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OUTLINE OF COMPARATIVE EMBRYOLOGY

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BY

AUTE RICHARDS

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THE HADDON CRAFTSMEN
CAMDEN, N. J

To

EDWIN GRANT CONKLIN

Investigator, Teacher,
Leader of Embryological Thought

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PREFACE

As the result of a number of years of experience in introducing students to the subject of embryology, the writer has found it desirable to depart from the common practice of studying chiefly the special embryology of the chick, pig, and perhaps of amphioxus and the frog, and to devote much attention to the principles of general embryology. This procedure is based upon the assumption that a broad background of general knowledge forms a desirable starting point for the study of special embryology to be taken up later. As comparative anatomy has proven to be the best basis for the later study of anatomy, so comparative embryology forms a proper basis for a study of special embryology. Unfortunately there has been no book in our language which brings together in a small compass the principles of general comparative embryology.

In this book it is the aim to set up the general principles of development so that the student may see how his special case—chick, starfish, or other form—is merely one of several types and what its relation to the other types may be. The writer holds that the most effective presentation of the subject matter of embryology is the laboratory study of desirable types, supported by text-book and "quiz" work on these forms and accompanied by the consideration from a comparative standpoint of the broad principles of the subject, such as types of egg structure, of cleavage, of gastrulation, and the critical consideration of classical embryological doctrines, etc. To present the material in brief form for this general part of a course in Comparative Embryology this volume is designed.

In Part One are taken up those phases of development which are passed through in the embryology of most higher forms of animals. A comparative study of these phases no doubt forms the basis of nine-tenths of the courses in this subject. Of the vast amount of material from which choice must be made, the decision as to whether any certain topic should be discussed has been based always upon a single criterion. It is sought to show only how the single-celled fertilized egg arrives at the multicellular condition characteristic of the fully formed, but undifferentiated, young organism in which the organ systems are established. In an outline of comparative embryology it is not possible to follow through all the details nor to repeat descriptions whose gen-

eral features apply to more than one kind of animal. Nor is it possible to follow through for these forms the later stages in their development and differentiation. If completeness were the goal, it would be necessary to include the special embryology of the many forms about which investigators would desire information, an obviously impossible task. It is hoped, however, that a basis is here laid for understanding the detailed embryology of any of the types and for working out later stages in whatever group the student's interest may lie.

Part Two considers numerous miscellaneous problems that have arisen in connection with the history of embryology. Here the choice of topics has been governed largely by questions asked by students both beginning and more advanced. No attempt is made to cover all the classical topics of embryology, nor to arrange the ones chosen in an order to develop any particular thesis. It is simply thought to satisfy in some measure student interest in them and to make them easily available. Discussions of many of them are not available in brief form in any English text, although they concern matters about which students of zoology should be informed, for they are chiefly problems of normal development.

The embryology of the present day is experimental embryology. Its aim is to secure an insight into the real nature of those steps by which a single-celled egg becomes a completed adult. An understanding of development in its true sense is its goal. Many aids to experiment used only by chemists or physicists in years gone by are now every-day tools of the embryologists, for descriptive embryology alone is inadequate to give a complete understanding of development. Yet experimental embryology runs the danger of considering only processes and those out of relation to structures. It is in structures that functions are inherent, for one cannot understand the workings of a machine with which he is unfamiliar. Morphology in its descriptive phases is therefore basic to a proper attempt to work out functional relations. Our admiration for the attempts to secure an intelligent conception of the mystery which underlies the pageant of development must not allow us to disregard the importance of a foundation of knowledge of descriptive embryology. Perhaps the errors which may be avoided by the study of descriptive embryology in its comparative phases may serve to recompense the student for the time lost from experimental embryology.

Undoubtedly numerous errors are to be found in this volume. Much that is discussed has been the subject of life-long study by many investigators and has concerned material collected from all over the world. To understand all adequately so that each may be mentioned correctly and weighted properly in a brief outline, is a task hardly to be accom-

plished, and many inconsistencies of treatment must be apparent to the reader. It must be borne in mind, however, that not an extended treatise but an outline of principles is the intent of the book, and the intent places many limitations upon what can be undertaken.

Acknowledgments are due for assistance in almost every section. The material treated in a work of this kind is by no means new, and it would be impossible to present any one phase of it without finding parallel treatment in some source of information available to students of embryology of this day. There is no claim to originality in the book, not even in the treatment of many chapters. The book will have served its purpose, however, if it appears to have sufficient pedagogical background and if it presents in sufficiently compact form those principles, many of which I think are not briefly summarized elsewhere in the English language, and which are needed to give students a basis for work in special embryology, either medical or experimental.

Korschelt and Heider's "Lehrbuch der vergleichenden Entwicklungsgeschichte" is a great treasure house of embryological learning to which constant reference has been made. In many places (particularly in Chapters VII and VIII, Part One) even their arrangement has been so much the best that only minor departures from it are to be found here. In others an entirely different organization of the same material is here adopted. But the debt to Korschelt and Heider is a very great one. I appreciate very much the gracious verbal permission to use their material freely which was given me by both Doctors Korschelt and Heider.

Drawings to illustrate the text are from many sources and not many are new. The writer is not impressed with the desirability of replacing pictures made by investigators on special forms with new ones when the former are well known and of proven value. The drawings in every case are accompanied by a legend giving the name of the investigators to whom indebtedness is due.

Permission has been granted by the following publishers to make use of the figures indicated either as modified, or in the case of numbers 1, 2, 3, 135, 136, 142, 153, 181 of the figures themselves. By Henry Holt & Co. permission has been given to use from Locy's "Biology and Its Makers," our figures 1, 2, and 3; to use from Kellicott's "General Embryology" our figure 5; from Kellicott's "Chordate Development" our figures 135, 136, 142, 144, and 181; and from Lillie's "Embryology of the Chick" our figures 151, 152. By P. Blakiston's Son & Co., Inc., permission has been given to use from Folsom's "Entomology with Special Reference to Its Ecological Aspects," 3rd edition, our figures 117, 161, and 162; and from Patten's "Embryology of the Pig," figure

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Especial acknowledgment for help in many ways including aid with the materials used and the reading of parts of the text is due to my wife, Mrs. Mildred Hoge Richards. My colleagues of the Department of Zoology of the University of Oklahoma, Doctor A. O. Weese and Doctor A. I. Ortenburger have assisted with advice and have read portions of the text. Professor J. F. Paxton of the Department of Greek has aided me with questions of terminology. And finally my assistants Mrs. Celeste Whaley Taft and Miss Kara J. Fullerton have given the most painstaking attention to a multitude of details. To all of these I am deeply grateful.

A. R.

PART ONE

GENERAL EMBRYOLOGY

CHAPTER I

HISTORICAL DEVELOPMENT OF EMBRYOLOGY

The science of embryology is scarcely a century old, dating from the work of Pander (1817) and von Baer (1827) whose investigations into developmental phenomena were more accurate than those of the earlier students and who were the first to give systematic form to the subject; but interest in and curiosity as to the events of the pre-natal period in animals or of the life and growth of an embryo, especially of the chick before hatching, goes back to the ancients, as evidenced by the contributions by Aristotle and Galen among the Greeks. Aristotle was the author of three works of great zoological importance, in one of which, "De Generatione Animalium," are given the results of his observations on the reproduction of various forms of animals as well as a description of the progressive stages in the development of an incubated egg. These observations were made directly upon the egg without the aid of lenses, and of course were very incomplete. The advance of embryological knowledge waited for the application of the lens to the study of life forms and processes which began some four hundred years ago. With the invention of the microscope the subject won fresh impetus until it became one of the major biological sciences when supported by exact cell studies in the last century.

Following Locy,* we may divide the progress during this time into periods as follows: (1) the period of Harvey and Malpighi; (2) the period of Wolff; (3) the period of von Baer; (4) the period from von Baer to Balfour; (5) the period of Balfour and comparative embryology; (6) the period of experimental embryology.

1. Harvey published in 1651 a treatise on embryology ("Exercitationes de Generatione Animalium") in which are recorded observations on the development of the chick and some facts of mammalian embryology. He also discussed the nature of development and deduced from observations the important principles that all animals come from ova and that the development is a new formation, not mere growth or enlargement. His study was made with simple lenses which did not enable him to trace the genesis of the embryo from its earliest stages. Malpighi, who was more of a microscopist than Harvey, outranks him as an

* Only the first five periods are recognized by Locy.

embryologist. His work on the development of the chick, published in 1672, shows Harvey's influence, but by means of excellent descriptions and many sketches carries the account of the development back to the close of the first day of incubation.

2. A century later than Harvey came the work of Caspar Friedrich Wolff, whose "Theoria Generationis" was first published in 1759. At that time embryology was dominated by the conception of preforma-

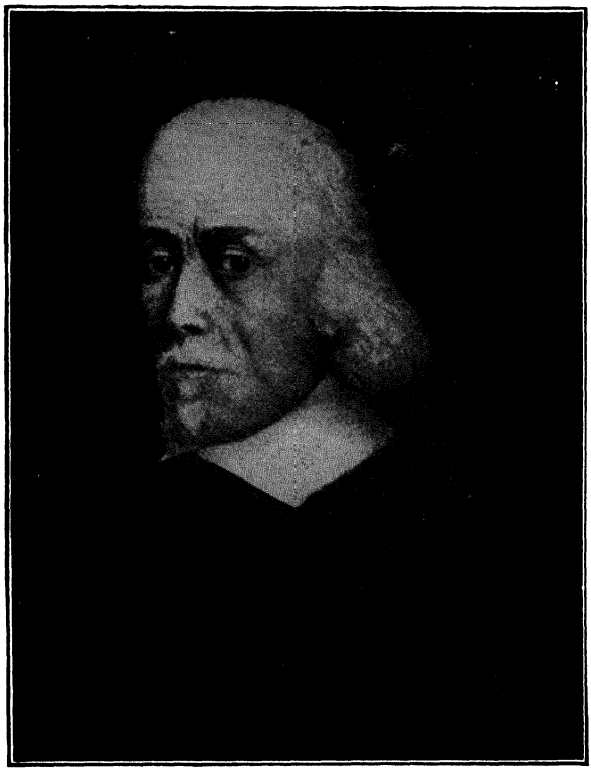


FIG. 1. William Harvey. (From Locy.)

tion which had gradually grown up around the observations of Malpighi, Leeuwenhoek, Boerhaave, and others. Although the point of view maintained by Wolff, that of epigenesis, had also been that of Harvey and Aristotle, he is to be regarded as the first active proponent of that doctrine. He was opposed by the great physiologist, Haller, and by Bonnet, a very prolific writer on the subject. Led by these men, the preformation-epigenesis controversy held the attention of embryologists to the close of the eighteenth century.

3. The period of von Baer really begins with the observations of his friend, Pander, first published in 1817. Pander established the presence of three layers in the early embryo from which the later organs are developed. Obviously this is a conclusive argument against the old preformation views. Von Baer, however, is responsible for the germ-

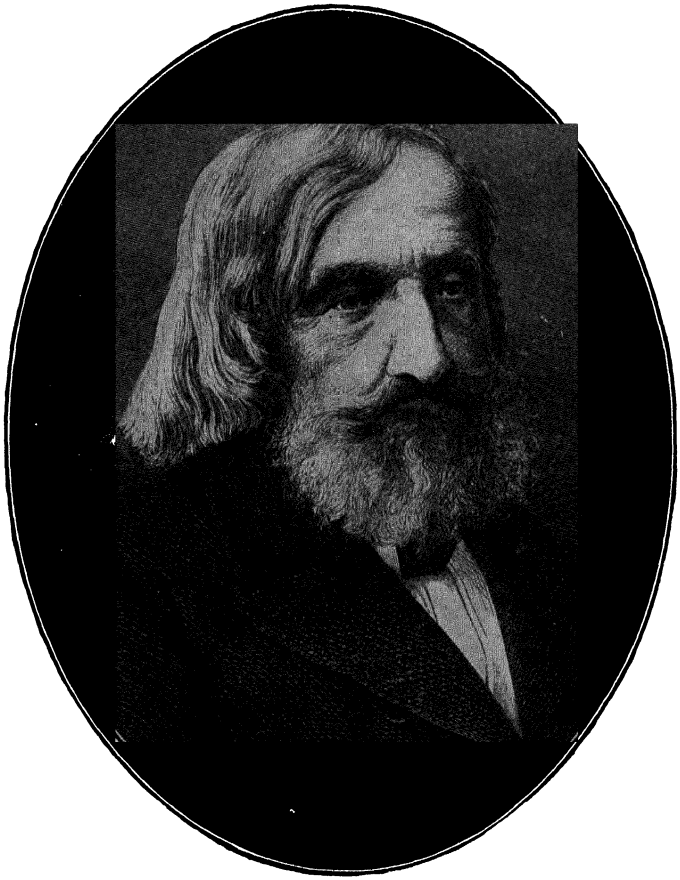


FIG. 2. Karl Ernst von Baer (From Locy.)

layer doctrine as a generalization, and for the exposition of the manner in which the layers give rise to the later organs of the embryo. He also discovered the egg in the ovary of mammals and of man and pointed out the similarity in the mode of origin of these animals to those lower in the scale. He was the first to recognize the notochord as occurring in all vertebrate animals and to point out the value of comparative embryology for anatomy and zoology. With von Baer the modern period of

embryology really begins. Von Baer is often spoken of as the originator of the recapitulation doctrine, and he did hint at it, but the formulation is due to Fritz Müller in 1863. Von Baer called attention to the greater similarity that exists between embryos of related groups than between adults. The younger the embryos the more alike are they. The recapitulation theory will be considered on a later page.

