ANIMAL GROWTH AND DEVELOPMENT

MAURICE SUSSMAN

FOUNDATIONS OF MODERN BIOLOGY

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For **Raquel**

Foundations of Modern Biology Series

The science of biology today is *not* the same science of fifty, twenty-five, or even ten years ago. Today's accelerated pace of research, aided by new instruments, techniques, and points of view, imparts to biology a rapidly changing character as discoveries pile one on top of the other. All of us are aware, however, that each new and important discovery is not just a mere addition to our knowledge; it also throws our established beliefs into question, and forces us constantly to reappraise and often to reshape the foundations upon which biology rests. An adequate presentation of the dynamic state of modern biology is, therefore, a formidable task and a challenge worthy of our best teachers.

The authors of this series believe that a new approach to the organization of the subject matter of biology is urgently needed to meet this challenge, an approach that introduces the student to biology as a growing, active science, and that also *permits each teacher of biology to determine the level and the structure of his own course.* A single textbook cannot provide such flexibility, and it is the authors' strong conviction that these student needs and teacher prerogatives can best be met by a series of short, inexpensive, well-written, and well-illustrated books so planned as to encompass those areas of study central to an understanding of the content, state, and direction of modern biology. The FOUNDATIONS OF MODERN BIOLOGY SERIES represents the translation of these ideas into print, with each volume being complete in itself yet at the same time serving as an integral part of the series as a whole.

Contents

7

8

What Is Development?

The essence of life is change. The essence of death is inertia. All of us learn this at an early age. We watch the waxing and waning of the seasons and observe a regular succession of changes in the living things about us. We watch the ant on a blade of grass, the fish in a bowl, the dog in our back yard, and find them in states of ceaseless activity. Some are short-term activities such as moving, eating, excreting, mating; some are long-term activities that become apparent only after the passage of weeks and months. Ultimately, we recognize these changes in ourselves as we grow and develop from babyhood to adulthood.

The biologist takes a more penetrating view of biological activity and distinguishes three classes of alterations:

1. Short-term physiological and morphological alterations. Many of these changes are quite familiar to us. For example, our body temperature may vary from hour to hour depending on whether we are awake or asleep, at work or at rest. The color, texture, and thickness of an animal pelt can vary rather significantly from winter to winter in the life of an animal, depending on the severity of each winter. A callus may form on a hard-working hand and disappear when the hand's owner takes up a more sedentary occupation. Sudden anger may increase the heart beat, divert blood from intestines to muscles, and augment the rate of breathing.

As still another example, if we expose a population of yeast cells, which

have previously been grown in the presence of glucose as an energy source, to a new sugar, maltose, the cells, in order to utilize it, must first synthesize an entirely new enzyme that splits the maltose into usable fragments. They can do this in a relatively short time. If the maltose were removed and replaced by glucose, the new enzyme would disappear from the cells and no record would be left of this interlude of physiological change.

All such activities have common properties. They do not occur in any regular rhythm but merely represent sporadic adjustments to specific environmental stimuli. They are usually reversible; that is, the progression of changes leaves the organism neither vastly different from what it was before nor unable to return to its former state.

2. *Long-term genetic and evolutionary changes.* Alterations in the genetic apparatus of an organism are called *mutations* and are inherited by the offspring. A single mutation may be minute in itself, perhaps leading only to the loss or gain of the capacity to synthesize a single enzyme, and its effect on the form or functioning of the organism may be correspondingly slight. If by virtue of the change, the mutant is more fitted to survive than its unaltered relatives (if it can breed faster, for example), it will flourish and the following generations will probably include a greater proportion of that mutant type. When successive mutations stand the test of natural selection, they can, over many generations, produce a variety of organism greatly different from its distant forebears. In the aggregate, these progressions contribute to the evolution of species.

Note how different these evolutionary changes are from the short-term changes mentioned before. First of all, a much longer time is involved, for the appearance of a new variety of organism in large numbers may require many generations of growth and reproduction. Second, the biologist who studies these phenomena must examine populations, not individual organisms, in order to follow the spread of genetic changes.

3. *Developmental events.* Developmental events are seen most dramatically in the growth patterns of higher animals. The fertilized egg undergoes a very orderly series of changes to become an embryo. The embryo develops further into the young animal, which in turn matures into an adult. The adult reaches its peak and then experiences a series of degenerative changes that ultimately lead to death. At maturity the animal releases eggs or sperm which originate yet another cycle of development.

The tempo of these phenomena is neither fast enough to be physiological nor slow enough to be evolutionary. Moreover, they are progressive; that is, they occur in a regular sequence with little variation, each leaving the organism different from its former state and unable to return to it. They begin before birth and end shortly after death, and in toto they represent the life cycle of the organism.

Developmental phenomena are not restricted to higher animals. Even the single cell goes through a corresponding progression of changes. Its life cycle begins when it arises from the division of its parent into two daughters and ends when it in turn divides in two. Between times, it grows in size and protoplasmic content, synthesizes cellular constituents in an orderly and well-regulated manner, and can, indeed, develop new *organelles* (a term used to designate any of the parts of a cell), and change its form and functioning drastically. Many higher protozoa and algae display complicated changes in this respect, and a few of these cycles will be described in succeeding sections.

Populations of cells also show developmental phenomena. For example, a culture of microorganisms has a life cycle of its own, as contrasted with those of the individual cells of which it is composed. As will be described in detail later, when we add an inoculum of bacteria to nutrient broth, the culture experiences an orderly succession of well-defined growth stages, reaches a stationary state and ultimately a period of decline during which the cells degenerate and die. Thus the bacteria en masse act in much the same fashion as the cells of a higher organism except that they are loosely distributed and largely independent, whereas the cells of an animal are dependent upon each other and are compressed into a compact organized structure. In fact, some sophisticated microorganisms exist for part of their culture cycle as independent cells, and during the remainder of it actually do come together and construct organized multicellular structures. A later chapter will describe these fascinating organisms.

A little higher on the scale, we come to the primitive animals, which display, on a limited level, the complicated series of developmental events that the higher animals show. One of the most interesting primitive groups in this respect, the coelenterates, will be discussed in detail.

The Central Problems of Developmental Biology

As we consider the life cycles mentioned above, three central problems of development become apparent and will be taken up here.

GROWTH

The egg starts as a single microscopic cell with one nucleus and by growth and replication produces an adult containing millions of cells. Needless to say, this represents a many-millionfold increase in protoplasmic mass. A number of questions come to mind when we think about this phenomenon. How is growth initiated? What raw materials are required for the synthesis of protoplasm? What specific chemical reactions

lead to the formation of nuclei, enzymes, cell walls, etc.? From where does the organism draw the energy needed to carry out these syntheses? Why does growth stop?

Another significant attribute of growth is the fact that all the parts of an organism increase in a carefully regulated manner. That is, if a cell doubles its size, the size of the nucleus, cytoplasmic constituents, cell wall, etc., all increase in proportion to one another. When a boy grows into a man, his organs increase harmoniously. Thus the length of his arm bears a fixed relation to his height, and within rather close limits this same relation holds in all other boys. There must, therefore, be regulatory mechanisms in cells and organisms that govern such relationships. What are these mechanisms?

CELLULAR DIFFERENTIATION

The millions of cells descended from the original fertilized egg are not all the same. Some are skin cells, others are nerve cells, still others are muscle cells, etc. They look different and perform decidedly different functions. When we think about the reproduction of, say, a bacterium or a rabbit, we normally expect the descendants of each to look and act very much like if not the same as the original bacterium or rabbit. Such is not the case, however, for the descendants of developing cells in an embryo. The processes by which these cells become specialized are collectively called *cellular differentiation*. These processes are still largely unknown and present a major challenge to developmental biologists.

Another mysterious aspect of cellular differentiation is the way in which the numbers of each cell type are carefully regulated. For example, you will never find one embryo with many brain cells and few liver cells or vice versa. Here again, as in the case of the boy and his arm, the various tissues and organs of the body bear a relatively fixed relation to one another. The mechanisms that control the proportions of differentiated cells are still to be discovered.

MORPHOGENESIS

A multicellular organism is not simply a bag of cells thrown together helter skelter. It is composed of organs and tissues, and, as has been mentioned, the parts of the animal are arranged in a specific pattern and bear definite relations to one another in terms of size and cellular content. Morphological regulation extends to the individual cell and its organelles. The establishment of this pattern and the processes whereby the adult organism takes its final shape is termed *morphogenesis* and requires explanation in physical and chemical terms.

The Relationship of Developmental Biology to Other Biological Disciplines

As the various aspects of developmental biology are considered in detail in later chapters, it will become obvious that this subject does not exist apart from the other biological disciplines, for development involves changes in the basic structure of an organism. Thus a rigorous description of developmental events requires excursions into gross and microscopic anatomy and into histology (which deals with tissue composition) and cytology (which deals with cell structure). And, since changes in the structure of an organism are brought about by new combinations of biochemical reactions and the new structure leads in turn to further changes in function, biochemistry and physiology must be included in developmental study. Finally, because development does involve change, it bears a direct relation to genetics. For example, when cellular differentiation occurs—i.e., when a single egg cell gives rise to a galaxy of cell types: nerve, muscle, skin, cartilage cells—we want to know if the genetic constitutions of these cells had to change in order to permit them to look and act this differently, and if so, what was the nature of the change.

As you see then, developmental studies are intimately involved with the rest of biology, and scientists who deal with developmental problems in their research must bring many different kinds of skills and training to bear upon such problems.

The Life Cycle of the Single Cell

A growing cell faces the problem of making enough of everything to construct two or more daughter cells. And it must divide the proceeds among them in such a way as to produce normal, well-functioning progeny. Most important, it has to endow each daughter cell with a complete set of genetic material. This material is carried in large part upon the chromosomes within the nucleus. However, some properties of organisms have been shown to be initiated through genetic determinants that reside in the cytoplasm and these too must be shared.[1]

Varieties of Cell Division

Nature has found several good solutions to the problem of sharing, enabling different methods of cell division to be employed in various types of organisms (Fig. 1).

1. Fission. The parent cell grows to approximately twice its original size and then splits into two more or less equal daughter cells. Protozoan and animal cells divide in this fashion, simply by pinching off two daughters at the middle and pulling apart the two halves. Bacterial and plant cells also reproduce by fission but have rigid walls and divide by constructing a cross wall.

2. Budding. The mother cell forms a little bleb at the surface which grows

[1] See Chapter 6 for a more detailed discussion of these matters.

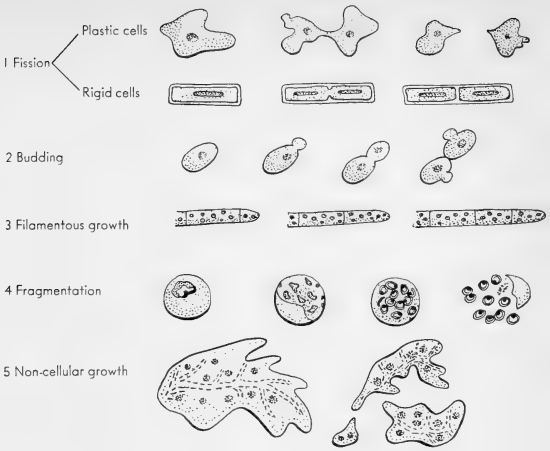


Fig. 1. Asexual reproduction. (1) Fission as it occurs in amoebae and bacteria. (2) Budding in yeast. (3) Filamentous growth in molds. (4) Fragmentation in *Plasmodium malariae*. (5) Noncellular growth in the myxomycetes.

rapidly to the approximate size of the mother and finally constricts off. It may separate completely from the mother or may remain attached. In the latter case, both mother and daughter may bud and so produce a chain of cells. This is the manner in which yeast cells divide.

3. Growth of filaments. The cells of fungi and some algae are linked together in thin hair-like fibers. Since growth can occur only at the tip of each filament, the tip elongates and a cross wall forms to yield a cell with a growing tip on its far end. Branching often occurs, when the growing tip bifurcates and both branches elongate in separate strands.

4. Fragmentation. The animal parasite, *Plasmodium malariae,* is one of a number of microorganisms that reproduce by fragmentation. The plasmodium grows inside a red blood cell. The parent nucleus divides or fragments into as many as 24 daughter nuclei and the cytoplasm coalesces about each of them. Separate walls are formed around the conglomerates of nucleus and cytoplasm, which are now called *merozoites.* The host red cell ruptures and releases the merozoites and each can now infect another red blood cell. (Incidentally, the well-known chills and fever of malaria are associated with the simultaneous release of merozoites from many blood cells.) The term fragmentation is a bit misleading because the parent organism does not break up into incomplete fragments, each of which reconstitutes a whole new organism. Rather it is as if a bacterium would synthesize enough protoplasm to create many new cells instead of just two. Many kinds of molds, algae, and protozoa form large numbers of spores[1] at certain stages of their life cycles. In principle, the process resembles that of *Plasmodium malariae.*

5. Noncellular growth. A few simpler organisms can grow extensively without cell division. A group called the mycetozoa consists of

[1] Special cells with thick walls that resist drying, heat, and radiation and which can lie dormant for long periods of time until permitted to germinate.

amoeboid masses of protoplasm that contain many nuclei. The protoplasm increases in amount and the nuclei divide as the organisms grow. Each protoplasmic mass can fragment (in the true sense) to yield two or more smaller masses. It should be noted that some higher animals can also fragment into small pieces, each of which can then reconstitute a whole new organism. Flatworms, starfish, and many others do this.

Biochemical Events in the Cell Division Cycle

In recent years, biologists have begun to study biochemical events during the cell division cycle. Some have refined analytical techniques to the point where they can study a single cell, weigh it (despite the fact that they must deal with weights of only millionths of a gram), and, by microchemical analysis, determine the amounts of the cell constituents (even though these are present in infinitesimal quantities). Other biologists have devised conditions to make cell populations divide synchronously. Cell populations are usually asynchronous; that is, at any instant some will be preparing to divide, some will have just completed division, and others will be in an intermediate stage. With synchronized cells, one need not apply very delicate and sensitive techniques for the study of a single cell, but instead can work with large samples of cells and thereby examine more easily the biochemical and morphological events that accompany each stage of the division cycle. This work has only begun, but ultimately we may hope to understand how the cell coordinates the synthesis of all its constituents. It is already clear from such studies that various cell constituents are synthesized during very specific phases of the division cycle. Figure 2 illustrates schematically the formation of deoxyribose nucleic acid (DNA), the primary component of chromosomes and the bearer of the cell's genetic constitution. As you can see, the DNA is synthesized during a relatively short period between successive mitoses. Contrary to what one might expect, no DNA is formed during the period when one sees the chromosomes actually divide.

NUCLEAR DIVISION

The primary method of vegetative (as opposed to sexual) nuclear division is that of *mitosis*. The particular stages need not concern us here. The significance of mitosis is that it is an almost foolproof way of ensuring that each daughter nucleus will possess a copy of each chromosome and, therefore, a complete set of the genetic material contained therein.

However, not all nuclei divide by mitosis. In some cells the nucleus does not assume the mitotic configuration, but merely stretches out into a

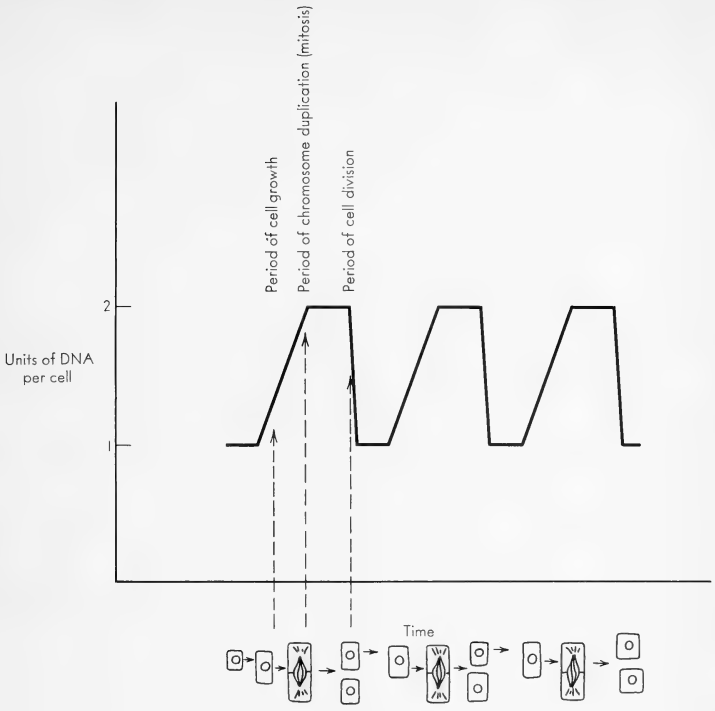


Fig. 2. DNA synthesis during the cell division cycle.

dumbbell shape. The dumbbell constricts in the middle and the two daughter nuclei pinch apart. This is called *amitosis*. Amitotic division is restricted to a few microorganisms and some cells of higher animals. As you may know, certain tissue cells grow only in the young animal and cease dividing in the adult, and it is interesting to note that their last few divisions are very often amitotic. Degenerating or abnormal tissue cells (e.g., tumors) also appear to divide amitotically.

EXTRA-NUCLEAR ELEMENTS

Some organelles of the cell seem to be partly or completely independent of the nucleus. For example, the long whip-like flagellum seen in many protozoa is formed from a small granule at its base called a blepharoblast or basal granule. When certain protozoa divide, the old flagellum disappears and the basal granule splits in two. Each daughter cell receives a daughter granule and at the completion of cell division

each granule forms a new flagellum. If by mischance one of the daughter cells does not receive a basal granule, it and its descendants will never again form a flagellum. Thus the basal granule is independent of the nucleus to the extent that the latter cannot replace the former after it once has been lost.

Other organelles (the gullets of ciliate protozoa, the chloroplasts of some algae, etc.) act in the same fashion, i.e., they duplicate at cell division and if lost from a cell cannot be regained. A most interesting case involves the mitochondria of yeast. You will recall that mitochondria are small bodies enclosed in membranes and distributed in the cytoplasm. They contain organized collections of enzymes involved in respiration. Recently it has been found that normally large yeast cells (which can respire) occasionally give rise by budding to dwarf cells (which cannot respire). And the descendants of these so-called "petite" cells are invariably petite even after hundreds of generations of growth. Thus, once lost, the capacity to respire can never be regained. During budding, the mitochondria concerned with respiration are normally shared between mother and daughter cell, but once in a while by mistake the bud receives none of them. Because these mitochondria seem to be independent, in the same sense as are flagellar granules, a cell bereft of them cannot respire nor can its descendants do so, the loss being irretrievable.

The nucleus itself, of course, is an autonomous organelle. That is, a cell deprived of its nucleus cannot create a new one. The loss of the nucleus is lethal for the cell; when anucleate, it can no longer reproduce.

In summary, the cell is not simply a bag of homogeneous material parcelled out in imprecise fashion during cell division. It is an assembly of highly structured organelles. Many of these (nucleus, basal granules, etc.) are autonomous in the sense that they control their own duplication. Thus, for cell division to leave the daughters with a fair share of all the constituents, the cell must make coordinated preparations, and organelles must be moved to the right places in the mother cell.

The Life Cycle of a Typical Unicellular Organism—Yeast

We choose common Baker's yeast, *Saccharomyces cerevisiae*, to exemplify the life cycle of simple plants and animals because it displays three features that are present in the cycles of most organisms within these groups:

1. The single cell as the unit of existence: In contrast to higher animals (i.e., organized assemblies of specialized cells that are dependent

upon each other for their collective existence), the single yeast cell is the total organism.

2. *Growth by vegetative (asexual) reproduction:* Yeast cells multiply asexually, a single cell giving rise to two by budding. Sexual fusion of gametes does occur under special conditions (see below) but its occurrence is not obligatory to growth.

3. *Sexual reproduction:* You will recall from your study of genetics that the higher plant or animal, consisting mostly of diploid cells (two of each kind of chromosome), contains a few haploid germ cells or gametes (one of each kind of chromosome). Two haploid gametes of the appropriate sexes can come together and fuse to yield a new diploid organism.

This process is called sexual fusion and serves a very important function. The diploid individual receives a set of chromosomes from each parent. By meiosis, it in turn produces gametes with a single chromosomal set which, by random assortment, is generally made up of some chromosomes from each of the original parents. Thus, the genes of the parents are shuffled together in the offspring and are reshuffled in the gametes produced by that offspring (Fig. 3). Sexual fusion, then, is a source of genetic recombination, producing new varieties by reshuffling the old. New varieties are important to a population, for they ensure that there will always be types that can take advantage of new environments and thereby make the species fit to survive under a variety of conditions.

To clarify this point, consider a population of yeast cells living in a moderately cool bit of dirt and plentifully supplied with glucose to grow on. With the coming of summer, the soil becomes hot and the glucose is replaced by a different sugar, galactose. Now suppose that the population had originally consisted of two types: cells that were resistant to heat but could grow only on glucose, and cells that were sensitive to heat but could grow equally well on galactose or glucose. Under the new conditions, type A could stand the heat but not the new sugar; type B could grow on the new sugar but could not stand the heat. Of course, type A might produce a mutant that could grow on galactose and type B a mutant that could resist heat, but these would be exceedingly rare events that might not come to pass, in which case the yeast population would be wiped out. But if sexual reproduction could occur, the chromosomes bearing the genes for heat resistance and the ability to grow on galactose would be shuffled together in the zygote, and the recombinant type would be able to survive. Figure 3 illustrates this.

Microorganisms such as yeasts and molds, protozoa, algae, and some bacteria are capable of sexual fusion. That is, diploid cells can by meiosis produce haploid gametes. These can be of different sexes (or mating types as they are called). Haploid gametes of the appropriate sexes can fuse to yield new diploid individuals.

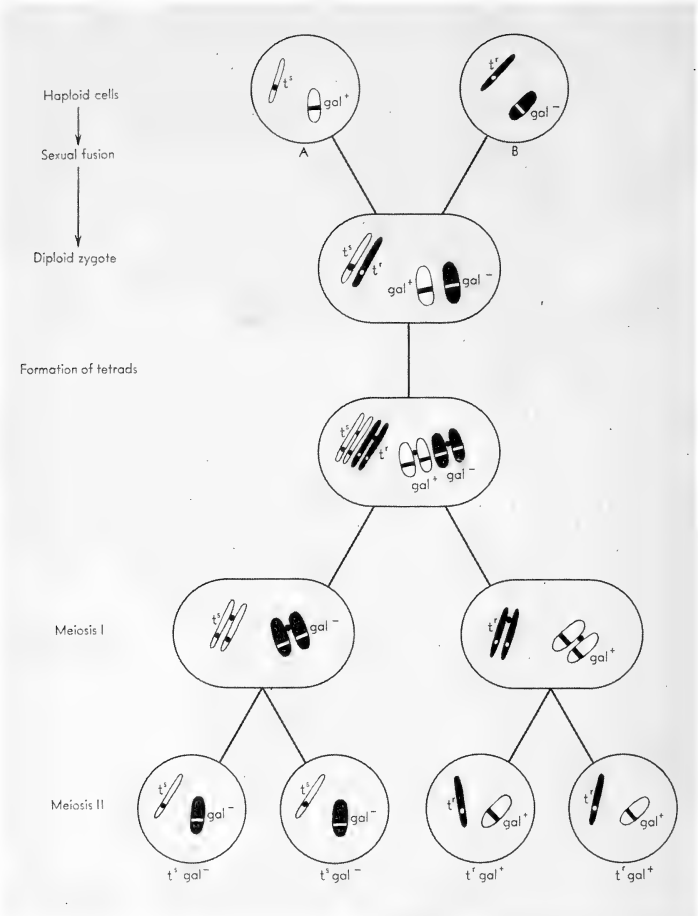
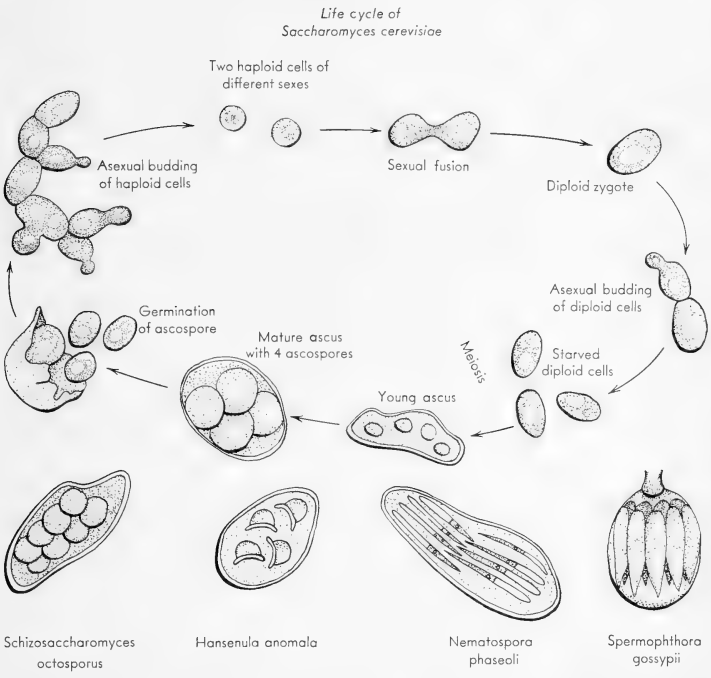


Fig. 3. Genetic recombination through sexual fusion. The gene for temperature sensitivity is indicated by the symbol t^s; t^r is its allele and denotes temperature resistance. We use gal^+ to denote ability to utilize galactose, gal^- to denote inability. Thus, cell A is heat sensitive and can utilize galactose; cell B is heat resistant but cannot utilize galactose. The chromosomes bearing these genes are shuffled together when cell A and cell B fuse to form the zygote. Each pair of chromosomes then duplicates to yield a quartet or tetrad. The nucleus then divides twice (meiosis I and II), and in the process reduces the quartets to pairs and the pairs to singles. The random assortment of chromosomes during meiosis I can produce a situation in which the same cell will receive both the gene for temperature resistance and the gene for galactose utilization; that is, a recombination of the parental traits will take place.

In higher animals, reproduction is tied to the sexual fusion of gametes. Human beings cannot bud or fragment. They must mate to reproduce their kind. In yeast cells, as in other microorganisms, sexual fusion is separate from reproduction. Yeast grow by asexual means, but under special conditions do employ sexual fusion to produce genetic recombinants (i.e., new varieties).

The life cycle of yeast is illustrated schematically in Fig. 4. The diploid individual is an elliptical cell. It reproduces asexually by budding and can do so indefinitely as long as it is supplied with nutrient materials and given conditions beneficial for growth. Under adverse conditions (when starved and unable to bud), the diploid cell undergoes meiosis to produce four haploid individuals. These are surrounded by thick walls and are resistant to drying, heat, ultraviolet radiation, etc. They are called

Fig. 4. The life cycle of yeast.



ascospores and are contained within a large sac or ascus. The ascus bursts, the ascospores are released and then germinate, yielding small round yeast cells, still haploid. Two haploid cells of the proper sexes [1] can come together and fuse once again to yield a large diploid individual. The cycle is complete at this point and can be repeated.

