corporis Fabrica* by Andreas Vesalius (1514–1564, a Belgian who became Professor of Anatomy at the University of Padua, where Fabricius later taught). Prior to this event Galen's anatomy was the authority. Vesalius was able to dissect human bodies and found that Galen was inaccurate in some instances. *De Humani Corporis Fabrica* has not only a complete description of gross anatomy but also beautiful illustrations by the Belgian artist Jan van Calcar (and not by Albrecht Dürer as has been suggested). This was the beginning of modern anatomy and is a straight path to Grey. Initially Vesalius had much opposition since even suggesting that such an ancient and respected authority as Galen might have erred was not in the best of taste. This opposition was primarily the attitude of the Church, which had a vested interest in the sanctity of (its) traditions and took severe measures to see that they were preserved.

A second accomplishment in 1543 was the publication of *De Revolutionibus Orbium Coelestrium* by the Polish physician, clergyman, and astronomer Nicholas Copernicus (1473–1543). Here is the beginning of modern astronomy. His hypothesis of heliocentrism lacked a convincing evidential base. It was only later that heliocentrism was made true beyond all reasonable doubt with the carefully collected data of the Danish astronomer, Tycho Brahe (1546–1601); and improvements in theory by the German astronomer, Johann Kepler (1571–1630); and the observations of the Italian physicist and astronomer Galileo Galilei (1564–1642). (Poland, Denmark, Germany, and Italy! science was truly international.)

The reaction of the church to this demotion of the earth, the site of God's creations, is well known. The Dominican monk, Giordano Bruno, who opposed all dogmatism and, in the main accepted the Copernican theory, paid for his intellectual independence by being burned at the stake in 1600. Luther had this to say about Copernicus "This fool wishes to reverse the entire science of astronomy; but sacred Scripture tells us that Joshua commanded the sun to stand still, and not the earth." Matters became truly serious after Brahe, Kepler, and Galileo had done their work. There was no escaping the conclusion that the earth rotates on its axis each day and circles the sun each year. Galileo had two trials by the Inquisition, and at the second

in 1633 made a public recantation of his belief in the heliocentric theory.

Galileo's crime was less what he said about the movement of celestial bodies and more that he challenged the authority of the Church, which held that the earth was the center of the universe. There are three good reasons why Copernicus, who after all was the author of the theory that got Galileo into trouble, largely escaped the wrath of the Church: he was careful to say that his notion "was just a theory," he dedicated his *De Revolutionibus* to the Pope, and most importantly he died very shortly after its publication.

For details of Galileo's accomplishments and persecution, both so important in the progress of science, see Bernardini and Fermi (1965), Campanella (1616), Drake (1957, 1970), Galilei (1615), Kaplon (1965), McMullin (1967), de Santillana (1955), and Shea (1972). For Redondis' recent and controversial reinterpretation of the Galileo affair see Dickson (1986).

The third notable event in 1543 was the recovery, translation, and publication of the works of the Greek physicist and mathematician, Archimedes (287–212 B.C.). He had made astonishing contributions to mathematics and mechanics, and he was a notable inventor. He viewed the universe itself as a gigantic machine, operating on mechanical principles. This was a liberating notion in the 16th century when the forces of nature were thought mystical and probably unknowable. The mechanics of Archimedes was to be basic to the work of Galileo and then to that of Newton.

So it seems appropriate to start the Scientific Revolution in 1543 with Vesalius, Copernicus, and Archimedes. Their works and the spirit they engendered would be part of the intellectual climate of Fabricius and Harvey, whose work in embryology has already been discussed. (History remembers Harvey less for his work on the embryology of the chick and more for *Exercitatio Anatomica de Motu Cordis et Sanguinis* of 1628. According to Frank (1980, p. xii) this was "the single most important discovery in the history of the physiological sciences—the circulation of the blood." It was also the beginning of physiology as an exact science.)

Another notable event that was truly part of the Scientific Revolution but precedes its traditional date of onset—1660—is the works of Sir Francis Bacon (1561–1626): *The Advancement of Learning* (1605) and *Novum Organum* (1620). (A discussion of Bacon's work will be found in III, pp. 591–596.)

When we do reach the 1660s, finally, there are two initial events that were to have profound influences for embryology—the foundation of the Royal Society in 1662 (III, p. 608) and discovery of cells by the Englishman Robert Hooke in 1663 (III, pp. 608–610). The first was to stimulate the development of science itself and the second was to provide, nearly two centuries later, a more fundamental level of embryological analysis.

For many the climax of the Scientific Revolution is to be found in the works of the Englishman Sir Isaac Newton (1642–1727): *Philosophiae Naturalis Principia Mathematica* and *Opticks* (1704). His genius ranged from mathematics, astronomy, mechanics, and light to the laws of motion and gravitation.

From the death of Newton, there has been steady progress in science, and in quantity at least it seems to adhere to Galileo's law of acceleration. Science as a way of knowing was here to stay.

> The men who founded modern science had two merits which are not necessarily found together: immense patience in observation, and great boldness in framing hypotheses. The second of these merits had belonged to the earliest Greek philosophers; the first existed, to a considerable degree, in the later astronomers of antiquity. But no one among the ancients, except perhaps Aristarchus, possessed both merits, and no one in the Middle Ages possessed either (B. Russell, 1945, pp. 527–528).

We can turn to Henry Power (1623–1668) for an understanding of the high hopes of the Scientific Revolution. He had recently been made a member of the Royal Society and in the conclusion of his *Experimental Philosophy* (1664) he addresses those "generous Virtuosi, and Lovers of Experimental Philosophy" as follows:

> Certainly this World was made not onely to be Inhabited, but Studied and Contemplated by Man; and, How few are there in the World that perform this homage due to their Creator? . . . It is Reason that transpeciates our Natures, and makes us little lower than the Angels . . . . There is a world of People indeed, and but a few men in it; mankind is but preserv'd in a few Individuals; the greatest part of Humanity is lost in Earth, and their Souls so fixed in that grosser moity of themselves (their Bodies) that nothing can volatize them, and set their Reasons at Liberty . . . .
>
> And this is the Age wherein all mens Souls are in a kind of fermentation, and the spirit of Wisdom and Learning begins to mount and free it self from those drossie and terrene Impediments wherewith it hath been so long clogg'd, and from the insipid phlegm and *Caput Mortuum* of useless Notions, in which it has endured so violent and long a fixation.
>
> This is the Age wherein (me-thinks) Philosophy comes in with a Spring-tide . . . . Me-thinks, I see how all the old Rubbish must be thrown away, and the rotten Buildings be overthrown, and carried away with so powerful an Inundation. These are the days that must lay a new Foundation of a more magnificent Philosophy, never to be overthrown: that will Empirically and Sensibly canvass the *Phenomena* of Nature, deducing the Causes of things from such Originals in Nature, as we observe are producible by Art, and the infallible demonstration of Mechanicks: and certainly, this is the way, and no other, to build a true and permanent Philosophy (pp. 183, 184, 192).

And they did.

THE CONTRIBUTION OF VIVIPAROUS FEMALES

Apart from the possible research of Cleopatra, embryological studies from earliest times to the 17th century were the work of males. As such they were well aware of the male's contribution to conception but that of the female was confusing. Aristotle had recognized several patterns of generation (see Peck's translation of *GA*, pp. lxxii and lxxiii). Oviparous females like the hen laid eggs from which the young hatch. Ovoviviparous females such as sharks and some snakes have eggs but these were retained in the body until hatching. Females of human beings and other mammals, however, puzzled Aristotle and many who followed him. They thought that menstrual blood or some other secretion of the female contributed to conception. Harvey refuted this notion because he could find nothing in the uteri of deer after mating that might be the beginning of the new individual. Nevertheless it was assumed that the female must contribute something.

The answer seemed to come with some observations of de Graaf (1672) on the mammalian ovary, which at the time was called the *testis muliebris* (=female testis). Its function, if any, was unknown. Harvey thought that it had no role in copulation or generation. De Graaf found that some ovaries had spherical structures and he suspected they might be the long sought mammalian eggs or be "egg nests" (Sarton, 1931, p. 232). They came to be called Graafian follicles. This made it seem more reasonable, to many at least, that Harvey's dictum, *ex ovo omnia,* might be correct. Subsequently it was established by von Baer that Graafian follicles are not eggs but structures in which the eggs are formed.

MALPIGHI

Marcello Malpighi (1628–1694), an Italian biologist, followed Harvey by a generation. He was a professor at the University of Bologna for many years. His scientific contacts, however, were mainly with the Royal Society of London with which he corresponded actively—describing his latest discoveries in great detail and receiving encouraging letters from the Secretary.

The Royal Society published his two main works on the development of the chick (1672, 1675). They consist of minute descriptions of what he could see not only with the unaided eye but also with magnification—he was one of the first biologists to use the rapidly improving microscopes of the day. He had a variety of instruments and according to Adelmann (1965, p. 830) was able to obtain magnifi-

cations as high as 143×. Malpighi had no deep interest in causal factors and, in this sense, he contributed little. His descriptive embryology, however, was masterful.

Malpighi found that he could remove the blastoderm from a chick egg and place it on a glass slide. This simple technique, followed to this day, made it far easier to use the microscope in making observations. Such preparations remained useful even after drying. Apparently he did not take advantage of Robert Boyle's (1666) discovery that embryos could be preserved in Spirit of Wine (85+ percent alcohol) thus being available for study at more convenient times, or for comparing different stages of development, or even for demonstrating the drama of development to friends.

One important problem for Malpighi was to ascertain the structure of the chick embryo at the very beginning of development. This is what he observed (the illustration referred to is shown here in Fig. 3; Adelmann's 1965 translation is used, with his identifications of structures shown in brackets):

> In eggs laid the previous day and not yet incubated the cicatricula [blastoderm] (as I observed last August when the weather was very warm) was of the size I have roughly sketched in figure I,A. In the center of it there was found a cinereous [ash colored] saccule (B) [nucleus of Pander] that was sometimes oval, sometimes another shape. This saccule or follicle floated in the liquor of the colliquament (C) [blastoderm and area pellucida], which closely resembled molten glass and was confined in an irregular pit [subgerminal cavity], so to speak; for this colliquament was surrounded by a white ring of solid material (D) [germ wall] like an embankment, whose outer portion was bathed by a molten, limpid humor (E) [in area opaca]. Then followed a substance of little width (F) [area vitellina internal, often variously lancinated and likewise immersed in the humor (G). In addition, there were other, larger, surrounding circles (H), formed of the same more solid material and separated by channels of fluid (I). These outer circles (H), in particular, Nature does not form in one manner, and the material by which they are extended is not always continuous. Within the saccule, when I later held it against the sunlight, I noticed the fetus (L) enclosed as if in an amnion; and its head, with the first filaments of the *carina* [primordia of central nervous system] appended to it, was clearly evident. Indeed, the loose and diaphanous texture of the amnion [area pellucida] frequently permitted one to look through it and see the enclosed animal. I have often opened the follicle with the point of a needle to release the animal confined there, but to no purpose, for it was so mucous and so very tiny that in every case it was lacerated by a light touch. It is therefore proper to acknowledge that the first filaments of the chick pre-exist in the egg (pp. 941, 943, 945).

History has seized upon that last sentence to place Malpighi among the preformationists. If one assumes that an unincubated egg has not begun developing, the conclusion is inescapable that at least the beginnings of the chick's body are preformed in the "undeveloped egg." If at least some structures could be seen with the crude microscopes of the 17th century, it was reasonable to assume that much more was there awaiting discovery.

Adelmann (1965) has suggested that it is not necessary to conclude that Malpighi was a preformationist. Even in Malpighi's time it was suspected that whatever interactions occurred between the male and female contributions to the new individual took place while the egg was still in the body of the female. Thus, development *might* begin before incubation. Malpighi mentioned that the eggs he studied had been laid the previous day and the observations were made in "August when the weather was very warm." It would seem rather surprising for Malpighi to write about the weather when describing embryonic development unless he believed such metereological data were important. Undoubtedly they were. It is almost certain that considerable development had oc-

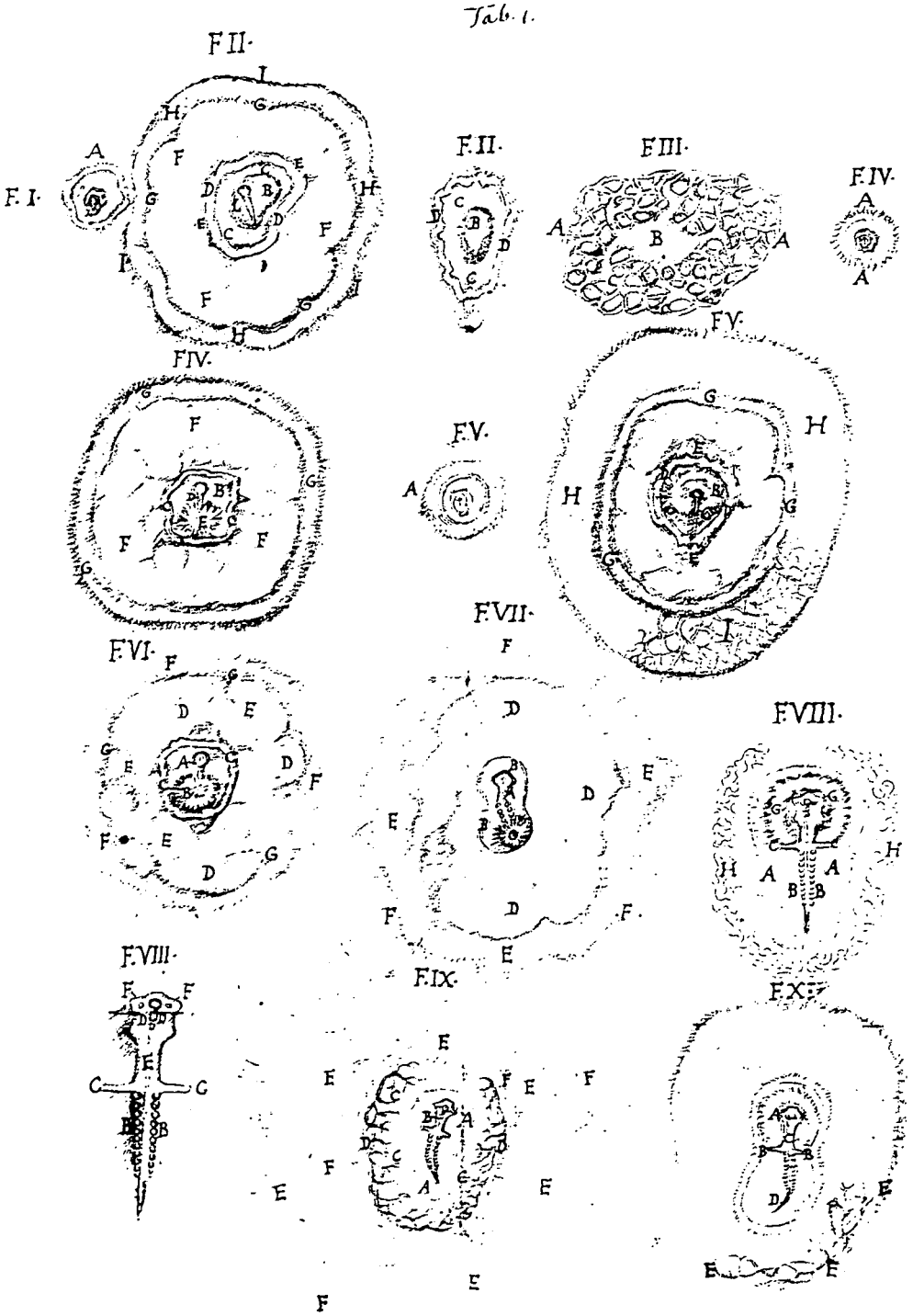


FIG. 3. Malpighi's drawings of the early development of the chick. "F I" is an unincubated egg in which Malpighi thought he saw the beginnings of the embryo. "F II" is after 6 hours of incubation; "F IV" and "F V" after 12 hours. The oldest shown, "F X," is about what we would call a 40-hour embryo. (From Adelmann, 1966.)

curred during that warm August day between the time the eggs were laid and Malpighi first studied them. A comparison of Malpighi's illustration of the unincubated egg with Huettner's (1941) figure 85 of an egg incubated 19 hours and figure 86, incubated 22 hours, suggests that a very warm August day can replace a brooding hen to some extent. This possibility is made more probable by the fact that the later stages described by Malpighi all seemed too advanced in terms of what later investigators found. (See also Hamburger and Hamilton, 1951, for an atlas of timed stages of chick development.)

Adelmann (1966) has provided us with an even more sumptuous and scholarly monograph on Malpighi than he did for Fabricius. It includes the originals and translations of Malpighi, a modern interpretation of what was known to Malpighi and other early students, a biography, and a remarkable account of the early history of embryology—a real *tour de force.*

* * * * *

Malpighi died in 1694 and the Royal Society, which had published his main works and corresponded with him for years, took note of the occasion by publishing an autopsy report (Phil. Trans. 19, pp. 467–471). This is quoted here as an aside—to give some notion of the state of biological science at the close of the 17th century.

> The Abdomen being opened, we found the Ventricle [here meaning the stomach], with the Guts, the Sweet-bread [pancreas], the Spleen and Liver, most sound, both as to colour and bigness; only the Bladder of the Gall abounded with a black Gall. The left Kidney had nothing amiss; but the right was twice as little, and had its *Pelvis* thrice as bigg; which discover'd the cause of the easie descent of the Stones. We found in the Bladder a little Stone, that seem'd to have fallen into it a few days before.
>
> When the Sternum was taken off, the Lungs appeared wither'd, with some mark of corruption on the backside. The Heart was bigger than ordinary, and the sides of the left Ventricle felt harder and thicker in some places than others; yet there was no Polypus found in either of the Ventricles, though there was ground to suspect it.
>
> At last the Skull being cut asunder, the true cause of his death was discovered, for the right Ventricle of the Brain contain'd almost two Ounces of extravasated Blood, and the left Ventricle was swell'd with a thick and yellow sort of Phlegm, which weigh'd more than an Ounce. Moreover the *Dura Mater* stuck closer to the Skull than is usual.

The advances in medical science had been sufficient by the end of the 17th century so that a proper diagnosis of the cause of Malpighi's death could be given.

> This proves that the conglobated Glands in the whole Body, had thrown into the Mass of Blood an Acid lymph, and that the conglomerated Glands of the Hypochondria [abdomen], especially those of the Liver had thrown into it a melancholy Humor, and that these two sorts of Humors being carried into the Vessels of the Brain, had dispos'd the Blood to coagulate there, and that having there corroded and broken the Tunicles [membranes] which serv'd for a stop to them, they had run into the Cavities, where they caused death without a Remedy.

Requiescat in pace.

A Two Millennial Summing Up

The study of development can be divided into two main categories: descriptive and analytical. Until recently the first has been primarily a morphological discipline. The course of development, from conception to maturity, was described in detail—how the embryo (apparently) changes and grows. Included was the characterization of whatever it is that parents contribute to their offspring at the time of conception. Analytical or experimental embryology is concerned with the mechanisms of embryological change, *i.e.*, how whatever it is that parents contribute to their offspring is converted into a new individual. Thus descriptive embryology is "What happens?" and analytical embryology is "How does it happen?"

What had Aristotle, Galen, Fabricius, Harvey, and Malpighi accomplished in descriptive and analytical embryology? Not much—nor could they. Aristotle was the one most interested in concepts and causes—analytical embryology; Malpighi the least. At the conceptual level one could have passed directly from Aristotle to the 18th century and lost almost nothing. But the embryologists were not uniquely unsuccessful. Progress was slow in all fields of biology and, for that matter, in all fields of science. Notable progress was made in only some aspects of physics and astronomy.

There are valid reasons for this conceptual stasis. Concepts must be based on data and during those long millennia the necessary data were unavailable. The data were to come from a then invisible level of analysis—the level of cells and their parts. First there had to be microscopes and then came knowledge of cells. Microscopes, though inadequate, became available in the late 17th century. On April 15, 1663 Robert Hooke had reported to the Royal Society his observations on cells in cork (III, pp. 608–610) yet nearly two centuries were to pass before cells became important in embryological explanations.

There seemed to be a few general principles of development that were true beyond reasonable doubt. All were known to Aristotle and this is a measure of the lack of significant conceptual progress. Here is the balance sheet.

1. Sexual reproduction, the interaction of males and females, is required in many species. It was assumed that there must be some material contribution but it was not known what it might be.

2. Both sexes influence the characteristics of the offspring but the mechanism of this influence was not understood. This means that not only was the basis of genetic continuity a complete mystery but so also were the mechanisms of transforming that basis into a new individual of the same type as the parents. There was no clear distinction between transmission of material and transformation of that material into a new individual.

3. The embryos of different species of the same major group, birds for example, resemble one another closely. There are even resemblances among various species of vertebrates—mammals, birds, and fishes.

4. Development appeared to be epigenetic, although Aristotle and later workers were unsure since microscopes were not adequate to show any minute beginnings.

Clearly the Scientific Revolution did not produce any vast improvement in the understanding of development. In fact, its effect on the life sciences as a whole was slight. Vesalius produced a better human anatomy than Galen's but no conceptual breakthrough was involved. Physiology started grandly with Harvey's observations and experiments on circulation, but thereafter progress was exceedingly slow.

One could argue that a knowledge of embryology did not have a high priority among scholars at that time and hence progress would be slow and episodic. True enough—there never was a critical mass of individuals concerned with development during those millennia.

But this cannot be the entire explanation since the same argument does not apply to medicine. There had always been many individuals with a deep concern for learning about human ailments and how to ameliorate them; yet progress was slow and seemingly the physicians were as perplexed as the embryologists at the end of the 17th century.

The following quotation, by an English physician Dr. James Cooke (1762), illustrates how much ideas in biology would have to change before modern understandings were to be possible. Cooke was a preformationist and an animalculist—one who believed that an already-formed body was located in the sperm, or animalcule. He was concerned with the fate of all those sperm which were present in semen but were not to be involved in conception:

> All those other attending Animacula, except that one that is conceived, evaporate away, and return back into the Atmosphere again, whence it is very likely they immediately proceeded; into the open Air, I say, the common Receptacle of all such disengaged minute sublunary bodies; and do there circulate about with other *Semina*, where, perhaps

> they do not absolutely die, but live a latent life, in an insensible or dormant state, like Swallows in Winter, lying quite still like a stopped watch when let down, till (they) are received afresh into some other male Body of the proper kind . . . to be afresh set on Motion, and ejected again in Coition as before, to run a fresh chance for a lucky Conception: for it is very hard to conceive that Nature is so idly luxurious of Seeds thus only to destroy them, and to make Myriads of them subservient to but a single one (quoted from Punnett, 1928, p. 506).

Not everyone would have accepted Cooke's analysis but this quotation suggests that the Middle Ages were alive and well in his thought patterns in the late 18th century. This quotation shows how much, of necessity, must be unlearned before progress was to become possible.

During the Scientific Revolution notable progress in conceptual science was being made only in the case of those natural phenomena that could be studied quantitatively. For example, primitive human beings had long observed the motions of stars and planets and had developed impressive predictive abilities. This line of analysis was extended in the Scientific Revolution when the data were used by Copernicus and Kepler. Data that suggested the laws of motion to Galileo and Newton were relatively easy to obtain, yet required genius to interpret, as were those that led Robert Boyle to see the relation of volume to pressure in gases. Newton's theory of gravitation, an undisputed stroke of true genius, again dealt with relatively simple relationships.

Progress in the separate sciences was to be, in a general way, inversely related to the complexity of the phenomena they were attempting to conceptualize and directly related to the ease with which relationships could be expressed in mathematical terms. Far more work had to be done before biology could enter a period of impressive and sustained progress.

It is important that students recognize that science can remain in a relatively sterile period such as those two millennia from Aristotle to Malpighi. Progress in science is usually presented to them as a series of consecutive discoveries that, if the time scale is omitted, suggest rapidity and inevitability. This is not so. Consider that most elegant feature of the Scientific Revolution, Newton's Theory of Gravitation. Once it had been formulated and applied to various phenomena, progress seemed to cease. Physicists today are still struggling to think further about gravity—what is "it" that seemingly pulls bodies towards one another in relation to their mass and distance apart. We know, most precisely, what gravity can do—not what it is.

Thus it should be noted that the seemingly inexorable advance of science is not a reflection of continuous progress in solving problems but of one advance now, another later. Progress should not be visualized as a host of parallel arrows but as a network with a very irregular advancing edge. One small area of that edge will be pushed out and only gradually will some of the adjacent areas be "pulled along." Progress in cytology and Mendelian genetics slowed until it was discovered that the data of one provided deep understanding of the other (III, pp. 653–660). Attempts to determine the age of the geological strata reached a stalemate until an advance in an entirely different field, radioactivity, provided new techniques and insights (I, pp. 487, 491–492, 513). Direct attempts to determine the nature of genes reached a dead end and further progress depended on advances in biochemistry. And embryology remained in an eddy until the equipment and techniques for studying cells became available.

One might say that embryologists were floundering. In fact, most scientists of all persuasions were. The early volumes of the *Transactions of the Royal Society* list the things considered at each meeting and these show the very elementary nature of the discussions and concerns. Those relating to biology were as follows: a description of an abnormal calf with no joints in the hind legs and with a three-parted tongue; Hooke's description of the appearance of

many things under his microscope, including a slice of cork; a test on whether or not one was unduly thirsty after eating viper flesh; suggestions for protecting ships' bottoms from being eaten by nauphagous ("ship-eating") worms; raising silkworms in Virginia; how to kill ratle-snakes in Virginia; the presence of shining worms in oysters; transfusions of blood between dogs and the suggestion that it would be good to know "whether those dogs, that have peculiarities, will have them either abolisht, or at least much impaired by transfusion of blood."

Yes, scientists were floundering but surely this is a necessary stage in the passage from ignorance to understanding. Floundering, at the very least, indicates activity and concern.

PREFORMATION AND EPIGENESIS

The resolution of the conflicting hypotheses, preformation or epigenesis, was the dominant theoretical problem of embryologists from the last quarter of the 17th century to the end of the 18th. This was also the first time that a sufficient number of individuals, a critical mass, was alive at the same time and so could engage in dialogue. One could now argue with the living instead of solely with the dead—a process of enormous importance in resolving issues, detecting errors, comparing techniques, and making scholarship seem worthwhile. Science is a social enterprise, obviously so today, thus it is necessary to have enough practitioners at any one time to interact effectively.

In its most restricted sense, preformation means that the parts of the adult exist as such, albeit much smaller, at the very beginning of development. Some preformationists, also known as "evolutionists," reported that they could see tiny organisms in eggs or in sperm. Although there is some doubt about Malpighi's position, as noted before, the following quotation from *De Formatione Pulli in Ovo* seems to represent the preformationist position:

> [When] studying attentively the genesis of animals from the egg, lo! in the egg itself we behold the animal already almost formed, and our labor thus is rendered fruitless. For, being unable to detect the first origins, we are forced to await the manifestation of the parts as they successively come to view (Adelmann, 1966, pp. 935, 937).

In epigenesis, on the other hand, the adult parts are not present at the beginning of development but appear seriatim as development proceeds even though the earliest stages of development can not be seen. Some embryologists, from Aristotle to Harvey, believed that epigenesis was the more probable hypothesis. Since neither hypothesis could be proven to be true beyond all reasonable doubt, cogent argument became the main method for defending one's position.

Today we tend to regard this effort to prove beyond all reasonable doubt that one or the other hypothesis is correct as possibly charming but probably silly. Neither is true. Those who debated preformation *vs.* epigenesis were concerned with the fundamental problem of differentiation. How could structures appear in the course of development from structureless material? What could be the stimulus that would convert structureless semen into heart, brain, legs, eyes and all the complex parts of the body? A 5th century B.C. Greek philosopher-scientist, Anaxagoras of Clazomenae (in Ionia), and some other philosophers, held that truly new things cannot originate. There could be no "coming-into-being out of non-existence" as Cornford (1930, p. 30) expresses it. Cornford quotes an ancient commentator who was not impressed with this view of Anaxagoras:

> Anaxagoras, finding an old doctrine [that of Parmenides] that nothing comes into being out of what in no way is, abolished coming-into-being and substituted for it a process of becoming distinct. He talked nonsense about all things being mixed with one another and becoming distinct as they grow. For in the same germ, he said, there are hair, nails, veins, arteries, sinews, bones. These are present in particles so small as to be invisible, but as

they grow they gradually become distinct. '*For how,*' he says, '*could hair come out of non-hair or flesh out of non-flesh?*'

The hypothesis of preformation circumvented the problem of differentiation—structure was present from the very beginning so there was no problem of deriving form from a formless beginning. Preformationism was based initially on an inability to see how epigenesis might work. Epigeneticists on the other hand based their hypothesis on observations, crude as they were, that seemed to show that new things did appear during development. Moreover, they were able to advance objections to preformation as in the case of hybrids. If the egg of a horse contained a preformed horse, how could one account for a mule? When different varieties of plants are crossed, how can the offspring be intermediate? If there is a strict preformation, how can there be any variation among offspring at all if they are raised under the same conditions?

But pure epigenesis also raised serious problems. One could argue that there must be some sort of preformation in the sense of there being a transfer of "information." Offspring do resemble their parents—rabbits do not hatch from hen's eggs. This transfer of information could be imagined to occur either at conception or later in the viviparous species. In oviparous species, however, especially those that broadcast their semen into the ocean, there could be no subsequent transfer of information from parent to offspring. So if there was some general rule that applied to all species, the transfer of information must occur at conception. Thus there must be preformed information whether or not there were preformed structures.

Therefore preformation *vs.* epigenesis is far from a trivial problem. It confounded philosophers from the earliest times and remained unresolved as the 17th century came to a close. Next we will trace how it was dealt with during the 18th century by those who came after Malpighi.

An aside. From now on we will use the terms "ova," or eggs for the female's contribution to the young and "sperm" for the male's contribution. In 1667 Leeuwenhoek reported that animalcules, later spermatozoa, were present in semen and suggested they were the active agent (III, pp. 614–615). Somewhat earlier, in 1651, Harvey had suggested that all life comes from eggs. Neither view was accepted by all until more than a century passed, but to avoid circumlocution, from here on these terms will be used.

DEDUCTIONS FROM THE HYPOTHESIS OF PREFORMATION

That profound philosophical difficulty of Parmenides, Anaxagoras, and later preformationists of how there could be a "coming-into-being out of non-existence" caused most embryologists of the late 17th and the entire 18th centuries to reject epigenesis.

However, some of the deductions from the hypothesis of preformation proved exceedingly troublesome. For example, if we assume that both ova and sperm have preformed bodies one might deduce that twins would result from each conception. But this is not true so how could one account for a single offspring? Could one imagine the fusion of two little heads, hearts, skeleton, and all the other complex parts of the body? One had to assume some sort of amalgamation otherwise twins should be the usual occurrence in human births.

This difficulty was circumvented with the assumption that *either* the sperm or ovum would contain the tiny body. In the case of human beings a homunculus, or "little man" was predicted. Not surprisingly this resulted in two schools of thought among the preformationists: the ovists who believed the homunculus to be only in eggs and the spermists (or animalculists) who believed the homunculus to reside in sperm. These were not silly aberrations of human thought but necessary deductions from the hypothesis.

How could these deductions be tested? By looking. There was a severely restricted problem here for the ovists. The true mammalian egg was not to be discovered

until 1827, by von Baer. The spermists did not suffer this restriction. Leeuwenhoek had reported that semen contains microscopic animalcules, later to be given the name "spermatozoa" by von Baer. These were examined with the crude microscopes of the day and, as predicted, found to contain tiny bodies. It was a necessary deduction, of course, that the tiny bodies in sperm would be species specific. And they were. Gautier d' Agoty (1750) claimed to see tiny chickens, horses, and donkeys in the semen (not sperm) of those species. Earlier Hartsoeker (1694) had provided an illustration of a severely cramped homunculus with a huge head and the fontanelle clearly indicated (Fig. 4). Hartsoeker made no claim that he had observed this homunculus—merely that if he could see it that is what it would look like. Others described sperm as being of two sorts—some with a male homunculus and others with a female homunculus.

These observations, or better imaginations, were not accepted by all—not even by some of the strict preformationists. The absence of acceptable verification, however, was not necessarily a serious problem. The fact that simple microscopes and simple techniques had revealed a rich and previously unseen world suggested that surely improved technology would greatly expand that world.

During the 17th and 18th centuries information about regeneration began to become available. Some animals were found to have astonishing abilities to replace lost parts. Strict preformationism, however, would preclude the possibility of regeneration. Yet it occurred and that knowledge led Hartsoeker to abandon preformationism.

Another deduction from preformation was so necessary and so improbable to many that it contributed to the rejection of the hypothesis. Let us adopt the ovists position and assume that the human egg has a completely formed homunculus—of a female. Then that homunculus must contain ovaries and those ovaries must have eggs with homunculi. Those homunculi again must have the next generation of homunculi and so on—like a set of Russian dolls. This deduction is a logical necessity from the hypothesis of preformation since the possibility of anything new appearing (epigenesis) is excluded.

FIG. 4. Hartsoeker imagined that the human sperm might look like this. This is not his observation but his hypothesis (from *Essai de dioptrique,* Paris, 1694).

One cannot imagine an infinite series of ever smaller and ever encased homunculi, so eventually the supply would be exhausted and the species would become extinct. It was suggested that the entire future of the human race was included in the successively encased homunculi in the ovaries of Eve. In more senses than one, the ovists thought of her as the Mother of Humanity.

This strict preformationist position was adopted by Malebranche (1672) late in the 17th century and continued with von Haller (1767) and Bonnet (1770) in the 18th century when it was the dominant hypothesis, and was given the name *emboîtement* ("encasing").

THE EPIGENESIS OF EPIGENESIS

The hypothesis of preformation accounted for a very great deal but it could not account for everything. Slowly efforts to refute it gained ground. In 1759 Caspar Friedrich Wolff published his *Theoria Generationis* based mainly on the chick. Wolff interpreted his observations as indicating a true epigenetic development. He worked in Germany, so presumably his eggs did not have to endure those hot August days that Malpighi mentioned. He observed embryos at a much earlier stage than Malpighi and saw no recognizable organs. Preformationists such as von Haller countered once again that just because structure could not be seen one could not conclude that it was not there.

But Wolff did make one strong and eventually convincing point. He emphasized that when organs first become clearly observable they are not in their final form. For example, the intestine of the chick embryo could be shown to start as a flat sheet and then become a tube. Epigenesis, therefore, was proven for individual structures. Thus is was not unreasonable to extend the hypothesis to development as a whole.

Other observations, such as those on plant and animal hybrids, could be accounted for more satisfactorily by the hypothesis of epigenesis than by that of preformation. Hybrids are generally intermediate. The hybrid individual would have been derived from the egg (assume the ovist position) of one type and, so, should not show any features of the other type.

In addition, how could preformationism account for variability within species? If Mother Eve really did have all the entombed members of humanity for all time in her ova, how could one account for the existing human races?

It might be interesting to see how your students evaluate the two hypotheses with the information given so far.

Then there was the seemingly well-established fact of spontaneous generation already mentioned in the discussion of Harvey. From the Greeks onward it was generally held that some organisms arose spontaneously—in decaying meat, from excrement, and in decaying food. Although in the century before Francesco Redi (1668) had made what were later to be regarded as definitive experiments, spontaneous generation of at least some lowly creatures was still accepted as "fact." Now, if organisms as complex as insects can arise spontaneously, the hypothesis of preformation becomes difficult to maintain. One cannot imagine that all meat contains preformed primordia of insects, which will start to develop once the meat begins to decay.

By the end of the 18th century epigenesis was slowly replacing preformationism as the dominant hypothesis. Embryologists were able to make better observations and the preformationist argument that "just because you can't see it doesn't mean that it is not there" carried less weight. Epigenesis seemed to explain more and require fewer improbable deductions. That deduction about Eve's ovaries was hard to accept as probable by those touched by the Enlightenment of the 18th century. If one ceases to believe in Eve, as many did, the demographic aspects of her ovaries do not present an insuperable problem.

The whole debate was, according to Sarton (1931, p. 317), a waste of time because

> the fine observational tradition of the seventeenth century [was] interrupted, or at any rate considerably slowed down for more than a century by discussions which were irrelevant, because they were too far ahead of the experimental data.

So, if we accept epigenesis, the awesome problem of differentiation still remains. The structure of embryological theory at the end of the 18th century remained roughly as it was formulated by Aristotle.

References: Galen to Wolff

Embryology. Adelmann (1942, *1966), Baxter (1976), Belloni (1974), Bowler (1971, 1973), Bylebyl (1972), Castellani (1973), Churchill (1970*a*), Cole (1930), Cooke (1762), Farley (1977, 1982), Gaissinovitch (1978), Gasking (*1967), Glass (1959), Haeckel (1905*b*, ch. 2), Harvey [1982], Heniger (1973), Highmore (1651), Hintzsche (1972), Maienschein (1986), Meyer (1936, 1938, 1939), Moore (1972*a*,

1972*b*), Needham (*1959), Oppenheimer (1955, *1967, 1975), Pagel (1967), Pilet (1970), Preus (1977), Punnett (*1928), Roe (1975, 1979, 1981), E. S. Russell (1917, *1930), Sandler (1973), Sarton (1931), Wallace (1970), Webster (1967), Wheeler (1899), Whitman (1896*a*, 1896*b*, 1896*c*), L. G. Wilson (1972), and Zanobio (1971).

Biology and science in general. Birch (1756–1757), Bodenheimer (1958), Butterfield (1957), Crombie (1952, 1953, 1959, 1963), Dampier (1929), Gardner (1965), A. R. Hall (1954), Haskins (1924), Hooykaas (1972), Koyré (1957, 1973), Lanham (1968), Locy (1915, 1925), Magner (1979), Mason (1953), Mayr (*1982), Mazzeo (1967), Nordenskiold (1928), Palter (1961), Radl (1930), Ritterbush (1964), B. Russell (1945), Sarton (1927–1948, 1952), Singer (*1950, *1959), Sirks and Zirkle (1964), de Solla Price (1975), Sprat (1722), Taton (1963), Thomson (1899), Thorndike (1923–1941), Whitehead (1925), Wightman (1951), and Woodger (1929).

THE 1800s—CENTURY OF DISCOVERY

We now cross a most important time-line in the development of science and enter the 19th century. This will be the century when humanity first began to truly understand, control, and predict the phenomena of science and nature. In roughly the first half of the century chemistry was to have its John Dalton (1766–1844), geology its Charles Lyell (1797–1875), and biology its Charles Darwin (1809–1882)—all Englishmen. There were to be radical changes in these three sciences whereas astronomy and physics, already with notable accomplishments, were to continue their rapid evidential and conceptual development.

The early 19th century was also to witness radical and irrevocable changes in the ways that people live. James Watt's (1736–1819) perfected steam engine of the late 18th century powered the Industrial Revolution and was the basis of George Stephenson's (1781–1848) locomotive engine. Again, both Watt and Stephenson were Englishmen. The arts of technology and transportation were unleashed.

Life in Western Civilization could never be the same again after the early 19th century. In field after field the impossible became possible—leading to the ultimate "impossibilities" of our own times. The renowned historian Fernand Braudel (1985) describes the crossing of this time boundary:

> Can it not be said that there is a limit, a ceiling which restricts all human life, containing it within a frontier of varying outline, one which is hard to reach and harder still to cross? This is the border which in every age, even our own, separates the possible from the impossible, what can be done with a little effort from what cannot be done at all. In the past, the borderline was imposed by inadequate food supplies, a population that was too big or too small for its resources, low productivity of labour, and the as yet slow progress in controlling nature. Between the fifteenth and the eighteenth century, these constraints hardly changed at all. And men did not even explore the limits of what was possible.
>
> It is worth insisting on this slow progress, this inertia. Overland transport, for example, very early possessed the elements which could have led to its being perfected. And indeed here and there, one finds faster speeds being reached because modern roads were built, or because vehicles carrying goods and passengers were improved, or new staging-posts established. But progress of this kind only became widespread by about 1830, that is just before the railway revolution. It was only then that overland transport by road became commonplace, regular, well-developed and finally available to the majority; so it was only then that the limits of the possible were actually reached. And this is not the only area in which backwardness persisted. In the end, the only real change, innovation and revolution along the borderline between possible and impossible came with the nineteenth century and the changed face of the world (p. 27).

And so it was with developmental biology though it was not until later in the 19th century that rapid and sustained progress

in the "how's" of development became possible. The first few decades saw a few outstanding embryologists building on the discoveries of previous workers and using the slowly improving technology of the time to make notable advances in descriptive embryology–the chick embryo continuing to be the material of choice. There were no startling breakthroughs and no radical new theories that directed research programs in new ways.

ANOTHER "FATHER" OF EMBRYOLOGY—VON BAER

One of these embryologists was Karl Ernst von Baer (1792–1876) an Estonian biologist. He and others of his time, such as his colleague, Heinrich Christian Pander (1794–1865) a Latvian, began to study chick embryos in better ways. Malpighi's method of removing the early embryo from the egg and placing it on a glass slide for study continued to be used, together with Boyle's suggestion for preserving (="fixing") embryos with alcohol or other substances. A method perfected by botanists for making thin slices of tissues with a very sharp razor was also employed. These thin slices, mounted on slides and studied with a microscope, revealed structures that could not be seen in whole mounts. This was long before microtomes were available and the slices were made freehand. That was not so difficult with plant stems, roots, and leaves for example, because of their rigid cell walls. It is not simple, however, to make very thin slices of a chick embryo even after fixing in alcohol. (Paraffin embedding was half a century in the future.)

Von Baer's main contributions are to be found in two monographs, *De Ovi Mammalium et Hominis Genesi* (1827) and *Über Entwicklungsgeschichte der Thiere: Beobachtung und Reflexion* (1828; a second, and unfinished, volume was published in 1837). When reading these next few pages remember that another decade would pass before the cell theory was applied to animal tissues.

Von Baer begins *De Ovi Mammalium et Hominis Genesi* with a discussion of earlier attempts to discover something in mammals that corresponded with the well known eggs of birds, fishes, reptiles, amphibians and many invertebrates. These eggs were large and readily visible. Where could the mammalian egg be, or did it really exist? In von Baer's time this was the state of theory and knowledge. Interest centered on the structure earlier described by de Graaf (see also Corner, 1958, ch. 10).

> It seems beyond question that the Graafian vesicle contributes something to the development of the ovum, because after conception it is changed into the corpus luteum. Among anatomists today two opinions prevail regarding the manner in which the ova arise from the vesicles. Some believe that the Graafian vesicles correspond exactly to the yolk of birds' eggs, and that in innate fluid surrounds the little membranes, and therefore that they are received from the tube in the form of an egg. Previously I myself supported this opinion, because of the conspicuous similarity between the ovaries of mammals and of birds as well as because of the development of the foetus. Others believe that the fluid of the vesicles is ejected, and that it forms the ovum in the tubes either by itself or mixed with male semen (O'Malley, 1956, p. 122).

Von Baer started with the known and then sought the unknown. That is, he began by studying early embryos in the uterus and then sought even earlier stages in the oviducts. Presumably the earliest eggs in the oviducts would resemble something in the ovary. If he discovered them, that would complete the link.

> When I examined the ovaries before incising them, I clearly distinguished in almost all the [Graafian] vesicles a whitish-yellow point which was in no way attached to the covering of the vesicle, but as pressure exerted with a probe on the vesicle indicated clearly, swam freely in its liquid. Led on more by inquisitiveness than by the hope of seeing the ovules in the ovary with the naked eye through all the coverings of the Graafian vesicles, I opened a vesicle, of which, as I said, I had raised the top with the edge of a

> scalpel—so clearly did I see it distinguished from the surrounding mucus—and placed it under the microscope. I was astonished when I saw an ovule, already recognized from the [Fallopian] tubes, so plainly that a blind man could scarcely deny it. It is truly remarkable and astonishing that a thing so persistently and constantly sought and in all compendia of physiology considered as inextricable, could be put before the eyes with such facility (O'Malley, p. 132).

Von Baer undertook to repeat these observations on other species—after all he was searching for the *mammalian* ovum. He was able to report in the 1827 monograph that he had

> compared the [Graafian] vesicles of cows, sheep, dogs, rabbits, the stag, porpoise and dolphin, as well as man, with [pigs], and I have persuaded myself that in all of these animals the structure is the same (O'Malley, pp. 134–135).

The 1827 monograph contains the first generally accepted description of the long sought mammalian egg but, as is frequently the case, discovery involves some error. This was his conclusion:

> *When we take the ovary and in general the maternal organism into consideration, the Graafian vesicle is thus the real egg of mammals.* However, as far as its development is concerned, it diverges widely from the egg of other animals; these are carried intact out of the ovary, and they not only provide for the fetus a place of development, they transform themselves into that fetus. In the mammals the *embedded vesicle* contains a more developed yolk and *behaves with regard to the coming embryo as the real egg. It might be called the fetal egg within the maternal egg.* The mammals have thus an egg within an egg, or, if this way of putting it may be allowed, an egg in the second power (Sarton, 1931, p. 322).

Sarton (p. 320) mentions von Baer's emotion at the moment of discovery:

> When he first saw the minuscule sphere of yolk which was the egg he had been dreaming of, he was so struck that he was obliged to rest himself before he had the courage to look a second time into his microscope; he was afraid of having been deluded by a phantom.

It was realized later that the true ovum is not the entire Graafian follicle but the much smaller structure within it. We can easily identify von Baer's "egg within the egg" as the mammalian egg and wonder why he did not reach the same conclusion—especially when he realized that the Graafian follicle remained in the ovary and became the corpus luteum. Sarton explains why (1931, p. 324):

> A man makes a great discovery and misinterprets it because he is hypnotized by earlier ideas. The history of science is full of similar examples. It shows that the most difficult thing of all is to see things as they are, without preconception. Few people are able to do it at all, and these few only by intermittence.

Von Baer's full page illustration is shown here as Figure 5. Figure IX shows the "Vesicula Graafiana" of a breeding sow enlarged ten times (reduced slightly from this in Fig. 5). The tiny spherical structure at the top, identified by line 8, is the "ovulum." After the ovum has been extruded the Graafian follicle becomes the corpus luteum. Figure XIV, immediately below shows the corpus luteum of a dog.

In the upper dark band is the large number "1" and just below it is a tiny white dot, which is a small ovarian egg. Below "2" is a mature egg. Both are from a dog and are *natural size.* Then come the truly exciting discoveries. Figure 3 is a dog's egg from the oviduct and Figure 4 is one from the uterus. Again these are natural size. The illustrations I–III immediately below the dark band are the same eggs magnified ten times and in I*–III* they are magnified 30 times.

Later stages are also shown. Figure 6 is a dog embryo of 12 days, again natural size. The beautiful drawing of Figure VII[a] is also of a dog embryo. The heart, ventral aorta, four aortic arches, and dorsal aorta are clearly shown as are the brain, eye, ear,

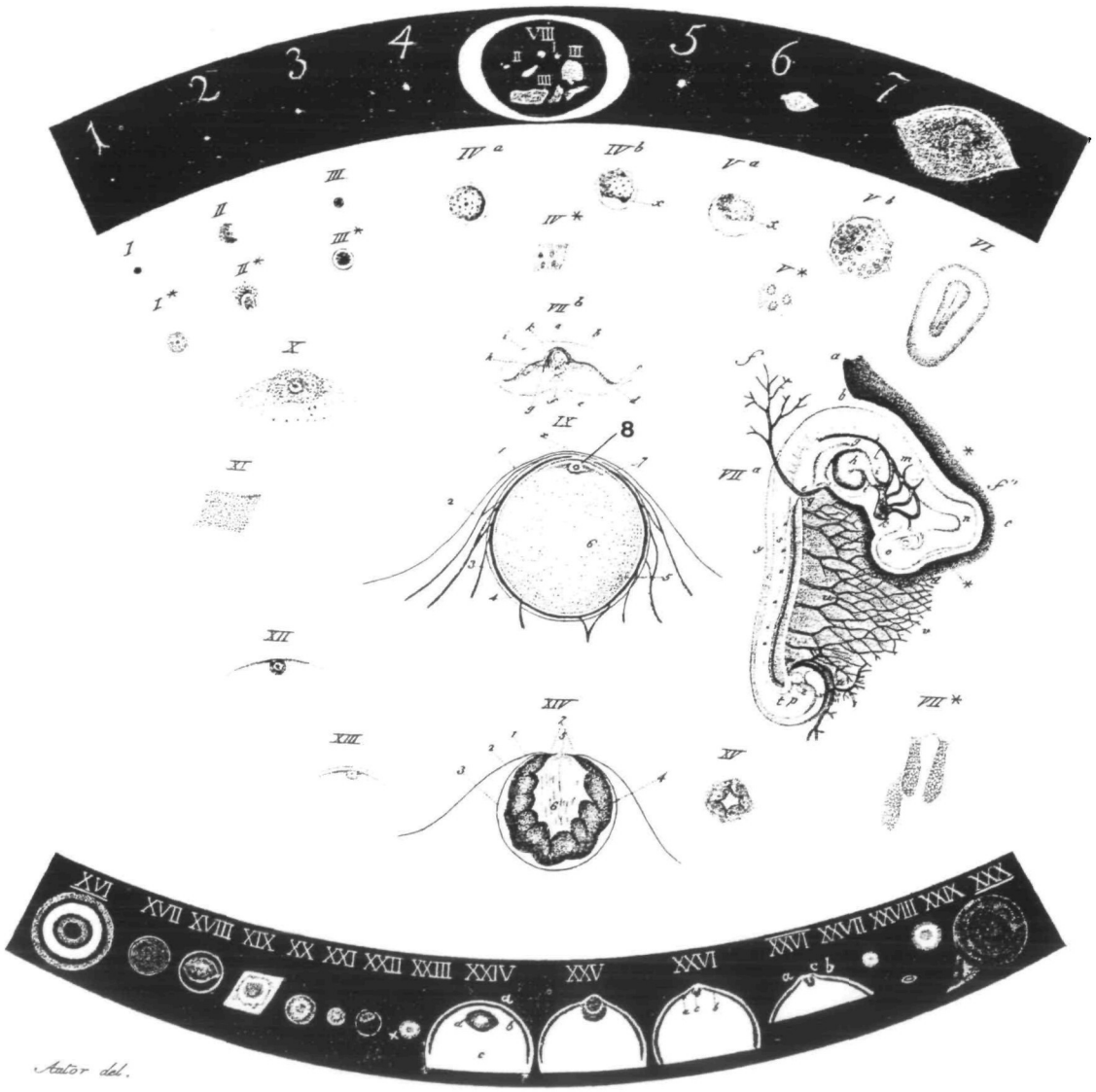


FIG. 5. Von Baer's (1827) illustrations of the mammalian ovum, 8 (retouched) in his figure IX, and various embryos.

spinal cord, somites and other structures. Figure XIII shows the human ovum magnified ten times. It is of interest to note that von Baer's first observations were made on a dog belonging to the professor with whom von Baer was working, and that the dog was sacrificed for that specific purpose.

The 1827 paper closes with four main conclusions. It is interesting to list them because they show what a dominant mind of the time regarded as important.

Every animal which springs from the coition of male and female is developed from an ovum, and none from a simple, formative liquid.

The male semen acts through the membrane of the ovum, which is pervious by no foramen, and in the ovum it acts first on certain innate parts of the ovum.

All development proceeds from the center to the periphery. Therefore the cen-

tral parts are formed before the peripheral.

The same method of development occurs in all vertebrate animals, beginning at the spine.

Note in the first conclusion the statement "every animal." Neither von Baer nor all the biologists since him have studied all animals. The important methodological principle here is that scientists assume that there are general rules that apply to natural phenomena—all is not chaos—and that one need but sample to find rules with broad applicability. This broad principle was not von Baer's. Harvey had made such a statement two centuries before.

The second conclusion is vague to the point of being meaningless yet, in the decades to come, fundamental advances in cytology, genetics, and embryology would emerge from studies on the interactions of sperm and ova.

The third conclusion is essentially correct and it was to be explained satisfactorily with the experiments of the Spemann school in the 20th century.

In the final conclusion von Baer agrees with many students of development beginning with Aristotle. A few decades later, observations of this sort, linked to the theory of evolution, were to change the way embryos were studied and to mold the conclusions reached.

VON BAER'S ACHIEVEMENTS

When we consider the achievements of von Baer we do not accept what he considered as important conclusions, as just listed, but emphasize his work in relation to later discoveries. The most notable was, of course, his detailed and abundantly confirmed description of the origin of the mammalian ovum from the Graafian follicle and its movement down the oviduct to the uterus, developing *en route*, was convincing.

> It is thus clear that v. Baer's great discovery did not come out of the blue sky,—no discovery ever does. The young Königsberg anatomist was well acquainted with the works of his predecessors. None of these had actually clinched the argument, and the best proof of it is that they had failed to convince anybody; they had not even completed their own conviction. Yet, thanks to their efforts the concept of mammalian egg had become plausible; it had been rescued from the oblivion to which Haller's absolutism had condemned it. Prejudices were not yet overcome, but the question was reopened and it was now possible for an intelligent and persistent man of science to investigate it anew, more thoroughly, and perhaps to solve it. This was v. Baer's achievement (Sarton, 1931, p. 319).

A second major discovery, often credited to von Baer, is reflected in his Figure VII^b, a cross section of a dog embryo magnified ten times. One can recognize embryonic layers. This conceptual advance was first made in 1817 by his colleague Pander. According to Oppenheimer (1967, p. 296),

> It is fully acknowledged that by demonstrating in terms of Pander's germ layers the true meaning of Wolff's concept of epigenesis, [von Baer] transformed embryology into a systematic and comparative science.

The concept was greatly elaborated by von Baer (1828), who extended it to include all major vertebrate embryos, and it gradually took on its classical form, that is, that embryos pass through a stage when they seem to be composed of three layers now known as ectoderm, mesoderm, and endoderm. The entire structure of the later embryo and adult is derived from these three layers.

Figure VII^b also shows the general features of a vertebrate embryo. The neural tube appears as a circle at top center. The tiny structure immediately below is the notochord, which von Baer discovered. He mentions that it is thinner than a hair and, in so doing, uses a scale unfamiliar to modern-day embryologists—we think of notochords only as they appear under the microscope where they seem to be about the same diameter as a half inch dowel. He describes the notochord as

> a streak which runs exactly in the axis of

> the future vertebral column and, therefore, of the entire fetus . . . . Because it is so slender, it can be recognized when it is first formed only if the water in which the embryo is being investigated is very free of yolk spherules . . . . This chord is obviously identical with the cartilaginous column found throughout life in the vertebral column of some cartilaginous fishes . . . . The notochord is not only the axis around which the first parts of the fetus form, but also the true measuring rod for the whole body and all the principal systems (quoted from Adelmann, 1966, pp. 1195–1196).

Von Baer did not get the relationship of notochord and vertebral column quite right—later it was found that the notochord is replaced by the vertebrae, not transformed into them.

Von Baer had a considerable interest in the general principles of development. He maintained, even as Aristotle had, that development in all vertebrates was essentially the same. He also maintained that early embryos are generalized and only later do they become specialized.

> *The more special develops from a more general type.* The development of the chick bears witness to this at every moment. In the beginning, when the back closes [*i.e.*, neural folds close], it is a vertebrate, and nothing more. When it constricts itself off from the yolk, and its gill clefts close and the allantois forms, it proves itself to be a vertebrate that cannot live in the water. Then later the two intestinal caeca form, a difference appears in the extremities, and the beak begins to appear; the lungs push upward, the rudiments of the airsacs are apparent, and we no longer can doubt that we are looking at a bird. While the character of the bird becomes still more evident through further development of the wings and airsacs, through fusion of the carpals, and so forth, the web between the toes vanishes and we recognize a land bird. The beak and feet proceed from a general shape to a particular one, the crop develops, the stomach has already divided into two chambers, the nasal shield appears. The bird attains the character of a gallinaceous bird, and finally, that of a domestic chicken (quoted by Oppenheimer, 1963, pp. 12–13).

Statements such as this were used by some in support of a rigid theory of recapitulation, which von Baer himself opposed.

Jane Oppenheimer (1963), in her fine study of von Baer, states that his "great contribution to embryology was a vast synthetic scheme," as shown by the following statement of von Baer:

> each step forward in development is made possible only by the preceding state of the embryo, nevertheless the total development is governed and directed by the whole essence of the animal that-is-to-be. And thus conditions at any moment are not alone absolutely determining for the future (p. 18).

But Oppenheimer points out that much of his "vast synthetic scheme" is what *we* read into von Baer. She offers this important caution, which we should always remember when the past is studied for its anticipations of the present:

> Although von Baer did comment, as we have seen, on sequences of events and conditions of the moment, he did not organize or arrange his remarks on them. When the excerpts quoted above are assembled out of context, as here, and placed in order, they suggest that von Baer had intimations of many important later concepts. But the reader must be warned to remember that such remarks are rare in the long volumes, not collected into a single chapter, and none were mentioned by von Baer in his summaries; they seem to have the quality of *obiter dicta* (p. 18).

Do we create the Fathers of Embryology in our own image?

Today von Baer is remembered primarily for two important discoveries and, as is so frequent in science, he was neither first nor entirely right in either.

One was the true egg of mammals. But, in fact an Englishman, William Cruikshank

(1797), had observed eggs in the oviduct of rabbits three days after mating. In addition, in 1824 Prévost and Dumas had published similar observations of an egg in the oviduct. Von Baer was aware of these anticipations but it was he who worked out some of the details of the relation of the Graafian follicles and eggs. He had erred, as noted before, in regarding the Graafian follicle as an egg and what we now know to be the true egg as "the fetal egg within the maternal egg."

His second main contribution has to do with the germ layers. Although Pander had first developed this concept, von Baer greatly extended it. He differed from later workers in recognizing four layers, counting the mesoderm as two because it splits later in development.

Nevertheless the accomplishments of von Baer were impressive and we are justified in ranking him, for his time, as the greatest embryologist since Aristotle. The fact that he could be so outstanding depended not only on his innate strengths but also on his opportunity to build on the publications and techniques of his predecessors ("He stood on the shoulders of Titans"). Science is a strongly accretive discipline and even a moderately gifted scientist can soon surpass the level of understanding and technical abilities of more illustrious predecessors. That much is expected.

But von Baer really did make a quantum leap. His monographs were far superior in detail and accuracy (but not in illustrations) to any work in embryology before his time. He was not overly concerned with theory but he was a vigorous opponent of the then currently exaggerated concept of recapitulation. His extensive knowledge of embryos led him to emphasize these points.

1. The embryos of different species belonging to a major taxonomic group resemble each other more closely early in development than they do as older embryos.

2. The embryos of higher species are like the embryos of lower species but not like the adults of lower species.

3. Thus if one compares the course of development of embryos of different taxonomic groups they are found to diverge progressively and not recapitulate different levels of organization.

Mayr (1982, pp. 469–479) has a fine discussion of this entire question. Remember that von Baer and others who speculated about embryos recapitulating a *scala naturae* did so before 1859 and Darwin's *On the Origin of Species.*

NINETEENTH CENTURY BIOLOGY: FROM UNITY TO FRAGMENTATION

One of the more striking aspects of early 19th century biology was its unified approach to problems. We have spoken of Harvey, Malpighi, von Baer and many others as "embryologists," but they were far more than that. Those general biologists, or naturalists in those days, did not approach the phenomena of life as geneticists, evolutionary biologists, cell biologists, or developmental biologists. Those disciplines, so distinct today, were then part of a conceptual whole. This unity came not from the recognition of fundamental principles but from the general lack of such principles. Those who studied embryos were interested in the material contributions of parents to offspring (cytology), what might be the "information" transferred (genetics), how the course of development related to the *scala naturae* (evolutionary biology), as well as the details of development itself (developmental biology).