4. The period following von Baer is one of rich biological achievement. The discovery of the cell, nuclei, mitosis, the publication of the evolution doctrine, the birth of histology and pathology, all wrought changes in the aspects of biology that necessarily had their influence on embry-

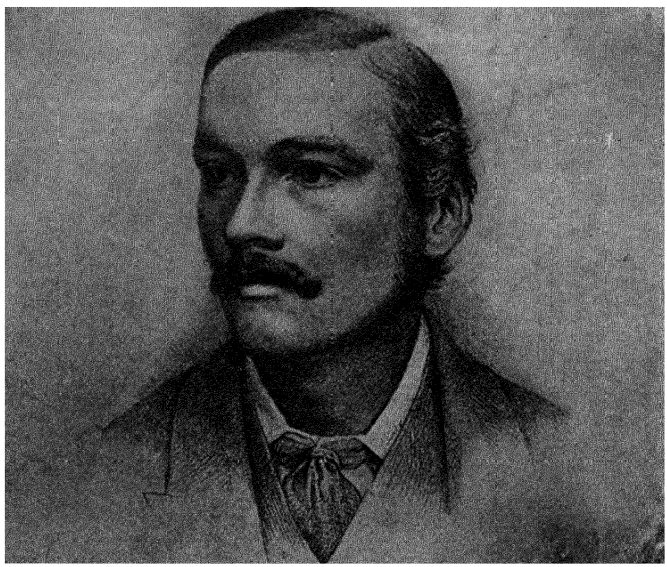


FIG. 3.—F. M. Balfour. (From Locy.)

ology. The facts of development were harmonized by the conception of evolution, and both comparative anatomy and paleontology threw light on the reasons for some of the obscure embryological phenomena discovered. This period saw established the relation of cells, tissues, and organs in the embryo, recognition of egg and sperm as cells, the process of fertilization, many details in the development of both vertebrates and invertebrates, and the beginnings of the doctrine of germinal continuity.

5. The period of Balfour was one of exact morphological research. Balfour was a young English embryologist (1851–1882) whose "Monograph on the Development of Elasmobranch Fishes" (1878), and whose "Treatise on Comparative Embryology" (1880–1881), won the highest

recognition. With improved microscopes and technique vast numbers of investigations have been undertaken since his time and the literature of the science greatly enriched. The newer science of cytology is a direct offshoot of the embryology of this period due to the pushing farther and farther back of the questions on the origin of germ cells and the fertilization and early development of the egg. One of the most notable advances of this period lay in the field of cell lineage. The investigations of the eighties and nineties established for the eggs of many animals a complete history from the one-celled egg to the larval form with definite fundaments of organs which later by specialization and elaboration make up the body of the adult. Thus is the organized character of the young embryo made clear even before the germ-layer stage. Thus also we know that in the organization of the egg there is a predetermination of the adult characters, a point of view which corresponds closely to neither of the old ideas of preformation and epigenesis.

6. While much of a morphological character remains to be learned by embryologists of the future, it is not to be supposed that the nature of the organic mechanism could be investigated only from that standpoint. With the increase of exact knowledge of the structure of the organism, we demand information as to its method of operation. To the developing animal must be applied not only the laws of physiology, with a correspondingly better understanding of the origin and development of these functions, but also the laws of physics and chemistry, and particularly physical chemistry must be appealed to. The study of development in the light of these laws is the dominant note of the embryology of the present day, and as an experimental science it is making remarkable strides forward. The present is the period of experimental embryology, although of course there are many problems of morphological character which are still to be solved.

EVENTS OF IMPORTANCE IN THE HISTORY OF EMBRYOLOGY

ANCIENT TIMES. Much information and many observations known to have been accumulated, but not recorded as a body of knowledge. Systems of medicine were developed both in Greece and in Egypt in Pre-Aristotelean times.

4TH CENTURY, B.C. Aristotle (384–322 B.C.) made first system of classification of animals and plants, published first accounts which are extant of natural history, anatomy, and embryology. Wrote "De Generatione Animalium."

Dissection of human body legalized in Alexandria.

2ND CENTURY, A.D. Galen compiled and brought up to date knowledge of anatomy and physiology, but without aid of human dissection.

16TH CENTURY. Vesalius (1514–1564) reformed teaching of anatomy, intro-

ducing dissection and replacing appeal to ancient authorities. "Structure of Human Body" published 1543.

1625. Fabricius' (1537–1619) treatise on development of chick published.

17TH CENTURY. Harvey (1578–1667) instituted experimental study of living animals. Announced discovery of circulation of blood in 1616; published "De Motu Cordis et Sanguinis" in 1628. Published in 1651 "De Generatione Animalium," an embryological treatise.

Invention of compound microscope had occurred by early years of seventeenth century and it was applied to biological uses by middle of century.

1645. Severinus (1580–1656) published "Zootomia Democritae" giving anatomical descriptions of comparative type of some vertebrates, and some developmental stages.

1665. Hooke (1635–1703) published "Micrographia."

1666. Redi experimentally disproved spontaneous generation of life among insects.

1669. Malpighi (1628–1694) published "Anatomy of Silkworm."

1672. Malpighi published two works on embryology: "De Formatione Pulli in Ovo," and "De Ovo Incubato."

Swammerdam (1637–1680) studied development of butterfly and by analogy to pupa thought earlier organs were preformed in egg (not published until 1737).

1675. Leeuwenhoek discovered spermatozoa (also attributed to Hamm, a medical student). Leeuwenhoek accordingly became a strong preformationist, adhering to the school of the "spermists."

18TH CENTURY. 1713. Leeuwenhoek discovered parthenogenesis in plants.

1735. Linnaeus (1707–1778) published first edition of "Systema Naturae." In 1753 he introduced the binomial system of nomenclature in "Species Plantarum," and in 10th edition of "Systema Naturae" in 1758.

1759. Wolff (1733–1794) published "Theoria Generationis" in defence of position of epigenesis.

1768. He published "De Formatione Intestinorum," the greatest masterpiece of embryology before von Baer.

1799. Cuvier (1769–1832) founded comparative anatomy.

19TH CENTURY. 1800. Bichat (1771–1801) founded histology.

1812. Cuvier founded vertebrate paleontology.

1817. Pander (1794–1865) recognized three primary body layers in chick.

1824. Prevost and Dumas saw segmentation in animal egg.

1828. Von Baer (1792–1876) published "Entwicklungsgeschichte der Tiere." Founded embryology as a science. Established the doctrine of germ layers. Made embryology comparative. Proposed a theory of recapitulation.

1831. Robert Brown (1773–1858) discovered the nucleus.

1835. Dujardin (1801–1862) discovered living matter, sarcode, in lower animals.

1838. Schwann (1810–1882) with Schleiden (1804–1881) founded cell theory.
1841. Remak (1815–1865) figured cell division (amitosis). His work and that of Kölliker laid foundations for a theory of cell division.
1846. Von Mohl (1805–1872) observed protoplasm in plants and named it.
1851. Newport observed entrance of sperm of frog into egg.
1856. Von Siebold (1804–1885) described parthenogenesis.
1858. Virchow (1821–1903) published "Cellular Pathology." *Omnis cellula e cellula.*
1859. Darwin (1809–1882) published "Origin of Species."
1861. Köliker (1817–1905) published general treatise on embryology. Studied segmentation of egg.
1861. Max Schultze (1825–1874) formulated protoplasm doctrine.
1863. Müller (1821–1897) propounded recapitulation doctrine.
1865. Sperms recognized as cells.
1866. Mendel (1822–1884) published studies on inheritance.
1866. Kowalevsky (1840–1901) showed that all animals pass through gastrula stage.
1862–1869. Pasteur (1822–1895) and Tyndall (1820–1893) disproved spontaneous generation.
1868. Use of stains in study of cells and protoplasm introduced.
1870. His (1831–1904) introduced use of microtome.
1873. Anton Dohrn (1840–1909) founded the Naples Zoological Station.
1874. Haeckel (1834–1919) proposed gastrea theory.
1874. Balfour and Foster published "Elements of Embryology."
1875. Flemming (1843–1905) discovered centrosome.
1875. Strasburger (1844–1912) first figured adequately chromosomes and cell division.
1878. Whitman (1842–1910) published "Embryology of Clepsine," which with Mark's (1847–) paper on "Maturation, Fecundation, and Segmentation of Limax" began the studies which became known as cell lineage in the next two decades.
1879. Flemming distinguished between direct and indirect types of cell division.
1880–1881. Balfour (1851–1882) published "Comparative Embryology."
1882. Flemming discovered longitudinal split of chromosomes.
1883. Roux (1850–1924) began studies of experimental embryology using frog's egg.
1891. Weismann (1834–1914) published theory of germ plasm.

Since 1890 the advances of embryology have been so numerous and so many investigators have been concerned that it is impossible now to choose between them as to historical significance.

Bibliographic Note

Among the more important accounts of the subjects contained in this chapter are the following: Locy, Osborne, Foster, Wilson, Gerould, Hertwig, and Nordenskiöld. These works are cited in full in the bibliography on page 406.

CHAPTER II

THE GERM-CELL CYCLE

The ontogeny of an organism includes its entire cycle of development from its earliest beginnings to old age and death. Embryology includes the first part of this cycle. Broadly considered, it may be held to include the development of the germ cells (gametogenesis) in their preparation for fertilization and cleavage, and most text-books treat of these matters. It is our purpose, however, in the present study to give but brief treatment to this phase of the subject, for general introductory courses in zoology commonly include a brief outline of gametogenesis, and a more thorough study deserves more treatment than can be given in a course in embryology.

Embryonic development in its narrower sense may be said to begin with fertilization of the egg and to consist of four periods. These are: first, cleavage; second, formation of germ layers; third, period of organ development; fourth, period of histological differentiation. It is to be noted that in many animals these four periods are not sharply separated from each other. The principles which are of importance from a comparative standpoint are chiefly illustrated by the first three of these periods.

The beginning of the life cycle of every organism is very closely related to the development of the parent, in that the cells from which the new organism comes are early set aside and from then on are to be distinguished from the other cells and organs of the body. Strictly speaking, the embryology of an animal would require us to trace the germ cells from their very first appearance on up through the stages of their development and to trace the formation of the matured sperm and ovum as well as the fertilization, cleavage, germ-layer formation and the subsequent stages, if we were to give a complete account of development. But it has come to be the practice to begin the embryological account with fertilization and cleavage, and we shall therefore deal with the germ-cell history only briefly, leaving the cytological details for other more exhaustive treatments.

Our knowledge of this development is a matter of the last half century, and so voluminous has the accumulated information become on the problems connected with the cell that it now constitutes an entirely

separate division of zoological science, namely cytology. The foundations of this science were laid in the last two decades of the nineteenth century by investigators who sought information concerning earlier and earlier stages of germ-cell development. These studies received a great impetus from the conception of the germ plasm which was published during the early part of the period by Weismann. The organism was thought of as consisting of two more or less opposing portions, a germ plasm (which functions as a hereditary vehicle and is passed on to the next generation as it gives rise to the new individual) and soma or somatoplasm (which is the remainder of the organism's body and is concerned with its individual well-being). It was thought by the earlier students that certain cells were thus the germ plasm, that is, the germ cells as contrasted with the body cells, while others had no part in the reproductive activities of the organism except in so far as they were necessary for nourishment and support.

This conception has been largely responsible for the attempts, now of many years' standing, to trace the origin of the germ cells back to the early blastomeres, that is, earlier than the period in which they can be recognized as part of the reproductive organ, the gonad. These attempts were productive of successful results in quite a number of cases in both vertebrates and invertebrates where it was found that the antecedents of the primordial germ cells could be recognized even in early cleavage divisions. In other cases, however, it has not been found possible to trace the history of the primordial germ cells back of their first appearance in the gonads. The origin of the primordial germ cells is an embryological problem of considerable importance in itself aside from its relation to the germ-plasm doctrine, and as such it is discussed in detail in a later chapter in this book. (See Part Two, Chapter II.)

Partly as a result of the inconclusive data from the study of the origin of the primordial germ cells in the animal kingdom as a whole, but more especially as a result of the work of recent years on heredity in which the importance of the chromosome has been made manifest, a new conception of the germ plasm has now showed itself to be more acceptable to many students of these problems than that of Weismann. According to this new view, each cell contains material which corresponds to both the germ plasm and soma, the chromatin representing the former and the cytoplasm the latter. This interpretation explains many facts which had previously proven difficult to understand and enables us to regard the origin of germ cells as a problem of embryology rather than as one whose chief interest is in relation to the transmission of a "germ plasm."