Many questions come to mind. For example, what specific environmental conditions induce a diploid cell to undergo meiosis and produce ascospores? What causes the ascospores to germinate into haploid cells? What happens when the haploid cells are kept separate and not allowed to meet other haploid gametes of the correct sexual type? Can they grow by budding just like diploid cells? How is it that the haploid gametes can be of different sexes and yet look alike? What is the physiological basis of sex in these organisms? How is sex inherited in yeast? Some of these questions have been answered through research. Many still remain unanswered.

The Life Cycle of *Paramecium Aurelia*

Figure 5 is a drawing of *Paramecium*, a favorite subject of study in elementary biology classes. Its large size (usually 150 microns x 50 microns [2]) and intricate structure attracted the attention of early biologists. In recent years its life cycle and genetics have been rigorously defined. We can do no more here than briefly summarize these findings.

SOME FACTS ABOUT *PARAMECIUM*

P. aurelia is a fresh-water organism that preys upon bacteria and algae. It is grown in the laboratory in association with a single bacterial species. In recent years a bacteria-free nutrient medium has been devised in which *Paramecia* can grow at a good rate. This medium is complex, consisting as it does of proteins and many amino acids, eleven vitamins including a derivative of vitamin D which is a requirement that is characteristic of higher animals, and purines and pyrimidines (needed for the synthesis of nucleic acids). *P. aurelia* possesses three nuclei. One is large (macronucleus) and two are small (the micronuclei). It has a complex, organized gut consisting of a mouth leading into a gullet lined with cilia

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[1] There are two sexes amongst yeast gametes, called + and —. Two + cells or two — cells cannot fuse. Only a + and — cell can. The two sexes look identical (in contrast to those of higher organisms) and can only be distinguished by their mating reaction. In each ascus two ascospores are of sex + and two are of sex —. From your knowledge of genetics, can you deduce the manner in which sex is inherited by yeast?

[2] A micron is 0.000001 meter. The average bacterium is about 2×0.5 microns.

which waft the food down to the esophagus and thence into the interior of the animal in the form of food vacuoles. The vacuoles circulate until digestion is complete. The waste products are eliminated through the anal pore. The surface of the animal is astonishingly intricate. It is covered by longitudinal rows of hexagons whose edges form distinct ridges.

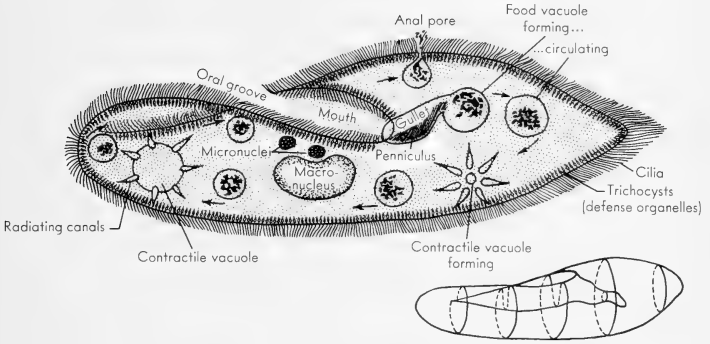


Fig. 5. A schematic drawing of *Paramecium*, showing the many organelles.

Each hexagon contains a basal granule from which a cilium arises. There are about 2,500 cilia and the ciliary pattern is inherited in a precise manner.

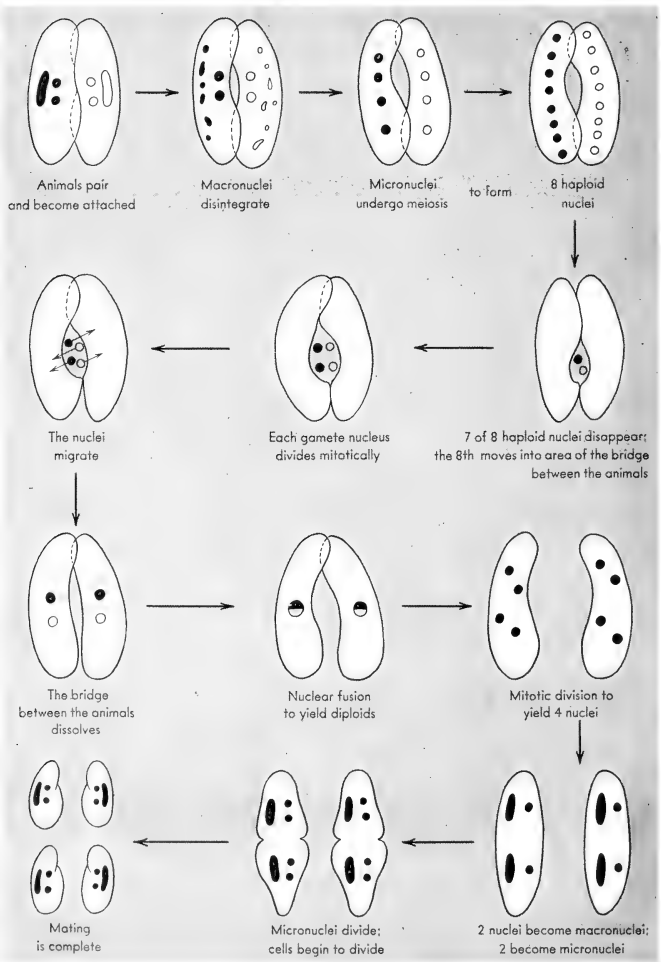
BINARY FISSION

Well-fed *Paramecia* reproduce about five times per day. The cell simply constricts at the middle and separates into anterior and posterior daughter cells. At first these are readily distinguishable as the front and rear ends, but they soon change into two identical and small but otherwise normal animals. Prior to the constriction, the three nuclei divide. The macronucleus elongates and the two halves pull apart. The micronuclei divide by normal mitosis. One product of each of the three nuclei passes to each daughter cell. A number of other cell structures duplicate during cell division. Buds protrude from the mouth and gullet of the dividing animal. The buds pass to the anterior daughter cell and produce a new mouth and gullet. The posterior daughter retains the old ones. The ciliary basal granules also bud off duplicates of themselves and these produce new cilia. The granules, cilia, and hexagonal ridges are then rearranged upon the daughter cells in their usual precise geometric pattern.

CONJUGATION

When culture conditions are adverse (notably, when the *Paramecia* are starved) these animals come together in pairs and undergo sexual union or *conjugation.* The steps in conjugation are outlined below (see Fig. 6).

Fig. 6. Conjugation in *Paramecium aurelia.*



1. Pairing. The animals collide anteriorly and also become attached at the region of the mouth. A bridge then forms between them. Mouths and gullets regress into very tiny rudiments and much of the cell contents becomes radically reorganized.

2. Nuclear changes. First, the macronuclei disintegrate into many small fragments which eventually disappear. Second, the diploid micronuclei undergo meiosis (i.e., each pair of chromosomes in the micronucleus duplicates to yield a quartet; the micronucleus then divides twice, reducing the quartets to pairs and thence to singles). In this manner, each micronucleus gives rise to 4 haploid nuclei, and the two micronuclei of each conjugating cell together produce eight. Seven of the eight disappear. Only the one nearest the bridge between the conjugant cells remains. This nucleus, still haploid, divides once more and now each cell has two gamete nuclei. One of these stays where it is. The other migrates through the bridge to the partner cell. The result is that the conjugant *Paramecia* have identical pairs of nuclei, each with one of its own and one of its partner's. The two haploid nuclei in each cell come together and fuse to yield a single diploid nucleus.

3. Post-conjugative changes. The newly formed diploid nucleus divides mitotically into two, and the two in turn produce four. One pair takes a position at the anterior end of the cell and one pair at the posterior end. The cell now constricts in the middle just as it does during asexual fission and produces two daughter cells, each with two nuclei. One of the two enlarges to form the new macronucleus. The other divides to yield the two micronuclei. Thus, four post-conjugate daughter cells eventually emerge from the two *Paramecia* that originally conjugated.

AUTOGAMY

Many organisms, particularly in the plant kingdom, are self-fertile, which means that they can undergo sexual congress alone and need no partner, i.e., they produce gametes that can fuse with each other in pairs to yield fertile zygotes. *Paramecium* is capable of self-fertilization and the process is designated *autogamy.*

The nuclear changes in an autogamous *Paramecium* are the same as those involved during conjugation. The macronucleus disappears and the micronuclei undergo meiosis to produce a total of eight haploid nuclei. Seven disappear. The eighth divides mitotically into two gamete nuclei and these fuse to yield a diploid zygote. The subsequent nuclear changes follow the pattern of those displayed by cells after conjugation. Figure 7 summarizes autogamy.

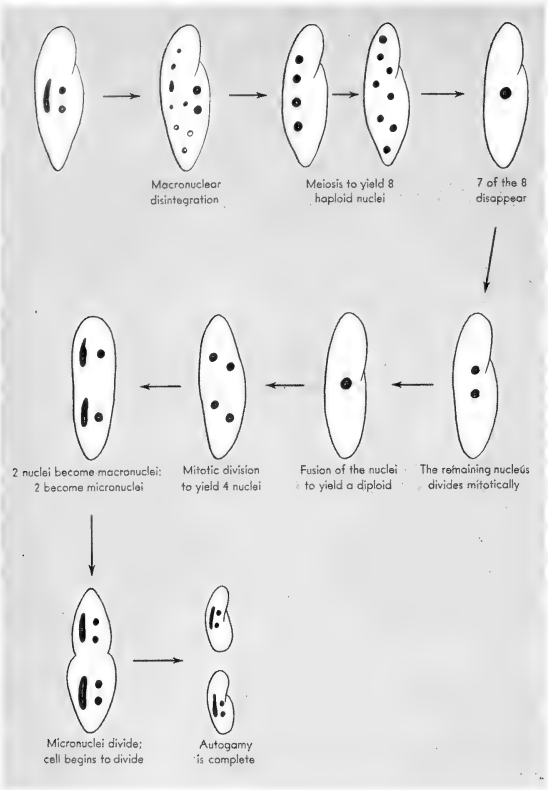


Fig. 7. Autogamy in *Paramecium aurelia.*

Persistent Rhythms in *Gonyaulax Polyhedra*: How Organisms Tell Time

Gonyaulax is a marine microorganism. It is single-celled, built like a protozoan, but contains chlorophyll and is capable of photosynthesis. It normally grows at the surface of the sea and can also be cultivated in the laboratory. In addition to being photosynthetic, *Gonyaulax* can luminesce (i.e., like fireflies and other insects, crustaceae, some molds, bacteria, and algae, it can operate biochemical reactions that release energy in the form of light instead of the more usual heat). It is one of the organisms responsible for the brilliant displays of "phosphorescence" seen at night on ocean waves. At least three important functions—photosynthesis, luminescence, and cell division—are carried out in a regular rhythm keyed to the marine habitat of the organism. *Gonyaulax* photosynthesizes by day, luminesces by night, and reproduces just before dawn.

For example, if a growing culture of *Gonyaulax* is exposed alternately to 12-hour periods of light and dark (i.e., a regular day-night cycle), cell division is confined to a very short time just before the end of the dark period. Thus the population increases like a staircase rather than steadily and continuously with time as do most microbial cultures. If now the culture is placed in constant dim light or total darkness, the population continues to mark off 24-hour cycles of rhythmic cell division. Thus the rhythm is persistent even though light, temperature, and other environmental conditions may be constant. (See Fig. 8.)

The same was found to be true of its luminescence. A culture of *Gonyaulax* was incubated during alternating 12-hour periods of light and dark. At frequent intervals, measurements were made of the intensity at which the cells luminesced, with an instrument somewhat like an ordinary

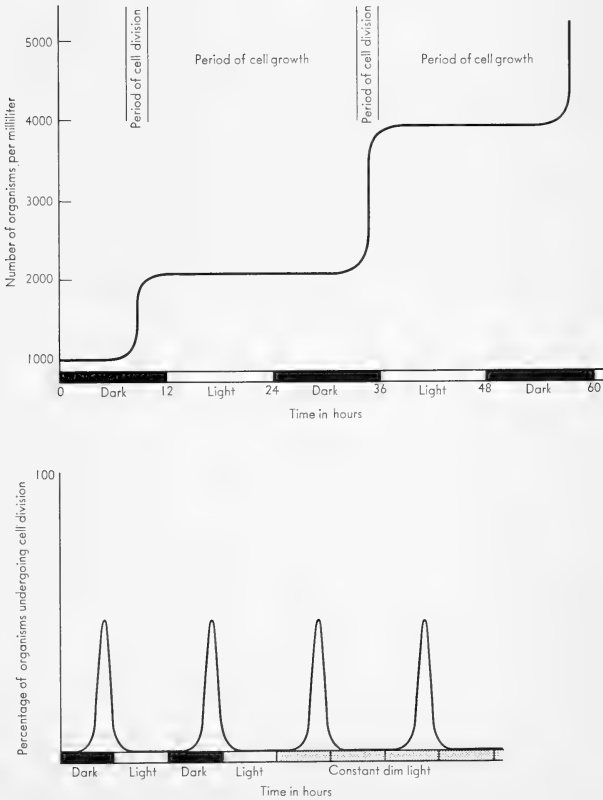


Fig. 8. Rhythmic cell division in *Gonyaulax*. The upper graph shows the growth curve of *Gonyaulax* as a staircase rather than as a continuous line, because the cells do not divide at all times of the day and night but only during a short period just before "dawn." The lower graph shows that even when the organisms are removed from the ordinary day-night cycle and placed in constant dim light, the 24-hour rhythm of cell division persists.

photographic light meter. It was found that the organisms luminesced only at night, reaching a peak of brightness at midnight and a low at noon (the upper part of Fig. 9). Then the animals were placed in constant dim light, and they still continued to mark off 24-hour cycles of luminescence over the course of many days. Finally, photosynthesis was

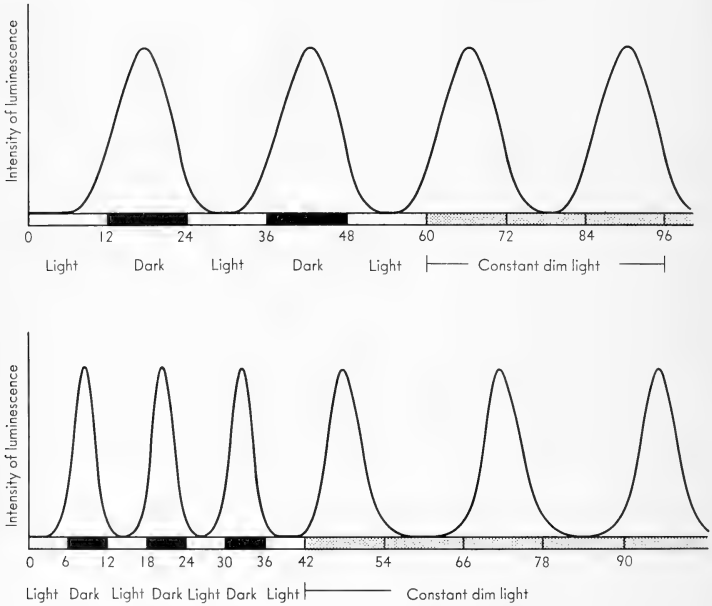


Fig. 9. Rhythmic luminescence in *Gonyaulax*. The upper curve shows that the rhythmic luminescence displayed during the usual day-night cycle persists even when the organisms are placed in constant dim light. The lower curve shows that although the luminescent rhythm can be altered by exposing the organisms to a different day-night cycle (in this case 6 hours of light, 6 of dark), it returns to the usual 24-hour rhythm as soon as the organisms are placed in constant dim light, and no memory of the unusual rhythm is retained.

also shown to be rhythmic even in constant dim light. The cells photosynthesized only during the daylight hours, reaching a peak at noon. Consider how remarkable must be the control mechanism that could force cells incubated in constant light to cease photosynthesis during the night hours even though night was as bright as day!

It was found that the rhythms could be altered in three ways. First, the frequency, normally 24 hours, could be changed over a wide range. For example, the organisms were exposed to alternating periods of 6 hours of light and 6 of dark and their rhythms speeded up until they repeated every 12 hours instead of 24. Other periods were attained by appropriate changes in the duration of light and dark periods. However, no matter how long the different regimen was imposed, as soon as they were returned to constant dim light, the cells resumed their 24-hour cycle (lower curve of Fig. 9). Second, the rhythm could be wiped out entirely by incubating the cells in constant bright light. In this condition, the cells photosynthesized all the time, reproduced asynchronously at any time, and luminesced at no time. Again if placed back in constant dim light or total darkness, they promptly resumed the normal rhythmic pattern.

Third, the rhythm could be reset. That is, the "clock" could be pushed ahead or set back. This happened when cells were incubated in constant darkness and suddenly were exposed to strong light for a minute or two. Before exposure the cells had marked off regular 24-hour cycles. After exposure they continued to do so but in a different time sequence, as if one suddenly moved the hands of a watch forward or backward. The light signal acted as the "reset" mechanism.

We have stressed these rhythmic activities because many living organisms are rhythmic, and the rhythms profoundly affect their development, indeed, are a part of it. Not all organisms operate a rhythmic cycle of 24 hours' duration. Some have a tidal rhythm, some a lunar (28-day) rhythm. Others have an annual rhythm, and a few have several different rhythms at the same time. Consider the following examples.

The fiddler crab, a common seashore denizen, changes color markedly. The change is rhythmic and keyed to the tide. The same is true of oysters and clams, who open and shut their shells according to a tidal rhythm. Some insects pupate according to a 24-hour rhythm. That is, they emerge from the pupal case at a certain time of day and only at that time. The mating habits of certain fish are rhythmic according to a lunar cycle. The hamster and rat show precise daily cycles of activity when placed in special running cages (with automatic tachometers) for long periods of time. The growth rates of some microorganisms show a similar daily rhythm. Finally, the menstrual cycle of the human female has a lunar rhythm. All of these, save the last, have been studied in the laboratory and show the same characteristics as the rhythms of *Gonyaulax*. Let us summarize these characteristics:

1. The rhythm is keyed to the physical environment of the organism (day, tide, lunar month, year, etc.) and probably enables the organism to feed or mate or protect itself more efficiently.

2. The rhythm is persistent and is repeated by the organism even under constant environmental conditions.

3. The rhythm can be temporarily wiped out or changed to a different time schedule by altering the environment. The change is only temporary, however, for the organism reverts to its original rhythmic habit once it returns to a normal environment.

4. The rhythm can be reset (i.e., pushed forward or backward as a clock is reset by appropriate signals in the environment).

Biologists interested in these problems are presently inquiring into the causes of rhythms. Some are proceeding on the assumption that organisms possess an internal "clock" which serves as the master control of all rhythmic action and are busily at work trying to find out the properties of the alleged clock. Others assume that there is no internal clock, that something in the physical environment (perhaps the fall of cosmic rays upon the earth) varies in a rhythmic manner which directly affects each metabolic reaction and so serves as the master control of rhythmic processes. A number of crucial experiments indicate that the first explanation is probably correct.

The Life Cycle of *Acetabularia*: Morphogenesis in a Microorganism

Acetabularia illustrates the capacity of some unicellular organisms to change their form drastically during their life cycle and to create very elaborate structures in doing so. Because of its relatively enormous size, it can reach a higher degree of complexity than most microorganisms.

Acetabulariae are green algae that live in tropical and subtropical marine waters. As shown in Fig. 10, the cell consists of (a) a large cap with leaf-like members arranged like the petals of a daisy, (b) a long stalk, (c) a root-like process at the bottom called the rhizoid. The stalk can be 4–6 cm long and the cap up to 1 cm in diameter in a mature individual. The growing cell contains a single, enormous nucleus usually situated in the rhizoid but sometimes found in the lower stalk.

The complete life cycle of *Acetabularia mediterranea* takes about 3 years. After one year of growth the cell consists of a rhizoid, which has sent out rootlets that hold the alga fast to the substratum, and a cylindrical stalk without a cap. In the autumn, the stalk dries up and falls off leaving the rhizoid which subsists on stored food reserves through the winter. The following spring a stalk grows out and forms a rudimentary cap before regressing once more. In the third year a fully-formed stalk and cap appear. At this time the single, huge, diploid nucleus undergoes meiosis

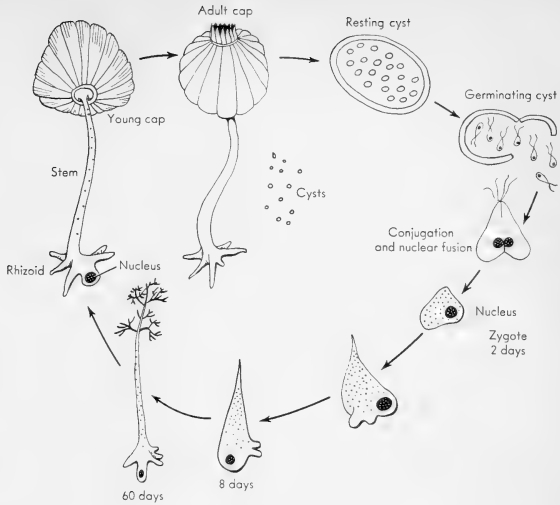


Fig. 10. Life cycle of *Acetabularia mediterranea* (after Brachet, *Biochemical Cytology*, p. 303). In the laboratory, unlike in the natural habitat, development of the adult structure from the zygote is direct, without regression, and takes only one year.

to yield haploid daughter nuclei. Subsequent mitotic divisions produce a great number of tiny haploid nuclei that stream away from the rhizoid into the cap where they are gathered into large bodies with thick walls called cysts. The cysts are eventually shed from the cap and lie dormant. By this time, each nucleus within the cyst is surrounded by cytoplasm and a cell membrane. They look like flagellate protozoa, being pear-shaped with two large flagella protruding from the front end. Eventually the lid of the cyst flips off, freeing the flagellate cells which swim about for a time and then conjugate in pairs. The two cells become one, the two haploid nuclei fuse to yield a diploid, and the zygote can then give rise after 3 years to an adult alga with cap, stalk, and rhizoid to complete the cycle.

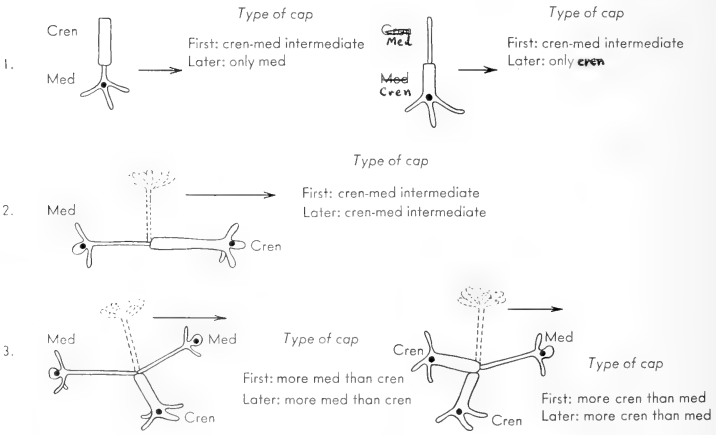
In nature, *Acetabularia* can regenerate lost parts. For example, the seasonal disappearance of the stalk is followed by the synthesis of a new stalk, and the loss of the adult cap by mechanical abrasion is followed by the formation of a new cap. The regenerative powers of *Acetabularia* have been employed to study its morphogenesis and in particular the role played by the nucleus in morphogenetic processes. One can ask whether or not the nucleus has to be present at all for regeneration to occur. It can in fact be shown that pieces of stalk lacking a nucleus (anucleate) can produce new cell membranes, chloroplasts, a perfectly normal new cap, and, in a few cases, even a new rhizoid—in short, everything but another nucleus. After a few months all these parts die. In contrast, pieces containing a nucleus can regenerate lost parts over and over again. These activities are accompanied by extensive synthesis of proteins and nucleic acids. Thus, we can conclude that an anucleate segment of *Acetabularia* has stored reserves of material and energy sufficient to construct a com-

plete adult at least once. However, to do so again and again requires a continual supply of energy and materials, and for this to occur, the nucleus must be present.

One can also ask whether the ability of a segment to form a new cap depends on the size or position of the segment. It turns out that long anucleate pieces of stalk can construct caps better, faster, and with greater frequency than short pieces, and pieces of stalk removed from near the cap have greater competence than pieces of equal length taken from positions near the rhizoid. These data have been given several interpretations. As an exercise, see if you can explain these phenomena and can design an experiment that would test the validity of your explanation.

The various species of *Acetabularia* display wide differences in the form of the cap (number, shape, and size of the leaves, etc.). This specificity, i.e., the power to control cap design, appears to reside in the nucleus. Grafts have been made between two species, *Acetabularia mediterranea* (med) and *A. crenulata* (cren). The following results have been obtained and are summarized in Fig. 11:

Fig. 11. Regeneration in *Acetabularia*. Experiment 1 shows that a piece of one species, containing a nucleus, when joined to a piece of another species, without a nucleus, at first forms an intermediate cap, but, in subsequent regenerations, forms a cap characteristic of the species that contributed the nucleus. Experiment 2 shows that two nucleated pieces not only form an intermediate cap at first but also in subsequent regenerations. Experiment 3 shows that when two pieces of one species are joined to a piece of the other species, all containing nuclei, the cap is intermediate but resembles more closely the species that contributed the two nuclei.



1. A rhizoid containing a nucleus (med) was joined to an anucleate segment of stalk (cren). After a few months of incubation a new cap was formed. Its appearance was intermediate between med and cren. If, however, the newly formed cap were cut off, a second cap was produced and this was a med-type cap with no remnant of cren characteristics. Furthermore, additional removals of the cap yielded only med-type caps. The reverse experiment (cren rhizoid with nucleus and a med anucleate stalk segment) also yielded at first a cap which was intermediate between the cren and med types. When this cap was removed, all subsequently formed caps were purely cren.

2. Two nucleate rhizoids, one of each species, were joined, cut end to cut end. At the junction a stalk grew out which formed a cap intermediate between med and cren. Any subsequent removals of the cap yielded regenerates still intermediate between the two species.

3. Two nucleate rhizoids of cren were joined, cut end to cut end. The cut end of a third nucleate rhizoid (med) was fused to the junction point of the first two. A stalk grew out from this point whose cap was intermediate in appearance but much more like cren then med. When the experiment was reversed, i.e., the two med rhizoids joined with one cren rhizoid, the cap was much more like med than cren.