Developmental biology was the most advanced and distinct of these four disciplines. The others gradually separated from the core of general biology. In the 1830s cytology was beginning to become a distinctive discipline. In the 1850s evolutionary biology would follow and, finally, genetics would become more independent and active in 1900.

There is a parallelism between some of von Baer's conclusions for different sorts of embryos, such as 1 and 3 just listed, and the different sorts of biologies. Both embryos and the biologies were much alike in their early stages and, as information was translated in the former and obtained for the latter, there was divergence among the embryos and among the branches of biology. Thus the embryos of the vertebrate classes are alike when very young but

become very different as adults. In the same way, genetics, cytology, evolutionary biology, and embryology all deal with the same grand problems of life and continuity. We observe an excessive fragmentation of biology today but it is not unreasonable to predict that by the year 2000, or hopefully earlier, the extraordinary advances in the discrete branches of biology will be the basis for a new and far more satisfying general synthesis.

THE CELLS OF EMBRYOS

Those aspects of cytology that are important to genetics were discussed in some detail in last year's essay (III, pp. 607–639, 653–664, 687–721). Much of that is also relevant to embryology but only the main points will be repeated here.

First, of course, was the realization that the bodies of embryos, as well as adults, are composed solely of cells or cell products. Cells were first described in a number of plants by Robert Hooke in 1665. As more and more plants were studied, it began to look as though a basic principle was emerging—the bodies of *all* plants are composed of cells or cell products. Cell walls were assumed to be restricted to plants. No such structures appeared to be present in animals but slowly it came to be realized that animal tissues did have some structures that were present also in plant cells. That is, apart from walls, one could recognize similarities. This point of view was pressed especially by Theodor Schwann (1839) who made a fundamental change in definition. He suggested that cells should be defined on the basis of containing a nucleus rather than on being surrounded by walls. That change made it possible to recognize cells as the basic units of structure of both animals and plants. Embryos proved to be excellent material for detecting cells and many of Schwann's illustrations were of them (III, p. 613, fig. 4).

The next landmark hypothesis was that advanced in 1859 by Virchow (1863). He suggested that cells do not arise *de novo,* as Schwann had believed, but only by division of preexisting cells (*omnis cellula e cellula*). Knowledge of cells continued to increase but later developments, especially those analyzing the nucleus, will be considered when we reach Wilhelm Roux.

TRUE BEYOND ALL REASONABLE DOUBT?

A goal of science is to be able to say that some statement about a natural phenomenon is "true beyond all reasonable doubt." Such statements become parts of a broad theory that both synthesizes available information and suggests new observations to make and experiments to do that will extend the theory.

Care must be taken to ensure that students understand the difference between statements that are already accepted as true beyond all reasonable doubt and those hypotheses that, no matter how reasonable, have yet to be extensively tested before they can be accepted as true beyond all reasonable doubt. Years, decades, centuries, or even millennia may intervene between these two positions. For example, students are sometimes left with the impression that Schwann and Virchow made discoveries that were immediately accepted by biologists. Not at all. Both were proposing hypotheses that were accepted as useful ways of looking at the microscopic structure of organisms only after very extensive observation and experimentation on many kinds of plants and animals. In fact, neither hypothesis was capable of absolute proof. Absolute proof would require that the bodies of all individuals of all species of organisms must be checked for their cellular nature and for the origin of those cells.

Such exhaustive tests are not required in biology any more than in chemistry. Chemists are not required to study all water in order to say that it is composed solely of hydrogen and oxygen in certain proportions. Scientists are required to do no more than study an adequate sample. Nature behaves as though there is uniformity in materials and processes and, once the limits of variation have been ascertained, repetition of observation and experimentation is pointless. Not for all time, of course. When better ways of collecting data become available, repetition is required. Statements about animal structure gained with the unaided eye had to

be checked first with simple lenses, then with compound microscopes, and now with electron microscopes.

As more and more organisms were studied, the hypotheses of Schwann and Virchow seemed to be true in most instances—but not in all. In some situations, such as the early embryos of insects and stages in the life cycle of slime molds, the body consists of nuclei in a cytoplasm not divided into discrete cells—a syncytium. As for the origin of cells, reports continued even into the 20th century of *de novo* formation but these have been shown to have been errors.

Thus there is a certain amount of wobble even in those biological statements that are the foundations of the science. This is not of any great concern since biologists have come to see that the phenomena of life can be described as themes with variations.

In the early 19th century there were very few statements about embryos that could be accepted as true beyond all doubt (excluding some exceptions). Aristotle in the 4th century B.C. and Harvey in the 17th century A.D. both believed that development was epigenetic but they only proposed, they did not prove. Acceptable proof came slowly following the observations of Wolff.

In fact, about all that could be said as true beyond all reasonable doubt in von Baer's time, the early 19th century, was:

1. Development is epigenetic, that is, there is differentiation with growth.
2. But there must be some sort of preformation that will account for the transfer of information from the parental to the filial generation.
3. There is considerable resemblance in the embryonic development of species within the same taxonomic group, the resemblances being greater in younger stages than in later stages.
4. The bodies of early embryos seem to be composed of layers from which the structures of the larva and adult are derived.

In addition to these few concepts, there was a rapidly increasing body of information on the normal development of a wide variety of organisms. This body of information was about to be incorporated into the most basic biological concept—organic evolution.

THE DEVELOPMENT OF CHARLES DARWIN

The paradigm shift of 1859 changed not only what biologists did but also provided a rationale for their research. The new paradigm was also able to offer a more satisfying explanation for much that had already been learned. In fact, the data themselves seemed to be awaiting some organizing theory and a simple idea provided it. But ideas are not simple until after they have emerged. Nevertheless many biologists must have wondered as did Thomas Henry Huxley (1888, p. 197):

> My reflection, when I first made myself master of the central idea of the "Origin" was, "How extremely stupid not to have thought of that!"

Darwinism provided a new way of thinking about a cluster of major biological phenomena related to embryology:

1. Living organisms seemed to form a continuum from the least complex to human beings—a Great Scale of Being or the *scala naturae.*
2. Embryos of species in the same taxonomic group resemble one another.
3. Recapitulation.
4. Homology.

Each of these phenomena was so striking and so pervasive that it was impossible not to think that there must be some underlying cause. Today we see all of this as a reflection of evolutionary change but that clarity of vision started only in 1859.

The recognition of types of organisms, or major groupings, must have been part of the way human beings looked upon the natural world from the very earliest of times. There were animals with hair, the mammals; animals with feathers, the birds; animals with scales that lived on land, the reptiles; animals generally with a smooth moist skin, the frogs; and animals with scales that lived in the water, the fishes. These were discrete groups without a closely graded series of intermediate forms. One could even lump all of these in a still grander group—animals with a backbone and many other similarities. Organisms

sharing similar features could be classified, that is, placed in taxonomic groups. What could be the basis of these groupings?

Although the taxonomic groups were discrete, nevertheless it was possible to arrange them in a scale of complexity and similarity. This "*scala naturae,*" or "chain of being" extended not only from human beings to the simplest organisms—protozoans and bacteria discovered by the early microscopists—but to lifeless matter as well. The *scala naturae* was a theological construct describing the immutable forms of life and non-life that had been created. It could be regarded as a ladder that found the least complex objects on the bottom rung and human beings at the top—just below the divine itself (Lovejoy, 1936; Eiseley, 1858; Ritterbush, 1964; Gould, 1977; Mayr, 1982).

Groups of organisms not only shared features when they were adults but Aristotle and all others after him realized that embryos were also alike in many ways. An equally important discovery was that the similarities were greater in younger than in older embryos.

By the early 1800s it seemed to some that there was a parallel between the *scala naturae* and the course of embryonic development and this led to the hypothesis of recapitulation. In its extreme form this meant that the embryos of higher forms (mammals for example) went through stages that resembled the *adults* of lower forms (fish, amphibians).

And finally there was the concept of homology. During the 18th century there had been a great increase in knowledge of the structure of organisms and some tantalizing phenomena were discovered. For example, it was found that the limbs of tetrapods seemed to be built on the same general plan. The arms seemed to have a basic plan of one proximal bone and two more distal; the hand had several bones forming a wrist, about five in the palm, and finally a few in each finger. Even the wings of birds and bats could be understood to be variations on this basic plan. The corresponding bones were said to be "homologous." That is, the humerus is really the "same thing" in different species.

It was recognized that homology is based on more than superficial resemblances. The wings of insects, for example, do not share the same basic structure. Wings of birds (or bats) and insects were said to be "analogous." Analogy, then, was restricted to functionally similar, but morphologically different, structures. Homology was restricted to morphologically similar structures that might be functionally similar (appendages of horses and frogs) or not (appendages of porpoise, bat, and monkeys).

And Darwin put it all together. In Chapter 13 of the *Origin* he sought to

> explain these several facts in embryology, [1] namely the very general, but not universal differences in structure between the embryo and the adult; [2] of parts in the same individual embryo, which ultimately become very unlike and serve for diverse purposes, being at this early period of growth alike; [3] of embryos of different species within the same class, generally, but not universally, resembling each other; [4] of the structure of the embryo not being closely related to its conditions of existence, except when the embryo becomes at any period of life active and has to provide for itself; [5] of the embryo apparently having sometimes a higher organisation than the mature animal, into which it is developed (pp. 442–443; I added the numbers in brackets).

The explanation of these five phenomena, which we hardly recognize as problems today, was not obvious in Darwin's time.

> There is no obvious reason why, for instance, the wing of a bat, or the fin of a porpoise, should not have been sketched out with all the parts in proper proportion, as soon as any structure became visible in the embryo (p. 442).

Yet when they first start to form in the embryo the wing and fin are nearly the same. They diverge later.

Darwin thought that this similarity of fin and wing and the other problems he listed could be explained on the basis of three

assumptions: first, evolution; second, that the modifications that occur in the course of evolution "may have supervened at a not very early period in life" (that is late in life); and third, "that at whatever age any variation first appears in the parent, it tends to reappear at a corresponding age in the offspring" (p. 444).

> Now let us apply these facts and the above two [he did not list evolution but it was implied] principles—which latter, though not proved true, can be shown to be in some degree probable—to species in a state of nature. Let us take a genus of birds, descended on my theory from some one-parent species, and of which the several new species have become modified through natural selection in accordance with their diverse habits. Then, from the many slight successive steps in variation having supervened at a rather late age, and having been inherited at a corresponding age, the young of the new species of our supposed genus will manifestly tend to resemble each other much more closely than do the adults . . . . We may extend this view to whole families or even classes. The fore-limbs, for instance, which served as legs in the parent-species, may become, by a long course of modification, adapted in one descendant to act as hands, in another as paddles, in another as wings; and on the above two principles—namely of each successive modification supervening at a rather late age, and being inherited at a corresponding late age—the fore-limbs in the embryos of the several descendants of the parent-species will still resemble each other closely, for they will not have been modified (pp. 446–447).

This line of reasoning can explain facts 1, 2, and 3, listed before—problems that Darwin puzzled about. Fact 4 refers to the lack of relation of embryos to their environment except when they are *active.* Active embryos are those that become free-living and secure their own food—in contrast with those of birds, for example, which have all the food they need stored in the egg. Many species of invertebrates have small eggs, with very little yolk. These eggs develop into free-living larvae that obtain their own food after a very short period. Many of these are microscopic, ciliated creatures having no resemblance to the adults they will become. Darwin looked upon them as having evolved the ability to develop quickly into a food-getting stage.

Darwin's fifth fact in need of explanation is that some embryos appear to be more complex than the adults. This made sense with an hypothesis of evolution but not otherwise. Many parasitic organisms, for example, have embryonic stages that are as complex as those of related free-living species but these develop into degenerate adults. It was difficult to explain why embryos of these parasites developed structures that later become reduced or lost unless it is assumed that the embryos are retaining the fundamental patterns of development characteristic of their taxonomic group.

Darwin's hypothesis of descent with modification, natural selection acting on the hereditary differences among individuals of a species, did far more than make some otherwise confusing embryological phenomena understandable. It accounts for the grand phenomenon of organisms belonging to sets or taxonomic groups.

> As all the organic beings, extinct and recent, which have ever lived on this earth have to be classed together, and as all have been connected by the finest gradations, the best, or indeed, if our collections were nearly perfect, the only possible arrangement, would be genealogical. Descent being on my view the hidden bond of connections which naturalists have been seeking under the term of the natural system. On this view we can understand how it is that, in the eyes of most naturalists, the structure of the embryo is even more important for classification than that of the adult. For the embryo is the animal in its less modified state; and in so far it reveals the structure of its progenitor. In two groups of animals, however much they may at present differ from each other in structure and habits, if they pass through the same or

> similar embryonic stages, we may feel assured that they have both descended from the same or nearly similar parents, and are therefore in that degree closely related. Thus, community in embryonic structure reveals community of descent. It will reveal this community of descent, however much the structure of the adult may have been modified and obscured; we have seen, for instance, that cirripedes [barnacles] can at once be recognized by their larvae as belonging to the great class of crustaceans. As the embryonic state of each species and group of species partially shows us the structure of their less modified ancient progenitors, we can clearly see why ancient and extinct forms of life should resemble the embryos of their descendants,—our existing species (pp. 448–449).

Here Darwin was applying his concept of evolution to the widely accepted but poorly understood hypothesis of recapitulation. But he was cautious in accepting recapitulation without reservation. He continues,

> Agassiz believes this to be a law of nature; but I am bound to confess that I only hope to see the law hereafter proved true. It can be proved true in those cases alone in which the ancient state, now supposed to be represented in many embryos, has not been obliterated, either by the successive variations in a long course of modifications having been supervened at a very early age, or by the variations having been inherited at an earlier period than that at which they first appeared. It should also be borne in mind, that the supposed law of resemblance of ancient forms of life to the embryonic stages of recent forms, may be true, but yet, owing to the geological record not extending far enough back in time, may remain for a long period, or for ever, incapable of demonstration.
>
> Thus, as it seems to me, the leading facts in embryology . . . are second to none in natural history . . . . Embryology rises greatly in interest, when we thus look at the embryo as a picture, more or less obscured, of the common parent-form of each great class of animals (pp. 449–450).

Darwin was so impressed with the data of embryology, together with that of morphology and classification (also discussed in his Chapter 13), that he concludes:

> Finally, the several classes of facts which have been considered in this chapter, seem to me to proclaim so plainly, that the inumerable species, genera, and families of organic beings, with which this world is peopled, have all descended, each within its own class or group, from common parents, and have all been modified in the course of descent, that I should without hesitation adopt this view, even if it were unsupported by other facts or arguments (pp. 457–458).

Darwin gave embryologists a mission of first-rate theoretical importance—the search for lineages in the minutiae of development. To be sure, embryos could do no more than reflect these lineages but, when fossil evidence was so meager, there was no alternative.

There had already been many triumphs that were known to Darwin. Barnacles were of special interest to him since he had produced the definitive monographs on these creatures. Barnacles are sessile animals enclosed in a shell. They resemble mollusks more than any other group of invertebrates. Cuvier, the most respected naturalist of the early 19th century, had included the barnacles in the Mollusca. It had been discovered, however, that the barnacle embryos develop into a larval type characteristic of the crustaceans. Darwin notes (p. 440),

> Even the illustrious Cuvier did not perceive that a barnacle was, as it certainly is, a crustacean; but a glance at the larva shows this to be the case in an unmistakable manner. So again the two main divisions of cirripedes, the pedunculated and sessile, which differ widely in external appearance, have larvae in all their several stages barely distinguishable.
>
> Among the vertebrates there were many

examples known to Darwin of embryos apparently recapitulating stages found in putative ancestors. Birds and mammals, with only a single aorta in the adult, have in the embryo the six pairs characteristic of fishes (I, pp. 499–501). Bird and mammalian embryos develop in succession a pronephros, mesonephros, the kidneys of the lower vertebrates, and finally their own adult metanephric kidney (I, pp. 503–504). Possibly the most dramatic example is that of the development of the malleus, incus, and stapes of the mammalian ear from the jaw bones of lower forms (I, pp. 498–499). This was predicted on the basis of careful embryological work, as being highly probable, long before paleontologists unearthed the mammal-like reptiles that provided absolute proof.

Thus by Darwin's time embryology had come to be more than the detailed study of successive stages in the development of organisms. The data of descriptive embryology could be used to suggest the course of evolution and to classify organisms into natural groups.

Homology was also clarified by embryology. The meaning of the "same thing" could now be understood. A common structure in an ancestor would change in the course of the evolution of different daughter species. The structure would still be the "same thing," although variously modified. In the case of hard structures, such as bones, it might be possible to trace the changes in fossils of different ages (jaw bones and ear ossicles, for example). Such would not be possible for soft parts (vertebrate kidneys and aortic arches) but often the embryos provided a clue. Homology, then, was defined as identity of embryonic origin.

RECAPITULATION

In the decades after Darwin, the fundamental theorem of evolutionary embryology was recapitulation. This concept was formulated well before Darwin and it expressed the relationship of embryogenesis to classification and to the *scala naturae.* When the Darwinian paradigm reinterpreted the *scala naturae* as the consequence of descent with modification, the data of embryology were reinterpreted as well.

The concept of recapitulation has had an incredible history and that history tells us nearly as much about the workings of science as about embryos. The concept occupied the center of theoretical embryology throughout the 19th century until it was displaced by the problem of the causes of differentiation. As Darwin suggested, many facts about development are inexplicable without the concept of recapitulation.

LOUIS AGASSIZ

It is fascinating to note how the concept of recapitulation, which itself suggests evolution, was used before the publication of *On the Origin of Species.* The case of Louis Agassiz (1807–1873) is especially interesting. He was a Swiss naturalist of great ability who spent much of his life in the United States. His famous *An Essay on Classification* was first published in 1857 as part of his *Contributions to the Natural History of the United States.* The *Essay* was republished in 1859—the year of the *Origin.*

It was well known in Agassiz's time that there is a general relation between the complexity of organisms and the time they first appear in the fossil record. This was a relationship that Darwin was to explain by his theory of evolution. Agassiz was never an evolutionist and he interpreted this relationship as an aspect of Creation. He described an especially striking example of the parallelism of complexity of living species and their fossil record in higher plants where

> we at once see how the vegetable kingdom has been successively introduced upon earth, in an order which coincides with the relative position its primary divisions bear to one another, in respect to their rank, as determined by the complication of their structure . . . . If the vegetable kingdom constitutes one graduated series [part of the *scala naturae*], beginning with the Cryptogams, followed by the Gymnosperms, and ending with the Monocotyledones and Dicotyledones, have we not in that series the

> most striking coincidence with the order of succession, as exhibited by the Cryptogams of the oldest geological formations, especially the Ferns, Equisetaceae, and Lycopodiaceae of the Carboniferous period, followed by the Gymnosperms of the Trias and Jura and the Monocotyledones of the same formation and the late development of the Dicotyledones? Here, as everywhere, there is but one order, one plan in nature (1859, p. 168).

There follows Section XXV, which is entitled "Parallelism between the geological succession of animals and the embryonic growth of their living representatives." Agassiz writes that

> Several authors have alluded to the resemblance which exists between the young of some of the animals now living and the fossil representatives of the same families in earlier periods (pp. 168–169).

These tended to be isolated cases yet Agassiz provided a number of examples from among the invertebrates and this conclusion is reached:

> It may therefore be considered as a general fact, very likely to be more fully illustrated as investigations cover a wider ground, that the phases of development of all living animals corresponds to the order of succession of their extinct representatives in past geological times. As far as this goes, the oldest representatives of every class may then be considered as embryonic types of their respective orders or families among the living (p. 174).

And the cause?

> It exhibits everywhere the working of the same creative Mind, through all times, and upon the whole surface of the globe (p. 175).

Thus in pre-Darwinian days it was recognized that the positions of organisms in the *scala naturae* might be parallel to the times of their first appearance in the fossil record, and to their patterns of development.

ERNST HAECKEL

Recapitulation became a truly baroque edifice in the hands of Ernst Heinrich Phillip Haeckel (1834–1919), a dominant personality in German science of the last half of the 19th century. The concept was eventually regarded as either wrong or useless, yet it remains true today that some extraordinary phenomena "make sense" on the basis of a more balanced statement of the concept. The rejection of the concept of recapitulation in the late 19th and early 20th centuries is probably a case, as Gould states (1977, p. 2), of "throwing the baby out with the bath water." I agree.

Haeckel's theory was proposed in his *Generelle Morphologie* of 1866, and revised in *Naturliche Schöpfungschichte* (1868; trans. 1876), and in *Anthropogenie* (1874; trans. 1905*a*, 1905*b*).

If the concept of recapitulation is accepted as a useful way of looking at some otherwise puzzling embryological phenomena rather than as a fundamental and relentless Law of Nature, it becomes a powerful heuristic device. But such a view demands that we modify Haeckel's striking formulation, "ontogeny recapitulates phylogeny," by adding "not quite" and "sometimes."

Haeckel did far more than formulate his aphorism—he attempted to provide a conceptual scheme for all of descriptive embryology. He provided an overall theory that described the history of the development of individuals and which paralleled Darwin's theory that described the history of the development of species. Furthermore he suggested a close relationship between the two.

> These two branches of our science—on the one side ontogeny or embryology, and on the other phylogeny, or the science of race-evolution—are most vitally connected. The one cannot be understood without the other. It is only when the two branches fully co-operate and supplement each other that "Biogeny" (or the science of the genesis of life in the widest sense) attains the rank of a philosophic science. The connection

between them is not external and superficial, but profound, intrinsic, and causal. This is a discovery made by recent research, and it is most clearly expressed in the comprehensive law which I have called "the fundamental law of organic evolution," or "the fundamental law of biogeny." This general law, to which we find ourselves constantly recurring, and on the recognition of which depends one's whole insight into the story of evolution, may be briefly expressed in the phrase: "The history of the foetus is a recapitulation of the history of the race"; or, in other words, "Ontogeny is a recapitulation of phylogeny." It may be more fully stated as follows: The series of forms through which the individual organism passes during its development from the ovum to the complete bodily structure is a brief, condensed repetition of the long series of forms which the animal ancestors of the said organism, or the ancestral forms of the species, have passed through from the earliest period of organic life down to the present day . . . .

Phylogenesis is the mechanical cause of ontogenesis. In other words, the development of the stem, or race, is, in accordance with the laws of heredity and adaptation, the cause of all the changes which appear in a condensed form in the evolution of the foetus.

The chain of manifold animal forms which represent the ancestry of each higher organism, or even of man, according to the theory of descent, always forms a connected whole. We may designate this uninterrupted series of forms with the letters of the alphabet: A, B, C, D, E, etc., to Z. In apparent contradiction to what I have said, the story of the development of the individual, or the ontogeny of most organisms, only offers the observer a part of these forms; so that the defective series of embryonic forms would run: A, B, D, F, H, K, M, etc.; or, in other cases, B, D, H, L, M, N, etc. . . . .

In the embryonic succession much is wanting that certainly existed in the earlier ancestral succession. If the parallel of the two series were complete, and if this great fundamental law affirming the causal connection between ontogeny and phylogeny in the proper sense of the word were directly demonstrable, we should only have to determine, by means of the microscope and the dissecting knife, the series of forms through which the fertilized ovum passes in its development; we should have before us a complete picture of the remarkable series of forms which our animal ancestors have successively assumed from the dawn of organic life down to the appearance of man. But such a repetition of the ancestral history by the individual in its embryonic life is very rarely complete. We do not often find our full alphabet. In most cases the correspondence is very imperfect, being greatly distorted and falsified (1905*b*, pp. 2–3).

Part of the defect in the alphabet of descent is to be expected:

> In this evolutionary appreciation of the facts of embryology we must, of course, take particular care to distinguish sharply and clearly between the primitive, palingenetic (or ancestral) evolutionary processes and those due to cenogenesis. By *palingenetic* processes, or embryonic *recapitulations,* we understand all those phenomena in the development of the individual which are transmitted from one generation to another by heredity and which, on that account, allow us to draw direct inferences as to corresponding structures in the development of the species. On the other hand, we give the name of *cenogenetic* processes, or embryonic *variations,* to all those phenomena in the foetal development that cannot be traced to inheritance from earlier species, but are due to the adaptation of the foetus, or the infant-form, to certain conditions of its embryonic development (1905*b*, p. 4).

Haeckel, along with most evolutionists at

the time, accepted both Darwinism and Lamarckism. The distinction he is making between palingenetic and cenogenetic sounds strange to modern ears but his examples show what he had in mind. He regards

> as *palingenetic* the formation of the two primary germinal layers and of the primitive gut, the undivided structure of the dorsal nerve-tube, the appearance of a simple axial rod [the notochord] between the medullary tube and gut, the temporary formation of the gill-clefts and arches, the primitive kidneys, and so on. All these, and many other important structures, have clearly been transmitted by a steady heredity from the earliest ancestors of the mammal, and are, therefore, direct indications of the presence of similar structures in the history of the stem.
>
> On the other hand, this is certainly not the case with the following embryonic forms, which we must describe as *cenogenetic* processes: the formation of the yelk-sac, the allantois, the placenta, the amnion, the serolemma, and the chorion (1905*b*, p. 4).

These structures listed as cenogenetic are characteristic of living mammals so, presumably, first evolved in them or in the mammal-like reptiles and, hence appeared late in phylogeny.

In the 1860s when Haeckel began to speculate about the phylogeny leading to the human species, the fossil record was mainly gaps (as it still is), accurate knowledge of chromosomal cytology, genetics and biochemistry was essentially nil, microscopists knew little about the biology of what we now call the prokaryotes, and even the mechanisms of evolutionary change were poorly understood. The most valuable biological information was to be found in comparative morphology and embryology. These two disciplines, therefore, provided the evidential basis for Haeckel's version of recapitulation.

One of Haeckel's early attempts (1876, vol. 2, p. 295) to imagine what the phylogeny from primitive monad to human being might be is shown here as Figure 6. Twenty-two major steps from Monera to Talking Man are recognized. The columns to the left of the ancestral stages show the geological time that those stages were thought to have first appeared, beginning with the Monera in the Laurentian (or Precambrian). The rightmost column shows the nearest living species to the ancestral stages.

Later (Haeckel 1905*a*) increased these ancestral stages to 30 as shown in Figures 7 and 8. The three rightmost columns show the relative values of the data ranging from 0, !, or I in cases of no supporting evidence to three horizontal I's, !!!, or III for the best.

Again his starting point is a group known as the Monera, a name that remains to this day in some schemes of classifications (R. H. Whittaker, 1969) as the Kingdom of the prokaryotes and so includes the bacteria, blue-green algae (and sometimes the viruses). Very little was known about the nature of these organisms in Haeckel's time. To him they consisted of "simple, homogeneous, albuminous matter (protoplasm)." In fact they were the simplest organisms that one could imagine. They were thought to be entirely without nuclei or other structures. They were assumed to have first appeared in the very earliest of PreCambrian times by spontaneous generation. The starting materials were assumed to be simple combinations of carbon, oxygen, hydrogen, and nitrogen.

The next stage is that of single celled animals, such as amoeba. In Figures 9–12 Haeckel (1905*a*) shows a comparison of the putative ancestors and the events in human ontogeny. Thus the first phylogenetic stage corresponds to the undivided human fertilized ovum, although he believed that there might be a remnant of a Monera stage since for a while the egg nucleus seems to vanish (although not understood at the time the germinal vesicle does break down and "disappear").

Stage 5 of Figure 6, Stage 6 of Figure 7, and Stage 3 of Figure 9 all represent a very important ancestral type in Haeckel's scheme—the Gastraeades. Embryologists were familiar with the common pattern of

ANCESTRAL SERIES OF THE HUMAN PEDIGREE.

M N = Boundary between the Invertebrate and Vertebrate Ancestors.

Epochs of the Organic History of the Earth.	*Geological Periods of the Organic History of the Earth.*	*Animal Ancestral Stages of Man.*	*Nearest Living Relatives of the Ancestral Stages.*
I. ARCHILITHIC OR PRIMORDIAL EPOCH	1. Laurentian Period 2. Cambrian Period 3. Silurian Period (Compare p. 22, and Plate XIV. and its explanation)	1. Monera (*Monera*)	*Protogenes* *Protamœba*
		2. Single-celled Primæval animals	Simple Amœbæ (*Automœbæ*)
		3. Many-celled Primæval animals	Communities of Amœbæ (*Synamœbæ*)
		4. Ciliated planulæ (*Planæada*)	Planula larvæ
		5. Primæval Intestinal animals (*Gastræada*)	Gastrula larvæ
		6. Gliding Worms (*Turbellaria*)	*Rhabdocœla* *Dendrocœla*
		7. Soft-worms (*Scolecida*)	? Between the Sea-squirts and Gliding worms
		8. Sack worms (*Himatega*)	Sea-squirts (*Ascidiæ*)
		M.....N	
		9. Skull-less (*Acrania*)	Lancelets (*Amphioxi*)
		10. Single-nostriled (*Monorrhina*)	Lampreys (*Petromyzonta*)
		11. Primæval fish (*Selachii*)	Sharks (*Squalacei*)
II. PALÆOLITHIC OR PRIMARY EPOCH	4. Devonian Period 5. Coal Period 6. Permian Period	12. Salamander fish (*Dipneusta*)	Mud fish (*Protopteri*)
		13. Gilled Amphibia (*Sozobranchia*)	(*Proteus*) Axolotl (*Siredon*)
		14. Tailed Amphibia (*Sozura*)	Water-newts (*Tritons*)
III. MESOLITHIC OR SECONDARY EPOCH	7. Trias Period 8. Jura Period 9. Chalk Period	15. Primæval Amniota (*Protamnia*)	?Between the Tailed-Amphibia and Primary mammals
		16. Primary Mammals (*Promammalia*)	Beaked animals (*Monotrema*)
		17. Pouched animals (*Marsupialia*)	Pouched rats (*Didelphys*)
IV. CENOLITHIC OR TERTIARY EPOCH	10. Eocene Period 11. Miocene Period 12. Pliocene Period	18. Semi-apes (*Prosimiæ*)	Lori (*Stenops*) Maki (*Lemur*)
		19. Tailed Narrow-nosed Apes	Nose aes Holy apes
		20. Men-like Apes or Tail-less Narrow-nosed Apes	Gorilla, Chimpanzee, Orang, Gibbon
		21. Speechless Men or Ape-like Men	Deaf and Dumb, Cretins or Microcephali
V. QUATERNARY EPOCH	13. Diluvial Period 14. Alluvial Period	22. Talking Men	Australians and Papuans

FIG. 6. Haeckel's correlations of geological age, putative human ancestors, and nearest living relatives (Haeckel, 1876, vol. 2, p. 295).

development in both invertebrates and vertebrates with eggs containing little yolk—the formation of a two-layered gastrula (Fig. 13). How was one to explain this seemingly fundamental pattern of development? Haeckel speculated that in very early geological times the ancestors of all metazoans had a body consisting of two layers enclosing a central cavity—the gut. This was his Gastraea Theory. Such creatures, the Gastraeades, were similar to the gastrula stage of the embryos of many living species and even to the adult stages of the most primitive living coelenterates.

Figure 14 shows the critical position of the Gastraeades in the phylogeny of the major groups of animals. Haeckel surmised the long evolutionary line to the mammals passed thereafter through worm-like stages and finally reached the chordates (Stage 8

A. Human Progonotaxis, First Half:

EARLIER SERIES OF ANCESTORS, WITHOUT FOSSIL EVIDENCE, PRE-SILURIAN

Chief Stages.	Ancestral Stem-groups.	Living Relatives of Ancestors.	Paleontology.	Ontogeny.	Morphology.
Stages 1–5: **Protist ancestors.** Unicellular organisms. 1–2: Plasmodomous protophytes. 3–5: Plasmophagous protozoa.	1. **Monera.** (Plasmodomia.) Without nucleus.	1. **Chromacea.** *(Chroococcus.)* *Phycochromacea.*	0	!?	I
	2. **Algaria.** Unicellular algæ.	2. **Paulotomea.** *Palmellacea eremosphæra.*	0	!?	II
	3. **Lobosa.** Unicellular (amœbina) rhizopods.	3. **Amœbina.** *Amœba leucocyta.*	0	!!	II
	4. **Infusoria.** Unicellular.	4. **Flagellata.** *Euflagellata zoomonades.*	0	?	II
	5. **Blastæades.** Multicellular hollow vesicles (cenobia).	5. **Catallacta.** *Magosphæra, volvocina, blastula.*	0	!!!	III
Stages 6–11: **Invertebrate metazoa ancestors.** 6–8: Cœlenteria, without anus and body-cavity. 9–11: Vermalia, with anus and body-cavity.	6. **Gastræades.** With two germ-layers.	6. **Gastrula.** *Hydra, olynthus, gastremaria.*	0	!!!	III
	7. **Platodes I.** *Platodaria* (without nephridia).	7. **Cryptocœla.** *Convoluta proporus.*	0	?	I
	8. **Platodes II.** *Platodinia* (with nephridia).	8. **Rhabdocœla.** *Vortex monotus.*	0	?	I
	9. **Provermalia.** (Primitive worms.) *Rotatoria.*	9. **Gastrotricha.** *Trochozoa trochophora.*	0	?	I
	10. **Frontonia.** *(Rhynchelminthes.)* Snout-worms.	10. **Enteropneusta.** *Balanoglossus cephalodiscus.*	0	!	I
	11. **Prochordonia.** Chorda-worms.	11. **Copelata.** *Appendicaria.* Chordula-larvæ.	0	!	II
Stages 12–15: **Monorhina ancestors.** Oldest vertebrates without jaws or pairs of limbs, single nose.	12. **Acrania I.** (Prospondylia.)	12. **Amphioxus larvæ.**	0	!!!	III
	13. **Acrania II.** More recent.	13. **Leptocardia.** Amphioxus.	0	!	III
	14. **Cyclostoma I.** (Archicrania.)	14. **Petromyzoa larvæ.**	0	!!!	II
	15. **Cyclostoma II.** More recent.	15. **Marsipobranchia.** Petromyzoa.	0	!	III

FIG. 7. The putative human ancestors among the invertebrates and lower vertebrates (Haeckel, 1905*a*, table 26).

B. Human Progonotaxis, Second Half:

LATER ANCESTORS, WITH FOSSIL EVIDENCE, BEGINNING IN SILURIAN PERIOD

Geological Periods.	Ancestral Stem-groups.	Living Relatives of Ancestors.	Paleontology.	Ontogeny.	Morphology.
Silurian.	16. **Selachii.** Primitive fishes. *Proselachii.*	16. **Natidanides.** Chlamdoselachus. Heptanchus.	I	! !	III
Silurian.	17. **Ganoides.** Plated-fishes. *Proganoides.*	17. **Accipenserides.** (Sturgeons.) Polypterus.	II	!	II
Devonian.	18. **Dipneusta.** *Paladipneusta.*	18. **Neodipneusta.** Ceratodus. Protopterus.	I	! !	II
Carboniferous.	19. **Amphibia.** *Stegocephala.*	19. **Phaneropranchia.** Salamandrina. (Proteus, triton.)	III	! ! !	III
Permian.	20. **Reptilia.** *Proreptilia.*	20. **Rhynchocephalia.** Primitive lizards. Hatteria.	III	! !	II
Triassic.	21. **Monotrema.** *Promammalia.*	21. **Ornithodelphia.** *Echidna.* *Ornithorhyncus.*	I	! ! !	III
Jurassic.	22. **Marsupialia.** *Prodidelphia.*	22. **Didelphia.** *Didelphys.* *Perameles.*	I	! !	II
Cretaceous.	23. **Mallotheria.** *Prochoriata.*	23. **Insectivora.** Erinaceida. (Ictopsida +.)	II	!	I
Older Eocene.	24. **Lemuravida.** Older lemurs. Dentition 3. 1. 4. 3.	24. **Pachylemures.** *(Hyopsodus +.)* *(Adapis +.)*	III	! ?	II
Neo-Eocene.	25. **Lemurogona.** Later lemurs. Dent. 2. 1. 4. 3.	25. **Autolemures.** *Eulemur.* *Stenops.*	III	!	I
Oligocene.	26. **Dysmopitheca.** Western apes. Dent. 2. 1. 3. 3.	26. **Platyrrhinæ.** *(Anthropops +.)* *(Homunculus +.)*	I	!	II
Older Miocene.	27. **Cynopitheca.** Dog-faced apes (tailed).	27. **Papiomorpha.** *Cynocephalus.*	I	!	III
Neo-Miocene.	28. **Anthropoides.** Man-like apes (tail-less).	28. **Hylobatida.** Hylobates. Satyrus.	I	! !	III
Pliocene.	29. **Pithecanthropi.** Ape-men (alali, speechless).	29. **Anthropitheca.** Chimpanzee. Gorilla.	II	! ! !	III
Pleistocene.	30. **Homines.** Men, with speech.	30. **Weddahs.** Australian negroes.	I	! ! !	III

FIG. 8. Continuation of Figure 7 (Haeckel, 1905*a*, table 27).

SYNOPSIS OF THE CHIEF SECTIONS OF OUR STEM-HISTORY

FIRST SECTION OF OUR PHYLOGENY.

Man's Invertebrate Ancestors.

First phylogenetic stage: **The Protists.**

Man's ancestors are unicellular protozoa, originally unnucleated monera like the chromacea, structureless green particles of plasm; afterwards real nucleated cells (first plasmodomous *protophyta*, like the palmella; then plasmophagous *protozoa*, like the amœbæ).

Second phylogenetic stage: **The Blastæads.**

Man's ancestors are round cœnobia or colonies of protozoa; they consist of a close association of many homogeneous cells, and thus are individuals of the second order. They resemble the round cell-communities of the magosphæræ and volvocina, equivalent to the ontogenetic blastula: hollow globules. the wall of which consists of a single layer of ciliated cells (blastoderm).

Third phylogenetic stage: **The Gastræads.**

Man's ancestors are gastræads, like the simplest of the actual metazoa (prophysema, olynthus, hydra, pemmatodiscus). Their body consists merely of a primitive gut, the wall of which is made up of the two primary germinal layers.

Fourth phylogenetic stage: **The Platodes.**

Man's ancestors have substantially the organisation of simple platodes (at first like the cryptocœlic platodaria, later like the rhabdocœlic turbellaria). The leaf-shaped bilateral-symmetrical body has only one gut-opening, and developes the first trace of a nervous centre from the ectoderm in the middle line of the back (Figs. 293, 294).

Fifth phylogenetic stage: **The Vermalia.**

Man's ancestors have substantially the organisation of unarticulated vermalia, at first gastrotricha (ichthydina), afterwards frontonia (nemertina, enteropneusta). Four secondary germinal layers develop, two middle layers arising between the limiting layers (cœloma). The dorsal ectoderm forms the vertical plate, acroganglion (Fig. 297).

Sixth phylogenetic stage: **The Prochordonia.**

Man's ancestors have substantially the organisation of a simple unarticulated chordonium (copelata and ascidian larvæ). The unsegmented chorda developes between the dorsal medullary tube and the ventral gut-tube. The simple cœlom-pouches divide by a frontal septum into two on each side: the dorsal pouch (episomite) forms a muscle-plate; the ventral pouch (hyposomite) forms a gonad. Head-gut with gill-clefts.

FIG. 9. Figures 9 through 12 form a series comparing phylogeny and ontogeny in an abbreviated series. Figures 9 and 10 compare pre-vertebrate phylogeny and ontogeny (Haeckel, 1905*a*, table 36).

in Fig. 6, Stage 11 in Fig. 7, and Stage 6 in Fig. 9). Even here there was little useful information from paleontology (Fig. 7) and the hypothetical phylogeny had to be based mainly on morphological data, with an assist from embryology. The fossil data became more useful for later hypothetical stages (Fig. 8) and it is interesting to compare Haeckel's analysis with present-day information. In Figure 6 the sequence to mammals passes through the ancestors of these still-living forms: amphioxus, lamprey, shark, bony fish, and amphibian. It was only later that he realized that the last part of

SYNOPSIS OF THE CHIEF SECTIONS OF OUR EMBRYOLOGY

FIRST SECTION OF OUR ONTOGENY.

Man's Invertebrate Forms.

First ontogenetic stage: **The Protozoa stage.**

The human embryo is a simple round cell, the cytula or stem-cell (first segmentation-cell, or fecundated ovum). Unicellular stage (unnucleated during caryolysis, afterwards nucleated and amœboid).

Second ontogenetic stage: **The Blastula stage.**

The human embryo consists of a round cluster of simple cells—segmentation-cells—like a colony of protozoa (a cenobium of social protozoa). It is a cenogenetic modification of the globular blastula, a hollow ball, the wall of which consists of a single layer of cells (blastoderm). The corresponding pure palingenetic form is still found in the amphioxus (Fig. 257 *c*).

Third ontogenetic stage: **The Gastrula stage.**

The human embryo is a round epigastrula, the cenogenetically modified gastrula of the higher mammals. It is composed of two layers of cells, the two primary germinal layers. The corresponding palingenetic form (archigastrula) is still found in the amphioxus (Figs. 257–260).

Fourth ontogenetic stage: **The Neurula stage.**

The human embryo assumes the bilateral-symmetrical form, and developes the first trace of the medullary tube (with the neurenteric canal) from the ectoderm in the middle line of the back. This is found in palingenetic form in the amphioxus (Fig. 260).

Fifth ontogenetic stage: **The Cœlomula stage.**

The human embryo is an oval bilateral embryonic disk (blastodiscus), in which we distinguish the four secondary germinal layers. Between the two limiting layers or the primary germinal layers two middle layers (the parietal and visceral layers of the simple cœlom-pouches) have spread out from the primitive mouth (or primitive streak). The dorsal ectoderm forms the medullary plate.

Sixth ontogenetic stage: **The Chordula stage.**

The human embryo has the structure of a simple unarticulated chordonium, the nearest living relatives of which are the copelata (appendicularia) and the ascidian larvæ. The unsegmented chorda developes between the dorsal medullary tube and the ventral gut-tube. The simple cœlom-pouches divide by a frontal septum into two pouches on each side: the dorsal pouch ("stem-zone") forms a muscle-plate, the ventral pouch ("parietal zone") corresponds originally to a gonad. Head-gut with gill-clefts.

FIG. 10. Compare with Figure 9 (Haeckel, 1905*a*, table 37).

the sequence should be amphibian—reptile—mammal (Fig. 8). Haeckel's sequence has stood the test of later discoveries well, at least so far as the vertebrates are concerned. The major difference is that the Selachii (Chondrichthyes) are thought to be derived from a very primitive group of bony fishes and are not themselves the most primitive fishes.

SACCULINA, BARNACLES, ASCIDIANS

More and more data seemed to suggest that early embryos may retain some relics of the basic structure of the group to which

SYNOPSIS OF THE CHIEF SECTIONS OF OUR STEM-HISTORY

SECOND SECTION OF OUR PHYLOGENY.

Man's Vertebrate Ancestors.

Man's ancestors are vertebrates, and have the form of an articulated individual or chain of metamera. The skin-sense layer is differentiated into horny plate and medullary tube. The skin-fibre layer has divided into corium-plate, muscle-plate, and skeleton-plate. From the gut-fibre layer we get the heart with the blood-vessels and the muscular wall of the gut. The gut-gland layer forms the chorda and the visceral epithelium.

Seventh phylogenetic stage: **The Acrania.**

Man's ancestors are skull-less vertebrates, like the amphioxus. The body is a series of metamera, as several of the primitive segments are developed. The head contains in the ventral half the branchial gut, the trunk the hepatic gut. The medullary tube is still simple. No skull, jaws, or limbs.

Eighth phylogenetic stage: **The Cyclostoma.**

Man's ancestors are jaw-less craniotes (like the myxinoida and petromyzonta). The number of metamera increases. The fore-end of the medullary tube expands into a vesicle and forms the brain, which soon divides into five cerebral vesicles. In the sides of it appear the three higher sense-organs: nose, eyes, and auditory vesicles. No jaws, limbs, or floating bladder.

Ninth phylogenetic stage: **The Ichthyoda.**

Man's ancestors are fish-like craniotes: (1) Primitive fishes (selachii); (2) plated fishes (ganoida); (3) amphibian fishes (dipneusta); (4) mailed amphibia (stegocephala). The ancestors of this series develop two pairs of limbs: a pair of fore (breast-fins) and of hind (belly-fins) legs. The gill-arches are formed between the gill-clefts: the first pair form the maxillary arches (upper and lower jaws). The floating bladder (lung) and pancreas grow out of the gut.

Tenth phylogenetic stage: **The Amniotes.**

Man's ancestors are amniotes or gill-less vertebrates: (1) Primitive amniotes (proreptilia); (2) sauromammals; (3) primitive mammals (monotremes); (4) marsupials; (5) half-apes (prosimiæ); (6) western apes (platyrrhinæ); (7) eastern apes (catarrhinæ): at first tailed cynopitheca, then tail-less anthropoids; later speechless ape-men (alali); finally speaking man. The ancestors of these amniotes develop an amnion and allantois, and gradually assume the mammal, and finally the specifically human, form.

FIG. 11. The vertebrate stages in human phylogeny. Continuation of Figure 9 (Haeckel, 1905*a*, table 36).

they belong. If so, one might predict that a study of the embryos of species that were "problems" so far as their relationships were concerned would be productive.

Sacculina was the name given to a bag-like structure that could be found attached to various species of crabs. So far as external appearances are concerned it could be a tumor, especially since branching roots of the sac actually penetrate the host's abdomen. Closer study showed that the sac contains reproductive organs and some muscle and nerve tissue. Thus *Sacculina* could be considered a parasite and the branching roots that enter the host could be the mechanism for obtaining food.

One can not deduce from the structure of *Sacculina* what its affinities might be. A study of the early embryos, however, gave the answer. The eggs were found to develop into a well known larval type—the nauplius larva with three pairs of appendages—that

SYNOPSIS OF THE CHIEF SECTIONS OF OUR EMBRYOLOGY

SECOND SECTION OF OUR ONTOGENY.

Man's Vertebrate Forms.

The human embryo represents an articulated individual or a series of metamera. The skin-sense layer is differentiated into horny plate and medullary tube. The skin-fibre layer has divided into corium-plate, muscle-plate, and skeleton-plate. From the gut-fibre layer we get the heart with the blood-vessels and the muscular wall of the gut. The gut-gland layer forms the chorda and the visceral epithelium.

Seventh ontogenetic stage: **The Acrania stage.**

The human embryo has substantially the organisation of a skull-less vertebrate, like the amphioxus. The body forms a series of metamera, as several of the primitive segments are differentiated. The head contains in the ventral half the branchial gut, and the trunk the hepatic gut. The medullary tube is still simple. No skull, jaws, or limbs.

Eighth ontogenetic stage: **The Cyclostoma stage.**

The human embryo has substantially the organisation of a jaw-less craniote (like the myxinoida and petromyzonta). The number of metamera increases, The fore-end of the medullary tube enlarges and forms a brain, which soon divides into five cerebral vesicles. At the sides of it appear the three higher sense-organs: olfactory pits, eyes, and auditory vesicles. No jaws, limbs, or lungs.

Ninth ontogenetic stage: **The Ichthyoda stage.**

The human embryo has substantially the organisation of a fish-like craniote. The two pairs of limbs appear in very rudimentary form, as fin-like buds: a pair of fore (breast-fins) and of hind (belly-fins) legs. Between the gill-clefts the gill-arches are formed: the first pair form the jaw-arches (upper and lower jaws). The lung (floating bladder) and pancreas grow out of the gut.

Tenth ontogenetic stage: **The Amniote stage.**

The human embryo has substantially the organisation of an amniote or gill-less vertebrate. The gill-clefts disappear or grow together. From the gill-arches are formed the jaws, hyoid bone, and the bones of the ear. The embryo is enveloped in two membranes (amnion and serolemma). The bladder developes from the body of the embryo, and forms the allantois (and afterwards, at a part of its periphery, the placenta). All the organs of the body gradually assume the mammal, and finally the specifically human, form.

FIG. 12. Compare with Figure 11 (Haeckel, 1905*a*, table 37).

is characteristic of many crustaceans. Later the nauplius larva transforms into the cypris larva, again a familiar crustacean larval type. After a period of independent life the cypris larva attaches to a crab, loses its appendages and most of its anatomy for that matter, and becomes the tumor-like structure of the adult *Sacculina*. Figure 15 shows a variety of nauplius larvae of crustaceans. As you can see, that of *Sacculina* fits the picture.

As noted before the story is similar for the barnacles, a large group of invertebrates. Since they are covered by a shell, many early naturalists considered them to be mollusks. A study of the embryos, however, showed that the barnacles are crustaceans, with a typical crustacean larval type (Fig. 15, D—*Lepas*).

The answer for the ascidians was different but the method of obtaining the answer was the same. The ascidians, or tunicates, are marine organisms. Most of the common ones look like an amorphous mass of

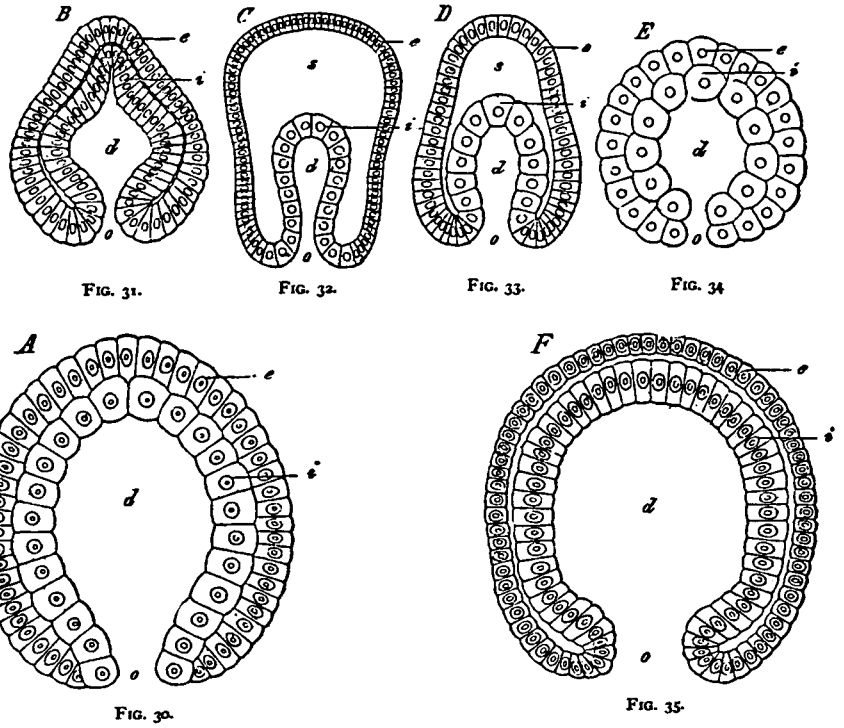


FIG. 31. FIG. 32. FIG. 33. FIG. 34

FIG. 30. FIG. 35.

FIG. 13. The Gastraead level of organization. A is a hypothetical simple two-layered animal, or Gastraead. It is similar to the gastrula stage of many metazoans having eggs with little yolk: the arrow-worm, *Sagitta* (B); the starfish, *Uraster* (C); the crab, *Nauplius* (D); the pond snail, *Limnaeus* (E); and the lancet, *Amphioxus* (F). In each figure *d* is the archenteron, *o* the mouth, *s* the blastocoel, *i* the endoderm, and *e* the ectoderm (Haeckel, 1905*b*, p. 63).

"something" that is attached to wharf pilings, rocks, etc. The adults consist mostly of a basket with perforated walls. Water enters an opening and passes through the walls of the basket and food particles are strained out. Again it would be difficult to classify these objects on the basis of the structure of the adult. The larvae provide the answer—they are chordates, with a nerve tube, pharyngeal gill slits, and a notochord.

Clearly ontogeny was a powerful tool for discovering relationships among organisms.

RECAPITULATION EVALUATED

Figures 6–12 and 13 represent a bold attempt by Haeckel to reduce to a single concept a very large amount of data—an attempt made when the data were most inadequate. He speculated far beyond the data available to him but in so doing he provided hypotheses for others to test and suggested studies that were worth doing. But this had serious disadvantages and in Oppenheimer's (1955, p. 15) opinion,

> What was damaging to science was Haeckel's fervency to oversimplify all morphology through his biogenetic law that "die Ontogenie ist eine Recapitulation der Phylogenie."

Nevertheless it is surprising how many of his ideas turned out to be correct in general—though rarely in detail. For example, his notions about the origin of life are not too different from the hypotheses of today. His attempt to interrelate all kinds of animals (Fig. 14) would not be accepted by all biologists today but, for that matter, no other scheme has achieved widespread approbation.

There are parallels between Darwin and Haeckel in the general acceptance of their

MONOPHYLETIC GENEALOGICAL TREE OF THE ANIMAL KINGDOM, BASED ON THE GASTRÆA THEORY

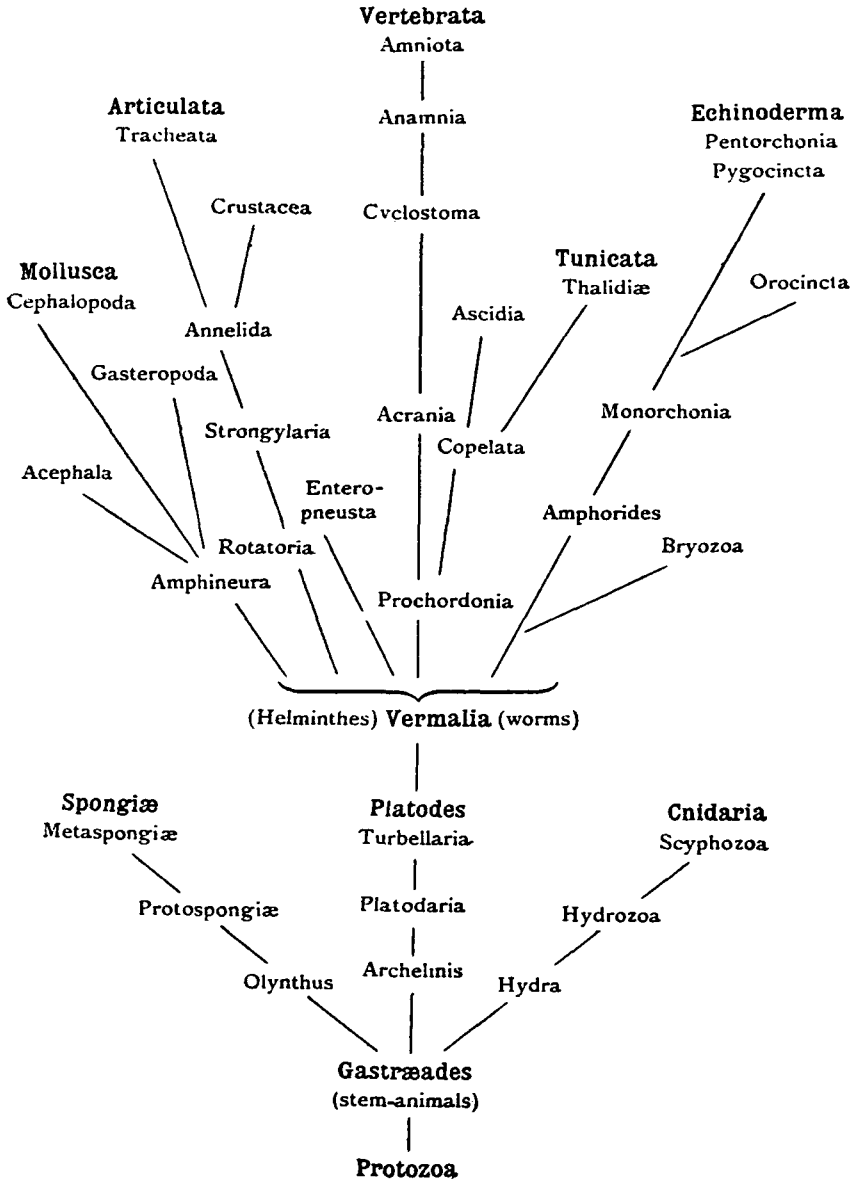


FIG. 14. Haeckel's hypothesis for the interrelations of the major groups of animals (Haeckel, 1905*a*, table 25).

main theses and the rejection of many of the details. Most biologists agreed that Darwin had shown beyond all reasonable doubt that evolution had occurred but his suggested mechanisms—spontaneous variations acted upon by natural selection—were thought improbable or impossible until well into the 20th century. Haeckel's

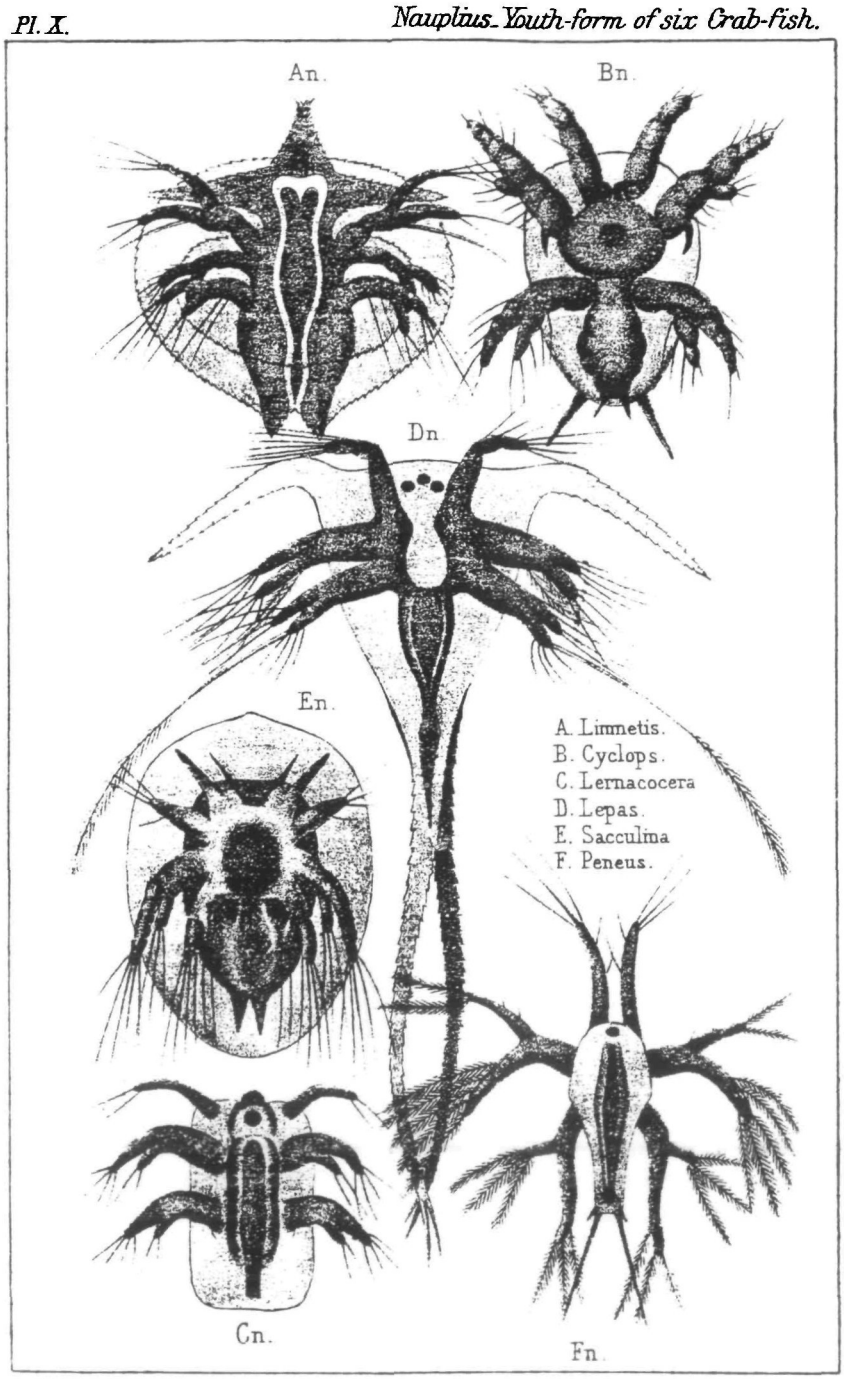


FIG. 15*a*. Larval and adult crustaceans. The early larval forms of six crustaceans with their three pairs of appendages, some obviously biramous. Compare with Figure 15*b*.