1. Cell Division in Gametogenesis

Regardless of their source or of the time of their first appearance, there are present in a developing gonad certain cells which are known as primordial germ cells. They remain in a quiescent stage during the early development of the organism, beginning their active development only when the somatic structures are well on the way to their adult condition. Somatic structures are produced by the differentiation of the cytoplasmic portions of the cells (giving rise to the familiar histological distinctions between tissues), and must obviously precede those activities which have for their end the reproduction of the organism. On the other hand, specialization of the nuclear structures involved in the function of mitotic cell division is responsible for reproduction and must wait until the proper development of the somatic structure has been attained.

a. Multiplication Period. At length the primordial germ cells begin a period of proliferation known as the multiplication period. This period varies in different forms, both as to its beginning and its duration, for it does not necessarily begin at the same stage in the life cycle, nor does it consist of the same number of cell divisions. It is said that in the grasshopper there are eight divisions in this period; thus each primordial germ cell would produce as a result 256 descendants. The cells undergoing the various divisions of the multiplication period are known as gonia; the male cells are spermatogonia and the female oögonia, alike in every fundamental respect.

b. Growth Period. At the end of the multiplication period, that is, with the formation of the primary oögonia or spermatogonia, the active proliferation of cells ceases and there follows a growth period. During this period without further division the cells increase in size and store up nutrient material, deutoplasm, to furnish them with energy for the further activities they are to undergo. In the case of the oögonia this material is the yolk, often present in large amount to furnish food for the embryo until it has become at least in part able to obtain sustenance from its surroundings. The amount and distribution of this yolk material is a very important factor in determining the type of cleavage and future development of the embryo, for cell division becomes increasingly difficult with the accumulation of inert yolk material. (This point should be kept in mind, for frequent use must be made of it in the study of cleavage types.) These cells are now primary spermatocytes or oöcytes.

c. Maturation Period. Two maturation divisions now succeed each other, usually with some degree of rapidity. They constitute the matu-

ration period during which nuclear events of the utmost importance from the standpoint of the future organism occur. In oögenesis as well

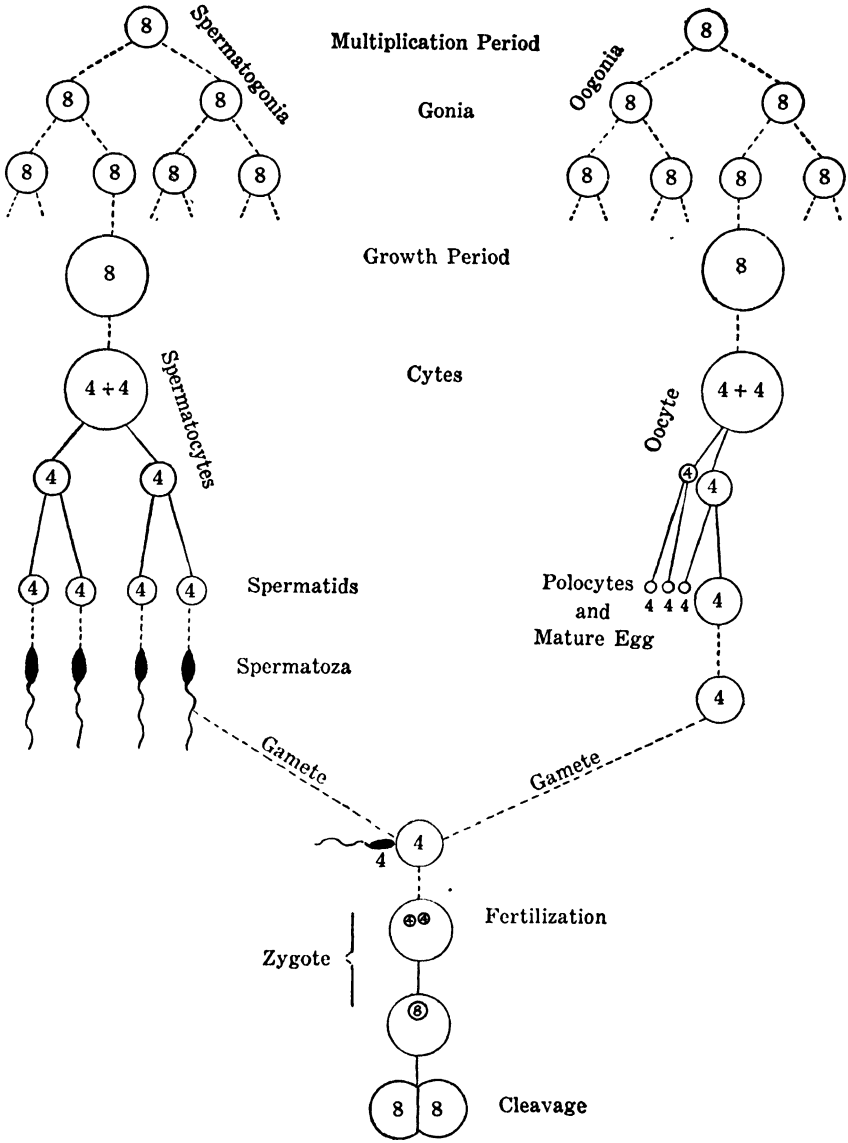


FIG. 4. Diagram to illustrate the processes of gametogenesis.

as spermatogenesis there are two divisions which are similar in all respects except one. In the maturation of male germ cells, the first

division results in two second spermatocytes which promptly divide again producing four spermatids from each primary spermatogonium. (In the grasshopper obviously there would be now 1024 descendants from each primordial germ cell.) These cells do not again divide but go through a series of changes (spermiogenesis) by which they become metamorphosed into functional spermatozoa with the characteristic structure of spermatozoa; these changes are cytoplasmic in character. In the developing female germ cells, however, maturation results in the production of one functional egg and three non-functional cells which are its equivalents from the standpoint of nuclear content and which are known as polocytes or polar bodies. This is accomplished as an immediate result of the fact that the first maturation spindle is short and occupies a position near the surface of the large oöcyte. Since the division plane cuts through the center of the spindle, a very small cell, the polocyte, is cut off from the larger "egg cell" which is now the second oöcyte. In the second maturation division both cells divide, the "egg cell" again unequally producing a matured egg and another polocyte, and the first polocyte two other polocytes. This size differentiation of the four cells which are descendants of the primary oögonia allows the normal nuclear events of maturation to go on unmodified, but concentrates the oöplasmic materials, which are necessary to the nourishment of the embryo, in one functional cell.

Gametogenesis is now completed. Sperm and egg are ready for the next step toward the production of a new organism, namely fertilization. By fertilization is meant the entrance of the sperm into the egg, but the process is very complicated and is completed only with the union of egg and sperm nuclei into a fusion or cleavage nucleus. Fertilization has two functions which are distinctly different: the initiation of cleavage or the development of the zygote, and the restoration of the chromatin material equivalent to that lost in reduction. This complicated process has been the subject of much study during the last two decades, especially from the viewpoint of experimental or physiological embryology, and many fundamental conceptions have resulted from this fascinating phase of embryological research.

2. Nuclear Events in Gametogenesis

The nuclear events during gametogenesis must also be understood, at least in outline. Their study has been the especial problem of cytology during the last quarter of a century.

a. Reduction. It may be stated as a general law that every species of organism is characterized by a definite number of chromosomes, and,

with a few exceptions which are not in contradiction to a full and adequate statement of the law, this number occurs in all the cells throughout the bodies of all the members of a particular species. This characteristic number is spoken of as the somatic or diploid number. In the history of the germ cells all the divisions of the multiplication period are found to have this characteristic number. During the maturation divisions, however, this number is halved, that is, reduction occurs, and the matured gametes have only half the number characteristic of the species. The union of the male and female gametes in fertilization effects a return to the somatic number. The process of reduction is often called meiosis and the divisions meiotic divisions. Commonly, although not at all of necessity, the first division is the reductional or heterotypic as distinguished from the somatic or homeotypic divisions. If the first is heterotypic the second is homeotypic, and conversely.

In those cases in which the *differences between reductional* and *somatic mitoses* are most clearly recognizable (that is, in those cases in which tetrads are formed), we may say that the explanation lies in two outstanding facts, namely, the peculiar prophase of the first division with its synaptic pairing of homologous chromosomes, and the entire absence of a prophase to the second division. The case of *Ascaris*, the roundworm, is one of the best known, and it may serve as an illustration of these processes. The somatic number of chromosomes in the common *Ascaris* is four. These are not looked upon as four separate and unrelated individual chromosomes, however, but as two pairs, for it has been shown in some animals that one member of each pair came from the male parent and one from the female in the preceding fertilization, and it is very probable that this is always the case. The members of a pair are spoken of as homologous chromosomes. Thus each individual derived from a fertilized egg contains in each of its cells two full sets of homologous chromosomes. This fact is to be contrasted with the well-substantiated observation that the germ cells contain but a single set of chromosomes owing to the process of reduction. It will be recalled that a typical mitotic division, whether in a germ cell or a somatic cell, involves in the prophase a condition which is really the climax of the entire set of mitotic events, namely the splitting of the spireme thread which is to condense to form the metaphase chromosomes. For this reason the metaphase chromosome may be looked upon as consisting of two halves, or chromatids, even if this condition sometimes is not easily seen under the microscope. That is to say, the ordinary chromosome is a bivalent one, a dyad. With these facts in mind we are now ready to inquire into the nature of synapsis.

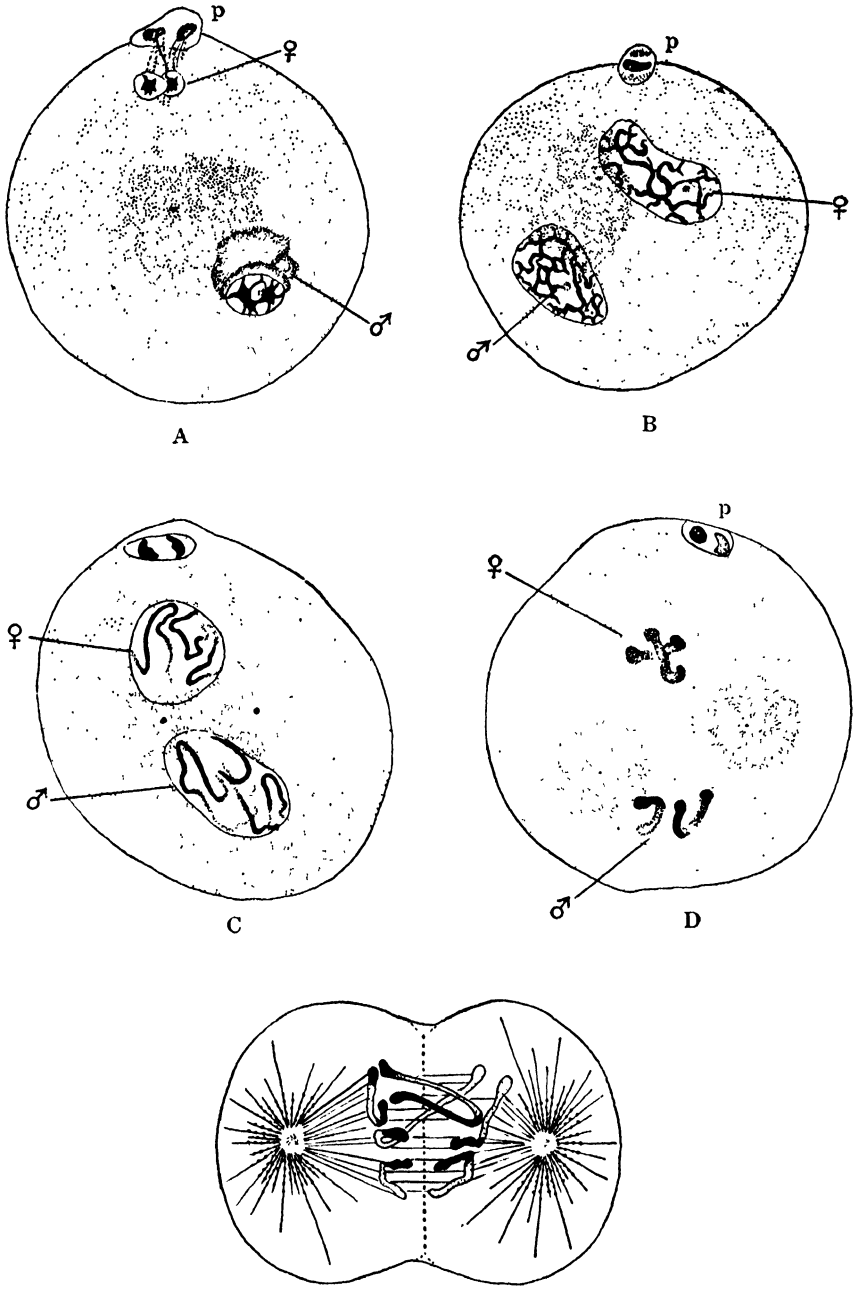


FIG. 5. Fertilization and cleavage of *Ascaris*. (Redrawn from Kellicott after Boveri.)