From these experiments we conclude that it is the nucleus which ultimately controls the morphogenetic activities of *Acetabularia*, presumably by directing the synthesis of substances that play specific roles in the construction of the cap. These materials are to some extent stored within the anucleate portions of the stalk but are not spread equally throughout. Thus, pieces of stalk taken from the apex have more of them than pieces taken from the base; longer stalk pieces have more of them than shorter pieces.

With these experiments as a background, it now is possible to inquire into the nature of the biochemical reactions that mediate morphogenesis in *Acetabularia* and into how the nucleus exerts control over them. Such problems represent an exciting challenge to young biologists and may lead to a general understanding of morphogenetic events in many organisms.

The Beginnings of Multicellular Organization

Living organisms are generally classified as unicellular (the individual is a single cell) or multicellular (the individual is an organized collection of specialized cells). Although such categories imply rigid distinctions, we must remember that discrete boundaries do not exist in nature but only in the minds of biologists. In reality, there is a middle ground occupied by organisms that are neither wholly unicellular nor wholly multicellular. For example, many protozoa, algae, fungi, and bacteria are transiently colonial; they come together for a time as loose clusters of quasi-independent cells with no organization or specialization. Others attain a primitive level of organization in which some cells of the colony become specialized for feeding, others for sexual reproduction, etc. A highly complicated level is attained by one such group, the cellular slime molds. During growth, the cells exist as orthodox unicellular forms, each living independently of its neighbors. After growth has ceased, the cells come together and cooperate to produce a single, organized, multicellular structure with well-differentiated tissues.

Many algae, fungi, and protozoa, and also one group of bacteria, are permanently colonial, because the cells do not separate after reproduction but instead adhere to one another. In some cases these become merely tangled amorphous masses of cells and we can see nothing except the cells that might be called individuals. In others a great deal of organization is attained. The individ-

uality of these cells is clearly submerged within the colony and we can begin to talk of the colony as the individual. Thus botanists are accustomed to call a colony of a higher mold a "plant" as if it were—as indeed it is—a single functional entity.

Multicellularity requires that nature must solve many problems relating first to the formation of the cell community and second to its proper functioning, problems that do not arise in isolated cells. Organisms that display primitive multicellular organization are of great interest to biologists because they illustrate nature's first efforts to develop an individual beyond the single cell. Furthermore, by learning the genetic and biochemical mechanisms of primitive morphogenesis, we hope to uncover principles that are general to all multicellular organisms, including higher plants and animals.

The Cellular Slime Molds

Figure 12 summarizes the life cycle of one species of cellular slime mold called *Dictyostelium discoideum*. The cycle is divided into four stages: growth, aggregation, migration, and fruiting-body construction. It starts with the germination of spores to yield amoeboid cells termed *myxamoebae*. The myxamoebae live in the soil or on the agar surface of a Petri dish, feed upon bacteria, and reproduce by binary fission. When

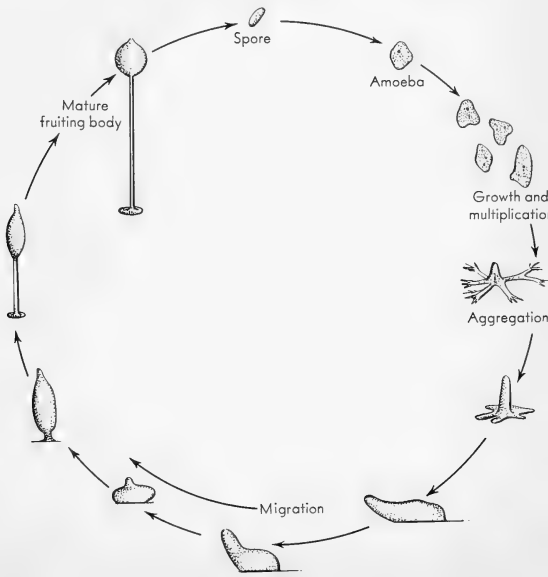


Fig. 12. The life cycle of *Dictyostelium discoideum*.

the supply of bacteria is exhausted, the cells stop growing and enter the aggregation stage. The myxamoebae stream radially toward central collecting points, and, as they reach the center, a conical mound of cells is built up. When all the cells have aggregated, each conical mound falls over on its side and is transformed into a worm-like slug up to a few millimeters in length. The slug migrates over the substratum and finally comes to rest. It then proceeds to construct a fruiting body with a round mass of spores at the top, a stalk enclosed in a cellulose sheath below, and a basal disc at the bottom. These constitute three clearly different cell groups. Ultimately the spores are cast off to repeat the cycle while the stalk cells and basal disc cells desiccate and die. Figure 13 shows photographs of an aggregation sequence, migrating slugs, and the construction of a fruiting body.

The cellular slime molds display the same attributes that more complicated developmental systems, i.e., embryos, do. These are categorized as cellular differentiation, cell interactions during development, and regulation.

CELLULAR DIFFERENTIATION

As we have mentioned, the fruiting body consists of three distinct cell groups: spores, stalk cells, and basal disc cells. These have radically different sizes, shapes, constitutions, and functions—as do the various tissue cells of a frog embryo. We can ask: (a) whether cells normally destined to become spores can develop instead into stalk cells? and (b) if a stalk cell or basal disc cell were removed from the fruiting body before desiccation and death and permitted to grow and reproduce, would its progeny develop normally, i.e., could they aggregate and build a fruit containing all three tissues?

The fate of a cell (i.e., whether it will become a spore or stalk cell or basal disc cell) appears to depend on the order in which it enters the

Fig. 13. Slime mold morphogenesis. Left, an aggregation sequence; top right, migrating slugs (from J. T. Bonner, *The Cellular Slime Molds*. Princeton: Princeton University Press, 1959, Plate II); bottom right, the stages of fruiting body construction—the photographs were taken appromixately 1½ hours apart (also from Bonner, Plate III).

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aggregate (Fig. 14). Cells that enter the aggregate first become the leading element of the migrating slug and ultimately the lower stalk of the fruit. Later arrivals take up progressively more posterior positions in the slug and become upper stalk cells and spores. Those entering last comprise the tail end of the slug and ultimately the basal disc of the fruit. One can tentatively imagine that the position of a cell in the aggregate and in the

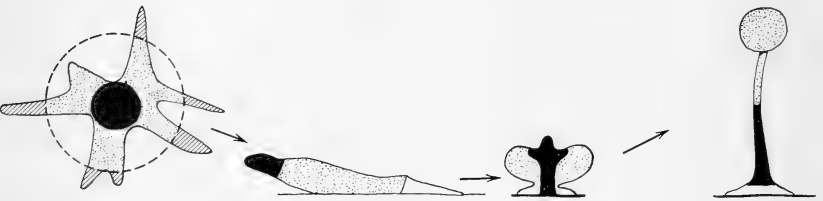


Fig. 14. The fate of the cells depends on the order in which they enter the fruit.

slug subjects it to a specific set of environmental conditions and of chemical signals imposed by its neighbors that forces it to become a spore or stalk cell or basal disc cell. These differences are reversible. Slugs have been cut into head and tail segments. Both could construct fruits separately. Although not completely normal, both contained all three types of cells even though the head segment would ordinarily have given rise to stalk only, while the tail segment would have yielded spores and basal disc cells but not stalk. In addition, stalk cells have been separated from the fruiting body before they desiccated and died and have been allowed to feed on bacteria and reproduce. The progeny of each such stalk cell were themselves capable of aggregating and constructing normal fruits with viable spores.

Another example of cellular differentiation is seen in the migrating slug. If the front end of the slug (about 10 per cent of the cells) is separated from the rear end, it continues to migrate, while the rear end stops in its tracks. If the front end is replaced, the two parts fuse, and migration of the whole slug proceeds once again in normal fashion.

These and other experiments demonstrated that two cell types, "leaders" and "followers," exist and that the slug is an organized entity. The biochemical mechanisms by which the leaders guide the migration of the followers and the manner in which cells become leaders and followers are still unknown.

Finally, there is a great deal of evidence to suggest that, in *Dictyostelium discoideum,* the formation of an aggregate requires the presence of a specially endowed cell, which has been called the "initiator cell." Thus at the time of aggregation, the population of myxamoebae is differentiated into initiator cells, which provide the signal to start aggregating (and in whose absence aggregation does not occur), and the responder cells, which answer the signal.

CELL INTERACTIONS DURING DEVELOPMENT

The development of a multicellular system is accompanied, indeed is largely directed, by a hierarchy of cell interactions. One cell or one tissue may stimulate or inhibit the development of another by exchange of appropriate chemical agents. It is this matrix of interactions that ensures the harmonious organization of the whole, i.e., that the components will develop at the right time, in the right place, and in the right amount. As we will see in Chapter 7, the use of cell interactions reaches its highest degree of complexity and refinement in vertebrate embryos. However, the beginnings of these control mechanisms operate in primitive organisms such as the slime molds.

A case in point is the way in which the cells aggregate. A large body of evidence has been accumulated to show that outlying cells are attracted and caused to aggregate by specific chemical agents produced at the center. (Attraction of cells by a chemical compound is called *chemotaxis.*) For example, if the myxamoebae are permitted to aggregate under a flowing stream of water (Fig. 15), a center forms, but the cells upstream are unaffected. Only those downstream are attracted by and move toward the center. This is just what you would expect if the center were producing a chemical agent that was being carried downstream by the current.

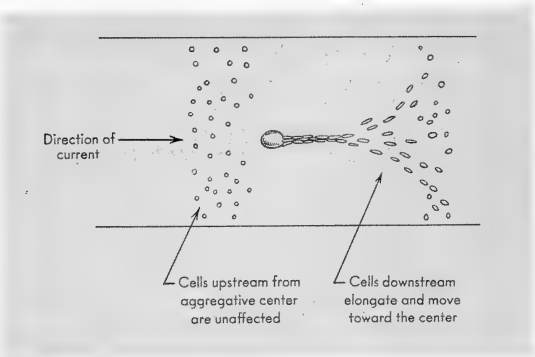


Fig. 15. Myxamoebae aggregate under a flowing stream.

REGULATION

This term refers to the ability of a morphogenetic system to turn out normal products of different absolute sizes. For example, individual frog embryos or tadpoles or adult frogs of a given species can vary markedly in size and yet be perfectly proportioned. That is, the sizes of the various parts (arms, legs, head, etc.) at each developmental stage bear precise relationships to the total length or total mass. Furthermore, if cells are surgically removed from a very young frog embryo, the remainder will develop into a very tiny but nonetheless normal tadpole, again demonstrating the same relationship of parts to whole.

Slime molds also display regulatory capacities. For example, myxamoebae packed together in a solid mass produce relatively large migrating slugs and fruiting bodies; some are as much as 5 mm in length and contain hundreds of thousands of cells. If sparsely distributed, the myxamoebae form tiny slugs and fruits a few tenths of a millimeter in length that contain a few hundred cells. Yet the gross proportions and the relative amounts of cells comprising the various parts of the fruiting body remain the same. This regulatory capacity is exhibited at a truly startling

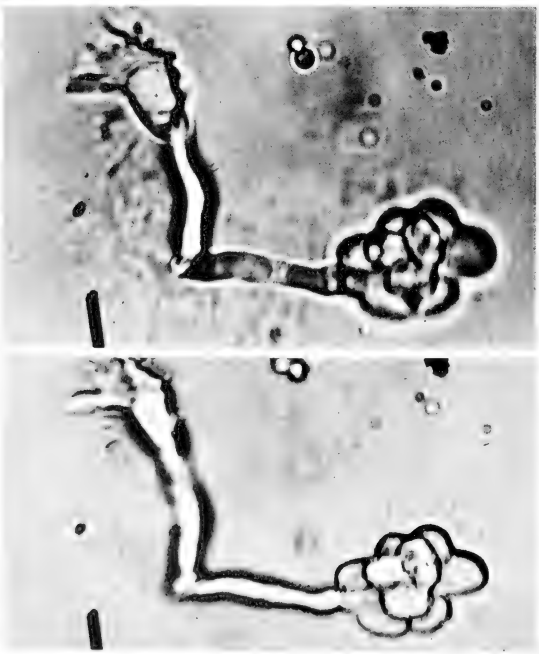


Fig. 16. Two views of a fruiting body formed by the "fruity" mutant of *D. discoideum*. This highly organized multicellular structure consists of 9 spores, 2 stalk cells, and 1 basal cell.

level by a mutant of *Dictyostelium discoideum* that can form normal fruits containing hundreds of thousands of cells or as little as 10–12 cells. Figure 16 shows an example of the latter, a fruiting body with 12 cells—9 spores, 2 stalk cells, and 1 basal disc cell. This exquisite miniature contains all the elements of a morphogenetic system—cellular differentiation, cell interactions, and regulation—but in a tiny packet of cells.

In summary, these are some of the lessons learned thus far from the study of slime molds:

a. That just as in the development of higher forms (vertebrate embryos, etc.), slime-mold development involves the appearance of new cell types (initiator and responder cells; leader and follower cells; spores, stalk and basal disc cells) which play a causal role in the construction of the multicellular whole. We would like to know how and when they arise and in what numbers, and what physiological mechanisms are responsible for the roles they play in morphogenesis.

b. That here as in other developmental systems, a matrix of cellular interactions helps to regulate the process. Chemical signals are passed between initiator and responder cells, between responder and responder during aggregation, and between leader and follower cells during migration of the slug. Finally, chemical signals tell some cells to become spores, others to become stalk cells, and still others to become basal disc cells in the fruiting body. We want to know what are these signals and how they act.

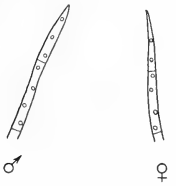
c. That normal development need not involve participation of huge numbers of cells. Instead, even as few as 10 cells can provide the components and the chemical interactions needed to construct a perfect fruiting body.

A Water Mold, *Achlya Bisexualis*

Achlya is an example of a permanently colonial organism. The cells grow in long, branched filaments which form a tangled mass. Some of these filaments can penetrate the substratum (a floating seed, a dead insect) to anchor the colony and absorb nutrient materials and thus serve as a primitive root system. Others grow as aerial stalks and produce special structures called *sporangia* that bear spores. The spores are cast off and germinate to yield new filamentous colonies. Some of the aerial filaments can also produce sexual structures, primitive counterparts of the male and female structures of plants. A single *Achlya* colony produces either male or female structures, not both. The fascinating aspect of this development is that the formation of male and female structures is triggered by hormones that pass between the colonies.

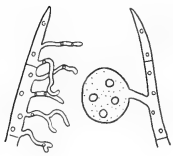
The sequence of events is shown in Fig. 17. When small pieces of male and female colonies are innoculated at opposite ends of the Petri dish, they grow toward each other as expanding mats of branched filaments (vegetative stage). As they approach each other: (1) the tips of

Fig. 17. Sexual hormones in *Achlya*.

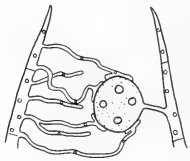


Male and female vegetative filaments

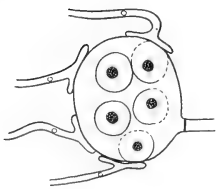
1. Female secretes Hormone A. Male reacts by producing antheridial initials.



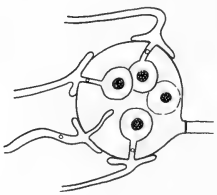
2. Having reached this stage, male secretes Hormone B. Female is induced to form the oogonial initial.



3. Female oogonial initial secretes Hormone C. This causes the antheridial initials to grow directly toward the oogonial initial and plaster themselves against it.



4. Male secretes Hormone D. That causes the cytoplasm to congeal about the oogonial nuclei and produce functional female gametes.



5. Finally, the antheridia, presumably directed by a female hormone, put out germ tubes that connect up with the female gametes and permit entry of the male nuclei.

the male filaments send out huge numbers of gnarled, branched shoots; (2) the female filaments expand at their tips into large sacs; (3) the male shoots grow in a directed manner toward the female sacs and plaster themselves around their surfaces; (4) cytoplasm congeals about the nuclei inside the female sacs, each aggregate of nucleus and cytoplasm being called an *oosphere*; (5) the male nuclei penetrate the sac through tiny tubes and each unites with a female nucleus to produce a zygote.

Male and female colonies must be grown together to yield sexual structures. Grown alone, they merely produce the usual filaments with sporangia. Furthermore, sexual structures begin to form before the two colonies actually come into physical contact. That is, they appear when the colony edges are still separated by several millimeters, and develop in a wave, back from the advancing edge of the colonies. Thus, we can conclude that (1) the presence of a male colony is necessary for the development of sex structures in a female colony and vice versa, and (2) the effects are mediated by agents, i.e., "sex hormones," that can diffuse through agar over considerable distances.

In addition, it has been found that each male and female *Achlya* colony produces several hormones that trigger successive stages in the development of the sexual structures. That is, a hormone A produced by the female vegetative colony induces the male to send out gnarled, branched shoots called *antheridial initials* (stage 1 of Fig. 16). Having done so, the male can now produce a hormone B that causes the female vegetative colony to produce the large sacs called *oogonial initials* (stage 2). The female can at this point only produce a third hormone C that causes the male shoots to grow in directed fashion toward the source of this hormone and to plaster themselves around the sacs (stage 3). Upon contact, the male produces a fourth hormone D that causes the female sacs to develop oospheres, and so on.

Attempts have been made to isolate the first of these hormones (A). Female *Achlya* was grown in 500 gallons of liquid culture medium, under conditions which permitted the fungus to pour out maximal quantities of hormone A. The medium was collected and concentrated. By exploiting various means of chemical purification, the intrepid biologists ended up with 0.0003 grams of material, still impure, but which could act upon the male *Achlya* when diluted to 1 part in 10,000,000,000,000 parts of water! Unfortunately, 0.0003 grams was too small an amount to permit chemical identification, and this gallant attempt had to be abandoned. Recently, however, the project has been resumed, this time starting with 10,000 gallons of *Achlya* culture! In addition, a similar hormone has recently been isolated from another water mold distantly related to *Achlya*. This hormone, given the romantic name *sirenin*, has been isolated, purified, and crystallized. Now chemical studies are being done to characterize the

agent and yield a structural formula. The importance of this work lies in the fact that, while hormone-triggered development is a common thing in fungi, none of the agents has been chemically identified and nothing is known about the biochemistry and physiology of these processes.

The Development of a Primitive Animal: The Coelenterates

A *tissue* is defined as a group of cells having similar structure and function and arranged in a compact, organized array. Thus, we talk of connective tissue or bone tissue or epidermal tissue. The revolutionary transformation of multicellular organisms from quasi-amorphous conglomerates of cells into structures with well-defined tissues was a significant step, for it enabled these tissues subsequently to combine into organs and organ systems and thus permitted the levels of complexity that higher animals have since attained.

The most primitive group of organisms still extant that displays a definite tissue organization is the Phylum *Coelenterata* (or *Cnidaria* as it is now called). This is a group of fresh-water and marine animals that includes *Hydra* (a favorite laboratory animal in elementary biology) and its relations, the sea anemones that abound on rocky coasts, the corals, the jellyfish, and the awesome Portuguese Man of War, whose toxin has paralyzed many unwary swimmers and led to death by drowning. A brief résumé of the general properties of these organisms illustrates their primitive condition:

1. The body is composed of only two tissues, the outer epidermis and the inner gastrodermis. These arise early in the development of the coelenterate embryo. In contrast, the tissues of all higher animals stem originally from three embryonic cell layers. Within the two coelenterate tissue layers are a number of cell types, some of which appear in Fig. 18.

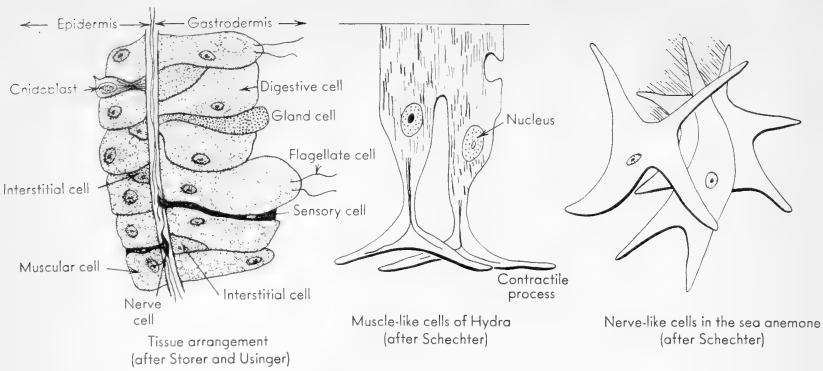


Fig. 18. Cell types found in coelenterates.

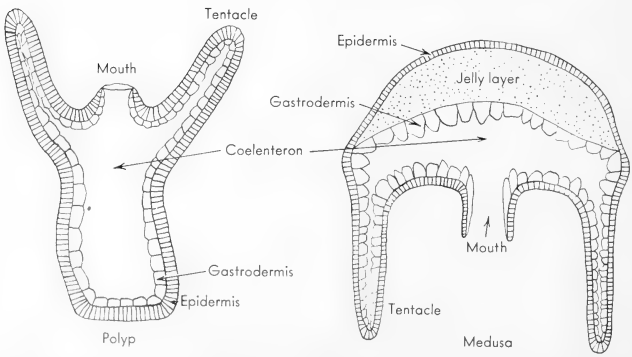
2. The body is essentially in the shape of a sack. That is, only one opening exists, and this serves as both mouth and anus. The internal lining of the sack (gastrodermis) serves to digest the food carried in through the mouth, but there is no suggestion of a digestive organ system.

3. There is no blood or circulatory system, no excretory or respiratory organs.

4. There is a diffuse network of nerve cells, but no central nervous system and only rudimentary sense organelles.

The adult coelenterates occur in two forms. One is the *polyp*, which has a tubular body with a closed end that is attached to the substratum. There it grows singly or in colonies. The second is the *medusa*, a free swimming, gelatinous body with a shape like an umbrella; its mouth hangs down from the concave undersurface. Figure 19 shows examples of these. They are variants of the same basic sack-like structure. In the polyp,

Fig. 19. Body plans of adult coelenterates.



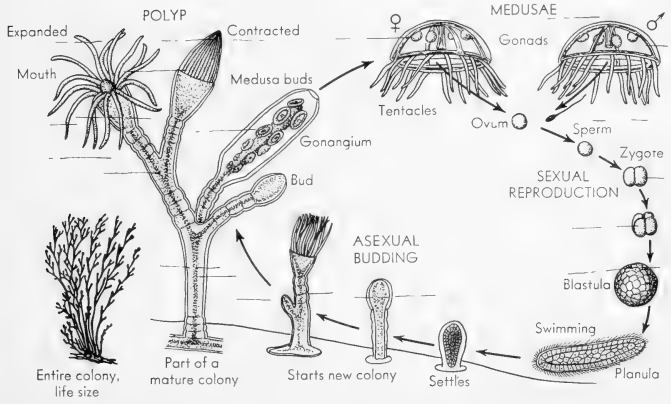
the sack (coelenteron) is elongated and narrow with its open end up. In the medusa, the sack is squat and rounded with its open end down. Some species appear both as polyps and medusae during their life cycles. In other species, one or the other adult form may be rudimentary and very short-lived or entirely absent.

In both forms, the mouth is surrounded by tentacles, and these contain special stinging cells called *cnidoblasts* (hence the name *Cnidaria*). Upon stimulation, they shoot out spear-like processes bearing powerful toxins to paralyze their prey (small crustaceans, insect larvae). Very little is known about the toxins. The few that have been studied appear to be proteins. When injected into rabbits, they produce neural damage and elicit the formation of antibodies, just as do certain snake venoms, tetanus toxin from bacteria, and other poisonous proteins.

Life Cycle of the Genus *Obelia*

Both polypoid and medusoid stages figure prominently in the life cycle of *Obelia*, which is summarized in Fig. 20. The polyps grow as colonies upon rocks and shells in shallow marine waters. The colony is fastened to the substratum by a mass of root-like runners that bear slender, branched stems, and from these extend polyps of two kinds: the feeding polyps equipped with mouth and tentacles, and the reproductive polyps, which lack all feeding mechanisms and are designed for one purpose—to produce medusae that bud off from the central cylinder and escape through the hole in the vase-shaped outer covering.

Fig. 20. The life cycle of *Obelia* (after Storer and Usinger).



The roots and stems of the colony are hollow cylinders composed of the same two tissue layers, epidermis and gastrodermis. The internal space, called the *coelenteron,* is filled with fluid, is continuous, and extends into the polyps. This means that the feeding and reproductive polyps and the cells in the walls of runners and stems are all in intimate contact, and all parts of the colony can be supplied with the food materials that enter through the feeding polyps.

Both kinds of polyp are initiated as buds on the stems. At first the bud is simply a protuberance of the stem wall that contains both tissue layers. The protuberance lengthens rapidly by cell division and by migration from other areas. Infoldings and constrictions of the tissue ultimately give rise to the organs that are characteristic of the feeding and reproductive polyps.

As we have mentioned, the medusae also are formed by budding from the central cylinder of the reproductive polyps. Fully formed, they are minute jellyfish, shaped like an umbrella and rimmed with tentacles. The mouth hangs down from the concave side and leads upward into the digestive cavity in the middle of the umbrella. They move by jet propulsion, water being forced out of the mouth, and feed upon crustacea and protozoa. They are of two sexes, male and female. Their gonads develop inside the digestive cavity, and eggs or sperm are propelled through the mouth. Fertilization takes place in the sea.

The fertilized egg promptly cleaves into two, the two into four, and so on until a hollow ball of cells is produced. This is called the blastula stage and corresponds to a similar stage in the development of vertebrate embryos (see Chapter 5). Some of the cells are forced into the interior of the ball and become the gastrodermis, while the cells that remain outside become the epidermis. This corresponds to the gastrula stage of embryogenesis, encountered in higher invertebrates and vertebrates, during which their three basic tissue layers appear. After tissue separation, the *Obelia* embryo elongates, and cilia appear over the outer surface. The constituent cells now begin to transform into sensory, gland, and muscle cells and cnidoblasts. At this point, embryogenesis is concluded, and the animal is called a *planula larva* (i.e., the immature, larval form of the adult polyp). The *planula* swims about for a period of hours to days and then attaches to a rock or shell. The attached end develops into a root-like runner, and the free end acquires a mouth and tentacles. Buds appear, the polyp colony is initiated, and the cycle is complete.

Polymorphism in the Coelenterates

Polymorphism (a variety of forms) occurs at two levels in the coelenterates. First, there is the basic difference in the adult structures (i.e.,

polyp and medusa). One can ask why it is that in an *Obelia* colony a bud on the stem of a feeding polyp gives rise to another polyp, while a bud on the cylinder of a reproductive polyp gives rise to a medusa. Are the constituent cells different, or is it merely that they inhabit different areas of the colony and so are subject to different environmental conditions? Actually the same sort of question can be asked about our own bodies; namely, why is it that the cells in one part of a human embryo develop into brain cells and in another part develop into liver cells? These questions will be taken up in detail in the chapter on cellular differentiation.