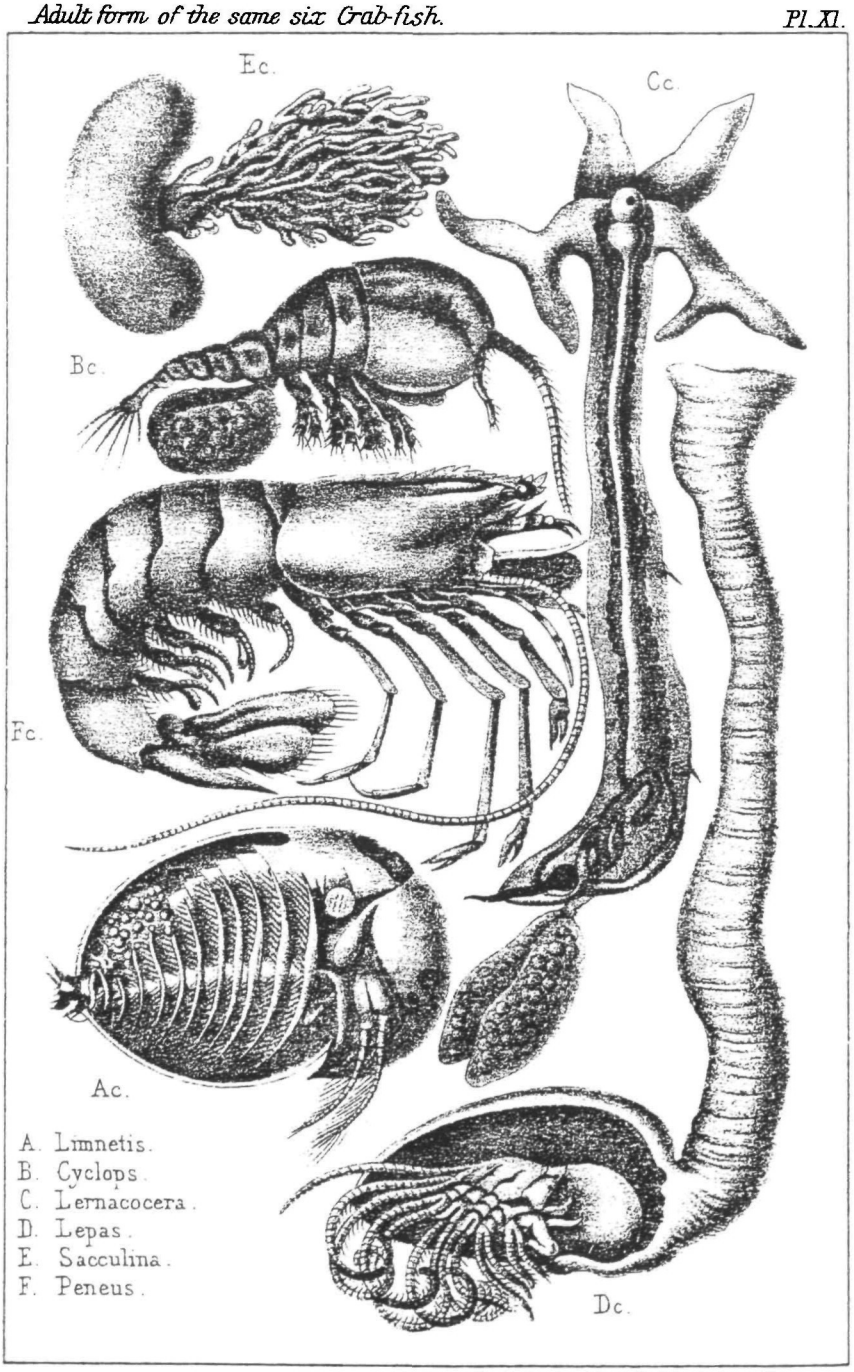


FIG. 15*b*. These are the adults of the larval forms shown in Figure 15*a*. In spite of their similar larval forms they differ greatly from one another (Haeckel, 1876).

synthesis of the data of descriptive embryology, evolution, and comparative anatomy is accepted in theory even though there has been a strong reaction against the details. Many have claimed that Haeckel believed that embryos recapitulate the *adult* stages of ancestors. I read Haeckel differently, possibly being biased by admiration for his attempts at synthesis. Surely one cannot gather from his discussion of the "Ichthyoda" stage in our phylogeny (Fig. 11) and in ontogeny (Fig. 12) that Haeckel is suggesting that we recapitulate that stage by swimming around in the amnion with pectoral and pelvic fins, a fishy tail, and encased in a scaly skin.

Gould (1977) has provided a magnificent synthesis of ideas related to recapitulation. He writes

> The theory of recapitulation played a fundamental role in a host of diverse disciplines; I suspect that its influence as an import from evolutionary theory into other fields was exceeded only by natural selection itself during the nineteenth century . . . . We grasp the importance of recapitulation only when we understand that it served as the organizing idea for generations of work in comparative anatomy, physiology, and morphology . . . . In my own field of paleontology, for example, it governed most studies in phyletic reconstruction from Haeckel's day right through the 1930s (pp. 115–116).

E. S. Russell (1917, pp. 312–313) offers another useful perspective:

> But evolutionary morphology for all practical purposes was a development of pure or idealistic morphology, and was powerless to bring to fruit the new conception with which evolution-theory had enriched it. The reason is not far to seek. Pure morphology is essentially a science of comparison which seeks to disentangle the unity hidden beneath the diversity of organic form. It is not immediately concerned with the causes of organic diversity—that is rather the task of the sciences of the individual, heredity and development. To take an example—the recapitulation theory may legitimately be used as a law of pure morphology, as stating the abstract relation of ontogeny to phylogeny, and the probable line of descent of any organism may be deduced from it, as a mere matter of the ideal derivation of one form from another; but an explanation of the reason for the recapitulation of ancestral history during development can clearly not be given by a pure morphology unaided. From the fact that the common starfish shows in the course of its development distinct traces of a stalk it is possible to infer, taking other evidence also into consideration, that the ancestors of the starfish were at one stage of their existence stalked and sessile organisms [perhaps resembling crinoids]. But this leaves unanswered the question as to how and why the starfish does still repeat after so many millions of years part of the organisation of one of its remote ancestors. Why is this feature retained, and by what means has it been conserved through countless generations? It is clear that the answer can be given only by a science of the causes of the production and retention of form, by a causal morphology, based upon a study of heredity and development.
>
> From the point of view of the pure morphologist the recapitulation theory is an instrument of research enabling him to reconstruct probable lines of descent; from the standpoint of the student of development and heredity the fact of recapitulation is a difficult problem whose solution would perhaps give the key to a true understanding of the real nature of heredity.
>
> To make full use of the conception of the organism as an historical being it is necessary then to understand the causal nexus between ontogeny and phylogeny.

Summary. But as we see it today, von Baer came closer to an acceptable concept than Haeckel did. Taking the best known examples, the chordates, we must admit that embryos do not in general recapitulate the adult stages of their ancestors. Chordate

embryos do share a common plan of development that has an early stage with notochord, dorsal nerve tube, and pharyngeal gill slits or pouches separated by gill arches (I, pp. 502–503). The adults of the lower chordate classes change less from this fundamental plan than the adults of the higher classes. The higher forms *retain* some fundamental features in their early development and then differentiate in their special ways. Thus the organ systems of the amphibians, reptiles, birds, and mammals can be understood as variations on a pattern based on the morphology of agnathans and primitive fish. Good examples are the pronephros–mesonephros–metanephros series of kidneys (I, pp. 503–504) and the jaw bones and ear ossicles (I, pp. 408–409).

It must be kept in mind that it is essentially impossible to show beyond all reasonable doubt that ontogeny does recapitulate phylogeny for structures that do not fossilize. Comparisons of living organisms cannot substitute for true ancestors. Thus tests of the hypothesis of recapitulation must be based on skeletons or other structures that form the fossil record. Mammalian ear ossicles are a case in point. Mainly on the basis of embryological observations, C. B. Reichert, in 1837, suggested their homologies to the jaw bones of lower forms (I, pp. 498–499). A century later this bold hypothesis was to be fully confirmed by the discovery of a series of fossils of the mammal-like reptiles.

The very difficult problem of why such "useless" structures as the notochord, gill pouches, or pronephros are recapitulated would not be answered until the organizer theory was developed.

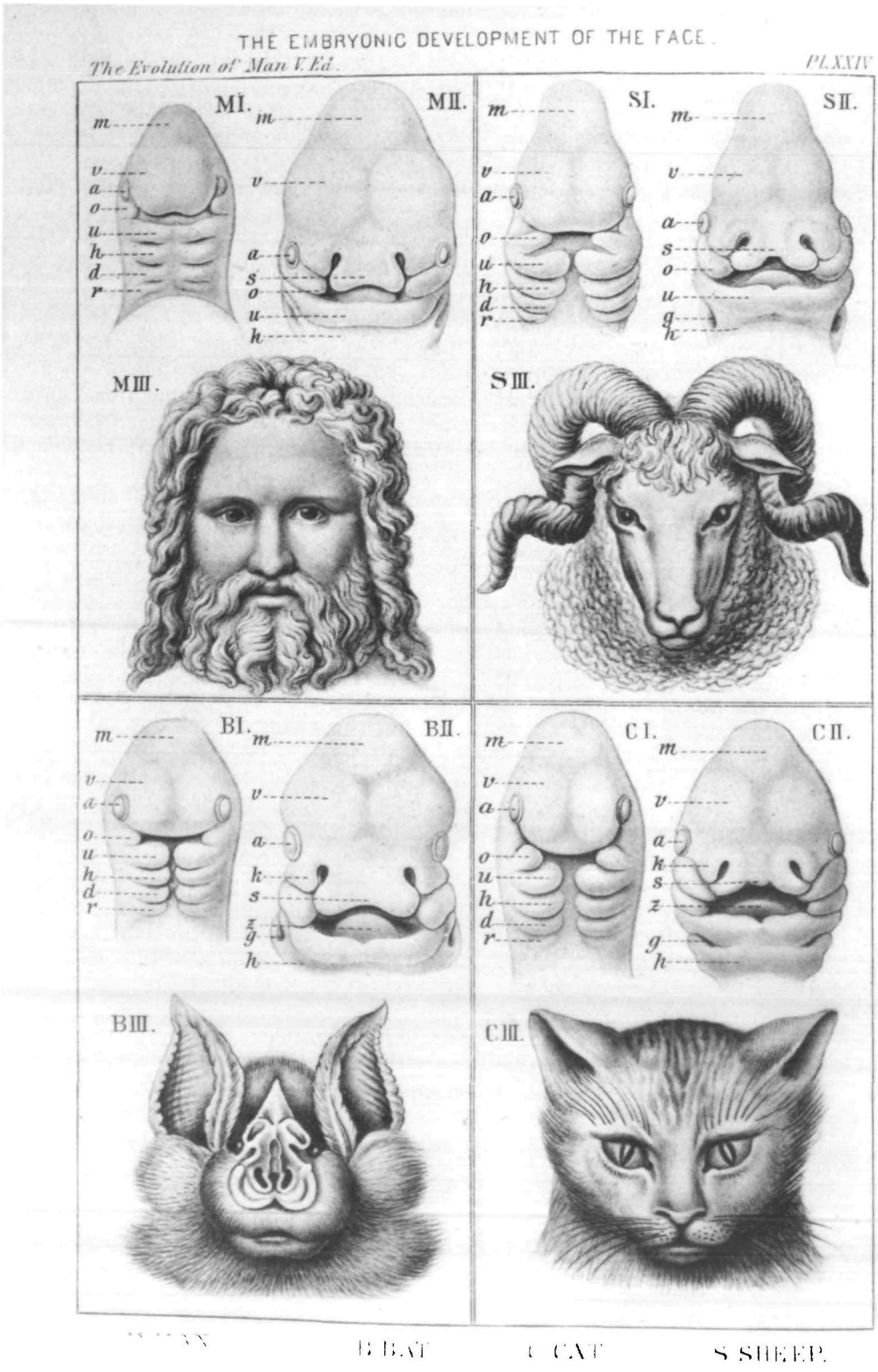
Haeckel's hypothetical prechordate ancestors did not find universal acceptance. Nevertheless his effort was eminently worthwhile. The problem is important and to this day there are no satisfactory answers or known techniques for obtaining them. At least Haeckel suggested how one *might* think about prechordate ancestors and we may have to settle for that. It is certainly reasonable to think that early metazoans may have passed first through a stage similar in general structure to a planula larva and then later through a stage similar to a gastrula. The chance of discovering a planula-like or gastrula-like ancestor in rocks laid down two billion years ago seems remote and, if such were found, to make a convincing argument that it was ancestral to the metazoan phyla would be almost impossible. But stranger and luckier things than that have happened.

There seems to be little question that Haeckel extended the concept of recapitulation well beyond the point where it could be tested in his day or even ours. It was an imaginative attempt and elements were soon regarded as improbable or wrong. Nevertheless the concept remains a powerful and useful way of trying to understand the structure of organisms. Data such as shown in Haeckel's illustration reproduced as Figure 16 are difficult to understand without the concept of recapitulation. So also are the many examples of vestigial structures. It is far simpler to account for the human appendix as a relic of an essential structure of an ancestor rather than as an object evolved mainly to assist the cash-flow problems of our surgeons.

So to paraphrase an earlier quotation of Stephen Gould's, "Let's not throw recapitulation out with Haeckel." In fact, we should keep both but be cautious in the manner in which they are used.

COMPARATIVE EMBRYOLOGY

In 1880–1881 Francis Balfour's two volume *A Treatise on Comparative Anatomy* was published. Apart from a briefer work on the same subject by Packard (1876), this was the first major synthesis since von Baer. In that half century enormous progress had been made in embryology. There were more students of development and better tools and methods: better microscopes, better histological techniques, and improved methods for obtaining and handling embryological material. As a result data became available on the developmental patterns of species belonging to all major groups of animals. Advances in Cell Theory allowed a deeper understanding of embryos—embryos were recognized as composed of cells, and the results of an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orderly process of cell division. The Theory of Evolution provided an explanatory hypothesis for the similarities as well as the variations in patterns of development. The data of embryology made so much sense in terms of evolution that they became one of the better evidences for evolution itself. And, in the absence of data from extinct ancestors, embryos were searched for evidences of phylogeny.

Quite apart from these research goals, there was the hope that uniformities, that is, rules that would hold for many different sorts of development, could be found. Had one known nothing of the varieties of ontogenies to be discovered, it might have been predicted that patterns of development would be as varied as patterns of adult morphology. Such proved not to be the case. The remarkable similarities in development of vertebrates, known to observers from Aristotle to Haeckel, was found to hold for invertebrates as well. There were, indeed, rules and the most diverse ends were achieved by variations on a fundamental pattern. It was discovered that, throughout the metazoans, one could usually recognize these major steps in development.

1. Development begins with the activation of an ovum by a sperm (sexual reproduction) or by other means (parthenogenesis).

2. The activated ovum divides repeatedly, and comparatively rapidly, about 8 to 12 times by mitosis. The result is a ball of cells, the blastula, with a cavity, the blastocoel.

3. There then ensues a rearrangement of cells with some moving inward forming a cavity, the archenteron. The cavity has an opening to the outside, the blastopore. This cup-shaped structure with an inner and outer layer is the gastrula (Fig. 13), which was regarded by Haeckel as the basic body plan from which all metazoans evolved (Fig. 14). The rate of cell division slows by the gastrula stage and remains slow for the rest of development.

4. The rearrangement of cells results in the formation of the embryonic layers. There are two of these in the coelenterates, an outer ectoderm and an inner endoderm. Other metazoans have an additional layer, the mesoderm, between the ectoderm and endoderm. When these layers first become definite their cells are essentially the same.

5. As development continues there is an increase in cell number, the cells become visibly differentiated, and there are structural rearrangements of the cells leading to the formation of organs and tissue layers.

6. Throughout the metazoans there is considerable uniformity in the structures developing from each germ layer. Typically the skin, nervous system, and some types of excretory organs are derived from the ectoderm; the lining of the alimentary canal and the associated organs are derived from endoderm; the circulatory system, muscles, connective tissue, and some types of excretory organs are derived from the mesoderm.

This commonality of patterns of development found its formal explanation in the theory of evolution and its derivative, recapitulation.

Nevertheless it was discovered that there are two strikingly different patterns of development—direct and indirect—that result in similar end products. One of the main reasons appeared to be the quantity of yolk in the ovum or the availability of food directly from the mother. The ova of some species, human beings and sea urchins for example, contain very small amounts of yolk—far less than is required to carry the embryo to the juvenile stage. Such embryos must rely on external sources of food—either capturing it themselves or obtaining it from mother.

The sea urchin mode of development,

←

FIG. 16. Early embryos and adults of four mammals. The embryos were much the same in spite of their very different destinies as von Baer and other embryologists had discovered (Haeckel, 1905*a*, p. 290).

which is common among the invertebrates, is for the embryo rapidly to reach a free-living larval stage, in this case a pluteus. The pluteus is a microscopic larva that obtains its food from the ocean. It bears no resemblance to the adult. It swims, feeds and grows. Eventually a complete restructuring of its anatomy, physiology, and life style begins—it undergoes metamorphosis into the adult sea urchin. This is called *indirect development,* since the embryo does not develop directly into an adult but passes through a larval stage very different in structure, physiology, and behavior from the adult.

Human beings and other mammals rely on nourishment from the mother. They differentiate into the juvenile form without a free-living, food-capturing larval stage. These embryos have *direct development.*

Direct development also occurs in birds but there the source of the food supply is different—the ovum contains sufficient food to carry the embryo to the juvenile stage.

The quantity of yolk not only determines the pattern of development in oviparous species but some of the details of early development as well. In species with little yolk, mitosis divides the entire embryo into cells of roughly equal size and a "typical" gastrula (Fig. 13) is formed. In many familiar frog species there is an intermediate quantity of yolk and the pattern of cleavage and gastrulation is much modified. In species with large amounts of yolk, such as the birds, the pattern of cleavage, gastrulation, and organ formation is so different from that of species with less yolk that much study was required before the basic homologies could be understood.

Patterns of direct and indirect development are obviously exceedingly different. It came as a distinct surprise to embryologists, therefore, to find that both patterns could occur in closely related species. Try to imagine how a strict recapitulationist would have interpretated these observations.

Most species of anurans of the United States and Europe—the ones that were studied first—have eggs with a modest amount of yolk and indirect development. The early embryo develops into a free-living tadpole, with gills and a tail, that seeks food in its aquatic environment. After a period varying from weeks to years, depending on the species, there ensues a drastic metamorphosis during which the tail is resorbed, limbs appear, the entire anatomy undergoes considerable modification, and a froglet hops out on land. These frog species, then, seem to recapitulate their fishy ancestors in their tadpole stage.

As more and more frog species were studied, however, it became clear that some have huge eggs and direct development, that is, the tadpole stage is entirely omitted and the embryo develops straight into a froglet (Duellman and Trueb, 1986, ch. 2; Salthe and Mecham, 1974). In these cases the anuran embryo is similar to birds in having enough yolk to last to the juvenile stage. Duellman and Trueb estimate that "direct development must have evolved independently in at least 12 groups" of anurans.

It must be emphasized that the adults of those species of frogs that develop directly and indirectly—seemingly fundamentally different patterns—may belong to the same genus and, hence, be very much alike. This same phenomenon exists in salamanders as well. *Desmognathus fuscus* has indirect development with a feeding larval stage whereas the closely similar *Desmognathus wrighti* has direct development.

It might be rewarding to explore with students where these data leave the concept of recapitulation. Should we conclude that *Desmognathus fuscus* and *Desmognathus wrighti* had basically different evolutionary histories?

This is a case where one must consider how anomalous data relate to the main body of data. When this is done there seems to be little reason to change one's opinion about the usefulness of the concept of recapitulation. The embryos of both species exhibit in their early stages the structures that make the concept of recapitulation a useful way to account for the data. They have the basic chordate structures: notochord, dorsal nerve tube, and pharyngeal

pouches. Their circulatory systems, jaw bones, and kidneys show the same modifications of the basic vertebrate body plan. The data are evaluated, therefore, and one bases relationships on those thought to be most important.

Thus, the patterns of direct and indirect development may not be so fundamentally different as we first thought. This conclusion is strengthened by the probability that direct development may have arisen independently those 12 times.

It bears repeating that the concept of recapitulation offers an explanation for only some aspects of development. These are the situations where vestiges of ancient patterns of development appear to have persisted. It must be accepted that these are generally restricted to the earliest stages of development. The concept is of value because it can reasonably account for some otherwise inexplicable observations.

We still have the problem, however, of discovering why any ancestral structures persist.

THOSE GERM LAYERS

The traditional treatment of embryology in beginning courses stresses the details of development. If the students are left with any organizing concept at all it is that the outside of the embryo is blue, the middle section red, and the inside yellow. Seemingly that concept has universal applicability for the organisms considered and, by inference, for those that were not.

And the Theory of Germ Layers has been one of the mainstays of descriptive embryologists as well as of students. The concept grew slowly from Wolff, Pander, and von Baer to its extensive development by Haeckel (1905*b*, ch. 10) and Lankester (1877). It has been almost as contentious as the concept of recapitulation. The arguments have been mainly about the applicability of the concept to embryos of different phyla and what is implied about the developmental potential of the layers themselves.

Let us consider those two aspects separately. If one is saying only that metazoan embryos consist of two (coelenterates) or three (the other major phyla) layers during an early embryonic stage, the concept has great heuristic value: "among the higher Metazoa there is then a wide correspondence between the germ layers as regards their fate and function in ontogeny" (Hyman, 1940, p. 270). It is conceptually satisfying to know that in animals differing greatly from one another the skin, the most anterior and most posterior parts of the alimentary canal, and the nervous system develop from the ectoderm; the muscles, connective tissues, skeletal and circulatory systems (if there are any), from the mesoderm; and, except for the two ends, the lining of the digestive system and its associated glands from the endoderm.

While "wide correspondence" exists between what the germlayers do, that does not signify that they are homologous. Nevertheless the origins of the three layers are so much alike throughout the vertebrates that it can be said that they are homologous by virtue of identity of embryonic origin. The conclusive data, origin from the same part of an ancestral species, will most likely never be available.

One can go one step further and entertain the hypothesis that there is a basic homology of germ layers throughout the Metazoa. In 1849 Thomas Henry Huxley started this line of inquiry and later wrote (1878, pp. 110–114) that the fundamental structure of a coelenterate consists essentially of

> a sac having at one end an ingestive or oral opening, which leads into a digestive cavity. The wall of the sac is composed of two cellular membranes, the outer of which is termed the *ectoderm,* and the inner the *endoderm,* the former having the morphological value of the epidermis of the higher animals, and the latter that of the epithelium of the alimentary canal . . . . The peculiarity in the structure of the body walls of the *Hydrozoa* [a Class of Coelenterata], to which I have just referred, possesses a singular interest in its bearing upon the truth (for, with due limitation, it is a great truth) that there is a certain similarity between the adult states of lower animals and the embryonic conditions of those of higher

> organization .... Thus there is a very real and genuine analogy between the adult Hydrozoön and the embryonic vertebrate animal; but I need hardly say it by no means justifies the assumption that the Hydrozoa are in any sense "arrested developments" of higher organisms. All that can justly be affirmed is, that the Hydrozoön travels for a certain distance along the same great highway of development as the higher animal, before it turns off to follow a road which leads to its special destination.

The mid 19th century argument, especially after Haeckel proposed his Gastraea Theory, for the homology of these germ layers was something like this. The basic structure of a hydrozoan is that of a double-walled vase, the central cavity being the enteron. The gastrula stage of an idealized vertebrate is essentially the same. No vertebrate embryo exhibits such an idealized structure but that difficulty can be explained, in part, as being due to yolk modifying development. The gastrula of amphioxus (Fig. 13F) does come close to the idealized two-layered vase. We may suspect, therefore, that the similarity of the two-layered hydrozoan body and the two-layered archetypal vertebrate gastrula makes tenable the homology of their germ layers.

That argument is far less compelling in the late 20th century. As more information became available, it was clear that the germ layers arise in many different ways in the metazoans. Therefore one cannot use identity of embryonic origin as proof of homology since the origins are not identical. One cannot maintain, for example that the mesoderm is the "same thing" throughout the bilateral phyla. To what extent can the cells on the outside of an earthworm be considered homologous to the cells on the outside of a starfish? Any answer is as dubious as would be any clear notion of how one would find out.

Once the germ layers have been formed, however, there is great uniformity in what they do, as Hyman noted. Therein lies their conceptual and pedagogical importance.

A second interesting problem has to do with the relation between what the germ layers form in the course of development and their innate abilities. Is there something "mesodermal" about the mesodermal cells, meaning that they produce only mesodermal organs, and that mesodermal organs are produced only by the mesodermal layer? Questions of this type can be formulated in hypotheses that can be tested. As we will learn later, the Spemann school found that there is no restriction on what mesodermal cells can form and mesodermal structures can be formed from other than mesodermal cells.

Other evidence of the non-specificity of the germ layers comes from studies of regeneration where in some cases the structures of the regenerated individual are derived from different germ layers than those from which they were first formed in embryonic development.

What does this tell us about the usefulness of the Theory of Germ Layers for students in first-year courses? Libbie Hyman, one of the greatest students of invertebrates of all time, answers that question as follows:

> It can scarcely be doubted that the later stages of development exhibit a certain similarity especially in the Bilateria [metazoans other than sponges, coelenterates, and ctenophores] and that in general each germ layer gives rise to certain definite organs. The doctrine of the homology of the germ layers may therefore be considered as broadly acceptable and if applied with caution may be used in interpreting embryological facts. It must always be borne in mind that a developing embryo is living, plastic, and modifiable, responding to changed conditions by morphological changes. Probably no development at present adheres to its original course, but all ontogenies have undergone changes, such as shortening of some stages, prolongation of others, precocious development of certain parts (heterochronism), and production of larval organs adapted to a free-swimming life. We may assume a general tendency toward cutting short and condensing stages no longer essential to the life of the embryo or to the development of future organs, and toward the pre-

> cocious or new appearance of useful parts. Every ontogeny is a compromise between an inherited ancestral mode of development and adaptive modifications and adjustments (1940, pp. 271–272).

In her fine study of germ-layer specificity Jane Oppenheimer (1940) makes it abundantly clear that the cells of germ layers do not have innate specificity. Apart from this however, the concept of germ layers is of some significance:

> It seems certain that the precise location of a cell during gastrulation in many forms, or the precise origin of its cytoplasm from the egg in others, is in many cases correlated with the type of its later activity; therefore in a certain sense the germ-layers are of topographic significance, since the cells pass through them in their orderly progression of movements. In a teleological sense, formation of germ-layers seems to be the embryo's method of sorting out its constituent parts. The essential point is, however, that this method is not the only method that the embryo can call upon to attain a specific end, and here as in many other cases in development the embryo can, when necessary, modify or abandon one method in favor of another . . . . The task of the student of the germ-layers then must become more than an attempt to discern how the embryo sorts its cells into one layer or another; it must become an elucidation of how wide the potencies of the germ-layers become subject to limitation to their normal accomplishments.

So, for the student the concept of germ layers should be considered no more than a map to guide the study of normal development; for the developmental biologist the germ layers should be the basis of experiments to throw light on the processes of differentiation.

The last half of the 19th century saw embryologists interpreting their observations on normal development in relation to the Theory of Evolution. Descriptive embryology, so interpreted, was the dominant paradigm. Frederick B. Churchill (1986, p. 7) has provided a fitting closing statement to our discussion of this era of embryology.

> When the historian of biology turns to nineteenth century embryology, he conjures forth an imposing structure. At one end of the century exist the exemplary observations of Pander and von Baer and at the other the dramatic experiments of Roux and Driesch. Firmly settled between these two opposing buttresses rises the towering edifice of classical descriptive embryology, solid in its discoveries, magnificent in its tracery and fine details, and as defiant of and removed from modern biology as a gothic cathedral is from today's secular world. Few can doubt the real achievements of those artisans who, in constructing this temple, eternally glorified the perseverance and perspicacity of descriptive biologists. From von Baer's discovery of the mammalian ovum, on through Rathke's analysis of the branchial arches, Müller's, Reichert's and Huxley's examination of the development of the vertebrate skeletal system and the exquisite descriptions of invertebrate development by Kowalevsky, Metchnikoff and Kleinenberg, and terminating with the monumental studies on the development of single organisms or organ systems by Götte, Balfour, Semper, His and scores of others, the spires of this cathedral rest on the surest of pillars.

Descriptive embryology of the 19th century was embryology looking outward—relating the phenomena of development to the basic biological concept of all time. Concurrently, however, there were tentative beginnings of another paradigm—analytical embryology. This is embryology looking inward—attempting to understand the causal relations in development. Analytical embryology is the paradigm that demands our attention today.

References: Descriptive embryology—von Baer to Haeckel

Adelmann (1966), Agassiz (1849, 1859), von Baer (1827, 1828), Balfour (1880–1881), Bather (1893), Baxter (1977), de

Beer (1958), Churchill (1970*b*, 1986), Di Gregorio (1984), Gardner (1965), Garstang (1922), Gasking (1967), George (1933), Haeckel (1866, 1868, 1874, *1876, *1905*a*), T. S. Hall (1951), O. Hertwig (1901), Horder *et al.* (1986), Huxley (1849, 1878), Hyman (1940), Kohlbrugge (1911), Kölliker (1861), Korschelt and Heider (1895), Lankester (1873, 1877), Lovejoy (1959), Magner (1979), Maienschein (1978), Mayr (1982), Meyer (1956), Oppenheimer (1940, 1955, 1957, 1959*a*, 1959*b*, 1963, 1964, 1966, *1967, 1970*a*, 1973), Ospovat (1976), Packard (1876), Reichert (1837), Rinard (1981), E. S. Russell (1917, 1930), Sarton (1931), Sedgwick (1894), Singer (1950), and E. B. Wilson (1896*a*, 1899).

THE AMPHIBIAN EMBRYO—EXTERNAL DEVELOPMENT

When the main reason for studying embryos began to switch from seeking to learn about evolution to an analysis of the causal factors in development, the amphibians were found to provide excellent material. Prior to that switch, it was important to have embryological data from a broad sample of organisms. For analytical embryology, on the other hand, it was necessary to use species with embryos that could survive experimental manipulations. The mature eggs of many common European and North American frogs and salamanders are usually about two or three millimeters in diameter, hence, large enough to be operated upon. Their embryos are hardy and heal well and recovery can usually be expected following operations and other experimental procedures. Each fertilized egg has a supply of yolk granules sufficient to carry the embryo to a free-living stage. This is a great advantage since the difficult problem of supplying an external source of food is avoided.

Much of the early data on the analysis of development came from amphibian embryos so it is necessary to give a short synopsis of the main events in their development. The following description is of embryos of *Rana pipiens* from Vermont. This species and other meadow frogs very similar to it are widely distributed in North and Central America. For several generations they have been extensively used in experiments. The external aspects of development, as shown in Figures 17–23, will be described first.

The rate of development depends on temperature—the embryos shown in the illustrations were kept at a constant temperature of 20°C. The numbers on each photograph give the time in hours after fertilization. Had the embryos been kept at 25°, development would have required about half the time, and if kept at 15° nearly twice as long. The lowest and highest temperatures for normal development are, respectively, about 5° and 28°. The embryos in the illustrations are magnified about 25 times.

Breeding. In the spring, spurred by warming days, moist nights, and hormonal changes, males and females congregate in ponds and swamps for a brief breeding period. At this time all of the mature ova of the females leave the ovary, pass into the coelom, and then enter the anterior openings of the oviducts. The ova move slowly along the oviduct where they are coated with thin layers of jelly. The ova accumulate in the posterior portion, or uterus, of each oviduct. When actual mating begins the male frog clasps the female in such a manner that his cloacal opening is directly over hers. The ova pass out of the female's body into the surrounding water and concurrently the male sheds sperm over them. The thin, almost invisible, jelly layers surrounding the fertilized ovum now imbibe water and begin to swell, eventually reaching a diameter about three times that of the egg. This jelly is at first sticky and adjacent eggs adhere to one another. As a consequence all of the eggs, which may number more than a thousand, stick together and form a large globular mass in which the embryos develop, each in its own jelly envelopes.

Meiosis and fertilization. Complex and important internal events have been occurring during this entire period (III, pp. 625–635). The ovarian egg has a very large nucleus, the germinal vesicle. (It is interesting to note the antiquity of some of the terms used in describing development. A

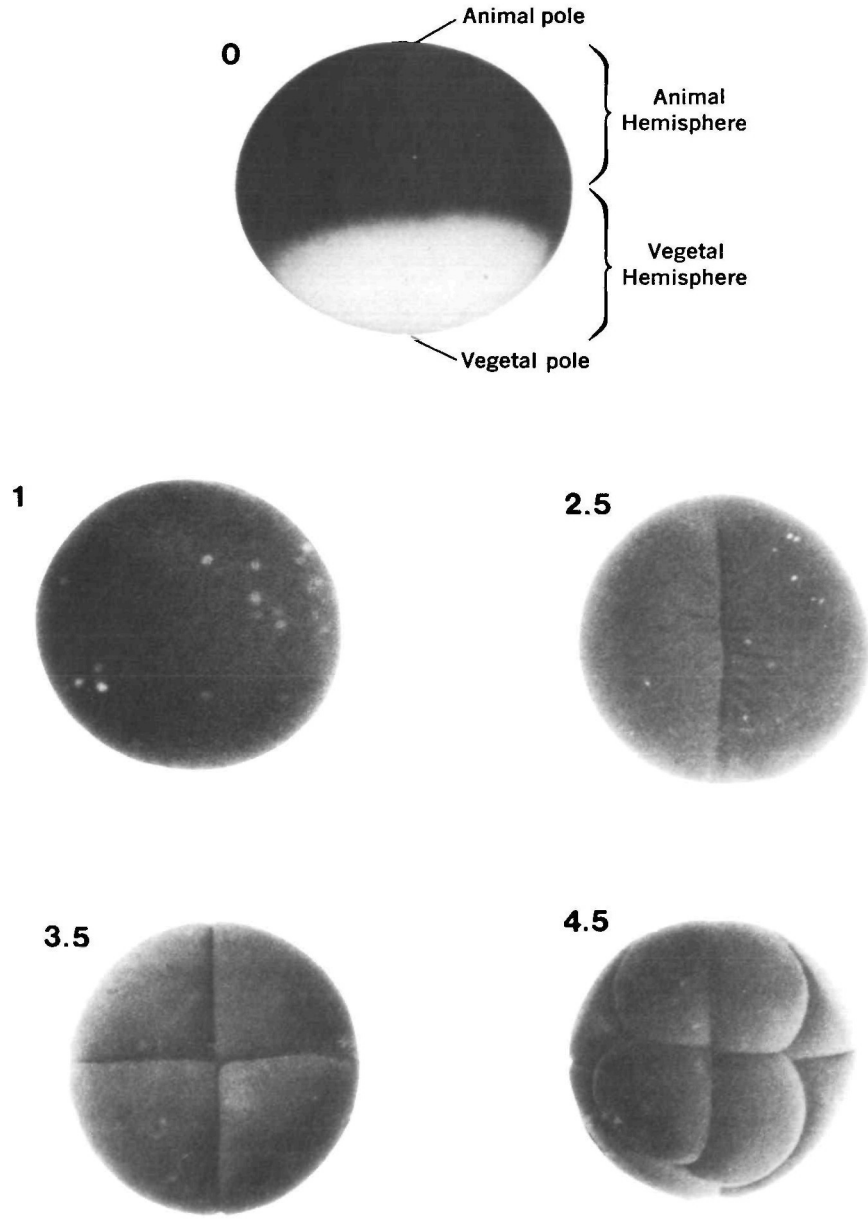


FIG. 17. Development of the frog's egg. Fertilization to eight cells. The 0-hour embryo is in side view; the other embryos are shown looking down on the animal hemisphere. The numbers to the upper left of each embryo in Figures 17–23 are the hours after fertilization at 20°C.

large spherical object was seen in ovarian eggs before it was realized that it was the nucleus. Since it occurred in the "germ" it was named the "germinal vesicle.") When the ova start to break out of their follicles in the ovary, meiosis begins. This involves the dissolution of the nuclear membrane. The first meiotic division occurs by the time the ovum has reached the upper portion of the oviduct and the first polar body is given off at that time. Metaphase of the second meiotic division is reached by the

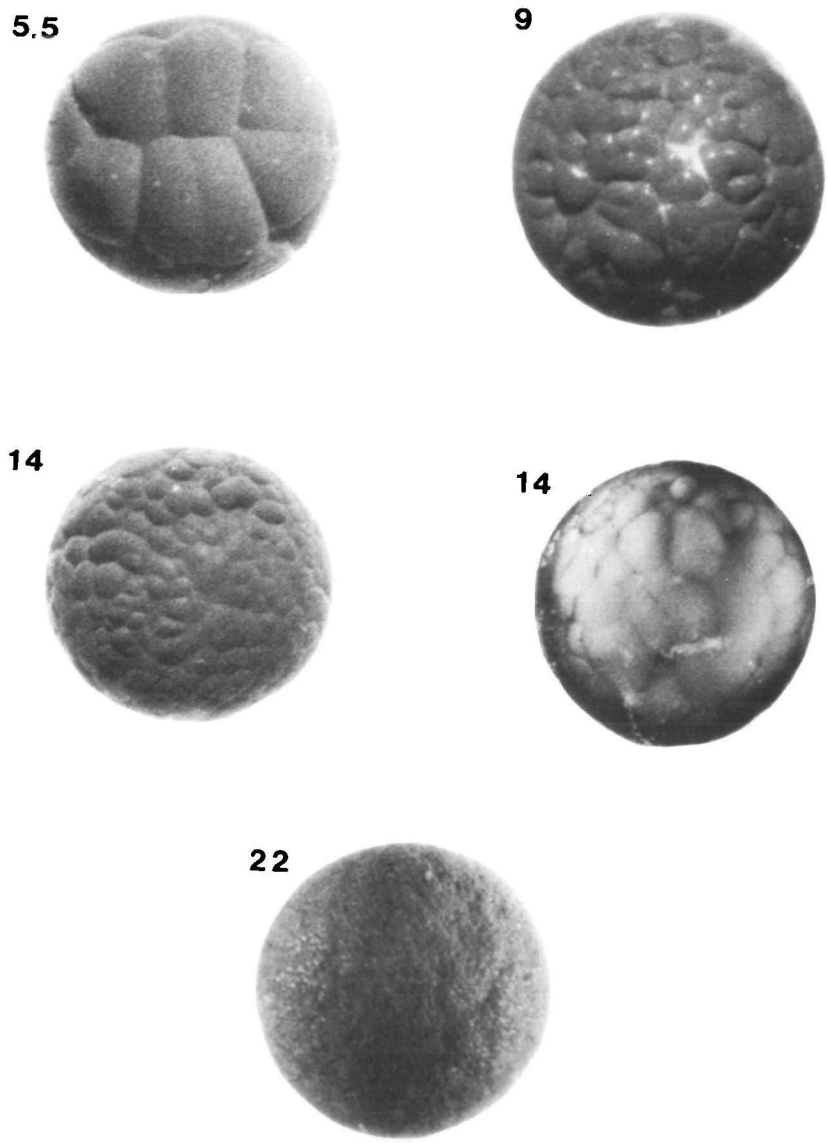


FIG. 18. Development of the frog's egg. Sixteen cells to late blastula. Animal hemisphere views except for the rightmost 14-hour embryo, which shows the vegetal hemisphere.

time the ova are in the uterus. Further nuclear changes are blocked at that stage.

A single sperm enters the ovum. Its head contains the paternal nucleus with the monoploid number of 13 chromosomes. A centriole is immediately behind the sperm head. It will become part of the first mitotic spindle. The entrance of the sperm removes the meiotic block and the second polar body is extruded in about a half hour. The maternal pronucleus now has the monoploid number of chromosomes. The two pronuclei move toward the upper center of the egg and there unite, restoring the diploid number of 26 chromosomes.

The uncleaved zygote. The just-fertilized ovum is a sphere approximately 1.7 mm in diameter. Somewhat more than half of the embryo, the animal hemisphere, is a dark chocolate-brown and the remainder, the

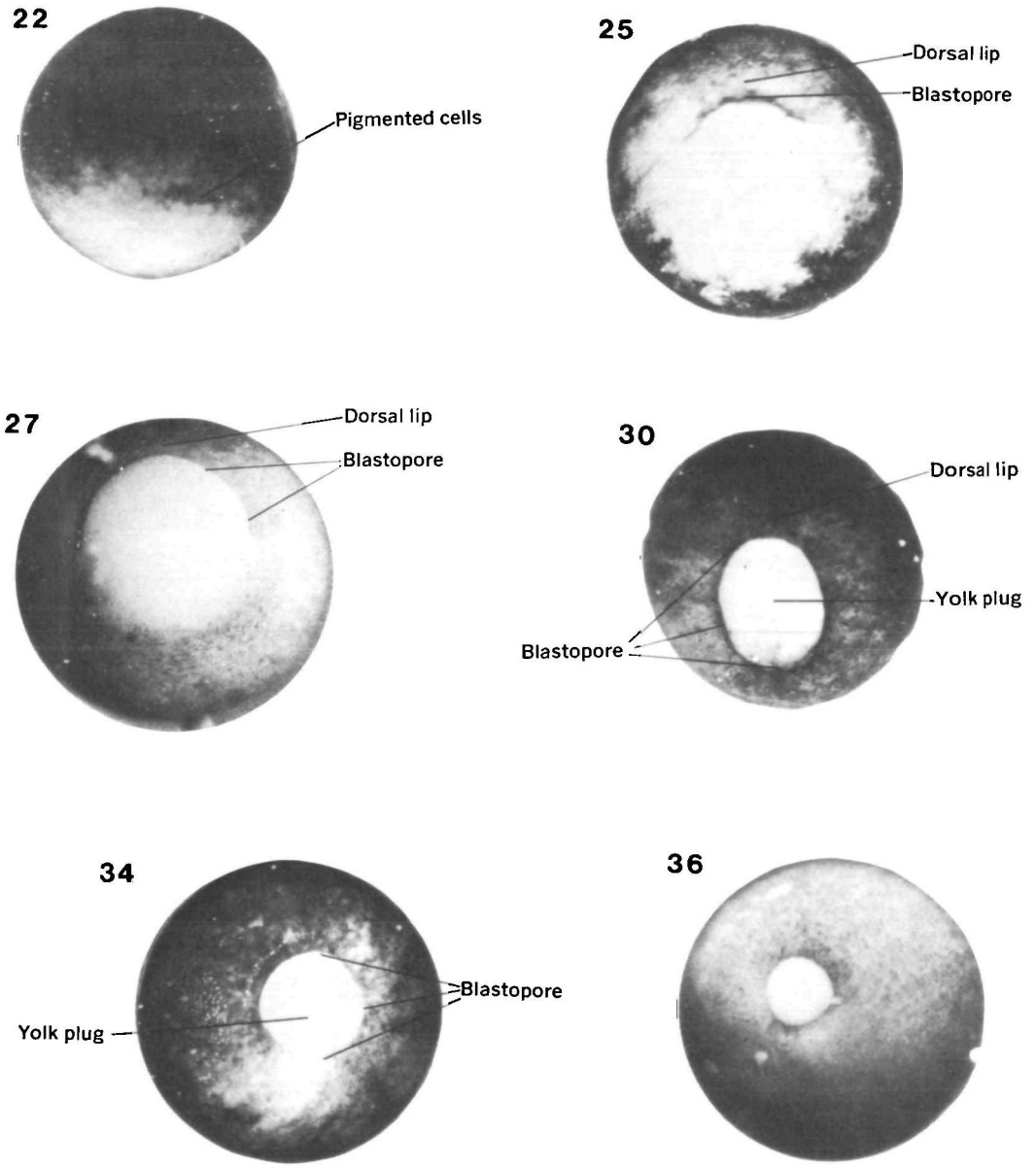


FIG. 19. Development of the frog's egg. Gastrulation. The 22-hour embryo is in side view; the others are ventral views.

vegetal hemisphere, is almost white (Fig. 17, 0 hours). The animal pole is in the center of the animal hemisphere. It is the site of polar body formation. The vegetal pole is 180° from the animal pole and in the center of the vegetal hemisphere. When the eggs are first deposited they are arranged at random in relation to their polarity. In about an hour after fertilization the swelling of the membranes surrounding the embryo leaves a space between the egg surface and the membranes. This allows the embryo to rotate and the heavier part, the yolky vegetal hemisphere, becomes bottommost. At this time when one examines a mass of eggs

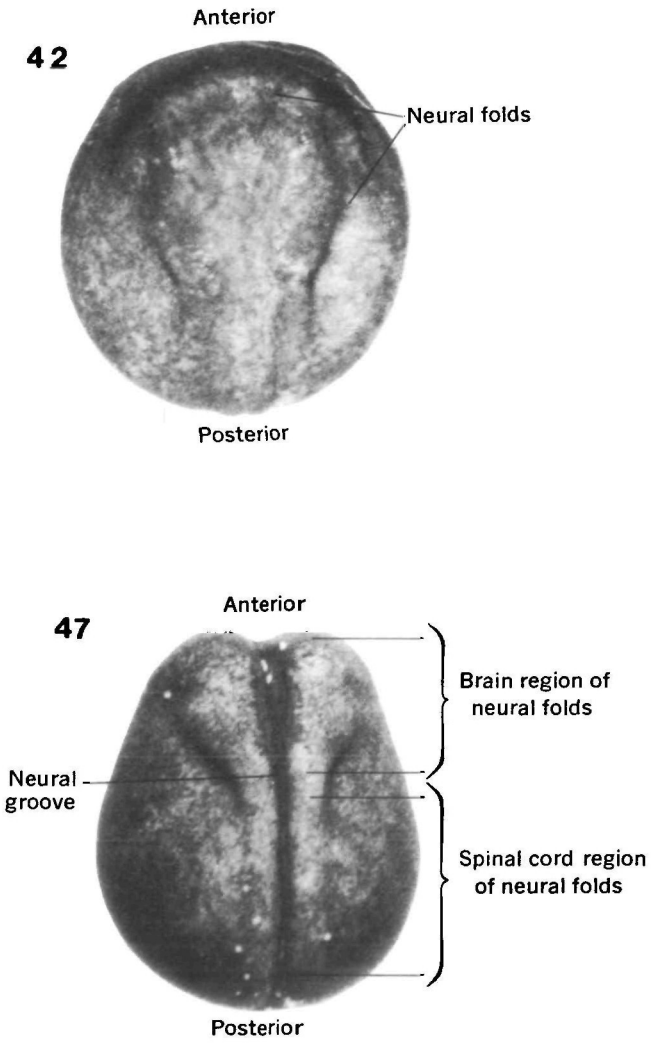


FIG. 20. Development of the frog's egg. Early and middle neurula.

from above the dark animal hemispheres will be all that is seen (Fig. 17, 1 hour) as all of the eggs will have rotated. If the egg mass is turned over, all one sees will be the white vegetal hemispheres. Quite quickly, however, the embryos rotate so the animal hemispheres are again uppermost.

Cleavage. (This is another antique term. The "cleaving" of the eggs into smaller parts was observed long before there was any concept of cells or cell division. It was most puzzling to early observers and it took about two centuries to understand what was going on.)

Two and a half hours after fertilization the first spectacular event that is externally visible occurs. A short groove appears in the animal hemisphere and it gradually lengthens to form the first cleavage furrow. The furrow slowly extends through the embryo until two cells are formed. Internally mitosis had begun before the cleavage furrow appeared. When the chromosomes are in telophase the furrow starts to form.

The second cell division begins at about 3.5 hours. The plane of this cleavage is again vertical and at right angles to the plane of first cleavage. Both cleavages pass through or very near to the animal and

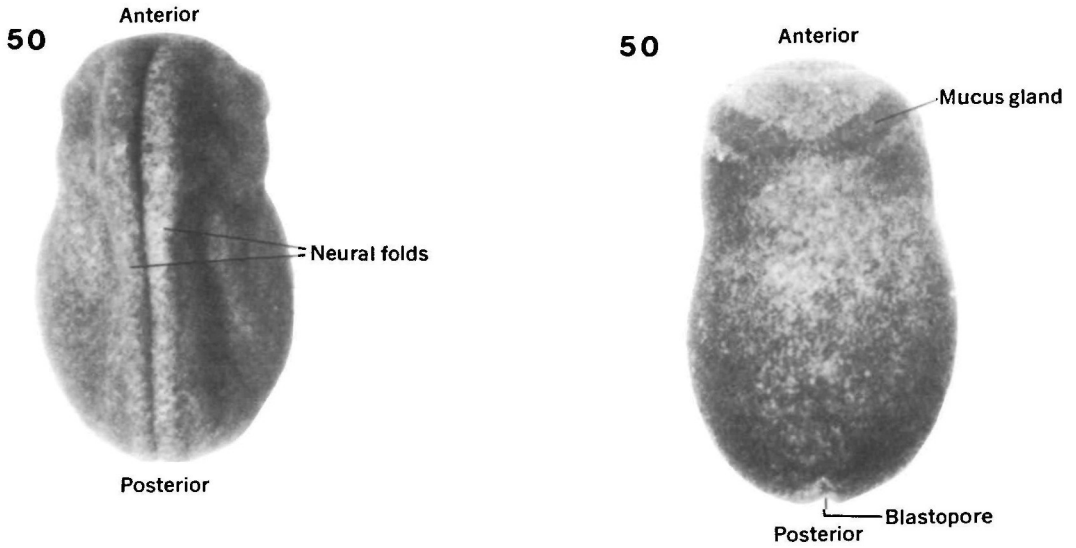


FIG. 21. Development of the frog's egg. Closing neural folds. Dorsal and ventral views of the same embryo.

vegetal poles. The third cell division occurs at about 4.5 hours (Fig. 17). The plane of this cleavage is horizontal. It does not divide the embryo equally, as the plane of cleavage is above the equator. Thus in the photograph one can see the four smaller uppermost cells and beneath them parts of four larger cells. The latter include all of the vegetal hemispheres and the lower part of the animal hemisphere.

When one examines a group of embryos that were fertilized at the same time and kept together, the synchrony of development is awesome. Each of the cleavages starts at almost exactly the same time and at the same place in the animal hemisphere. In fact this synchrony is true of all early development. Each stage is reached at almost the same time in all embryos. It is as though each has an internal clock that was started at the same time and ticks along together with the other clocks. Each must have some sort of biological clock but as yet we know very little about what it is and how it works.

This developmental precision could not have gone unnoticed by embryologists in the 19th century and it would have pressed upon their minds that development, in part at least, must be like a machine. Development seemed precise, uniform, and predictable. When a biological phenomenon has those features one suspects constant cause and effect relationships and of course entertains the hope that they are ascertainable through proper observations and experiments.

The process of cell division continues and soon the intervals between divisions increase. The embryo is divided into smaller and smaller cells and there is no obvious increase in size. We must remember that no food is entering the embryo. Its energy source consists of the yolk granules within each cell. As these are used in metabolism, the dry weight of the embryo decreases. Oxygen diffuses through the jelly layers and enters the embryo and carbon dioxide and some waste materials take the opposite path.

Figure 18 shows this slow decrease in cell size. The 5.5-hour embryo is in the 16-cell stage and by 9 hours there are more than 100 cells. The two photographs in the second row are of a 14-hour embryo. The left one shows the small cells of the animal hemisphere. That embryo was then turned over and the photograph on the right shows the much larger vegetal hemisphere cells. By 22 hours the animal hemisphere cells have become much smaller.

The embryos from 9 to 22 hours are

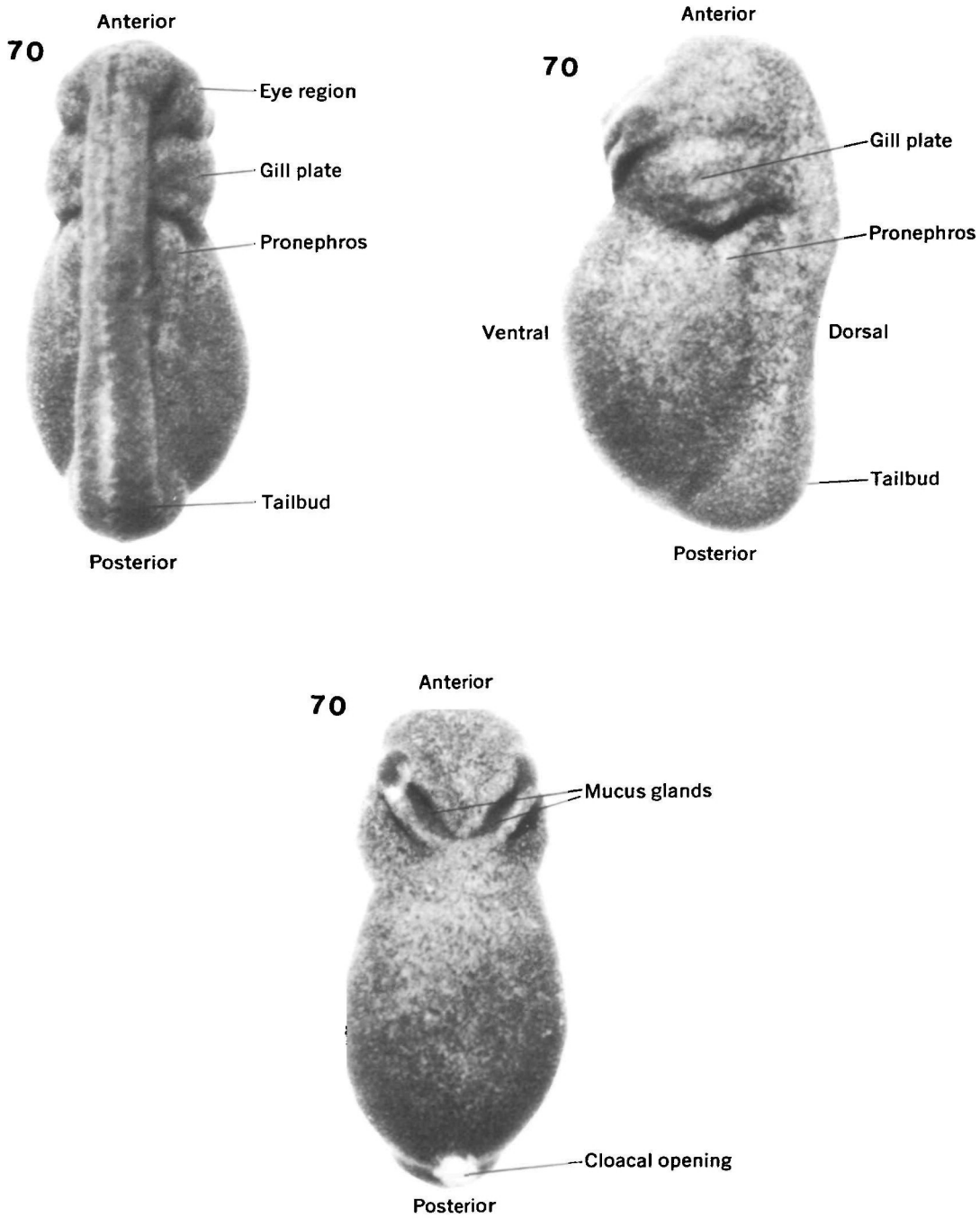


FIG. 22. Development of the frog's egg. Tailbud embryo shown in dorsal, lateral, and ventral views.

blastulae. The blastula stage is characterized by an internal cavity, the blastocoel, which, when fully formed, occupies most of the interior of the animal hemisphere. More will be said about it later when we consider the internal events of early development.

All of the cells of the late blastula are

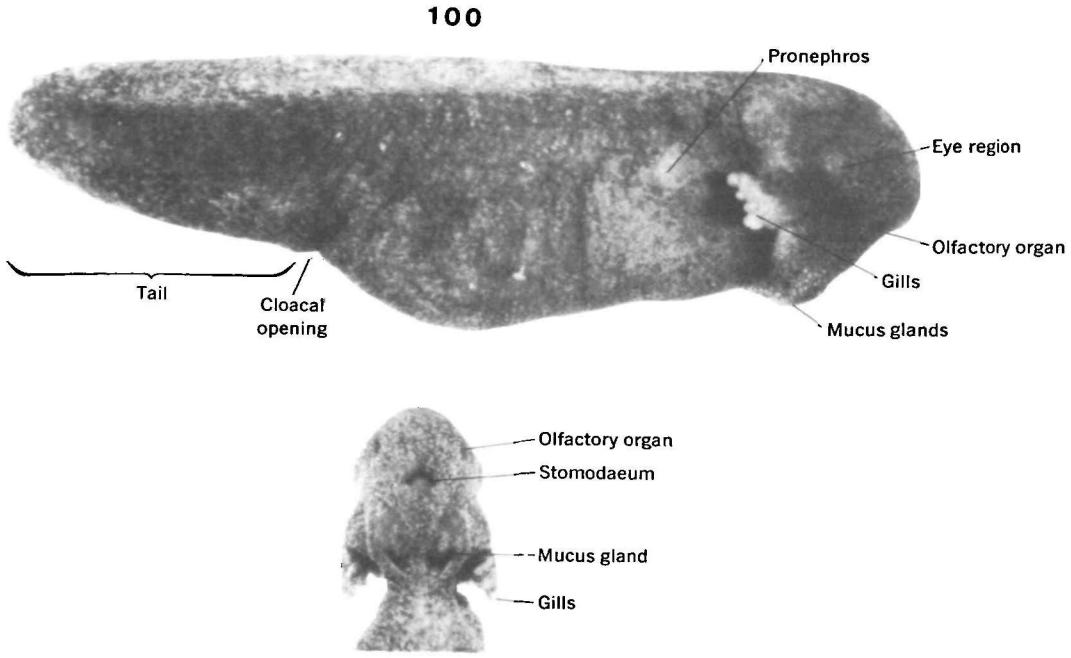


FIG. 23. Development of the frog's egg. Just-hatched larva shown in lateral view and a ventral view of the head.

essentially the same apart from the differences in pigmentation and size. There is a gradient in size that runs from the smallest cells at the animal pole to the largest cells at the vegetal pole. The density of yolk granules follows the same gradient.

Thus the blastula has only a single axis, that extending from animal to vegetal pole. There is no right and no left, that is, no landmark on the sides. If we compare the blastula with the earth, we could recognize a North Pole (animal pole) and a South Pole (vegetal pole). That would enable us to determine the latitude of any position on the blastula but the lack of differences along the sides makes the determination of longitude impossible.

Gastrulation. When the 22-hour blastula is turned over, one observes a narrow groove of pigmented cells in the vegetal hemisphere just below the equator (Fig. 19). By 25 hours this groove has become deeper and extended laterally. The groove itself is the blastopore and its formation marks the beginning of gastrulation. The cells immediately above the blastopore are called the dorsal lip of the blastopore. They will play an extraordinary role in development.