A, spermatozoon entering the egg as the second maturation division is taking place, B, pronuclei going into prophase and division of sphere and centrosome beginning; C, D, showing further advance toward E, the first cleavage division.

b. Synapsis. By synapsis is meant the union in the first maturation prophase of the two homologous members of each chromosome pair to form a single chromosome, although one which has a valence of four, as

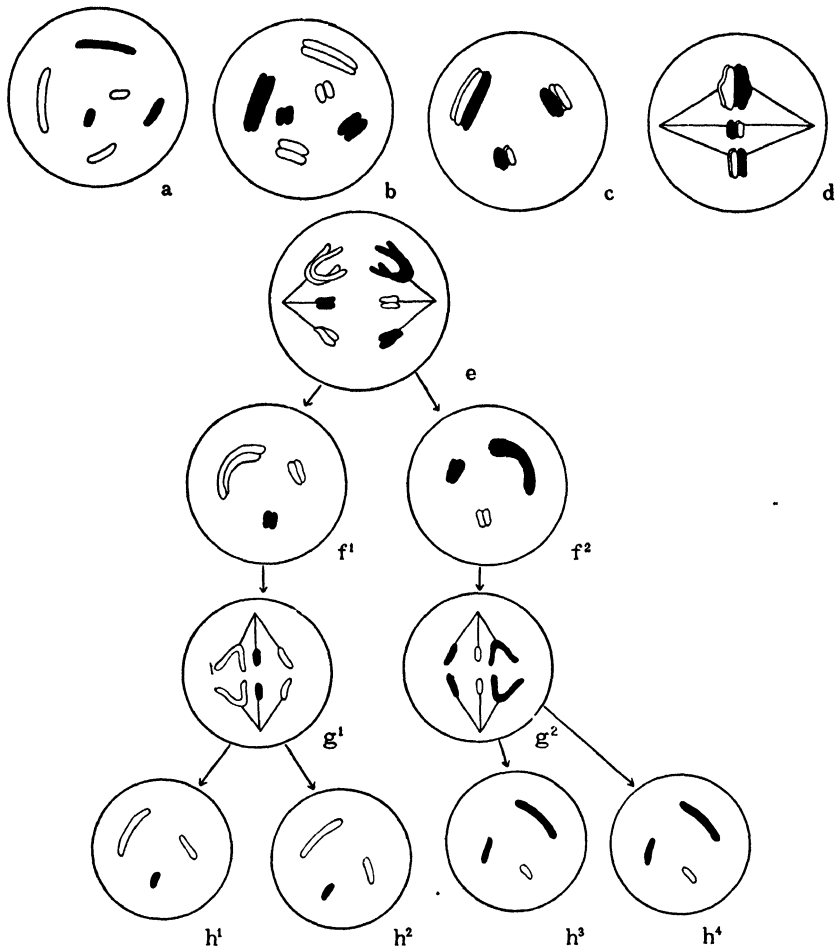


FIG. 6. Diagram of tetrad formation and subsequent reduction. (Suggested by diagrams from Sharp.)

a, chromosome conditions in spermatogonia and at beginning of first maturation prophase; b, synapsis in prophase of first maturation prophase (first spermatocyte), c, tetrads ready for the division; d, metaphase of first maturation division; e, anaphase of d; f, second spermatocytes produced by the division of e; g, division of second spermatocytes; h, resulting distribution of chromosomes as they go into the four spermatozoa.

contrasted with the preceding bivalent condition. Since two somatic chromosomes, each consisting of two chromatids, are involved in the formation of this new chromosome, it is called a tetrad. It is evident

that by synapsis a reduction in the number of chromosomes, but not in the amount of chromatin, has been effected. It is the function of the two maturation divisions to complete this reduction in amount by distributing the four component parts of each tetrad to the four different spermatozoa, or, in the case of oögenesis, to the egg and the three polar bodies.

As a result of synapsis it appears that there are in each first spermatocyte or oöcyte the reduced number of chromosomes in the form of tetrads, or quadrivalent chromosomes. Each tetrad consists of four chromatids, $aa'bb'$, two from each of the homologous mates, A and B. If, in the succeeding metaphases and anaphases, the bivalent chromosomes (dyads) which are the result of the division of the tetrad are each composed of two chromatids derived from the same one of the constituent synaptic mates, that is, a and a' in one chromosome and b and b' in the other, the division is said to be reductional or heterotypic. In other words, in a reducing division whole chromosomes are separated. In this case the second division would be an equational division for the equivalent half chromosomes would necessarily be separated as in any ordinary mitosis. That is, the bivalent aa' is now divided into a and a' and bb' into b and b', and the maturation with the consequent distribution of the four parts of the tetrad to the four germ cells is completed. If, on the other hand, the bivalent chromosomes resulting from the division of the tetrads consist of half of each synaptic mate, that is a and b in one and a' and b' (or of course a and b' or a' and b may be linked together), then the first division is equational. The second division would then be reductional and as in the previous case would result in the distribution of the four component parts of the tetrad to the four matured germ cells.

In these cases in which tetrads are formed there is no resting stage preceding the second maturation division, but the dyads without a reorganization arrange themselves on the second spindle. There is a large number of cases, however, in which actual tetrads are not formed owing to the failure of the usual split of the spireme to appear during the early stages of the first division. In this case the first division is always reductional; there is a pause between the first and second divisions during which the chromosomes undergo some reorganization; and the splitting which was delayed takes place. It is evident, therefore, that precisely the same result is achieved in the two cases, whether or not tetrad formation occurs. That is, there are formed from each spermatocyte or oöcyte of the first order four cells, each with half the number of chromosomes characteristic of the species. These four cells are mature spermatozoa or one functional egg and three polocytes or polar bodies.

3. Fertilization

It has already been pointed out that there are two totally different functions served by fertilization, namely, the one concerned with the hereditary mechanism in which the diploid number of chromosomes is restored, and the other which sets into operation those processes leading to cleavage and the further development of the embryo. The first of these functions is accomplished in the conjoining of the male and female pronuclei. The sperm with its reduced number of chromosomes enters the egg, and its subsequent union with the female pronucleus which also contained the reduced number produces in the zygotic nucleus so formed the full number of chromosomes. The second function is accomplished in a very complicated series of processes of physico-chemical nature. Much of the research in experimental embryology of the last two decades has dealt with the physico-chemical aspects of the activation of the egg,

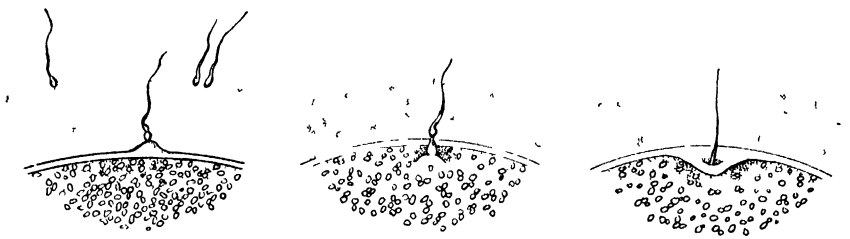


FIG. 7 Entrance of spermatozoon into egg of the starfish *Asterias glacialis*. (Redrawn from Conklin after Fol.)

and results have been obtained which have given real insight into the fundamental nature of the living organism.

The morphological aspect of fertilization is well known from the studies of Lillie on *Nereis*, of Wilson on *Toxopneustes*, of Kostanecki and of Wierzejski on *Physa*, of Boveri on *Ascaris*, and of many others. In most animals the entire sperm enters the egg, but there are many others, as in the sea-urchin and the starfish, in which the tail or at least most of it remains outside. The important elements of the sperm that enter the egg and have a function in fertilization are the head which is equivalent to the nucleus, the central body or the structures derived from it, the acrosome which is derived from the Golgi apparatus of the spermatid, and some chondriosome material. Of course the most important of these is the nucleus, but historically the central body also has been the subject of much discussion in connection with fertilization. The sperm enters rather largely through the activity of the cortical layer of the egg and by a fairly definite path makes its way toward the egg pronucleus. Immediately upon entrance, however, it rotates so that the middle piece

precedes in the advance to the female pronuc'eus. The sperm aster appears from this region, and it is the usual condition that it should become the aster of the cleaving egg. It divides and forms the spindle

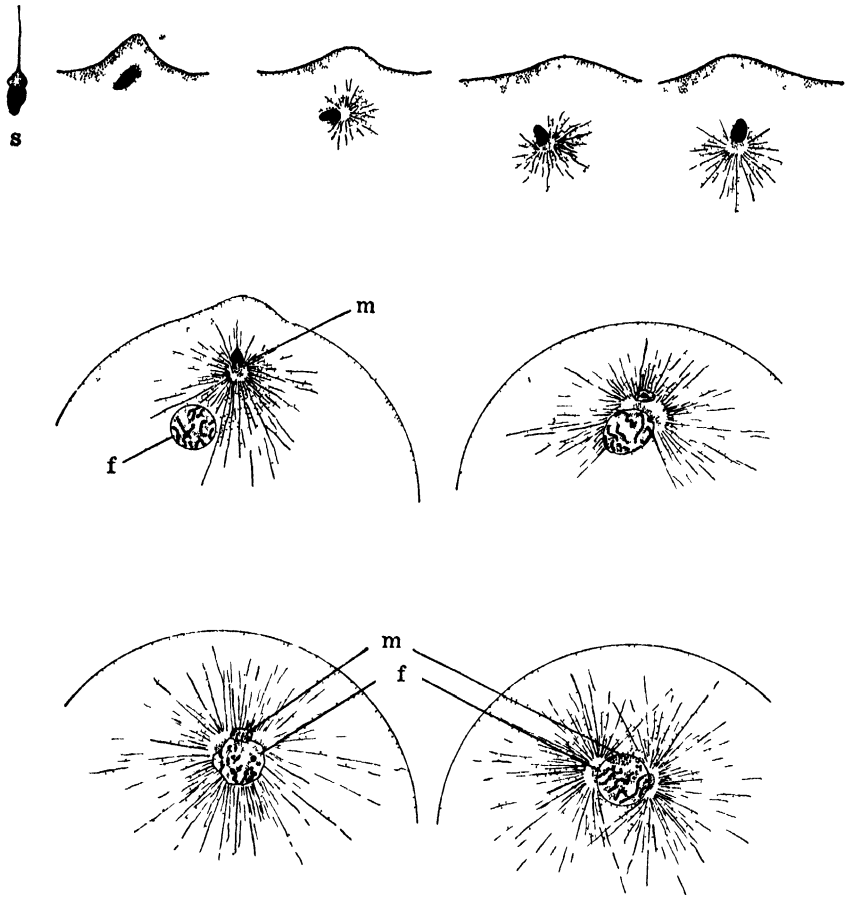


FIG 8. Steps in fertilization of the sea urchin, *Toxopneustes* (Redrawn from Conklin after Wilson)

s, mature spermatozoon, m, transformation of spermatozoon into male pronucleus, f, female pronucleus

between the two approaching pronuclei. It is not the rule that the egg aster contributes to the formation of a cleavage spindle.

The entrance of the sperm occurs at different times with respect to the extrusion of the polar bodies in different eggs. At one extreme is the condition as found in the sea-urchin and in the coelenterates in which both maturation divisions are completed before the sperm can

enter. At the other extreme is the *Ascaris* type in which the sperm enters the egg before either polar body has been extruded; in some cases it may be before the germinal vesicle has been broken down, in others while the first maturation mitosis is in the metaphase stage. Nematodes, flatworms, molluscs, some annelids and crustaceans belong to the *Ascaris* type. There are of course intermediate stages between these two extremes.

The series of processes involved in fertilization may be said to be completed when the two pronuclei have closely approached each other and a cleavage spindle is formed between them. There is seldom an actual fusion of the pronuclei as such; rather they lie side by side upon the developing first cleavage spindle, and at the end of this first division the chromosomes of the components are intermingled and are no longer to be distinguished as from the two parents.

We are thus back at the stage from which we started and cleavage is the next step in ontogeny.

Bibliographic Note

Among the more important accounts of the subjects contained in this chapter are the following: Wilson, Sharp, Kellicott, Cowdry. These works are cited in full in the bibliography on page 406.

CHAPTER III

EGG AND CLEAVAGE TYPES

Of the four stages of embryonic development, cleavage, germ-layer formation, period of development of organs, and of histological differentiation, the period of cleavage is the most constantly recognizable and the most distinct. It consists of a succession of cell divisions taking place in a very regular manner and having for their purpose the distribution of the egg protoplasm into a great many single cells. Every organism above the protozoa consists of many cells, and every one starts as a single-celled egg. The first problem of development for the fertilized egg, therefore, is that of the regular distribution of its substance in such a manner that the organs of the later stages may normally arise from the appropriate material. Since divisions follow one another with considerable rapidity during cleavage the daughter cells never have time to grow to the size of the mother cell. The cleavage cells or blastomeres normally divide into two cells each, thus setting up a normal rhythm of 2, 4, 8, 16, 32 cells, etc. Even in the simplest cases, however, this rhythm cannot long persist, for with the beginning of differentiation processes certain cells lag behind in the divisions and irregularities thus come in.

Cleavage usually results in spherical blastomeres which are in contact only at the point where the cytoplasmic division last cut through. Owing to the tension and other physical factors, this condition at once passes and the blastomeres come to lie closely pressed together with only a furrow remaining to show where the separation really is. The furrow naturally falls perpendicular to the spindle axis of the last division. Often the plane of the first furrow corresponds to the median plane of the future embryo.