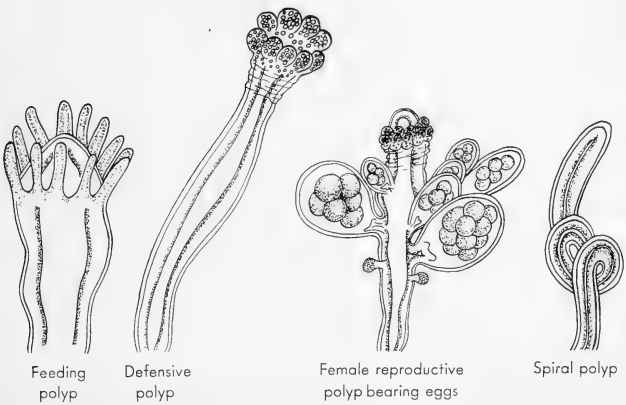
The second level of polymorphism is exemplified in *Obelia* by the specialization of the polyps. Another genus, *Hydractinia*, displays an even greater range of differences in polyp structure, as Fig. 21 shows. *Hydractinia* colonies live on the surface of a specific type of snail shell but only when inhabited by hermit crabs. Three types of polyp develop:

1. Feeding polyps. These correspond in essential features to the feeding polyps of *Obelia*.

2. Defensive polyps. These are concentrated at the lip of the snail shell. They lack a mouth and their tentacles are reduced to small knobs arranged in two circular rows. The stems have considerable musculature and can whip about fiercely, thereby permitting the cnidoblasts on the tentacle knobs to discharge a concentrated volley upon contact with the foe.

3. Reproductive polyps. In *Hydractinia*, the medusa stage is absent. Instead, the reproductive polyps produce sex cells directly. There are two

Fig. 21. Polymorphism in *Hydractinia*.



sexes (a single colony produces reproductive polyps of one sex only). The male and female varieties are quite differently formed, as seen in Fig. 20.

Regeneration of Polyps and Medusae

The decapitation of a polyp is followed within a few days by the appearance of a new head at the cut end complete with mouth and tentacles. If a segment is cut from a polyp stem or from a root-like runner and incubated in water, the isolated piece can give rise to a new polyp. Similarly, a piece taken from a medusa, if large enough, can reorganize itself into a complete functional medusa with all parts normal and intact.

The regeneration is specific. That is, a piece of tissue taken from a polyp regenerates a polyp. A piece taken from a medusa regenerates a medusa. In a recent study, it has been shown that this specificity extends even to the kind of polyp from which the piece of tissue was taken. Cut stems taken from a *Hydractinia* colony regenerated true to type. Stems of feeding polyps regenerated only feeding polyps. Those from reproductive polyps regenerated only reproductive polyps. Two conclusions are permissible from these data:

1. A complete, functional, adult coelenterate contains many cell types (i.e., cnidoblasts, epidermal cells, gland cells, muscle cells, nerve cells, etc.). These results show that even a small piece of coelenterate tissue either contains all the necessary types or can give rise to them by appropriate transformation of one cell type to another.
2. The pieces of tissue appear to "know" from what kind of adult they came and to be able to reorganize themselves accordingly.

Compare these results and conclusions with those gained from the study of *Acetabularia* regeneration described in Chapter 2. Please note that they are essentially identical, save that in one case we deal with a structure whose parts are divided up into cellular packets, while in the other we deal with one whose parts are not so divided but are nevertheless organized in a specific manner.

Regeneration by stem segments has other interesting features that are summarized in Fig. 22. First, the rate and extent of new head development depends on the part of the stem from which the segment is taken. If a polyp stem is cut into serial segments of equal size, the one from the end nearest the previous head (distal end) develops fastest and most completely, while that from the lowest (proximal) end is the most laggard. In other words, along the stem there is a gradient of potency for the production of new heads. In addition, one part of a stem in producing a new head can prevent another part from so doing. For example, a stem segment

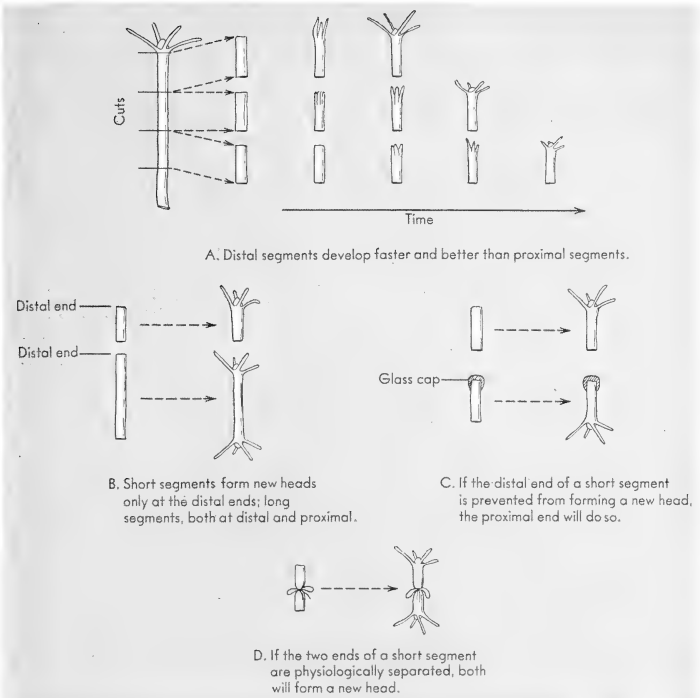


Fig. 22. Regeneration of polyps by stem segments.

of moderate length develops a new head at only one cut end, and this is invariably at the distal end. However, if we prevent the distal end from regenerating by slipping a glass cap over it, then the proximal end will form the head (although slower than the distal end would have done it). This interaction can be relieved in two ways: first, by making the stem segment so long that the two ends are not in close contact; second, by separating the two ends physiologically. The latter can be accomplished by tying a string tightly around the middle of the segment so as to prevent the flow of materials and cells between the ends. When these measures are taken, heads form at both ends without any evidence of competition between them.

This type of phenomenon is commonly encountered in developmental systems. A case in point is the development of the hind limb in a chick embryo. As described in Chapter 5, the limb originates as a small protuberance from a specific point along the flank of the embryo. Areas surrounding this point can also be shown to be capable of forming the

protuberance and ultimately a complete limb. We can ask, therefore, why it is that the limb forms where it does and why other areas that have the potency for limb construction normally do not grow one.

A system in which many parts can develop in a certain way but where only one part actually does so is called a *morphogenetic field,* which will be discussed in detail in the next section. The dominance of one part of the field over another maintains regularity of development. It ensures that a coelenterate polyp will have one head and that it will be where it ought to be, i.e., at the top of the stem. It ensures that a chicken will have one tail, two wings, and two hind limbs in the right places. Moreover, it makes for versatility, for if by accident the dominant area of a morphogenetic field is destroyed, a neighboring area has the potency to take its place. Thus the top of the coelenterate stem and only the top normally forms a head. But if by accident the head is destroyed, a new one can be constructed from underlying tissues.

Morphogenetic Fields

The concept of a morphogenetic field had its modern inception in the work of Professor C. M. Child, a famous American embryologist. Dr. Child was the first to emphasize the fact that in such fields one area will gain dominance over another by its superior capacity to form a given structure and by its ability to inhibit neighboring areas from doing so. He and his students provided many examples of field development in the regeneration of coelenterates, flatworms, and starfish and in the formation of embryos. Thus we speak of head fields, limb fields, tail fields, heart fields, etc., to denote this kind of development. Attempts have been made to describe morphogenetic fields in mathematical terms in an effort to understand the processes by which dominance is established. A simplified version of the mathematical argument is given below.

Consider a group of coelenterate stem cells that can form a head. We divide this group into two separate areas. The speed at which the stem cells in an area will construct a head should depend on their inherent capacity (i.e., developmental potential) to be converted into head cells and also on the number of cells available in the area for head construction. This can be written formally as:

$$\text{rate of head development} = (\text{developmental potential}) \times (\text{number of stem cells}) \qquad 1$$

The rate of development in area I might be greater, equal to, or less than that of area II, depending on the relative developmental potential and on the numbers of cells that comprise each area. However, a difference in developmental potency is not enough to create a morphogenetic field,

since, if the two areas were isolated from each other, each would construct a head at its characteristic rate and neither would dominate the other. It is necessary for the two areas to be able to interact upon one another.

Suppose the developing head cells can pour out materials that inhibit others from becoming head cells. First of all, the cells in area I that had already been incorporated into the developing head would inhibit the remaining stem cells in that area from changing into head cells. The degree of inhibition would depend on the inherent sensitivity of the area I stem cells toward being inhibited by their own head (autosensitivity) and on the number of head cells that are producing the inhibitory materials. This inhibition would have to be subtracted from the rate of head development defined in statement 1. Thus,

$$\begin{pmatrix}\text{rate of head}\\ \text{development}\\ \text{in area I}\end{pmatrix} = \begin{pmatrix}\text{developmental}\\ \text{potential}\\ \text{of area I}\end{pmatrix} \times \begin{pmatrix}\text{number of}\\ \text{stem cells}\\ \text{in area I}\end{pmatrix} - \begin{pmatrix}\text{auto-}\\ \text{sensitivity}\\ \text{in area I}\end{pmatrix} \times \begin{pmatrix}\text{number of}\\ \text{head cells}\\ \text{in area I}\end{pmatrix} \qquad 2$$

According to this statement, the rate of development would be very high at the beginning of head formation. Then, as the head grew larger and more and more cells were incorporated into it, they would produce inhibitory materials and detract materially from the rate at which additional stem cells would be incorporated into the head. Meanwhile, the number of stem cells would decrease as they were converted into head, and the first term on the right side of the above equation would decrease. Eventually, the two terms on the right side would cancel out, and the rate of head development would become zero (i.e., the head would now be complete). In the case under consideration, the rate would fall to zero before all the stem cells had been converted into head cells, since a coelenterate head would look pretty silly without a stem to support it.

Now suppose the two areas are in contact with each other (for example, if the two have a common coelenteron and are bathed by the same body fluid). Then the head cells of area II would inhibit the stem cells in area I in the same fashion as described above. That is, the rate of area I development will be impeded by the inherent sensitivity of area I stem cells to the inhibitory products [1] of area II head cells (*heterosensitivity*)

[1] I have described the interaction between areas I and II as the result of the production of inhibitory materials. We could equally well account for it by assuming that the areas compete for a common pool of nutritive materials that serve to stimulate development. Were this the case, area II would inhibit area I by depriving it of these materials and vice versa. Actually, the mathematical argument turns out to be the same whether we put it in terms of inhibition or deprivation.

and by the number of head cells in area II. The complete description of the rate of head development in area I would then become:

$$\text{rate of head development in area I} = \begin{pmatrix}\text{developmental potential in area I}\end{pmatrix} \times \begin{pmatrix}\text{number of stem cells in area I}\end{pmatrix} - \begin{pmatrix}\text{autosensitivity in area I}\end{pmatrix} \times \begin{pmatrix}\text{number of head cells in area I}\end{pmatrix} - \begin{pmatrix}\text{heterosensitivity in area I}\end{pmatrix} \times \begin{pmatrix}\text{number of head cells in area II}\end{pmatrix} \quad 3$$

The same sort of equation can be written for the rate of head development in area II.

We can now ask how area I can gain dominance over area II and prevent it from forming a head, and we see that this can be done in a number of ways:

1. Area I could have a much higher developmental potential or a much greater number of cells. In either case, it would form a head so fast and begin pouring out inhibitory materials so soon that area II wouldn't have a chance to get started.

2. Area I might be very resistant to auto-inhibition (i.e., the inhibitory materials might be carried away by the body fluid circulation quickly, whereas area II might be very sensitive (i.e., the inhibitory materials might not be carried away).

3. Area I might not be very heterosensitive, while area II might be extremely so.

4. Even were developmental potentials, sensitivities, and cell numbers equal, area I could gain dominance by starting head formation first. The head start (forgive me) would permit it to inhibit area II.

Any one of these conditions, or a combination of them, would serve to establish the dominance of area I.

At present, none of the biochemical and genetic mechanisms that create differences in developmental potential or that exert inhibitory effects have yet been identified for any morphogenetic field. Thus, this kind of developmental study is in its infancy.

The Development of the Vertebrate Embryo

Nature's job in *embryogenesis* is to create a young, functioning, multicellular organism. Her raw materials are (1) an egg, a single cell, large with respect to sperm but tiny with respect to the finished product that is to come, and (2) a sperm containing little more than a nucleus which, when joined with the egg nucleus, will provide the instructions needed to produce the embryo. She solves the problems of embryogenesis by the following strategems:

1. Cleavage: the fertilized egg is first subdivided into a large number of smaller cells, because little self-contained packets are easier to control and alter than a single, undivided mass of protoplasm.

2. Gastrulation: the cleaved cells are moved about so as to create three basic tissue layers. These become further subdivided and give rise to the tissues and organs of the functional adult.

3. Organ formation: each subdivision of the three original tissue layers becomes a semi-autonomous system within which cells appear by division, are grouped into tissues, and finally become functional entities (kidney, spleen, brain, etc.). Thus, many changes occur simultaneously within the subsystems of the developing embryo, and they must be linked in such a way as to operate harmoniously, in the right place at the right time. This is accomplished in part by having chemical signals pass between developing subsystems so that one of them

can trigger or curtail the next and therefore provide chronological order to what would otherwise be chaos.

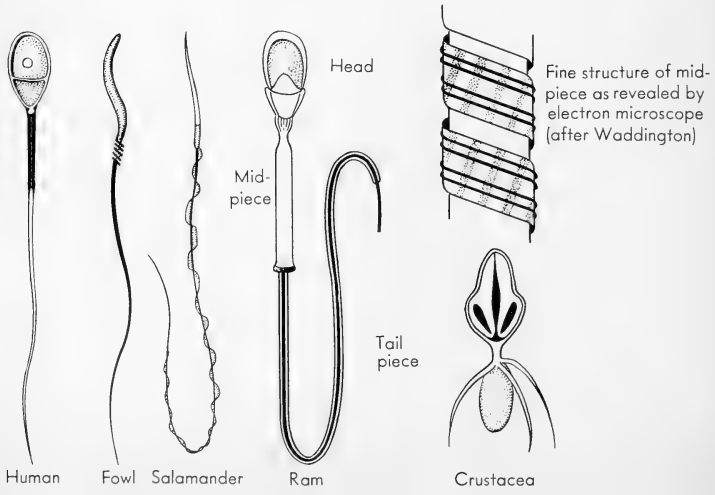
In the rest of this chapter we shall consider these processes, as well as the earlier phases of embryogenesis, i.e., the formation and union of the gametes, in some detail.

The Early Stages of Embryogenesis

THE FORMATION OF GAMETES: THE SPERM

The first step in the recipe for roast chicken is: find your chicken. Similarly, nature's instructions to a sperm are: first, find your egg. To accomplish this, the mature sperm is a stripped-down model equipped with organs of locomotion and with a high rate of metabolism so that it can generate energy to move rapidly over relatively great distances. Once in contact with the egg, it must deliver a nucleus containing a haploid set of chromosomes, one-half the complement of the future embryo. Figure 23 is a drawing of the sperm of several animals and includes a reconstruction of what we see in the electron microscope when we look at the midpiece of ram sperm. This should give you some idea of the sperm's structural complexity. Various sperm may look very different, but they all contain these basic parts:

Fig. 23. Spermatozoa.



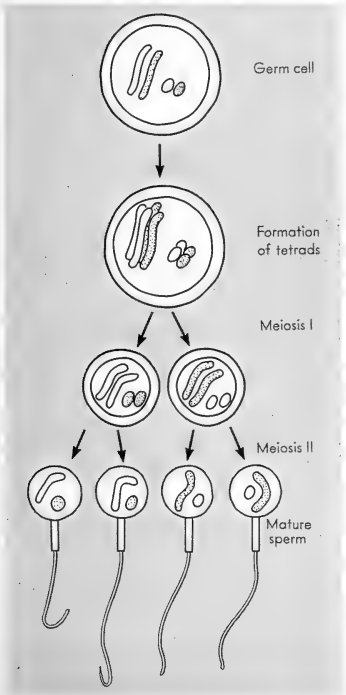
1. The head. This portion is taken up almost completely by the sperm nucleus.

2. The midpiece. This part contains material which will be put to use after the sperm penetrates the egg. It aids in the fusion of the egg and sperm nuclei and in the cleavage of the fertilized egg into the first two daughter cells.

3. The tail. This can be long or short, flexible or stiff. It may vibrate, whip, or rotate in a screw-like motion depending on the species. It is the locomotor organelle.

The ancestors of the sperm start out as ordinary diploid individuals. The germ cells, as they are called (both in males and females), arise early in the development of the embryo. They appear to be irreplaceable, because when embryos are treated in a manner that destroys or removes (by ultraviolet irradiation or surgery) the germ cells, the resultant adult organisms are sterile.

Fig. 24. Maturation of the sperm.



Gametogenesis, the process by which the diploid germ cells give rise to haploid sperm or eggs, is properly within the domains of cytology and genetics but will be reviewed briefly here. Figures 24 and 25 are schematic summaries of the maturation of male and female gametes. For simplicity, we imagine that the diploid nucleus contains only two pairs of chromosomes. Each chromosome duplicates, yielding a quartet or tetrad of chromatids. The cell itself then divides into two, each with a pair of sister chromatids. This is *meiosis I.* These cells in turn divide without chromosomal duplication to yield four *(meiosis II)* so that the daughter nuclei end up with two chromosomes rather than the original four and are therefore haploid. In the case of sperm, the daughter cells are changed from ordinary-looking cells into functional gametes after meiosis II.

THE FORMATION OF GAMETES: THE EGG

The egg has three functions to perform:

1. To supply a nucleus containing half the chromosomal complement of the future embryo.
2. To supply almost all the cytoplasm upon union with the sperm.
3. To supply food reserves that will enable the embryo to develop to a stage where it can begin to feed upon exogenous materials.

Because it must do all these things, the animal egg is a cell of giant size. Consider for example, the size of a hen's egg. Even that of the mammal, .05–.25 mm, is considerably larger than ordinary body cells.

The egg is covered by a protective envelope: in birds, by an inorganic shell; in amphibians, by a jelly coat made up of polysaccharide and protein of high molecular weight. In mammals, a single layer of very small protective cells cover the egg surface. The egg proper is bounded by one or more membranes. A single haploid nucleus resides within, together with cytoplasmic constituents whose fine structure, revealed by the electron microscope, does not seem to be different in kind from the constituents of other cell types. Finally, there is yolk, the food supply for the embryo. This is a heterogeneous mixture of fat droplets, granules, and small bodies, bounded by membranes. Some of these are highly pigmented and, because the yolk is organized material, can be seen to occupy definite regions of the egg and to be parceled out amongst the daughter cells in a definite manner.

There are great differences among eggs of the animal species with respect to yolk content. For example, the eggs of marine animals contain relatively little yolk because the embryo begins active feeding at a very early stage. What yolk there is in marine eggs is distributed homogeneously and not concentrated in a particular region. In contrast, the eggs of birds contain enormous amounts of yolk. For example, the hen's egg contains a tiny disc of cytoplasm sitting on top of an enormous mass of yolk. Insect eggs also contain large amounts of yolk, which is situated at the center of the egg and surrounded by a thin outside layer of cytoplasm. In these forms, the embryo undergoes considerable developmental changes before emerging as an actively feeding organism; hence the large supply of food reserves. At the top of the evolutionary scale are the mammals. A mammalian embryo develops in a sense as a parasite inside its mother and receives a continuous supply of food materials from her through the circulatory system, much as if it were just another of her internal organs. A large yolk reserve is therefore unnecessary, and mammalian eggs resemble the primitive marine eggs in this respect.

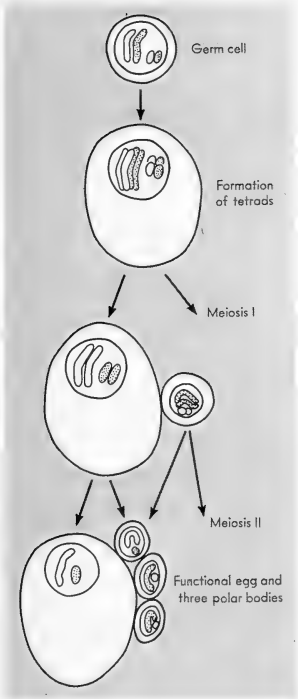


Fig. 25. Maturation of the egg.

The maturation of the egg, like that of the sperm, proceeds via two meiotic divisions from a small diploid parent cell (Fig. 25). The parent cell chromosomes duplicate, and the cell then grows to enormous size. The proteins, fats, nucleic acids, and carbohydrates that comprise the yolk are synthesized within the egg or are received from without (contributed by so-called "nurse" cells). When the final size is attained, the egg nucleus goes through meiosis I and two cells appear. The cell division is very unequal, however, and the lucky nucleus finds itself with most of the cytoplasm and yolk, the unlucky nucleus with very little. Meiosis II follows and now four haploid cells are present. Again, because of a very unequal division, one of the daughter cells contains practically all the yolk and cytoplasm. This, then, is the mature egg, and its three tiny sisters (called polar bodies) shortly degenerate and disappear. Figure 24 is a schematic summary of the process.

In most cases, developing eggs reach stage C and then lie dormant until fertilization, at which time the process is completed and the surviving female gamete nucleus unites with the sperm nucleus. In a few cases the egg is arrested at stage B and very few reach stage D before fertilization.

THE LIFE SPAN OF GAMETES

A mature egg must be fertilized within a short time, if it is to be fertilized at all. Thus, human eggs generally remain functional for about 12–24 hours after their release from the ovary. The eggs of sea urchins, which are shed into the water, last for 48 hours, but the eggs of most invertebrates as well as fish and amphibia must be fertilized within a few minutes of being shed. The life spans of other animal eggs are of the same order of magnitude as those of human eggs.

Sperm are generally just as short-lived as eggs. However, in some cases (bees, bats) the sperm can remain viable for years within the female genital tract. The study of sperm preservation has recently gained great economic importance because of the use of artificial insemination for livestock breeding. In the case of cattle, a stud bull is induced to service an artificial vagina (a water-jacketed rubber tube with a collecting bottle at one end and the bull at the other). About 4.5 ml of semen is collected per ejaculation, and the bull can be used for service up to 10 times in a two-hour period. The semen is cooled slowly to a temperature just above freezing and can be stored for seven days at most. It is diluted before use and injected into the uterus of a cow in heat, through a pump. A good stud bull can be used to impregnate literally thousands of cows in a single season. It should also be noted that many human females fail to conceive by natural means even though the gametes of both husband and wife are perfectly viable. In such cases, artificial insemination of the wife with her mate's semen is generally successful in inducing pregnancy.

FERTILIZATION

The act of fertilization can be divided arbitrarily into three phases: (1) penetration of the egg by the sperm, (2) activation of the egg, and (3) the union of egg and sperm nuclei.

PENETRATION. Contact between sperm and egg is generally the result of random collision. The discharge of huge numbers of sperm by the male increases the chance that at least one such collision will occur. Upon contact, the sperm is bound tightly to the outer surface of the egg. This may be accomplished by chemical reactions between proteins at the surfaces of both gametes called *fertilizins*. Fertilizin, isolated from eggs, causes sperm to become sticky and to clump together, while anti-fertilizin obtained from the sperm does the same thing to eggs. The substances are specific and act only upon gametes of the same species. Thus, fertilizin and anti-fertilizin may increase the chance of an effective and lasting contact between egg and sperm. However, the evidence that the fertilizins actually do operate during fertilization is still equivocal.

The sperm that is now bound to the egg must penetrate its membrane. Material extracted from sperm has been shown capable of dissolving the egg membrane by enzymatic action. Presumably the intact sperm employs the enzyme to dissolve a hole big enough to permit entrance. In mammals, the egg is surrounded by a layer of tiny follicle cells cemented together by a substance called hyaluronic acid. Mammalian semen contains an enzyme, hyaluronidase, that dissolves this cement and permits the sperm to slip through. Many pathogenic bacteria use this same device to slip through body tissues and invade all parts of the organism.

ACTIVATION. As mentioned previously, the egg as it develops in the female stops at one or another stage of meiosis and lies dormant until activated by contact with the sperm. A number of characteristic changes then occur:

1. Completion of meiosis. The nuclear membrane breaks down and the egg nucleus completes the meiotic process (in eggs where it is incomplete).

2. Fertilization cone. In some eggs a conical projection pushes up through the surface jelly from the egg membrane. It "captures" the sperm at the jelly surface and by contraction draws it down toward the egg. Meanwhile, the sperm dissolves a portion of the membrane to permit ingress.

3. Block to polyspermy. Penetration by the first sperm makes most eggs almost instantaneously impervious to the entrance of any others. The mechanism of this reaction is still unclear. In a few species, however (some reptiles and insects), the entrance of more than one sperm is a normal event. One of the sperm nuclei fuses with the egg nucleus and the others degenerate and disappear.

4. Rearrangement in egg contents and physiology. Upon fertilization the egg changes its shape, generally veering toward the spherical. The permeability of the egg membrane to the ingress of salts and other substances rises markedly. Cytoplasmic materials migrate to new areas. The axes of the future embryo (dorsal-ventral, anterior-posterior) are fixed.

Many eggs can be activated in the absence of sperm. Among treatments found successful are: X-ray and ultraviolet irradiation, introduction of foreign protein by a needle, heat shock, addition of certain salts and acids. The activated egg proceeds through the changes noted above and goes on to cleave and construct an embryo. The eggs of certain organisms (sea urchins, starfish, frogs, bony fishes, rabbits) can develop perfectly normally without fertilization by sperm and give rise to viable adults. The process, called *parthenogenesis*, is carried out routinely in nature by colonial insects including bees. Although only the egg nucleus is present, the cells of a parthenogenetic embryo need not remain haploid. In some cases the activated haploid egg duplicates its chromosomes without cell division, thereby producing a diploid, homozygous individual.