Gastrulation is a process that leads to a complete rearrangement of the cells of the embryo. Many of those on the outside of the blastula will move to the interior. This process of moving in at the lips of the blastopore is called invagination. Figure 13 shows examples of gastrulation in embryos with very little yolk. This process is greatly modified in the frog because of its large, relatively yolky egg but the events are fundamentally the same.

The blastopore of the 25-hour embryo leads into a tiny archenteron. Notice also that the pigmented surface appears to be enlarging. Compare its extent in the 22- and 27-hour embryos. The dark cells of the animal hemisphere are actually moving downward and the lighter cells of the vegetal hemisphere are moving to the interior as the animal hemisphere cells cover them. By 27 hours the lateral lips of the blastopore have extended to the sides and by 30 hours they have finally met to form a 360° blastopore. The area of light-colored cells has become much smaller and they form

the yolk plug. Gastrulation continues until, by 36 hours, the animal hemisphere cells have almost overgrown the embryo and the only original vegetal cells seen on the outside are those of the ever-smaller yolk plug. Finally the yolk plug itself moves to the interior and the blastopore is reduced to a tiny slit, marking the end of gastrulation. Now the dark animal hemisphere cells cover the entire surface. The cells have become so small that at moderate magnification they are invisible. The embryo has a superficial resemblance to an uncleaved egg when viewed from the top—it is black and "structureless." You can guess how confusing this stage was to some of the earlier observers.

With the formation of the dorsal lip of the blastopore at 25 hours, we are finally able to specify longitude. Therefore, once gastrulation has begun, we can describe any spot on the surface of the embryo in terms of its latitudinal distance from the animal pole and its longitudinal distance from the dorsal lip. Why one would even wish to do such a thing may not be clear now, but it soon will be.

At the end of gastrulation there is still essentially no obvious cellular differentiation beyond that of the blastula. The diameter of the late gastrula is about the same as that of the uncleaved egg. The rate of metabolism has increased and, since there is still no external source of food, the dry weight is less than before. There are two cavities in the embryo—the vanishing blastocoel and the enlarging archenteron. These are filled with fluid.

Neurulation. The next prominent external change is the beginning of the formation of the nervous system. It is usually surprising to students to find that, as in the frog, their brains actually start developing on the outside of the body. Figures 20 and 21 show the sequence of events. In the 42-hour embryo the neural folds appear as low ridges on the dorsal side of the embryo. These folds are formed as paired structures, extending from each side of the blastopore region anteriorly to where they connect in the region that will become the head. By 47 hours the folds are more elevated and begin to close and by 50 hours the folds touch along their entire length. The folds close in such a manner as to form an internal tube, the neural tube. The walls of the broad anterior portion of the neural folds will become the brain and the walls of the more posterior portion will become the spinal cord. The bore of the neural tube remains even in adult life as the neurocoel.

The embryo at 42 hours is still spherical but it begins to elongate as neurulation proceeds. This growth in length is not the same in all areas. This can be illustrated by what happens in the central nervous system. At 47 hours the lengths of the brain and spinal cord regions of the neural folds are roughly the same. Later in development the spinal cord area will increase in length much more than the brain.

When the 50-hour embryo is turned over (Fig. 21, right) one can see the beginnings of still another structure, the mucus glands. This structure secretes a sticky mucus that enables the larva to attach to various objects.

Tailbud stage. After another day of development there are more external changes (Fig. 22). The elevated ridge along the back contains the brain and spinal cord. Paired bulges in the brain indicate the place where the eyes are forming. Large swellings behind the eye region are the beginnings of the gills. Still farther back a small swelling marks the site where the pronephros is beginning to form. On the ventral side the mucus glands are better developed.

The 100-hour embryo. It is not too difficult to extrapolate from the 70-hour tailbud stage to the embryo of 100 hours (Fig. 23). By 100 hours the embryo has reached the point where the precursors of all its organ systems have formed and some are beginning to function. For example, circulation has begun and close examination shows blood cells moving through the gills. Externally the embryo has begun to resemble a tadpole. The eyes are present as bumps on the side of the head but they are not yet functional—the overlying skin is still deeply pigmented. The olfactory organs are paired pits at the anterior end. Between them is another pit, the stomodaeum. This pit will break through to the primitive alimentary

canal, forming the mouth. There is a well-developed tail, that is, a portion of the body posterior to the cloacal opening.

The embryo hatches from its jelly envelopes at about this time. Most of its yolk will have been consumed but, before all the yolk has gone, the young creature will have begun to find and eat food in the pond where it is living.

Thus in a period of about four days, at 20°C, the frog-to-be will have started as a single cell and, with cell division, differentiation and growth, produced a larva with all of its organ systems beginning to form and some already functioning. The epigenetic changes will have occurred with a clock-like precision that continues to awe the observer today as much as it did when amphibian embryos were first studied. This is truly an astonishing phenomenon, all the more impressive to observe because our own development occurs in the almost inaccessible interior of the body—unavailable for easy study.

Although amazing external changes have occurred in the developing embryo, they are not as numerous nor as great as what is happening internally—our next topic.

THE AMPHIBIAN EMBRYO—INTERNAL DEVELOPMENT

In all complex animals the vital organ systems are internal—protected by a skin and often by scales, bone, chitin, feathers, shells, or similar structures. In the vertebrates all of these organ systems develop internally as well. This was a difficult problem for early embryologists since techniques were not available for studying these important internal events. However, by the late 19th century, techniques had been developed for imbedding embryos, after fixation, in paraffin wax and then making thin sections. These could be mounted on glass slides, stained, and studied with a microscope. It even became possible to make serial sections of embryos, *i.e.*, beginning at one end and making thin slices of the entire embryo. The slices were then mounted in order on slides and the end result would be hundreds of slices of the entire embryo. One then had the task of deducing the whole internal structure from these thin sections.

An embryo in serial section is static and cannot provide a complete story of the movements of cells to their final sites where they produce the various structures. One can not determine, for example, how the archenteron forms from looking at slides. Does it involve an invagination of cells from the outside or is it a matter of new cells being formed at the advancing edge of the archenteron?

Consider the events in the early development of the frog (Fig. 19). The changes from 22 to 36 hours can be explained as the downgrowth of the dark-colored animal hemisphere cells over the light-colored vegetal hemisphere cells. Alternatively, the events could be explained equally well by assuming that the light-colored cells slowly become pigmented.

How could one decide between these two hypotheses? Some early experimental embryologists sought an answer by pushing a needle through the jelly membranes and killing some of the cells on the surface of the embryo. One could then trace the movements of the scar for as long as it lasted, which often was not very long. Nevertheless, experiments of this sort made it seem true beyond all reasonable doubt that cells on the outside did move down from the animal hemisphere.

By the 1920s the experimental analysis of the development of the amphibian embryo had reached the stage where it was necessary to know, with a high degree of accuracy, the direction of movement of the various parts of the embryo during gastrulation.

The basic problem was to be able to describe accurately all positions on the embryo and to be able to trace these positions throughout early development. We have seen already that in a late blastula one can determine position only in terms of distance from the animal pole. To return to our analogy with the earth, if we were told only that Philadelphia was 50° from the North Pole, we would have no way of knowing whether it was in Spain, Turkey, Russia, China, or the United States.

It is only when the dorsal lip of the blas-

topore appears at the onset of gastrulation that, by analogy, we have the zero meridian of Greenwich. The ability to determine distance from the animal pole and from the dorsal lip means that any position on the surface of the early gastrula can be described accurately.

But experimentalists needed to know not only where a given group of cells might be at the onset of gastrulation but where these same cells would be at various times thereafter. Would they be in the same place or would they have moved?

THE FATE MAP

It took a German embryologist, Walther Vogt, many seasons of painstaking observation and experimentation to provide an acceptable answer. The term "seasons" is employed because the amphibian embryos used in his experiments came from breeding adults and the breeding season was restricted to a few weeks during the year.

Vogt sought to define the location in an early gastrula of the cells that would later become the three germ layers: ectoderm, mesoderm, and endoderm. That is, he wished to determine the eventual fate of all of the cells. The results could then be expressed by a map-like diagram of an early gastrula showing where the cells would be in a later embryo. Such a diagram is called a Fate Map.

Vogt found that the cells destined to form each layer occurred together at the onset of gastrulation and that each remained as a unit throughout gastrulation. He found also that, within the limits of his ability to measure position, cells in different embryos behaved in precisely the same manner.

The technique for making the observations necessary for the construction of a fate map is as follows. A layer of wax is put in a small dish and a small pit, about the size of an early gastrula, is made in the surface (Fig. 24, top). Tiny pieces of agar are stained with a variety of vital dyes and placed in the sides of the pit. The jelly membranes are removed from an early gastrula, leaving only the vitelline membrane, and the embryo is pushed into the pit. It is held in place by a tiny piece of bent cover glass.

Some of the dye would diffuse from the agar and stain the cells on the outside of the embryo. Differently colored vital dyes were used, thus allowing individual spots on the embryo to be differently stained. After exposure to the dyes, the embryo would be removed from the pit and a drawing immediately made of the position of the colored spots. At frequent intervals thereafter the same embryo was studied and sketched. Some of the colored spots were invaginated. In these cases it was necessary to dissect the embryo and ascertain the position of each spot.

The three diagrams at the bottom of Figure 24 show one of Vogt's experiments. Eight colored spots, 1 through 8, were placed on the embryo along the meridian that passes through the animal pole and the dorsal lip of the blastopore. A short time later, Vogt found that spot 7 had moved to the interior and that spot 6 was now the dorsal lip (diagram *a*). In the middle gastrula, *b*, only spots 1–4 remain on the outside. They did not remain in that position, however, but became stretched to cover a much larger portion of the surface of the late gastrula, as shown in *c*.

After performing hundreds of experiments of this sort, Vogt was able to prepare a fate map (Fig. 25) of the early gastrula of the European toad, *Bombinator*. He studied other amphibians as well. He found that the cells that form the three germ layers were laid out on the surface of the early gastrula as shown.

This is a complex diagram and is difficult to understand when first seen. However, it is a valuable conceptual scheme for understanding how the germ layers, and later the organ systems, are formed. It will be a point of reference in describing further development. Some general introductory remarks about Figure 25 may be useful.

The presumptive ectoderm, that is, the cells that will form the ectoderm later in development, occupies nearly all of the animal hemisphere. Two main subdivisions are delimited in Figure 25: the presumptive neural tube, an area consisting of those cells that will eventually form mainly the brain, spinal cord, and optic cup; and the presumptive epidermis, which occupies

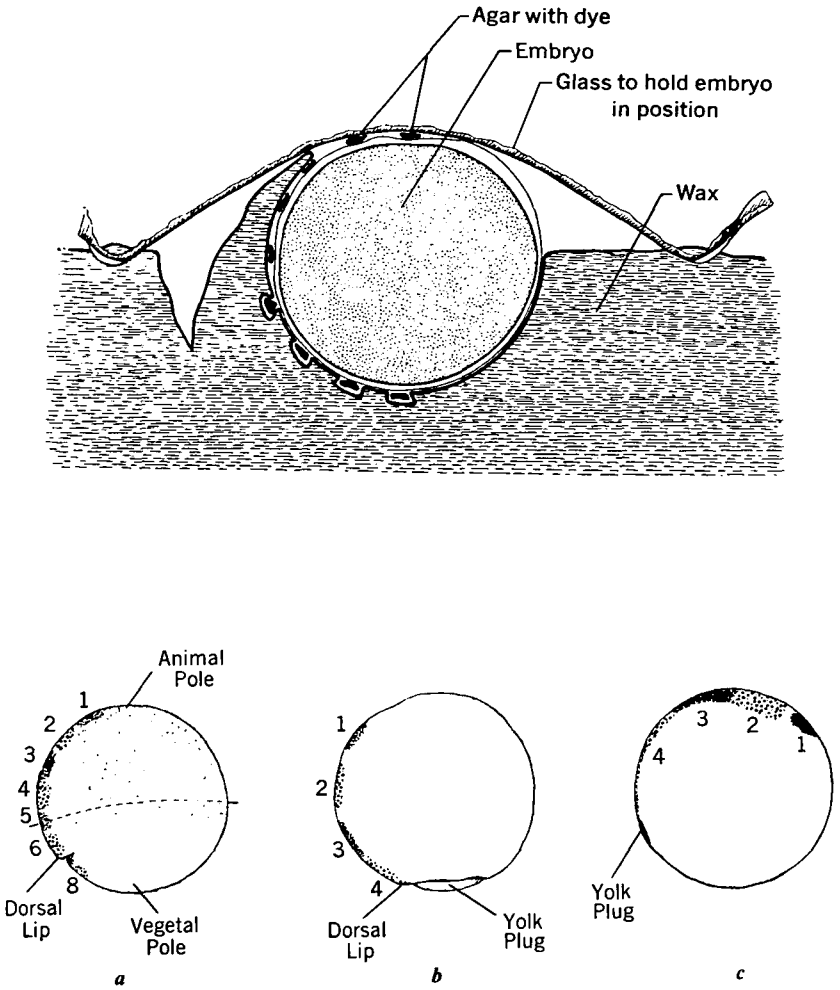


FIG. 24. Vogt's technique for staining embryos is shown in the upper figure. The three lower figures show one of the experiments. The vitally stained spots have been given numbers. *a* is an early gastrula with the dorsal lip below 6; spot 7 had already invaginated. *b* is a mid gastrula and spots 5, 6, 7, and 8 have invaginated. *c* is a late gastrula and only spots 1, 2, 3, and 4 remain on the outside. Compared to their positions in *a*, they have spread considerably. Note the positions of all these spots with the fate map in Figure 25. (From Vogt, 1925 and 1929.)

about a quarter of the surface of the early gastrula and will eventually spread to form the entire epidermis covering the embryo and later the adult.

The presumptive mesoderm forms a band of cells surrounding the embryo in the equatorial region. It, too, consists of two main areas: the cells immediately above the dorsal lip will form the notochord; the remainder of the presumptive mesoderm will form the muscular, skeletal, circulatory, reproductive, and excretory systems as well as connective tissue and coelomic epithelia.

The presumptive endoderm occupies much of the vegetal hemisphere. Its cells will form the lining of the alimentary canal and structures derived from it such as the liver, pancreas, and bladder.

The presumptive mesodermal and presumptive endodermal cells that are on the outside of the early gastrula will all be invaginated to the interior during gastrulation. The division between what goes in

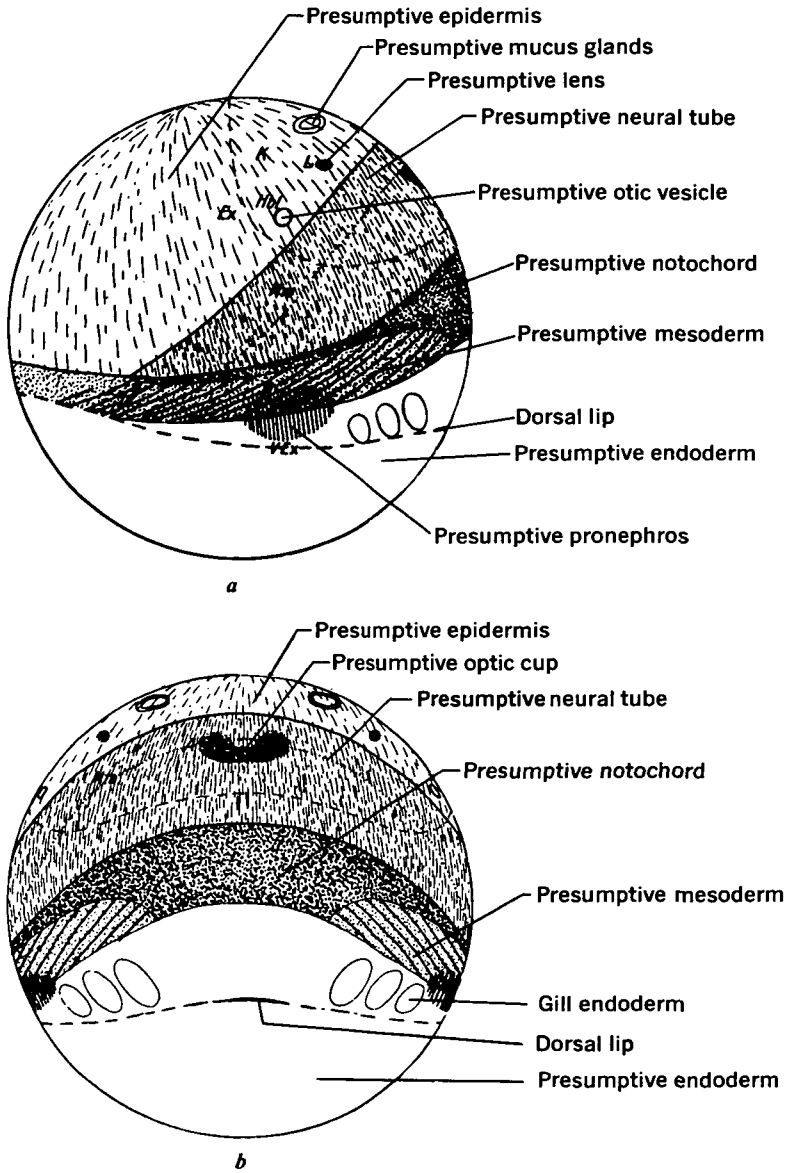


FIG. 25. Vogt's Fate Map for *Bombinator* (Vogt, 1929).

and what stays out is shown in Figure 25 by the line that separates the presumptive ectodermal areas from the presumptive mesoderm. Note also the dotted line that starts at the dorsal lip and extends around the embryo. That is the line that marks the region of invagination.

Yes, these events are complex—but wait. When we look at diagrams of embryos it will become clearer. Vogt's discoveries are important for us since they are basic to understanding the experiments from Roux to Spemann that have thrown so much light on the underlying causes of differentiation.

CELL MOVEMENTS DURING GASTRULATION

Before gastrulation cell division divides the embryo into many cells, and a small

cavity, the blastocoel, appears in the animal hemisphere. In the 12-hour blastula the blastocoel is still small and the surrounding cells are many layers thick (Fig. 26).

By 22 hours (Fig. 27) the blastocoel occupies most of the animal hemisphere and its roof is only a few cells thick. The pigmented cells that foreshadow the site of the dorsal lip have appeared by this time, though the photograph does not show them. It is now possible to use Vogt's fate map to demarcate the positions of the presumptive germ layers. Using the fate map shown in side view (Fig. 25, top), an imaginary slice of the embryo has been made through the meridian that includes the animal and vegetal poles and the dorsal lip. This slice is diagrammed in Figure 27*b*. Most of the blastocoel roof consists of presumptive epidermis and anterior to this are the presumptive neural tube and notochord. The dorsal lip will form at about 4 o'clock. The presumptive notochord cells are in the area above the dorsal lip and they are continuous with a band of the other mesodermal cells that extends entirely around the embryo. Essentially all of the vegetal hemisphere is presumptive endoderm.

Further movements of the cells are shown in Figures 28 through 32, which should be studied in relation to the whole embryos shown in Figures 18 through 22.

The archenteron of the 30-hour embryo (Fig. 28) is a thin cavity opening to the outside through the blastopore. It appears to be pushing ahead of it a wall of cells that encroach upon the blastocoel. The growth of the archenteron is rapid and by 34 hours (Fig. 29) it has almost reached the anterior end and by 36 hours it has done so (Fig. 30). Its leading end continues to push anteriorly, then ventrally, and finally posteriorly, ending in a slight bulge that will form the liver diverticulum—in the 47-hour neurula (Fig. 31). This process continues and by 55 hours the archenteron begins to look more like a tube and less like a huge cavity (Fig. 32). The blastocoel, while still present at 47 hours (Fig. 31) is soon obliterated.

The archenteron continues to be open to the outside through the blastopore. (This opening does not show in all of the photographs of the embryos.) The blastopore will close eventually and shortly thereafter the anus will break through near the place where the blastopore closed. The mouth will break through at the anterior end of the archenteron.

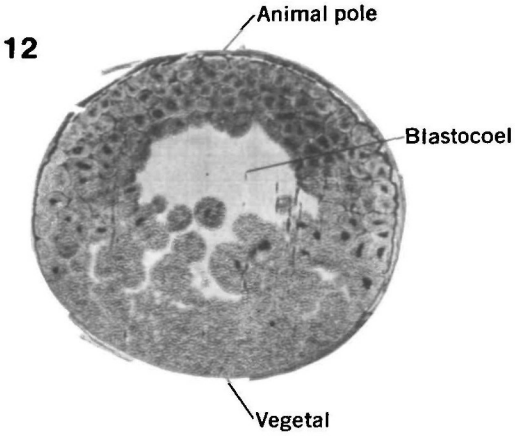


FIG. 26. Development of the frog's egg. Cross section of an early blastula. The numbers to the upper left of the embryos in Figures 26–34 indicate the hours after fertilization.

In the 30-hour embryo (Fig. 28) the presumptive notochord cells are moving inward to form the dorsal wall of the archenteron. This ingression continues until the entire area is inside by about 36 hours and it almost reaches the anterior end of the embryo in the 55-hour late neurula (Fig. 32). The rearrangement of the presumptive notochord area involves a considerable change in shape. In the fate maps of Figure 25 the presumptive notochord cells appear as a band extending across the embryo. During gastrulation these cells move to the mid-line and are stretched in an anterior direction.

The presumptive neural tube area undergoes a similar change in shape. In the fate maps of Figure 25 this area also extends across the embryo. Again the gastrulation movements change the long axis to anterior–posterior. In the 47-hour embryo (Fig. 31) a portion of the neural folds shows at the anterior end. If this seems to be a strange place, look at the whole embryo in Figure 20. A slice in the mid-line (sagittal section) would go between the two neural

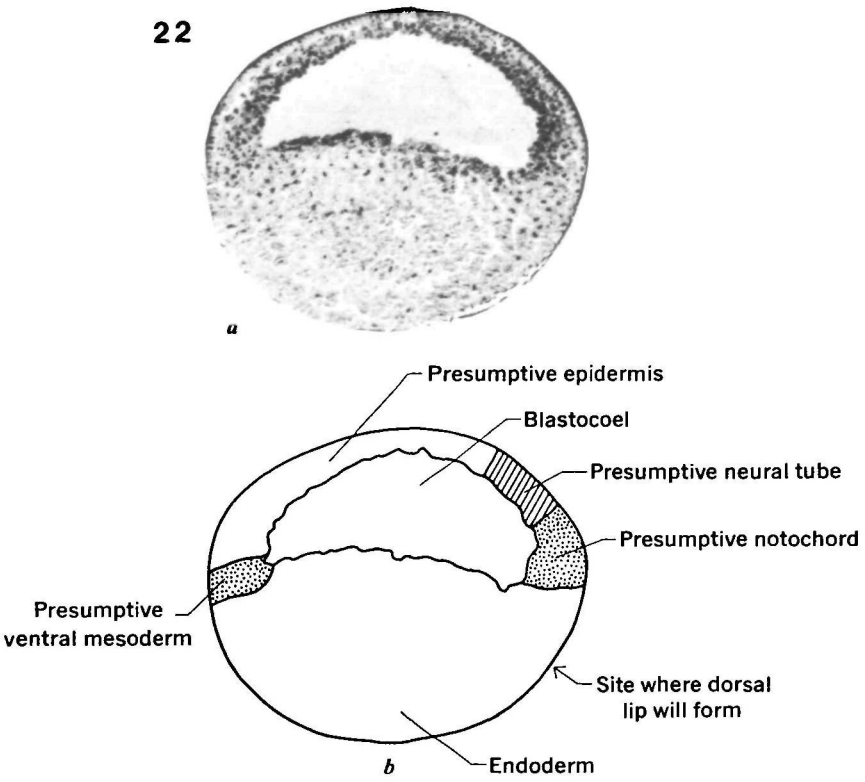


FIG. 27. Development of the frog's egg. Cross section and interpretative diagram of a late blastula.

folds and cut them only at the very anterior end where they are joined. By 55 hours (Fig. 32), however, the folds have closed and then a mid-line section will cut through the neural tube.

Embryos are three dimensional and the two dimensional longitudinal mid-line sections of Figures 27 through 32 tell us nothing of what is occurring on the sides of the embryo. It is necessary to use cross sections, that is, slices across the long axis of the body, for a better understanding of the embryo's structure.

Figure 33 shows what the 47- and 50-hour embryos look like when sectioned at right angles to the plane of the previous sections. The neural folds are about to close at 47 hours. The notochord is in the dorsal mid-line below the neural folds. To either side of the notochord the mesoderm extends a short distance. The huge archenteron is surrounded by endodermal cells.

The 50-hour embryo shows the final and fundamental distribution of the germ layers of a vertebrate embryo. The ectoderm has formed the neural tube and the epidermis that surrounds the embryo. The middle layer is mesoderm. It consists of the notochord on the mid-line beneath the neural tube flanked by the lateral mesoderm, which by this time extends as a thin layer entirely around the body. The inner layer, the endoderm, surrounds the archenteron.

By a day and a half later, at 80 hours (Fig. 34), there have been considerable further developments. The cross section of the head shows that the neural tube has enlarged to form the brain and from its ventro-lateral walls the optic cups have grown out. The optic cups will form the retina, the light-sensitive portion of the eye. The epidermis adjacent to the optic cup forms the lens. The notochord does not appear in this very anterior section. Figure 32 shows why.

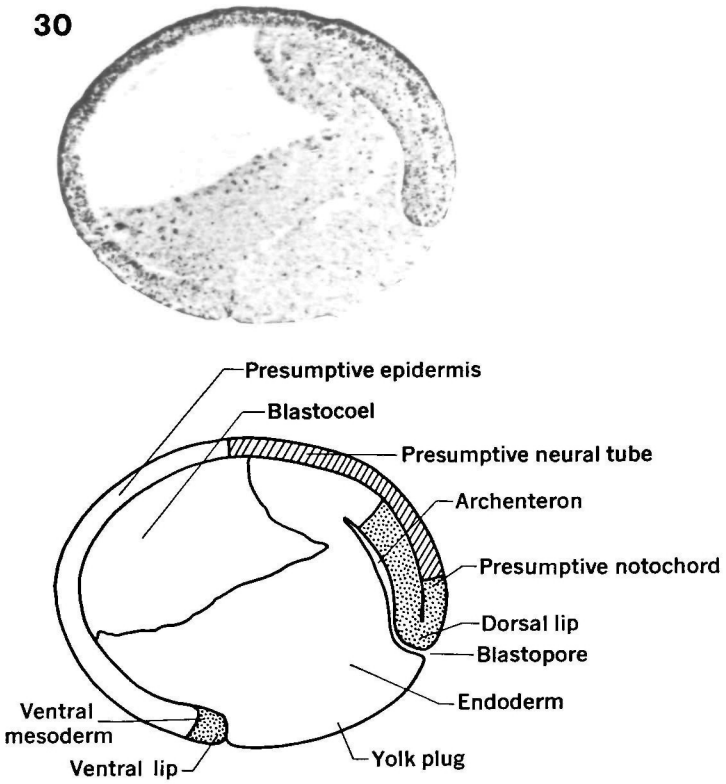


FIG. 28. Development of the frog's egg. Sagittal section and interpretative diagram of a 30-hour gastrula.

A section made in the heart region of the same embryo shows additional structures. The neural tube at this level is the hindbrain, which will form the medulla. Otic vesicles have been formed from the outer ectoderm and will differentiate into the inner ear. The heart is forming as a delicate tube beneath the archenteron. The cavity surrounding it is the pericardium, which is part of the coelom.

The cross section of the middle of the body shows an additional structure—the pronephros. It is the first stage in the development of the excretory system. The mesoderm on either side of the nerve tube and notochord has differentiated into the myotomes or somites, which will form the voluntary muscles and parts of the skeleton. The more ventral mesoderm will eventually split along its length and the cavity so formed will be the coelom.

This brief survey of early development of the amphibian embryo will provide a basis for understanding the experiments that, beginning in the 1850s, sought to explain differentiation. Now that we have surveyed *what* happens, we can try to understand *how* it happens.

THE DAWN OF EXPERIMENTAL EMBRYOLOGY

The books that students read and the university lectures they attend cannot fail to leave the impression of the inevitability of progress in science. Practitioners of science know better. Every important discovery is a rare event that is preceded by a series of failures.

A lesson that can be learned from the study of progress in embryology is how slow progress has been and how exceedingly difficult it has been to understand the underlying mechanisms that transform the relatively simple zygote into a complex adult. In fact, there was no effective experimentation from Aristotle in 4th century B.C.

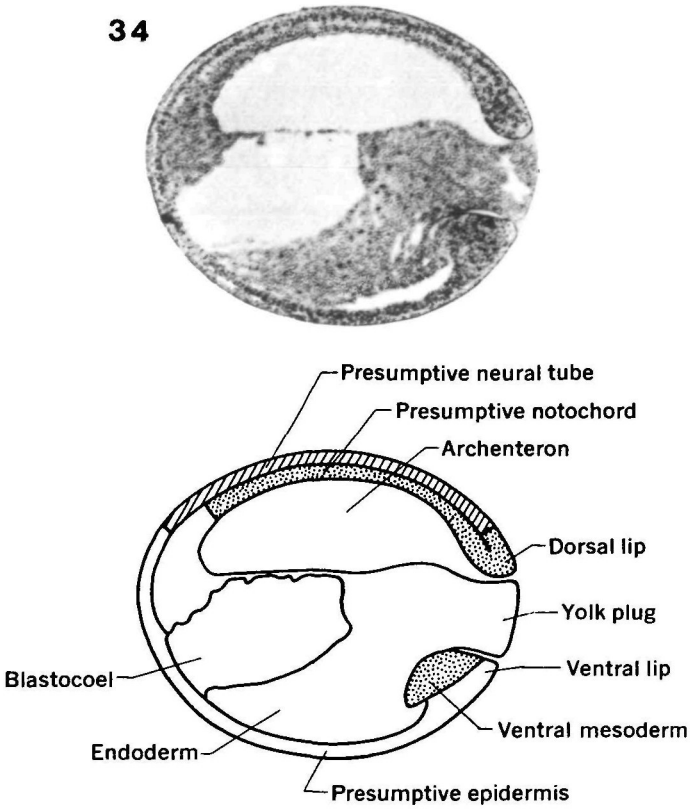


FIG. 29. Development of the frog's egg. Sagittal section and interpretative diagram of a 34-hour gastrula.

to George Newport in mid-19th century Victorian England. Why these long centuries of stasis? There is a simple answer: no one knew how to ask a useful question.

Once again it is helpful to students if they are asked, now that our long survey of descriptive embryology has been concluded, "How would you go about seeking ways to understand the mechanisms of development?" "What are some of the important problems that should be solved?" The answers are far from obvious.

GEORGE NEWPORT

Horder *et al.* (1986, p. xix) credit George Newport (1802–1854) with performing "the first experiment on embryos: point of sperm entry determines axis of developing embryo." There had been many sorts of crude experiments on embryos long before Newport but we have in him a person with a clear notion of what he wished to do and great skill in making observations and performing experiments. Experimental embryology did not begin with Newport but with him it most surely took a quantum leap.

Newport was primarily interested in fertilization and the factors influencing it (1851, 1853, 1854). First he studied the ovarian eggs of frogs, noted the breakdown of the germinal vesicle, described the passage of the ova through the body cavity into the oviducts, and their storage in the uterus. He found that the jelly layers deposited while the ova passed through the oviducts were necessary for fertilization. He stripped semen from the males and carried out many experiments on the relation of sperm concentration and motility to fertilization as he tried various temperatures and chemical solutions to find their effects on fertilization.

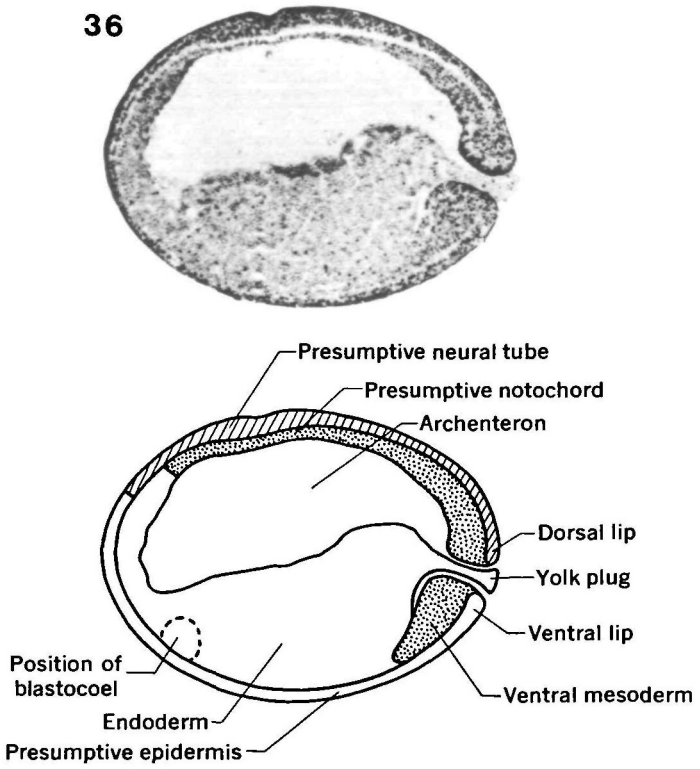


FIG. 30. Development of the frog's egg. Sagittal section and interpretative diagram of a 36-hour gastrula.

At first he did not accept other reports or his own observations as indicating that the sperm actually enters the ovum. Eventually he did so and he is now generally regarded as the first person to offer conclusive proof of this fundamental event.

The fertilized egg of a frog is an enormous sphere compared to sperm. Therefore the chance of observing sperm penetration is slight since one would be searching a huge surface (as it would appear under a microscope) for a tiny event. Newport successfully solved this problem by controlling the point of sperm entry. He prepared a sperm suspension and then dipped the point of a pin into it. The pin was then touched gently to the jelly membranes as he looked through the microscope. In order to facilitate these observations,

> I employed a glass cell to contain the egg whilst it was examined, with the view of keeping it in one position, and preventing the movement derived from accidental causes: it is made of a section of a piece of barometer tube, from one-eighth to one-fourth of an inch deep and three lines [a "line" is 2.2 mm, or $\frac{1}{12}$ of an inch] in diameter in the clear, which is cemented on a plate of glass of convenient size. This piece of apparatus, which I name a *tube-cell,* is of a size sufficient to contain only a single egg after its covering is fully expanded. For the purpose of making an observation, the egg is to be placed in the centre of the cell, immediately after removal from the body of the frog, and before it has come into contact with any fluid; by this proceeding the gelatinous envelopes adhere so firmly to the glass as to render the egg almost or quite immoveable, when the jelly expands on the subsequent addition of water. In order that the proper focal distance of high magnifying powers may be obtained, I commonly use a cell which allows the object-glass [= objective] to

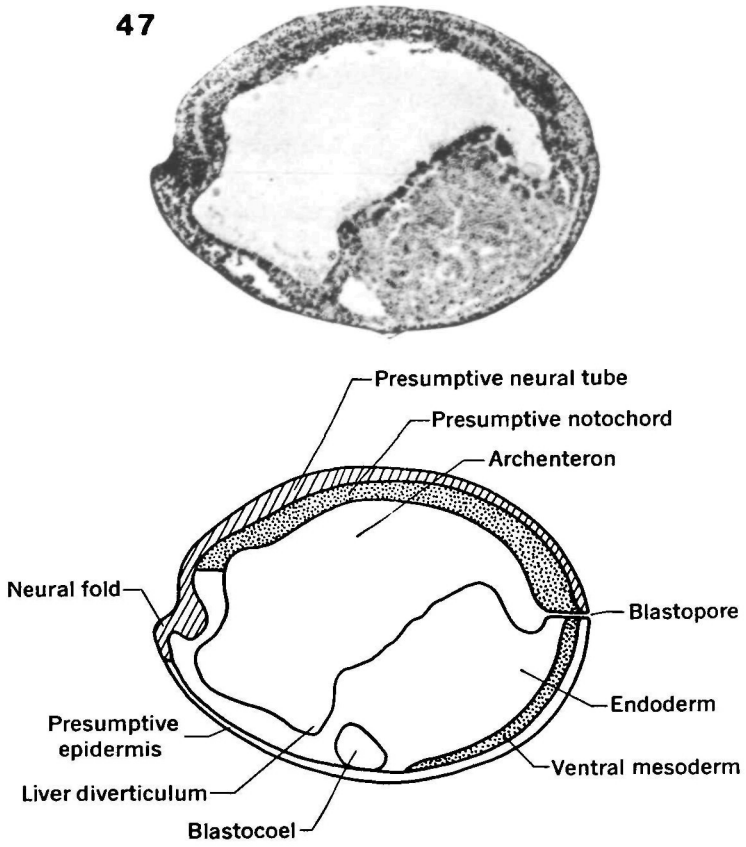


FIG. 31. Development of the frog's egg. Sagittal section and interpretative diagram of a 47-hour neurula.

be immersed in the fluid. As this cell admits light on every side, it is well adapted for viewing the penetration of the spermatozoon into the egg envelopes . . . (1854, p. 230).

Newport also made a *cistern box*, with flat sides so that he could study eggs from the side—the curved glass of the tube-cell gave considerable distortion.

The ability to immobilize the embryos permitted Newport to make some very important observations about the polarity of the embryo.

On the correspondence of the primary cleft of the Yelk with the axis of the future Embryo.

I have been long aware that the axis of the embryo was in the line of the first cleft [cleavage] of the yelk, but my endeavour to show this was not always satisfactory, in consequence of the difficulty of making the egg keep in a given position, whilst it was free to move; but since I have employed the tube-cell I have obtained the desired evidence with great ease. The results of the following observations will support my statement.

Obs. 1.—I took an egg that had just divided for the first time, and placed it in a glass cell only sufficiently large to contain it when the jelly was fully expanded, and filled the cell with water. The dorsal surface turned uppermost, as usual, consequently I had under my eye the whole surface; and could watch the changes with the microscope. I marked the plate of glass supporting the cell with a line parallel to the primary cleft of the yelk, and indicated the position of the

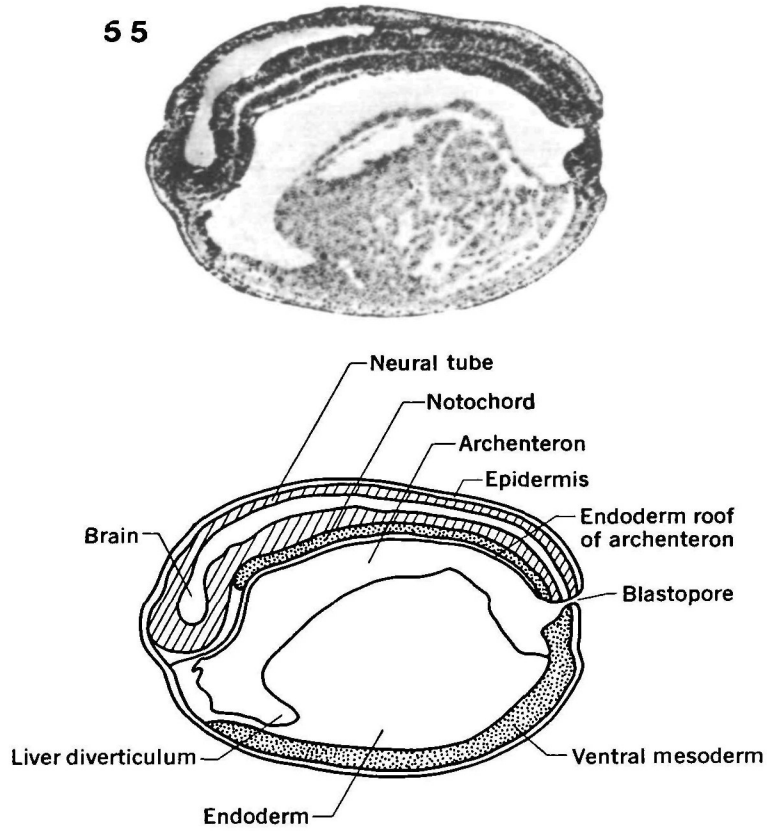


FIG. 32. Development of the frog's egg. Sagittal section and interpretative diagram of a 55-hour neurula.

ends of the sulcus [furrow] by other marks. The whole was placed in a temperature of 60° Fahr.

At the time of the closing-in of the dorsal laminae [neural folds], I found the correspondence between the axis of the embryo and the line of the first cleft to be exact . . . .

Obs. 2.—Nine eggs were put in separate cells on March 11th, and when segmen-

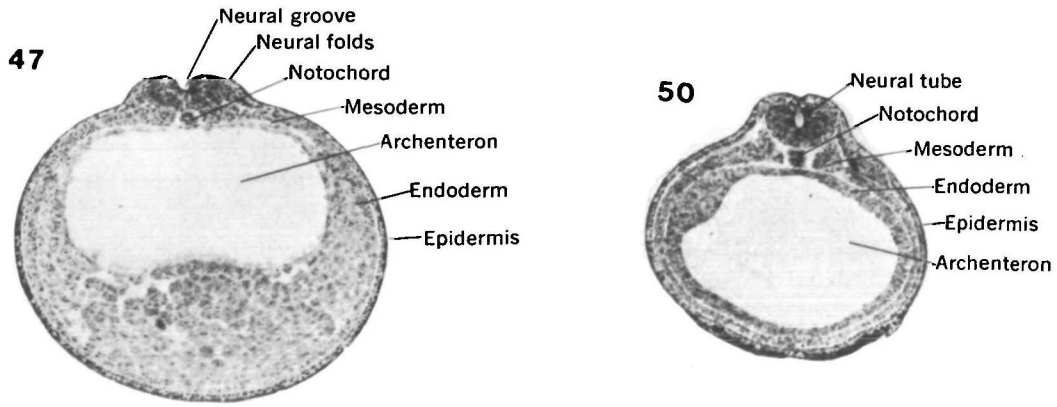


FIG. 33. Development of the frog's egg. Cross sections of a mid and late neurula.

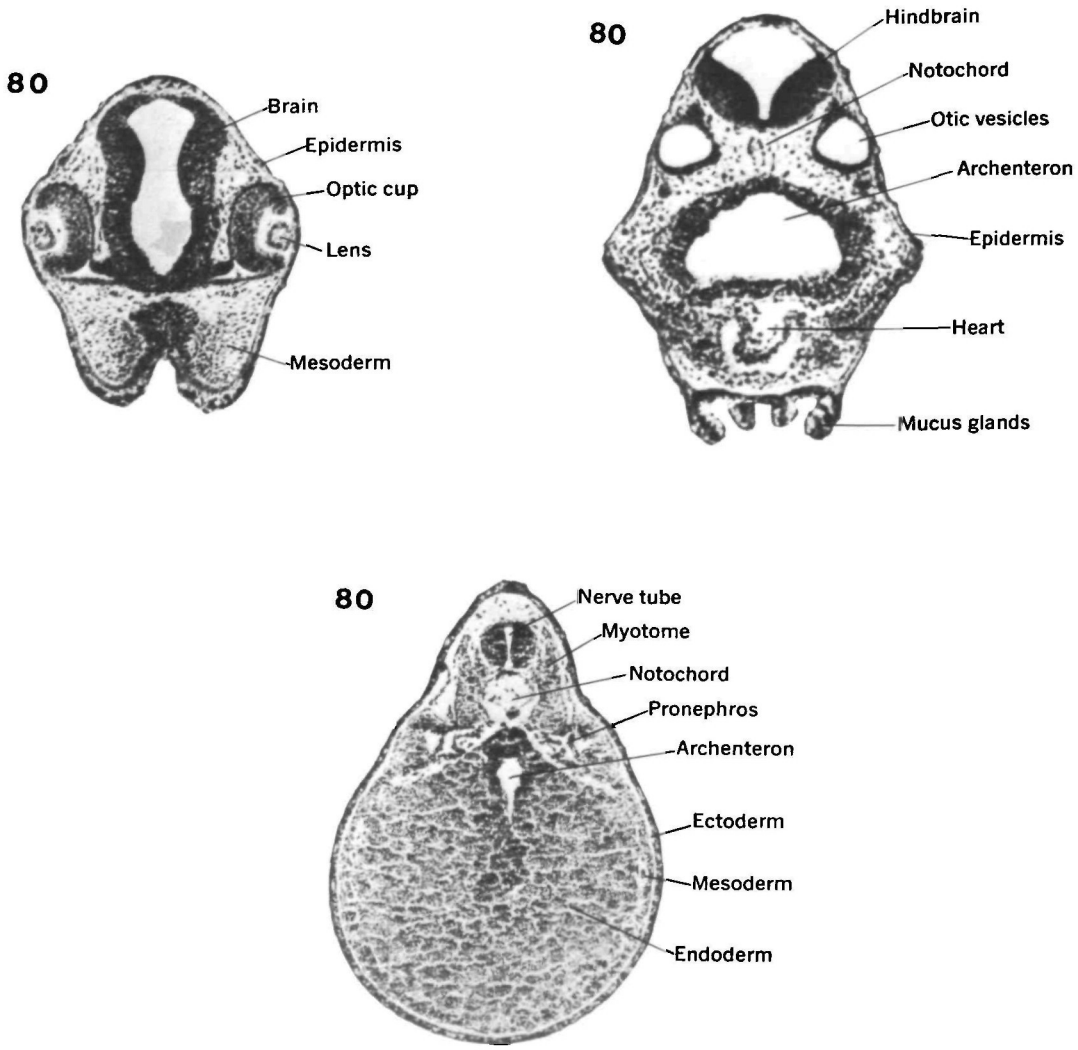


FIG. 34. Development of the frog's egg. Cross sections of a tailbud embryo in the eye, ear, and trunk regions.

tation began, the line of the first cleft was carefully marked on the glass in the manner before explained. One of the eggs was abortive . . . .

March 14. In each of the eight instances the axis of the body is more or less precisely in the line of the sulcus: thus in five it was in the exact line, in one about five degrees to the left, in another about three degrees to the left, and in the remaining one more to the left of the given line (1854, pp. 241–242).

Now comes the remarkable observation that makes it most appropriate to recognize Newport as the first experimental embryologist.

On the power of the Spermatozoon to influence in artificial impregnation the direction of the first cleft of the Yelk.

In connection with the influence of the spermatozoon on the egg, I determined to try whether the artificial application of that body to different parts of the egg's surface could affect the position of the first cleft of the yelk.

Obs. 1.—Several eggs were placed, March

29, in separate tube-cells, with each turned on its side so that both the dark and white surface were exposed. Very recent spermatic fluid was then applied, by means of a pin's head, to the lower part of the dark surface, and the cell was carefully marked close to the spot, to show where the egg was touched . . . . April 3. Each egg has formed an embryo, and in each instance with the head to the side of the egg touched.

Obs. 2.—Four eggs were placed in separate cells as before, and only two became fruitful. In one the primary cleft was in the precise line of the spot touched, although the egg subsequently diverged to the left; and the head corresponded to the part fecundated. In the other the cleft was about ten degrees to the left of the part impregnated, and the head was also turned to the part touched with fluid.

Obs. 3.—Four other eggs were taken, but two of them were sterile; and in the development of one the head deviated remarkably from the usual position. The first cleft in one (*a*) was about six degrees to the right; and in the other (*b*) about five degrees to the left of the point touched. Both formed embryos: in one (*a*) the head was at the end of the cleft nearest the point touched, but in the other (*b*) at the end furthest from the same point. The peculiarity in the last experiment I cannot explain; possibly there might be some want of precision in conducting it.

Similar experiments were repeated four other times, and the results showed that the first cleft of the yelk is in a line with the point of the egg artificially impregnated, and that the head of the young frog is turned toward the same point (1854, pp. 242–243).

Newport was always careful to mention any deviation from what he had come to expect, but he states that the deviation never exceeded 15°. Today it seems remarkable that the results are so consistent. After all, one might not expect the sperm to enter the ovum at exactly the spot touched with the sperm-laden pin.

That ended Newport's career—he became ill after a collecting trip in a swampy area near London and died. In fact the 1854 paper was completed after his death by a friend.

One can only speculate how Newport would have built on this fundamental discovery to continue his experiments. Not only had he established a causal relationship between the entrance point of the sperm, the plane of first cleavage, and the primary axis of the embryo, but he could control that relationship. This ability to control development in such a basic way made possible the experimental analysis of differentiation. One could begin to ask meaningful questions and have some hope of being able to answer them.

It is important to note that a breakthrough in experimental science frequently comes as a result of observations having little to do with the problem being explored. Newport was initially mainly interested in fertilization. The tube-cells that he constructed to observe sperm penetration better held the embryo in a fixed position. This same experimental setup proved to be valuable in another way—to make possible his observations associating the entrance point of the sperm, first cleavage and the embryonic axis.

Experimental embryology was underway. Well, not quite. Newport's remarkable discoveries were not to be extended for several decades. Darwin was shortly to capture the interest of embryologists and experimentation was to receive little attention. In fact, the data that were essential for the further analysis of development were to come from the study of cells, not from the study of embryos.

DEVELOPMENT, HEREDITY, AND THE CHROMOSOMES

The greatest puzzle of all in the mid-1800s was, How can the fertilized ova of two species, which may seem identical, develop into two adults that differ greatly from one another? Whatever the nature of heredity might be, surely it must be fundamental in determining the pattern of development. With the passage of each decade the possibilities of spontaneous

generation seemed ever less likely. It seemed more and more probable that living organisms could come only from living organisms and that cells could come only from cells. Thus, whatever its nature, inheritance must be associated with the dividing cells of the developing individual and with the cells of reproduction—ova and sperm.

Schwann's and Schleiden's paradigm of the Cell Theory, plus the great improvements in methods for studying cells, resulted in a burst of cell research beginning in the 1870s and lasting to the turn of the century. There is a lengthy discussion of the results in last year's Essay (III, pp. 609–639) so there is no need to repeat the details here. These are the general conclusions.

1. The reproduction of somatic cells results in the formation of daughter cells identical with each other and with the parent cell.

2. That means that the structures of cells must also reproduce or be synthesized. For both the cell and its parts there must be a doubling and then division.

3. Some cell structures appear to be passively allocated to the daughter cells.

4. On the other hand chromosomes appear to be divided by a complex and precise mechanism—mitosis. Each chromosome replicates and then one daughter chromosome goes into each daughter cell.

5. In some species, at least, the number of chromosomes appears to be constant.

6. This number remains constant from generation to generation: hence, there must be some mechanism for maintaining this constancy. The mechanism was found to consist of two modified nuclear divisions—meiosis.

7. Meiosis in males was found to occur just prior to the formation of mature sperm. In females it may begin either at the time of ovulation or just after fertilization. Meiosis consists of two divisions the result being a halving of the number of chromosomes. Thus, after meiosis has been completed, both ovum and sperm have the monoploid number of chromosomes.

8. The fusion of a monoploid female pronucleus and a monoploid male pronucleus at fertilization restores the diploid number in the new individual.

9. Although there were many variations in different kinds of animals, the basic patterns of mitosis and meiosis were found to be remarkably constant throughout the animal kingdom.

10. These complex, remarkable, and nearly universal mechanisms for maintaining nuclear and chromosomal constancy, plus a few crude experiments, made probable the hypothesis that the nucleus, and more specifically the chromosomes, play an important role in heredity.

A caution should be entered at this point. The concept of the chromosomes as the physical basis of inheritance is so firmly embedded in the way we think today that we tend to forget that a hundred years ago that was not the case. Some biologists did regard that notion as a probable hypothesis but many still treated heredity as an abstract idea and not as a phenomenon closely associated with known structures of the cell. Other biologists at that time thought more in terms of the idioplasm hypothesis of Carl von Nägeli, who proposed that the physical basis of inheritance consisted of an invisible network that extended throughout all cells (III, p. 636). Some thought of the idioplasm as Darwin's gemmules (III, pp. 596–605) linked together in an organized structure. The hypotheses of chromosomes *versus* idioplasm as the substance of inheritance were not mutually exclusive, since the idioplasm was thought to spread through the nucleus as well.

E. B. WILSON: STATING THE PROBLEM

In the 1870s when the experimental analysis of development began to attract more investigators, there was a need to define the problem. What it was has been said well by E. B. Wilson. Although the following quotation was written later (1900) he expressed a point of view that would have been much the same two decades earlier.

> Every discussion of inheritance and development must take as its point of departure the fact that the germ is a single cell similar in its essential nature to

any one of the tissue-cells of which the body is composed. That a cell can carry with it the sum total of the heritage of the species, that it can in the course of a few days or weeks give rise to a mollusk or a man, is the greatest marvel of biological science. In attempting to analyze the problems that it involves, we must from the onset hold fast to the fact, on which Huxley insisted, that the wonderful formative energy of the germ is not impressed upon it from without, but is inherent in the egg as a heritage from the parental life of which it was originally a part. The development of the embryo is nothing new. It involves no breach of continuity, and is but a continuation of the vital processes going on in the parental body. What gives development its marvelous character is the rapidity with which it proceeds and the diversity of the results attained in a span so brief.

> But when we have grasped this cardinal fact, we have but focussed our instruments for a study of the real problem. *How* do the adult characteristics lie latent in the germ-cells; and how do they become patent as development proceeds? This is the final question that looms in the background of every investigation of the cell. In approaching it we may well make a frank confession of ignorance; for in spite of all that the microscope has revealed, we have not penetrated the mystery, and inheritance and development still remain in their fundamental aspects as great a riddle as they were to the Greeks . . . . The real problem of development is *the orderly sequence and correlation of . . . phenomena toward a typical result.* We cannot escape the conclusion that this is the outcome of the organization of the germ-cells; but the nature of that which, for lack of a better term, we call "organization," is and doubtless long will remain almost wholly in the dark (pp. 396–397).

Yet something could be said about that organization. Since the egg is part of the parent, as Wilson emphasized, its organization must be a part of the organization of the parent. The egg has, therefore, "something" of the parents. That inherent something will be encased in a single-celled zygote and the problem becomes the mechanisms that convert the zygote to adult.

THE HYPOTHESIS OF GERMINAL LOCALIZATION

In 1874 William His (1831–1904) attempted to say something about organization. His hypothesis of germinal localization, or as it was to be called later, cytoplasmic localization, became fundamental in analytical embryology. He worked mainly with chick embryos and his problem was the eternal one—if the body of the chick is not preformed in the germ, what is? He suggested that, if the parts were not preformed, whatever is responsible for them is present at the beginning of development.

> It is clear, on the one hand, that every point in the embryonic region of the blastoderm must represent a later organ or part of an organ, and, on the other hand, that every organ developed from the blastoderm has its preformed primordium in a definitely located region of the flat germ-disc . . . . The material of the primordium is already present in the flat germ-disc, but it is not yet morphologically marked off and hence not directly recognizable. But by following the development backwards we may determine the location of every such primordium even at a period when the morphological differentiation is incomplete or before it occurs; logically, indeed, we must extend this process back to the fertilized or even the unfertilized egg. According to this principle, the germ-disc contains the primordia of the organs spread out in a flat plate, and, conversely, every point of the germ-disc reappears in a later organ; I call this *the principle of organ-forming primordial-regions.* (In E. B. Wilson's translation [1900, p. 398] of His's paper "germ" was used in two ways, one meaning embryonic, as in "germ-disc," the other referring to the substances necessary for the formation of organs. For the latter I have

substituted "primordia" for the sake of clarity.)

Today it may be hard to understand why His's hypothesis was thought important. Would not one expect that the parts of the older embryo and adult come from the substance of the zygote? What other possible source could there be? However, His was saying something more important, namely, that the organization of the egg consists of the localization of the factors, unknown but presumably material, that are responsible for the development of the parts of the embryo and adult. Thus the zygote was not to be regarded as a totally unorganized bit of protoplasm but of having some *substances*—not force or immaterial organizing principle—that were the *sine qua non* for differentiation. His was suggesting that by careful observation one could prepare a fate map of the chick embryo much as Vogt was to do a half century later for the amphibian embryo (Fig. 25).

Although His spoke of the "principle" of organ-forming germ-regions, "hypothesis" would have been a better term—he suggested, he did not prove. Nevertheless, his hypothesis was a useful way to think of the egg's organization and it suggested experimental approaches to Roux and others.

WILHELM ROUX UND *ENTWICKLUNGSMECHANIK*

Analytical embryology became a full-fledged program of experimentation in the hands of the German biologist Wilhelm Roux (1850–1924). He was a gifted, vigorous, outspoken, and dedicated scientist who was prominent—even in the Germany of his famous teacher, Ernst Haeckel. Roux's main hypotheses were to require much modification and many of his experiments proved to be defective but with brilliance and perseverance he raised the questions that brought experimental embryology into full flower. He initiated and for years was the editor of the first important journal devoted to analytical embryology—*Wilhelm Roux' Archiv für Entwicklungsmechanik,* which began in 1894–1895 and continues to this day.

Together with his compatriot, August Weismann, he developed the first important hypothesis of differentiation from which deductions could be made and then tested by observation and experiment. The Roux-Weismann hypothesis (usually called "theory") was based mainly on the observations, experiments, and interpretations of Roux plus some theoretical elaboration by Weismann.

Roux's key paper for the discussion that follows was published in 1888 and can be found, in translation, in Willier and Oppenheimer (1964). The following quotations are from that source. Roux posed the problem as follows:

> The following investigation represents an effort to solve the problem of self-differentiation—to determine whether, and if so how far, the fertilized egg is able to develop independently as a whole and in its individual parts. Or whether, on the contrary, normal development can take place only through direct formative influences of the environment on the fertilized egg or through the differentiating interactions of the parts of the egg separated from one another by cleavage (p. 4).

Roux was posing fundamental questions. The first one, whether or not the development of an egg requires specific stimuli from the environment, may seem strange to us today. It was not strange in the 1880s. Botanists had been describing the many diverse effects of the environment on the growth and differentiation of plants. Light had a pronounced effect on the production of chlorophyll, the rate of growth, the pattern of growth, leaf retention or loss, and seemingly just about everything plants did. Gravity, temperature, wind, moisture, and soil chemistry all had great effects on plant growth and development. Roux sought to determine if frog embryos were similarly affected by these environmental factors by rotating the embryos constantly so that gravity, light, heat, and magnetic forces would not be able to exert an effect from a constant direction. The embryos developed perfectly normally.

> We can conclude from this that the typical structures of the developing egg and

> embryo do not need any formative influence by such external agencies for their formation, and that in this sense the morphological development of the fertilized egg may be considered as self-differentiation (p. 4).

Having answered his question for the embryo as a whole, Roux sought to ask the same question for its parts. The very fact that he was able to ask such a question at all depended not only on his work but also on the work of those who had preceded him or who were his contemporaries. We must never forget this most important aspect of scientific work. The questions that can be asked at any time relate to the state of the field, which means that others have prepared the groundwork for the scientist's research. For example, in the 1880s there were exciting new discoveries in cell biology, especially about chromosomes. It was becoming ever more important to solve the problem of inheritance. A huge amount of information was available about development and His had postulated that differentiation depended on the presence of determinants for the structures of the embryo.

All of this information is general and could not suggest to Roux what he should do the next morning when he went to his laboratory. However some very specific facts that he had learned suggested the possibility of a truly impressive experiment that, if successful, would throw great light on the age old problem of the causes of differentiation.

Roux reported that he had discovered some fascinating rules involving the early development of frog embryos. The first of these was that the plane of first cleavage coincides with the median plane of neurulae and later embryos. There was even the possibility of this being a rule of broad applicability because others had found the same thing to hold true for such different embryos as those of bony fish and ascidians. Newport had discovered this long before and Roux notes,

> It is worth mentioning that observations pertinent to this matter had already been recorded in the posthumous papers of G. Newport, published in 1854. These aroused no notice at the time and were not discovered again until later (p. 6).