This last fact, along with others which indicate that there are, at least on many eggs, structural features that link up the uncleaved egg with the future organs developing from its various special regions, raises the question of the promorphology of the ovum. This matter is correlated with the preformation discussion to which reference is made elsewhere. It is often possible to trace the origin of organs or cell groups to very early blastomeres or even to the single-cell stage, a study which is known as cell lineage, and there are often in the egg marks of structural

differences by which regional differentiation is indicated. The presence, for example, of yolk at the vegetative pole of the egg distinguishes the ectodermic and the endodermic portions at once. In ascidian eggs there are differences in the consistency of the protoplasm and in the pigments

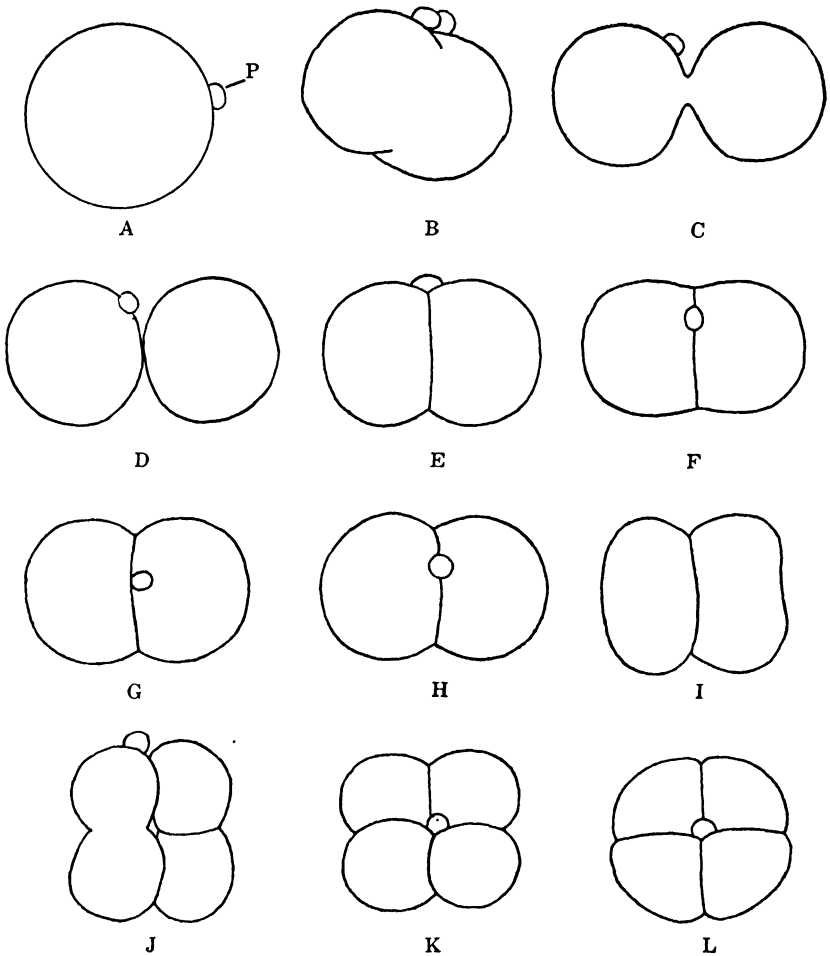


FIG. 9. The progress of the first cleavages of the snail, *Planorbis*, showing the manner in which the furrows cut through forming two spherical blastomeres, which then draw together, substituting a plane of contact for the point of contact.

present, so that with the entrance of the sperm there can be distinguished four regions, namely, a clear protoplasmic cap at the animal pole, a darker area at the vegetative, and in between two crescents, one yellow and one light gray. There are thus localized certain organ-forming sub-

stances in four different regions, and these will give rise to very different parts of the embryo. For these reasons we say that there exists in the eggs of many species a predelineation of the structures which are to be developed. This is of course contrary to the assertion sometimes made that the blastomere is similar to the entire egg, is a small picture of the whole. This statement is true only in the sense that the egg commonly shows little evidence of histological differentiation, and the same is at least superficially true of the early blastomeres. That the resemblance is only superficial, however, is also borne out by the nuclei of certain forms. In *Ascaris* one of the two nuclei of the 2-cell stage undergoes a process known as chromatin diminution, becomes smaller, and stains more lightly than the other; this is indicative of the separation of the germ line from the soma.

As cleavage progresses the tension which keeps the entire protoplasmic mass nearly spherical imparts to the individual cells a tendency to approach each other and press together. Largely owing to this tendency the blastomeres arrange themselves at the periphery of the mass and leave a cavity at the center, known variously as a cleavage cavity, segmentation cavity, blastocoele, or primary body cavity. It is filled with fluid or with a gelatinous secretion. In eggs containing large amounts of yolk, however, the cavity is crowded out of the center and often greatly restricted by this deutoplasmic accumulation. The cellular surface of the embryo at this stage is known as a blastoderm, whether it involves the entire surface or is limited to a small area of it.

Cleavage may be said to begin with the formation of the first segmentation spindle. This is a more accurate statement than that it follows fertilization, for in the case of eggs which develop parthenogenetically there is of course no fertilization. It is more difficult to say when it closes, for any limit which we may set has only relative value. Although some cases do not conform, it may be said that the end of cleavage is reached with the establishment of a definite size relation between nucleus and cytoplasm (the nucleo-cytoplasmic ratio), the particular ratio being that which is normal for that particular stage of that particular species. It is of course to be noted that where differentiations due to yolk, etc., are present there cannot be a single nucleo-cytoplasmic ratio for an entire embryo. It is perhaps better to say that cleavage is ended when a typical blastula is formed consisting of the first primary germ layer with the cells arranged in an epithelial layer.

It must be borne in mind by the analytical student of developmental phenomena that three distinct kinds of processes are involved in the transformation of the egg into a larva. These are cell division, growth, and differentiation—processes which are not only distinct, but to a certain extent antagonistic, the last two never taking place at the same

time with the first, and only to a minor extent together. (Differentiation is here used in the sense of histological differentiation, for of course in another sense its processes take place by means of cell divisions.) We may regard the actual growth processes, that is, increase in bulk, as anabolic, and obviously cell division is largely katabolic, from which it follows that the two cannot take place as dominant factors in the same cell at one time. Their independence in this relation does not mean, however, that they are not dependent on each other, for if they do not maintain their proper balance further development on the part of the organism as a whole cannot occur.

Before discussing the types of cleavage it should be pointed out that development is possible in some cases without regular cleavage. This really amounts to a delay in cell division as contrasted with nuclear division. Several nuclear divisions take place in succession without the cleavage of the cell body; at a later stage the cell areas are cut off around the nuclei all at one time. Examples of such development are found in the alcyonarian *Clavularia* (Kowalevsky and Marion), *Renilla* (Wilson), *Alcyonium* (Hickson), *Tealia* (Appellöf), and *Cucumaria glacialis* (Mortensen).

It should also be made clear that a classification of cleavage types has nothing of the evolutionary significance which the systematic grouping of animals has, nor can it be made a basis for systematic classification. Although cleavage types are in general stable and are coextensive with certain animal groups, yet there are very notable departures in this regard. It will be seen by an inspection of the table below that the cephalopods have a different type of cleavage from other molluscs, that the scorpions cleave differently from others of their class, and that many other exceptions also exist. Indeed, there are instances in which nearly related species have different methods of cleavage, and the extreme modification of this sort is perhaps seen where two types of cleavage occur in the very same species. An example of this last case is seen in *Polyphemus oculus*, whose summer eggs undergo regular holoblastic cleavage, whereas in the winter eggs a type of meroblastic cleavage occurs which strongly suggests conditions found in the superficial cleavage of some insect eggs. These are, of course, extreme cases which merely serve to show how variable cleavage forms may be, doubtless as adaptive responses to the conditions of development, without having any special phylogenetic or taxonomic significance.

1. Classification of Egg Types

On the basis of their structure, particularly as modified by the amount of yolk present, Balfour divided eggs into three groups, alecithal, telolecithal, and centrolecithal.

Alecithal, or better *homolecithal*, eggs (also called *isolecithal*) are those in which there is relatively a small amount of deutoplasmic material uniformly distributed throughout the egg. Such eggs cleave regularly into equal-sized blastomeres. Echinoderm eggs are examples.

Telolecithal eggs have a clear axial structure, the protoplasm at the animal pole containing very little yolk, while the vegetative half of the egg is rich in yolk. The frog egg may be taken as an example. The blastomeres at the vegetal pole, therefore, are distinctly larger by reason of their large yolk content, and the cleavage cavity is pushed toward the animal pole. Such eggs necessarily have unequal cleavage, if the furrows are able to cleave the yolk at all.

In another type of telolecithal eggs the accumulation of yolk is so great that it cannot undergo cleavage. The protoplasm is thus limited by the yolk to a small disc at one pole of the egg while the yolk fills the rest of the space. The eggs of birds and of teleost fishes furnish excellent examples. When cleavage takes place it cuts through the disc only, and is therefore discoidal.

Telolecithal eggs are thus of two sorts: holoblastic, those in which the entire egg cleaves, although unequally; and meroblastic, in which only part of the egg mass can be divided, and that by either discoidal or superficial cleavage. The terms "partial" and "total" cleavage also apply to these types. Between these two types there are of course intermediate conditions shown by various eggs. These two extremes lead to two types of embryo formation; in the holoblastic egg the entire egg goes to form the embryo body, but in the meroblastic type the germ disc is all that is involved, and the yolk is in the form of an appendage, the yolk sac.

Centrolecithal eggs are richly yolk laden. The cleavage nucleus in a cytoplasmic area is located at the center of the egg surrounded by the yolk. A protoplasmic layer, however, covers the surface and is connected to the central mass by fine plasmic threads. Some centrolecithal eggs are so definitely oriented that the position in which they are laid indicates exactly the axes of the future embryo, but in general the primary axes are not well developed in eggs of this type, although bilaterality is to be noted. Cleavage begins by the plasma island in the center multiplying through several nuclear divisions, thus forming a syncytium. These "blastomeres" now move radially toward the periphery and unite with the plasma layer which is at once cut up into cytoplasmic areas of similar size, each supplied with a nucleus. The central yolk does not divide, or if division starts it is incomplete. This constitutes superficial cleavage, the blastoderm forming about the yolk which fills entirely the cleavage cavity (if one might use that expression).

2. Classification of Cleavage Types

Dependent upon the amount of yolk they contain, the first divisions of eggs are total or partial. Eggs having total cleavage are said to be *holoblastic*; those having partial cleavage are *meroblastic*. It is also customary to classify types upon the basis of the planes of symmetry which

Egg Structure	Cleavage	Germ Formation	Cleavage Types	Examples
Homolecithal	Equal	Holoblastic	1. Radial	Porifera Cnidaria Echinoderma
			2. Disymmetrical	Ctenophores
			3. Bilateral	Nematodes Rotifers Ascidians Amphioxus Petromyzontidae Amphibia Higher mammals
Telolecithal	Inequal		4. Spiral	Polyclads Nemerteans Annelids and molluscs (except cephalopods)
Centrolecithal	Discoidal	Meroblastic	5. Discoidal	Scorpion Cephalopods Pyrosomes Myxinoids Elasmobranchs Teleosts Gymnophionans Reptiles Birds Monotremes
	Superficial		6. Superficial	Arthropoda (except scorpions) Some other scattered forms among coelenterates

are to be observed in the dividing egg. Thus in radial cleavage there is uniform distribution around the egg axis, but in disymmetrical cleavage there are two centers of symmetry, and in bilateral cleavage a single plane only will cut the egg in equal halves. In spiral cleavage the plane of the spindle in the first divisions turns in a spiral with reference to the egg axis. These four cleavage types are holoblastic. Meroblastic eggs may be divided into superficial and discoidal. In the former case the blastodisc forms around the outside of the egg but involves practically the entire surface whereas in the latter only a small disc undergoes segmentation, owing to the extensive amount of yolk present. These relationships are shown by the accompanying table as are the animal groups to which they belong. We shall consider these types in order.

3. Classification on the Basis of Predelineation

Before considering the details of these different modes of cleavage it is desirable to mention certain qualities of eggs by which they are related to the later developing stages. With reference to the degree of predelineation shown, Conklin has recognized two types of eggs as follows:

a. Eggs with Determinative Cleavage. The best examples are the eggs of annelids, mollusces (except the cephalopods), and the ascidians. In eggs of these groups after fertilization, but before cleavage, there can be recognized in the one-cell stages regions or definite areas in the egg protoplasm, the organ-forming regions of His. These regions are distinguishable in various eggs by different means, among which are differences in yolk content, presence or absence of pigment, clear or granular condition of the cytoplasm, and the presence of very fine granules which can be demonstrated by definite staining methods. The centrifuge has also proved useful in detecting different substances in the egg which as cleavage advances become segregated into different blastomeres. (*Cf.* the work of Lyon, Lillie, Conklin, etc.) The study of the cell lineage of such an egg shows that these blastomeres are the beginnings of definite organs of the embryo. In other words the egg substances which are thus localized into regions are determined for the production of definite organs of the later stages. During the progress of the investigations which established the determinative character of certain eggs there was a great deal of discussion, and even controversy, as to whether blastomeres were totipotent, that is, interchangeable, or constituted a mosaic work, each being capable of taking only its own special place. We now understand that eggs which have determinative cleavage have an operative mechanism of great precision, and that correspondingly they have lost much of their power of regulation. Because of their content of organ-forming substances they are apparently destined for a particular fate,

and can undergo very little regulation. These statements of fact for determinative eggs do not apply to eggs of the next type.

b. Eggs with Indeterminate Cleavage. The problem of determination, after all, is a problem of differentiation. In eggs of this indeterminative type differentiation sets in only relatively late after the embryo consists of many cells. In the preceding case the marks of differentiation are recognizable from the very first. In indeterminative eggs, there are no organ-forming regions, little histological differentiation is found, and the blastomeres appear to be alike. Such eggs are simple in structure and are accompanied by high capacity for regulation. Examples are: vertebrates, some insects, most arthropods, cephalopods, most echinoderms. In them the power of regulation is much greater than in the determinative type, and there is much evidence to indicate that the fate of a blastomere is often a function of its position, or that the blastomeres are totipotent and without special "prospective significance." In Part Two the chapter on the determination problem gives the evidence in more detail.