FUSION OF NUCLEI. The sperm head and midpiece enter the egg, membranes disappear, and the sperm chromosomes arrange themselves on a spindle constructed by material in the midpiece (Fig. 26). Sperm and egg nuclei migrate toward each other and the egg chromosomes arrange themselves on the spindle. The pattern now resembles that seen in meta-

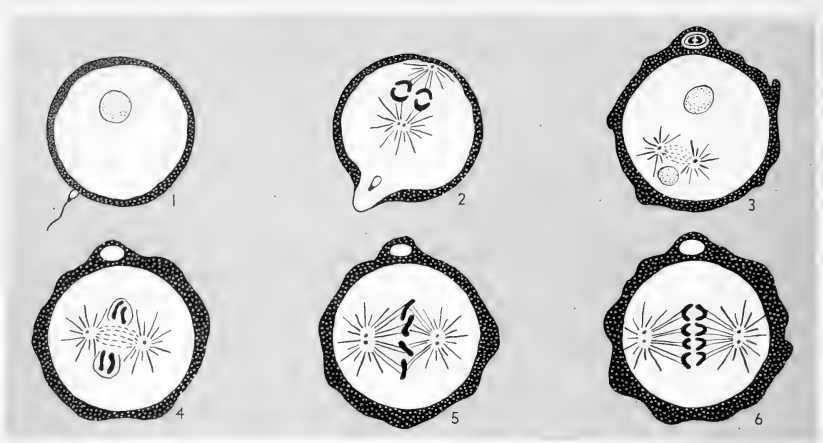


Fig. 26. Fertilization (after Wilson). (1) Sperm contacts egg. (2) Sperm head and midpiece enter egg through fertilization cone; egg nucleus proceeds through meiosis II. (3) Sperm nucleus enlarges; midpiece constructs spindle and asters; meiosis II of egg completes to yield polar body and functional, haploid egg nucleus. (4) Sperm and egg nuclei migrate toward each other across midpiece spindle; nuclear membranes break down. (5) Chromosomes dispose themselves upon mitotic spindle. (6) Chromosomes duplicate and mitosis draws to a close in preparation for first cleavage.

phase of mitosis. The chromosomes duplicate, the first cleavage of cells occurs, and two daughter cells, now diploid, emerge. The egg has become an embryo.

Cleavage

In all eggs, fertilization is followed by a period of extensive cell division without an increase in size. The fertilized egg is cleaved repeatedly into smaller and smaller cells. The early divisions are generally synchronous; thus the egg yields two cells, the two produce four, the four, eight, and so on. From this time on, synchrony may disappear and different parts of the embryo may cleave at different rates. At the end of the cleavage stage, the embryo, now called a *blastula*, is a ball of thousands of small cells (blastomeres).

Although there is no size increase during this period, a considerable amount of synthesis occurs. The cleavages are regular mitotic divisions so that chromosomes and total nuclear content must be duplicated in the daughter cells. This means extensive synthesis of deoxyribose nucleic acid plus other nuclear constituents. In addition, a great deal of ribose nucleic acid and proteins, including enzymes, are formed in the cytoplasm. The energy and raw materials for these processes come, of course, from the supply of yolk.

Cleavage performs at least three functions in embryonic development:

1. It provides an adequate number of cells, i.e., building blocks for the future organization of tissues and organs.
2. It lays the groundwork for the gross design of the embryo (dorsal-ventral axis, anterior-posterior axis, etc.) by shifting around the material (both cytoplasm and yolk) of the egg and compartmentalizing this material within separate cells.
3. It brings nuclear and cytoplasmic materials into balance. The egg starts as a cell with a single nucleus and an enormous amount of cytoplasm. During cleavage, total cytoplasmic content does not change markedly but thousands of nuclei appear as cell division proceeds.

The pattern of cleavage is determined in large part by the amount of yolk in the egg. In eggs that contain a little yolk that is homogeneously distributed, cleavages are equal; that is, the daughter cells are always the same size. Figure 27a shows this type of cleavage in *Amphioxus*. Eggs with a large amount of inhomogeneously distributed yolk cleave unequally. Figure 27b illustrates this type of cleavage in the frog. The yolk in the frog egg is concentrated in one hemisphere (the vegetal), and decreases in a sharp gradient toward the opposite (animal) hemisphere. The first two cleavages split the frog egg longitudinally to produce four cells shaped like the segments of an orange. The third cleavage is transverse and separates the yolky (vegetal) half from the non-yolky (animal) half. The split is unequal, since the four animal blastomeres are much smaller than the four vegetal ones. Subsequently, the yolky cells cleave far more slowly than the non-yolky ones. The result is that in the completed blastula, the dorsal part contains many small cells while the ventral part contains fewer but larger cells. In fish and birds, the extreme of yolkiness is reached. The cytoplasm and nucleus of the egg sit atop a huge ball of inert yolk. The yolky area does not cleave at all and only the little cap on top divides into cells (Fig. 27c).

The specific appearance of cleaving cells is much the same in all embryos. A groove (called the cleavage furrow) appears at one point of the egg. For example, in the frog the first furrow appears at the animal pole. The furrow then deepens and extends downward on both sides. The two ends meet at the vegetal pole. The furrow then extends inward radially, finally constricting the egg into two sister blastomeres.

In most cases, as the cells cleave, a cavity appears in the middle of the ball of cells. This is the *blastular cavity*. Thus, by the end of the cleavage stage, the embryo is a spherical or flattened hollow ball generally one cell thick. The blastomeres can vary in size, yolk content, and cytoplasmic organization, but no tissues and certainly no organs yet exist.

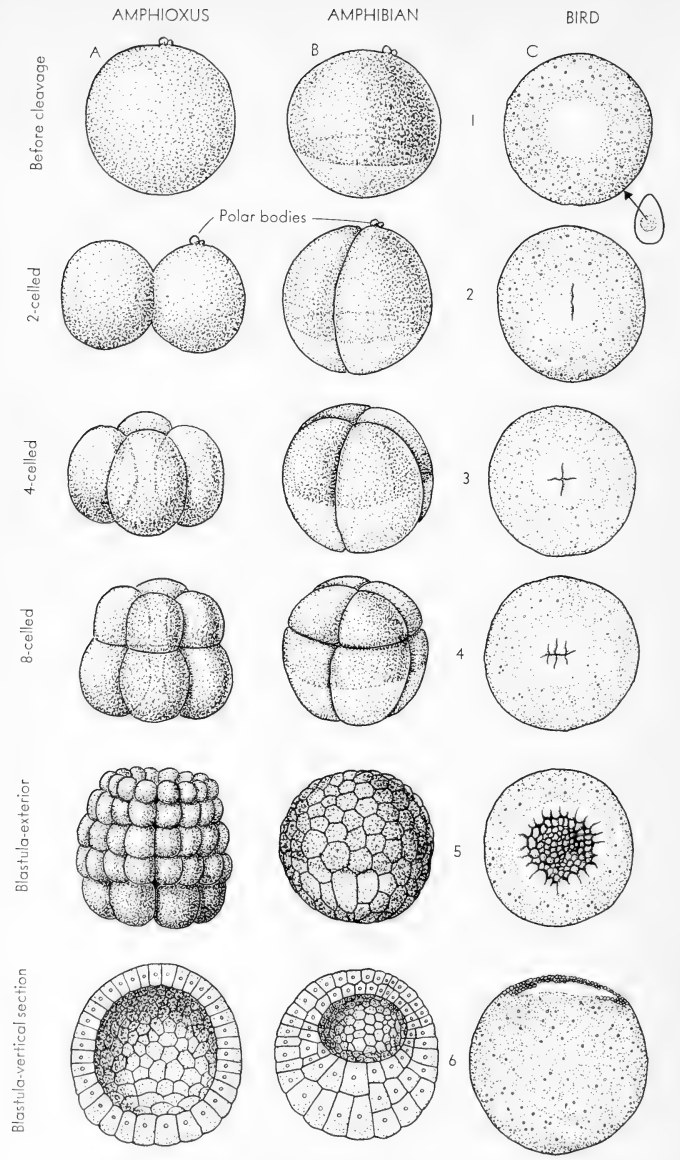


Fig. 27. Patterns of cleavage in *Amphioxus*, frogs, and birds (from Storer and Usinger).

The early cleavages are regular phenomena that occur at specified times after fertilization. Thus in the egg of the frog, *Rana sylvatica,* incubated at 18°C, the first cleavage (longitudinal) occurs at 2.5 hours after fertilization, the second (also longitudinal) occurs at 3 hours, the third (transverse) at 4.5 hours, the fourth (again longitudinal) at 5.5 hours, leaving the embryo as a still solid ball consisting of two hemispheres of eight cells each, the dorsal being smaller than the ventral. The blastular cavity soon appears and produces a hollow ball with a thin roof and a thick floor. By 21 hours the blastula is complete and gastrulation commences.

Gastrulation

At the beginning of gastrulation, the embryo is a hollow ball of cells. By the end of gastrulation, the embryo consists of three basic tissue layers: an outer layer (*ectoderm*), an inner layer (*endoderm*), and an intermediate layer (*mesoderm*). Gastrulation, then, involves a series of integrated cell movements that lead to the formation of these discrete layers. Because the movements are quite complex, they are hard to follow. Investigators have observed them by time-lapse movie photography and by marking areas of the blastula with carbon particles or dyes and following the path of motion. In this way they have been able to map the origins of the three tissue layers back to cells of the early blastula stages. The gastrular movements of many species have now been studied and show that they have achieved a variety of solutions to the problem of establishing three tissue layers. Three mechanisms of gastrulation will be discussed: in *Amphioxus,* in the frog, and in the chick.

AMPHIOXUS

Amphioxus or the Lancelet is a primitive chordate, a marine animal resembling the fishes. As seen in Fig. 27a, cleavage is virtually equal and produces a blastula whose vegetal-pole cells are only slightly larger than those at the animal pole. Gastrulation begins when the cells at the vegetal pole move to the interior (Fig. 28). As they press upward, the blastular cavity grows progressively smaller and finally disappears when the vegetal-hemisphere cells press up against the animal-hemisphere cells. The embryo is now a double-walled cup such as might be made if we pressed upon one side of a hollow rubber ball. The external wall is the ectoderm and will ultimately give rise to the epidermis, certain external organs, and the nervous system. The internal wall is the endoderm, and the hollow U-shaped cavity is called the *archenteron.* Surrounded by endodermal

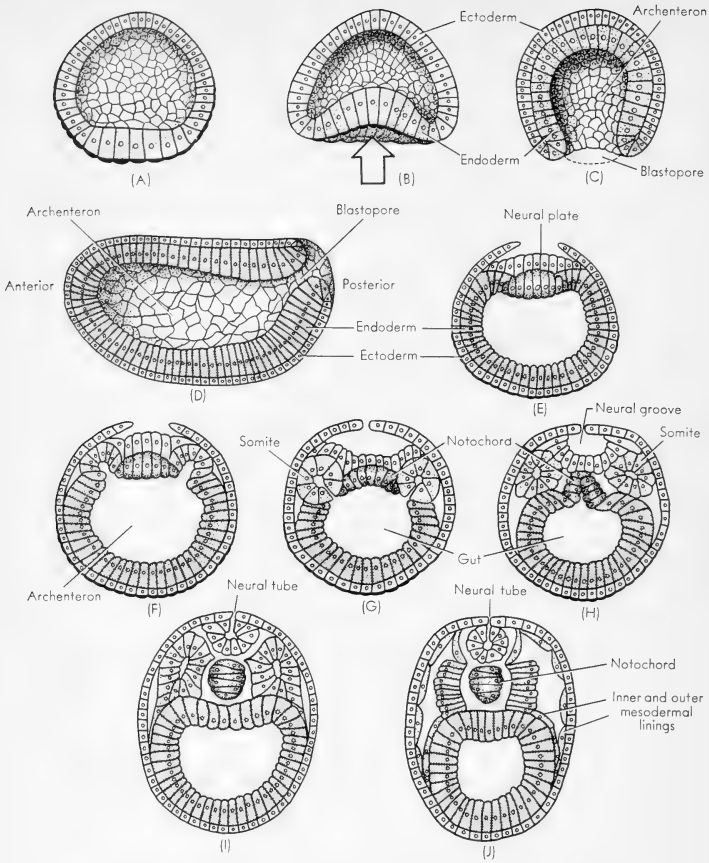


Fig. 28. Gastrulation and later embryonic stages in *Amphioxus* (after Villee).

cells, it is the primitive digestive tract (gut). The embryo now lengthens and the site of the old animal pole becomes the anterior end of the embryo; the site of the old vegetal pole becomes the posterior.

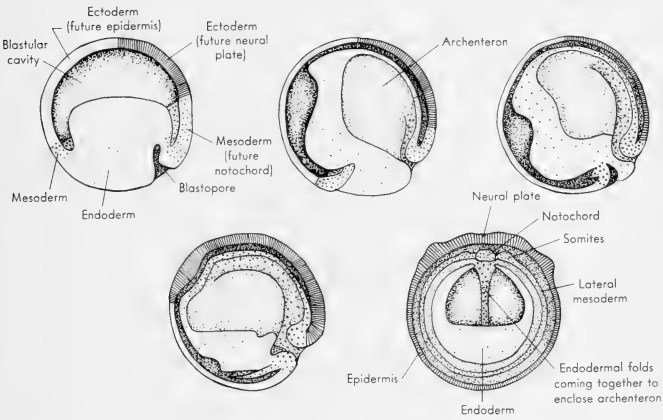
The gastrula is still only two-layered and the third layer (mesoderm) appears at this time. Three long tubes of cells are pinched off from the endoderm. The middle one becomes the *notochord*, a flexible skeletal rod which persists in amphioxus as its axial supporting member. (In higher chordates, the notochord appears during embryogenesis but is later surrounded or replaced by the vertebral column.) The lateral tubes grow by cell division into large tissue masses that are divided into segments along the length of the embryo and are called *somites*. These expand and plaster

themselves exteriorly up against the ectoderm to become the second layer of skin and interiorly against the endoderm to become the outer lining of the gut. Eventually internal organs such as the gonads, kidneys, etc. will pinch off from the mesoderm and inhabit the internal body cavity that lies between the mesodermal linings. With the formation of the third basal layer, the gastrula is complete.

THE FROG

At the end of the cleavage stage, the frog embryo is a hollow ball with a thin roof and a thick floor. A small dimple appears near the vegetal pole when large yolky cells leave the surface of the egg and begin to press inward. This process of inward movement is called *invagination.* As more cells invaginate, the dimple becomes a crescent-shaped groove curving downward toward the vegetal pole. The groove is called the *blastopore* and has an upper (dorsal) and a lower (ventral) lip (Fig. 29).

Fig. 29. Gastrulation stages in the frog.



The sheet of cells from the upper surface of the blastula moves downward and converges upon the blastopore. As the cells reach the dorsal lip, they turn inward and enter the interior of the embryo. Similarly, the ventral cells invaginate over the ventral lip of the blastopore. As the presumptive (future) mesoderm folds inward over the dorsal lip, it separates from the endoderm that entered before it. The mesoderm plasters itself against the outer layer of ectoderm and continues to move away

from the blastopore and up toward the animal pole. The detached endodermal cells move to the middle of the embryo and form a long trough-shaped structure. The edges of the trough move up and around, finally meet, and the trough is converted into a tube. The mesoderm breaks up longitudinally into three masses: a central tube, the notochord (this later disappears and is replaced by the vertebral column), and two lateral masses which later segment and become somites.

THE CHICK

At the end of cleavage, the chick blastula is a small cap of cells several layers thick that sits on top of the yolk. A thin cavity appears within the blastoderm, so that at completion it is a flattened hollow ball. Despite a great deal of descriptive study, it is not clear whether the lower layer of cells simply splits off from the upper layer or whether cells at the edges of the cap move underneath it and join at the center to form the lower layer. In any case, the upper layer of cells is the source of both ectoderm and mesoderm; the lower layer is the endoderm.

A long narrow groove called the *primitive streak* appears on the surface of the blastodisc and runs from the approximate center back toward one edge. This edge will become the posterior of the embryo and its opposite will be the anterior. Cells stream toward the primitive streak and then turn under to spread out in a sheet between the ectoderm and the endoderm. This intermediate layer is mesoderm and when it is fully formed, gastrulation is complete. Note that the embryo is still only three isolated sheets of cells like a layer cake. Eventually the blastoderm in the anterior and posterior regions and at the sides will get tucked under the embryo. When the two infoldings meet in the middle, the ectoderm will have been transformed from a single sheet of cells into a complete outer covering. The endoderm will also have been tucked under to produce a long hollow tube, the gut. The mesoderm at the same time gives rise to notochord and somites. Figure 30 is a schematic representation of these changes.

THE SUBSEQUENT DEVELOPMENT OF ECTODERM, MESODERM, AND ENDODERM

An embryo develops in one sense much as a delta-choked river travels to the sea. That is, it starts as one big channel and then splits up into several smaller ones that in turn branch and rebranch. The original embryonic channel is the fertilized egg. The three intermediate channels are ectoderm, mesoderm, and endoderm. The diagram opposite summarizes these ramifications.

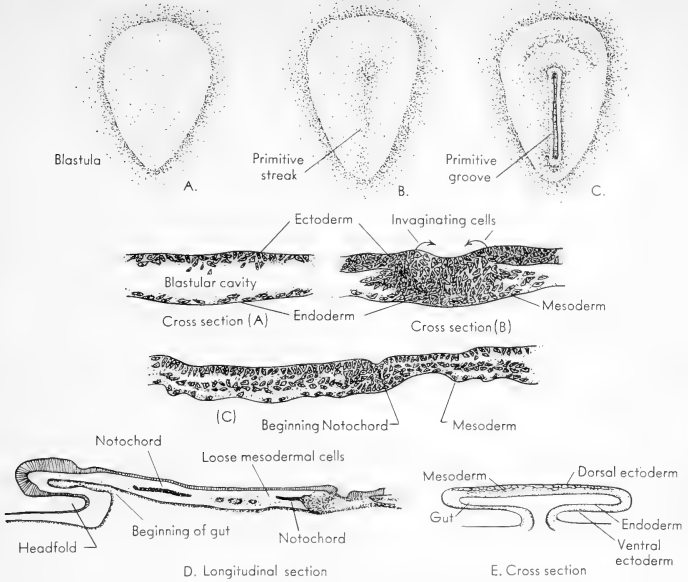
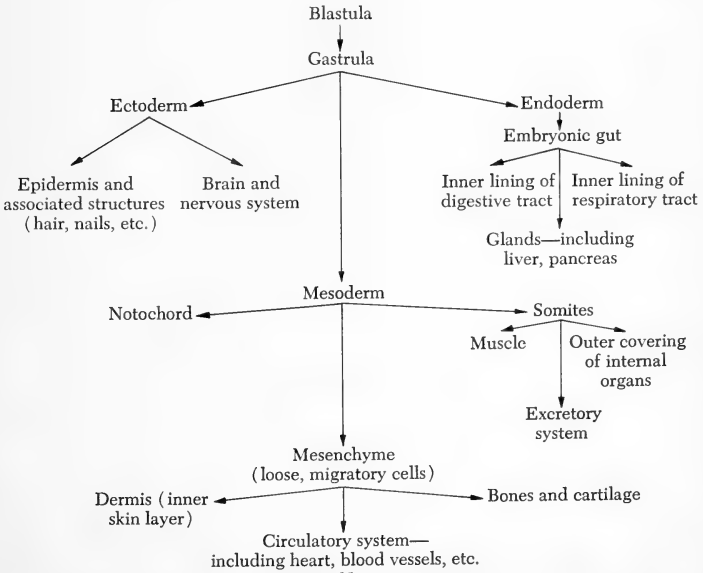


Fig. 30. Gastrulation in the chick.



Most of the research carried out so far on gastrulation has been descriptive, consisting of efforts to follow the patterns of the cell movements, to trace the origins of the three basic tissue layers back into the blastula and even to the fertilized egg, and to account for the tissues and organs that are produced in the later embryonic stages. Now, biologists can begin to ask questions at the mechanistic level. For example, what is the motive force for gastrular movements? That is, are the cells pushed from behind or pulled from in front, or does each cell move as an individual at the same rate as its neighbors? Why is it that the cells move to specific areas of the embryo? What motivates somite cells to plaster themselves against ectoderm and endoderm respectively? By what mechanism do endoderm cells move up and around so as to produce a tube-like gut? Are these simply random movements that are channeled into specific patterns by the shape of the embryo, or are the cells attracted or repelled by specific chemical stimuli and then move toward or away from the places where these substances are produced?

As you can see, many problems of biological interest involving gastrulation still remain unsolved and are open to investigation.

Organ Formation

Toward the end of gastrulation, ectodermal cells immediately in front of the blastopore (or in front of the primitive streak of the chick and mammal) begin to divide rapidly. The resultant crowding forces some cells beneath the surface, and in this manner a thick plate of ectoderm appears. This is the *neural plate*. The wave of cell division proceeds anteriorly and the neural plate finally extends along the entire dorsal line of the embryo. Now the edges of the plate become elevated into folds and the center of the plate is depressed. This produces a groove which steadily deepens as the neural folds come together along the midline. Finally the upper margins of the folds touch and fuse and the groove is now formed into a tube, the neural tube, which is overlaid with a continuous layer of ectoderm. Figure 31 shows these foldings in cross section, and Fig. 32 shows the external appearance. As described below, the neural tube is the rudiment of the central nervous system, including the brain and the spinal cord. The embryo has reached the neurula stage.

While the neural system takes shape, other parts change as well. Externally, the embryo elongates and becomes sculptured into head and trunk regions. Limb buds, tail buds, and gill slits appear. Internally, the mesoderm splits into notochord and somites; the endoderm takes a long trough-like shape and rolls up into a tube, the primitive gut, which is open at the rear through the anus and later at the front through the mouth.

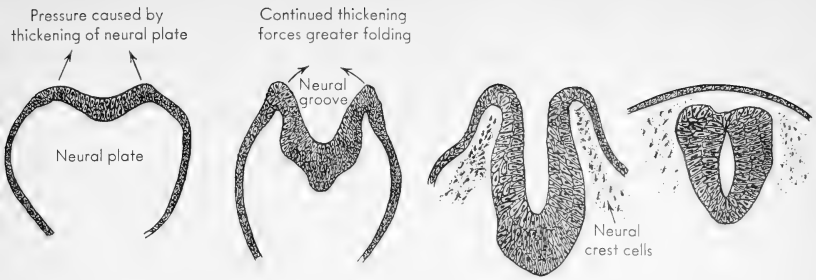


Fig. 31. Neural tube formation.

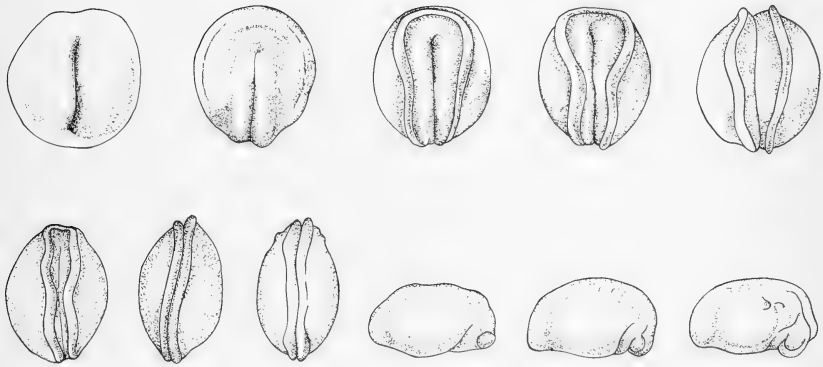


Fig. 32. Changes in the external form of the amphibian embryo, from the appearance of the neural fold to the completion of the neural tube.

DEVELOPMENT OF THE CENTRAL NERVOUS SYSTEM

Originally the neural tube is wider at the front end of the embryo. This width is enhanced by local swellings that produce three distinct bulges. These are the forebrain, midbrain, and hindbrain. Now the forebrain bulges laterally and two cup-like rudiments appear. Simultaneously the surface ectoderm (the epidermis) folds inward to meet the optic cups. Folds are pinched off and ultimately develop into eye lenses. The ears and nostrils also appear at first as epidermal infoldings. In the meantime the spinal cord undergoes considerable growth and cell division. Mesodermal cells migrate toward the cord and aggregate around it and the underlying notochord. They transform into cartilage and ultimately into bone tissue, giving rise to a hollow vertebral column with associated ribs, etc.

The spinal-cord cells are at first large and rounded. They now assume typical neuron shapes, spinning out long fibers. The cells in the ventral part become motor neurons, and their fibers penetrate peripheral

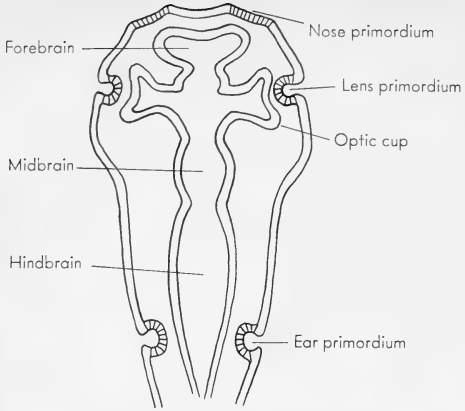


Fig. 33. The development of the brain and associated sense organs (after Waddington).

organs and tissues. Cells that had previously been crowded out of the neural folds (the neural crest cells in Fig. 31) migrate down toward the cord and aggregate into cell masses called *ganglia.* These cells become sensory neurons and send dendrites to connect up with the dorsal part of the spinal cord and axons into surrounding tissues. In this manner the complete sensory-motor network is constructed.

The growth of the nerve fibers and their penetration into peripheral tissues is a fascinating subject. The process has been studied: (a) in the normally developing embryo, (b) by cutting functional nerves in order to follow the regeneration of a fiber from the old stump, and (c) by separating embryonic nerve cells and growing them in an artificial medium (usually on a blood clot surrounded by blood serum and other constituents found by experience to be delectable to a nerve cell). The presumptive nerve cell body appears to send out an amoeboid process; the nucleus remains within the cell body. Protoplasm flows into the process causing it to move ahead while still connected to the cell body by a thin protoplasmic strand. This is the fiber. Special cells called Schwann cells then flatten out and wrap themselves around the fiber in much the same way that insulation is wrapped around an electrical conductor.

The first fibers to penetrate into surrounding tissues are called *pioneer fibers* and these are followed by others which apply themselves to the pioneer and form a cable. Many problems involving the mechanism of nerve growth remain unsolved. For example, how does a fiber know to what specific organ or tissue it must go, and how does it know when it has gotten there? How do the follower fibers duplicate the precise route of the pioneer fiber? Why do only fibers of similar function collect into cables? What is the control system which ensures that certain tissues will receive many sensory and motor fibers while others will be innervated only sparsely?

DEVELOPMENT OF THE DIGESTIVE AND RESPIRATORY SYSTEM

At first the gut is a simple tube stretching from mouth to anus. At the anterior end, five folds appear on each side which penetrate and finally pierce the mesoderm and make contact and fuse with the outer ectoderm. Slits appear where endoderm and ectoderm meet. These are the gill slits. In fish they remain as functional organs, but in higher vertebrates they disappear and the component tissues are transformed into other organs. The lungs also originate from the anterior gut, first as a simple outpocketing which soon branches into two bag-like structures.

Similar pocketings, extensions, and local swellings in the posterior portion of the gut result in the formation of stomach, intestines, liver, etc.