This neglect of Newport's discovery cannot be explained away, as in the case of Mendel's work, as the consequence of his results being published in an obscure journal. *The Philosophical Proceedings of the Royal Society of London* was the most prestigious scientific journal in the English language. No notice was taken of Newport's discovery in 1854 because no one had the remotest idea of how to profit by it. The field was "not ready."

Roux confirmed, for the most part, Newport's other discovery of the relation of the point of sperm entrance to the plane of first cleavage and the future polarity of the embryo. But Roux found another relationship: shortly after fertilization a broad crescent in the lower part of the animal hemisphere, opposite the point of sperm entry, loses some of its dark pigment and becomes the gray crescent. The gray crescent persists at most for a few cleavages. By preventing the embryos from changing position, Roux found that the dorsal lip of the blastopore appears where the gray crescent had been.

There seemed, therefore, to be these relations. 1. The sperm enters the ovum. 2. The gray crescent forms 180° from the sperm's entrance point. 3. The plane of first cleavage is in the meridian of the entrance point of the sperm and the animal pole. 4. The plane of first cleavage bisects the gray crescent. 5. The dorsal lip forms where the gray crescent had been. 6. The anterior–posterior axis of the embryo forms in relation to the dorsal lip. Later, when the neural folds form, the blastopore will be at their posterior end.

Thus, as Newport had observed and Roux confirmed, the plane of first cleavage divides the embryo into a right and left half. Roux saw the possibility of testing His's hypothesis.

TESTING THE HYPOTHESIS OF HIS

If we assume that the hypothesis of His—that the primordia are absolutely necessary for the formation of the parts of the

embryo—is true, then this deduction follows logically:

> *If some of the primordia can be destroyed, and the embryo still be able to develop to some extent, the structures normally determined by those primordia must be absent.*

Since the primordia were hypothetical structures it was impossible to identify and then manipulate them. Roux sought to achieve that end, however, in an indirect way. This involved a subsidiary hypothesis and this deduction:

> *If the plane of first cleavage divides the embryo into a right and left half, each half must contain the primordia for that specific half. Therefore, the destruction of one blastomere would also destroy the primordia for half of the body.*

After trying various methods Roux destroyed one cell of the two-cell stage with a hot needle.

> I heated the needle by holding it against a brass sphere for a heat supply, heating the sphere as necessary. In this case only a single puncture was made, but the needle was ordinarily left in the egg until an obvious light brown discoloration of the egg substance appeared in its vicinity . . . I now had better results; they were as follows. In about 20% of the operated eggs only the undamaged cell survived the operation, while the majority were completely destroyed and a very few, where the needle had possibly already become too cold, developed normally. I thus developed and preserved over a hundred eggs with one of their halves destroyed, and, of these, 80 were sectioned completely (p. 9).

And the experimental analysis of development was underway. In the 20 percent where the untreated cell survived, various results could be expected,

> For example, abnormal processes might intervene which would lead to bizarre structures. Or the single half of the egg, which, after all, according to many authors, is a complete cell with a nucleus completely equivalent in quality to the first segmentation nucleus, might develop into a correspondingly small individual. These authors see in the mechanism of indirect nuclear segmentation [*i.e.*, mitosis], on my authority as it were, only a contrivance for qualitative halving. I have repeatedly and clearly opposed this opinion. But instead of the possible surprises as postulated above an even more amazing thing happened; the one cell developed in many cases into a half-embryo generally normal in structure, with small variations occurring only in the region of the immediate neighborhood of the treated half of the egg (p. 12).

Figure 35 shows some of the results. In embryo *a* the left blastomere had been killed but the right blastomere lived and formed a half blastula. In embryo *b* the right blastomere had been killed, was later sluffed off, and the living side rounded up and produced an embryo with a single neural fold and with the mesodermal layer extending from the notochord around the left side of the embryo only. There is what may be described as half an archenteron, though it is hard to recognize half a hole. What was one to conclude?

> In general we can infer from these results that each of the first two blastomeres is able to develop independently of the other and therefore does develop independently under normal circumstances . . . . All this provides a new confirmation of the insight we had already achieved earlier that developmental processes may not be considered a result of the interaction of all parts, or indeed even of all the nuclear parts of the egg. We have, instead of such differentiating interactions, the self-differentiation of the first blastomeres and of the complex of their derivatives into a definite part of the embryo . . . . The development of the frog gastrula and of the embryo initially produced from it is, from the second cleavage on, a mosaic of at least four vertical pieces developing independently (pp. 25–28).

Figure 36 is a schematic representation of

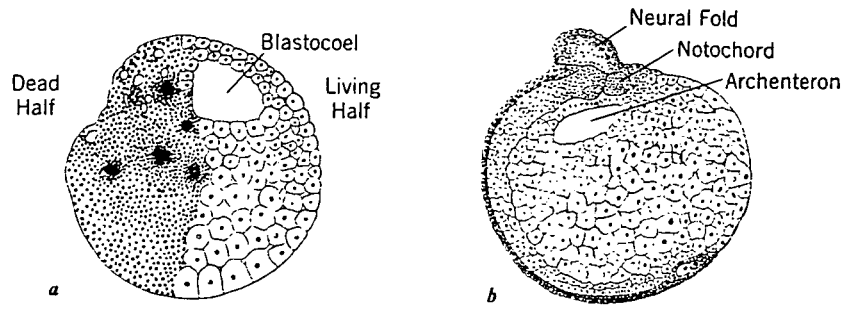


FIG. 35. Half-embryos obtained after killing one blastomere of the two-cell stage of a frog embryo. In A the dead half remains. In B it has been sluffed off. (Roux, 1888, p. 113.)

Roux's interpretations after it came to be assumed that the determinants were associated with the nucleus. It shows the segregation of the determinants that produces "a mosaic of at least four vertical pieces developing independently."

These results can be taken as a dramatic and convincing test of our original two deductions and so Roux's hypothesis for the localization of determinants is made more probable—as is, of course, His's hypothesis that there are primordia, or determinants, for differentiation.

DIFFICULTIES WITH THE HYPOTHESIS

It is hard to overemphasize the importance of Roux's hypothesis for the rapidly developing field of experimental embryology. However, important ideas in science must be tested in a variety of ways and by many different scientists before they can be accepted. Such requirements help to eliminate faulty hypotheses and faulty experiments. Roux's ideas were center stage for at least a decade but eventually they had to be drastically altered because

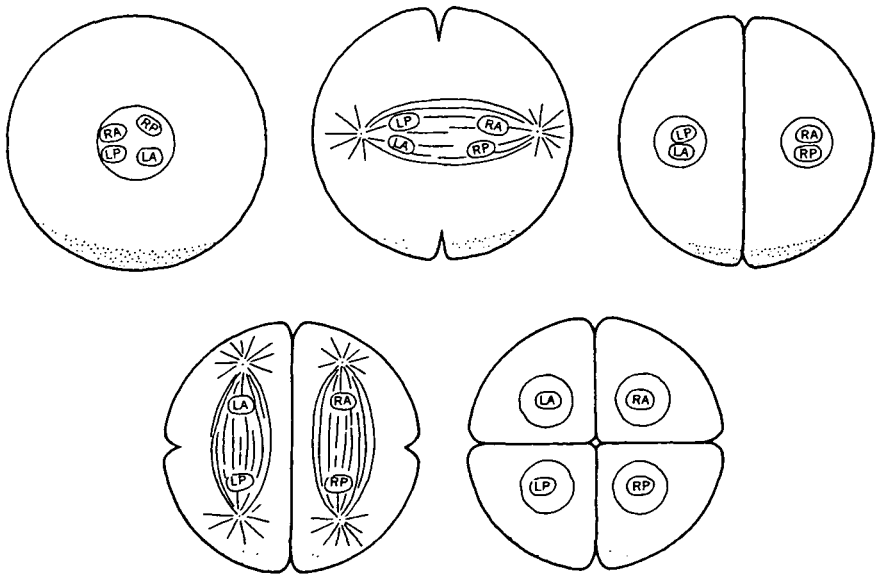


FIG. 36. Schematic representation of Roux's revised hypothesis for the segregation of determinants during cleavage. LA = left anterior determinants; RA = right anterior determinants; LP = left posterior determinants; RP = right posterior determinants.

of what he and others discovered. These later developments will be summarized now, out of place so to speak, and then we will return to the main line of analysis pretending to be ignorant of what is to come.

Roux's hypothesis appeared to be fully confirmed by the development of the half-embryos up to the neurula stages. Some of the embryos, however, were kept and what was surely a most discouraging phenomenon was observed: the half-embryo gradually formed a whole embryo. Roux called this "postgeneration."

The simplest interpretation of postgeneration would be that there had been no destruction of the determinants for one side. It had been assumed that each cell of the two-cell stage had the determinants *only* for that half. Certainly development to the neurula stage seemed to indicate that the determinants for the operated side had been destroyed. If so, they could not have "come to life" and produced a whole embryo. Yet they must have been preserved in some way since postgeneration would have been impossible without them.

Roux developed a subsidiary hypothesis to account for postgeneration but the fact that he had to do so greatly weakened his original hypothesis. He held, in effect, that there were two sorts of determinants. The main sort consisted of the determinants that were divided *qualitatively* during cell division and specified the organs and parts of the embryo. In addition, another sort of determinant was held in reserve. It was divided *quantitatively* and kept intact the complete set of determinants. Later in development, if a part were lost, this reserve set made it possible for there to be a complete regeneration.

This *deus ex machina* solution was without much merit. It was proposed to save the original hypothesis and it seems impossible that experiments could be devised to test it.

Roux himself reported some experiments that made the original hypothesis questionable when he found that embryos could be obtained, without the need for any postgeneration, from single cells of the two-cell stage. (He called these "hemio-oholoplasten" embryos—a term that, seemingly, was not widely accepted and used.)

Eventually the data made it less and less likely that Roux's hypothesis was correct and others began to suggest another, namely:

> *There is no segregation of determinants in the early cleavage stages.*

If this alternative hypothesis is true, it would still be necessary to explain the results Roux obtained in the embryos developing to the neurula stage—results that were confirmed when others repeated Roux's experiments. One possibility is that these half-embryos might be the result of the presence of the dead cell affecting the development of the living cell. If so, the following deduction can be made:

> *If one cell of the two-cell stage is removed rather than destroyed, the remaining cell should produce a complete embryo.*

Various ways of performing this experiment were tried and the clearest results were obtained by McClendon (1910). He found it possible to remove one blastomere at the two-cell stage by sucking one out with a tiny pipette. The single remaining cell developed and produced a complete, though small, embryo. Morgan (1897, pp. 111–122) reports on the early attempts to confirm Roux's claims and provides a later (1927, ch. 18) account of the clearing up of the problem.

For the time being we will ignore these final clarifications since they were unknown and Roux's experiments and conclusions played an important role in the analysis of development.

SCRAMBLING THE EGGS

In 1884 Pflüger found that, if uncleaved frog eggs were compressed between two plates of glass, the planes of cleavage could be modified. This technique allowed the experimenter to study the role of cleavage in later development. It proved to be so valuable that it was employed by many workers who used it on a wide variety of eggs.

Figure 37 shows one such experiment in which an uncleaved frog egg, in side view,

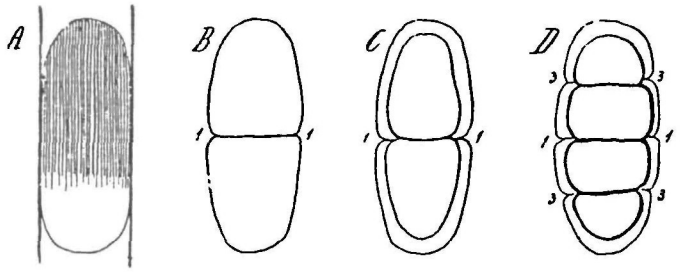


FIG. 37. The effects of pressure on the planes of cleavage. The animal hemisphere is shaded in A. Orientation is the same in B–D. The numbers 1 and 3 indicate the planes of first and third cleavage. The second cleavage plane is vertical (C) but is not numbered. (From Jenkinson, 1909, p. 36.)

is compressed by two plates that are parallel to the animal pole–vegetal pole axis. The animal hemisphere is shaded. "B" shows the plane of first cleavage extending horizontally from one glass plate to the other. In an uncompressed embryo this cleavage would have been vertical. "C" shows second cleavage, which is vertical. In "D" the third cleavage again is horizontal. The result is a very strange eight-cell stage.

It may seem surprising but there is a simple explanation for these abnormal cleavage planes: the mitotic spindle takes a position with its long axis parallel to the two pressure plates. In "B" and "D" the spindles would have been parallel and vertical and in "C" parallel and horizontal.

This technique allowed one to control the positions of the early cleavage planes and so scramble the relative positions of the blastomeres. This afforded a test of Roux's hypothesis for the localization of the primordia of the embryo's structures and their qualitative separation by cell division (Fig. 36). Recall that Roux maintained that the frog embryo is "from the second cleavage on a mosaic of at least four vertical pieces developing independently." One could further test his hypothesis by the following deduction:

> *If regional determination is a consequence of the separation by the process of cleavage of the determinants, or primordia, for future differentiation, any modification of the planes of cleavage should be followed by specific modifications of development.*

However, when these pressure experiments were performed normal embryos developed.

These experiments appeared to be fairly conclusive in showing that Roux's hypothesis was probably wrong. A precise pattern of cleavage, dividing those hypothetical determinants, was not a requirement for normal development. There was, however, another possibility. It could be maintained that the determinants are localized in specific portions of the uncleaved egg and that they are not segregated by cell division. They would eventually be in the proper place no matter what the earlier planes of cleavage had been. The role of cleavage would then be solely to divide the original egg into many smaller cells, and cleavage would not be a primary mechanism for differentiation—the embryo would still be a mosaic of independently developing parts.

FURTHER TESTS OF THE HYPOTHESIS OF MOSAIC DEVELOPMENT

Despite such results, and the difficulty in explaining postgeneration, Roux's dramatic experiments and seemingly reasonable hypothesis were having a great influence. If his conclusions could be established as true beyond all reasonable doubt, embryologists would be well on the way to solving the problem of differentiation. It was hoped, of course, that a general explanation was at hand and not one that applied only to a single species of European frog. The hypothesis of mosaic development remained the prime concept of the last quarter of the 19th century and many investigators, working on many sorts of organisms, sought to test it further. The

resulting studies fell into two main classes: Roux was right; Roux was wrong. There were dramatic verifications and rejections. We will start with one of each.

A Harmonious Equipotential System

Four years after Roux's seminal paper, Hans Driesch (1892) sought to confirm or deny Roux's hypothesis using a European sea urchin, *Echinus microtuberculatus*. He, too, started with the hypothesis of His's organ-forming primordial areas and, as had Roux, sought to separate the cells of the two-cell stage.

Driesch's methods were considerably different. Instead of killing one of the cells, he put about 100 embryos in a small tube with a little sea water. Then he shook the tube violently for about five minutes or more. Some of the two-cell embryos were found to have broken through their membranes and the blastomeres had separated. From many experiments he was able to obtain for study about 50 isolated cells from the two-cell stage.

How would they develop? The first check could be made of the cleavage stages. In normal embryos the planes of the first two cleavages are vertical and the third horizontal, as in the frog. The result is eight cells of almost equal size. The fourth cleavage, however, is very different. The four vegetal cells divide very unequally to give four large macromeres and four very small micromeres. The four animal hemisphere cells divide about equally.

Would the isolated blastomeres of the two-cell stage produce micromeres and, if so, at which division? If they behaved as whole normal embryos, they would form micromeres at the fourth division. If they behaved as though they were still part of a whole embryo, the micromeres would be formed at their third division (the isolated cells would, of course, have already gone through the first division and then been shaken apart). Driesch found that the first two cleavages were equal, giving four cells looking just like a half of a normal embryo. The third cleavage of the isolated cells produces micromeres and the result was an embryo that was identical with half of a normal 16-cell embryo.

So far, the isolated cells were behaving just as His and Roux would have predicted. In fact they continued to exhibit mosaic development, forming a half blastula, that is, one that resembled a cup in being open on one side.

That was the evening of the first day and, having observed the experimental embryos all day, Driesch went to bed. What would the morrow bring when he knew that normal embryos would gastrulate and develop into pluteus larvae? By now he was down to 15 experimental embryos, some of the others having been preserved for study or died. He wondered if those remaining would be half gastrulae and half pluteus larvae.

Three had formed *fully normal* pluteus larvae except for their small size. Apparently Driesch was astonished but not overjoyed with this discovery. It seemed to him "almost a step backward along a path considered well established."

How could he account for these results? Driesch suggested that, after all, frogs are not sea urchins. Since this answer did not seem adequate, he thought that maybe the difference was that Roux had not really isolated blastomeres at the two-cell stage. He had killed one cell, but it remained in contact with the one living one. Possibly the dead one was having an inhibitory effect.

It seemed possible to Driesch that the two sorts of embryos would have behaved in the same manner if the blastomeres in both had been fully isolated. (His guess was correct for, as we have already seen, years later McClendon and others were to succeed in removing one cell of the two-cell stage of frog embryos and find that normal larvae would result.)

These results were extremely difficult to deal with. When the cells of the two-cell stage remained together, each would produce half of an older embryo. Yet when these same kinds of cells were separated from one another, after developing as half-embryos through the blastula stage, they regulated and formed entirely normal pluteus larvae. One had to assume that there is some overall control exerted by the whole embyro over its constituent parts, that is,

the embryo is not a complete mosaic of self-differentiating parts.

Thus, there must be some harmonious control by the entire embryo of its equipotential parts—a hypothesis that led Driesch to regard the developing sea urchin embryo as a "harmonious equipotential system." But this was not to be true of some other invertebrate embryos.

CTENOPHORES

The ctenophores are beautiful, medusa-like, marine invertebrates. Their bodies are of glass-like transparency. They move slowly through the ocean water propelled by eight rows of comb plates, which are a series of paddle-like structures that, in fact, serve as paddles.

Several investigators had isolated the blastomeres of ctenophore eggs, among them Driesch and Morgan (1895). They used *Beroe ovata* in which the first three cleavages are vertical, producing an eight-cell stage with the blastomeres almost in a flat plane. Morgan (1897, pp. 129–130) summarized their results:

> When the first two blastomeres are separated from each other by a sharp needle or cut apart by a pair of small scissors, each continues to cleave as a half, *i.e.* as though it were still in contact with its fellow-blastomere. When the organs appear in the larva, only half the full number of rows of swimming-paddles appear. Each row, however, has its full complement of paddles. The invagination of ectoderm to form the "stomach" is very excentric in the half-larva, but forms a *closed* tube running from the mouth-opening to the excentric sense-plate. In several respects, therefore, the larvae were distinctly half-larvae. But in other respects they were more than half-larvae. The endodermal cells of the normal larva arrange themselves into four hollow pouches, and the "stomach" invagination passes in the central line of the four pouches. In the half-larva, on the contrary, the endodermal mass forms more than two pouches (*i.e.* more than half the number in the whole larva). Two distinct pouches are present and in addition, generally, a third smaller pouch is formed . . . .
>
> The isolated one-fourth blastomere [that is, one blastomere from the four-cell stage] segments also as a part of a whole, and develops in some cases into a one-fourth larva, having only *two rows* of paddles (*i.e.* one-fourth the normal number), but with *two* endodermal pouches . . . . The three-fourth embryos [three cells of the four-cell stage] develop six rows of paddles and *four* endodermal pouches . . . .
>
> The results show, however, beyond question, that, even when isolated from its fellow, the one-half blastomere may give rise to a larva that is in many respects only one-half of the normal larva.

The fact that the isolated blastomeres cleaved as though they were still part of a whole embryo, and that the number of rows of comb plates seemed to indicate strictly mosaic development, deeply impressed embryologists. Later experiments indicated (but did not make certain as it turned out; see Reverberi, 1971, pp. 85–103) that each isolated cell from the eight-cell stage would produce a larva with one row of comb plates. That was about as mosaic as one could get.

REGULATIVE AND MOSAIC DEVELOPMENT

Clearly sea urchin and ctenophore embryos were different. It appeared that there were two fundamentally different patterns of development—mosaic and regulative. The first was a pattern of independently developing parts and the latter a pattern of parts that could regulate and form more than they were normally destined to do. The ctenophore embryo was taken as an example of mosaic development and the echinoderm egg as an example of regulative development.

Regulative development was a disturbing notion. What could be the controlling mechanism that restrained the individual cells of the two-cell sea urchin embryo and molded them into parts of a single organism but released those same cells when isolated and allowed each to form an entire organism? It had all seemed so clear and

intellectually satisfying if development were, as Roux suggested, fixed from the onset. It had been equally satisfying, long before, when it was accepted that the embryo was preformed in the ovum (or sperm) and equally disturbing when finally it was shown convincingly that development is epigenetic.

The concepts of preformation and mosaic development avoided the central problem of development—how can novelty arise? The concepts of epigenesis and regulative development must come to grips with that central problem.

Driesch puzzled about the implications of his discoveries, that backward step as he saw it, and he eventually abandoned experimental science and devoted full time to philosophy.

DEVELOPMENT IN AMERICA

Experimental embryology began as a European, and mainly German, science. Newport had been forgotten and the field was dominated by Wilhelm His, Eduard Pflüger, Wilhelm Roux, Hans Driesch, Gustav Born, Oscar and Richard Hertwig, Alexander Kowalewski, Curt Herbst, and Edouard van Beneden. Most of these individuals were associated with universities, and many trained graduate students.

Universities are traditionally the nurseries of science, and in the last quarter of the 19th century a few American universities began to develop programs in biology. Johns Hopkins was the most notable example. It became possible to be trained as a professional embryologist in the United States. Beginning with Charles Otis Whitman, who however had studied with Rudolf Leuckart in Leipzig, a vigorous school of American embryologists developed that by the 1880s was engaged in outstanding research. The group included Edward Beecher Wilson (1856–1939), Thomas Hunt Morgan (1866–1945), Edwin Grant Conklin (1863–1952), and Ross Granville Harrison (1870–1959). All had studied with William Keith Brooks (1848–1908) and Henry Newell Martin (1848–1896) at Johns Hopkins. These four students did much to make embryology and genetics more exact sciences.

The origin of the American school of embryologists was not an example of mosaic development, with Europe and America showing self-differentiation. The fledgling American school had the benefit of the vast literature of descriptive embryology, all European, and its members visited the European laboratories and the marine station at Naples where they met the outstanding European embryologists. Thus the Americans became part of international experimental embryology.

AMPHIOXUS

In the summer of 1892 E. B. Wilson (1893) worked at the Statzione Zoologica, a marine biological laboratory at Naples, and repeated Driesch's experiments, using the cleavage stages of amphioxus (=*Branchiostoma*). His basic technique was the same—that of shaking the blastomeres apart. This is what he found:

> An isolated ½ blastomere [that is, one cell of the two-cell stage] undergoes a cleavage identical with, or approximating to, that of a normal embryo. It produces a normally-formed blastula [in contrast with the sea urchin] and gastrula of half the normal size, and finally may give rise to a half-sized dwarf larva exactly agreeing, except in size, with the normal larva up to the period when the first gill-slit is formed . . . .
>
> An isolated ¼ blastomere may undergo a cleavage nearly or quite identical with that of a normal ovum, but often varies more or less widely from it. It may give rise to a ¼ blastula and ¼ gastrula, differing from the normal only in size. The [larval] stage, with a notochord, is rarely attained and no normally constituted ones were observed . . . .
>
> The ⅛ blastomeres are of two sizes (micromeres and macromeres) which, as far as could be determined, do not differ essentially in mode of development. The isolated blastomere segments in a form approaching that of a complete ovum but *never identical with it.* In rare cases a ⅛ blastula is formed . . . but the gastrula stage is never attained (pp. 587–589).

Wilson then provides an analysis of the Roux-Weismann hypothesis, especially as it applies to postgeneration and to regeneration in general. These two phenomena were very difficult to explain on the basis of that hypothesis. Wilson suggested that the hypothesis was based on two main assumptions—both false.

> The first assumption relates to the causes of histological differentiation. It is assumed [by Roux] that in normal development differentiation is primarily determined by the nature of cell-division, karyokinesis [= mitosis] being conceived as qualitative in character in such wise that cells of different prospective value receive correspondingly different forms of idioplasm [the hereditary material, whatever it might be] at the moment of their separation. Every cell, therefore, has an independent power of self-determination inherent in the structure of its idioplasm, and this in turn owes its character to the nature of the mitosis by which the cell-nucleus arose. The entire ontogeny is, therefore, compared by Roux to a mosaic work; it is essentially a whole arising from a number of independent self-determining parts . . . .
>
> The second of the Roux-Weismann assumptions is logically necessitated by the first in view of the phenomena of regeneration. Obviously these phenomena are inexplicable under a theory of strictly qualitative division. Both Weismann and Roux, therefore, assume that during cell-division each cell may receive, in addition to its specific form of idioplasm, a portion of unmodified idioplasm afforded by purely quantitative division. This unmodified idioplasm . . . remains latent in normal development . . . . Injury to the ovum . . . acts as a stimulus to the latent idioplasm, which thereupon becomes active, and causes a repetition of the original development.
>
> Considered as a purely formal explanation, the Roux-Weismann hypothesis is perfectly logical and complete. Its weakness lies in its highly artificial character; for both of its two fundamental postulates—viz: qualitative nuclear division, and accessory latent idioplasm—are purely imaginary. They are complicated assumptions in regard to phenomena of which we are really quite ignorant, and they lie at present beyond the reach not only of verification, but also of disproof (pp. 605–606).

It is not sufficient to demolish one explanatory hypothesis without providing another so Wilson provided one. He adopted a line of reasoning that he modified over the years and that became, I believe, a paradigm of differentiation that remains central to this day. He proposed to give "a simpler and more natural interpretation of the facts" that was similar to the views already adopted by Oscar Hertwig and Driesch. He assumed that mitosis

> is not qualitative, but purely quantitative; that at every cell-division the daughter cells, whatever their prospective character, receive exactly equal kinds, as well as amounts, of nuclear material . . . [differentiation is] a result of *physiological* changes in the idioplasm, *subsequent to cell-division,* such that certain of the idioblastic units [equivalent to today's genes] remain latent, while others become active and determine the specific form and activities of the cell. Finally, the physiological specialization of the idioplasm is brought about by the interaction of the cell with its fellows in the cell-complex . . . [many experiments have shown that the] embryo develops as a whole, as a unit, and demonstrate the truth of the principle urged by Whitman, Hertwig and others, that "the organism, as a whole, controls the formative processes going on in each part" (pp. 606–607).

Thus the results of Driesch on isolated sea urchin blastomeres and of Wilson on amphioxus found a ready explanation. If all cells receive the same hereditary materials and if embryos act as wholes, a single cell from the two-cell stage should behave as a whole.

But this does not hold true as development progresses. Wilson's experiments

showed that for blastomeres isolated up to the eight-cell stage, "their power of development progressively diminishes as the cleavage advances" (p. 608). This was thought to be a consequence of the following:

> As the ontogeny advances the idioplasm of the cells undergoes gradual and progressive *physiological* modification (brought about by the interaction of the various parts of the embryo), without, however losing any of its elements. The isolation of a blastomere restores it in a measure to the condition of the original ovum and the idioplasm, therefore, tends to return to the condition of the original germ-plasm and thus to cause a repetition of the development from the beginning.

But as development continues the idioplasm becomes progressively modified.

> By the 8-celled stage [in amphioxus] it is incapable of returning to the original state, and the normal type of cleavage is no longer repeated . . . . The specialization of the idioplasm, like that of the cell as a whole, appears to be a cumulative process that results in a more and more fixed mode of action . . . . The independent, self-determining power of the cell, therefore, steadily increases as the cleavage advances. In other words: *the ontogeny assumes more and more of the the character of a mosaic-work as it goes forward. In the earlier stages the morphological value of a cell may be determined by its location. In later stages this is less strictly true and in the end the cell may become more or less completely independent of its location, its substance having become finally and permanently changed* (pp. 606–610).

Wilson pushes the analysis back to the beginning of development by suggesting that we regard

> ontogeny as a connected series of interactions between the blastomeres in which each step conditions that which succeeds. The character of the whole series depends on the first step, and this in turn upon the constitution of the original ovum . . . . The entire series of events is primarily determined by the organization of the undivided ovum that forms its first term, and, as such, conditions every succeeding term (pp. 613–614).

CELL LINEAGE

Few observations speak so forcefully for the importance of the organization of the ovum as those on cell lineage, which were one of the main contributions of the American school in the 1890s and early 1900s. Cell lineage is the description of the history of each embryonic cell. Beginning with the uncleaved egg, the products of every division are traced until the rudiments of the embryonic organs have become distinct.

Suppose that a hypothesis we are testing requires that we know the origin of every cell in an early embryo, that is, the ancestors of each cell back to the uncleaved zygote. Let's take the frog embryo as an example to show what might be done and some of the problems that would arise.

We would begin with the uncleaved egg, as in Figure 17. First cleavage divides the embryo into two identical cells yet, because of the presence of a gray crescent, we could distinguish them. Thus, if we view the embryo from the gray crescent side, we could call one blastomere "right" and the other "left." (Had there been no gray crescent, there would be no way to differentiate the first two cells.) In the case of the frog egg we would know, thanks to Newport and Roux, that if we view the egg from the gray crescent side, the right blastomere will form the right half of the embryo and the left blastomere the left half of the embryo.

The second cleavage divides the embryo into an anterior-right blastomere, posterior-right blastomere, posterior-left blastomere and anterior-left blastomere (Fig. 36). The third cleavage is horizontal and forms an upper-right-anterior blastomere, lower-right-anterior blastomere, upper-right-posterior-blastomere, lower-right-posterior blastomere, etc. And by this eight-cell stage it is clear that a better system of identifying embryonic cells is essential.

Moreover by this time the gray crescent is difficult to recognize and, unless the cells

are vitally stained, we could no longer work out their lineages. Frog embryos are unsuitable for another reason. They are opaque, which makes it impossible to observe cells in the interior.

Only if individual cells can be identified throughout early development is it possible to trace their lineage. Such embryonic cells must differ in some way from one another, either in size, coloration, or position. As embryologists coursed up and down the animal kingdom looking for suitable embryos to study, they found many, especially those of marine invertebrates, with distinctive patterns of coloration and cleavage, and with different sizes of blastomeres. Some embryos were even transparent, allowing one to observe cells of the interior.

Therefore nature was providing naturally stained eggs that could serve the same purpose as Vogt's vitally stained embryos. The patterning of pigmentation of the eggs was found not to be a random affair but part of a basic organization. The planes of cleavage were constant in relation to the pigmented areas, and in many cases the differently colored regions of the egg seemed to have a fixed relation to the germ layers and to the structures that they would form.

This visible organization of certain kinds of eggs at the very beginning of development made it difficult to regard a just-fertilized ovum as an amorphous mass of protoplasm awaiting the directing influences of either idioplasm, determinants, gemmules, nuclei, chromosomes, or whatever. One could not deny organization when it was so striking and constant in what it was and did.

There was, however, an opposing view stated earlier by Pflüger. He held that the uncleaved ovum is *isotropic,* that is, there is no axial organization and all parts of the cytoplasm are equivalent. This hypothesis appealed to many investigators who were impressed by Driesch's experiments on sea urchins and some other experiments in which two eggs were fused and found to produce a single embryo.

Things were confusing! There appeared to be experimental proof for nearly every conflicting hypothesis.

Clepsine

One of the first of the painstaking studies of cell lineage was done by Whitman (1878) on the embryos of *Clepsine complanata* (now *Glossiphonia complanata*), a leech. The first two cleavages of *Clepsine* produce four cells of equal size, which Whitman called *a, b, c,* and *x.* At the next division these divide to give four very small cells and four large ones. The four small cells are the progenitors of the ectoderm. The following cleavages become irregular. The cell derivatives of *x* were able to be followed and were found to give rise to the mesoderm and the nervous system. In fact, Whitman found that entire organ systems could be traced back to their origin in pairs of cells, called teloblasts. One pair gave rise to the mesoderm bands, another pair to ventral nerve cord, another to the trunk nephridia, and so on.

Although Whitman was only doing descriptive embryology, in this case carried out in great detail, he also related his observations to the explanatory hypotheses of the time:

> In the fecundated egg slumbers potentially the future embryo. While we cannot say that the embryo is predelineated, we can say that it is predetermined. The "Histogenetic sundering" of embryonic elements begins with the cleavage, and every step in the process bears a definite and invariable relation to antecedent and subsequent steps . . . . It is, therefore, not surprising to find certain important histological differentiations and fundamental structural relations anticipated in the early phases of cleavage, and foreshadowed even before cleavage begins.
>
> The egg is, in a certain sense, a quarry out of which, without waste, a complicated structure is to be built up; but more than this, in so far as it is the architect of its own destiny (pp. 263–264).

Whitman expressed a point of view that His had proposed in 1874 and Roux held a few years later: the parts of the future

embryo existed as primordia from the very beginning and the course of development is determined, not regulative.

NEREIS

In 1892 E. B. Wilson published a magnificent study of the cell lineage of the marine polychaete worms, *Nereis limbata* and *Nereis megalops.* These he collected in the Eel Pond behind the Marine Biological Laboratory at Wood's Holl (as it was called then; it later became Woods Hole). He referred to the "epoch-making researches" of his friend Whitman, who had become the director of the MBL, noting:

> That an entire system of organs, such as the ventral nerve-cord, or trunk-nephridia could be traced back to a single blastomere was a fact so extraordinary that many morphologists, Balfour among them, at first refused to credit Whitman's statements . . . . Whitman's researches showed that the material for complicated adult organs might be so condensed and accelerated in development as to be set apart by a single stroke, as it were, in the early stages of cleavage, long before the establishment of the gastrula (p. 368).

Wilson also was working as a descriptive embryologist, yet his findings were to be of great importance in analytical embryology. He studied *Nereis* in the hopes of learning more about the homologies of the germ layers.

First cleavage cuts across what will become the future longitudinal axis of the embryo, dividing the egg into a small anterior cell, called *AB,* and a large posterior cell, called *CD* (Fig. 38A). The second cleavage coincides with the median plane of the future body and it produces four large macromeres: *AB* dividing into *A* and *B* and *CD* into *C* and *D* (Fig. 38B, C). Third cleavage (Fig. 38D) is horizontal and unequal. Each large macromere gives off a small micromere. This first quartet of micromeres Wilson designated as a^1, b^1, c^1, and d^1.

Each micromere does not come off directly above a macromere. Instead each comes off in a slightly clockwise direction. This pattern is known as "spiral cleavage."

It might be suspected that this clockwise movement of the micromeres is merely their sliding into the grooves between the almost spherical macromeres but this is not the case. Before the third cleavage began the spindle in each macromere was slanted in a clockwise direction as much as 45°. Spiral cleavage is a reflection of the embryo's basic organization, not a device for convenient packaging of the blastomeres.

The fourth division is also unequal and horizontal. This time the spindles of the macromeres slant in the opposite direction and a second quartet of micromeres comes off in a counterclockwise direction. At the same time the first quartet of micromeres divides.

At the fifth division, the third quartet of micromeres comes off the macromeres in a clockwise direction.

These first three quartets of micromeres form the entire ectoderm.

At the next division, which is no longer synchronous throughout the embryo, the *D* macromere divides into a large cell, still called *D,* and a smaller cell, d^4 (the bottom embryo in Fig. 38; this has been simplified by omitting the divisions of the micromeres). That d^4 was to become famous, because localized in that small cell was the entire material that would form mesodermal structures.

> The formation of the second somatoblast [= d^4] ends the spiral period of development, and it is a very significant fact that the close of this period marks also the complete differentiation, not only of the germ-layers, but also of many of the protoblasts from which the adult organs arise. The segregation of the embryonic material is in fact so nearly completed, that this last spiral stage may be taken as a new point of departure. The embryo now consists of thirty-eight blastomeres (pp. 392–393).

with their fates as summarized in Figure 39.

Wilson used terms ending in "blast" to designate "a blastomere of the segmenting

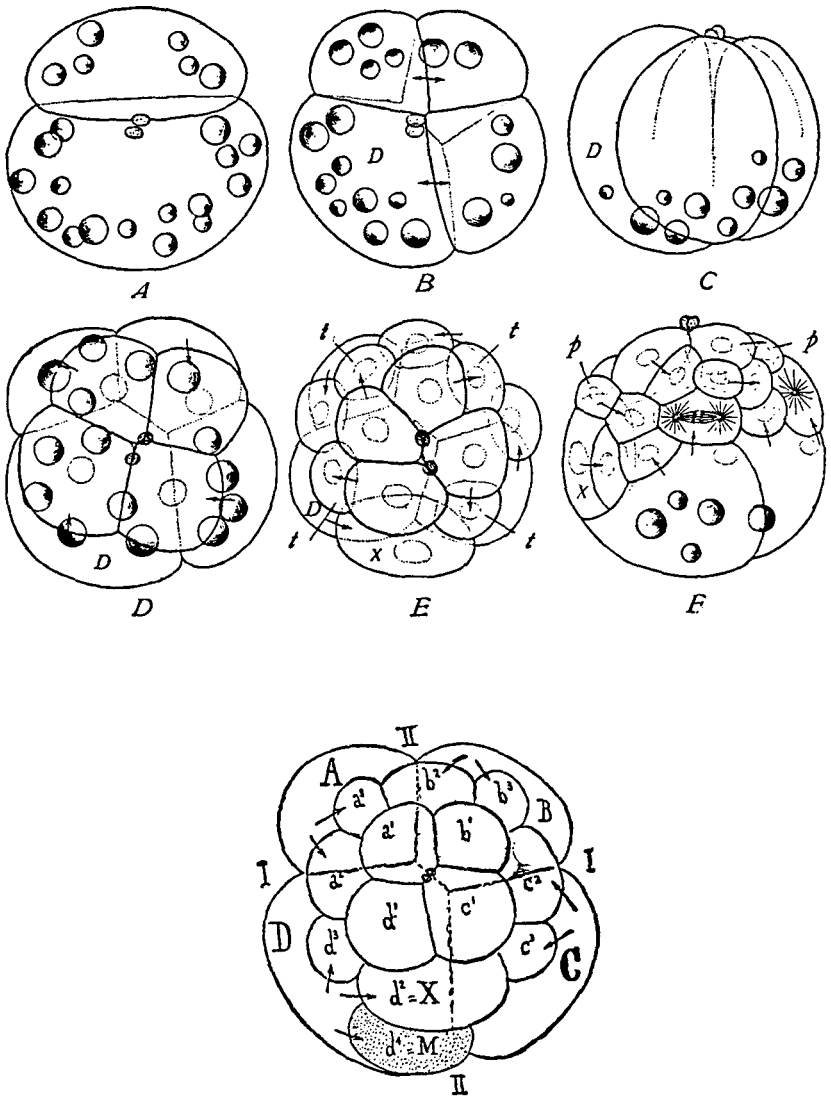


FIG. 38. Early cleavage in *Nereis*. All except C and F are animal pole views; they are side views. A. The two-cell stage; the circles are oil drops. B. Four-cell stage. C. Side view of four-cell stage. D. Eight-cell stage; the first quartet of micromeres has come off clockwise. E. Sixteen-cell stage; the blastomeres marked "t" will form part of the prototroch; "X" will form the nerve cord and some other structures. F. Side view of the 29-cell stage. The lower figure is a simplified drawing showing the micromeres as they come off in quartets but omitting their subsequent divisions; the mesoblast is d^4 or "M." (Upper six figures from Wilson, 1900, p. 369; lower figure from Wilson, 1892, p. 378.)

egg which is the parent-cell of a definite part or organ" (1900, p. 446). For example, the micromeres are "ectoblasts" because they will form the ectoderm.

It is worth examining Wilson's chart (Fig. 40) that shows the complete cell lineage of *Nereis*—not so much for the details but for the amount of work that was required. The eggs are tiny, 0.12 to 0.14 mm in diameter, and the optical equipment available at that time could not match that available today. The time of breeding was most inconvenient—after dusk. The adult males and females swarm at the surface of the water where they can be netted and then placed in separate dishes. Back in the laboratory

4. The macromeres *A*, *B*, *C*, *D*, or entomeres . . . . . = Entoblast.
4. The first group of micromeres, a^1, b^1, c^1, d^1,
8. The products of the trochoblasts, $a^{1.1.1}, a^{1.1.2}, b^{1.1.1}, b^{1.1.2}, c^{1.1.1}, c^{1.1.2}, d^{1.1.1}, d^{1.1.2}$,
4. The four intermediate girdle-cells, $a^{1.2}, b^{1.2}, c^{1.2}, d^{1.2}$,
4. The rosette-cells, $a^{1.3}, b^{1.3}, c^{1.3}, d^{1.3}$,
3. The three smaller secondary micromeres, $a^{2.1}, b^{2.1}, c^{21}$,
3. The stomatoblasts, $a^{2.2}, b^{2.2}, c^{2.2}$,
3. The first somatoblast (X) and its progeny (x^1, x^2),
4. The four tertiary micromeres (a^3, b^3, c^3, d^3),

} Ectomeres = Ectoblast.

1. The second somatoblast or mesomere . . . . . = Mesoblast.

38

FIG. 39. The fates of the blastomeres of *Nereis*. (Wilson, 1892, p. 393.)

when males and females were put together spawning would occur. Observations on the embryos began about 9 P.M. and continued throughout the night.

In order to trace every cell Wilson had to view the cleavage stages from all angles but manipulating the eggs was quite difficult because of their very small size. He solved this problem in an interesting manner. He placed tiny pieces of wax on a microscope slide so that they would support one edge of a cover glass. The cover glass would thus be at a slight angle. The eggs with a drop of ocean water were then drawn up into a pipette and squirted under the cover glass. The eggs arranged themselves in a single layer and they could be turned by gently moving the cover glass.

Wilson made 92 beautiful colored drawings of the embryos and these were lithographed in Frankfurt-am-Main and published in eight plates as part of his *Nereis* paper in the *Journal of Morphology*—at that time the most prominent zoological journal in the United States. This is one of the very great studies in embryology and is well worth examining not only for what is said but for its beautiful illustrations and the evidence of dedicated, careful and difficult work. (I knew Wilson when I was an undergraduate at Columbia University. And after he died most of his papers were discarded but I rescued his original drawings for the *Nereis* paper. Years later I gave them to the library of the American Philosophical Society for their archival collection in American Biology.)

Wilson was able to describe the cell lineage of *Nereis* completely. What was to be concluded?

> The cleavage of the ovum takes place with a precision and regularity which oft-repeated examination only renders more striking and wonderful [even after being up all night!] . . . . The entire ontogeny gives the impression of a strictly ordered and predetermined series of events, in which every cell-division plays a definite *role* and has a fixed relation to all that precedes and follows it (p. 377).

One might gather from these remarks that Wilson believed that development in *Nereis* is strictly of the mosaic type. The complexity and rigidity of the cleavage patterns would seem to indicate that such was the case. Did the *Nereis* embryo consist of an assemblage of determined and self-differentiating cells?

The studies of both Roux and Driesch had appeared shortly before this work of Wilson. He suggested that the difference between the development of "isolated" blastomeres of the frog and isolated blastomeres of the sea urchin was only that the regeneration of the missing half occurred much earlier in the sea urchin. In contrast with many others, he did not take an extreme view and maintain that development must be either mosaic or regulative. He interprets the experiments of Roux and Driesch as proving that:

> In normal development each of the blastomeres is profoundly influenced by the other; that the cell is not an isolated

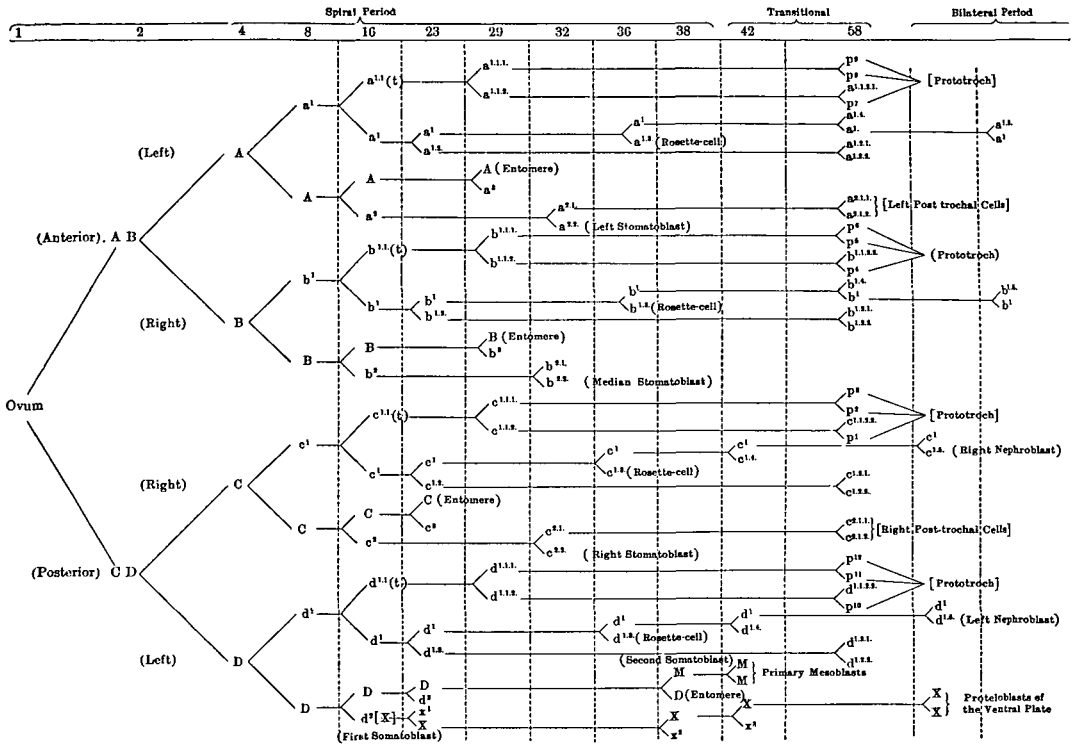


FIG. 40. The cell lineage of *Nereis*. (Wilson, 1892, p. 382.)

mechanism whose mode of action is wholly predetermined in its molecular structure. It proves in fact that the form of cell-division is determined by two factors. The first factor is the inherited tendency of the cell to pursue a definite course, a tendency which we may assume exists by virtue of a corresponding molecular or protoplasmic structure. The second factor is the influence upon the cell of other cells in the colony. When the second factor is removed or modified, the first is correspondingly modified, and a complete readjustment takes place. I can see no logical halting point in the application of this principle (p. 447).

In thinking about Wilson's conclusions remember that when he wrote there was almost no useful knowledge of either inheritance or cell physiology.

Wilson took a strong stand against the Roux-Weismann hypothesis of qualitative nuclear division, having tested it by subjecting unsegmented eggs of *Nereis* to pressure in order to obtain abnormal distributions of the nuclei (1900, pp. 411–412).

> If unsegmented eggs be subjected to pressure . . . they segment in a flat plate, all of the cleavages being vertical. In this way are formed eight-celled plates . . . . If they are now released from pressure, each of the cells divides in a plane approximately horizontal, a smaller granular micromere being formed above, leaving below a large clear macromere . . . . The sixteen-cell stage, therefore, consists of eight deutoplasm-laden macromeres and eight protoplasmic micromeres (instead of four macromeres and twelve micromeres, as in the usual development). These embryos developed into free-swimming trochophores containing eight instead of four macromeres . . . . In this case there can be no doubt whatsoever that four of the

> entoblastic nuclei were normally destined for the first quartet of micromeres, from which arise the apical ganglia and the prototroch. Under the conditions of the experiment, however, they have given rise to the nuclei of cells which differ in no wise from the other entoderm-cells. Even in a highly differentiated type of cleavage, therefore, the nuclei of the segmenting egg are not specifically different, as the Roux-Weismann hypothesis demands, but contain the same materials even in the cells that undergo the most diverse subsequent fate.

SPIRAL CLEAVAGE AND HOMOLOGY

Most embryologists in the 1890s, even the experimentalists, were still influenced by the Haeckelian paradigm and Wilson sought to relate his study of *Nereis* to studies on other embryos with spiral cleavage. At the very same time that he was working at Woods Hole another Hopkins graduate, E. G. Conklin was there studying cell lineage in a mollusk, the limpet *Crepidula*. The two of them made the astonishing discovery that the details of early cleavage in the annelid worm *Nereis* and the mollusk *Crepidula* were nearly identical. In both the three quartets of micromeres came off in the typical pattern of spiral cleavage: the first quartet clockwise, the second counterclockwise, and the third clockwise. But the truly startling discovery was that both formed a d^4 cell from which all mesodermal structures are derived in later development.

It is hard not to conclude that annelids and mollusks, phyla that differ so widely in the structure of their adults, retain some "ancestral reminiscences" (as Wilson, 1898, later called them) in the details of their early development. The concept of homology seems to apply.

Wilson pointed out (1892, pp. 439–443) that not only do some annelids and mollusks have the same pattern of spiral cleavage but so does a platyhelminth, the polyclad *Discocoelis*.

> Up to a late stage in the spiral period (twenty-eight cells) every individual blastomere and every cell-division is represented by a corresponding blastomere and a corresponding cell-division in the embryo of the polyclade, and in that of the gastropod [*Crepidula*].

So, if identity of patterns of cleavage can be taken as a criterion of homology, this would again appear to be a clear case.

But if one asks another question—is the origin of the mesoderm the same—the answer is not obvious. Figure 41 shows the cleavage pattern of annelids, mollusks, and polyclads. First note the great similarity of D, a mollusk, and E, an annelid. The three quartets of micromeres have been given off and the four macromeres are shown at the bottom. The important thing to notice is the origin of the mesoderm—shown as the shaded cell, M or d^4. The polyclad is shown in C and it is obvious that the pattern of cleavage is similar to that of annelid (E) and mollusk (D). In the polyclad, however, the origin of mesoderm, shown as shaded cells, is different.

> In the polyclade the first group of micromeres gives rise to the entire ectoblast, the second and third groups to the mesoblast, the macromeres to the entoblast. In the mollusk and annelid, on the other hand, the second and third groups of micromeres give rise to the ectoblast, like the first set, and the mesoblast arises subsequently. This remarkable divergence between the polyclade on one hand and the mollusk and annelid on the other is a fact of capital importance, for it proves that cells having precisely the same origin in the cleavage, occupying the same position in the embryo, and placed under the same mechanical conditions, may nevertheless differ fundamentally in morphological significance. We cannot escape the conclusion that the cell possesses a definite hereditary tendency upon which primarily its nature depends, however much its outward form or mode of division may be affected by the mechanical conditions of its environment in the body; and full weight must be given to this heredity in every attempt to interpret the origin and meaning of cleavage-forms (p. 441).

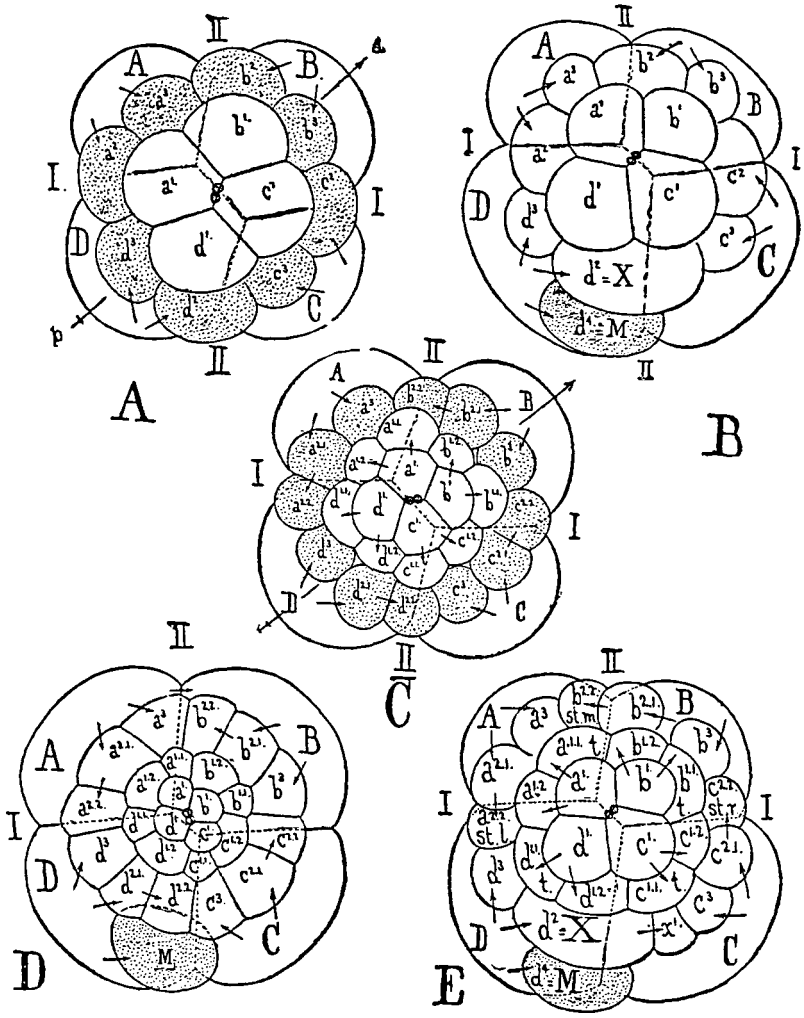


FIG. 41. Cleavage patterns in a polyclad (A, C), a mollusk (D), and annelid (B, E). Animal pole views. The cells that will form the mesoderm are shaded. In mollusks and annelids (D and E) the mesoderm is derived from a single cell, d^4, or M. In the polyclad (A and C) it is formed from the second and third quartets of micromeres. (Wilson, 1892, p. 440.)

What can one conclude, then, about the homologies of cleavage patterns and of the mesoderms? The answers depend, of course, on how homology is defined. If we define homology as the inheritance of the putative homologous structures from a common ancestor we will probably never know the answer—the likelihood of our being able to work out the early embryology of the presumed Precambrian ancestors is not promising. If we define homology as being strict identity of embryonic origin, we have to say that the mesoderm in polyclads is not homologous with the mesoderm in annelids and mollusks. That answer is not acceptable to many morphologists since there are other, and important, reasons for assuming that all mesoderms are homologous.

Wilson, always one to think deeply about the implications of his research and that of others, devoted one of the famous "Wood's Holl Lectures" (1895) to the embryological criterion of homology.

> The puzzling facts reviewed . . . leave no escape from the conclusion that embryological development does not itself afford at present any absolute criterion whatever for the determination of homology. Homology is not established through precise equivalence of origin nor is it excluded by total divergence . . . . But it does not by any means follow that the embryological method must be abandoned as a means of investigating homologies. The most skeptical critic of the recapitulation theory cannot deny that the embryological evidence is often of the clearest and most convincing character.

What, then, should the basic criterion be?

> Obviously it is the standard of Owen, viz., the structure and structural relations of the developed organs; it is the standard of comparative anatomy . . . . We must primarily take anatomy as the key to embryology, and not the reverse. Comparative anatomy, not comparative embryology, is the primary standard for the study of homologies, and hence of genealogical descent (pp. 113–114).

But can more be said about those embryological similarities that appear to indicate homologies? Wilson emphasizes that developmental stages do not remain unchanged in evolution but are capable of being modified much more than is generally thought. The fact that some aspects of development seem to be ancient is not surprising because:

> They point to the conclusion that the events of ontogeny are essentially adaptive, and that the persistence of ancestral reminiscences in development or of similarities in the development of homologous parts is in some way connected with the persistence of ancestral conditions of development (p. 121).

What should we conclude? It may be best not to make any strict conclusions about homology and merely note that, as in the examples given by Wilson, some members of the great phyla Mollusca, Annelida, and Platyhelminthes have a common pattern of spiral cleavage that apparently has an inherited basis. The most economical hypothesis is that it does reflect a common ancestry. In the case of the origin of the mesoderm, we can accept that in both annelids and mollusks it has a common and highly unusual embryological origin. Again, the most economical hypothesis is that it is a pattern inherited in common. The problem with the polyclads is more difficult. For many reasons it is useful to think of the mesoderm as homologous in the three phyla but that a genetic change has slightly altered the precise point of its origin in the polyclads—or alternatively that the polyclads represent the primitive condition and that it was the common ancestor of annelids and mollusks that underwent the genetic change.

In any event these difficult puzzles do have a possible answer as reflections of form and function in ancestors that lived at times so remote as to be nearly beyond human comprehension. Yet we do have a conceptual scheme that allows us to relate a variety of natural phenomena—a scheme that can be modified on the basis of new information and new hypotheses. Otherwise the commonality of spiral cleavages and d^4 cells is really not very interesting at all.

Styela

One of the more remarkable cases of the visible organization of the uncleaved ovum and the early cleavage stages was provided by Conklin (1905). He had gone to the Marine Biological Laboratory at Woods Hole intending to study maturation of the egg and fertilization in the ascidian *Ciona intestinalis*. The adults proved difficult to obtain early in the season so he switched to two other ascidians, *Molgula manhattanensis* and *Cynthia partita* (now *Styela partita*), but quickly settled for the latter:

> The very first lot of the living eggs of *Cynthia* which I examined showed a most remarkable phenomenon and one which modified the whole course and purpose of my work; for there on many of the unsegmented eggs, which were of a slaty-gray color, was a brilliant orange-yellow spot, which in other eggs appeared in the form of a crescent or band. Further

> observation showed that this crescent became divided into two equal parts at the first cleavage and that it could be followed through the later cleavages and even into the tadpole stage. I therefore, for a considerable portion of the summer, devoted myself to the study of the living eggs of *Cynthia*.

And no wonder. Conklin had struck embryological gold and he was the careful and capable person worthy to develop the strike. He followed the changes from ovarian egg to fully formed larva. Only the early events will be described now (Fig. 42).

The mature oocyte has a large transparent germinal vesicle. The interior of the oocyte consists of a mass of gray yolk and the periphery contains a yellow pigment. When the germinal vesicle ruptures at the onset of meiosis, it liberates a quantity of clear material. At fertilization the sperm enters near the vegetal pole and this starts a dramatic rearrangement of the cytoplasm. The yellow cytoplasm (which appears black in the photographs of Fig. 42 of Conklin's beautiful colored plates) and the clear cytoplasm (from the germinal vesicle) flow toward the point of sperm entry where the yellow cytoplasm forms a peripheral cap and the clear cytoplasm is in the interior. This movement leaves the gray yolky material in the animal hemisphere where it surrounds the maturation spindle in the area where the polar bodies will form.

The yellow cytoplasm next moves to form an equatorial crescent extending about 180 degrees around the posterior part of the ovum. The clear cytoplasm moves toward the center of the ovum.

The first cleavage furrow bisects the yellow crescent. The clear cytoplasm and the yolky cytoplasm switch positions—so that the clear cytoplasm occupies the animal hemisphere and the yolky cytoplasm the vegetal hemisphere.

Conklin discovered that at the close of first cleavage these distinctively colored regions of the embryo have a precise relationship with the structures that would form subsequently. The fate of the yellow crescent is to form muscles and mesenchyme, the fate of the gray yolky cytoplasm is to form endoderm, and the clear cytoplasm of the animal hemisphere will form ectodermal structures. Conklin could even distinguish the area that would form the neural plate and the notochord (Fig. 43).