Bibliographic Note

Among the more important accounts of the subjects contained in this chapter are the following: Korschelt and Heider, Conklin, Morgan in "Experimental Embryology," Cowdry's "General Cytology" (especially the article by Conklin on "Cellular Differentiation"). These works are cited in full in the bibliography on page 406.

CHAPTER IV

HOLOBLASTIC TYPES OF CLEAVAGE

I. RADIAL CLEAVAGE

The eggs of the Porifera, of all the coelenterates, except the ctenophores, and of the echinoderms have radial cleavage. Formerly amphioxus was given as a type of this class, but it has been shown that the older accounts were in error, and that its cleavage is really bilateral.

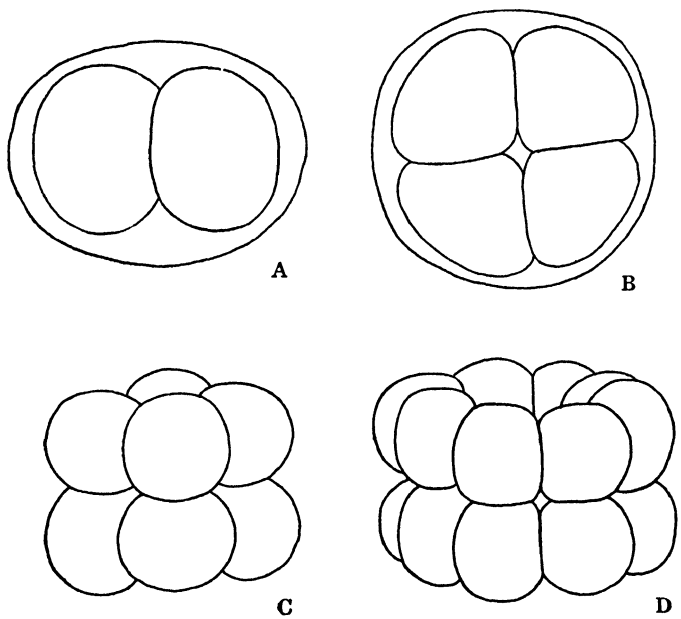


FIG. 10. The cleavage of sea cucumber *Synapta digitata*. (Redrawn from Korschelt and Heider after Selenka.)

A, 2-cell stage; B, polar view of 4-cell stage; C, D, lateral views of 8- and 16-cell stages.

Radial cleavage includes eggs of both homolecithal and telolecithal types, although of the latter of course only those which have holoblastic cleavage belong here. In the typical and simple cases such as that of *Synapta* (which as described by Selenka may be taken as most nearly corresponding to the ideal form of radial cleavage), the division planes

succeed each other in regular alternation dividing the egg first meridionally and then equatorially into equal blastomeres. It very nearly corresponds to the Sachs' principle of the alternation at right angles of successive planes of cell division. The first furrow is meridional and divides the egg equally. The second furrow is also meridional and

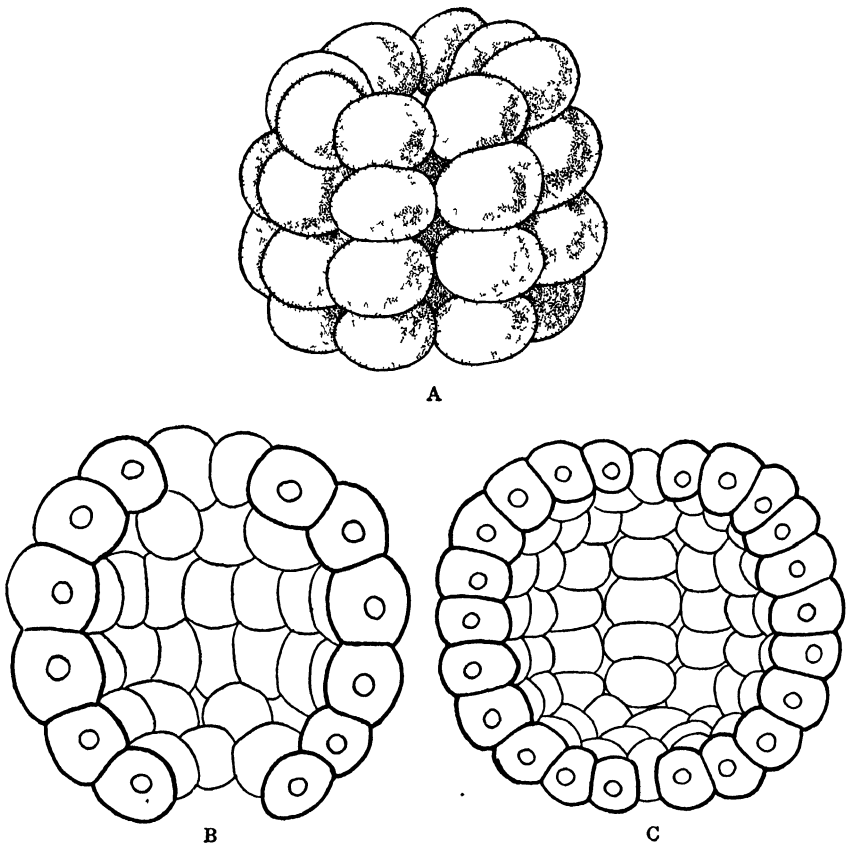


Fig. 11 Continuation of Fig. 10 32-, 64-, and 128-cell stages of *Synapta digitata*

divides each of the first two blastomeres equally. The third furrow is equatorial and the blastomeres are equal, making eight cells arranged in two rows about the two poles. By this time a tiny cavity is discoverable at the center of the mass which is to become the cleavage cavity or blastocoele. The furrows of the next cleavage are meridional but at an angle of 45 degrees to the first and second. The furrows of the fifth are again latitudinal, dividing all the blastomeres equally. Subsequently the divisions keep their regular alternation for a period, forming a blastula

in which the cells are arranged in horizontal and vertical rows. With a shift of the blastomeres about the pole by which the opening there is closed, a spherical blastula consisting of a single layer of cells (a typical coeloblastula) is formed.

The cleavage of many jellyfishes, though in general radial, undergoes many modifications due to yolk distribution, loose arrangement of cells, and to other factors.

Radial cleavage undergoes some interesting modifications in some of the sea-urchins, notably *Strongylocentrotus lividus*, as shown by Boveri (1901). In this form cleavage is unequal and differential. The egg is of a clearly determinative type, a jelly canal indicating the position in which the polar bodies will be given off. It is the place at which, in the oöcyte stage, there is uniformly distributed an orange-yellow pigment which, upon fertilization of the egg, at once rearranges itself as a band about the lower half of the egg, leaving a cap without pigment about the vegetal pole. This cap is destined to produce in subsequent cleavages small cells known as micromeres, a phenomenon which in itself is striking since in most eggs the cells at the vegetative pole are commonly larger than those of the animal half. The egg nucleus is not limited to the animal half of the egg, nor is the spermatozoon restricted to the jelly canal.

The first two cleavages are meridional and equal, resulting in four blastomeres which receive equal amounts of the three kinds of egg materials, the pigment-free animal half, the pigment zone, and the clear polar cap. With the next cleavage which is nearly equal (latitudinal) the four animal blastomeres receive but a small portion of the pigment, the remainder being in the four blastomeres of the vegetal half of the egg. Now follows a peculiar differential cleavage, the upper four cells dividing meridionally while the lower four are unequally divided latitudinally. This results in the immediate segregation of the pigment-free cap into four vegetative cells, the micromeres. The descendants of these are later to form the primary mesoderm.

In producing the next stage (32 cells) the eight cells of the animal half (mesomeres) divide equally and latitudinally producing two wreaths of eight cells each. The four larger pigmented macromeres divide meridionally, and the four micromeres of the unpigmented cap latitudinally and unequally. Subsequent divisions tend to equalize the size differences and the blastula is regular and consists of equal-sized cells, but from the substance of the mesomeres comes the ectoderm, except that of the lower pigmented area which is contributed to the secondary mesoderm; from the macromeres come the endoderm and secondary mesoderm; and the primary mesoderm is from the micromeres.

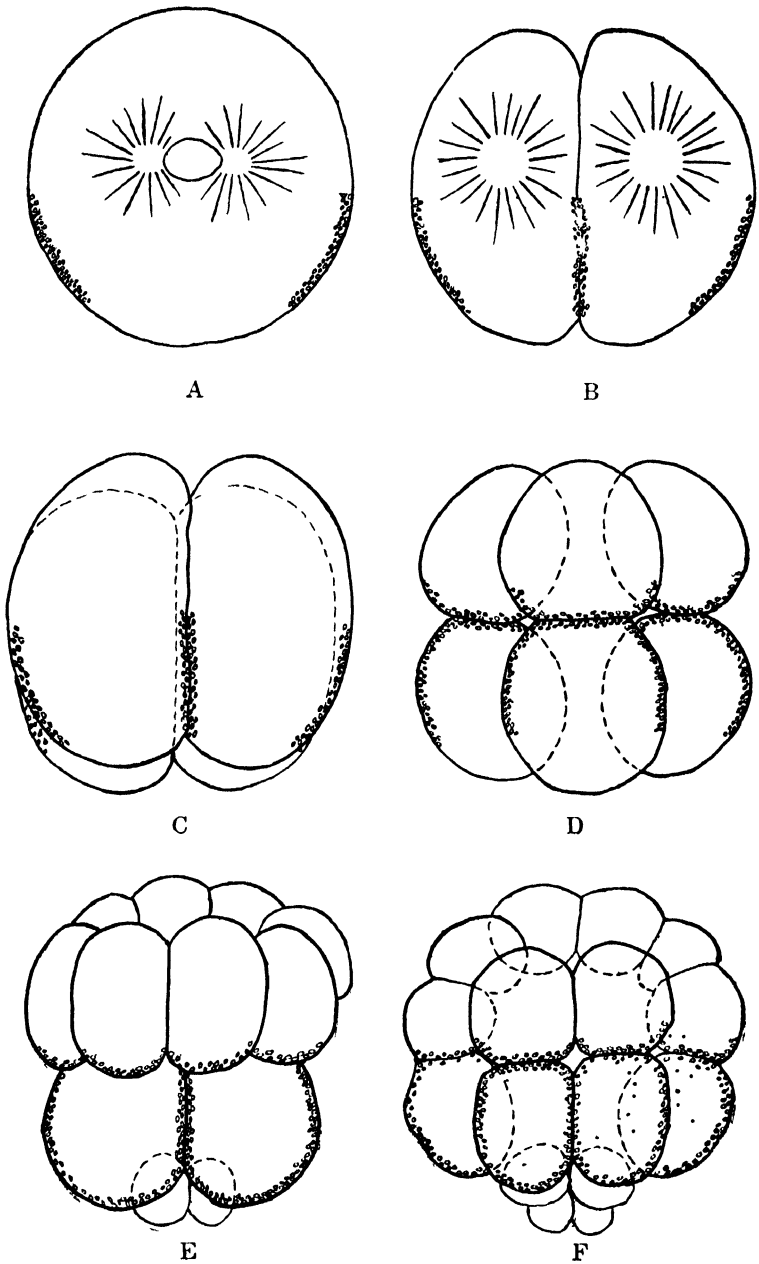


FIG 12 Cleavage of sea-urchin *Strongylocentrotus* (Redrawn from Korschelt and Heider after Boveri)

Lateral views showing the three zones and their distribution in the blastomeres

II. DISYMMETRICAL CLEAVAGE

The ctenophores are the sole representatives of the disymmetrical type of cleavage, and to the average student of embryology the fact

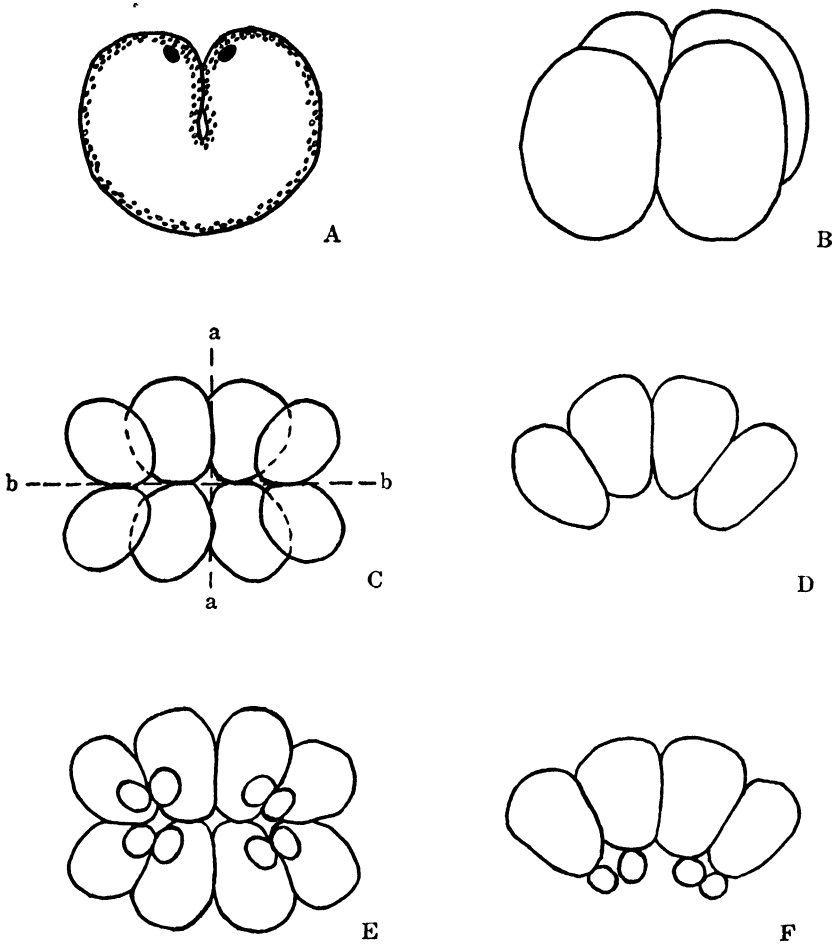


FIG. 13. Cleavage of *Beroe*. (Redrawn from Korschelt and Heider after Zeigler.)