DEVELOPMENT OF MESODERMAL STRUCTURES

By the end of gastrulation, the mesoderm makes up the bulk of embryonic tissue. The notochord is the first mesodermal organ to appear, and the remainder of the mesoderm is spread out as a thin sheet lying between the ectoderm and endoderm. This sheet breaks up into longitudinal strips. The thick strips lying on both sides of the notochord become

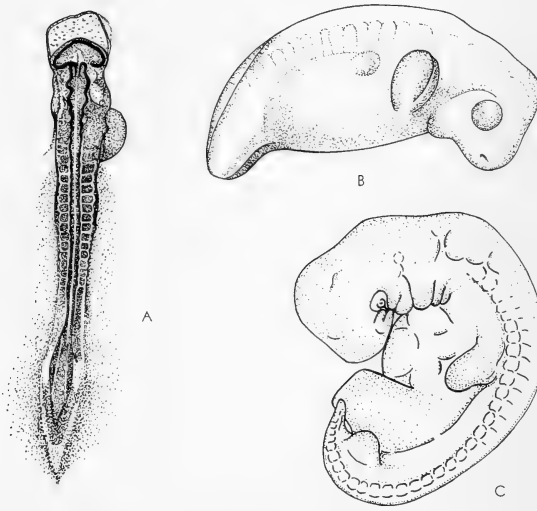


Fig. 34. Advanced embryos: (A) the chick, (B) the salamander, and (C) the human.

segmented into blocks of tissue called somites. The somite cells migrate toward the notochord and the spinal cord and form the vertebrae (as described previously) and also skeletal muscles. They also spread upward to underlie the outer ectoderm and become part of the skin and inward to become the outer covering of internal organs. Portions of the mesoderm lateral to the somites pinch off as longitudinal strips of tissue and give rise to the urogenital tract, including kidneys, gonads, etc. The heart and circulatory system also originate from the non-somite mesoderm.

DEVELPOMENT OF LIMBS

The limbs first appear as slight external swellings (limb buds) which rapidly elongate. The bud is simply a mass of loose mesodermal tissue capped by an ectodermal cover. As the limb elongates, the mesodermal cells grow rapidly in number and come together into a series of tight aggregates corresponding to the skeletal elements of the adult limb. They then are transformed into cartilage and finally into bone tissue. The remaining mesoderm forms muscles and blood vessels. Meanwhile, the external sculpturing of the limb, joints, digits, etc. is accomplished.

Biochemical Embryology

We are accustomed to the fact that the specialized cells of a multicellular organism have vastly different structures and functions. I have mentioned before, but cannot stress too greatly, the fact that these are simply outward manifestations of differences in metabolism, in the spectra of chemical reactions which in turn reflect what enzymes and enzyme concentrations these cells possess. Therefore, merely to describe the acquisition of new structure by a developing embryo would be incomplete without a concomitant catalog of biochemical changes.

As a result of biochemical research over the past thirty years, a multitude of enzymes has been discovered, purified, and characterized. The reactions that they catalyze have been studied in the test tube to the point where we can specify the reacting molecules, the products, and the chemical mechanisms by which reagents are transformed into products. These reactions, each representing a relatively minor chemical alteration, have been integrated into comprehensive reaction chains and cycles that explain the broad pathways of breakdown and synthesis of cell components and of energy generation, transport, and utilization. This in turn has led to a rational basis for understanding the nutritional requirements of organisms (i.e., the starting materials needed for synthesis and energy generation). In addition, the chemistry of the major cell components, pro-

teins, nucleic acids, polysaccharides, etc., has been elucidated, and the relationships between the structure of cell organelles and their biochemical functions have begun to emerge.

Until recently, however, most biochemical studies have been done with bacteria harvested after they had stopped growing or with adult animal tissues and cells (liver or muscle slices, etc.). Consequently, they could tell us only about the biochemical machinery of a cell at one instant in time, so to speak. But with our present biochemical understanding, it is now possible to examine how the biochemical apparatus changes as the organism proceeds upon its developmental course. At this early stage, some of the directions taken by students of biochemical embryology are outlined below:

1. Chemical characterization of the egg. For example, we can ask: do the components of an egg differ in kind or quantity from those of an ordinary cell? Do the organelles (nucleus, mitochondria, ergastoplasm, etc.) have the same chemical composition and biochemical activities as they do in ordinary cells? What are the components of yolk? How are they utilized?

2. Biochemistry of spermatozoa. What are the energy requirements for sperm locomotion? What substrate is used (the sugar, fructose)? How (via the glycolytic cycle, as explained in the monograph on cell physiology, and therefore much like other cells)? A very interesting question is how the sperm midpiece synthesizes the protein fibers that make up the spindle apparatus for the first cleavage.

3. Cleavage. A great deal of work has already been done, particularly with the sea-urchin blastula. As mentioned previously, the egg starts out with very disproportionate amounts of nuclear and cytoplasmic materials. As cleavage proceeds, the ratio of the two rises to a normal balance. We want to know about the chemical details of the shift and of the controlling mechanisms. We want to know why the first cleavages are synchronous and why later on some parts of the blastula cleave faster than others.

4. Nutrition of the embryo. For example, early chick embryos have been separated from their yolk supply and placed on agar medium containing amino acids, vitamins, mineral salts, and glucose as an energy source. The embryos developed in normal fashion under these conditions and formed a full complement of organs. We can ask: what are the minimum nutritional requirements for normal embryonic development? What are the biochemical pathways of energy generation and how is the energy employed for cellular differentiation, morphogenesis, and growth? If we omit a specific essential nutrient, does all embryogenesis cease or are only certain organs affected?

5. Enzymic changes during development. What enzymic changes accompany the transformation of unspecialized cells into an organized tissue or organ, i.e., which enzymes appear, which disappear, which increase or decrease in concentration, what are the control mechanisms? The cataloging of enzymes and enzyme concentrations is now a routine procedure and can be accomplished with ease and precision.

6. The appearance of tissue-specific substances during embryogenesis. For example, we can follow the development of the heart in the early embryo by looking for the presence of heart-muscle myosin, a contractile protein unique to this muscle tissue. How to distinguish between heart myosin and the myosins of other muscles? We can purify the heart myosin and inject it into rabbits. Because chick heart myosin is a foreign substance to the rabbit, the animal can make antibodies that will react specifically with chick heart myosin. Thus the rabbit serum containing these antibodies can be employed as a specific indicator of the contractile protein, and we can answer questions such as at what time the myosin appears and from where, how much, and in what cells, at a stage long before the actual heart is evident as a discrete organ.

You will notice that the above categories are heavily punctuated by question marks. This is as it should be, for chemical embryology is in its infancy and not nearly at the level of sophistication that one would like. Here, perhaps more than any other, is an area open to fruitful investigation by physics- and chemistry-minded young students.

Cellular Differentiation

Professor Curt Stern, one of the fathers of modern genetics, in his article [1] entitled "Two or Three Bristles," has a superb discussion of cellular differentiation that is designed to be read by scientists who are not biologists; it can easily be understood and will be greatly appreciated by serious beginning students of biology.

To illustrate the problems inherent in cellular differentiation, Dr. Stern describes the formation of bristles upon the abdomen of the fruit fly:

> In some regions there arise short or long outgrowths—the bristles—strong and wide at the base and gently tapering to a fine point. Narrow grooves, as in fluted columns with a slightly baroque twist, extend along their lengths. A short stalk fits each bristle into a round socket within the body armor so that the bristle can be moved within this articulation . . . The bristles are tiny sense organs, perhaps sensitive to the fluctuations of air pressure when the fly is in flight.

Dr. Stern goes on to describe the cellular structure of the bristle organ. It consists of three cells: the bristle cell itself, which secretes the tapered outgrowth; the socket cell that secretes a socket-like ring of hard chitin into which the base of the bristle fits; and, below these two, a sensory nerve cell that is linked to the bristle by a short nerve fiber and whose other long nerve fiber connects up with the central nervous system, thereby communicating to it stimuli felt by the bristle.

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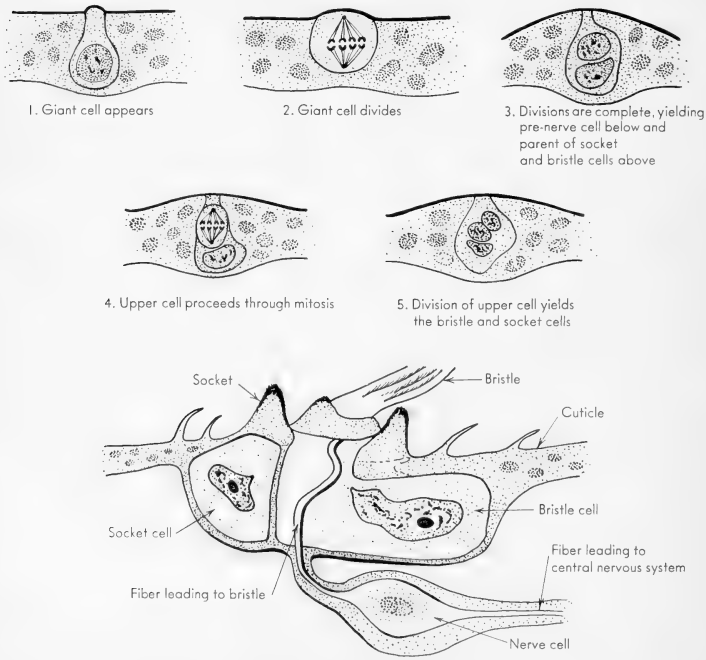
[1] Published in *American Scientist*, April 1, 1954.

The three bristle organ cells come from a single ancestor. At first, the abdominal epidermis is simply a continuous sheet of many identical cells. At a fixed stage in the development of the young adult from the immature larva, single cells within the sheet grow to giant size. Division occurs and one of the daughter cells is transformed into a sensory nerve cell. The other daughter divides once more and one of these becomes the bristle-forming cell, the other the socket-forming cell. See Fig. 35 for the lineage.

It should be noted that a fixed number of giant cells appear in the abdominal epidermis and always at specific points so as to yield a precise pattern. There are mutant fruit flies, however, that display different numbers and patterns of bristles, and as one would expect in these organisms, the number and positions of the giant cells differ from the norm. In addition, some mutants produce bristles of different shape and size or produce none at all. These abnormalities, too, can be traced back to specific events in the early development of the fly. For example, in one of the types that produce no bristles, the giant cells arise at the right time and place and

Fig. 35. Development of the bristle organ (adapted from C. Stern).



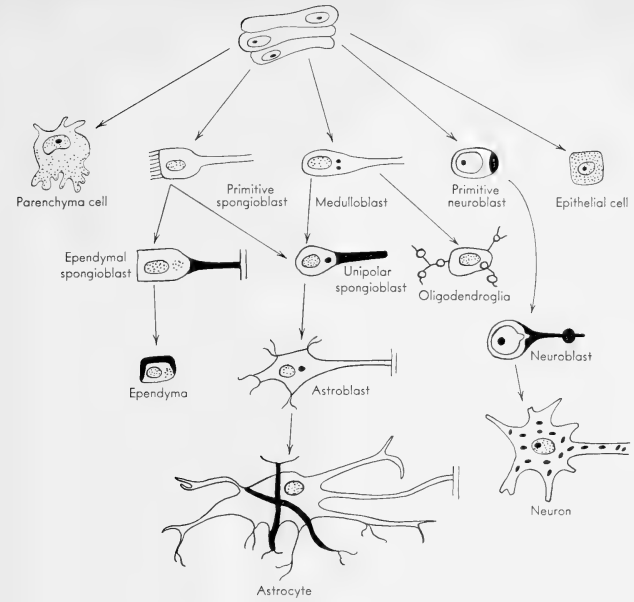


Fig. 36. The main cell types that arise from neural epithelial tissue (after P. A. Weiss).

each divides into two. One of the daughters as usual becomes a nerve cell and the other divides as it should. But instead of yielding a bristle and a socket cell, two socket cells appear!

This work raises many questions. What genetic and biochemical processes cause one cell among many suddenly to become a giant? Why does it appear here and not there? Why, having produced three daughter cells, does cell division stop? What causes three cells coming from the same parentage to be transformed into totally different individuals?

Cellular differentiation, as we see it in bristle formation, occurs in every developmental system, whether it be slime mold, coelenterate, or human embryo. Each stage of development is accompanied by the appearance of new cell types which play a direct role in the formation of the adult structure. Some of these cells, like the giant bristle cell in the fruit fly, perform a specific act and then do one of three things: die, divide, or simply become unimportant for further development, in which case they can be ignored. Others, like the nerve cell in the bristle organ, persist and play a role in the subsequent functioning of the adult. Figure 36 summarizes a more complicated series of cellular differentiation in the vertebrate embryo. All the cell types shown arise in the neural tube in the region of the brain. They are descended from ectodermal cells that in-

habited the dorsal area of the embryo after gastrulation. Only those cells that remain in the inner core of the neural tube continue to reproduce actively and to resemble their parents. Those cells that are pushed toward the periphery cease dividing and are transformed into the types illustrated in Fig. 36. It should be realized that the drastic morphological changes shown are reflections of equally drastic changes in the metabolic capabilities of these cells.

The extent to which differentiated cells can differ from one another can be appreciated from current work on the reaggregation of tissue cells. Cartilage tissue was cut from a chick embryo and the cells were dispersed by treatment with an enzyme that destroyed the material cementing them together. A suspension of the loose cells in nutrient broth was then pipetted into a glass chamber. The cells promptly reaggregated and became cemented together so as to construct a tissue of uniform texture, a perfectly normal cartilage tissue. When dispersed kidney cells and cartilage cells from the chick were intermixed and pipetted into the chamber, the two cell types ignored each other. They formed separate aggregates, the one producing something approaching normal kidney tissue and the other apparently normal cartilage. In other words, the kidney and cartilage cells recognized their mutual differences and refused to become cemented one to the other in a mixed tissue. Now, dispersed cartilage cells from chick and mouse embryos were mixed. The two kinds of cells could be distinguished by size and nuclear structure. The cells came together and were cemented into a single cartilage tissue in which chick and mouse cells were homogeneously interspersed. Mouse kidney and chick cartilage cells ignored each other, as did the homologous chick mixtures. Two conclusions are permissible:

1. With respect to at least one criterion (the ability to stick together when they meet and so form a stable aggregate and later a homogeneous tissue), chick cartilage cells are very much like mouse cartilage cells and very unlike chick kidney cells. Thus, in this instance, embryological differences within the same animal can transcend evolutionary differences between two animals as diverse as a bird and a mammal.

2. The ability of two cells to be cemented together is a very specific process. While the mechanisms are still unknown, apparently the same ones are used regardless of species (otherwise mouse and chick tissues could not stick together).

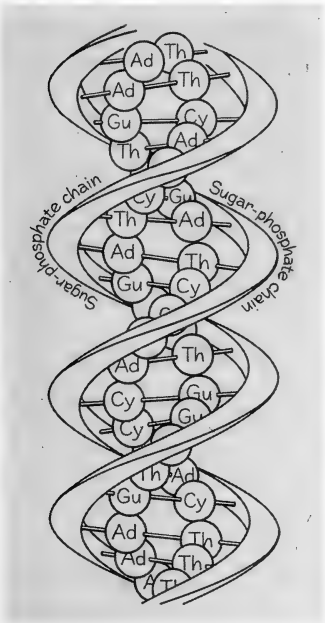
The Genetic Basis of Cellular Differentiation

When we say that parent cells give rise to progeny that display a wide difference in structural and metabolic properties, it is the ears of the

geneticist that perk up most sharply. For the possibility arises that the genetic endowment of the progeny has been altered. In actuality, cellular differentiation can be explained in two ways from the geneticists' viewpoint. To understand the explanations, it is necessary to review the modern concept of heredity.

The genetic endowment of a cell is simply a collection of macromolecules that contains a set of instructions for making the remainder of the cellular constituents. The instructions are provided in the form of a code. The separate letters of the code are represented by the small building blocks of which the macromolecules are composed. The order in which the building blocks are strung together determines the meaning of the built-in message. A strand of deoxyribose nucleic acid (DNA) exemplifies macromolecules of this type (Fig. 37). Its backbone is a chain of alternating sugar (deoxyribose) and phosphate molecules. Attached to each sugar is one of four molecules: Adenine (A), Guanine (G), Thymine (T), or Cytosine (C). Thus the sequence, ATTCG . . . , would have a meaning different from TATCG. . . . It is these sequences, biologists think, that must constitute the message. The nature of the code is one of the most exciting problems in present-day biology, and we all await with impatience the Rosetta stone that will unravel the genetic language.

Fig. 37. The structure of DNA according to Watson and Crick.



Most of the genetic information is in the nucleus, borne by the chromosomes in the form of genes or, if you will, separate sentences of the message. But it is equally clear that the cytoplasm also contains macromolecules bearing information not present in the nucleus (see Chapter 2).

How is the genetic information employed in making the rest of the cellular constituents? The present view, supported by strong but not conclusive evidence, is that the separate genetic sentences built into genes or extra-nuclear elements determine the order of the amino acids

in corresponding protein molecules. (A protein of molecular weight 10,000 contains about 75 amino acids linked together in chains. If a chain were composed of a series, alanine-glycine-tryptophane-alanine-valine, it would have unique properties directly attributable to the particular order of the amino acids.) In other words, the gene controlling the hydrolysis of the sugar, lactose, determines the order of amino acids in a particular protein. The unique amino acid sequence permits this protein to act as an enzyme, to combine specifically with lactose and to split it. Other genetic determinants guide the synthesis of still other enzymes. Ultimately, the remainder of the constituents and products of a cell arise through the action of these enzymes.

To recapitulate, then, the genetic material of a cell has two jobs to perform:

1. Each coded macromolecule must guide the synthesis of an exact copy so that when the cell divides each daughter will have a complete set of genetic instructions.
2. Each coded macromolecule must guide the synthesis of a specific enzyme or enzymes.

Genetic information provides the cell with the potential for making a variety of cell constituents and for performing many functions. However, the fact that a cell is genetically equipped to carry out a particular activity does not guarantee that it will do so, for the external environment in which it lives also tells the cell what it can and cannot do. Three examples are provided to show what we mean by this statement:

a. *Euglena* is a protozoan-like organism that is capable of photosynthesis and contains about 10 chloroplasts per cell. When grown in the dark, where it generates energy not by photosynthesis but by oxidation of organic nutrients, the chloroplasts disappear. The instructions for making chloroplasts are still intact, but whether or not the cell does make them is decided by the light; when *Euglena* is once again exposed to light, chloroplasts are again formed.

b. The bacterium, *Escherichia coli,* can synthesize the amino acid, arginine, from simple starting materials via a series of stepwise reactions. It therefore does not require an external supply of arginine for growth. When, however, arginine is supplied to the cell in high concentration, it inhibits the formation of an enzyme, Ornithine transcarbamylase, which catalyzes a step in the synthesis of arginine. The absence of the enzyme causes the cell to stop making arginine, and it uses only the external supply. Thus *E. coli* is told by its environment not to synthesize its own arginine although it retains the genetic instructions that will enable it to

do so whenever the external supply of arginine is exhausted and it can again synthesize the missing enzyme.

c. The lymphoid tissue of an animal exposed to diphtheria toxin will form antibodies (proteins that specifically neutralize the toxin). Although the genetic potential for making antibodies is ever present, only exposure to the foreign toxin can trigger antibody synthesis.

In brief, then, what a cell can do is determined by its genetic endowment, i.e., the instructions provided by its genetic material. The geneticist terms this endowment the *genotype*. The cell's actual activity is the result of the interaction between the genetic potential of the cell and the environment in which the cell finds itself. What the cell does (i.e., its structure and metabolic capacities) is termed the *phenotype*.

Faced with the fact that during the development of a multicellular organism, daughter cells can gain new capacities or lose old ones and so become different both from their parents and from their sisters, we can ascribe the change to one or both of two processes:

1. The daughter cell receives from the parent its genetic endowment intact and unchanged. However, the environment in which it must live is different from that which the parent cell faced. Consequently, it synthesizes cell constituents that its parent did not and vice versa. The altered metabolism leads to morphological differences as well.

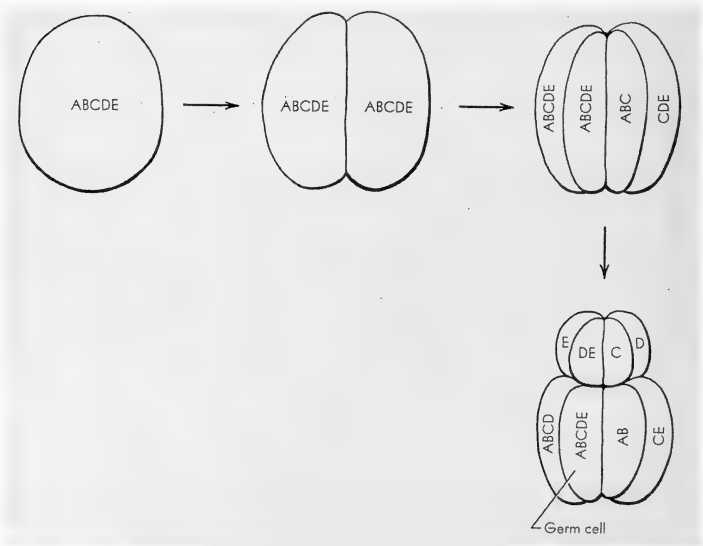
2. The genetic endowment of the daughter cell is different from that of the parent. Thus the act of cellular differentiation would involve a change in the primary genetic material and the alteration would be inherited by the progeny, if any, of the differentiated cell. The genetic macromolecules being altered, the enzymes constructed under their direction would be altered in quantity or kind. In this manner, the altered genotype becomes amplified into an altered phenotype.

This second process, though not given in chemical terms, was implicitly contained in an early theory offered by A. Weissmann in 1900. Weissmann proposed that, when a fertilized egg cleaved, daughter cells did not receive identical sets of genetic determinants. Instead, these were parceled out in a regular fashion depending on the location of the cell in the embryo, so that different cells received different genetic endowments. The difference in genetic content would account for cellular differentiation. The parceling out according to location would insure normality of form. Figure 38 is a summary of this scheme in which the original egg cell contains all the genetic elements necessary to make a complete embryo. After cleavage, its descendants not only contain different sets of genetic elements, but the necessary cell types end up in the right places in

the embryo. Weissmann realized that the embryo would ultimately become an adult animal, that it would produce eggs or sperm, and that these would necessarily have to contain all the genetic elements to make the next generation of embryos. He made provision for this by adding the qualification that during cleavage a few cells would inherit a complete set of genetic elements (see Fig. 38) and that these would become the gametes of the future adult.

In order to test this hypothesis, biologists permitted egg cells to cleave into 2, 4, 8, 16, or more cells and then removed some of them. If all the cells had inherited a complete set of instructions for making an embryo, the loss of a few cells would not be expected to matter. In contrast, if most of the cells received an incomplete set of instructions, the removal of some cells would remove certain instructions that the other cells lacked and an incomplete embryo would result. Experiments of this kind have tended to group embryos into two categories. If we permit a fertilized egg to cleave into two blastomeres and then separate them, each will, it is true, produce a complete embryo. However, if the egg happens to be from a clam, starfish, sea urchin, or comb jelly, this capacity disappears rapidly. That is, once the egg has cleaved into 8 or 16 or 32

Fig. 38. A schematic illustration of Weissmann's hypothesis (the segregation of genetic elements).



cells and some of these are removed, the remaining cells form an incomplete embryo, with specific parts missing according to which of the cells were removed. (This is called *mosaic* development.) In contrast, the embryos of frogs, salamanders, and chicks are more malleable. Portions of the early frog embryo can be removed without reducing its capacity to turn out a normal product. The remaining cells simply take over the functions of the missing ones. (This is called *regulative* development.) But even here, if we wait long enough and permit the embryo to develop far enough, removal will result in incomplete development. We might conclude from the above that, during development, the cells remaining after excision had lost the capacity to replace the missing contingent. But we can equally well imagine that by the time the excision is made, the environmental conditions no longer permit the transformation of a cell from one type to another.

In recent years an elegant set of experiments has shown that cell nuclei taken from late embryonic stages are no longer equivalent to the nucleus of the egg cell from which they were derived. Frog eggs were enucleated; that is, the nucleus was removed with a microtool. It was replaced with a nucleus taken from a cell at the blastula or gastrula or neurula stage of frog embryogenesis. In other words, the egg cytoplasm was combined with a nucleus representative of cells that had already become differentiated. This synthetic egg was then allowed to develop in order to determine the degree of normality and completeness achieved.

When a blastula nucleus was added to the enucleated egg, a normal embryo resulted. When nuclei from later stages were employed, the resulting embryos were grossly abnormal. Most important, the kind of abnormality observed depended on the part of the embryo from which the nucleus had been taken. Thus, an endoderm nucleus added to the enucleated egg gave rise to an embryo whose structures, derived from endoderm, were normal, but whose other structures were abnormal. A mesoderm nucleus yielded an embryo whose mesodermal development was normal, but whose other parts were abnormal. For reasons we have not space to deal with here, these experiments and the ones mentioned earlier do not conclusively demonstrate that a change takes place in the genetic elements of cells undergoing differentiation, but they certainly do not argue against this thesis.

Conclusion

We can now define cellular differentiation in a general way. We know that the development of an organized multicellular structure always involves the appearance of new cell types, different from their parents

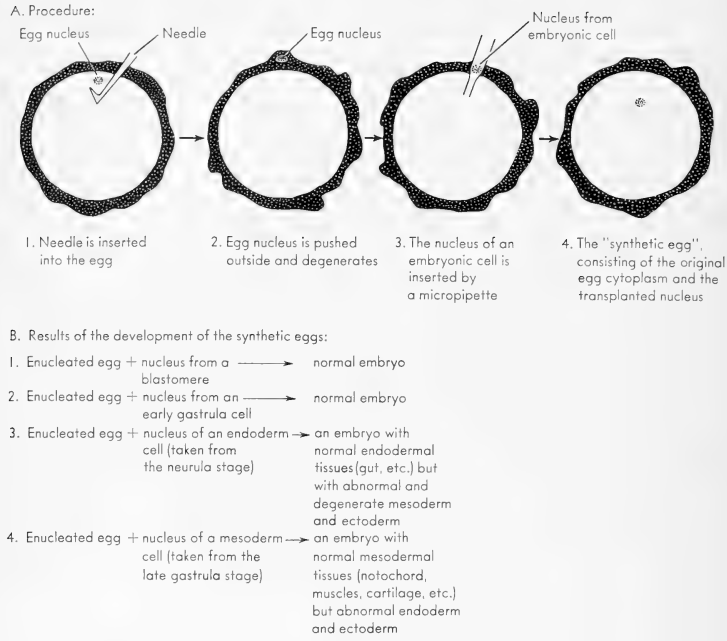


Fig. 39. Transplantation of embryonic nuclei.

and from their sisters. If these new cell types can be shown to play a causal role in the construction of the multicellular being and/or its subsequent functioning, such cells are said to be *differentiated,* and the processes by which they came to be different are collectively called *cellular differentiation.*

The question arises, what kind of information do we have to provide before we can be satisfied that a single act of cellular differentiation has been adequately described and understood? The categories of necessary information are outlined below.