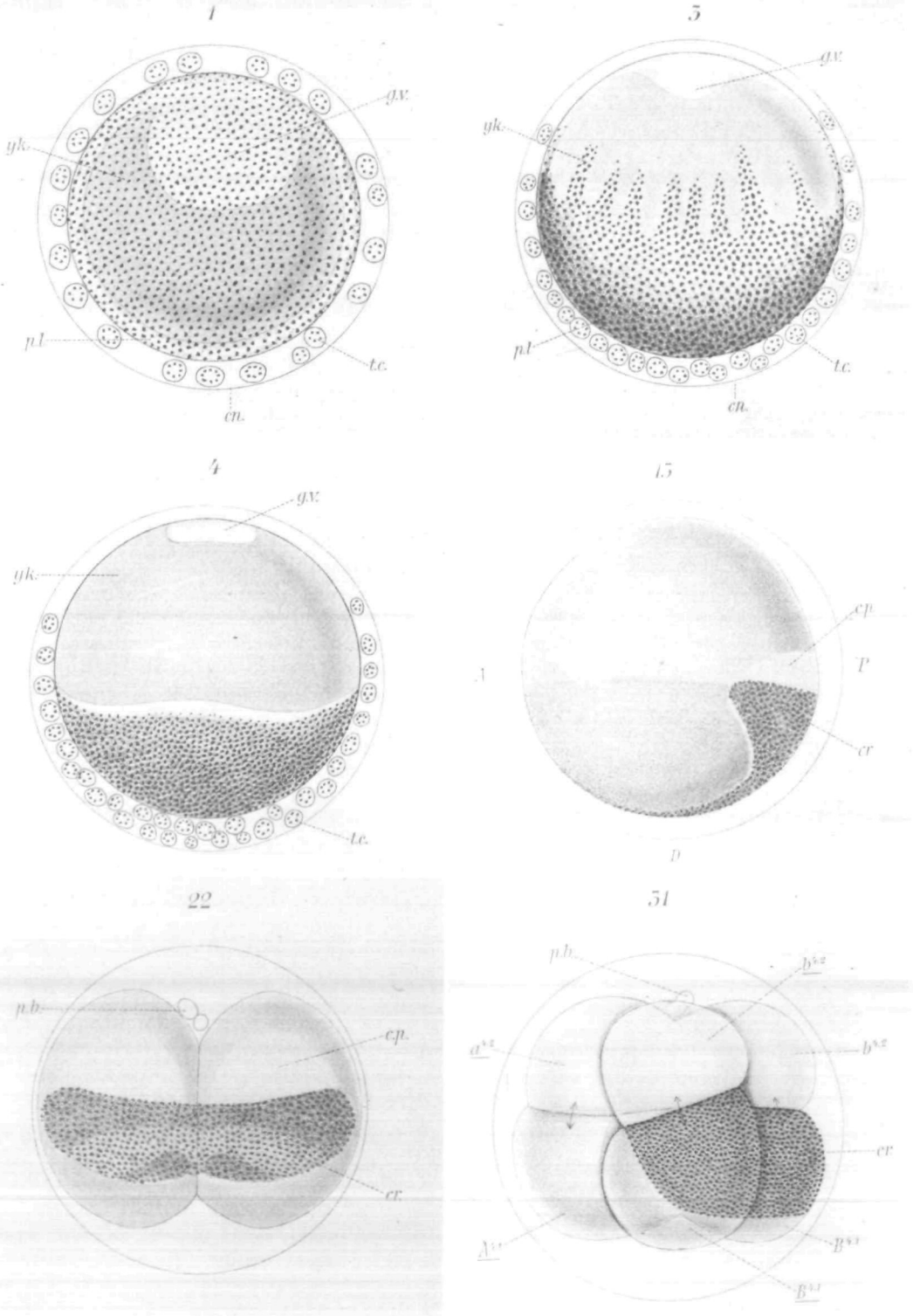
The striking aspect of these observations on the embryo of *Styela* is not that the positions of the structures-to-be are already fixed at the very beginning of development. The same is probably true for the frog's egg. *Styela* is notable because it has pigments that correspond to the boundaries of the germ layers that will form and this allows the embryologist to trace these areas in the course of development.

These studies of Whitman, Wilson, Conklin, and many others on cell lineage demonstrated that the mature ovum is a complex and highly organized structure. It is far from the isotropic cell hypothesized by Pflüger. Whitman had believed that although "we cannot say that the embryo is predelineated we can say that it is predetermined."

All one can really conclude from these studies, however, is that in the course of normal development identifiable regions of the very early embryo develop into specific structures of the older embryo. One cannot say that those regions can only form those structures of the older embryo. Neither can we say that the structures of the older embryo can be formed only by those delineated parts of the early embryo.

A careful distinction must be made between *fate* (prospective significance) and *capacity* (prospective potency). Fate means what an area of a younger embryo will form in a later embryo. Capacity means what the cells of that area of the younger embryo are able to do under a variety of experimental conditions.

The fate and capacity of an area of an early embryo may be the same. Such a situation would be where the region is irreversibly determined, that is, it self-differentiates into a specific later structure. Alternatively, that region of the early embryo might, under different conditions, have the capacity to produce much more than its normal fate would suggest, that is, it would have the capacity to regulate.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Thus the distinction between mosaic development and regulative development, which has been applied to the whole embryo, must also be applied to the different regions of the embryo. Problems of this sort, and especially the determination of prospective potency, could be solved only by experimentation.

Embryologists undertook to isolate blastomeres and conduct other sorts of experiments intended to discover the interrelations of embryos and their parts. For example, what is the significance of the colored areas of the egg of Conklin's *Styela*? Does the yellow crescent represent the actual material necessary for the formation of mesoderm? Or is this colored area an indicator of the presence of other substances—the real organ-forming substances? Is the d^4 cell of *Nereis* and *Crepidula* the exclusive source of material required for the mesoderm to develop?

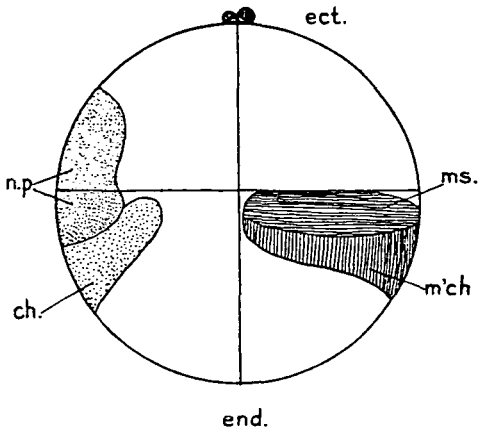


FIG. 43. Conklin's fate map for the ascidian embryo at the end of first cleavage. After the figure was drawn he realized that the presumptive chorda and the presumptive mesenchyme meet—hence, forming a complete equatorial band that separates the presumptive ectoderm above and the presumptive endoderm below. With this correction there is a striking resemblance of the fate maps of ascidian and amphibian as shown in Figure 25. (Conklin, 1905, p. 108.)

DENTALIUM AND *PATELLA*

The repertoire of techniques available to experimental embryologists at the turn of the century was limited and very crude. One could push hot needles into blastomeres or shake them apart. It was found that when cleavage stages of some marine invertebrates were placed in sea water without calcium ions the blastomeres separated, which meant that the isolation of blastomeres was made much easier. Simple hand centrifuges enabled one to stratify the more fluid parts of uncleaved eggs. It was discovered that some embryos could be cut with a scalpel.

Since there were not many experimental techniques available, embryologists adopted a strategy common in biology—search for organisms that differ from those that have already been studied in the hope of finding a new pattern of development—an experiment that nature had done—that might provide new information and new insights.

One interesting variant that nature provides is the presence of polar lobes in the early cleavage stages. Polar lobes are formed in many invertebrate embryos. They are non-nuclear structures that push out from blastomeres and then flow back into them. They are outgrowths of the vegetal hemisphere that appear to be a mechanism for redistributing cytoplasmic materials in the early cleavage stages.

Polar lobes are found in the embryos of the mollusk, *Dentalium*. Figure 44, from Wilson's classic study (1904*a*), shows the

←

FIG. 42. The organization of the ascidian egg. These black and white photographs were made from Conklin's natural color illustrations. The pale yellow pigment of the living egg appears here as black. 1 is an unfertilized egg with the germinal vesicle (g.v.) beginning to break down; test cells (t.c.) are beneath the chorion (cn); the area shaded is the gray yolk (yk) and the egg is surrounded by a layer of clear protoplasm (p.l.). 3 shows an egg 5 minutes after fertilization; the yellow pigment and the clear protoplasm are collecting in the vegetal hemisphere. 4 shows the yellow pigment and the clear protoplasm entirely in the vegetal hemisphere. In 13 the yellow pigment and the clear protoplasm have formed crescents in the posterior part of the egg. First cleavage is underway in 22 and the crescents are bisected. 31 shows the eight-cell stage with the yellow crescent material restricted to the two posterior vegetal hemisphere blastomeres. (From Conklin, 1905.)

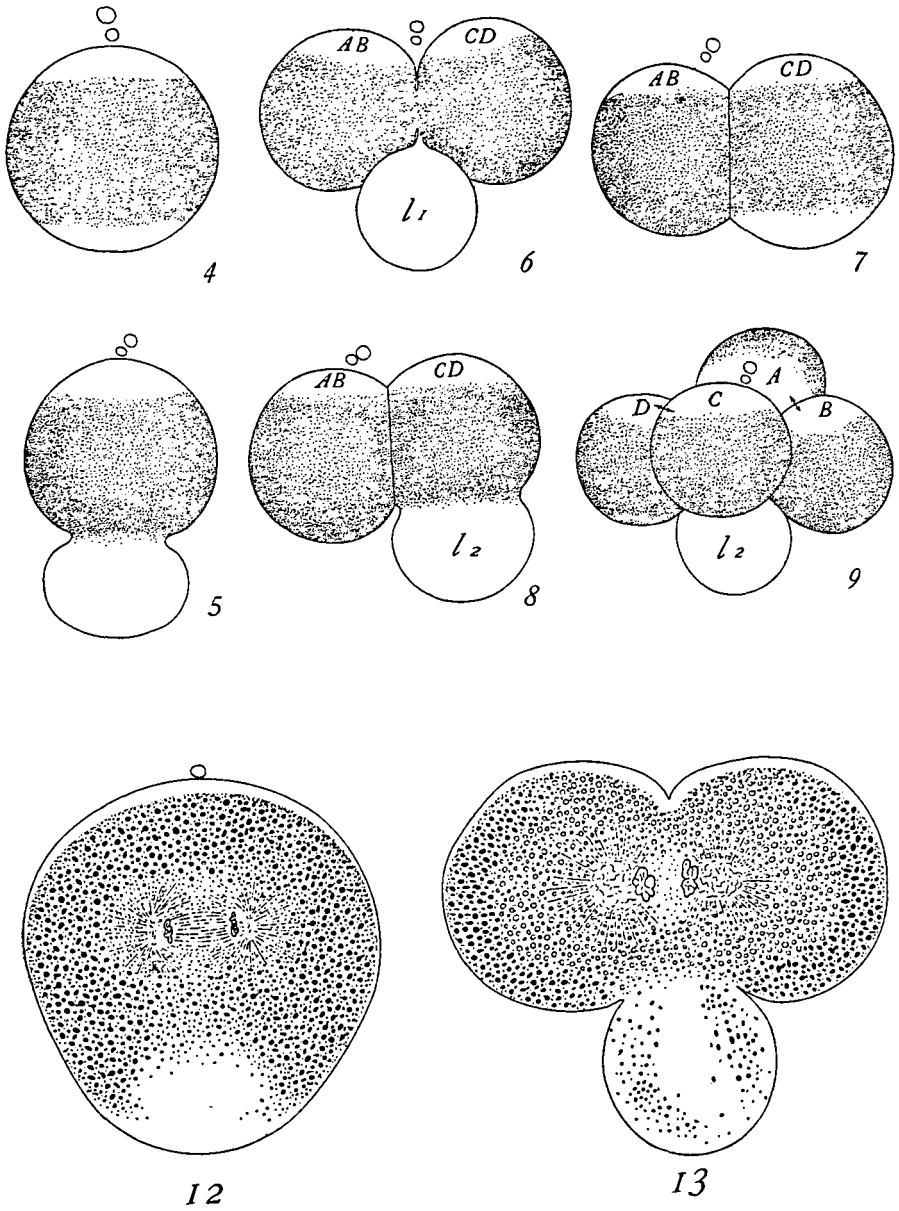


FIG. 44. Polar lobe formation in *Dentalium*. (Wilson, 1904*a*, pp. 6, 9.)

events up to the four-cell stage. When the eggs are shed from the ovary, they are divided into three zones: a clear cytoplasm at the animal pole, a central portion reddish in color, and another clear area at the vegetal pole. An embryo one hour after fertilization is shown in Figure 44, 4. The central pigmented area and the two clear areas are evident as are the polar bodies at the top.

Before first cleavage the first polar lobe forms at the vegetal pole, as shown in Figure 44, 5. It contains essentially all of the clear cytoplasm of the vegetal hemisphere. In embryo 6 cleavage is underway and the plane is such that the first polar lobe is

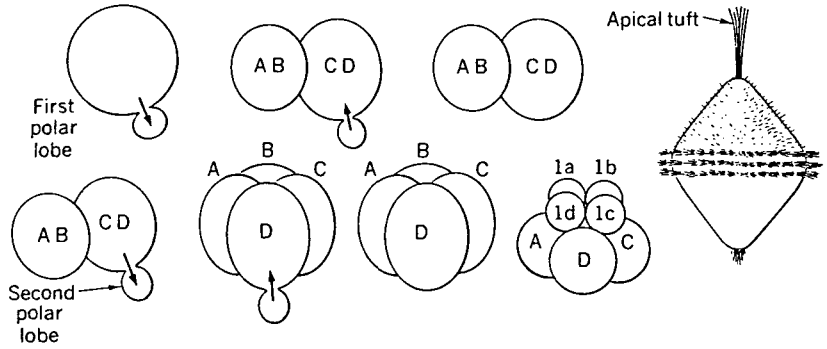


FIG. 45. *Dentalium.* Early cleavage stages and the trochophore larva.

attached to only one blastomere, called the *CD* blastomere. The first polar lobe is then withdrawn into *CD* and the completed two-cell stage is shown as embryo 7. Blastomere *AB* has clear cytoplasm only in the animal hemisphere but *CD* has it not only there but also in the vegetal hemisphere—the contents of the first polar lobe. As a result, *CD* is larger than *AB*.

A second polar lobe forms from the vegetal hemisphere of *CD*, as shown in embryo 8. Second cleavage occurs as in 9, and at its completion the contents of the second polar lobe are incorporated in the *D* blastomere.

Two drawings of fixed and sectioned embryos before first cleavage have been completed and are shown at the bottom of Figure 44. Embryo 12 has a thin cap of clear cytoplasm at the periphery of the animal hemisphere and the clear cytoplasm of the vegetal hemisphere is starting to form the first polar lobe. Embryo 13 shows the first polar lobe fully formed. The mitotic spindle is shown in both sectioned embryos—in the center of the egg, far removed from the polar lobe.

At third cleavage the *D* blastomere forms the third polar lobe, which then flows right back into *D*. Before this cleavage starts the clear cytoplasm near the animal pole moves clockwise and when the cells divide it becomes incorporated into the first quartet of micromeres.

These events are diagrammed in Figure 45.

In Dentalium the freshly discharged egg, prior to maturation or fertilization, shows a definite segregation of visibly different materials which accurately foreshadows a corresponding distribution of these materials among the blastomeres during cleavage (Wilson, 1904*a*, p. 17).

Wilson underscored that sentence since it was describing, once again, the remarkable organization of the mature ovum.

EXCISING POLAR LOBES

A trochophore larva is formed in a day. It is top-shaped with an apical tuft of long, stiff cilia and an equatorial band—the prototroch—of three rows of motile cilia, a ciliated pretrochal region and a non-ciliated post-trochal region (Fig. 46, embryo 29).

Wilson sought to learn the significance of the polar lobes by cutting them off from the blastomeres with a scalpel and observing subsequent development. When he cut off the first polar lobe the second polar lobe failed to form. Otherwise the cleavages were normal. After 24 hours, however, a larva was formed that was a disaster (Fig. 46, embryo 32). It had three rows of prototrochal cilia that were larger than normal. The pre-trochal region is present—it can be identified by its covering with short cilia. The apical tuft is absent and so is the entire post-trochal region—the embryo ends at the prototroch. Embryo 29 is an unoperated control of the same age shown for comparison.

Embryo 36 (Fig. 46) had its second polar lobe removed. It also formed a larva with an exaggerated prototroch and no post-trochal region. It does, however, possess a

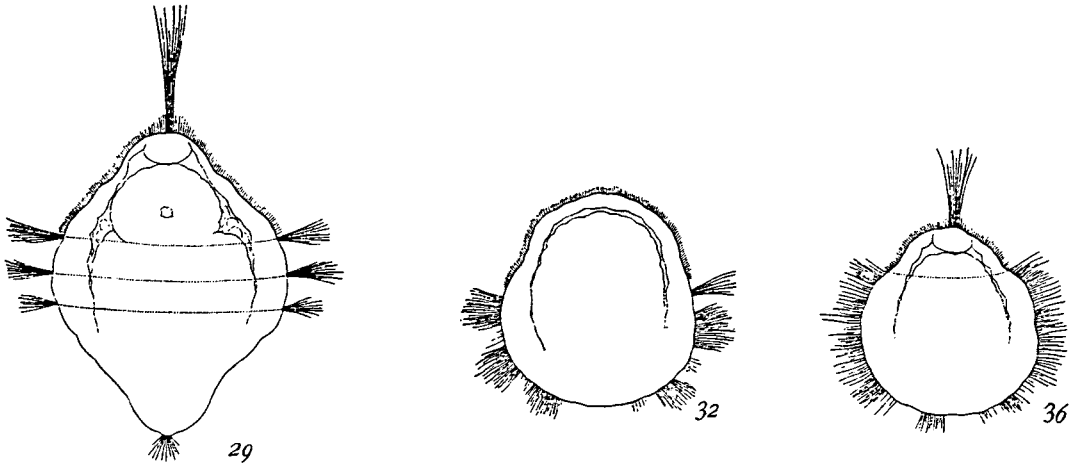


FIG. 46. Polar lobe elimination experiments. 29 is a normal trochophore larva. 32 is a larva that had its first polar lobe removed. 36 is a larva that had its second polar lobe removed. (Wilson, 1904*a*, p. 24.)

normal apical tuft. What sorts of hypotheses could your students propose for the determinants of the apical tuft and post-trochal region on the basis of the information so far?

Wilson was much impressed with the importance of the polar lobes especially since

> the amount of material removed with the polar lobe . . . is wholly disproportionate to the effect produced. The polar lobe includes less than one-fifth the volume of the egg; yet its removal does not merely cause a structural effect of like extent, but inhibits the whole process of growth and differentiation in the post-trochal region (pp. 56–57).

Figure 47 shows the results of Wilson's experiments in isolating blastomeres. His caption gives the details. When the blastomeres were cut apart at the two-cell stage the results were strikingly different. Embryos 45 and 46 are isolates from the same two-celled embryo. Embryo 45 developed from the *CD* blastomere and has an apical tuft, a ciliated pre-trochal region, a prototroch of normal size, and the non-ciliated post-trochal region. Embryo 46 developed from the *AB* blastomere and is the same as embryos from which the first polar lobe is removed (Fig. 46, embryo 32).

Wilson then isolated blastomeres at the four-cell stage. Embryos 47 and 48 are both from a separated *CD* blastomere. Embryo 47, from the isolated *D* blastomere, is fairly normal, having both an apical tuft and a post-trochal region. Embryo 48, from the isolated *C* blastomere, is about the same as the isolated *AB* blastomere (embryo 46) or as an embryo from which the first polar lobe had been removed (Fig. 46, embryo 32).

Finally when he isolated the micromeres after the third cleavage, an important new bit of information was obtained. Embryo 49 (Fig. 47) developed from the 1*d* cell and embryo 50 from 1*c* of the same embryo. Only the 1*d* cell produces an embryo with an apical tuft.

With this additional information your students should be able to use these data and specify where the substances required for the apical tuft and the post-trochal region are localized. Wilson's experiments are splendid for asking questions of this sort: if removal of the first polar lobe results in a larva without the apical tuft or the post-trochal region, what would you predict would be the development of the isolated *CD* and *AB* blastomeres of a normal two-cell embryo (*i.e.*, one from which the polar lobe has *not* been removed!)?

Putting all these data together, Wilson

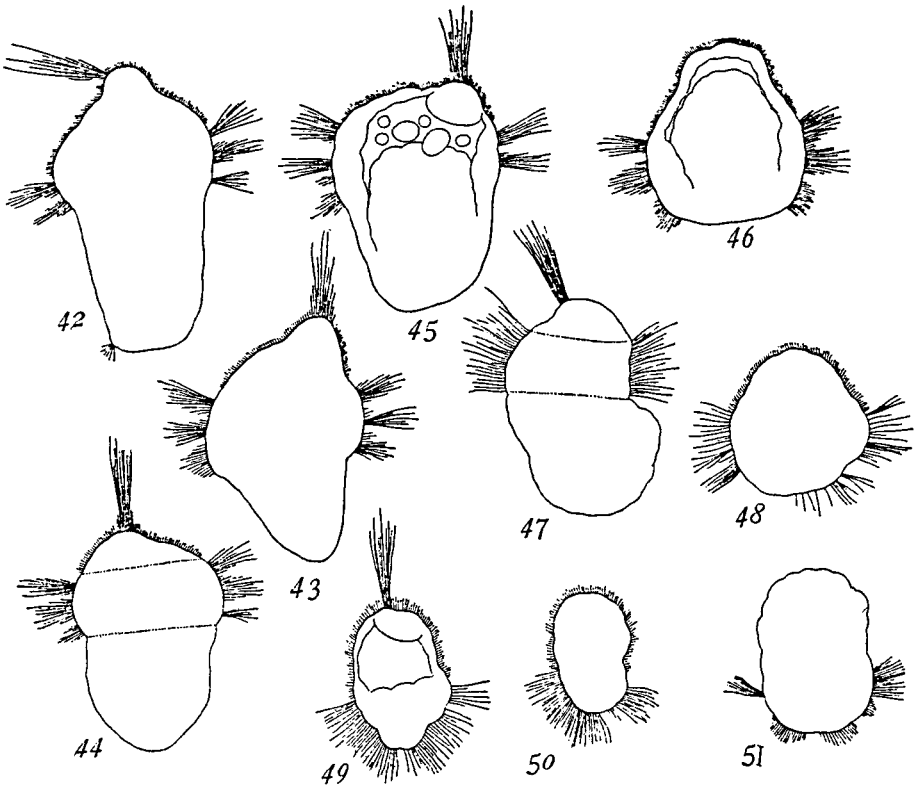


FIG. VII.

Larvae from isolated Blastomeres.

42, 43, 44, Various forms of larvae from isolated CD halves, 24 hours; 45, 46, twin larvae from the isolated CD and AB halves of the same egg, 24 hours; 47, larva from isolated D-quadrant, 24 hours; 48, larva from isolated C-quadrant of the same egg, 24 hours; 49, larva from isolated posterior micromere, 1d, of 8-cell stage, 24 hours; 50, larva from isolated micromere, 1c, of the same egg, 24 hours; 51, one-fourth larva from one of the small quadrants (A, B or C), 72 hours.

FIG. 47. Isolation of blastomeres. (Wilson, 1904*a*, Fig. VII.)

concluded that the substances in the egg that are necessary for the post-trochal region to develop are originally in the clear cytoplasm of the vegetal hemisphere of the uncleaved egg. They are then successively located in the first polar lobe, the *CD* blastomere, the second polar lobe, and finally in the *D* blastomere.

Similarly, the materials necessary for the apical tuft are first in the vegetal hemisphere, then successively in the first polar lobe, *CD* blastomere, *D* blastomere, and then the 1*d* micromere.

CYTOPLASMIC DETERMINATION

As noted before, the polar lobes do not contain a nucleus so the substances, or determinants, responsible for the apical tuft and post-trochal region must be cytoplasmic and they must be present in the egg before fertilization. Does this conclusion mean that the determinants are unrelated to genes? Almost certainly not. The hypothesis that will be developed later is that genes control the synthesis of the determinants while the ovum matures in

the ovary. Essentially this conclusion was reached by Wilson more than three-quarters of a century ago.

> My observations demonstrate conclusively, I think, both the mosaic character of cleavage in these eggs, and the definite prelocalization of some of the most important morphogenic factors in the unsegmented egg. The *Dentalium* egg shows, even before it breaks loose from its attachment in the ovary, and long before even the initial changes of maturation, a visible definite topographical grouping of the cytoplasmic materials. This is proved by the experiments to stand in definite causal relation to the subsequent differentiation of the embryo in such wise that the removal of a particular cytoplasmic area [he had also cut off parts of eggs] of the unsegmented egg results in definite defects in the resulting embryo that are not restored by regenerative or other regulative processes within the time-limits of the experiment [*i.e.*, there was none of Roux's post-generation] (p. 55).
>
> The conclusion is therefore unavoidable that the specification of the blastomeres in these eggs is due to their reception, not of a particular kind of chromatin, but of a particular kind of cytoplasm; and that the unsegmented egg contains such different kinds of cytoplasm in a definite topographical arrangement (p. 56).

But his final conclusion is that all is ultimately under nuclear control:

> It therefore appears possible, not to say probable, that every cytoplasmic differentiation, whether manifested earlier or later, has been determined by a process in which the nucleus is directly concerned, and that the regional specifications of the egg-substance are all essentially of secondary origin (p. 64).

Wilson's experiments on *Dentalium* were done at the Naples Zoological Station between February and August of 1903, a period in his life when his long-held view of the importance of the nucleus, and specifically of the chromosomes, in inheritance—including development, of course—was prominent in his mind. His close friend Th. Boveri had recently published his experiments on dispermic sea urchin embryos, which showed that normal development depends on a balanced set of chromosomes (III, pp. 662–663). But more importantly, his student W. S. Sutton had just published his remarkable papers linking chromosomes and Mendelian inheritance (III, pp. 653–662). In a few years Wilson was to essentially abandon embryological work and devote his full energies to establishing the cytological basis of genetics (III, pp. 673–677). At the same time, Thomas Hunt Morgan, his colleague at Columbia University, would soon be making genetics an exact science (III, pp. 678–720).

Mosaic Development, Regulative Development—Neither or Both?

If we ask "What were the Big Questions?" that concerned experimental embryologists during the last decades of the 19th century and the first one of the 20th, we will find that few were new. The dominant question was whether early development could be best described as mosaic or regulative. That was no more than an extension of the age-old debate over preformation *vs.* epigenesis. Studies of the organization of the mature ovum, the pattern of early cleavages, cell lineage, and the isolation of blastomeres were all designed to ascertain the degree to which the parts of the ovum were irreversibly determined, that is, irrevocably committed to a specific developmental pattern, or regulative, that is, with the capacity to do more than their normal fate would indicate.

These questions attempted to dissect the fundamental phenomena of development in order to understand differentiation better. They could not be answered by watching normal embryos develop. In a normal embryo the fates of the parts of an embryo and what the parts actually do are identical. The fate (prospective significance) of a part and the capacity (prospective potency) of that part, however, may differ widely. Capacity must be determined by subjecting the part to various abnormal situations.

Thus one cannot ask "What is the capacity of a single blastomere from the two-cell stage of an *Echinus* embryo?" and obtain an answer by watching development. All one could determine would be that half of an embryo produces half of a larva. However, if we isolate that blastomere, we learn that it has the capacity to do all that an entire embryo can do.

Tentative answers to these questions were obtained for the major groups of animals by the turn of the century. It was possible to describe embryos as being mosaic, regulative, or some mixture of these basically different patterns. These characterizations related to the early cleavage stages only since it was generally understood that, eventually, all embryos reached a mosaic stage where the parts would self-differentiate.

Wilson (1904*b*), in a companion paper to the one on *Dentalium* just considered, found that another mollusk, *Patella,* was also strictly mosaic. Together with these mollusks, the ctenophores, polyclads, and annelids were thought to be strongly mosaic. The amphibians and echinoderms were thought to be intermediate and amphioxus was regarded as the most regulative in the early cleavage stages.

As mentioned before, sometimes nature performed experiments for the experimental embryologists. For example, it seemed highly probable that *Homo sapiens* is a regulative species when it was realized that identical twins or identical triplets are derived from a single fertilized egg. That indicates that we are regulative at least up to the end of second cleavage.

Teratology provided other data. The frog embryo shown in Figure 48 had some developmental accident and ended up with two normal-sized heads. When this embryo was sectioned each head was found to have a normal brain, eyes, otic capsules, olfactory organs, and other head structures. Had this embryo been normal all of its cells would have produced some specific part. In the double headed embryo, however, some of those cells were channelled in a different direction—indicating that their capacity was greater than their expected fate.

FIG. 48. A two-headed frog embryo. Spontaneously produced.

Before trying to make sense of this diversity there are two critical bits of information that, although of the greatest importance, are not usually emphasized. The first is that even the most regulative embryos tend to become mosaic at some stage in their development, as noted before. The second is that, in the regulative species even though one blastomere of the two- or even the four-cell stage can produce a normal larva, one is not justified in concluding that *any* half an egg can produce a whole embryo. Here nature might be misleading the egg shakers. In all eggs that had been studied it was realized that the unfertilized egg is organized to some degree. There was often a difference in pigmentation of animal and vegetal poles, frequently there was a gradient in the quantity of yolk granules in cells and, wherever it was possible to test, the polar bodies formed in a specific area of the ovum. (There is a third important fact that will be developed later, namely, that in even the most strictly mosaic species, their mosaicism is a transitory state—the annelid worms, for example, have remarkable powers of regeneration when they are adults.)

Thus it seemed beyond question that there are organized differences along the animal pole–vegetal pole axis. If the cleavage planes were at random with respect to the A–V axis, valid conclusions about the capacity of half-eggs, produced by a cleavage at any angle, could be drawn from the isolation of blastomeres experiments. But that is not what happens. The plane of the

first cleavage is parallel to this A–V axis so the blastomeres are not receiving a random half of the contents when the egg divides.

All one can conclude from the development of isolated blastomeres of the two-cell stage is that a half-embryo cut by cleavage along the A–V axis will develop in a certain way. We cannot conclude that any half-embryo—such as one derived from an egg that cleaved horizontally to give an animal hemisphere cell and a vegetal hemisphere cell—will develop the same way. That notion occurred to those shaking the eggs apart. Driesch wondered if the *Echinus* egg had cleaved horizontally instead of vertically, would he have obtained the same result? He suspected the answer would be "no" and there were some data suggesting that answer. It was to remain for Hörstadius, a half century later, to provide the answer in some most elegant experiments.

In spite of all the variations among the embryos and vituperations among the scientists (some held firmly to the hypothesis that regulative development was the rule; others held firmly to the hypothesis of mosaic development), it did seem possible to provide a conceptual scheme to cover all embryos. By 1900 Wilson had developed such a scheme:

> The cytoplasm of the ovum possesses a definite primordial organization which exists from the beginning of its existence even though invisible, and is revealed to observation through polar differentiation, bilateral symmetry, and other obvious characters in the unsegmented egg . . . . [These] promorphological features of the egg are as truly a result of development as the characters coming into view at later stages. They are gradually established during the preembryonic stages, and the egg, when ready for fertilization, has already accomplished part of its task by laying the basis for what is to come (pp. 384, 386).
>
> In *Amphioxus* the differentiation of the cytoplasmic substance is at first very slight, or readily alterable, so that the isolated blastomere, as a rule, reverts at once to the condition of the entire ovum . . . . In the snail and ctenophore we have the opposite extreme to *Amphioxus*, the cytoplasmic conditions having been so firmly established that they cannot be readjusted, and the development must, from the onset, proceed within the limits thus set up.
>
> Through this conclusion we reconcile, as I believe, the theories of cytoplasmic localization and mosaic development with the hypothesis of cytoplasmic totipotence [and regulative development]. Primarily the egg-cytoplasm is totipotent in the sense that its various regions stand in no fixed relation with the parts to which they respectively give rise, and the substance of each of the blastomeres into which it splits up contains all of the materials necessary to the formation of a complete body. Secondarily, however, development may assume more or less of a mosaic-like character through differentiations of the cytoplasmic substance . . . . Both the extent and the rate of such differentiations seem to vary in different cases; and here probably lies the explanation of the fact that the isolated blastomeres of different eggs vary so widely in their mode of development. When the initial differentiation is of small extent or is of such a kind as to be readily modified, cleavage is *indeterminate* in character and may easily be remodelled (as in *Amphioxus*). When they are more extensive or more rigid, cleavage assumes a mosaic-like or *determinate* character, and qualitative division [of the cytoplasm], in a certain sense, becomes a fact (p. 423).
>
> It is important not to lose sight of the fact that development and differentiation do not in any proper sense first begin with the cleavage of the ovum, but long before this, during its ovarian history. The primary differentiations thus established in the cytoplasm form the immediate conditions to which the later development must conform; and the difference between *Amphioxus* on the one hand, and the snail or ctenophore on the other, simply means, I think, that the

initial differentiation is less extensive or less firmly established in the one than in the other.

[Thus] we reach the following conception. The primary determining cause of development lies in the nucleus, which operates by setting up a continuous series of specific metabolic changes in the cytoplasm. This process begins during ovarian growth, establishing the external form of the egg, its primary polarity, and the distribution of substances within it. The cytoplasmic differentiations thus set up form as it were a framework within which the subsequent operations take place in a course which is more or less firmly fixed in different cases (pp. 424–425).

The data available to Wilson supported the hypothesis that all eggs and even some embryos begin as highly regulative and then gradually become mosaic. The various species differ in the time when ovulation and fertilization occurs in relation to this transition from the regulative mode to the mosaic. That time comes early in amphioxus and late in *Dentalium* and *Patella.*

THE END OF AN ERA

When Wilson and others were reaching these conclusions, the next paradigm of experimental embryology was being formulated. It would be concerned not so much with the development of isolated parts of embryos as with the interactions among the parts. We will consider two examples: the work of Hörstadius on the sea urchin and that of the Spemann school on amphibian organizers.

Interest in the earlier problems did not cease, however. Old experiments were repeated with better techniques and better information. For the most part the results of the pioneers were confirmed but there was a general trend for finding the regulative eggs to be somewhat more mosaic and mosaic eggs to possess some regulative ability. The details can be found in a fine monograph edited by Reverberi (1971).

WORKING TOGETHER: *PARACENTROTUS*

During the 1920s and 1930s Sven Hörstadius, a Swedish experimental embryologist, performed a remarkable series of experiments on the eggs and embryos of the sea urchin, *Paracentrotus lividus.* He was skilled at operations on the minute embryos and his results, and their interpretation, are one of the main contributions to developmental biology in this century. He provided a fine summary of his work in 1973 (see also Hörstadius, 1939; Waddington, 1956; Giudice, 1973; Reverberi, 1971).

Only one aspect of this work will be considered here—the one showing that normal development requires the interaction of the parts of the embryo. This phenomenon is not encountered to any great extent in the mosaic eggs with their determinate cleavage and self-differentiating parts.

Echinoderm embryos have been favorite materials for experimental embryologists from the time of Driesch to the present. Mature *Paracentrotus* ova have a pigmented equatorial band that serves as a convenient landmark. As is true with so many species, the first two cleavages are meridional and the third is equatorial. The resulting eight cells are of approximately equal size (Fig. 49C).

The fourth cleavage, giving 16 cells, is vertical in the animal hemisphere, the result being a single layer of eight cells. The cleavage plane in the vegetal hemisphere is horizontal and unequal—resulting in four large macromeres and four small micromeres (Fig. 49D).

We will note the fifth cleavage only for what happens to the macromeres—they divide horizontally into two layers–called an_1 and an_2 (Fig. 49E).

Figure 49 is really a fate map for *Paracentrotus.* The boundaries of the cells, and their corresponding regions in the uncleaved egg (Fig. 49A), are differentiated so they can be traced throughout cleavage and up to the pluteus larval stage (Fig. 49M, N). The unbroken black line at the animal pole of the egg will become the an_1 layer. When traced through to an early larva (Fig. 49L) it is seen to form the ecto-

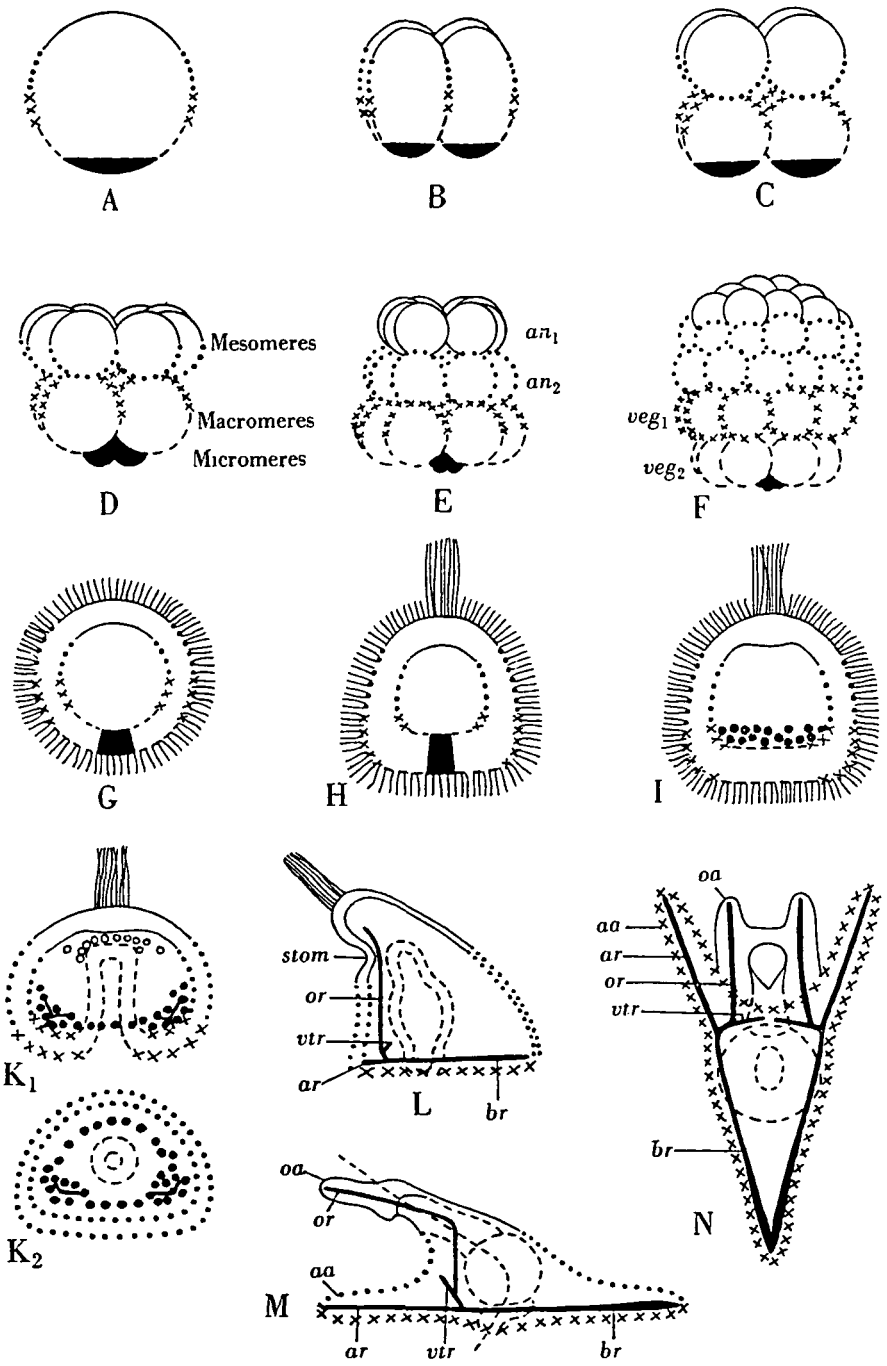


FIG. 49. Normal development of the sea urchin embryo. See text for details (Hörstadius, 1939).

dermal covering of the upper part of the larva, the apical organ, and the stomodaeum.

The dotted lines show where the next layer of cells, an_2, come from and what their fate will be. It forms the epidermis (ectoderm) of the lower sides of the embryo (Fig. 49L). The parts of the early cleavage

embryos that will form layer veg_1 are shown as crosses. This is also an ectodermal layer and its fate is to form the epidermis of the base of the early larva (Fig. 49L). Layer veg_2, shown in dashed lines, consists of the presumptive endodermal cells plus some mesoderm and it will form the archenteron, the secondary mesenchyme, and the coelom. At the region of the vegetal pole one finds the micromeres, shown solid black. Hörstadius reported that they form the primary mesenchyme and the larval skeleton (Fig. 49L, M, N).

The aspect of Hörstadius' work that forms an important part of our analysis is his experiments on separating embryos horizontally. This led to some important insights about development—showing that the egg and early embryo may have concentration gradients of animalizing and vegetalizing materials and, furthermore, that normal development is not so much a question of the parts involved but whether or not there is a proper balance of these two hypothetical types of substances. The animalizing substances are assumed to be necessary for the development of structures normally formed from the ectodermal areas. The vegetalizing substances are assumed to be necessary for the formation of those parts normally derived from the presumptive mesoderm and endoderm.

Hörstadius concludes that his data can be explained better by assuming that there are two gradients. Some investigators believe that the experimental data can be explained just as well by assuming one gradient of a single substance (Child, 1941, pp. 142, 240). We will assume that there are two.

The hypothetical animalizing substances are assumed to be in highest concentration at the animal pole and lowest at the vegetal pole. For convenience let us assume that they have a concentration of 5 in an_1, 4 in an_2, 3 in veg_1, 2 in veg_2 and 1 in the micromeres. In contrast, the vegetalizing substances are assumed to have the highest concentrations, let us say 5, in the micromeres and then decreasing one number per layer until they have a value of 1 in an_1. Let us also assume that normal development is possible only when the concentrations of the animalizing and vegetalizing substances are roughly equal in the whole embryo or fragment produced experimentally. Thus in a normal embryo, if we sum the values from an_1 to the micromeres, there will be a total of 15 animalizing units ($5+4+3+2+1=15$) and the total will be the same for the vegetalizing substances ($1+2+3+4+5=15$). (This system of giving arbitrary values to the hypothetical substances is not Hörstadius' but mine—it proved most helpful to students in introductory biology.)

The hypothesis that development is related to these substances can be tested by cutting the embryo horizontally between an_2 and veg_1 (Fig. 49F). The animal hemisphere half would have $5+4=9$ animalizing units and $1+2=3$ vegetalizing units. That ratio of 9 animalizing to 3 vegetalizing units is far from equal. The vegetal hemisphere half would have 6 animalizing and 12 vegetalizing units.

The results of such an experiment are shown in Figure 50. The upper row illustrates the blastulae derived from the animal hemisphere halves (consisting of $an_1 + an_2$—both presumptive ectoderm). When the blastula stage is reached, the apical organ, instead of being of normal size (Fig. 49H), may be expanded to cover nearly the entire embryo. The embryos A_1 through A_4 show the range of results, the majority being like A_1 or A_2. The A_4 type usually arises from embryos where the cleavage plane is somewhat lower than usual and so includes some of the material that would normally be in veg_1. The second row shows the limits of development of the animal halves. Most show no signs of gastrulation but those derived from A_4 may show slight invaginations, as in A_8.

The bottom row shows the development of the lower half ($veg_1 + veg_2$ + the micromeres; that is, one layer of ectoderm, one mainly of endoderm, and the micromeres, which form the primary mesenchyme and the skeleton). These plutei usually have an enlarged gut, poorly developed arms or none at all, and often no mouth. Earlier they usually lacked the apical organ.

These results seemed to support the hypothesis. The half with the hypothesized

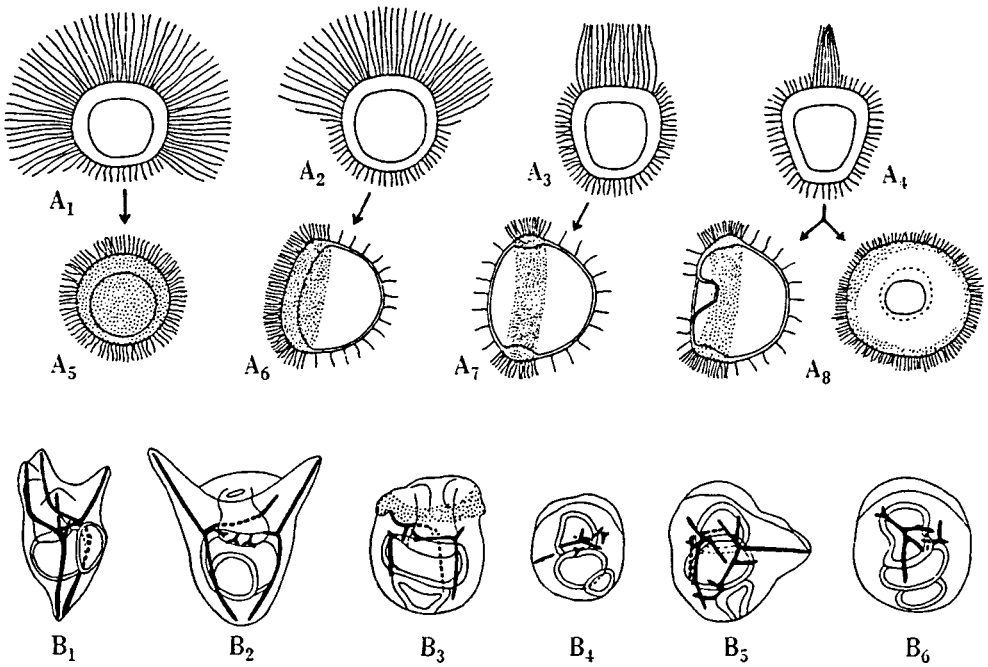


FIG. 50. Development of isolated animal (A's) and vegetal (B's) hemispheres of sea urchin embryos. See text for details (Hörstadius, 1939).

high level of animalizing substances and low level of vegetalizing substances does produce an animalized embryo unable to gastrulate. The vegetal half with the presumed high level of vegetalizing substances and low level of animalizing substances does seem to have exaggerated vegetal-type developments.

Such vegetalized embryos were not new. Since the turn of the century it has been known that, when normal echinoderm embryos are raised in sea water to which a small amount of a lithium salt has been added, they produce abnormal embryos with exaggerated vegetal structures.

Hörstadius' gradient hypothesis was tested in many other ways. He developed the techniques to separate the individual layers at either the 32- or 64-cell stages and combine them at will. These are some of the results (with our hypothetical values for the animalizing and vegetalizing materials in parentheses).

1. $an_1 + an_2$ = blastula with large apical tuft; almost never any gastrulation (9 animalizing and 3 vegetalizing units). Figure 51, A_1.
2. $an_1 + an_2 + veg_1$ = apical tufts normal but almost never any gastrulation. These three layers consist of the entire ectoderm (12 animalizing and 6 vegetalizing units). See Figure 51, B_1.
3. $an_1 + an_2 + veg_2$ = normal apical tuft. Reasonably normal pluteus larva (11 animalizing and 7 vegetalizing units). Figure 51, D_1.
4. $an_1 + an_2 + veg_1$ + micromeres = normal development (13 animalizing and 11 vegetalizing units). Figure 51, E_1.
5. $an_1 + an_2$ + micromeres = normal development (10 animalizing and 8 vegetalizing units). Figure 51, F_1.

Thus there is normal development when the ratio of animalizing to vegetalizing substances are close: 13/11, and 10/8; an intermediate condition when the ratio is 11/7; and abnormal development when the ratios differ markedly: 9/3 and 12/6. (If one desires, the values assumed for each layer can be adjusted so that normal development occurs when the sums of the animalizing and vegetalizing substances are approximately equal.)

Note that in experiment 3 the addition

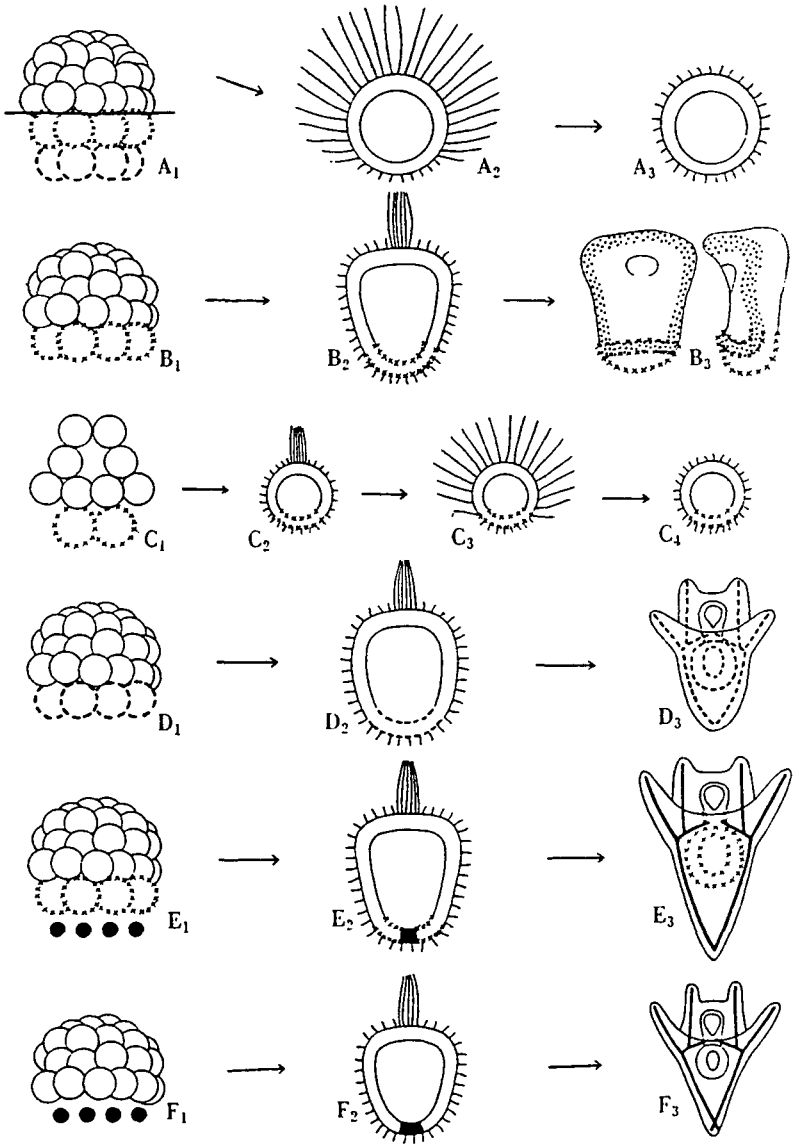


FIG. 51. Development of combinations of cell layers of sea urchin embryos. See text for details (Hörstadius, 1939).

of a single vegetal hemisphere layer, veg_2, is enough to balance the animalizing influences. A fairly normal pluteus is obtained even though a third of the presumptive ectoderm (veg_1), and the micromeres that normally form the primary mesenchyme and the skeleton are missing. Other cells, however, are able to alter their normal fates and produce the structures that the missing layers would have formed in a normal embryo.

Experiment 4 shows a similar result. The layer that would normally form the endoderm, veg_2, has been removed. The embryo remaining consists only of the presumptive ectoderm and the presumptive primary mesenchyme. Nevertheless an archenteron is formed.

The embryos of experiment 5 are much the same, except that they have lost a third of their presumptive ectoderm.

These results, plus many more not listed, lead to many important conclusions:

1. Earlier experiments by Driesch and

others on the isolation of blastomeres suggested that, although the pattern of cleavage and blastula formation indicated that the sea urchin embryos were partially mosaic, yet normal pluteus larvae were obtained indicating that the early embryos could regulate. The conclusion, therefore, was that half-embryos have the capacity to produce whole larvae. However these half-embryos were all obtained the same way—separation of blastomeres along the plane of first cleavage.

2. But now we find not just *any* half will suffice. When the blastomeres of the two-cell stage are isolated each will have the entire range of substances that are localized along the A–V axis. However, if the half-embryo is obtained by an equatorial cut, isolating the animal hemisphere and the vegetal hemisphere, as in the experiments just described, development is abnormal.

3. Thus the experiments on the isolation of halves show that the sea urchin embryo is mainly of the regulative type when the separation is along a meridian plane (the animal pole–vegetal pole axis) but largely mosaic when the separation is along an equatorial plane.

4. Although development can be explained by assuming concentration gradients of substances distributed along the A–V axis, these substances are not localized to specific areas. Any one of the five tiers of cells—an_1, an_2, veg_1, veg_2, and the micromeres—can be eliminated and a normal larva result. *Thus the development of a part depends on the entire embryo.* That is, the development of the part is regulated in such a manner that the end result is as normal as the entire fragment will permit. This hypothesis can be traced back to the late 1800s and it expresses the view of those who accepted the hypothesis of regulative development. Hertwig, writing in 1893, expressed it thus:

> Since every elementary part (*i.e* cell) arises through the division of the germ, or fertilized egg, it contains also the germ of the whole, but during the process of development it becomes ever more precisely differentiated and determined by the formation of cytoplasmic products according to its position with reference to the entire organism (blastula, gastrula, etc.) (quoted from Wilson, 1900, p. 415).

There are many morals to be learned from this research on sea urchin embryos. Probably the most important for students is that the "facts" of science are to be accepted only for the precise phenomena they are assumed to describe. Sea urchin embryos were the model for regulative development, a "fact" based on the development of halves obtained by the separation of blastomeres along the meridian extending from animal pole to vegetal pole. This "fact" is replaced by a better "fact" when experiments produce halves by isolation of animal and vegetal hemispheres.

We no longer can describe sea urchin embryos as "regulative" or "mosaic" but must specify which conditions and which parts are being discussed.

Driesch was not wrong; his statements were incomplete. Since the questions he asked were fundamental to our understanding of development, others sought to repeat his experiments. When they used his techniques they usually obtained his results. Hörstadius was able to ask the question in a different way and he obtained a different answer that expanded our understanding of early development.

Another moral: the test of a single deduction rarely establishes an hypothesis as "true beyond all reasonable doubt."

THE THEORY OF AMPHIBIAN ORGANIZERS

In the early 1920s a new paradigm began to attract notice. This was the line of work started by the German embryologist Hans Spemann (1869–1941), which sought to discover how the parts of an embryo influence one another. This led to the hypothesis that one part of an embryo, the *organizer,* can influence the differentiation of another part, the *reacting tissue.*

The hypothesis of organizer action was tested in many ways, with the embryos of many species, and by many experimenters. The hypothesis was abundantly confirmed and, since it accounts for a great variety of

developmental phenomena, we can promote it to a "theory."

THE FORMATION OF THE NEURAL TUBE

The cells of an amphibian embryo in the late blastula stage are essentially the same throughout the entire embryo. To be sure there is a gradient of increasing size, with the smallest cells at the animal pole and the largest at the vegetal pole. There is also a gradient in the concentration of yolk granules, with the least number in the cells at the animal pole and the most in those at the vegetal pole. The animal hemisphere cells are packed with melanin granules whereas those of the vegetal hemisphere are relatively pigment free. Apart from these differences there is nothing to suggest the widely divergent destinies of the cells of different regions.

The conversion of the single-celled zygote into the many-celled late blastula is brought about by cleavage with little or no visible differentiation of the cells: they just get smaller. During gastrulation the cells become rearranged and the sites of the three presumptive germ layers can be located (Fig. 25). This recognition of germ layers, however, is based almost entirely on the location of the cells and not their appearance. Subsequently the slow process of cellular differentiation results in visibly different cell types—muscle cells, leucocytes, neurons, gland cells—that form the tissues and organs of the embryo.

The first system developed in an amphibian embryo is the nervous system, so it is not surprising that it engaged the interest of embryologists. Although in the interior of the adult, it appears on the outside of the early embryo. At the end of gastrulation a flattened area, the neural plate, becomes visible—extending anteriorly from the closed blastopore. Neural folds appear at the edges of the neural plate, move to the center, and fuse along their crests, forming a tube that lies under the outer epidermis (Figs. 20, 21, 33).

When embryos of these stages are examined in sectioned material, we find that by the time the neural plate is forming gastrulation movements have brought a sheet of presumptive notochord cells into a position below the neural plate (Figs. 27–31).

Repeated observation would show that these events always occur in normal development—as Vogt's fate map indicates (Fig. 25). The neural tube forms in a constant way with respect to the positions of the blastopore, archenteron, and polarity of the embryo. These constant relations must be important because, if something always happens in the same way, it is assumed that it is a fixed phenomenon, presumably with cause-effect relationships.

Thus our problem is to understand how, at the end of gastrulation, those presumptive ectodermal cells that are in the area above the roof of the archenteron become the neural tube, whereas the rest of the presumptive ectodermal cells, which look identical, becomes the epidermal covering of the body—brains *vs.* skin are very different fates. In our own case the difference is quite spectacular. One set of presumptive ectodermal cells becomes so changed that it can think about the epidermis; the epidermal cells can never think about the brain at all.

HYPOTHESES, DEDUCTIONS, TESTS

So we ask: "Why these different fates?" The answer can only come from experimentation but, as usual, there is that awesome problem of knowing what to do—that is, how to ask a question that is answerable. The first thing we might try is to ask those questions of the 1890s once again—"Is the part mosaic or regulative?" Two alternative hypotheses to explain how presumptive neural tube cells of the early gastrula become the neural tube suggest themselves.

Hypothesis 1. The presumptive neural tube cells of an early gastrula possess an inherent capacity to form neural tissue. They are determined, that is, they have within themselves all that is necessary to differentiate into a neural tube.

Hypothesis 2. The presumptive neural tube cells of an early gastrula do not possess an inherent capacity to form neural tissue. That is, they are still in a regulative stage and

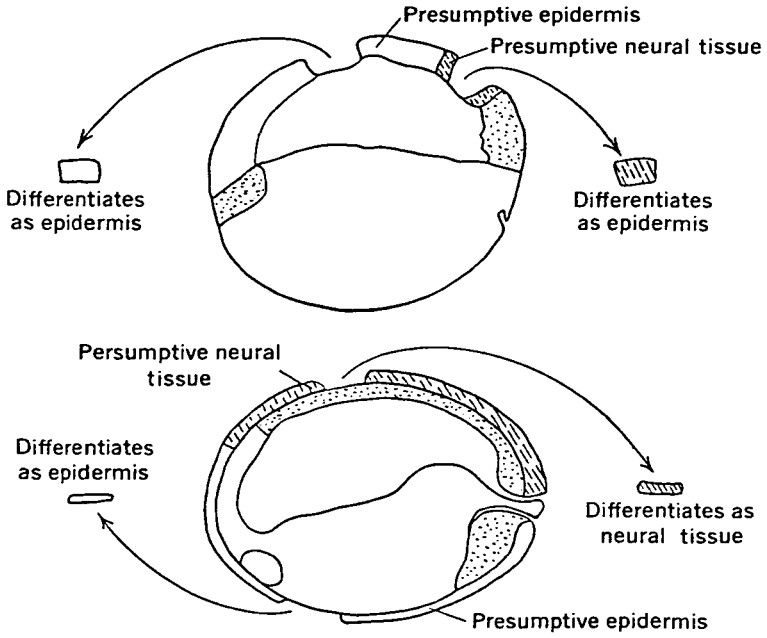


FIG. 52. Explantation of presumptive neural tissue and presumptive epidermis in an early (above) and late (below) gastrula. Refer to Figures 27 and 30 for full labels.

influences from outside the presumptive neural tube area are necessary for them to differentiate into a neural tube.

We can start by testing the first hypothesis. That is, we will provisionally accept that hypothesis 1 is true, make deductions, and then test the deductions.

If the presumptive neural tube cells are already determined and possess within themselves all that is necessary for the differentiation of a neural tube, this deduction follows logically:

> The presumptive neural tube cells should be able to differentiate into a neural tube if they are separated from the remainder of the embryo.