A, second division. The vegetative pole is the upper, as in other figure showing lateral view; B, 4-cell stage; C, 8-cell stage from animal pole, aa, median plane; bb, transverse plane; D, same from lateral view; E, F, 16-cell stages from animal pole and from side view respectively.

that they constitute a remarkable exception to the general rules is their greatest interest. Although the peculiarities of these forms early stimulated a great deal of investigation, Ziegler's findings (1898) form the basis of our present understanding of this type of development.

The eggs of ctenophores have an ectoplasmic surface layer and an inner vacuolated yolk mass; the nucleus lies in the ectoplasm near the vegetative pole of the egg where the polar bodies are given off (Hatschek). In no other case in the animal kingdom is this type of egg organization found. The first and second cleavage furrows begin at the vegetative pole, advancing meridionally to the animal pole, dividing the egg equally. The first furrow corresponds to the median plane of the adult and the second to a plane connecting the two long tentacles; each blastomere is therefore one-fourth (one quadrant) of the body.

The third division is spoken of as diagonal. It runs obliquely from near the animal pole to the lateral part of the vegetative half of the egg,

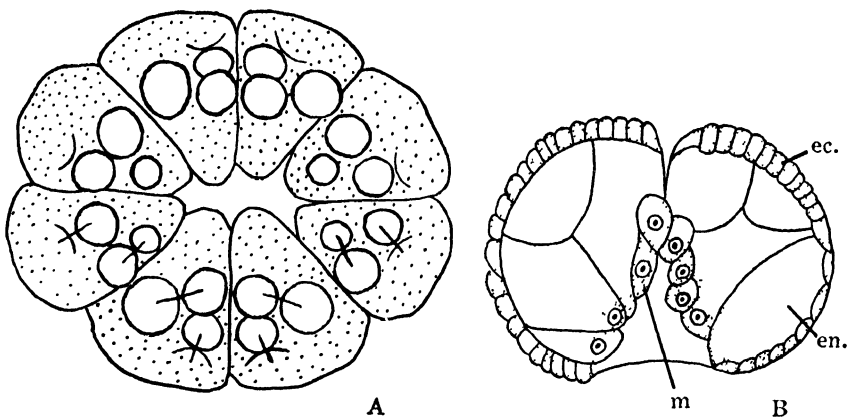


FIG. 14. A, continuation of Fig. 13. 40-cell stage of *Beroe*; B, gastrula of *Callianira bialata*. (After Metchnikoff.)

ec. outer cells, ectoderm; en. large inner cells, endoderm; m. small inner cells, mesoderm

giving rise to a curved plate of eight cells of which the four larger middle ones are called submedian and the four somewhat smaller ones are the subtentacular cells. This curved plate, concave on the animal side, is thus divided symmetrically in two directions, but is elongated in the tentacle plane, thus giving the impression that there are two centers of symmetry, one in each half, right and left, of the embryo. This impression is accentuated in the next stage in which eight micromeres in two distinct groups are given off on the concave side (animal half) of the embryo. The 32-cell stage is reached as each of the eight macromeres gives off another micromere and each of the old micromeres divides. The micromeres of the submedian octants divide equally, those of the subtentacular ones very unequally.

By subsequent divisions the micromeres come to form an ectodermal cap which gradually grows over the macromeres, now increased to 16.

The cap of ectomeres closes up late at the animal pole, a point at which the sense organ later develops. In the formation of the ectodermal cap of micromeres gastrulation is accomplished and the endoderm is marked off from the ectoderm. Endoderm formation in the animal kingdom may be accomplished by several methods which are described in Chapter VII. Although invagination is the most common of these methods it is by no means a necessary process in endoderm formation. In this case it plays no part at all, for the large macromeres that are the primordia of the endoderm are simply overgrown by the micromeres. This process is known as epiboly. The epibolic gastrula of *Callianira* is shown in Fig. 14*B*. For further details in the development of ctenophores the student is referred to the descriptions of Ziegler, Metchnikoff, and others.

III. BILATERAL CLEAVAGE

Bilateral cleavage is so called because of a bilateral arrangement of the egg substances which is very often recognizable before cleavage, indeed before fertilization in some cases, and certainly early in cleavage. Examples include rotifers, nematodes, and those vertebrates which have holoblastic cleavage. Thus it occurs in eggs which belong to homolecithal and to holoblastic telolecithal types, and either equal or decidedly unequal cleavage may result. It will be recalled that telolecithal eggs may have holoblastic or meroblastic cleavage depending on the degree in which the yolk mass forces the cytoplasm to be localized on one side. In the extreme cases discoidal cleavage results, which is thus related to the bilateral types. On the other hand it will later appear that the discoidal type is also related to the superficial type.

1. Amphioxus

As a first type amphioxus may be chosen. It was formerly the custom to class amphioxus as of the radial type. This is perhaps to be ascribed to the fact that there is some variability in the behavior of different eggs. Cerfontaine carefully restudied the case, however (1906), and found that in the majority of cases the following description holds true. Since his work, nevertheless, it should perhaps be pointed out, as Dean has done, that the cleavage of amphioxus may be polymorphic, that is, in one case radial and in another bilateral.

The egg of amphioxus is clearly bilateral in structure before cleavage. The animal pole is marked by the second polar body, the first being given off before the formation of the vitelline membrane and so lost. It is said that the animal pole is the point of attachment of the oöcyte to the germinal epithelium. If the egg is oriented according to the planes of the future embryo the animal pole is found to be in the center of the

antero-ventral region and the vegetal in that of the postero-dorsal. In the anterior half of the egg may be distinguished a clear mass of yolk-free cytoplasm, in which the nucleus lies, although part of this mass extends a little into the posterior half on the ventral side; most of the cytoplasm of the posterior half of the egg is well filled with yolk globules. Yolk granules also are found in the animal half outside the clear cytoplasmic area. The distinction of yolk-free and yolk-laden halves of the egg is indicative of its bilateral symmetry. The telolecithal character of the egg is peculiar in that the yolk mass is not only in the vegetative half, but toward the

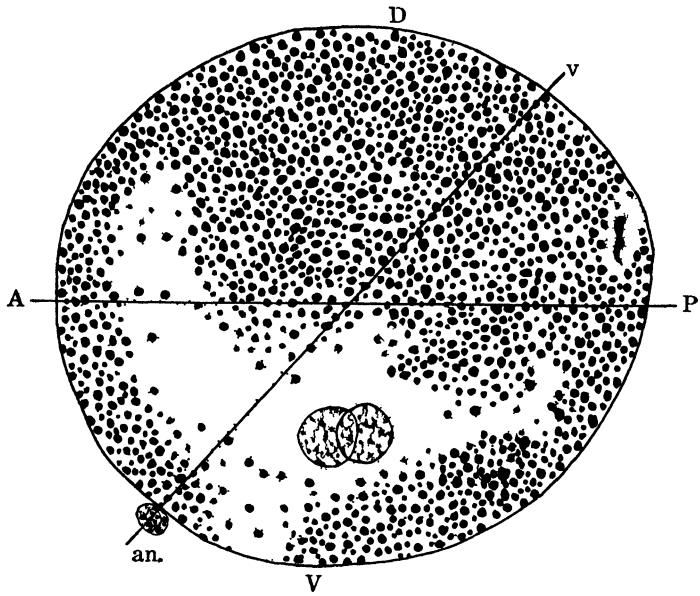


FIG. 15. Median section through egg of amphioxus arranged to show relation of egg axis to future orientation of body. (After Cerfontaine.)

an.-v. egg axis terminating in the two poles, A, P, D, V, the future anterior, posterior, dorsal and ventral regions of the egg.

posterior end. The first furrow is meridional and corresponds to the median plane, dividing the egg into right and left halves. The second furrow is meridional, but slightly unequal blastomeres are separated by it. The antero-dorsal are the smaller. The third furrow is latitudinal, cutting off four micromeres at the animal pole. Owing to the inequality of the 4-cell stage there are really four pairs of cells of different sizes in the 8-cell stage.

The 16-cell stage is not reached by meridional furrows as is to be expected. The four micromeres each divide by a furrow more or less at right angles to the median plane, and the four macromeres by a parallel

furrow. Thus there arise two plates of eight cells each, those of the animal half curving from front to back, while the others curve from right to left.

From this stage on, development becomes irregular, and characteristic differentiations appear. The typical coeloblastula characteristic of

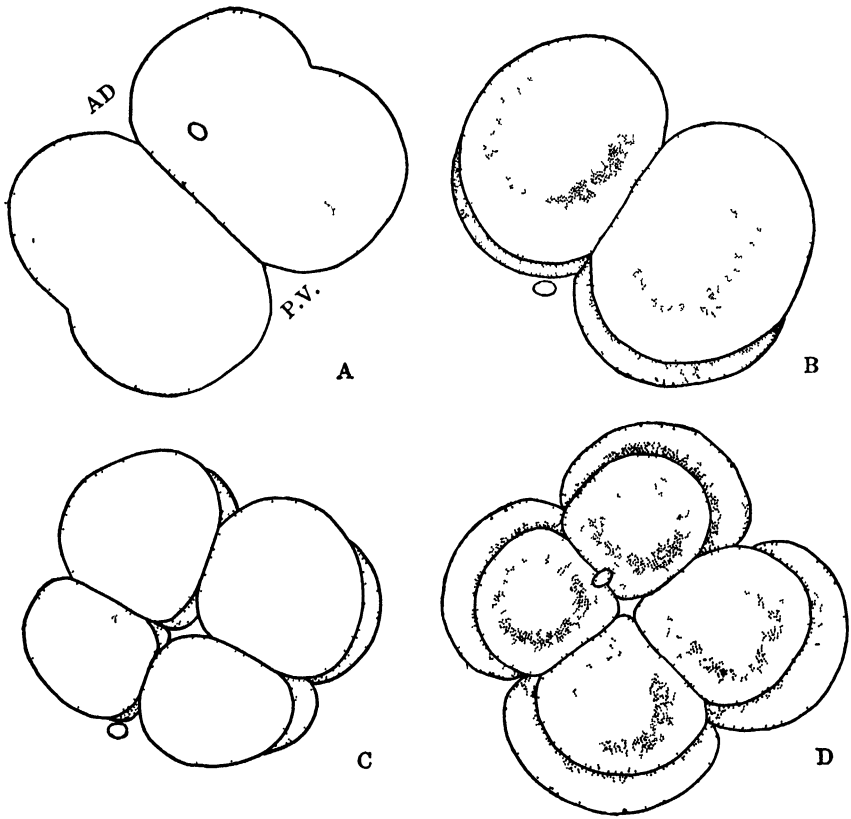


FIG. 16 Cleavage of amphioxus (Redrawn from Korschelt and Heider, after Cerfontaine)

A, second cleavage, B, 4-cell stage from left side C, D, 8-cell stage from side view and from animal pole

amphioxus is formed with smaller ectoderm-producing cells at the animal pole and the larger endoderm-forming ones at the vegetative. By invagination, epiboly, and involution the gastrula is formed.