1. Describe the new phenotype. It is not enough to say that the differentiated cell differs from its parent or sister cells. We must show how it differs. Which enzymes are present in the new cell type and are absent or greatly diminished in the old, and vice versa? Which specific structures in the cell have been altered? This is one of the points of de-

velopmental biology where cytology and cell physiology both enter.

2. Account for the function of the differentiated cell. As stated in the definition, the new cell type is meaningful because it contributes in some specific manner to the construction of the multicellular organism (i.e., without the appearance of the new cell type, the normal development of the multicellular structure or the normal functioning of the adult derived from it would be impossible). We must show the specific role played by the new cell type and how its altered metabolism and structure control or trigger later developmental processes in the rest of the organism.

3. Account for the number of each type of cell. We must be able to examine a very small sequence in the development of the multicellular organism, pinpoint precisely the time at which a particular new cell type appears, count the number of them in the cell population, and account for the number that we see by studying the rate at which they appear and the rate, if any, at which they disappear.

4. Determine the manner in which the new cell type arose from its parent. Here we return to the question broached in an earlier section. We must determine whether, in a specific act of cellular differentiation, the new cell type possesses genetic instructions different from those of its parent or whether, faced with a new environment, it does different things from what its parent did but uses the same old set of instructions. Cell genetics in recent years has provided ways of distinguishing between these alternatives and also has provided methods to determine in each case some of the specific mechanisms involved.

As you see, the description of even one step of cellular differentiation is a tall order. At present no such step has been completely elucidated in any organism and, in fact, even the most precise descriptions so far are very primitive and not very quantitative. Consequently, the elucidation of processes of cellular differentiation is one of the most exciting challenges in modern biology and presents the hope that, by studying them, we can uncover new mechanisms of cell variation.

Cell Interactions during Growth and Morphogenesis

We have seen the complexities that attend the construction of a multicellular organism, whether it be slime mold fruiting body or vertebrate embryo. Many new cell types arise and must maintain fairly rigid numerical proportions. Cells must migrate to specific areas of the aggregate and must be integrated into tissues and organs. All these processes are underlain by a bewildering array of biochemical activities that must occur in strict chronological order.

How does the system maintain a balance of kind, number, place, and time? Obviously, each subunit of the organism cannot be permitted to develop autonomously, disregarding other subunits. Each, therefore, must receive information from other parts about what these have been doing in the immediate past and about what it may do and must not do in the immediate future. We will now concern ourselves with the exchange between cells of chemical messengers that trigger, assist, control, and inhibit developmental events.

Many Cells Do What Few Cannot Do

The simplest example of this kind of interaction is seen in cultures of microorganisms or dispersed animal or plant cells growing in a liquid nutrient medium, a topic we will take up in detail in the next chapter and briefly describe here. Such cells, when inoculated into fresh medium, experience a lag of vari-

able duration before they can begin to reproduce actively, a lag that results mainly from the fact that each cell must accumulate threshold concentrations of key compounds (vitamins, amino acids, etc.) before cell division can commence. Yet as fast as the cell produces these compounds, they pour out into the medium by diffusion through the cell membrane. As the external concentrations of these key substances rise, the rate of diffusion out of the cell decreases, enabling the internal concentrations to increase and finally reach the necessary thresholds. The time needed to saturate the external environment with the critical substances and so permit internal accumulation will depend on how fast the substances can be produced. Obviously, if each cell produces them at a constant rate, two cells will produce twice as much per unit time as will one and therefore do the job in half the time, i.e., many cells inoculated into a given volume of fresh medium can begin growing very quickly, whereas a few cells in the same medium would take a much longer time or might never get started at all.

Cooperative interactions also occur between embryonic cells. For example, if we cut out a large piece of dorsal ectoderm from a chick or mouse embryo and cultivate it in a dish with appropriate nutrients, the tissue will yield nerve cells just as it would if left in the embryo. If, however, the single large piece is cut up into many small pieces and each is cultivated separately, no nerve cells appear. The mere act of cutting does not affect the result, because if the pieces are not separated but are cultivated together in a compact mass, they produce nerve cells as efficiently as before. Thus, many cells can do what few cannot, in this case become transformed into specialized nerve cells.

Inductive Interactions

We have already dealt with one very fine example of induction—namely, the production of male and female sex organs in the water mold, *Achlya* (see Chapter 3). The properties of this system reflect perfectly the general features of all inductive morphogeneses.

1. The male sex organelle will not develop when the plant is grown in isolation, but only when induced to do so by the presence of the female plant. The development of the female sex organelle is similarly dependent on the presence of the male (i.e., *one cell group induces another to develop along a specific path*).

2. *The induced cells must be at the correct stage of development in order to be receptive to the inductive signal.* For example, after the female plant has produced the large sacs, oospheres are induced to form in re-

sponse to an inductive stimulus from the male. If too long a time elapses before receiving the signal, the female ceases to be responsive and will no longer produce oospheres.

3. *The inducing cells must also be at the correct developmental stage.* For example, only after the gnarled, branched shoots of the male *Achlya* have grown out toward the female sac and plastered themselves around it can the male produce the hormone which induces oosphere formation.

THE EMBRYONIC ORGANIZER

The most complex inductive mechanisms are found in vertebrate embryos; one example is the *embryonic organizer* demonstrated by Dr. H. Spemann, one of the founders of modern embryology. Spemann was interested in the problem of whether fragments of an embryo could contain or produce all the cell types necessary for the construction of a complete, normal adult (see Chapter 6). He cut salamander embryos in half at various stages of development and found that, before gastrulation, both halves yielded normal adults but that after gastrulation both developed aberrantly. To fix the point at which the capacity for normal development was lost, Spemann cut embryos in half at various times during gastrulation and, to his surprise, one of the halves invariably gave rise to a complete, normal embryo, while the other yielded a degenerate, amorphous cell mass. Upon closer examination, he found that the half yielding a normal product was always the one that contained the dorsal lip of the blastopore [1] and that the half that degenerated always lacked the dorsal lip. It appeared from these results that: (1) cells from the dorsal lip have the power to organize or initiate the construction of the embryo, and (2) no other cells can do this (if the dorsal lip is absent, therefore, embryonic organization does not occur).

To confirm that dorsal-lip cells (called *prospective chorda-mesoderm*) were the inducers of the embryonic organization, Spemann cut out pieces of this tissue and implanted them in the ventral sides of other gastrulas (in contrast to the position that chorda-mesoderm normally occupies, i.e., under the dorsal ectoderm). Two results were observed:

1. At the site of implantation, a small but perfect embryo developed as a sort of Siamese twin of the host embryo. Brain, spinal cord, and associated organs were present.

[1] You will recall from Chapter 5 that the blastopore is the crescent-shaped hole through which the cells invaginate from the surface to the interior of the embryo. Cells at the upper or dorsal lip of the blastopore move inside and take up residence directly under that part of the ectoderm that will later give rise to the central nervous system. They become the central part of the mesoderm and give rise to notochord and somites.

2. The implanted chorda-mesoderm went ahead and developed precisely as it would have had it been in its normal position in the embryo; that is, it constructed a notochord and somites. In contrast, any other part of the gastrula, when implanted in this manner, did not develop as it would have if it had not been moved, but instead developed in accordance with its new locale.

The second result showed that indeed there was something special about dorsal-lip tissue. The first result revealed that dorsal-lip cells could induce overlying ectoderm to form brain, nerve cord, and associated parts. When that part of the dorsal-lip tissue that normally underlies the brain was implanted, it induced brain development very well and tail development very poorly, and when posterior chorda-mesoderm was implanted, it induced tail very well and brain very poorly. Thus the chorda-mesoderm is a specific inducer.

The main question, of course, is what compound or compounds does the chorda-mesoderm supply to the overlying ectoderm to produce the organization of the ectoderm tissue? Unfortunately, any of a wide variety of substances from many sources has been found to do this, so wide in fact that the induction appears to have very little specificity. It is almost as if the ectoderm at this stage of development is a gun primed to shoot in a fixed direction and needing only a touch on the trigger to set it off. This "sensitivity" raises two other very interesting questions.

1. Although it is true that the embryologist can make ectoderm organize itself by treating it with a wide variety of agents, in the actual embryo only dorsal-lip cells can do this. What, then, passes between the chorda-mesoderm and the overlying ectoderm? To answer this question, we label the constituents of dorsal-lip cells with radioactive carbon or sulfur or phosphorus and thus determine what materials pass from the "hot" mesodermal cells to the "cold" ectodermal cells.

2. If it is true that the embryologist can cause ectoderm to organize itself by applying many agents, why is it in the actual embryo that only one part of the ectoderm normally becomes organized into brain and spinal cord? Why aren't secondary embryos caused to pop out all over the place, having been induced by any of the multitude of materials that normally leak out of cells? Here we return to the problem of the *morphogenetic field* discussed in Chapter 4.

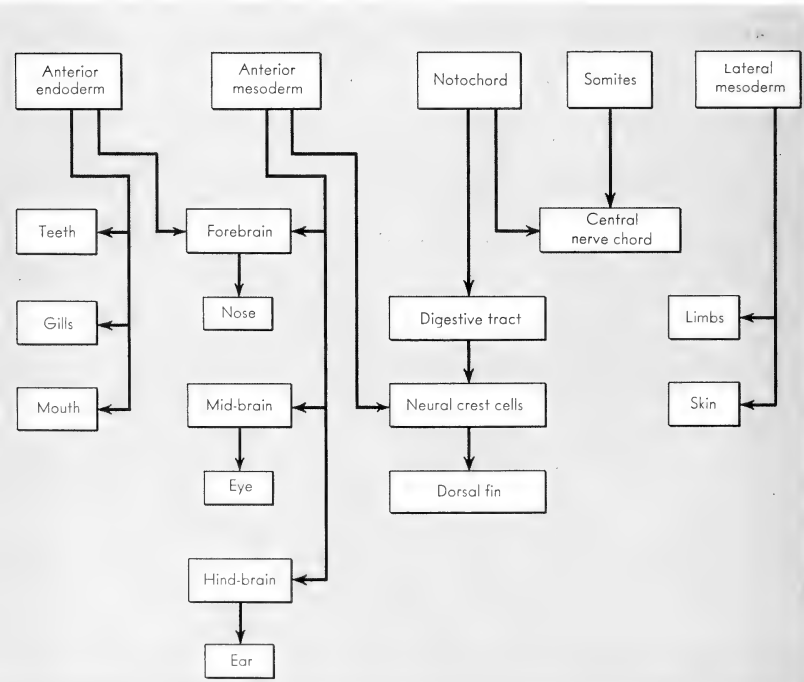
SECONDARY INDUCERS IN EMBRYOS

As we just described, dorsal-lip tissue induces overlying ectoderm to transform into brain and nerve cord, while it itself forms notochord and

somites. From this point on, many contiguous tissues provide each other with inductive signals that trigger the formation of additional organs. In general, the proof that such inductions operate has fallen into two categories: investigators have shown (a) that if tissue A is removed or physically separated from tissue B, tissue B will not construct a specific organ as it usually does; and (b) that if tissue A is moved to a neighboring spot occupied by cells closely allied to B, the organ in question will form at the new site occupied by tissue A and not at the old.

Figure 40 is a schematic and partial summary of the manifold inductive relationships uncovered in this manner. In some cases, these inductions have, by and large, proved to be much more specific than that supplied by the embryonic organizer. However, little of the rigorous biochemistry needed to shed light on them has been performed so far.

Fig. 40. Inductive relationships between different parts of the developing embryo (after Holtfreter). Arrows point from the inducing tissues to the induced organ that ultimately appears. Bear in mind that this array of inductions is far from complete.



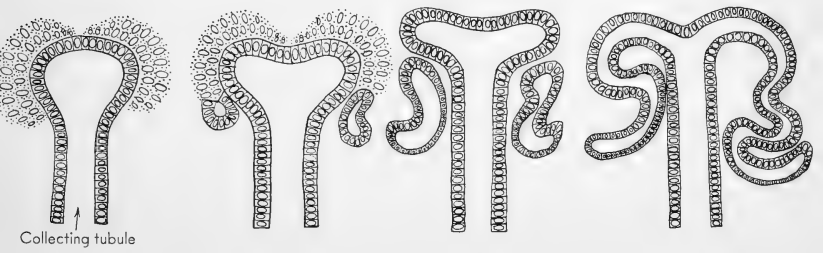
Synergistic Inductions

Synergistic inductions are those in which two different tissues mutually interact so that each causes the other to develop in a way that it would not do when alone. For example, the developing kidney contains many small S-shaped *secretory tubules* connected to a fan-like network of branched *collecting tubes* which serve to transport material collected by the secretory tubules into the ureter to be voided as urine. Figure 41 is a schematic diagram of kidney-tubule development. The tubes and tubules originate from two different rudimentary tissues, starting at about the eleventh day of embryonic life; these are the *nephrogenic cord,* a loose collection of mesodermal cells, and the *ureteric bud,* a compact tubular tissue also derived from mesoderm. The development of the secretory tubules from the former and the collecting tubes from the latter has been found to depend on the intimate association of both. Removal of either from the embryo stops the development of the other.

It is possible to separate the ureteric bud and the associated nephrogenic cord from a mouse embryo and cultivate the tissues on a blood clot overlain with a nutrient solution. Under these conditions, tubes and tubules develop as they do in the embryo. If the two tissues are separated from each other and cultivated, tubes and tubules never develop. Many unsuccessful attempts have been made to extract material from either tissue, that, when added to a culture of the other, would make it develop in normal fashion. If, however, the two tissues are separated by a very thin membrane with tiny holes, the synergistic induction does occur. Although the membrane is thick enough to prevent the two kinds of cells from touching each other across the membrane, blobs of cytoplasm could conceivably pass between the two. When opposed across thicker membranes, synergistic development does not occur.

This type of induction, termed *direct-contact induction,* is a common phenomenon in developmental systems. It requires the reacting tissues to

Fig. 41. Formation of kidney tubules (after Arey).



remain in intimate association for long periods of time, in contrast to the "*Achlya*" type of induction, which occurs when the reacting tissues are separated by a considerable distance. Many chemical explanations can be imagined to account for induction by direct contact but, as in much of what we have already described, the necessary biochemical analyses are only just beginning.

Inhibitory Interactions

As we stressed in Chapter 5, all morphogenetic fields involve inhibition—i.e., cells in one part of the field develop in a certain manner and simultaneously inhibit the surrounding cells from doing so. This is the case in hydranth development in coelenterates, bud development in plants, the construction of the head, heart, limbs, and eyes in vertebrate embryos, etc. As we mentioned in Chapter 5, inhibition can conceivably exist at two levels:

a. Where competition for a common store of materials in the environment enables only the cells that receive the greatest supply to develop.
b. Where the production of material by one cell inhibits another.

Known Mechanisms of Cell Interaction

Space limits us to only a few examples of known mechanisms by which one cell can influence the growth and development of another. We have drawn these from experiments with microorganisms which, being simpler to manipulate and easier to grow under defined conditions, permit more rigorous analyses at present. Unfortunately, these experiments involve only single celled organisms and thus their interactions can serve merely as models for similar interactions among the cells of higher plants and animals.

SELECTIVE INHIBITION DURING BACTERIAL GROWTH

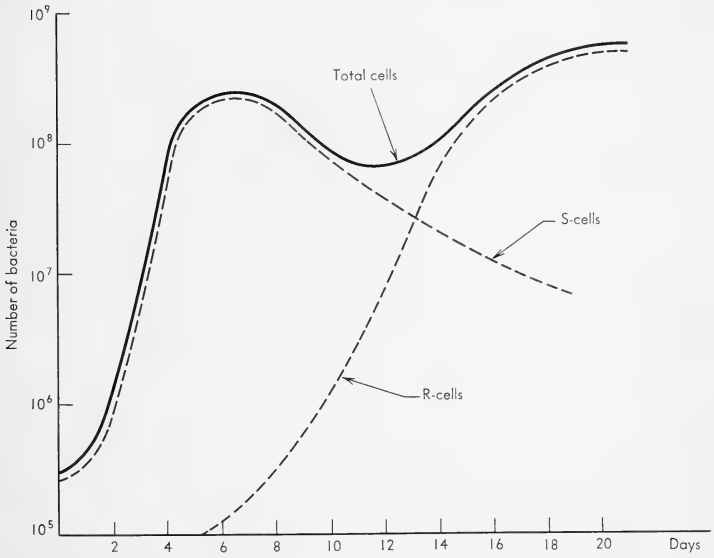
The bacterium *Brucella abortus* causes aborting fever in cattle and Brucellosis in man. When *B. abortus* is taken from the blood of a diseased animal and grown on an agar medium, it forms a colony with a very even outline and mucoid texture, because each cell in the colony is surrounded by a polysaccharide capsule. This type of colony is called "smooth," and the constituent cells are called S-cells. During the growth of S-cells, rare

mutations occur that prevent the mutant and its offspring from synthesizing a capsule. The mutants are called R-cells, and since the colonies derived from them possess an uneven outline and wrinkled appearance, they are called "rough."

If we inoculate a flask of nutrient broth with S-cells, we find that by the time growth has ceased, the population is no longer composed only of S-cells but also contains a great majority of R-cells. Figure 42 summarizes the results. The total population grows normally until the fourth day of incubation, then it dips quickly, begins to grow again, and finally reaches a stationary state. Until the fourth day, the population is made up almost completely of S-type cells. After 4 days, these decline rapidly. R-cells make their appearance coincidentally with the decline of S-cells, increase rapidly, and by 20 days make up over 90 per cent of the population.

As the bacteria grow, intracellular substances leak into the broth; one of these substances is the amino acid, alanine ($CH_3 \cdot CH \cdot NH_2 \cdot COOH$). The appearance of alanine coincides with the decline of the S-cells. The S-cells are very sensitive to alanine and die in its presence, while R-cells are resistant to alanine and can grow normally. Thus by the fourth day, the

Fig. 42. Selective inhibition during growth of *Brucella abortus* cells.



S-cells have produced enough alanine to be poisoned by it. In the meantime, a few R-cells have arisen by mutation. Since the S-cells cannot grow, the R-cells do and eventually dominate the population. The proportion of R- and S-cells is controlled at any time by the levels of alanine.

The interaction between the two cell types, therefore, is not simply the result of an exchange of materials between the two. The S-cells, by acting upon themselves (i.e., committing suicide), permit the R-cells to do something they ordinarily could not have done, namely grow. (Had the S-cells not died out, there would have been no opportunity for the R-cells to grow.)

TRANSFORMATION OF CELL TYPES *DIPLOCOCCUS PNEUMONIAE*

The bacterium *D. pneumoniae* gives rise to mutant types that are different in many ways from the parental stock. For example, we can isolate mutants that are resistant to penicillin or streptomycin (when the parent type is sensitive to these antibiotics), mutants that can degrade sugars that the parent type is incapable of degrading, and mutants that require various compounds for growth which the parent type can make for itself.

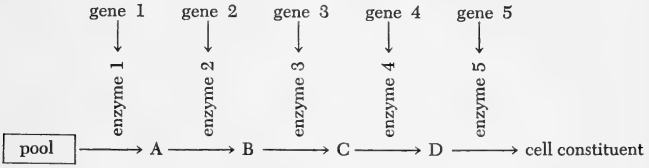
Highly purified deoxyribose nucleic acid (DNA) has been prepared from penicillin-resistant mutant cells. When the sensitive parental type is exposed to this DNA, a large proportion of the cells are transformed into the penicillin-resistant type, and what's more, they pass on this new capacity to their offspring. The DNA can only cause the exposed cells to acquire capacities that are possessed by the cells from which the DNA was obtained (i.e., DNA from penicillin-sensitive bacteria never transforms the exposed cells to penicillin-resistant varieties). In essence, then, it is possible to extract genetic information from one cell and introduce it into another. The macromolecule bearing this information can be incorporated into the genetic apparatus of the treated cell and thus the information is passed on to the progeny.

SYNERGISTIC GROWTH BY BIOCHEMICALLY DEFICIENT MUTANTS

We deal here indirectly with some of the most important genetic experiments of our time, which were conducted upon the bread mold *Neurospora crassa*. Among other things, they provided convincing proof that genes act by controlling the synthesis of specific enzymes. *Neurospora crassa* can be grown on a relatively simple medium containing glucose as a source of carbon and energy, ammonium chloride as a source of nitrogen, a few mineral salts, and two vitamins. Mutants were isolated, that, unlike

the parental type, had to be supplied with various compounds in order to grow because they could not synthesize the compounds; some examples are histidineless mutants (which cannot synthesize histidine and will not grow unless supplied with it) and Thiamineless, Riboflavinless, and Tryptophaneless mutants, and many others. These are called *biochemically deficient mutants.*

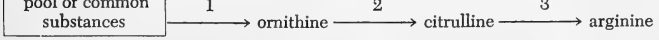
In all cases, the mutant lacks a single enzyme necessary for the synthesis of a particular cell constituent. Amino acids, vitamins, etc., are generally synthesized in stepwise fashion from a pool of common, simple substances in a manner shown symbolically below:



The absence of enzyme 2 would eliminate the reaction A ⟶ B and thus prevent the production of intermediates B, C, D, and the final product. If gene 2 in the nucleus mutated to a non-functional form (i.e., one unable to direct the synthesis of enzyme 2), the mutant cell would be unable to synthesize the final product and would grow only when it was supplied in the culture medium. Two predictions can be made for such mutants and they turn out to be true. These predictions are:

1. That the mutant lacking enzyme 2 could still grow if supplied with the final product, or with intermediates B, C, or D, since the cell can convert these into the final product.
2. That intermediate A would accumulate in the mutant cell, since the reaction A ⟶ B is blocked. Because these intermediates are generally of low molecular weight and are readily diffusible, they not only accumulate inside the mutant cell but usually seep out of the cell into the surrounding medium.

Synergistic growth of deficient mutants occurs because of this osmotic property. As an example, we shall consider biochemically deficient mutants of the bacterium *Escherichia coli,* which are all argininеless (unable to synthesize the essential amino acid, arginine). Arginine is normally synthesized via the following pathway:



Three kinds of mutants have been isolated:

Mutant 1 could grow if supplied with ornithine, citrulline, or arginine (i.e., the enzyme that catalyzes reaction 1 is missing).

Mutant 2 could grow if supplied with citrulline or arginine, but *not* if supplied with ornithine. However, ornithine accumulated in the cells and seeped out into the medium (i.e., the enzyme-catalyzing reaction 2 was missing).

Mutant 3 could grow only if supplied with arginine, *not* with ornithine or citrulline. However, citrulline accumulated in the cells and seeped out into the medium (i.e., the enzyme-catalyzing reaction 3 was missing).

If, as shown in Fig. 43, the three mutant types are streaked on agar containing a tiny amount of arginine, synergistic growth occurs. Mutant 3 grows very sparingly until the arginine in the medium is used up, and then it stops. It continues, however, to generate energy by oxidation and to carry out a wide variety of biochemical reactions, including the synthesis of citrulline, which accumulates and seeps out into the medium. Mutant 2, growing nearby, can convert the citrulline to arginine. Since mutant 3 makes a large quantity of citrulline, mutant 2 can grow very well. If the mutant 2 cells do not lie next to mutant 3, they grow very sparingly, but they still can metabolize and accumulate ornithine. Since mutant 1 can

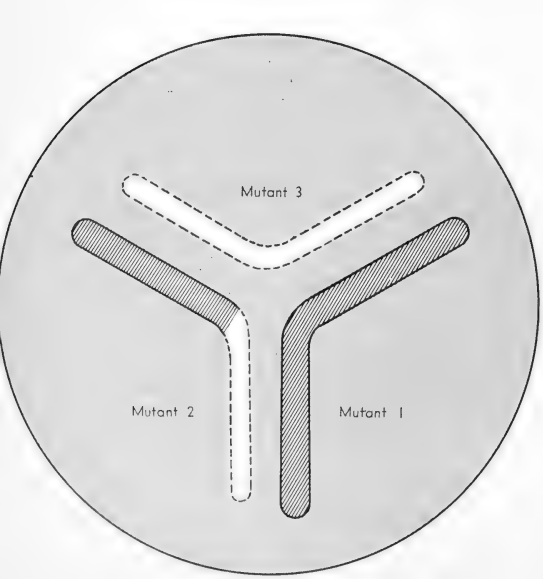


Fig. 43. Synergistic growth by three mutant types of *E. coli*.

convert both citrulline or ornithine to the needed final product, it grows very well if it is streaked next to either mutant 2 or mutant 3. In multicellular organisms, the various tissues may well influence the growth of neighboring cells in just this manner. There are numerous examples in embryonic development of the effect of one organ or tissue upon the growth of another.

Conclusion

All cells in nature interact with one another. Sister microbial cells compete with each other for food and space, and sometimes exert direct inhibitory effects on each other in order to survive the competition. They can also assist each other by exchange of metabolites. Genetic information can be transferred from one to another and produce new capacities in the recipients. In multicellular forms, where the constituent cells are packed together in a compact mass, opportunities for cell interaction are very great and, since developmental activities must be timed perfectly, are even more necessary than in single-celled organisms. The kinds of interaction can be classed as follows:

a. population-density effects—where many cells of the same kind cooperate to do something that few together could not do.
b. inductive interactions.
c. synergistic inductions.
d. inhibitory interactions.

The vehicles of interaction seem to fall into these categories:

a. Interactions promoted by diffusible agents between cells separated from one another. Such agents can be carried by simple diffusion or transported through the circulatory system.

b. Direct-contact interactions between the effector and effectee cells. Such systems probably require intimate contact because the material transferred is very unstable and will not survive transport over great distances or because large amounts of cytoplasm are exchanged and several substances must travel as a unit.

c. Contact interactions without exchange of material. The surface of one cell reacts with that of another to create drastic alterations of the surface, which are then reflected by secondary changes in the metabolism of the cell.

Growth and Form

How an organism grows and the processes, both internal and external, that control and limit growth are fundamental biological problems. A wide variety of living organisms has served as objects of study. Much has been learned by examining the growth of populations of microorganisms—bacteria, protozoa, algae, and fungi. In recent years, biologists have been able to remove tissues of higher animals and grow them outside the body (tissue culture). In some cases, it has been possible to disaggregate such tissues and to cultivate the cells as dispersed individuals exactly as if they were microorganisms. Even the cells of human beings (tumor cells, kidney cells, etc.) can be so treated and have been found to obey the same rules of growth and nutrition as their more primitive brethren. Intact higher animals have been grown under controlled conditions in which the natural diet is replaced by mixtures of known chemical compounds delivered in measured amounts. The rat is a favorite laboratory animal for this purpose, but other animals such as the fruit fly (*Drosophila*) and the university undergraduate (voluntarily) have been used.