We now have to devise experimental means of verifying or denying the deduction. One such experiment was performed by Johannes Holtfreter (born 1901), a student of Spemann. Pieces of the blastocoel roof of an early gastrula are cut out and cultured in a dilute salt solution. No external source of food is required since each cell has many yolk granules. Such *explants* remain alive for days—many more than are necessary for the control embryos to gastrulate and form the neural tissue. Explants were taken from two areas—the presumptive neural tube area and the presumptive epidermis. The experiment is shown in Figure 52, top diagram.

The results of many experiments were the same: neither type of explant differentiated as neural tissue. Both formed only simple epidermal-like cells.

If these results can be accepted as an adequate test of the deduction, we must conclude that hypothesis 1 has not been supported. The experiment can be criticized, of course, as having resulted in injury to the excised piece of the blastocoel roof. This possibility can be partially ruled out since self-differentiation by other explants is possible, as we will soon see.

The evidence from this first experiment suggests that the presumptive neural tube cells have not been determined by the onset of gastrulation, since they are unable to self-differentiate. Nevertheless, they must become determined within a day because at that time they do form a neural tube.

Holtfreter now did the experiment at the end of gastrulation but before there was any indication of the neural plate. This experiment is shown in the bottom diagram of Figure 52 and the results were dramatically different: neural tissue was formed.

What could be the cause? The cells of the presumptive neural tube explant were older at the end of gastrulation, they contained fewer yolk granules, and they were smaller. They also were in a different environment. In the first experiment, before being explanted, the outer surface of both explants faced the outer environment while the inner surface faced the blastocoel. In the second experiment done at the end of gastrulation, before being explanted, the presumptive neural tube cells were above the presumptive notochord cells whereas the presumptive epidermal cells were above the presumptive endoderm. This might be significant.

Holtfreter found, quite by accident, another way to test hypothesis 1. In some experiments designed for an entirely different problem, early gastrulae were placed in water to which extra salts had been added, then the membranes surrounding the gastrula were removed, and the embryos were rotated so the animal hemisphere was down. Under these conditions gastrulation movements were abnormal. The presumptive ectoderm cells did not move down over the vegetal hemisphere but tended to pull away from the rest of the embryo. The result was a dumbbell-shaped embryo known as an exogastrula. In extreme cases the presumptive ectodermal cells formed an irregular mass connected by only a thin strand of cells with the presumptive endodermal and mesodermal cells.

A diagrammatic representation of the differentiation of these exogastrulae is shown in Figure 53. Development of the two parts was very different. The presumptive endoderm and presumptive mesoderm differentiated into heart, muscle, parts of the alimentary canal, and other organs normally formed from these two layers. These layers were able to self-differentiate. In marked contrast, the presumptive ectoderm remained essentially undifferentiated. There was no trace of a nerve tube.

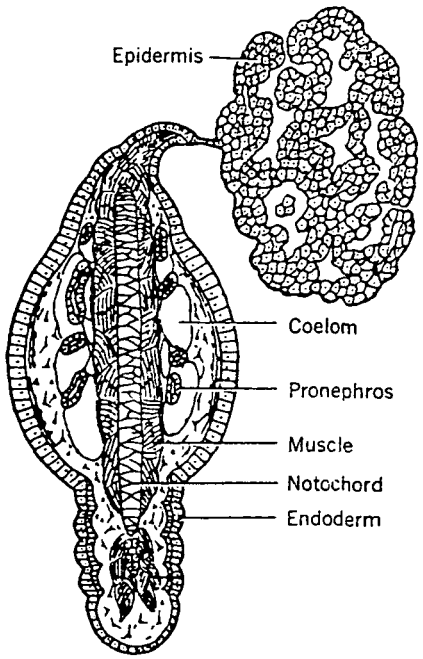


FIG. 53. The differentiation of the parts of an exogastrula (Holtfreter, 1933*b*, p. 406).

Exogastrulae are strange not only in this separation of presumptive ectoderm from the other regions but in the abnormal movements of the other two presumptive regions. The embryo turns inside out. As a result the mesoderm is inside the endoderm and the *lining* of the archenteron faces outward (Fig. 53).

Once again, the data indicate that the presumptive neural tissue is undetermined at the onset of gastrulation. We know, however, that it is determined by the end of gastrulation. Thus some change must occur in the interval between the early gastrula and the late gastrula. This change, however, does not occur in the presumptive neural tube tissue during the time it is an explant or part of an exogastrula—its cells do not become determined. We might suspect, therefore, that the change is due to influences from other parts of the embryo; and this would mean almost certainly influences from either the presumptive mesoderm or presumptive endoderm, or both.

That fits our second hypothesis, which implies that the presumptive ectoderm is completely undetermined at the onset of gastrulation and that some outside influence results in part of it being determined to become neural tissue. Since only part of the presumptive ectoderm becomes neural tissue, the stimulus from outside must be localized. If this is the case, the following deduction can be made:

> If the relative positions of the animal hemisphere, which contains the presumptive ectoderm, and the rest of the embryo are altered, the position of the neural tube should be altered accordingly.

An experimental test of this deduction was made by Spemann. He cut off the upper part of the animal hemisphere of an early gastrula, rotated it 180°, and stuck it back on the lower portion of the embryo. The two parts healed and the embryo went on to form a normal larva, not in relation to the presumptive regions of the animal hemisphere but of the ventral part.

Figure 54 shows the experiment. The upper figures are normal, unoperated embryos. The fate of the presumptive ectoderm is shown. As Vogt had established, the presumptive notochord area (stippled in the figure) is above the dorsal lip and the presumptive neural tube above that. The lower two figures show the operation. The animal hemisphere was cut along the dashed line and then rotated. As a consequence, the presumptive neural tube area is now 180° from its normal position and the presumptive epidermis is adjacent to the presumptive notochord. The operated embryo continues to develop but the neural folds appear in their normal position *with reference to the dorsal lip of the blastopore.* This means that the presumptive epidermis formed the nerve tube and the presumptive neural tube cells formed epidermis!

This experiment shows that the differentiation of the presumptive ectoderm is greatly influenced by the ventral part of the embryo. But what part? The constant relation of the dorsal lip of the blastopore to the position of the neural plate and neural tube, both in normal development and in the experiment on rotation of the animal hemisphere, suggests that the dorsal lip might be involved. The dorsal lip is the place where the presumptive notochord cells turn in, forming the archenteron roof, and come to lie beneath the presumptive neural plate. Recall that in the first experiment (Fig. 52) the presumptive neural tube tissue became determined after the presumptive notochord cells moved under it to form the archenteron roof.

These experiments and their analysis suggest a variation on hypothesis 2.

> *Hypothesis 2a. The presumptive neural plate cells of an early gastrula do not possess an inherent capacity to form neural tissue. Instead, the presumptive neural plate cells become determined as a result of stimulation by the presumptive notochordal cells of the archenteron roof.*

If this hypothesis is accepted as true, the following deduction can be a test of it.

> If the dorsal lip cells are removed from a donor embryo and grafted into a host embryo, and if they are able to invaginate, a nerve tube should be produced from the overlying presumptive ectoderm of the host.

This difficult (at the time) experiment was performed in 1924 by Hilda Mangold, when she was a student of Spemann. It is one of the classics of embryology, winning a Nobel Prize for Spemann in 1935 (Hilda Mangold had died shortly after the experiments were performed).

The operation is shown at the top of Figure 55. In order to recognize the origin of the cells, embryos of two species of salamander were used. In one species the embryos are nearly white and in the other they are brownish. A small piece of tissue was removed from the dorsal lip region of the donor embryo and then transplanted to a site 180° from the host's dorsal lip.

The host, therefore, had two dorsal lips—its own and the donor's. Invagination occurred at both. Because of the difference in pigmentation of the two species, it could be established that the dorsal lip cells of the donor invaginated. At the time the host's neural folds were forming (the

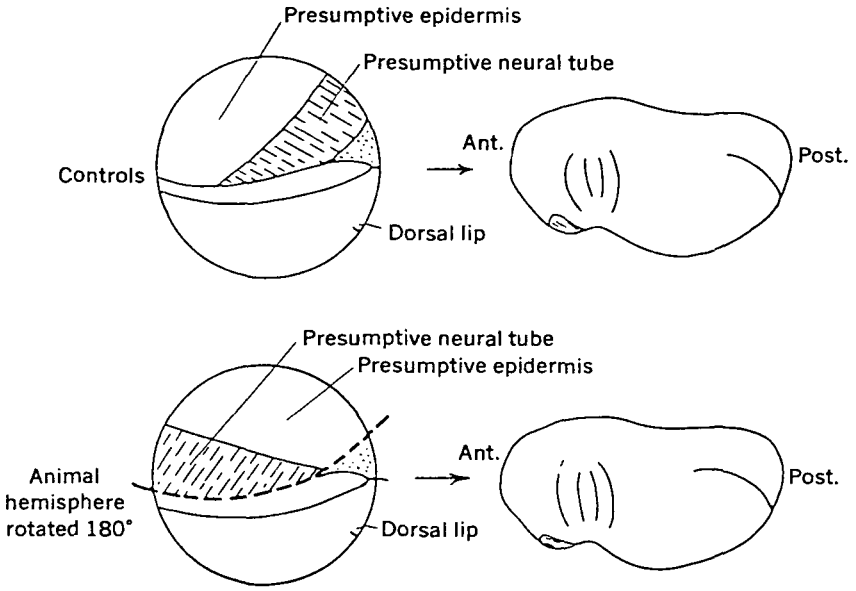


FIG. 54. Experimental rotation of the animal hemisphere. Refer to Figure 25 for full labels.

primary embryo), neural folds also appeared above the region where the donor dorsal lip cells had invaginated (the secondary embryo). The sectioned embryo is shown at the bottom of Figure 55. The secondary embryo is essentially normal.

An important question now confronts us. Is the secondary embryo formed from the donor tissue, host tissue, or both? Again, we can tell because of the difference in pigmentation of host and donor tissue. The answer is both. The donor tissue forms the archenteron roof of the secondary embryo, which later becomes the notochord. It also forms other structures, mainly mesodermal. The neural tube, however, is formed almost entirely from host cells. Thus cells that normally would form epidermis now form a nerve tube.

Spemann and Mangold had shown that the presumptive notochordal cells that invaginate at the dorsal lip and form the roof of the archenteron have a profound effect on development. They spoke of these cells as the *organizer* and their action on the undetermined ectodermal cells as *induction.* Induction is not restricted to events in only the secondary embryo but is a phenomenon of normal development.

The experiments so far described suggest that in normal embryos the neural tube is formed under the influence of the organizer. At the beginning of gastrulation, the organizer region consists of the cells above the dorsal lip corresponding roughly to the presumptive notochordal region of Vogt's fate map (Fig. 25). This region invaginates to form the roof of the archenteron. The roof of the archenteron then induces the overlying ectoderm to form a neural tube. Without this inductive influence these cells will form only simple epidermis.

We now have a theoretical basis to interpret the experimental results from explanation of tissues, exogastrulation, and the rotation of the animal hemisphere.

When presumptive neural plate cells from an early gastrula are explanted, they will never be induced by the organizer and hence cannot form neural tissue.

The same is true of exogastrulae—the presumptive ectoderm is never in contact with the organizer. In this case, however, Holtfreter made some most interesting observations. He found that by varying the culture conditions he could obtain partial exogastrulae. In these instances the presumptive ectoderm that was in contact with the presumptive mesoderm and endoderm was induced to form neural tissue.

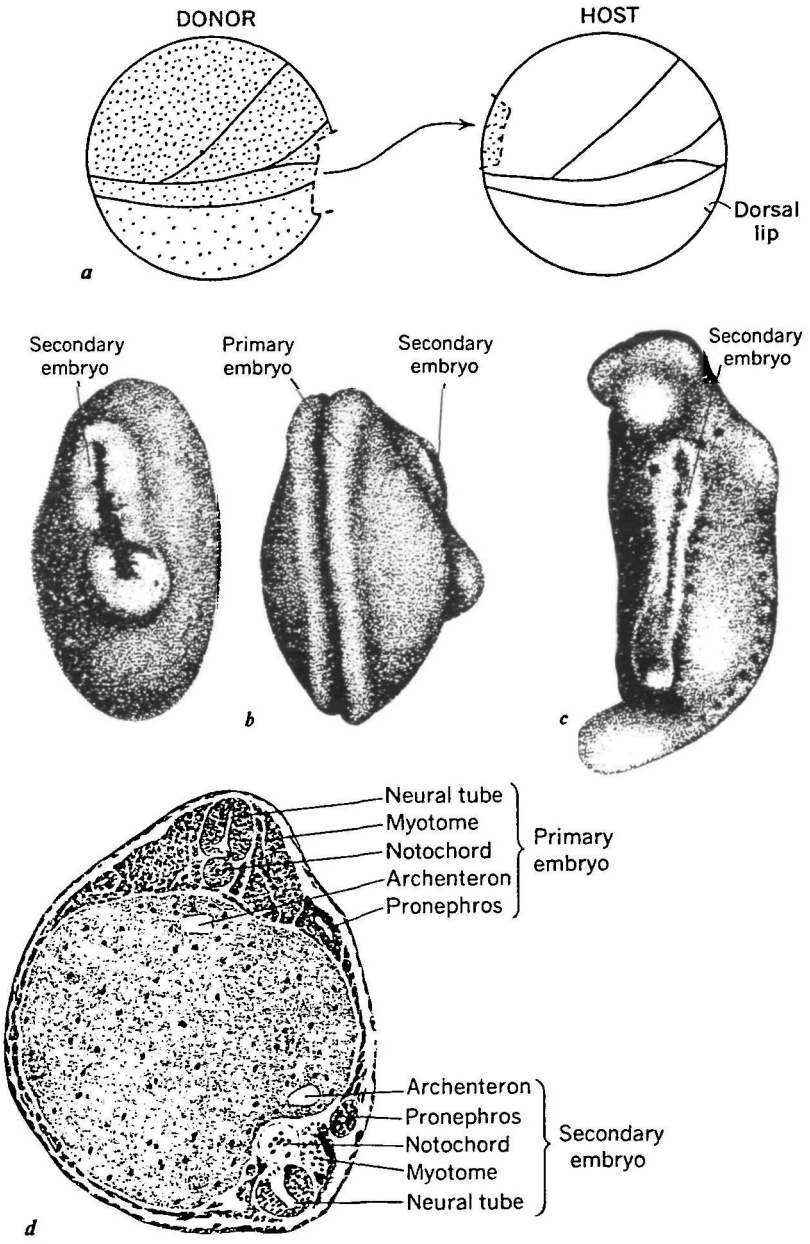


FIG. 55. The dorsal lip transplantation experiment of Spemann and Hilda Mangold. *a* is a diagram of the operation—see Figure 25 for full labels. *b* and *c* show the secondary embryos. *d* is a cross section showing the structure of the primary and secondary embryos (*b*, *c*, and *d* modified from Spemann and Mangold, 1924).

The experiment on rotating the roof of the blastocoel has a similar explanation. The original presumptive neural tube area was moved to a position where the archenteron roof would not make contact with it—and it remained as epidermis. The presumptive epidermis, however, came to be situated over the archenteron roof and it was induced to form a neural tube.

SECONDARY ORGANIZERS

It was soon found that there is not just one organizer, the one associated with the

tissue of the dorsal lip which later forms the archenteron roof, but many. Organizers were discovered for the mouth, heart, eye, lens, otic vesicle, olfactory organs, pronephros, and many other structures. (In fact there is much evidence to suggest that the primary axial organization of the early embryo is controlled by the invaginated material that forms the walls of the archenteron.) Secondary organizers act subsequently to but in the same manner as the primary organizer, that is, undetermined cells of the embryo are induced. Once these cells are determined they can self-differentiate.

The formation of the optic cup and lens of the eye can serve as an example.

THE FORMATION OF THE EYE

Vogt's fate map (Fig. 25) shows the amphibian eye as having a dual origin. The bottom diagram shows the presumptive optic cups in the middle of the presumptive neural tube area. The lenses, however, are the small ovals above, to the right and left, in the presumptive epidermis area. They are shown and labelled in the upper diagram.

The complete eye has the lens centered, which is of course necessary for normal vision. An off-centered lens would be useless. When we remember the complicated movements of the presumptive areas during gastrulation and neurulation, one can only marvel that the processes are so precise that the lens always ends up exactly where it should. But there is more to the story.

Shortly after the closure of the neural folds, the optic cups begin to grow laterally from the floor of the brain (Fig. 34). When the optic cup reaches the epidermis, a lens begins to form from the inner layer of the epidermis opposite the middle of the optic cup. Subsequently the outer layer of the epidermis, still full of pigment granules and quite opaque in Figure 34, begins to clear and form the cornea.

Experiments have shown that, in some species at least, the optic cup acts as an organizer that induces the head epidermis to form a lens.

The optic cup area itself seems to be induced by the archenteron roof. That is, the primary organizer not only induces the overlying ectoderm to form a neural tube but also induces a regional specificity.

The experiments designed to throw light on the formation of a lens are performed as follows. When the optic cups are beginning to form, a slit is made in the head epidermis and the optic cup on one side is cut off. The epidermis is pushed back in position and heals in a few minutes.

The embryo is allowed to develop for two days and then fixed and used for serial sections. The optic cup on the unoperated side (we have an experimental and control animal in a single individual!) is found to have produced a normal eye with lens. On the operated side, however, the brain is found to have healed and there is no optic cup at all. The brain cells, therefore, could not regulate to replace the excised optic cup. Of greater significance, however, is the fact that there is no lens on the operated side. Thus, in the absence of an optic cup, lens differentiation does not occur. This result suggests that the optic cup may be the organizer for the lens.

The next experiment supports that conclusion. The optic cup is removed when it is first starting to form and placed under the epidermis of the trunk region. The wound heals (amphibian embryos are just wonderful in this way) and, at the time a lens normally forms, the epidermis over the transplanted optic cup forms a lens. Thus it seems true beyond all reasonable doubt that, in the species used, the optic cup induces the overlying epidermis to form a lens. That trunk epidermis would normally have continued to differentiate as epidermis but this experiment shows that it still has the ability, or competence in the language of embryologists, to do more than its fate suggests.

Students are sure to ask about that eye back in the flank, which may appear to be entirely normal. "Does that eye enable the tadpole to see where it has been or, at least, who is sneaking up behind it?" No, the transplanted eye never makes the proper nerve connections. (This is a good place to reinforce the notion that we "see" with our brains, not our eyes.)

THE REACTING TISSUE

These descriptions of the induction of neural tubes and lenses have emphasized the role of the inducing agent. This may have given the impression that the reacting tissue is passively molded by the organizer. This is not the case. The ability of tissue to respond to organizers is limited in several ways.

Age is one limitation. The experiments described before showed that any portion of the presumptive ectoderm of an early gastrula can be induced to form a neural tube but this competence is short lived. At or about the stage when the neural folds close the presumptive epidermis is no longer capable of being induced by the archenteron-roof organizer. However, it is still competent to respond to other organizers—the optic cup, for example.

Tissue specificity is another limitation. The type of experimentation shown in Figure 52 has been extended to all parts of the early gastrula. Explantation is a test of the degree to which a tissue has been determined at the time of explantation and hence the extent to which it can self-differentiate. Such tests show that the presumptive ectoderm of an early gastrula has not been determined. If similar explantation experiments are done with the presumptive notochord and adjacent mesodermal regions of an early gastrula, another result is obtained. Both kinds of explants, although too small to produce organs, differentiate into notochordal, neural, and some other tissue types. These cells, therefore, are partially determined. They can form differentiated tissues but they are not completely determined—or they would form only what their fate suggests. There are problems with endodermal explants, as the cells tend to fall apart, but indirect evidence suggests that the presumptive endoderm is probably fully determined.

The ability of tissues to respond, their competence, can be tested in other ways. When small pieces of an early gastrula are transplanted to various parts of the body of an older embryo, such as a neurula, one discovers another important property of the reacting tissue. If pieces of presumptive ectoderm are transplanted, they are found to participate in the formation of whatever structure is present in the region where they are placed (Fig. 56). If transplanted to the heart region, heart tissue is formed; to the liver region, liver; to the kidney region, kidneys; to the brain region, brain. The same is true of the presumptive chorda-mesoderm as well.

The presumptive ectoderm and presumptive chorda-mesoderm, therefore, do not exhibit germ-layer specificity. Seemingly the cells of those regions do not know to which germ layers they belong.

Figure 57 summarizes the embryological state of the parts of an early gastrula. Much of the work is that of Holtfreter, who has been preeminent in adding to our understanding of amphibian development. His figure *a* is a fate map, essentially the same as Vogt's (Fig. 25). Note the special symbols for each presumptive area since they are repeated in *b* and *c*. Figure *b* shows the ability of explants from each region to self-differentiate. Figure *c* shows the capacity of the cells of each area to respond when transplanted to older embryos (as in the competence experiments shown in Fig. 56). The cells of most of the embryo can participate in the formation of any structure or tissue. Figure *d* shows the distribution of the dorsal lip organizer.

Genetic specificity is another limitation. The dorsal lip transplantation experiments of Spemann and Hilda Mangold involved two species of salamanders, then known as *Triton taeniatus* and *Triton cristatus*. The embryos of *taeniatus* are pigmented; those of *cristatus* are pale. These pigmentation differences can even be detected in histological preparations. Thus when a *cristatus* dorsal lip was transplanted to *taeniatus*, it was possible to say that the *taeniatus* ectoderm had formed the neural tube.

But which kind of neural tube? Was it a *taeniatus* neural tube or a *cristatus* neural tube? That is, does the structure of the induced neural tube conform to the species of the host or the species of the donor? That question cannot be answered, since the neural tubes of the two species are identical in shape and overall appearance. What is required is a system where the

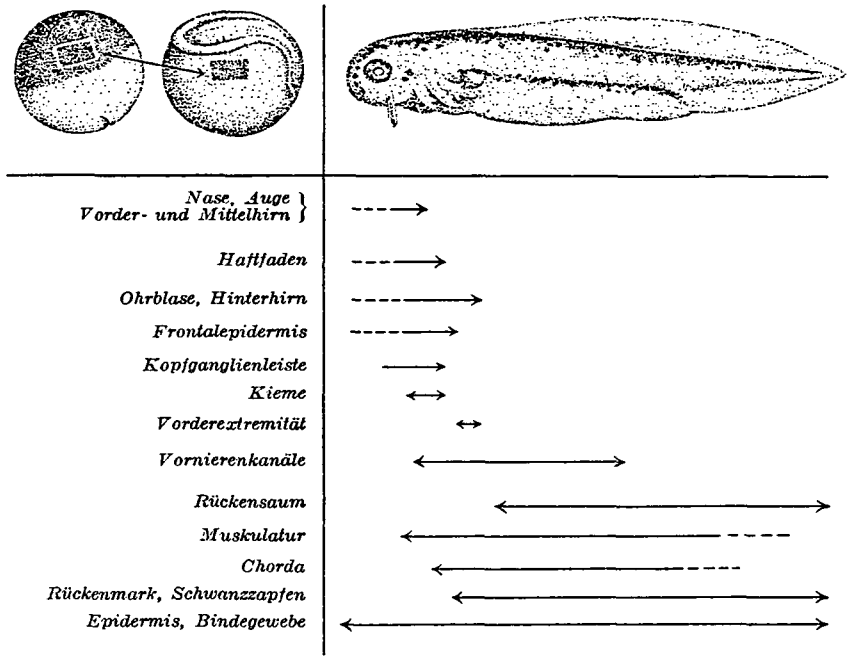


Abb. 68. Regionale Verbreitung der aus Gastrulaektoderm hervorgegangenen Differenzierungen auf dem Wirt (determinierende Felder der Neurula).

FIG. 56. Testing the competence of the presumptive ectoderm of an early gastrula. Gastrula tissue of a salamander was transplanted to various sites in an older embryo where it formed structures appropriate to the location in the host. The lines with arrows show the structures, listed at the left, that were induced in the donor tissue. Thus a line drawn directly down from the two spots just anterior to the larva's gills shows that the transplanted ectoderm can form olfactory organs, eyes, forebrains, midbrains, balancers, ears, hindbrains, frontal epidermis, neural crest, gills, pronephric ducts, muscles, epidermis, and connective tissue when placed at that site on the host. (Holtfreter, 1933*a*, p. 759.)

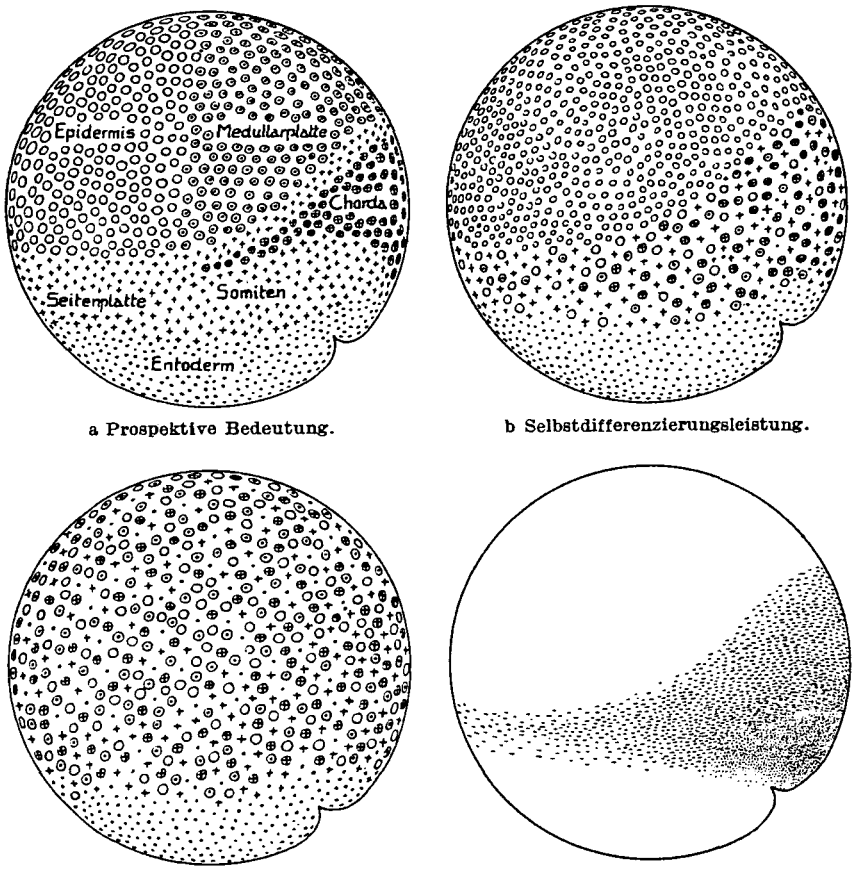
induced structure is recognizably different in host and donor.

Again nature supplied the material. The mouth regions of frog and salamander larvae differ greatly. The frog larval mouth is bordered by black, horny jaws and rows of tiny teeth (these are formed by the ectoderm and have no relation to the true jaws and teeth). The salamander larva lacks both ectodermal jaws and teeth; its mouth is just a hole in the head.

Since in young frog and salamander embryos it is possible to interchange the ectoderm of the region where the mouth will form, we have the prospect of answering the question: "If a mouth region is induced, will it be characteristic of the host or of the donor species?"

The results of such experiments are clear cut. The frog ectoderm on the salamander embryo is induced by the salamander mouth-region organizer to form a mouth. That mouth is of the frog type—with those horny jaws and teeth. In the reciprocal experiment the salamander ectoderm on a frog host produces a salamander mouth.

Other experiments of this sort have been tried and a general rule emerges: the tissue responds in accordance with its specific genetic constitution. Competent tissues can react to organizers but they must do so their own way. One is left with the impression that organizers are general stimuli and that the end result of their action is modulated by the genetic limitations of the reacting tissue. In normal embryos, of course, there is no problem—both the organizing tissue and the reacting tissue are from the same individual and hence have the same genes. It is only under



a Prospektive Bedeutung. b Selbstdifferenzierungsleistung.

c Prospektive Potenz. d Primärer Induktorbezirk.

Abb. 32a—d. Schemata der Determinationsverhältnisse im frühen *Gastrula*-Stadium der Urodelen.

FIG. 57. The developmental state of early gastrula cells. *a* shows the fate, that is, the presumptive regions (compare with Fig. 25); the symbols repeat in *b* and *c*. *b* shows the ability of explanted bits of tissue from various parts of the gastrula to self-differentiate when explanted. *c* shows the capacity, or competence, of cells to form structures when transplanted to various regions of older embryos. *d* shows the distribution and relative potency of the primary organizer. (Holtfreter, 1936, p. 406.)

experimental conditions that unite inducing and reacting tissues of different genetic types that we uncover this principle of the limitation of the reacting tissue's response.

RECAPITULATION AGAIN

The extensive and detailed work on the experimental embryology of amphibians made important contributions to our understanding of recapitulation. For example, one can easily understand, why that "useless" structure, the notochord, forms in the amphibian larva only to be replaced by the vertebral column in the adult. The experimental evidence points to the vital role of the notochordal area or the roof of the archenteron in the induction of the central nervous system. Other experiments have shown that it plays the same role in all other vertebrates. The notochord, therefore, although of transitory importance as a skeletal element, is part of the basic organization of the vertebrate embryo. It is present in all vertebrates because it is necessary if the embryo is to get past the gastrula stage.

There is a similar group of experiments for the pronephros, which is also recapitu-

lated in all vertebrate embryos. It is functional in the amphibian larva but is replaced by the mesonephros, which is the functional kidney of the tadpole and adult frog. Chick embryos start with a pronephros, but it is never functional. Their functional embryonic kidney is the mesonephros; and their adult kidney is the metanephros. Why bother with that useless structure, the pronephros? It turns out it isn't useless at all—when the pronephric duct is cut in either amphibian or chick, the mesonephros fails to develop. Like the notochord, the pronephros plays a vital, though brief, role in embryonic development even though it has no functional role in the adult.

The discovery of the inductive role of some recapitulated structures allows us to reevaluate that concept which was so puzzling, and so important, to 19th century embryologists and morphologists. There were serious disagreements but these were largely of our own making. Two fundamental errors were made: first, it was assumed that structures such as the notochord or the pronephros are "useless," and, second, that development and evolution were considered to be so demanding that inefficiencies would be rapidly eliminated by natural selection.

Both assumptions are at least partially wrong. Now we understand that some of those recapitulated "anomalies" are parts of the fundamental mechanisms of development. These mechanisms are built into the gametes under the direction of the parental DNA. The organization of the ovum, for example, will be what has proved successful for the lineage over time—success here being measured by survival and efficient reproduction. If the vertebrates early on evolved a system whereby the presumptive notochordal cells of the archenteron act as an organizer for the central nervous system, there is every reason for it to be preserved, not eliminated, by natural selection.

There is a pseudo-problem, however, of why it is necessary for the presumptive notochordal cells to actually differentiate histologically into a notochord. Why not just have those cells act as the organizer without going to the "trouble" of histological differentiation? An equally valid question is, "Why not differentiate as notochordal cells?"

This becomes a problem because of our second erroneous assumption: developmentally and evolutionarily this must be the best of all possible worlds. Yet we are wrong to assume that evolution must produce the most efficient patterns of development and adult life. Natural selection is stringent only to the degree that enables the species to "get by," or "good enough is good enough." Were this not the case we might be presumptuous enough to expect all evolutionary lineages to be leading to *Homo sapiens,* as was indeed believed by some pre-Darwinian evolutionists.

If we grant that the second assumption is erroneous, we should expect that some ancestral reminiscences would remain as part of the baggage of inherited developmental patterns. These would be structures with no detected importance in development, and theoretically some might be of no importance at all. They could remain because they are so innocuous that there are no selective pressures to eliminate them. Of course, we must remember that these ancestral reminiscences are the exception.

Our final conclusion: Structures *are* recapitulated and there are good reasons why they should be. It is largely the recapitulation as envisioned by von Baer—the sharing of a common pattern of development by the diverse organisms of a natural group. That being the case, it is inevitable that to some degree ontogeny gives the appearance of recapitulating phylogeny but, to an even greater degree, ontogeny recapitulates ontogeny.

THE NATURE OF THE ORGANIZER

Clearly the dorsal lip organizer is of great importance in development, so not surprisingly there was eagerness to know what it was. Questions of this sort were asked in the 1930s when endocrinologists were discovering more and more hormones and were able to purify some of them. Could the organizer be a hormone-like sub-

stance? One could hypothesize that the roof of the archenteron might secrete a hormone-like substance that caused the overlying ectoderm to form a nerve tube?

The organizer was found to be widely distributed. The structures in other vertebrates—fish, reptiles, birds, and mammals—equivalent to the dorsal lip of amphibians acted as organizers when tested on amphibian embryos. This was exciting, but an even more exciting discovery was that pieces of the dorsal lip, or of the archenteron roof, could be killed by heat or chemical means and still induce undetermined ectoderm to form neural tissue. This was so important because it showed that the organizer was a stable chemical substance and that meant it might be possible to extract and purify the active principle.

But soon things started to get out of hand, or at least out of theory. Not only would dead dorsal lips induce but so would dead tissue from any part of an amphibian gastrula. Earlier experiments to determine the extent of tissue that could serve as an organizer had shown that such ability is restricted largely to the presumptive notochordal region and the presumptive endoderm above the dorsal lip in living embryos (Fig. 57). There was no organizing ability in living presumptive ectoderm but now, when killed, there was.

It was also discovered that tissues of many invertebrates, none of which possess a notochord or dorsal nerve tube, would also induce when killed. What was equally baffling was the finding that dead adult tissues, such as kidney or liver, could induce.

And the list became ever more bizarre: silica, kaolin, methylene blue, steroids, egg albumin, and polycyclic hydrocarbons were all found to have inductive power.

Some investigations suggested that these substances are not really organizers but are acting as toxic substances that somehow stimulate amphibian embryonic cells to form neural tissue. Although this is not a satisfying explanation, at the present time, there is none other.

There is no question that some tissues having no obvious relation to archenteron roofs are potent organizers. The liver of adult mice or guinea pigs, especially if treated with alcohol, can induce head structures in amphibian embryos. On the other hand, guinea pig kidney is a potent inducer of trunk structures.

The problem seems insoluble. There is simply no way at present to specifically identify the substance in the archenteron roof that causes the overlying ectoderm to form a neural tube if such a wide variety of other substances have the same effect. If one is searching for a substance, there must be some way of identifying it. The original test was the ability of the archenteron roof to induce neural tissue in competent ectoderm. But since essentially any tissue when killed will induce, one is left with no way of screening for the *real* organizer substance.

We must await new ideas and new techniques.

And what did its discoverer, Hans Spemann, think of the nature of the organizer? He was not even willing to think of it in chemical terms. This is how he concluded his monograph *Embryonic Development and Induction* (1938):

> There still remains, however, an explanation which I believe to owe the reader. Again and again terms have been used which point not to physical but to psychical analogies. This was meant to be more than a poetical metaphor. It was meant to express my conviction that the suitable reaction of a germ fragment, endowed with the most diverse potencies, in an embryonic "field," its behavior in a definite "situation," is not a common chemical reaction, but that these processes of development, like all vital processes, are comparable, in the way they are connected, to nothing we know in such a degree as to those vital processes of which we have the most intimate knowledge, viz., the psychical ones. It was to express my opinion that, even laying aside all philosophical conclusions, merely for the interest of exact research, we ought not to miss the chance given to us by our position between the two worlds. Here and there this intuition is dawning at present. On the way to the

> high new goal I hope to have made a few steps with these experiments (pp. 371–372).

That is from one of the most influential biologists of the early 20th century. It's hard to known what to say—except that apparently no one has followed his line of thought. Shades of Driesch!

References: Experimental embryology

The field and its publications are vast and the following references are intended to be no more than ways to begin a search. The emphasis is on the literature of "classical" experimental embryology.

General works. Alberts *et al.* (1983), Barth (1964), Berrill (1971), Bertalanffy (1933), Bonner (1963, 1982), Brachet (1950, 1957), Brachet and Mirsky (1959), Child (1941), Conklin (1924), Dalcq (1938, 1957), Davenport (1908), *Davidson (1986), Davidson and Firtel (1984), DePomerai (1985), Dürken (1932), *Edelman (1985), Giudice (1973, 1986), Goldschmidt (1955), Goss (1969), Graham and Wareing (1984), Hamburger (1960), Harrison (1969), Hertwig (1900, 1901), J. Huxley and de Beer (1934), Jenkinson (1909, 1917), Just (1939), Kumé and Dan (1968), Lash and Whittaker (1974), Lillie (1919), Løvtrup (1974), Metz and Monroy (1985), Moore (1972*a*, 1972*b*), Morgan (1897, 1901, 1927, 1934), Nakamura and Toivonen (1970), Needham (1931, 1942), Nieuwkoop *et al.* (1984), Preyer (1885), Przibram (1908), Raven (1958, 1959, 1961), *Reverberi (1971), Rose (1970), Sawyer and Showman (1985), Saxen and Toivonen (1962), Schleip (1929), Slack (1983), *Spemann (1938), Waddington (1952, *1956, 1962), Weismann (1891–1892, 1893), Weis (1939, 1968), Wilkins (1986), Wigglesworth (1959), Willier *et al.* (1955), Wilson (1900, 1928).

Papers, mainly those cited. Briggs and King (1952), Conklin (1897, 1905, 1933), Curtis (1962), Driesch (1892, 1894), Driesch and Morgan (1895), Flickinger (1966), Gurdon (1962, 1963), Gurdon and Brown (1965), Gurdon and Woodland (1968), Holtfreter (1933*a*, 1933*b*, 1936, 1968), Hörstadius (1939, 1973), McClendon (1910), Pasteels (1964), Pflüger (1884), Roux (1888), Spemann and Mangold (1924), Whitman (1878, 1887, 1888, 1894), Whittaker (1940), Willier and Oppenheimer (1974), Wilson (1892, 1893, 1894, 1895, 1896*a*, 1896*b*, 1898, 1904*a*, 1904*b*).

History and biography. Allen (1971, 1976, 1978, 1985), Churchill (1968, 1975), Clarke (1974), Coleman (1965), Ebert (1985), Gabriel and Fogel (1955), Gilbert (1978), Gross (1985), T. S. Hall (1951), Hamburger (1984), *Horder *et al.* (1986), Maienschein (1978, 1983, 1985), Monroy and Groeben (1985), Moore (1983, 1986), Olby (1972), *Oppenheimer (1967, 1970*b*, 1971*b*), Pi Suner (1956), Sandler and Sandler (1985), Waddington (1975), *Willier and Oppenheimer (1974), Woodger (1929, 1948).

PUTTING IT ALL TOGETHER

Or can we? There has been a general feeling that developmental biology, almost alone among the biological sciences, is yet to achieve a satisfactory conceptual coherence.

A generation ago, Joseph Needham (1959, p. 240) observed that

> . . . strictly evolutionary dominance in embryology did not last on into the twentieth century. The unfortunate thing is that nothing has so far been devised to put in its place. Experimental embryology, Morphological embryology, Physiological embryology, and Chemical embryology form today a vast range of factual knowledge, without one single unifying concept, for we cannot yet dignify the axial gradient doctrines, the field theories and the speculations on the genetic control of enzymes, with such a position. We cannot doubt that the most urgent need of modern embryology is a series of advances of a purely theoretical, even mathematico-logical, nature. Only by something of this kind can we redress the balance which has fallen over to observation and experiment; only by some such effort can we obtain a theoretical embryology suited in magnitude and spaciousness to the wealth of facts which contemporary investigators are accumulating day by day.

Needham did not accept his own challenge—he went on to his magnificent project of chronicling the history of science and technology in China.

The Biological Revolution that started in 1952 with Watson and Crick had little immediate large scale effect on developmental biology. According to Medawar (1965):

> Embryology is in some ways a model science. It has always been distinguished by the exactitude, even punctilio, of its anatomical descriptions. An experiment by one of the grand masters of embryology could be made the text of a discourse on scientific method. But something is wrong; or has been wrong. There is no *theory* of development, in the sense in which Mendelism is a theory that accounts for the results of breeding experiments. There has therefore been little sense of progression or timeliness about embryological research. Of many papers delivered at embryological meetings, however good they may be in themselves . . . one too often feels that they might have been delivered five years beforehand without making anyone much wiser, or deferred for five years without making anyone conscious of great loss.
>
> It has not always been so. In the 1930's experimental embryology had much the same appeal as molecular biology has today . . . [this] was mainly due to the 'organizer theory' of Hans Spemann, the theory that differentiation in development is the outcome of an orderly sequence of specific inductive stimuli . . . .

But efforts to discover the chemical properties of the organizer failed and

> Wise after the event, we can now see that embryology simply did not have, and could not have created, the background of genetical reasoning which would have made it possible to formulate a theory of development.

These rather dismal opinions of developmental biology should be taken less as an evaluation of the field and more as an evaluation of working scientists. What is already known to them, no matter how magnificent, is of lesser interest than the tantalizing unknown. For the working scientist the great discoveries are valued more for guiding new research than for synthesizing that of the past. This has been true for Darwinian evolution, Mendelian genetics, Morgan's genetics, and the capstone set by Watson and Crick.

Therefore, no matter what the discovery, there is always a burning need to understand biological phenomena at a more basic level. Reductionism is so powerful a force in biology that we tend to overlook the fact that we may have satisfying explanations at one level even though there is always more to be discovered at more "basic" levels. The discovery that blood circulates has proved to be a sufficient answer, even to this day, for many physiological and medical questions. The discovery of the heart's pacemaker enriches the basic discovery—it does not make it less adequate. Similarly we can accept the knowledge that light, expressed as day length, affects the breeding behavior and migration of birds without having to know whether light consists of particles, waves, or both.

I suspect that we know more about developmental biology than we are willing to admit and that Horder (Horder *et al.*, 1986, p. xvi) may have a wiser vision:

> The meaningless of this question ["What is Life?"] to us now makes one wonder what equivalent pseudo-questions may be influencing our priorities today. It may well be that the very idea of 'the unsolved problem of embryology' is one such; the phrase itself almost presupposes a particular form of solution and invites particular forms of research, tending to invoke images of a single, all-revealing experiment or discovery. In fact it may be the case that we already have the data we need to arrive at an understanding of embryology, and the approach to such a subject lies in the direction of integration and rearrangement of a complex of existing concepts.

What might we imagine a "basic theory

of development" to be? Would it be something as conceptually simple as Mendelian genetics or as that all atoms are composed of a nucleus, mainly of protons plus neutrons, with set numbers of electrons whirling about them?

I believe that we must accept the fact that there can be no simple theory of development, any more than there can be a simple theory to embrace all of anatomy, or physiology, or behavior—unless we can be satisfied with "Development is the functioning of genes in embryonic cells."

And that, of course, is what embryology is. But embryologists are interested primarily in the products of gene action, not in the gene themselves. A variant of this basic concept applies to all life. So one answer to Horder's question "What is Life" is "Life consists of the activities of genes in organized cellular and subcellular systems." I suspect that those who ask that question hope for a different answer—possibly along the lines of Spemann's belief quoted before—that life is not only a manifestation of matter and energy but that there is something "more." Possibly there is but so far it eludes the most sophisticated methods and machines of science.

A Conceptual Basis for Developmental Biology

An attempt will now be made to construct *a* (not *the*) conceptual framework for developmental biology. It will be based largely on the hypotheses and data of those "grand masters of embryology," as Medawar referred to them, as synthesized by E. B. Wilson (1928, especially chapters 13 and 14), plus a modicum of updating based on subsequent observations and experiments.

A conceptual framework has value in relation to its ability to arrange data and ideas in a comprehensible and comprehensive system of thought. It is a way of looking at the natural world and finding associations among the diverse and seemingly unrelated phenomena. A conceptual framework for developmental biology will help students organize the various facts and ideas relating to embryonic development and to see developmental biology in its relations to biology as a whole. Needless to say there are many possible conceptual schemes for developmental biology. None will be fully correct nor fully complete—nor is such possible. A conceptual framework has value even when incomplete, or even partly inaccurate, since it provides a base to explore new puzzles.

The following numbered statements are of various types. Some are concepts, strictly speaking, others are not. Some are so well established that they are listed almost without comment, whereas others are expanded. The first two groups of statements relate embryos to life in general and to the history of life.

Concepts Relating to Cells

1. *Embryos are cellular.* They are living systems and so exhibit the basic properties of life, one of which is to be composed of cells. Hence, what cells can do embryos can do.

2. *Cells are complexly organized systems that consist of many interdependent and interacting parts.* Basic to all cell activities is the genetic code of DNA.

3. *The life and reproduction of cells is controlled by DNA.* Cells have the ability to replicate themselves and their DNA, events associated with mitotic cell division.

4. *The replication of DNA, nearly always precise, is subject to occasional error, or mutation.* The near constancy of DNA replication preserves the adaptations that have been built into the genome over the ages. The existence of rare errors is the basis of new adaptive possibilities.

Concepts Relating to Evolutionary History

5. *The origin of new adaptations is a consequence of natural selection acting on inherited variations.* This is the principal mechanism of evolutionary change.

6. *Evolution has favored those organisms with mechanisms for reproduction.* Theoretically individual organisms might be immortal but the forces of evolution have ignored that option.

7. *Reproduction involves the transfer of a cellular portion of the parent's body to a new individual(s).* Thus genetic continuity is always associated with reproduction.

8. *Reproduction is under Malthusian control.* Every species has the theoretical possibility of increasing to a population of infinite size. The resources required for life, however, are not infinite. Nevertheless there is a tendency for each species to increase to the limits of the carrying capacity of its environment.

9. *This tension between the increase in population size* vs. *an environment with finite resources puts a selective advantage on those individuals that can exploit new environments or exploit old environments in new ways.* This is the origin of the diversity of life—of today as well as of the past.

10. *The evolution of multicellularity has been one of the most successful adaptations.* Since most organisms of more than microscopic dimensions are multicellular, it is reasonable to conclude that this is the only effective solution that evolution has been able to devise for large and complex organisms. Increased size and complexity are associated with new ways of obtaining the resources required for life. With many cells there is the opportunity for groups of them to become specialized for different functions—that specialization making them more efficient in performing some function essential for the whole organism. But specialization means the loss of the ability to perform all of the functions required for the life of individual cells and the need for the cells with one type of specialization to depend on those cells with other types of specialization. In large, complex, cellular organisms the function of individual cells is for the welfare of the individual as a whole, just as the function of the individual must be for the benefit of the cells themselves.

11. *The evolution of large, complex organisms consisting of numerous highly differentiated types of cells requires new patterns of reproduction.* In comparison, single-celled organisms reproduce by dividing in half, the chromosomes undergoing mitosis. The daughter cells then grow to full size. Hence in single-celled organisms cell division and their reproduction is the same—and the requirement for genetic continuity is met.

This is not possible for multicellular organisms. In order to reproduce they cannot just split in half like an amoeba. They have evolved two basic ways of transferring parts of their body to their offspring—asexual reproduction and sexual reproduction.

12. *The forces of evolution have put a premium on sexual reproduction.* This involves the development of special cells, the gametes, in which the parental genes are segregated and independently assorted in an almost infinite number of combinations. At the same time meiosis halves the number of chromosomes that carry the genes. The union of eggs and sperm, when derived from different individuals, results in a still greater variety of genetic types. Some of these new genetic types of individuals might be able to invade new environments or be better competitors in their old environment. If so, their numbers would increase.

13. *Asexual reproduction, that is without the fusion of gametes, involves the formation of a new individual on the parent body, followed by the detachment of the new individual.* In some species, fragmentation of the body is followed by the regeneration of each fragment to form a whole individual. In both cases rigorous genetic continuity results, since the cells of the offspring are identical with those of the parent. Species that reproduce solely by such methods are regarded as evolutionary "dead-ends," since there is no possibility of genetic recombination.

CONCEPTS RELATING TO DEVELOPMENT

14. *Since sexual reproduction by multicellular organisms results in the formation of a single-celled zygote, complex mechanisms for converting that zygote into the multicellular adult with its diversity of differentiated cell types are required.* Thus development is required. The principal events in development are an *increase in cell number,* the *rearrangement of cells,* and, finally their *differentiation* and association as tissues and organs.

15. *Mitotic cell division is the universal way that individuals increase the number of their cells.*

(Since this discovery was made so long ago, in the 1850s, it has become completely incorporated in our thought patterns. Had the discovery been made in 1900, it would

have been recognized as the embryological equivalent of Mendel's Law of Segregation—his First Law—and there would be fewer criticisms of embryology's lack of theory.)

16. *The embryo's cells become rearranged, usually drastically, and in their final positions become the primordia of the future structures.* There is essentially no variation in these cell movements in different embryos of the same species.

(Had these regularities been discovered in 1900, they would have been known as the Second Law of Development and note would have been made of the fact that this Second Law, plus the First, are as broadly applicable as Mendel's two Laws.)

CONCEPTS RELATING TO DIFFERENTIATION

Differentiation is the prime problem of developmental biology and a consideration of it will conclude this essay. Suggestions for a conceptual framework for this aspect of development will be based on these four axioms.

A. Cells are the biological units of structure and function.

B. Genes control the cellular syntheses and through them cell structure and function.

C. The gene-controlled cytoplasm can exert feedback control over gene activity.

D. Individual organisms are integrated systems that have overall control of their separate parts.

Axioms A and B are restatements of concepts 1–4 and are so well established that no more need be said about them.

Axioms C and D require explanation so far as their relations to ontogeny are concerned and they will be divided into several numbered concepts.

17. *The mature ovum is highly structured and has localized cortical and cytoplasmic determinants that largely control early development.*

Care must be taken here not to regard genes and cytoplasm as separate and antagonistic entities. They are two aspects of a functioning whole and totally dependent on one another. There is, however, a biological chain of command. The specificity of cells, organs, individuals, and species depends ultimately on the information encoded in their DNA. But the products of gene action—cellular substances and activities—may have feed-back control of the genes themselves. This cytoplasmic control is of enormous importance in early development.

Evidence for the importance of the cytoplasm accumulated in the late 19th century. A case that greatly influenced contemporary thought was Boveri's discovery that the chromosomes of the nematode, *Ascaris,* destined for the cells of the somatic tissues differ greatly from those destined for the gametes. Those of the germ line retain their form whereas those that will be in somatic cells fragment into many tiny chromosomes and, in fact, parts of the original chromosomes are eliminated (chromosomal diminution):

> By an ingenious study of centrifuged and double-fertilized eggs Boveri was able to establish the fact that the process of diminution is not an autonomous act on the part of the chromosomes but is induced by their cytoplasmic surroundings in the egg, a conclusion of fundamental importance for our general conceptions of development (E. B. Wilson, 1928, pp. 323–328).

A recognizably different cytoplasm, called the pole plasm, is present in a specific portion of the eggs of some insects. Nuclei that enter this zone are incorporated into cells that become gametes. If nuclei are prevented from entering the pole plasm, the embryos develop into adults that produce no gametes. It is possible to manipulate the nuclei to establish the fact that any nucleus forced to enter the pole plasm will become part of a gamete (E. B. Wilson, 1928, pp. 320–322).

Numerous examples are now known of the effects of the cytoplasm on the nucleus but, for our purposes, a dramatic example provided by Gurdon and Brown (1965) for the frog *Xenopus* will suffice. They studied the production of ribosomal RNA in early development. Essentially none is produced before gastrulation but thereafter the rate of synthesis increases rapidly. One can

interpret this to mean that the rRNA genes are "turned off" before gastrulation and "turned on" thereafter. Using the techniques of nuclear transfer they removed an rRNA synthesizing nucleus from a neurula and injected it into an enucleated uncleaved ovum. Development began and the question was "Will the nucleus continue to synthesize rRNA or will its genes be turned off and synthesize none?"

One group of experimental embryos was allowed to develop to blastulae and then the amount of their rRNA measured. None had been synthesized. Another group of embryos was allowed to develop to the neurula stage and then their rRNA production measured. These had resumed rRNA production.

Thus the neurula nuclei had, in the cytoplasm of an early embryo, behaved as a nucleus in a normal early embryo. Then at the normal time—the cytoplasm's normal time—rRNA production began. We could say that those turned-on genes of the neurula were turned off by the cytoplasm of the early embryo and turned-on again at the normal time (see also Gurdon and Woodland, 1968, for more examples).

In mature ova and early embryonic cells the molecules responsible for the basic organization are situated mainly in the cortex. When we recall that early development is striking in the constancy of its events, it is not surprising that organization is built into the relatively stable cortex compared with the more fluid cytoplasm.

There is evidence of some organization in the more fluid cytoplasm as well. Wilson's observations on the determinants of the apical tuft of *Dentalium* suggest most strongly that the determinants were located first near the vegetal pole and then, in a few cleavages, were localized in cells near the animal pole. It would be hard for such shifts to occur in the cortex.

Most of the data, however, indicate that the determinants are to be found in the cortex. Those strikingly different pigmented areas of the cortex of *Crepidula* and other ova are so closely associated with the formation of specific embryonic structures that one suspects that they are at least markers, and may be the determinants in some cases.

The surprising results obtained by centrifuging eggs pointed to the importance of the cortex. Fertilized eggs could be centrifuged until the cytoplasm was divided into layers of materials differing in density—all the yolk granules at the bottom and all the oil drops at the top, for example. Nevertheless such embryos developed normally or almost so. However, when greater centrifugal force was used, enough to disrupt the pattern of the cortex, then abnormalities were observed.

The importance of the cortex was emphasized by Just (1939) and dramatically demonstrated by Curtis (1962), who showed that the cortex of the gray crescent of the amphibian, *Xenopus,* could be transplanted and induce a secondary embryo. Thus the determinants, or the primary organizer, that Spemann and Mangold found in the dorsal lip are already present well before the onset of gastrulation (see also Pasteels, 1964).

Cytoplasmic localization was well known to the grand masters of embryology but this was not always understood by others. After 1900 the rapid rise of genetics, compared with the measured tread of embryology, left many biologists with the opinion that cells, especially those of embryos, were somewhat leaky bags of assorted molecules awaiting instructions from the genes. New discoveries found the genes doing more and more things and soon nothing seemed to be left for the cytoplasm. Thus what was clear to E. B. Wilson and others by the early 1890s ceased to be part of a general theory of development.

Part of the problem was the apparent simplicity of ova. There is no question but that many mature ova appear to be "simple cells." Apart from a few mosaic eggs, with their visibly differentiated cortex, ova seem to consist of a cell membrane, a nucleus, assorted granules, oil droplets, and yolk granules, all uniformly distributed throughout the cell. There was little to compare with the complexity of the highly differentiated cells of adult organs and tissues. Many protozoans appeared to be more

complex than ova. Since these ova looked simple, researchers felt that they must be simple.

Simple ova made for complex problems so far as a hypothesis for differentiation was concerned. What mechanisms could convert a simple, undifferentiated, homogeneous, generalized egg into an embryo? It was hard to see how novelty could arise from such a beginning (our old problem with epigenesis again). One could imagine mitotic cell division dividing that simple cell into a ball of identical simple cells. Once there was a ball—a solid blastula, perhaps—there would be the possibility of an external stimulus. Some of the cells would be on the outside and others on the inside. One might suspect that those two regions would be stimulated in different ways. The outer cells would have a better oxygen supply and the possibility of eliminating carbon dioxide and other wastes more readily. Finally, if that ball of cells dropped to the ocean floor and stuck, there would be a top and bottom. One could imagine the formation of an individual with a structure similar to Haeckel's *Olynthus.* Our organism would be radially symmetrical, differing in latitude but not longitude.

18. *The organization of the ovum is largely determined by stimuli from without.* It appears that the mature ova of all animal species are organized to a considerable degree by the time of ovulation. This basic organization is established under the influence of maternal genes. While the ova are in the ovary they are not isolated individuals. They are cells of the mother's body, formed from preexisting maternal cells, and supplied with the requisites for life. Since ova are cells of the adult female we should find it no more of a problem to accept that they are organized than to accept that the mother's neurons, kidney cells, or cells of the Islets of Langerhans are highly organized.

If we accept that the ovum has already taken many steps along the route of becoming a new individual during oogenesis, a difficult technical problem emerges for the developmental biologist—ovarian eggs cannot be manipulated with the same ease as early embryos of amphibians, sea urchins, or ctenophores. Hence clues had to be sought through correlations between the organization of the oocyte and external conditions. Many were found. For example, in many species of marine invertebrates the basic polarity—the animal pole–vegetal pole axis—is determined by the position of the egg in the ovary. Another example comes from insects, many of which have elongate ova and the long axis of these often parallels the main axis of the adult body.

The egg that seems to have the least organization at the time of ovulation is that of the marine alga, *Fucus* (D. M. Whittaker, 1940). Almost immediately, however, a protuberance appears on the undivided egg and at first cleavage the egg divides into two unequal cells (Fig. 58). The fate of these two cells is established at this time. The larger cell becomes the thallus and the one with the protuberance becomes the rhizoid. So far as Whittaker could tell, the formation of the protuberance, which sets future development, is due to some external influence. He suspected this when he noticed that in groups of cells the protuberance formed inward, as shown in Figure 58. This suggested that maybe the concentration of some substance produced by the cells was the stimulus. In addition, tests of various environmental components, such as pH, light, and temperature, showed that the site of the protuberance could be manipulated at will (Fig. 58).

By experimental means, therefore, a fundamental step in differentiation could be controlled. Genes that happen to be allocated to the cell with the protuberance will become active in the formation of the rhizoid. Those entering the other cell at the first mitotic division will participate in the formation of the thallus. It would appear, therefore, that what genes do can be affected by external factors working through the cytoplasm in which they function.

Another example of external stimuli affecting the organization of the early embryo relates to the origin of bilaterality. This is the reason why those observations of Newport, Roux, and others on the

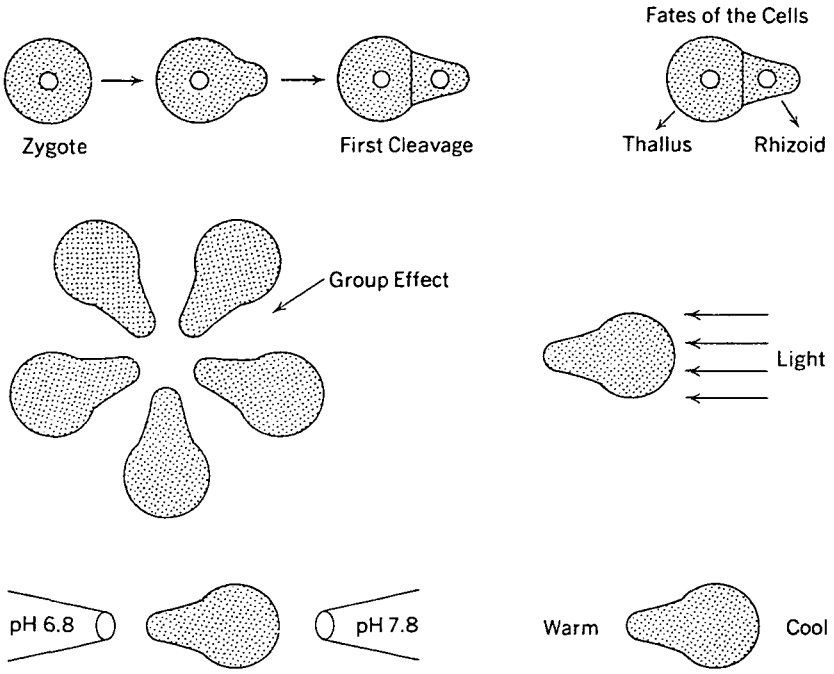


FIG. 58. The early development of *Fucus* and some of the factors that influence the formation of the protuberance.

entrance point of the sperm were so electrifying. Here was a stimulus that not only determined the position of a gray crescent but also marked the plane of first cleavage, the position where the dorsal lip was to appear, and finally the anterior–posterior axis of the embryo and adult.