The first lesson learned was that the term "growth" has several meanings and that an organism may grow in one sense but not in another. Growth, under one interpretation, is an increase in the number of cells, which is found by counting the total cell population or a measured sample from it. Growth can also be described as an increase in protoplasmic

material, which we find by determining the weight or volume changes in an organism. Or we can pick out some constituent of protoplasm that is always a constant proportion of the whole, such as the nitrogen content or protein content. If either were to increase, we would know to what extent the total protoplasmic mass had increased.

It should be noted that both cell number and protoplasmic content do not always increase together. For instance, cell division can occur without any increase in protoplasm; the result is a greater number of smaller cells. Alternatively, protoplasm can be synthesized in the absence of cell division, in which case the cells grow larger but not more numerous. However, isolated growth of this kind can go on only under exceptional conditions and for a relatively short time. Ultimately cell division must cease without protoplasmic increase and vice versa.

Nutrition

The act of growth, when it involves an increase in protoplasm, requires the synthesis of a wide variety of cell constituents—nuclei including chromosomal material, centrioles, nucleoli, etc., mitochondria and other cytoplasmic organelles, thousands of enzyme molecules, cell membranes and other structural material. These require the synthesis of macromolecules such as proteins, nucleic acids, and polysaccharides in which many subunits are linked together. The subunits themselves, amino acids, sugars, fatty acids, vitamins, etc., must be synthesized from still simpler compounds or must be assimilated from the environment.

Two basic needs must be met if these activities are to be accomplished—the need for energy and the need for raw materials. Cells obtain energy by the oxidation of materials obtained from the environment. Sugars are the prime fuels for this purpose, but many other oxidizable compounds can be employed by one or another kind of cell. The energy thus made available is employed for the synthetic reactions. At their simplest levels, the raw materials for the synthesis of protoplasm are the elements of which cells are composed. The major components are carbon, hydrogen, oxygen, nitrogen, sulfur, phosphorus, magnesium, manganese, iron, and traces of many other elements as well. The elements must, however, be supplied in usable form. For example, the amino acid, alanine, $CH_3CH_2{-}NH_2\ COOH$, is essential for the synthesis of proteins. No known organism can synthesize alanine if it is merely supplied with elemental carbon, hydrogen, oxygen, and nitrogen. But some cells can do so by converting nitrogen from the air to ammonia and combining the ammonia with pyruvic acid as follows:

$$NH_3 + 2H + CH_3CO\ COOH \longrightarrow CH_3CHNH_2{\cdot}COOH + H_2O$$

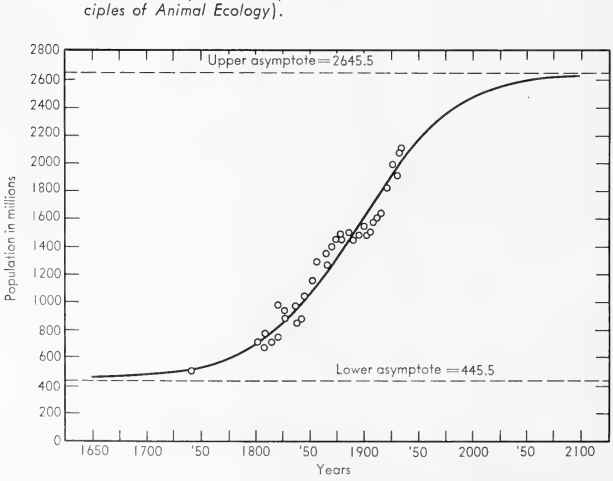
Other cells cannot convert N_2 to NH_3, but if given NH_3 and pyruvic acid they can make alanine. Still other cells, which do not possess any of the enzymes that catalyze these reactions, must be supplied with alanine itself if they are to grow. The diversity of nutritional requirements results from the inability of cells to synthesize one or another protoplasmic constituent.

The S-Shaped Curve of Growth

Figure 44 shows growth curves which, as you can see, are S-shaped. The abscissa is time—minutes, days, hours, years, depending on the organism studied. The ordinate is growth and can describe the number of cells in a bacterial culture, the number of human beings on earth, the size or weight of a sunflower seedling or a rat, the size or weight of the heart or the brain. In other words, the ordinate is a measure of the growth of a population or a single organism or any of its parts and, when growing under optimal conditions, all show precisely this sort of growth curve. Many questions come to mind when we look at this curve. Why does growth start? Why does it stop? Why is the curve S-shaped?

In this discussion of the factors affecting growth, we shall deal with

Fig. 44a. The growth curve of the human population of the world. The circles indicate census totals, the solid line the mathematically fitted, smooth, S-shaped curve (after Pearl and Gould, from Allee, et al., *Principles of Animal Ecology*).



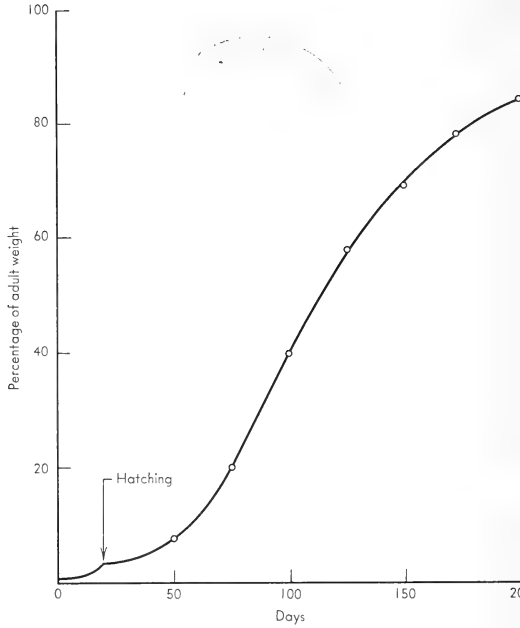
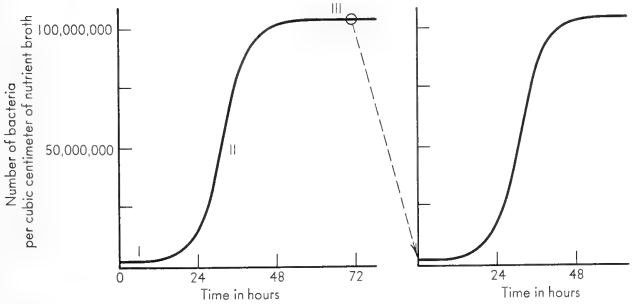


Fig. 44b. Growth of the chick during its embryonic and post-hatching periods (redrawn from Weiss and Kavanau).

Fig. 44c. Bacteria are introduced into fresh nutrient broth. At intervals, a sample is abstracted to determine the number of bacteria per cubic centimeter of broth. Three phases of the resultant growth curve are recognized: I—lag phase; II—logarithmic phase; III—stationary phase. If, now, bacteria from the stationary phase are used to inoculate another flask of nutrient broth, the growth pattern repeats itself.



a culture of bacteria because it is the easiest to understand, and conclusions reached from studies of it are applicable to all organisms, plant and animal alike.

Imagine that we introduce a few hundred bacterial cells into a cotton-stoppered flask containing sterile, nutrient broth (called the "medium"). Every hour or so we abstract one cubic centimeter of the broth and determine the number of living bacterial cells. Figure 44c shows the complete curve that would be obtained.

The curve is divided into three parts: I—the period called the *lag phase* during which the cells prepare for growth; II—the period of actual growth, called the *exponential or logarithmic phase*; III—the period in which growth ceases and the population enters the *stationary phase*. None of these phases is of set duration. That is to say, the time a culture spends in each phase depends on the particular species of bacteria and the conditions under which they are grown.

LAG PHASE

The lag phase is a period of rapid protoplasmic growth and a period of preparation for the cell division that is to come. Thus, it is a time when the cells become larger but remain constant in number.

We may ask why cells must prepare for cell division. Why can they not enter the exponential phase immediately? The answer is that a cell in the exponential phase is somewhat like an assembly line operating full-blast. Parts are fabricated on the line in consecutive stages or are received from outside contractors and these are ultimately put together to yield the finished product. The prime requirement for an efficient assembly line is that all the operations mesh harmoniously. Supplies must arrive in the right place, at the right time, and in the right amounts, and the operations along the assembly line must proceed at equivalent speeds. If, then, we start a culture with cells that are already in the exponential phase (i.e., the "assembly lines" are already set up and operative) and supply them with everything they need, the lag phase should be eliminated, and this is just the result that is obtained. In contrast, if we start a culture with cells that are not in the exponential phase, they require time to prepare for growth. We will now take up the preparations that are necessary to generate the exponential phase.

RESYNTHESIS OF ENZYME SYSTEMS REQUIRED FOR GROWTH. A cell that has already passed the exponential phase is no longer faced with the problems of growing, only with those of survival; it must protect itself against metabolic waste products and other toxic substances that may have built up in the environment and must conserve whatever raw materials and energy that it still may have left. In so doing, it may lose some enzymes that are no longer needed and synthesize others that are required for its

survival. If now such cells are exposed to a fresh nutrient medium in which growth is once again possible and survival no longer a problem, they must reverse the course of events mentioned above. But this involves extensive protoplasmic reorganization and a further synthesis of enzymes and other macromolecules. All this takes time; hence the lag phase.

THE SYNTHESIS OR ASSIMILATION OF RAW MATERIALS FOR GROWTH. Each original cell must stock up on the chemical subunits (amino acids, sugars, fatty acids, vitamins, etc.) that are needed to construct enough protoplasm to make two cells. If the nutrient supply were rich and these compounds already present in the environment, the job would be simple and the lag phase correspondingly short. If the nutrient supply were poor, the missing subunits would have to be synthesized from simpler materials in the environment. The job, then, would be more difficult and the lag phase correspondingly longer. The lag phase can also depend on how many cells are actually present, for each is a little factory geared to the production of the subunits, and the more factories there are, the faster they can be synthesized and the shorter will be the preparatory period. In the jargon of the bacteriologist, the cells "condition the medium." Moreover, like a well-functioning assembly line, each original cell that has stocked up on enough subunits to create two new cells also insures the continuity of supply so that the two in turn will, without delay, be able to collect or synthesize enough subunits to make four, and so on. Thus, no further lag is apparent and growth can proceed apace. To summarize, then, the lag phase is influenced by:

1. The richness of the nutrient supply.
2. The number of cells preparing for growth.
3. The "physiological state" of the cells.

THE EXPONENTIAL PHASE

We now come to the stage of active growth and reproduction and the peculiar S-shaped curve. To understand it we need merely consider the properties of populations that are growing actively under optimal conditions:

1. The more organisms there are, the more there can be. In other words, offspring must have parents and the more parents, the more offspring.

2. Most of the organisms in such a population reproduce during their life cycles. Very few are barren.

3. The generation time is constant. In other words, the time for each organism to emerge, mature, and reproduce is approximately the same as long as the population continues to grow.

To return to our bacterial culture, the above statements mean (1) that during the exponential phase each bacterial cell gives rise to two, the two to four, the four to eight, etc., and (2) that it takes the same amount of time for one to yield two as for two to yield four, etc. Suppose the generation time were one hour. Then the population would, on the average, double once every hour. If we draw a graph in which the number of cells is plotted against time, we get the curve shown in Fig. 45. This is the first part of the S-shaped curve. If now the period of active growth draws gradually to a close—cells begin to die rather than reproduce and those that can reproduce take longer than an hour to do so—the second part of the S-shaped curve appears.

If growth were unlimited, the growth curve would simply go up and up at an ever increasing rate, and it is interesting to speculate about what would happen to our planet if the growth of, say, bacteria were unlimited. One species of bacteria, *Escherichia coli,* can divide every 20 minutes in an optimal environment. Thus, if at time zero there were one cell, at 20 minutes there would be 2, at 40 minutes 4, at 60 minutes 8, and so on. The series 1, 2, 4, 8 can be expressed as 2^0 (=1); 2^1 (=2); 2^2 (=4); 2^3 (=8); note that the exponents represent the number of generations of growth that have occurred. Therefore, the number of cells present after n generations of growth would be 2^n. Since the generation time of *Escherichia coli* is 20 minutes, there could be 3 generations per hour, or $24 \times 3 = 72$ generations per day. In other words, were growth not limited, one bacterial cell would give rise in 24 hours to $2^{72} = 40{,}000{,}000{,}-$

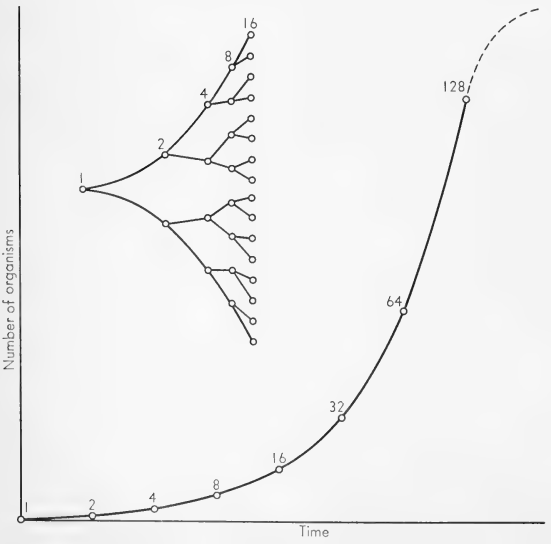


Fig. 45. The first part of the S-shaped curve is generated when every organism gives rise to progeny (in this case by binary fission) and the generation time is constant.

000,000,000,000,000 cells. A mass of bacterial cells this size would weigh 8 million pounds. We would quite literally be up to our ears in bacteria.[1]

Time for Organisms To Double Their Mass

Escherichia coli	20 min
Fly larva	13 hr
Silk worm	68 hr
Rabbit at birth	6 days
Pig at birth	6–7 days
Sheep at birth	10 days
Guinea pig at birth	18 days
Horse at birth	60 days
Man at birth	180 days

The same arguments hold for higher animals. As anyone knows who has had experience with rabbits, they may differ from bacteria to the extent that you need at least two (of the appropriate sexes) to get more, but they are the same as bacteria in that the more rabbits you have the more rabbits you will surely get. Therefore, rabbit colonies or any other animal population, when well fed and given enough space, display S-shaped curves of growth. It should be noted that two rabbits do not necessarily, and in fact hardly ever do, produce only four rabbits and four only eight. The fact that with each generation the number of organisms more than doubles would not change the shape of the curve but would merely make it steeper.

THE STATIONARY PHASE

As already mentioned, toward the end of the exponential curve, more and more cells begin to die and those that survive take longer and longer to reproduce. Ultimately the rate of cell death increases so much and the rate of cell growth decreases so much that cells die as fast as new ones appear and the total number of living cells remains constant. This is the stationary phase.

Why does the population stop growing? What limits growth? There are at least two general causes:

1. The supply of an essential nutrient becomes exhausted. If an organism requires a particular nutrient for growth and the supply gives out, the organism will cease growing no matter how much there remains of any other nutrient. Whether the exhausted nutrient happens to be a vitamin or an amino acid or a source of energy, or a metal ion or oxygen, the result will be the same. The cessation of growth may be subtle, as in

[1] See the section at the end of the chapter for a detailed discussion of growth kinetics.

a bacterial culture, or very dramatic, as when populations of Alaskan lemmings exhaust their food supply and in the course of frantic migration commit mass suicide by leaping into the ocean.

2. *The environment becomes too toxic for further growth.* Organisms, as they grow, pour out metabolic waste products and thus pollute the environment. As long as the number of organisms is small, the concentration of wastes will be low and will not poison them. When the number is large, the rate of waste production will rise and the concentration in the environment will increase to a toxic level and lower the vitality of the organisms to the extent that they can no longer grow or perhaps even survive.

We should mention a special case under this second category that is particularly pertinent for animal populations, namely the stoppage of growth not by toxic materials but by epidemics caused by parasites. Epidemics are like nuclear chain reactions. That is, the host population must rise above a critical density before the epidemic can take place. (Can you construct an explanation of this?) Thus, when an animal population does grow to great numbers, an epidemic can strike and reduce the population drastically. Then another cycle of growth will begin. Biologists have charted such growth cycles for many wild animal populations.

Growth of the Multicellular Organism

A population of microorganisms is a collection of discrete cells that are largely independent of each other for their existence. The higher animal is itself simply a collection of discrete cells that is organized into a compact whole, but the cells are closely dependent upon each other for their continued existence. To the degree that the animal is simply a collection of discrete cells, it grows according to the same rules as does a microbial culture. But the interdependence of cells in the animal body brings complications:

1. All parts of the animal do not grow at the same rate. Some tissues do not grow at all once the embryo is formed. Others grow very slowly. Still others grow very rapidly. The total organism, however, which is the resultant of all the individual cells and tissue, does grow precisely as do populations of microorganisms, i.e., in an S-shaped curve.

2. All parts of the organism do not stop growing simultaneously. In fact, some tissues continue to grow during the entire lifetime of the animal. However, the total organismic mass does remain relatively constant once maturity is reached (in the absence of pathological changes).

3. The growth of one part of an animal can be and usually is controlled by the activities of another part. A prime example of this is the dependence of many tissues upon secretions of the pituitary gland.

The Limitations of Size and Form

There are obviously great variations in the sizes of living things, particularly between different species but often even within them. Many factors govern size. Among them are the following:

1. Genetic constitution. Mendel did some of his first experiments with the dwarf and giant varieties of pea plants and showed that the two differed by a single pair of genes. Such genetic determinants act by affecting the rates of critical metabolic processes.

2. Nutrient supply and toxicity. As shown in the previous section, the growth of populations is limited by these factors. The same is true of the growth of a single cell or a single multicellular organism. Any environmental condition that affects either factor can also affect size and form.

3. The ratio of surface to volume. Imagine an organism, perfectly spherical in shape, which starts life with a radius of 1 centimeter and grows to a radius of 10. Being spherical, its volume would be $\frac{4}{3}\pi r^3$ and its surface $4\pi r^2$. At the beginning, the volume would have totaled $\frac{4}{3} \times 3.14 \times (1)^3 = \frac{4}{3} \times 3.14 \times 1 = 4.2$ cubic centimeters (cm^3). After the growth period, the volume would be $\frac{4}{3} \times 3.14 \times (10)^3 = \frac{4}{3} \times 3.14 \times 1000$ or 4200 cm^3. Thus, the volume of the organism would have increased by $4200/4.2 = 1000$ times. The surface at the beginning would have been $4 \times 3.14 \times (1)^2$ or 12.56 square centimeters (cm^2) and at the end $4 \times 3.14 \times (10)^2 = 4 \times 3.14 \times 100$ or 1256 cm^2. Thus, its surface would have increased by 1256/12.56 or only 100 times. In other words, when an organism increases in size, the volume increases much faster than its available surface area. The point of this is that if an organism experiences a thousandfold increase in protoplasmic volume, it is going to require a thousandfold more food to sustain itself and it is going to have to get rid of a thousandfold more waste products. But food enters and wastes leave through the surface of this organism which, as we have seen, increases much less than its volume. Consequently, this limits the size that the organism can attain. Different species have overcome this limitation by:

1. Changing their shape in order to decrease the disparity between volume and surface as the two increase during growth.
2. Using their food supply more efficiently or by devising ways to make its entrance more rapid.
3. Devising biochemical tricks for cutting down on the production of toxic wastes or by speeding the exit of wastes out of the system.

4. Structural limitations. Nature is an engineer and an architect. She builds conservatively and according to sound engineering principles. She pays attention to strength of materials and patterns of stress. She believes that form should follow function. The man to realize these ideas most strongly and to express them most convincingly in terms of mathematical rigor was a great biologist named D'Arcy Thompson, who wrote a book on the subject entitled *Growth and Form.*

Equations That Describe Growth

Many attempts have been made to describe mathematically the growth of a population of cells or of multicellular individuals. It is not difficult to arrive at an equation that can generate an S-shaped curve. What is difficult is to invest the constants of this equation with biological meaning, that is, to interpret them as being due to specific physiological mechanisms that initiate, maintain, and terminate growth. Generally, we start with the growth-affecting processes we know about (production of diffusible subunits, production of toxic wastes, exhaustion of the nutrient supply), then describe them in mathematical terms, and derive the proper equation relating the number or mass of organisms to the passage of time. The extent to which the equation (called the logistic equation) accurately describes growth, as measured in specific organisms, tells how much we really know about growth processes. The extent to which it does not, tells us how much is not known but, more important, provides a testing ground for the inclusion of additional physiological processes that we suspect may affect growth. The following paragraphs contain in abbreviated form the arguments that led to the derivation of the logistic equation. It is not expected that you will understand the subject completely from this exposition, but I hope you will appreciate from it that a rigorous, quantitative approach to biological phenomena is possible and represents a fertile field of investigation for the mathematically-minded student.

The first part of the S-shaped curve, the period of so-called exponential growth, is simple to derive. It is described by the equation:

$$\log_e \frac{N}{N_0} = kt \qquad 1$$

where N is the number of organisms at time t, N_0 is the starting number, k is a constant, and t is time. As mentioned previously, the growth of a population of cells that divide by binary fission follows the series 1, 2, 4, 8, 16, . . . and these can be stated as powers of 2, i.e., 2^0, 2^1, 2^2, 2^3, . . . in which the exponents refer to the number of generations of growth that

have occurred. Therefore, starting with one cell and after n generations of growth, the number of cells would be 2^n. If we started with 1000 cells instead of 1, the series would be 1000, 2000, 4000, . . . i.e., 1000×2^0, 1000×2^1, 1000×2^2, . . . and after n generations, $N_0 2^n$, where N_0 is the starting number. The general statement of the number N of organisms would be:

$$N = N_0 2^n \qquad 2$$

Let us assume that these cells reproduce once every two hours (a constant generation time). Then the population would have undergone one generation of growth in 2 hours, two in 4 hours, three in 6 hours, and so on. In short, the number of generations would be proportional to time, and this is stated algebraically as $n = k't$ where k' is a constant. Equation 2 can be restated as:

$$N = N_0 2^{k't} \qquad \text{or} \quad \frac{N}{N_0} = 2^{k't} \qquad 3$$

Taking logarithms of both sides, we get:

$$\log_e \frac{N}{N_0} = (\log_e 2) k't$$

and if we put $k'(\log_e 2)$ equal to a new constant, k, the equation becomes:

$$\log_e \frac{N}{N_0} = kt \qquad 4$$

which is identical with equation 1. Remember that the only two assumptions used to derive this relationship are that 1 organism gives rise to 2 and that the generation time is constant. Obviously, the shorter the generation time, the faster would the population grow and the higher would be the value of k. Suppose the cells do not divide by binary fission but, instead, each cell gives rise to 3. Then the growth would be 1, 3, 9, 27, 81, . . . a series in powers of 3, and the equation would become:

$$\log_e \frac{N}{N_0} = (\log_e 3) k't$$

Only the value of k in equation 4 would change, but the basic equation would remain the same. In other words, whether 1 organism gives rise to 2 or 3 or more, or whether 2 organisms must cooperate to yield 3 or 4 or more does not change the equation but only the value of the constant.

But equation 4 as written does not describe the entire growth sequence. Note that as time passes (t gets larger), N/N_0 would get larger

and never reach a limit, i.e., growth would never stop. To show how an automatic brake can be added to this equation, we derive it in another and more general way. Consider that growing organisms display two properties:

a. To get any progeny at all, you must start with at least one individual or, in the case of higher animals, with at least two of the right sexes (i.e., there is no spontaneous generation of life).

b. The more parents you have, the more progeny you will get. In other words, the rate of increase in the number of organisms is proportional to the number already present.

Now rate of increase is simply the change in the number of organisms per unit time, or:

$$\frac{\text{total change in number of organisms}}{\text{total change in time}}$$

We symbolize this as $\Delta N/\Delta t$. Since the rate of increase is proportional to the number, N, of organisms already present, we can write:

$$\frac{\Delta N}{\Delta t} = kN \qquad 5$$

This equation can be solved by the integral calculus. That is, the relation between N and t can be deduced from the way in which N changes with time. By these means (the mechanical manipulations are unimportant here), we obtain the same equation as already has been derived, i.e., equation 4.

Equation 5 can be elaborated to account for the stoppage of growth. What we want is to have the rate of growth start out high and give us the first part of the growth curve and then to decline steadily to zero, at which point the number of organisms would remain stationary. For some organisms that do not remain stationary after cessation of growth but actually die out, we would want the rate of growth to become negative after a time (a negative rate of increase is itself a decrease). These qualifications are accomplished by making k not a constant but a variable. For example, one way of doing this is to set k equal to $(a - bN)$, where a and b are constants. Equation 5 would then become:

$$\frac{\Delta N}{\Delta t} = (a - bN)\mathrm{N} \qquad 6$$

At the start, when the number of organisms was very small, bN would be negligible, and the rate of growth would be substantially equal to aN

(i.e., would be like equation 5) and would yield the first part of the S-shaped curve. As growth proceeded and N became larger, bN would become larger and begin to detract appreciably from a. Thus the rate of growth would progressively decrease. When N became so large that bN equalled a, the term $(a - bN)$ would be zero, the rate of growth would be zero, and growth would cease. The constant, b, could be used to describe the decrease in the food supply or accumulation of toxic wastes that inhibit growth. In the case of the growth of organisms that serve as prey for other organisms, b could be used to describe the way in which the predator cuts down the growth of the prey (by killing off potential parents). In this case, b would not be a constant but would depend on the number of predatory organisms. Finally, b might be used to account for epidemics that kill substantial numbers of organisms and would be treated in much the same way as if a predator were feeding upon the population. If we were studying the growth of an organ, say the number of liver cells in a human, b might also be used to describe the action of hormones from other parts of the body upon the liver cells.

Selected Readings

Popular discussions of many of the investigations described in these chapters can be found in the back issues of *Scientific American.* Ask your school librarian for a list of the articles.

Brachet, J., *Biochemical Cytology.* New York: Academic Press, 1958. A very advanced study of cell development.

Brachet, J., *Chemical Embryology,* 2nd ed. New York: Interscience, 1950. An advanced discussion of the subject.

Butler, E., and D. Rudnick, eds., *The Symposia of the Society for the Study of Growth and Development.* Princeton: Princeton University Press, 1953 to present. Contains a wide variety of general reviews of developmental biology. These discussions are at an advanced level.

Waddington, C. H., *Principles of Embryology.* London: Allen & Unwin, 1956. A good elementary embryology text.

Willier, B. H., P. A. Weiss, and V. Hamburger, *Analysis of Development.* Philadelphia: Saunders, 1955. A comprehensive advanced treatise on embryology.

Wilson, E. B., *The Cell in Development and Heredity.* New York: Macmillan, 1925. A true classic, as fresh and illuminating as if it had been written yesterday. It should, however, be reserved by the serious student until his senior year for full appreciation.

Index

Index

D

E

F

G

T

V

W

Y

Z