19. *Each cell receives a complete set of genes and different ones of these are expressed in different ways in different embryonic cells, controlled in part by specific cytoplasmic molecules of both cortical and non-cortical regions.*

The Roux-Weismann hypothesis of qualitative nuclear division was short lived and most of the grand masters came to accept the hypothesis that all cells receive the same set of genes—and what the cells did with them in very early development depended largely on the cytoplasm. This hypothesis was hard to prove because one could not at that time study the genetics of somatic cells.

Nevertheless the indirect evidence was fairly good. The data on regeneration of planarians and hydroids seemed to indicate that all cells retained the full genetic capability of the species.

The isolation of blastomeres of regulative eggs showed that a full set of genes goes to each cell, at least for the first few cleavages. When differences among the chromosomes of somatic cells was recognized, it became possible to trace them throughout successive mitotic divisions and find that all somatic cells have the same set of chromosomes. This individuality of the chromosomes (III, pp. 653–657) was evidence that all cells are genetically equivalent.

None of these earlier investigations was wholly convincing and it was not until Briggs and King (1952) and later Gurdon (1962) perfected methods for transferring nuclei from older embryos and differentiated cells that better data were available. The technique is shown in Figure 59. Cells from a blastula, or a later embryo, or even an adult can be dissociated and then injected into an enucleated ovum. Normal development occurs in varying percentages of the cases, depending on the source of the nuclei. In some cases sexually mature adults have been obtained from these fatherless embryos.

That even some nuclei from differen-

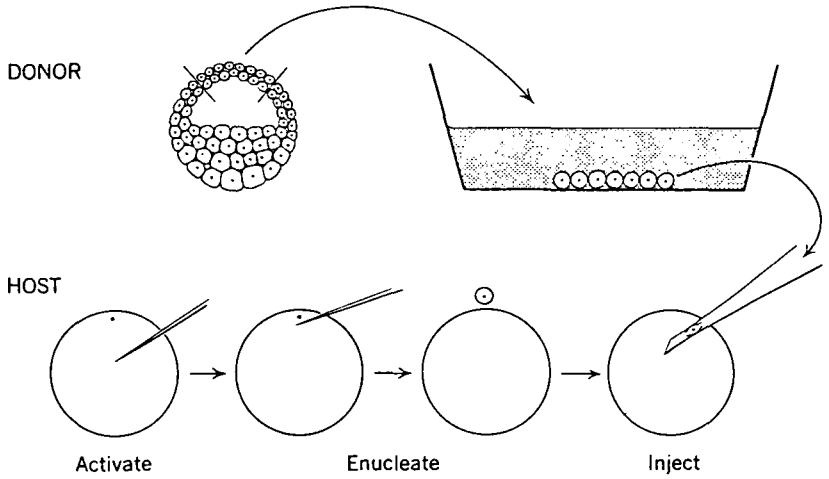


FIG. 59. The Briggs and King method of transferring nuclei. A piece of an older donor embryo, in this case the roof of the blastocoel, is removed and placed in a solution that causes the cells to disassociate. A donor egg, fresh from the uterus, is then activated by a jab with a glass needle and its nucleus removed by flicking it out with a glass needle. A single cell donor is drawn into a pipette and then injected into the host embryo. No sperm are involved and the host develops with the injected donor nucleus.

tiated cells have the ability to support normal development is taken as evidence that any nucleus can do so.

However, one cannot conclude from such results that all somatic nuclei are undifferentiated. One can only conclude that they are not irreversibly differentiated. It is beyond argument that the nuclei of differentiated cells are differentiated. The fact that an erythrocyte of a frog synthesizes hemoglobin, but not pepsinogen and a stomach cell synthesizes pepsinogen, but not hemoglobin shows that different genes are active in the two cell types.

20. *Embryos are integrated systems with the whole having overall control of the parts.* There are innumerable examples of the whole embryo or adult organism controlling its parts and the mechanisms are varied. Holtfreter's experiments on the transplantation of pieces of early gastrula ectoderm to older embryos and finding that each developed according to its surroundings is a fine example (Fig. 56). Hörstadius's combinations of different cell layers in *Paracentrotus* find their explanation less in the specific layers involved than in the portions of the gradients they contain. Each blastomere of the two-cell stage of amphioxus or *Echinus* has the capacity to produce an entire embryo but that capacity is restrained when each is part of a whole embryo.

The fate of an embryonic cell reflects its position in the whole embryo rather than its innate capacity.

Some of the more dramatic examples of the control of the whole over its parts come from experiments on regeneration. Consider the case of a planarian flatworm cut in half—across the long axis of its body. Each half will undergo an extensive reorganization and produce a complete planarian. The events in regeneration can be explained by assuming the presence of a gradient (Child, 1941). The "high" point of the gradient is the head end and there is a gradual decline in its effect until we reach the tail.

When the body is cut crosswise the anterior half will regenerate a tail at its hind end. The posterior half will regenerate a head at its front end. The cells at the posterior end of the anterior half and the cells at the anterior end of the posterior half were adjacent before they cut so they should have been as similar to one another as is possible to imagine. Nevertheless their fates in regeneration are entirely different. The conclusion is inescapable that the newly regenerating whole is controlling what happens in its parts.

That last statement reflects a truly extraordinary biological phenomenon. Let us consider some of the implications. A planarian when cut begins to regenerate and stops when its body is complete. What stops this regeneration? Why does it not continue as a cancerous growth forever? Each fragment must have the complete information on "How to make a whole planarian" and also a mechanism to shut off regeneration when the complete body has been formed. In the case of planarians the marvel is not only that the lost part is restored but that each fragment is totally reformed. The entire structure is altered in each fragment so that at the end of regeneration a perfect, though small, planarian is the result. (There is no feeding or growth during this period.)

THE BASIC PATTERN OF ONTOGENY

These 20 concepts can provide a framework to organize the data of developmental biology.

At the time of fertilization the ovum is a highly structured system with the determinants for the early stages of development arranged in a definite order, mainly in the egg cortex. The ovum was part of the adult female and its genetically determined organization was laid down during oogenesis.

This system is set in operation by the entrance of a monoploid sperm and its fusion with the monoploid egg nucleus.

The diploid zygote undergoes a series of cleavages that divide not only the original cytoplasm of the ovum but also the cortex into a number of cells. Mitosis gives each cell a full set of genes but they will come to occupy cells that differ in their specific cytoplasms and differ especially in their cortex.

These cytoplasmic localizations and cortical differences among the cells result in different genes being activated in different cells. This is the initial cause of cell differentiation.

Once the embryonic cells have begun to differentiate, the interactions among them will lead to further differentiations.

The position of cells, from the earliest stages, determine their fate—that is, the structures they will form. Every portion of the later embryo is to be found in a specific location in the embryos of earlier stages.

A rearrangement of cells occurs during gastrulation and at its close the cells are in groups that will then differentiate into the structures in an exact manner. This differentiation often involves the control of one group of cells by another, as in the amphibian organizers.

At the time of fertilization the eggs of some species have pronounced regional differences of cortex and cytoplasm. These are the mosaic eggs with their determined parts which, when isolated, will usually self-differentiate. By contrast, the cells of the regulative species are less determined. In any event even these eventually reach the mosaic stage.

Even in the most strictly mosaic species, however, the period of strict determination may be transitory. For example we find that annelids and mollusks, which have highly mosaic eggs, regain a high degree of regulative ability when they are adults. Then they can regenerate whole bodies from parts.

Thus we can account for differentiation by assuming that the genes of the zygote find themselves in an environment where different genes are activated by cortical and cytoplasmic molecules that were, themselves, produced and ordered under the influence of the maternal genes. Differentiation is not just a matter of genes and cortex and cytoplasm interacting with each other but also of the genes of one generation controlling those of the next generation during the period of very early development.

I believe that we do have a conceptual framework that will account for embryonic development. Some of the basic concepts cannot be rigorously defined—the control of the whole over the parts, for example—but there is no doubt whatsoever that such control exists.

Thus the grand masters have left us a framework that allows us to comprehend what has been discovered and that can serve as a basis for further analysis at the level of cell and organism. It can, as well, serve to extend the analysis to the molecular level.

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REFERENCES

Adelmann, Howard B. 1942. *The embryological treatises of Hieronymus Fabricius of Aquapendente.* 2 vols. Cornell Univ. Press, Ithaca.

Adelmann, Howard B. 1966. *Marcello Malpighi and the evolution of embryology.* 5 vols. Cornell Univ. Press, Ithaca.

Agassiz, Louis. 1849. *Twelve lectures on comparative embryology.* Henry Flanders, Boston.

Agassiz, Louis. 1859. *An essay on classification.* Longmans *et al.*, London. Reprinted 1962 by Harvard Univ. Press, Cambridge.

Alberts, Bruce *et al.* 1983. *Molecular biology of the cell.* Garland, New York.

Allen, G. E. 1971. Edwin Grant Conklin. Dictionary Scientific Biography 3:389–391. Scribners, New York.

Allen, G. E. 1976. Edmund Beecher Wilson. Dictionary Scientific Biography 14:423–436.

Allen, Garland E. 1978. *Thomas Hunt Morgan: The man and his science.* Princeton Univ. Press, Princeton.

Allen, G. E. 1985. Heredity under an embryological paradigm: The case of genetics and embryology. Biol. Bull 168(suppl.):107–121.

Aristotle. [1910]. *The works of Aristotle.* Volume IV. *Historia animalium.* Clarendon Press, Oxford. (Thompson trans.)

Aristotle. [1912]. *The works of Aristotle.* Volume V. *De Partibus animalium. De motu. De incessu animalium. De generatione animalium.* Clarendon Press, Oxford. (Ogle, Farquharson, and Platt trans.)

Aristotle. [1928]. *The works of Aristotle.* Volume VIII. *Metaphysica.* Clarendon Press, Oxford. (Ross trans.)

Aristotle. [1943]. *Generation of animals.* Loeb Classical Library. Harvard Univ. Press, Cambridge. (Peck trans.)

Aristotle. [1955]. *Parts of animals. Movement of animals. Progression of animals.* Loeb Classical Library. Harvard Univ. Press, Cambridge. (Peck and Forster, trans.)

Aristotle. [1957]. *On the soul. Parva naturalia. On breath.* Loeb Classical Library. Harvard Univ. Press, Cambridge. (Hett trans.)

Aristotle. [1965–1970]. *Historia animalium.* 2 vols. Loeb Classical Library. Harvard Univ. Press, Cambridge. (Peck trans.)

Aristotle. [1972]. *Aristotle's De Partibus Animalium I and De Generatione Animalium I.* Clarendon Press, Oxford. (Balme trans.)

Artz, Frederick, B. 1965. *The mind of the Middle Ages: A.D. 200–1500.* 3rd ed. Alfred Knopf, New York.

Baer, Karl E. von. 1827. *De ovi mammalium et hominis genesi.* Leopold Vossi, Lipsiae. Reprinted 1966 by Culture et Civilisation, Bruxelles and by Sarton (1931). English trans. O'Malley (1956). See also Meyer, 1956.

Baer, Karl E. von. 1828. *Über Entwicklungsgeschichte der Thiere. Beobactung und Reflexion.* Borntrager, Königsberg. Reprinted 1967 by Culture et Civilisation, Bruxelles.

Baldry, H. C. 1932. Embryological analogies in presocratic cosmogony. Classical Quarterly 26:27–34.

Balfour, Francis M. 1880–1881. *A treatise on comparative embryology.* Macmillan, London. 2 vols. 2nd ed. 1885 "reprinted without alteration from the first edition."

Balss, H. 1936. Die Zeugungslehre und Embryologie in der Antike. Quellen u. Studien zur Gesch. der Naturw. u. der Med. 5:193–274.

Barth, Lucena J. 1964. *Development: Selected topics.* Addison-Wesley, Reading.

Bather, F. A. 1893. The recapitulation theory in palaeontology. Natural Science 2:275–281.

Baxter, A. L. 1976. Edmund B. Wilson as a preformationist: Some reasons for his acceptance of the chromosome theory. Jour. History of Biology 9:29–57.

Baxter, A. L. 1977. E. B. Wilson's "destruction" of the germ-layer theory. Isis 68:363–374.

Beatty, John L. and Oliver Johnson. 1977. *Heritage of western civilization.* 4th ed. Prentice-Hall, Englewood Cliffs.

de Beer, Sir Gavin. 1958. *Embryos and ancestors.* Clarendon Press, Oxford.

Bekker, Immanuel. 1831–1871. *Aristotelis opera, edidet Academia regia borussica.* Reimer, Berlin.

Belloni, L. 1974. Marcello Malpighi. Dictionary Scientific Biography 9:62–66. Scribners, New York.

Bernardini, G. and L. Fermi. 1965. *Galileo and the Scientific Revolution.* Fawcet Premier, New York.

Berrill, N. J. 1971. *Developmental biology.* McGraw-Hill, New York.

Bertalanffy, Ludwig von. 1933. *Modern theories of development. An introduction to theoretical biology.* Oxford Univ. Press, London.

Birch, Thomas. [1756–1757]. *The History of the Royal Society of London.* A. Millar, London. Reprinted 1968 by Johnson Reprints, New York.

Bodenheimer, Frederick S. 1958. *The history of biology: An introduction.* Dawson, London.

Bonner, John T. 1963. *Morphogenesis: An essay on development.* Atheneum, New York.

Bonner, John T. (ed.) 1962. *Evolution and development.* Springer-Verlag, New York.

Bonnet, Charles. 1770. *La palingénésie philosophique.* Philibert and Chirol, Geneva.

Bowler, P. J. 1971. Preformation and pre-existence in the seventeenth century: A brief analysis. Jour. History Biol. 4:221–244.

Bowler, P. J. 1973. Bonnet and Buffon: Theories of generation and the problem of species. Jour. History Biol. 6:259–281.

Boyle, R. 1666. Of preserving birds taken out of the egge, and other small faetus's. Phil. Trans. Royal Soc. 1:199–201.

Brachet, Jean. 1950. *Chemical embryology.* Interscience, New York.

Brachet, Jean. 1957. *Biochemical cytology.* Academic Press, New York.

Brachet, Jean and A. Mirsky. 1959. *The cell: Biochemistry, physiology, morphology.* Vol. 1. Academic Press, New York.

Brainerd, Charles J. 1978. *Piaget's theory of intelligence.* Prentice-Hall, Englewood Cliffs.

Braudel, Fernand. 1985. *The structure of everyday life.* Vol. 1. Harper & Row, New York.

Brehaut, Ernest. 1912. *An encyclopedist of the Dark Ages: Isidore of Seville.* Columbia Univ. Press, New York. Reprinted by Burt Franklin, New York.

Briggs, R. and T. J. King. 1952. Transplantation of living nuclei from blastula cells into enucleated frog's eggs. Proc. Nat. Acad. Sci. 38:455–463.

Burns, Edward M. *et al.* 1986. *World civilizations.* 2 vols. 7th ed. Norton, New York.

Butterfield, Herbert. 1957. *The origins of modern science: 1300–1800.* Free Press, New York.

Bylebyl, J. 1972. William Harvey. Dictionary Scientific Biography 6:150–162. Scribners, New York.

Campanella, Thomas. [1616]. *The defense of Galileo.* Reprinted by Arno Press, New York. 1975.

Castellani, C. 1973. Spermatozoan biology from Leeuwenhoek to Spallanzani. Jour. History Biol. 6:37–68.

Cherniss, H. 1951. The characteristics and effects of presocratic philosophy. Jour. History Ideas 12: 319–345.

Child, Charles M. 1941. *Patterns and problems of development.* Univ. Chicago Press, Chicago.

Churchill, F. B. 1968. August Weismann and the break from tradition. Jour. History Biology 1:91–112.

Churchill, F. B. 1970*a*. The history of embryology as intellectual history. Jour. History Biol. 3:155–181.

Churchill, F. B. 1970*b*. Francis Maitland Balfour. Dictionary Scientific Biography 1:420–422. Scribners, New York.

Churchill, F. B. 1975. Wilhelm Roux. Dictionary Scientific Biography 11:570–575.

Churchill, F. B. 1986. Weismann, Hydromedusae, and the biogenetic imperative: A reconsideration. *In* T. J. Horder *et al.* (eds.), *A history of embryology.* Cambridge Univ. Press, New York.

Clarke, E. 1974. George Newport. Dictionary Scientific Biography 10:39–40. Scribners, New York.

Cole, Francis J. 1930. *Early theories of sexual generation.* Oxford Univ. Press, Oxford.

Coleman, W. 1965. Cell, nucleus and inheritance: An historical study. Proc. Amer. Philosophical Soc. 109:124–158.

Conklin, E. G. 1897. The embryology of Crepidula, a contribution to the cell-lineage and early development of some marine gasteropods. Jour. Morph. 13:1–226.

Conklin, E. G. 1905. The organization and cell-lineage of the ascidian egg. Jour. Acad. Nat. Sci. Philadelphia. 2nd ser. 13:1–119.

Conklin, E. G. 1924. Cellular differentiation. *In* Edmund V. Cowdry (ed.), *General cytology: A textbook of cellular structure for students of biology and medicine.* Univ. Chicago Press, Chicago.

Conklin, E. G. 1933. Mosaic *vs.* equipotential development. Amer. Nat. 67:289–297.

Cooke, James. 1762. *A new theory of generation, according to the best and latest discoveries in anatomy, further improved and fully displayed.* London.

Corner, George W. 1958. *Anatomist at large. An autobiography and selected essays.* Basic Books, New York.

Cornford, F. M. 1930. Anaxagoras' theory of matter. I, II. Classical Quarterly 24:14–30, 83–95.

Cornford, Francis M. 1952. *Principium sapientiae: The origins of Greek philosophical thought.* University Press, Cambridge.

Cornford, Francis M. 1957. *From religion to philosophy: A study in the origins of Western speculation.* Harper & Row, New York.
Cremonini, Cesare. 1596. *Explanatio proemii libororum Aristotelis de physico auditu.* Patavii (Padua).
Crombie, Alistair C. 1952. *Augustine to Galileo: The history of science, A.D. 400–1650.* Falcon Press, London.
Crombie, Alistair C. 1953. *Robert Grosseteste and the origins of experimental science.* Oxford Univ. Press, Oxford.
Crombie, Alistair C. 1959. *Medieval and early modern science.* 2 vols. Doubleday, Garden City.
Crombie, Alistair C. (ed.) 1963. *Scientific change.* Heinemann, London.
Cruikshank, W. 1797. Experiments in which, on the third day after impregnation, the ova of rabbits were found in the fallopian tubes; and on the fourth day after impregnation in the uterus itself, with the first appearances of the foetus. Phil. Trans. Royal Soc. London 87:196–214.
Curtis, A. S. G. 1962. Morphogenetic interaction before gastrulation in the amphibian *Xenopus laevis*—the cortical field. Jour. Embryol. Exp. Morphol. 10:410–422.
Dalcq, Albert M. 1938. *Form and causality in early development.* Cambridge Univ. Press, Cambridge.
Dalcq, Albert M. 1957. *An introduction to general embryology.* Oxford Univ. Press, London.
Dampier, Sir. W. C. 1929. *A history of science and its relation with philosophy and religion.* Cambridge Univ. Press, Cambridge.
Darwin, Charles. 1859. *On the origin of species by means of natural selection.* John Murray, London.
Davenport, Charles B. 1908. *Experimental morphology.* Macmillan, New York.
Davidson, Eric H. 1986. *Gene activity in early development.* 3rd ed. Academic Press, New York.
Davidson, Eric H. and R. A. Firtel (eds.) 1984. *Molecular biology of development.* Alan R. Liss, New York.
DePomerai, David. 1985. *From gene to animal: An introduction to the molecular biology of animal development.* Cambridge Univ. Press, New York.
Dicks, D. R. 1959. Thales. Classical Quarterly N.S. 9:294–309.
Dickson, D. 1986. Was Galileo saved by plea bargain? Science 233:612–613.
Di Gregorio, Mario A. 1894. *T. H. Huxley's place in natural science.* Yale Univ. Press, New Haven.
Downey, Glanville. 1962. *Aristotle: Dean of early science.* Franklin Watts, New York.
Drake, Stillman. 1957. *Discoveries and opinions of Galileo.* Doubleday, Garden City.
Drake, Stillman. 1970. *Galileo studies: Personality, tradition, and revolution.* Univ. Michigan Press, Ann Arbor.
Driesch, H. 1892. Entwicklungsmechanische Studien. I. Der Werth der beiden ersten Furchungzellen in der Echinodermenentwicklung. Zeit. wissenschaft. Zool. 53:160–178.
Driesch, H. 1894. *Analytische Theorie der organischen entwicklung.* Wilhelm Engelmann, Leipzig.
Driesch, H. and T. H. Morgan. 1895. Zur Analyse der ersten entwicklungsstadien des ctenophoreneises. I. Von der Entwicklung einzelner Ctenophorenblastomeren. Arch. entwicklungsmech. org. 2:204–215.
Duellman, William and Linda Trueb. 1986. *Biology of the amphibians.* McGraw-Hill, New York.
Düring, I. 1966. *Aristoteles, Darstellung und Interpretation seines Denken.* Carl Winter, Heidelberg.
Dürken, Bernhard. 1932. *Experimental analysis of development.* Norton, New York.
Ebert, J. E. 1985. Cell interactions: The roots of a century of research. Biol. Bull. 168(suppl.):80–87.
Edelman, Gerald M. (ed.) 1985. *Molecular determinants of animal form.* Alan R. Liss, New York.
Eiseley, Loren. 1958. *Darwin's century: Evolution and the men who discovered it.* Doubleday, Garden City.
Fabricius. See Adelmann, 1942.
Farley, John. 1977. *The spontaneous generation controversy from Descartes to Oparin.* Johns Hopkins Univ. Press, Baltimore.
Farley, John. 1982. *Gametes and spores: Ideas about sexual reproduction, 1750–1914.* Johns Hopkins Univ. Press, Baltimore.
Farrington, Benjamin. 1949. *Greek science. I. Thales to Aristotle. II. Theophrastus to Galen.* Penguin Books, Harmondsworth.
Flavell, John H. 1985. *Cognitive development.* 2nd ed. Prentice-Hall, Englewood Cliffs, NJ.
Flickinger, R. A. 1966. *Developmental biology: A book of readings.* Wm. C. Brown, Dubuque.
Frank, Robert G., Jr. 1980. *Harvey and the Oxford physiologists; scientific ideas and social interaction.* Univ. California Press, Berkeley.
Frankfort, Henri *et al.* 1977. *The intellectual adventure of ancient man.* Univ. Chicago Press, Chicago.
Gabriel, Mordecai L. and S. Fogel. 1955. *Great experiments in biology.* Prentice-Hall, Englewood Cliffs.
Gaissinovitch, A. E. 1978. Caspar Friedrich Wolff. Dictionary Scientific Biography 15:524–526. Scribners, New York.
Galen. [1916]. *On the natural faculties.* Loeb Classical Library. Harvard Univ. Press, Cambridge.
Galen. [1968]. *On the usefulness of the parts of the body.* 2 vols. Cornell Univ. Press, Ithaca.
Galilei, Galileo. 1615. Letters to the Grand Duchess Christina. See Drake (1957) or Beatty and Johnson (1977) vol. 2., pp. 42–58.
Gardner, Eldon J. 1965. *History of biology.* 2nd ed. Burgess, Minneapolis.
Garstang, W. 1922. The theory of recapitulation: A critical restatement of the biogenetic law. Jour. Linn. Soc. London, Zool. 35:81–101.
Gasking, Elizabeth B. 1967. *Investigations into generation 1651–1828.* Johns Hopkins Univ. Press, Baltimore.
Gasking, Elizabeth B. 1970. *The rise of experimental biology.* Random House, New York.
Gautier d'Agoty, Jacques. 1750. *Zoogénésie, ou génération de l'homme et des animaux.* Paris.
George, T. N. 1933. Palingenesis and paleontology. Biol. Rev. 8:107–135.
Gilbert, S. F. 1978. The embryological origins of the gene theory. Jour. History Biol. 11:307–351.
Giudice, Giovanni. 1973. *Developmental biology of the sea urchin embryo.* Academic Press, New York.
Giudice, Giovanni. 1986. *The sea urchin embryo: A*

developmental biological system. Springer-Verlag, New York.

Glass, B. 1959. Maupertius, pioneer of genetics and evolution. *In* Bentley Glass, O. Temkin, and W. L. Strauss, Jr., *Forerunners of Darwin 1745–1859.* Johns Hopkins Press, Baltimore.

Goldschmidt, Richard B. 1955. *Theoretical genetics.* Univ. California Press, Berkeley.

Goss, Richard J. 1969. *Principles of regeneration.* Academic Press, New York.

Gould, Stephen J. 1977. *Ontogeny and phylogeny.* Harvard Univ. Press, Cambridge.

de Graaf, René. 1672. *De mulierum organis generationi inservientibus tractatus novus, demonstrans tam homines et animalia, caetera omnia quae vivipara dicuntur, haud minus quam ovipara, ab ovo originem ducere.* Leiden.

Graham, C. F. and P. E. Wareing (eds.) 1984. *Developmental control in animals and plants.* Blackwell Scientific Pub. Co., Palo Alto.

Grene, Margorie. 1963. *A portrait of Aristotle.* Univ. Chicago Press, Chicago.

Gross, P. R. 1985. Laying the ghost: Embryonic development in plain words. Biol. Bull. 168(suppl.):62–79.

Gurdon, J. B. 1962. Adult frogs derived from nuclei of single somatic cells. Dev. Biol. 4:256–273.

Gurdon, J. B. 1963. Nuclear transplantation in amphibia and the importance of stable nuclear changes in promoting cellular differentiation. Quart. Rev. Biol. 38:54–78.

Gurdon, J. B. and D. D. Brown. 1965. Cytoplasmic regulation of RNA synthesis and nucleolus formation in developing embryos of *Xenopus laevis.* Jour. Mol. Biol. 12:27–35.

Gurdon, J. B. and H. R. Woodland. 1968. The cytoplasmic control of nuclear activity in animal development. Biol. Rev. 43:233–267.

Guthrie, William K. C. 1962. *A history of Greek philosophy.* Vol. 1. Cambridge Univ. Press, Cambridge.

Haeckel, Ernst. 1866. *Generelle Morphologie der Organismen: Allgemeine Grundzüge der organischen Formen-wissenschaft, mechanisch begründet durch die von Charles Darwin Reformirte Descendenz-Theorie.* Reimer, Berlin.

Haeckel, Ernst. 1868. *Naturliche Schöpfunggeschichte.* Reimer, Berlin. For translation see 1876.

Haeckel, Ernst. 1874. *Anthropogenie: Keimes- und Stammes-Geschichte des Menschen.* Engelmann, Leipzig. For translation see 1905*a, b.*

Haeckel, Ernst. 1876. *The history of creation.* 2 vols. Appleton, New York. Translation of 1868.

Haeckel, Ernst. 1905*a. The evolution of man.* 2 vols. Putnam's, New York.

Haeckel, Ernst. 1905*b. The evolution of man.* Eckler, New York. There are many differences, mainly in illustrations and tables, between this and the above. Translation of 8th edition of *Anthropogenie.*

Hall, Alfred R. 1954. *The Scientific Revolution 1500–1800.* Beacon Press, Boston.

Hall, Thomas S. 1951. *A source book in animal biology.* McGraw-Hill, New York.

von Haller, Albrecht. 1767. *Opera anatomica minora.* Vol. 2. *Ad generationem.* Grasset, Lausanne.

Hamburger, Viktor. 1960. *A manual of experimental embryology.* Rev. ed. Univ. Chicago Press, Chicago.

Hamburger, V. 1984. Hilda Mangold, co-discoverer of the organizer. Jour. History Biology 17:1–11.

Hamburger, V. and H. L. Hamilton. 1951. A series of normal stages in the development of the chick embryo. Jour. Morphol. 88:49–92.

Hamilton, Vernon and M. D. Vernon (eds.) 1976. *The development of cognitive processes.* Academic Press, New York.

Harrison, Ross G. 1969. *Organization and development of the embryo.* Yale Univ. Press, New Haven.

Hartsoeker, Nicolaas. 1694. *Essai de dioptrique.* Anisson, Paris.

Harvey, William. 1651. *Anatomical exercises on the generation of animals.* Reprinted in *The works of William Harvey.* Johnson Reprint Corp., New York. 1965. See next entry also.

Harvey, William. [1982]. *De generatione animalium.* Trans. by G. Witteridge. Blackwell, Oxford.

Haskins, Charles H. 1924. *Studies in medieval science.* Harvard Univ. Press, Cambridge. 2nd ed. 1927.

Haskins, Charles H. 1927. *The renaissance of the twelfth century.* Harvard Univ. Press, Cambridge.

Heniger, J. 1973. Antoni van Leeuwenhoek. Dictionary Scientific Biography 8:126–130.

Hertwig, Oscar. [1900]. *The biological problem of today: Preformation or epigenesis? The basis of organic development.* Macmillan, New York.

Hertwig, Oscar. 1901. *Text-book of embryology of man and the mammals.* 3rd ed. Macmillan, New York.

Highmore, Nathaniel. 1651. *The history of generation, examining several opinions of divers authors, especially that of Sir Kenelm Digby in his discourse of bodies.* J. Martin, London.

Hintzsche, E. 1972. (Victor) Albrecht von Haller. Dictionary Scientific Biography 6:61–67. Scribners, New York.

His, Wilhelm. 1874. *Unsere Körperform und das Physiologische Problem ihre Entstehung.* Vogel, Leipzig.

Holtfreter, J. 1933*a.* Der Einfluss von Wirtsalter und verschiedenen Organbezirken auf die Differenzierung von angelagertem Gastrulaektoderm. Wilhelm Roux' Archiv Entwicklungsmechanik 127:619–775.

Holtfreter, J. 1933*b.* Organisierungsstufen nach regionaler Kombination von Entomesoderm mit Ektoderm. Biol. Zentralblatt 53:404–431.

Holtfreter, J. 1936. Regionale Induktionen in xenoplastisch Zusammengesetzten Explantaten. Wilhelm Roux' Archiv Entwicklungsmechanik 134:466–549.

Holtfreter, J. 1968. The emergence of order in developing systems. Symp. Soc. Dev. Biol. 27:ix–xx.

Hooke, Robert. 1665. *Micrographica: Or some physiological descriptions of minute bodies made by magnifying glasses with observations and inquiries thereupon.* Martyn and Allestry, London. Reprinted 1971 by Dover, New York.

Hooykaas, R. 1972. *Religion and the rise of modern science.* Scottish Academic Press, Edinburgh.

Horder, Timothy J., J. A. Witkowski, and C. C. Wyle (eds.) 1986. *A history of embryology.* Cambridge Univ. Press, Cambridge.

Hörstadius, S. 1939. The mechanics of sea urchin development, studied by operative methods. Biol. Rev. 14:132–179.

Hörstadius, Sven. 1973. *Experimental embryology of echinoderms.* Clarendon Press, Oxford.

Huettner, Alfred F. 1941. *Fundamentals of comparative embryology of the vertebrates.* Macmillan, New York.

Huxley, Julian S. and G. R. de Beer. 1934. *The elements of experimental embryology.* Cambridge Univ. Press, Cambridge.

Huxley, T. H. 1849. On the anatomy and affinities of the family of the Medusae. Phil. Trans. Royal Soc. London 139:413–434.

Huxley, Thomas H. 1878. *A manual of the anatomy of invertebrated animals.* Appleton, New York.

Huxley, T. H. 1888. On the reception of the "Origin of Species." *In* Francis Darwin (ed.), *The life and letters of Charles Darwin,* vol. 2, pp. 179–204. John Murray, London.

Hyman, Libbie H. 1940. *The invertebrates: Protozoa through Ctenophora.* McGraw-Hill, New York.

Inhelder, Bärbel and J. Piaget. 1958. *The growth of logical thinking from childhood to adolescence.* Basic Books, New York.

Inhelder, Bärbel and J. Piaget. 1964. *The early growth of logic in the child.* Harper and Row, New York.

Jaeger, Werner. 1948. *Aristotle: Fundamentals of the history of his development.* 2nd ed. Clarendon Press, Oxford.

Jenkinson, J. Wilfred. 1909. *Experimental embryology.* Clarendon Press, Oxford.

Jenkinson, J. Wilfred. 1917. *Three lectures on experimental embryology.* Clarendon Press, Oxford.

Jones, William T. 1952. *A history of Western philosophy.* Harcourt, Brace, New York.

Just, Ernst. 1939. *The biology of the cell surface.* Technical Press, London.

Kaplon, Morton (ed.) 1965. *Homage to Galileo.* MIT Press, Cambridge.

Knowles, David. 1962. *The evolution of Medieval thought.* Random House, New York.

Kohlbrugge, J. H. F. 1911. Das biogenetische grundgesetz. Eine historische studie. Zool. Anz. 38:447–453.

Kölliker, Albert. 1861. *Entwicklungsgeschichte des Menschen und der höhern Thiere.* Wilhelm Engelmann, Leipzig.

Korschelt, E. and Karl Heider 1895. Text-book of embryology of the vertebrates.

Koyré, Alexandre. 1957. *From the closed world to the infinite universe.* Johns Hopkins Press, Baltimore.

Koyré, Alexandre. 1973. *The astronomical revolution: Copernicus, Kepler, Borelli.* Cornell Univ. Press, Ithaca.

Kottek, S. S. 1981. Embryology in Talmudic and Midrashic literature. Jour. History Biology 14: 299–315.

Kudlein, F. and L. G. Wilson. 1972. Galen. Dictionary of Scientific Biography 5:227–237.

Kumé, Matazo and K. Dan. 1968. *Invertebrate embryology.* NOLIT, Belgrade.

Lanham, Url. 1968. *Origins of modern biology.* Columbia University Press, New York.

Lankester, E. R. 1873. On the primitive cell-layers of the embryo as a basis of genealogical classification of animals, and on the origin of vascular and lymph systems. Ann. Mag. Nat. Hist. ser. 4, 11:321–338.

Lankester, E. R. 1877. Notes on the embryology and classification of the animal kingdom: Comprising a revision of speculation relative to the origin and significance of the germ-layers. Quart. Jour. Microscopic Sci. 17:399–454.

Lash, Jay and J. R. Whittaker (eds.) 1974. *Concepts in development.* Sinauer Associates, Stanford.

Lillie, Frank R. 1919. *Problems of fertilization.* Univ. Chicago Press, Chicago.

Locy, William A. 1915. *Biology and its makers.* 3rd ed. Holt, New York.

Locy, William A. 1925. *The growth of biology.* Holt, New York.

Lones, Thomas E. 1912. *Aristotle's researches in natural science.* West, Newman, London.

Longrigg, J. 1976. Thales. Dictionary Scientific Biography 13:295–298. Scribners, New York.

Lovejoy, Arthur O. 1936. *The Great Chain of Being: A study of the history of an idea.* Harvard Univ. Press, Cambridge.

Lovejoy, A. O. 1959. Recent criticisms of the Darwinian theory of recapitulation: Its grounds and its initiator. *In* Bentley Glass *et al.* (eds.), *Forerunners of Darwin: 1745–1859.* Johns Hopkins Press, Baltimore.

Løvtrup, Søren. 1974. *Epigenetics: A treatise on theoretical biology.* Wiley, New York.

Lurie, E. 1970. Jean Louis Rodolphe Agassiz. Dictionary Scientific Biography 1:72–74. Scribners, New York.

McClendon, J. F. 1910. The development of isolated blastomeres of the frog's egg. Amer. Jour. Anatomy 10:425–430.

McMullin, Ernan (ed.) 1967. *Galileo: Man of science.* Basic Books, New York.

Magner, Lois N. 1979. *A history of the life sciences.* Dekker, New York.

Maienschein, J. 1978. Cell lineage, ancestral reminiscence, and the biogenetic law. Jour. History Biol. 11:129–158.

Maienschein, J. 1983. Experimental biology in transition: Harrison's embryology, 1895–1910. Studies History Biol. 6:107–127.

Maienschein, J. 1985. First impressions: American biologists at Naples. Biol. Bull. 168(suppl.):187–191.

Maienschein, J. 1986. Preformation or new formation—neither or both? *In* Horder *et al.,* 1986, pp. 73–108.

Malebranche, Nicolas. 1672. *De la recherche de la vérité.* André Pralard, Paris.

Malpighi. See Adelmann, 1966.

Mason, S. F. 1953. *A history of the sciences: Main currents of scientific thought.* Routledge and Kegan Paul, London.

Mayr, Ernst. 1982. *The growth of biological thought: Diversity, evolution, and inheritance.* Harvard Univ. Press, Cambridge.

Mazzeo, J. A. 1967. *The design of life: Major themes in the development of biological thought.* Pantheon, New York.

Medawar, P. B. 1965. A biological retrospect. Nature 207:1327–1330.

Metz, Charles B. and A. Monroy (eds.) 1985. *Biology of fertilization.* 3 vols. Academic Press, New York.

Meyer, Arthur W. 1936. *An analysis of the De Generatione Animalium of William Harvey.* Stanford Univ. Press, Stanford.

Meyer, A. W. 1938. The discovery of the earliest representation of spermatozoa. Bull. Inst. History Medicine 6:89–110.

Meyer, Arthur W. 1939. *The rise of embryology.* Stanford Univ. Press, Stanford.

Meyer, Arthur W. 1956. *Human generation: Conclusions of Burdach, Döllinger, and von Baer.* Stanford Univ. Press, Stanford.

Monroy, A. and C. Groeben. 1985. The "new" embryology at the Zoological Station and at the Marine Biological Laboratory. Biol. Bull. 168(suppl.):35–43.

Moore, John A. 1972*a*. *Heredity and development.* 2nd ed. Oxford Univ. Press, New York.

Moore, John A. 1972*b*. *Readings in heredity and development.* Oxford Univ. Press, New York.

Moore, J. A. 1983. Thomas Hunt Morgan—the geneticist. Amer. Zool. 23:855–865.

Moore, J. A. 1987. Edmund Beecher Wilson, class of '81. Amer. Zool. (In press)

Morgan, Thomas H. 1897. *The development of the frog's egg: An introduction to experimental embryology.* Macmillan, New York.

Morgan, Thomas H. 1901. *Regeneration.* Macmillan, New York.

Morgan, Thomas H. 1927. *Experimental embryology.* Columbia Univ. Press, New York.

Morgan, Thomas H. 1934. *Embryology and genetics.* Columbia Univ. Press, New York.

Morsink, Johannes. 1982. *Aristotle on the generation of animals: A philosophical study.* Univ. Press of America, Washington.

Nakamura, O. and S. Toivonen. 1970. *Organizer—a milestone of a half-century from Spemann.* North-Holland, Amsterdam.

Needham, Joseph. 1931. *Chemical embryology.* 3 vols. Cambridge Univ. Press, London.

Needham, Joseph. 1942. *Biochemistry and morphogenesis.* Cambridge Univ. Press, London. Reprinted and revised 1966.

Needham, Joseph. 1959. *A history of embryology.* 2nd ed. Abelard-Schuman, New York.

Neugebauer, Otto. 1957. *The exact sciences in antiquity.* Princeton Univ. Press, Princeton.

Newport, G. 1851. On the impregnation of the ovum in the Amphibia (First series). Phil. Trans. Royal Soc. 1851, pp. 169–242.

Newport, G. 1853. On the impregnation of the ovum in the Amphibia (Second series, revised). Phil. Trans. Royal Soc. 143:233–290.

Newport, G. 1854. Researches on the impregnation of the ovum in the Amphibia; and on the early stages of the development of the embryo (Third series). Selected and arranged from the author's mss, by George Viner Ellis. Phil. Trans. Royal Society 144:229–244.

Nieuwkoop, Pieter D. and J. Faber. 1956. *Normal tables of Xenopus laevis (Daudin).* North-Holland, Amsterdam.

Nieuwkoop, Pieter, A. G. Johnen and B. Albers. 1984. *The epigenetic nature of early chordate development.* Cambridge Univ. Press, New York.

Nordenskiöld, Erik. 1928. *The history of biology: A survey.* Tudor Publishing Co., New York.

O'Connor, Daniel J. (ed.) 1964. *A critical history of Western philosophy.* Free Press, New York.

Olby, R. 1972. Wilhelm August Oscar Hertwig. Dictionary Scientific Biography 6:337–340. Scribners, New York.

O'Malley, C. D. 1956. On the genesis of the ovum of mammals and of man by Karl Ernst von Baer. Isis 47:117–153. English trans. of von Baer (1827).

Oppenheimer, J. M. 1940. The non-specificity of the germ-layers. Quart. Rev. Biol. 15:1–27. Also in Oppenheimer, 1967.

Oppenheimer, J. M. 1955. Analysis of development: Problems, concepts and their history. *Also,* Methods and techniques. *In* Benjamin H. Willier *et al.* (eds.), *Analysis of development.* Saunders, Philadelphia. Also in Oppenheimer, 1967.

Oppenheimer, J. M. 1957. Embryological concepts in the twentieth century. Survey of Biological Progress 3:1–46. Also in Oppenheimer, 1967.

Oppenheimer, J. M. 1959*a*. Embryology and evolution: Nineteenth century hopes and twentieth century realities. Quart. Rev. Biol. 34:271–277. Also in Oppenheimer, 1967.

Oppenheimer, J. M. 1959*b*. An embryological enigma in the *Origin of Species. In* Bentley Glass *et al.* (eds.), *Forerunners of Darwin: 1745–1859.* Johns Hopkins Press, Baltimore. Also in Oppenheimer, 1967.

Oppenheimer, J. M. 1963. K. E. von Baer's beginning insights into causal-analytical relationships. Devel. Biol. 7:11–21.

Oppenheimer, J. M. 1964. Questions posed by classical descriptive and experimental embryology. *In* John A. Moore (ed.), *Ideas in modern biology.* Doubleday, New York. Also in Oppenheimer, 1967.

Oppenheimer, J. M. 1966. The growth and development of developmental biology. *In* M. Locke (ed.), *Major problems of developmental biology.* Society for Develop. Biol. 25 Symposium: 1–27.

Oppenheimer, Jane M. 1967. *Essays in the history of embryology and biology.* MIT Press, Cambridge.

Oppenheimer, J. M. 1970*a*. Karl Ernst von Baer. Dictionary Scientific Biography 1:385–389. Scribners, New York.

Oppenheimer, J. 1970*b*. Theodor Boveri. Dictionary Scientific Biography 2:361–365. Scribners, New York.

Oppenheimer, J. M. 1971*a*. Aristotle as a biologist. Scientia July–August 1971, pp. 1–10.

Oppenheimer, J. 1971*b*. Hans Adolf Eduard Driesch. Dictionary Scientific Biography 4:186–189. Scribners, New York.

Oppenheimer, J. 1973. Recapitulation. *In* P. P. Weiner (ed.), *Dictionary of the history of ideas,* 4:56–59. Scribners, New York.

Oppenheimer, J. M. 1975. Reflections on fifty years of publications on the history of general biology and special embryology. Quart. Rev. Biol. 50: 373–387.

Ospovat, Dov. 1976. The influence of Karl Ernst von Baer's embryology, 1828–1859: A reappraisal in light of Richard Owen's and William B. Carpenter's "Palaeontological application of 'Von Baer's Law'." Jour. History Biol. 9:1–28.

Owen, G. E. L., D. M. Balme, and L. Minio-Paluello. 1970. Aristotle. Dictionary Scientific Biography 1:250–281.

Packard, A. S. P. 1876. *Life histories of animals, including man, or outlines of comparative embryology.* Holt, New York.

Pagel, W. 1967. *William Harvey's biological ideas. Selected aspects and historical background.* Karger, Basel.

Palter, Robert M. (ed.) 1961. *Toward modern science: Studies in ancient and medieval science.* Noonday Press, New York.

Pasteels, J. J. 1964. The morphogenetic role of the cortex of the amphibian egg. Advances in Morphogenesis 3:363–388.

Peters, Francis E. 1968. *Aristotle and the Arabs: The Aristotelian tradition in Islam.* New York Univ. Press, New York.

Pflüger, E. 1884. Ueber die Einwirkung der Schwerkraft und anderer Bedingungen auf die Richtung der Zelltheilung. Pflüger's Archiv 34:607–616.

Piaget, Jean. 1954. *The construction of reality in the child.* Basic Books, New York.

Piaget, Jean. 1977. *The development of thought: Equilibrium of cognitive structures.* Viking, New York.

Piaget, Jean and B. Inhelder. 1969. *The psychology of the child.* Basic Books, New York.

Pilet, P. E. 1970. Charles Bonnet. Dictionary Scientific Biography 2:286–287. Scribners, New York.

Pi Suner, August. 1956. *Classics in biology.* Philosophical Library, New York.

Power, Henry. 1664. *Experimental philosophy in three books.* Roycroft, London. Reprinted 1966 by Johnson Reprint, New York.

Preus, A. 1970. Science and philosophy in Aristotle's *Generation of Animals.* Jour. History Biology 3:1–52.

Preus, Anthony. 1975. *Science and philosophy in Aristotle's biological works.* Georg Olms, New York.

Preus, A. 1977. Galen's criticism of Aristotle's conception theory. Jour. History Biol. 10:65–85.

Prêvost, J. L. and J. B. Dumas. 1824. De la generation dans les mammifères et des premiers indices du dévelopment de l'embryon. Annales sciences naturelles 3:113–138.

Preyer, W. 1885. *Spezielle Physiologie des Embryos.* Grieben, Leipzig.

Przibram, Hans. 1908. *Embryogeny: An account of the laws governing the development of the animal egg as ascertained through experiment.* Cambridge Univ. Press, Cambridge.

Punnett, R. C. 1928. Ovists or animalculists. Amer. Nat. 62:481–507.

Radl, E. 1930. *The history of biological theories.* Oxford Univ. Press, Oxford.

Raikov, Boris. 1968. *Karl Ernst von Baer 1792–1876; sein Leben und sein Werk.* Leipzig.

Randall, J. H., Jr. 1940. The development of scientific method in the School of Padua. Jour. History of Ideas 1:177–206.

Randall, John H., Jr. 1960. *Aristotle.* Columbia Univ. Press, New York.

Raven, Chr. P. 1958. *Morphogenesis: The analysis of molluscan development.* Pergamon, New York.

Raven, Chr. P. 1959. *An outline of developmental physiology.* 3rd ed. Pergamon, New York.

Raven, Chr. P. 1961. *Oogenesis: The storage of developmental information.* Pergamon and Macmillan, New York.

Redi, Francesco. 1668. *Esperienze intorno alla generazione degl' insetti.* Florence. See also M. Bigelow (1909); Schwartz and Bishop (1958), vol. 1:405–410; Gabriel and Fogel (1955).

Reichert, C. 1837. Ueber die Visceralbogen der Wirbelthiere in Allgemeinen und deren Metamorphosen bei den Vögeln und Säugethiere. Müllers Archiv 1837:120–222.

Reinard, R. G. 1981. The problem of the organic individual: Ernst Haeckel and the development of the biogenetic law. Jour. History Biol. 14:249–275.

Renner, John W. *et al.* 1976. *Research, teaching, and learning with the Piaget Model.* Univ. Oklahoma Press, Norman.

Reverberi, G. (ed.) 1971. *Experimental embryology of marine and fresh-water invertebrates.* North-Holland, Amsterdam.

Ritterbush, Philp C. 1964. *Overtures to biology: The speculations of eighteenth century naturalists.* Yale Univ. Press, New Haven.

Roe, Shirley A. 1975. The development of Albrecht von Haller's views on embryology. Jour. History Biol. 8:167–190.

Roe, S. A. 1979. Rationalism and embryology: Caspar Friedrich Wolff's theory of epigenesis. Jour. History Biol. 12:1–43.

Roe, S. A. 1981. *Matter, life and generation: 18th century embryology and the Haller-Wolff debate.* Cambridge Univ. Press, Cambridge.

Rose, Sylvan Meryl. 1970. *Regeneration: Key to understanding normal and abnormal growth and development.* Appleton-Century-Crofts, New York.

Rosen, Hugh. 1977. *Pathway to Piaget: A guide for clinicians, educators, and developmentalists.* Postgraduate International, Cherry Hill, NJ.

Ross, William D. 1930. *Aristotle.* 2nd ed. Methuen, London.

Roux, W. 1888. Beiträge zur Entwicklungsmechanik des Embryo . . . . Virchows Archiv 114:113–153, 289–291.

Roux, W. 1895. *Gesammelte Abhandlungen über Entwicklungsmechanik der Organismen.* William Engelmann, Leipzig.

Russell, Bertrand. 1945. *A history of Western philosophy.* Simon and Schuster, New York.

Russell, Edward S. 1917. *Form and function.* Dutton, New York.

Russell, Edward S. 1930. *The interpretation of development and heredity.* Clarendon Press, Oxford.

Salthe, S. N. and J. S. Mecham. 1974. Reproductive

and courtship patterns. *In* Brian Lofts (ed.), *Physiology of the Amphibia.* Vol. 2:309–521. Academic Press, New York.

Sandler, I. 1973. The reexamination of Spallanzani's interpretation of the role of the spermatic animalcules in fertilization. Jour. History Biol. 6:193–223.

Sandler, I. and L. Sandler. 1985. A misconstruction of Mendel's work. History and Philosophy of the Life Sciences 7:3–70.

de Santillana, Giorgo. 1955. *The crime of Galileo.* Univ. Chicago Press, Chicago.

Sarton, George. 1927–1948. *Introduction to the history of science.* 3 vols. in 5. Williams & Wilkins, Baltimore.

Sarton, G. 1931. The discovery of the mammalian egg and the foundation of modern embryology. Isis 16:315–378. Included facsimile of von Baer, 1827.

Sarton, George. 1952 *A history of science; ancient science through the Golden Age of Greece.* Harvard Univ. Press, Cambridge.

Sawyer, Roger H. and R. M. Showman. 1985. *The cellular and molecular biology of invertebrate development.* Univ. South Carolina Press, Columbus.

Saxen, Lauri and S. Toivonen. 1962. *Primary embryonic induction.* Prentice-Hall, Englewood Cliffs.

Schleip, W. 1929. *Die Determination der Primitivenentwicklung.* Akademische Verlag, Leipzig.

Schmookler, Andrew B. 1984. *The parable of the tribe: Problems of power in social evolution.* Univ. California Press, Berkeley.

Schwann, Theodor. 1839. *Mikroskopische Untersuchungen über die Uebereinstimmung der Struktur und dem Wachsthum der Thiere und Pflanzen.* Reimer, Berlin. Translated by Henry Smith as *Microscopical researches into the accordance in structure and growth of animals and plants,* 1847, Sydenham Society, London.

Schwartz, George and Philip W. Bishop. 1958. *Moments of discovery.* 2 vols. Basic Books, New York.

Science as a Way of Knowing. 1984. *I. Evolutionary biology.* Amer. Zool. 24:419–534.

Science as a Way of Knowing. 1985. *II. Human ecology.* Amer. Zool. 25:375–641.

Science as a Way of Knowing. 1986. *III. Genetics.* Amer. Zool. 26:573–913.

Sedgwick, A. 1894. On the law of development commonly known as von Baer's law; and on the significance of ancestral rudiments in embryonic development. Quart. Jour. Micr. Sci. 36:35–52.

Shea, William R. 1972. *Galileo's intellectual revolution.* Macmillan, New York.

Shumway, W. 1932. The recapitulation theory. Quart. Rev. Biol. 7:93–99.

Singer, Charles. 1922. *Greek biology and medicine.* Clarendon Press, Oxford.

Singer, Charles J. 1950. *A history of biology.* Rev. ed. Schuman, New York.

Singer, Charles. 1960. *A short history of scientific ideas to 1900.* Clarendon Press, Oxford.

Sirks, Marius J. and C. Zirkle. 1964. *The evolution of biology.* Ronald Press, New York.

Slack, J. M. W. 1983. *From egg to embryo: Determinative events in early development.* Cambridge Univ. Press, New York.

de Solla Price, Derek. 1975. *Science since Babylon.* Yale Univ. Press, New Haven.

Smith, Anne B. 1982. *Understanding children's development.* Allen and Unwin, Boston.

Spemann, Hans. 1938. *Embryonic development and induction.* Yale Univ. Press, New Haven.

Spemann, H. and H. Mangold. 1924. Über Induktion von Embryonalanlagen durch Implantation artfremder Organisatoren. Roux' Arch. Entwicklungsmechanik 100:599–638.

Sprat, Thomas. 1722. *The history of the Royal Society of London.* 3rd. ed. Chapman, London.

Taton, René (ed.) 1963–1966. *History of science; ancient and medieval science from the beginnings to 1450.* 4 vols. Basic Books, New York.

Taylor, Henry O. 1951. *The medieval mind; a history of the development of thought and emotion in the Middle Ages.* 4th ed. Harvard University Press, Cambridge.

Thompson, D'Arcy W. 1922. Natural science. *In* Richard W. Livingstone (ed.), *The legacy of Greece.* Clarendon Press, Oxford.

Thompson, D'Arcy W. 1940. *Science and the classics.* Oxford Univ. Press, Oxford.

Thomson, John A. 1899. *The science of life: An outline of the history of biology and its recent discoveries.* Stone, Chicago.

Thorndike, Lynn. 1923–1941. *History of magic and experimental science.* 8 vols. Macmillan, New York.

Uschmann, G. E. 1972. Ernst Heinrich Philipp August Haeckel. Dictionary of Scientific Biography 6:6–11.

Virchow, Rudolph. 1863. *Cellular pathology as based upon physiological and pathological histology.* Dewitt, New York. Reprinted 1971 by Dover, New York.

Vogt, W. 1925. Gestaltungsanalyse am Amphibienkeim mit örtlicher Vitalfärbung. Vorwort über Wege und Ziele. I. Methodik und Wirklungsweise der örtlichen Vitalfärbung mit Agar als Farbträger. Roux' Archiv 106:542–610.

Vogt, W. 1929. Gestaltungsanalyse am Amphibienkeim mit örtlicher Vitalfärbung. II Teil: Gastrulation und Mesodermbildung bei Urodelen und Anuren. Roux' Archiv 120:385–706.

Waddington, Conrad H. 1952. *The epigenetics of birds.* Cambridge Univ. Press, Cambridge.

Waddington, Conrad H. 1956. *Principles of embryology.* Macmillan, New York.

Waddington, Conrad H. 1962. *New patterns in genetics and development.* Columbia Univ. Press, New York.

Waddington, C. H. 1975. Hans Spemann. Dictionary Scientific Biography 12:562–569. Scribners, New York.

Waerden, Bartel van der. 1961. *Science awakening.* Oxford Univ. Press, New York.

Wallace, W. A. 1970. Albertus Magnus. Dictionary Scientific Biography 1:99–103. Scribners, New York.

Webster, C. 1967. Harvey's *De generatione*: Its origin and relevance to the theory of circulation. British Jour. History Science 3:262–274.

Weismann, August. 1891–1892. *Essays upon heredity*

and kindred biological problems. 2 vols. Clarendon Press, Oxford.

Weismann, August. 1893. *The germ-plasm: A theory of heredity.* Scribner's, New York. Reprinted 1974, AMS Press, New York.

Weiss, Paul. 1939. *Principles of development: A text in experimental embryology.* Holt, Rinehart and Winston, New York.

Weiss, Paul. 1968. *The dynamics of development: Experiments and inferences.* Academic Press, New York.

Wheeler, M. W. 1899. Caspar Friedrich Wolff and the *Theoria Generationis.* Biol. Lectures Wood's Holl 1898:265–284.

Whitehead, Alfred N. 1925. *Science and the modern world.* Macmillan, New York.

Whitman, C. O. 1878. Embryology of Clepsine. Quart. Jour. Micr. Sci. 18:215–315.

Whitman, C. O. 1887. A contribution to the history of the germ-layers in Clepsine. Jour. Morph. 1:105–182.

Whitman, C. O. 1888. The seat of formative and regenerative energy. Jour. Morph. 2:27–49.

Whitman, C. O. 1894. The inadequacy of the cell-theory of development. Biol. Lectures Wood's Holl 1893:105–124.

Whitman, C. O. 1896*a*. Evolution and epigenesis. Biol. Lectures Wood's Holl 1894:205–224.

Whitman, C. O. 1896*b*. Bonnet's theory of evolution: A system of negations. Biol. Lectures Wood's Holl 1894:225–240.

Whitman, C. O. 1896*c*. The palingenesis and the germ doctrine of Bonnet. Biol. Lectures Wood's Holl 1894:241–272.

Whittaker, D. M. 1940. Physical factors of growth. Growth Suppl. 1940:75–90.

Whittaker, R. H. 1969. New concepts of kingdoms of organisms. Science 163:150–160.

Wigglesworth, V. B. 1959. *The control of growth and form: A study of the epidermal cells in an insect.* Cornell Univ. Press, Ithaca.

Wightman, William Persehouse Delisle. 1951. *The growth of scientific ideas.* Yale Univ. Press, New Haven.

Wilkins, Adam S. 1986. *Genetic analysis of animal development.* Wiley, New York.

Williams, W. C. 1972. Thomas Henry Huxley. Dictionary Scientific Biography 6:589–597.

Willier, Benjamin H. and J. Oppenheimer. 1974. *Foundations of experimental embryology.* 2nd ed. Hafner, New York.

Willier, Benjamin H., P. A. Weiss, and V. Hamburger (eds.) 1955. *Analysis of development.* Saunders, Philadelphia.

Wilson, E. B. 1892. The cell-lineage of Nereis. A contribution to the cytogeny of the annelid body. Jour. Morph. 6:361–480.

Wilson, E. B. 1893. Amphioxus and the mosaic theory of development. Jour. Morph. 8:579–639.

Wilson, E. B. 1894. The mosaic theory of development. Biol. Lectures Wood's Holl 1893:1–14.

Wilson, E. B. 1895. The embryological criterion of homology. Biol. Lectures Woods Hole 1894:101–124.

Wilson, E. B. 1896*a*. The embryological criterion of homology. Biol. Lectures Wood's Holl 1894:101–124.

Wilson, E. B. 1896*b*. On cleavage and mosaic work. Archiv Entwicklungsmechanik 3:19–26.

Wilson, E. B. 1898. Considerations on cell-lineage and ancestral reminiscence. Annals New York Acad. Sci. 11, no. 1:1–27.

Wilson, E. B. 1899. Cell-lineage and ancestral reminiscence. Biol. Lectures Wood's Holl 1898:21–42.

Wilson, E. B. 1900. *The cell in development and inheritance.* Macmillan, New York. The first edition was published in 1896.

Wilson, E. B. 1904*a*. Experimental studies on germinal localization. I. The germ-regions in the egg of Dentalium. Jour. Exp. Zool. 1:1–72.

Wilson, E. B. 1904*b*. Experimental studies on germinal localization. II. Experiments on the cleavage-mosaic in Patella and Dentalium. Jour. Exp. Zool. 1:197–268.

Wilson, E. B. 1928. *The cell in development and heredity.* 3rd ed. Macmillan, New York. The third edition was published in 1925 and in 1928 reprinted with corrections.

Wilson, L. G. 1972. Galen. Dictionary Scientific Biography 5:227–237.

Wolff, Caspar F. 1759. *Theoria generationis.* Halae ad Salam.

Woodbridge, Frederick J. E. 1965. *Aristotle's vision of nature.* Columbia Univ. Press, New York.

Woodger, Joseph H. 1929. *Biological principles: A critical study.* Routledge & Kegan Paul, London.

Woodger, J. H. 1948. Observations on the present state of embryology. Symposia Soc. Exp. Biol. 2:357–365.

Zanobio, B. 1971. Girolamo Fabrici [= Fabricius]. Dictionary Scientific Biography 4:507–512. Scribners, New York.