2. Ascidians

The bilateral cleavage of the ascidians has been studied by many embryologists, but Conklin's work on *Cynthia, Ciona,* and *Mogula* is

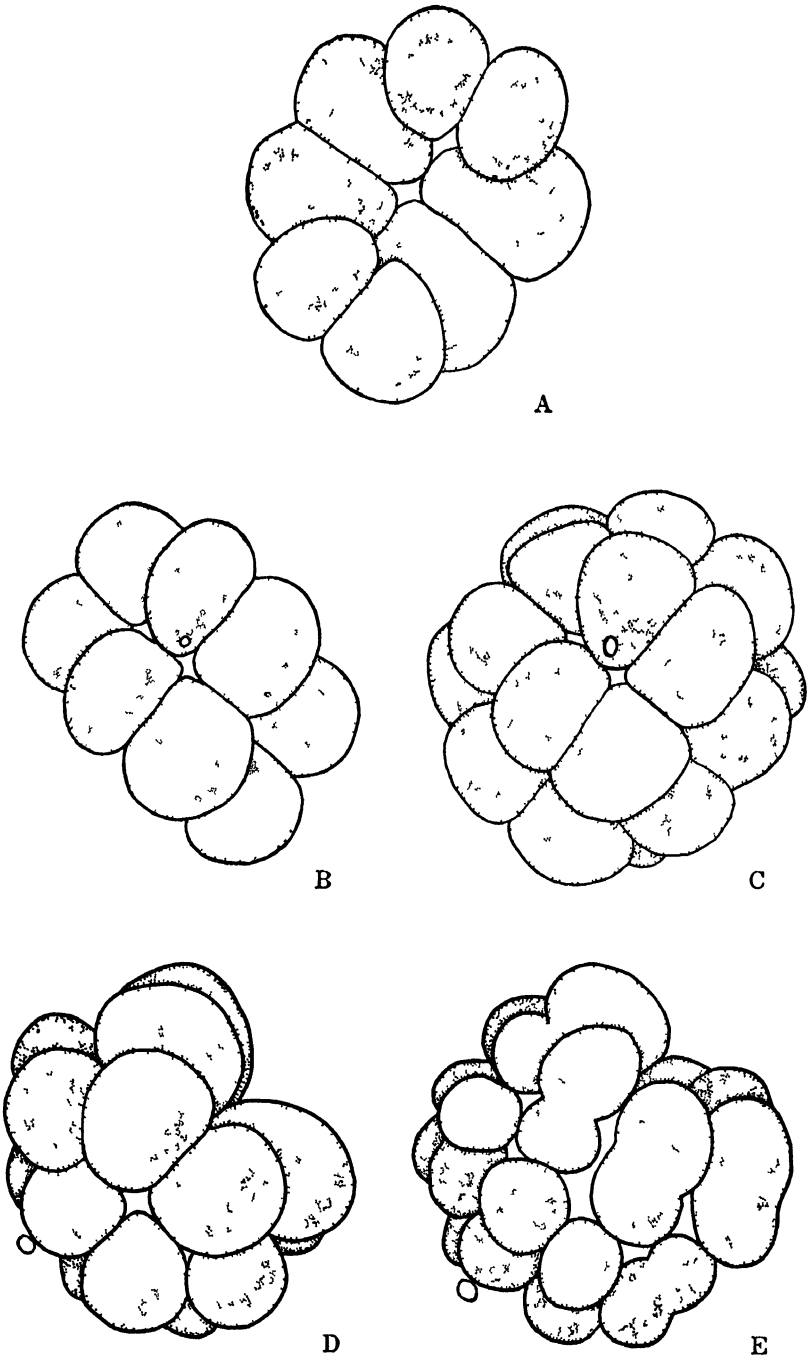


FIG 17 Continuation of Fig 16 A, vegetative half, and B animal half of 16-cell stage C same from animal pole, D, same from left side, E, fifth cleavage from left side

probably the most complete. These eggs have typical determinative cleavage, with definite organ-forming regions which are recognizable by color difference of the cytoplasm.

The newly extruded egg surrounded by a layer of test has a cortical layer of exoplasm in which is uniformly distributed a yellow pigment. Soon after fertilization the polar bodies are given off and peculiar rearrangements of substance take place. About five minutes after fertiliza-

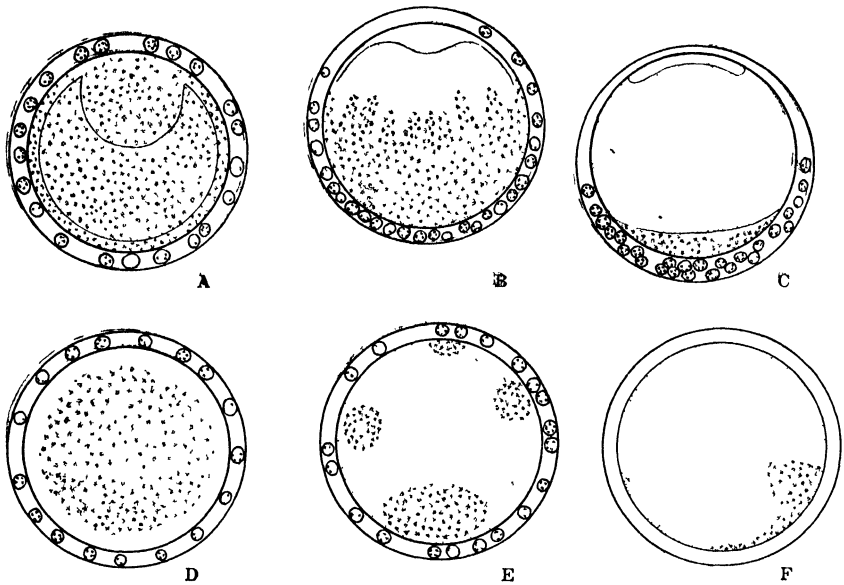


FIG. 18. The egg of *Cynthia partita*. (After Conklin.)

A, unfertilized egg with germinal vesicle, central gray yolk mass, peripheral yellow protoplasm, and test cells; B, five minutes after fertilization showing streaming of yellow protoplasm to vegetative pole; C, streaming of yellow protoplasm nearly completed, clear protoplasm lies beneath it and is visible at its upper edge. On the top of the egg the germinal vesicle is still seen; D, the light crescentic area at the vegetative pole represents the male pronucleus; the unstippled area is the gray central yolk mass of A; E, polyspermic egg; F, formation of yellow crescent (heavily stippled) with the light gray crescent above it.

tion the yellow pigment, mesoplasm, may be seen streaming toward the vegetal pole, leaving behind the gray yolk mass, endoplasm, which fills most of the cytoplasm of the egg. The yellow mesoplasm arranges itself in a crescent in the posterior half and will, later in development, give rise to mesoderm. The large germinal vesicle contains material in addition to that which goes to form the cleavage nucleus, which is liberated at the time of formation of the maturation spindle. This material, the clear protoplasm, is the ectoplasm of the fertilized egg; it shifts its position to lie in a clear gray crescent next to the yellow crescent but on the side of

the animal pole. It is the neurochordal anlage (or fundament from which will arise the neural canal and notochord). The animal pole is surrounded by ectoplasm from which ectoderm arises, and the vegetative by endoplasm producing endoderm.

The first division, a meridional one, divides the egg and likewise each of the organ-forming regions into two equal halves. Since there is but a

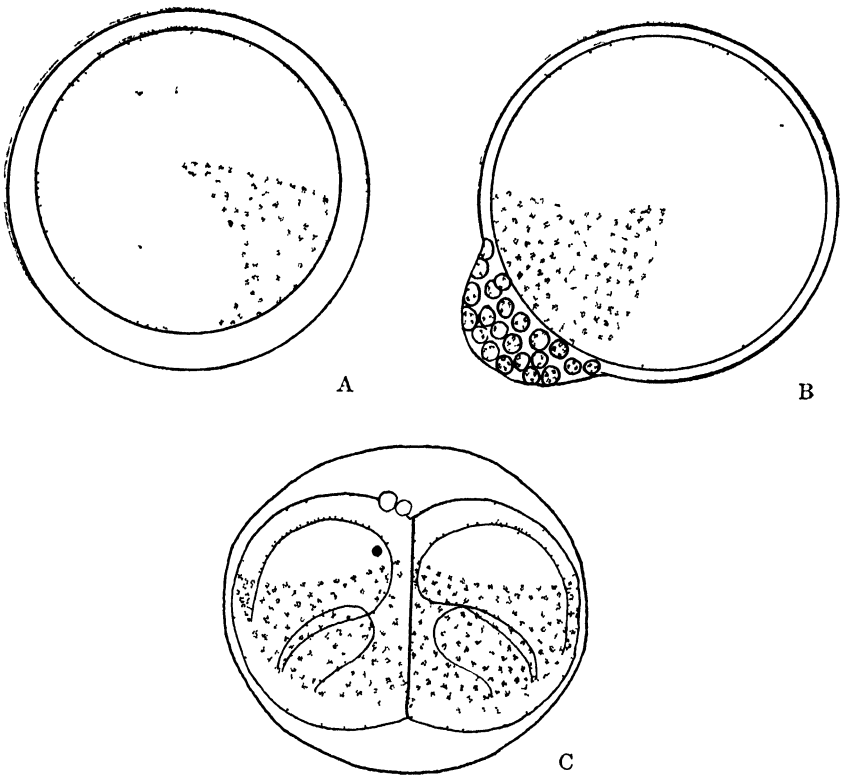


FIG 19 A, continuation of Fig 18 B, showing aggregation of test cells over the crescent and the protrusion of the chorion, C, 2-cell stage with formative substances outlined Uniform stipples, yolk, light stipples, clear protoplasm enlarged and extended toward the animal pole, grouped stipples, yellow crescent material

single plane which can do this it is evident that the cleavage is bilateral in character. The separation of the egg substance into organ-forming regions, which had started during the first cleavage, is completed now, although it must be recognized that it is never as sharp as the boundaries of the regions shown in the usual diagrams. It is not possible, however, that living protoplasms could be entirely and sharply separated from each other while in a single cell. Indeed, we may regard the process of

cleavage as having the especial function of distributing these substances into the appropriate cells.

The second division is meridional and displays the beginning of inequality. The orientation of the ascidian egg is similar to that of amphioxus. Therefore, two antero-dorsal cells are separated by this division from two postero-ventral ones. It is customary to designate the anterior cells by the letter *A* and the posterior by the letter *B*. In each case the right blastomere is indicated by underlining the letter

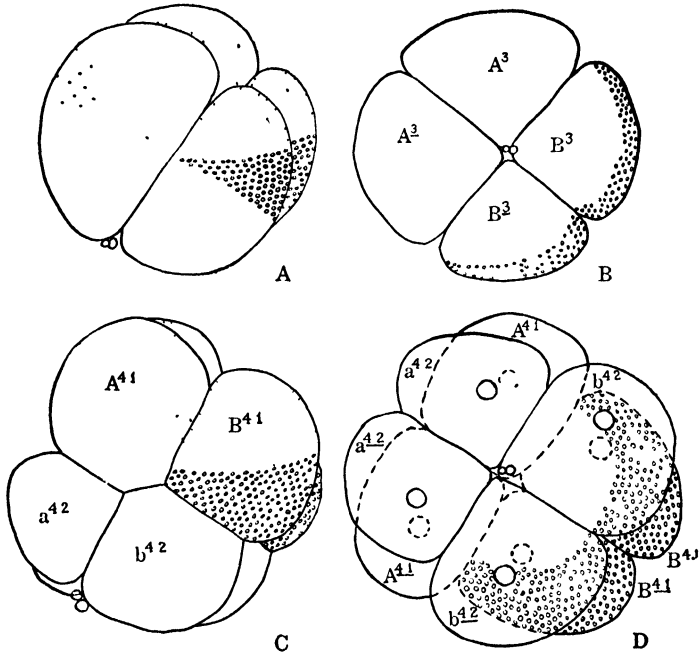


FIG. 20. Cleavage of *Cynthia* (Redrawn from Korschelt and Heider, after Conklin) A, 4-cell stage from left side, B, from animal pole, C, 8-cell stage from left side, D, from animal pole.

as $\underline{A}^3$ or $\underline{B}^3$. The exponent ^{3} indicates that this is the third cell generation, counting the single-celled egg as 1. The division of A^3 results in two blastomeres which are designated $A^{4\,1}$ and $A^{4\,2}$. The exponent ^{4} indicates the fourth cell generation, and the ^{1} or ^{2} after the period shows that it is the first or second cell from the vegetal pole. These principles of nomenclature are used throughout the cell lineage of the ascidian.

The third division is somewhat unequal and latitudinal, resulting in 4 cells of the animal half of the embryo ($a^{4\,2}$, $b^{4\,2}$, $\underline{a}^{4.2}$, $\underline{b}^{4.2}$) which are smaller than the corresponding ones of the vegetative half ($A^{4.1}$, $\underline{A}^{4.1}$, $B^{4\,1}$, $\underline{B}^{4\,1}$). Due to a shift in the position of the spindles of the animal

half which converge toward the plane, the nuclei of $a^{4.2}$ and $\underline{a}^{4.2}$ lie nearer to each other than do those of $A^{4.2}$ and $\underline{A}^{4.2}$. We may take the A or the anterior half of the embryo as the type; the cells of the posterior half are to be regarded similarly.

The next division is therefore not strictly meridional as is to be expected. The spindles of the small animal cells ($a^{4.2}$, $\underline{a}^{4.2}$) lie nearly parallel to the median plane, but those of the broader animal cells are more transversely placed. Thereupon the cells $a^{5.3}$ and $a^{5.4}$ seem to be behind each other, while $b^{5.3}$ and $b^{5.4}$ are side by side. In the vegetative half the converse relations hold: $A^{5.1}$ and $A^{5.2}$ lie side by side and $B^{5.1}$

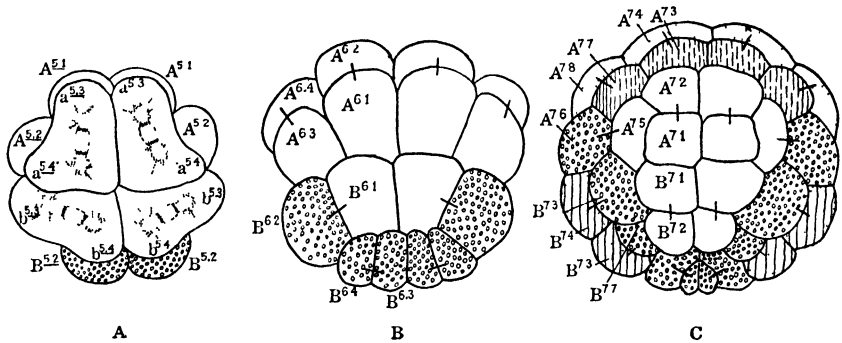


FIG. 21. Later stages of *Cynthia* cleavage. (Redrawn from Korschelt and Heider, after Conklin.)

A, the fourth cleavage seen from animal pole; B, vegetative half of embryo in 32-cell stage; C, same in 64-cell stage.

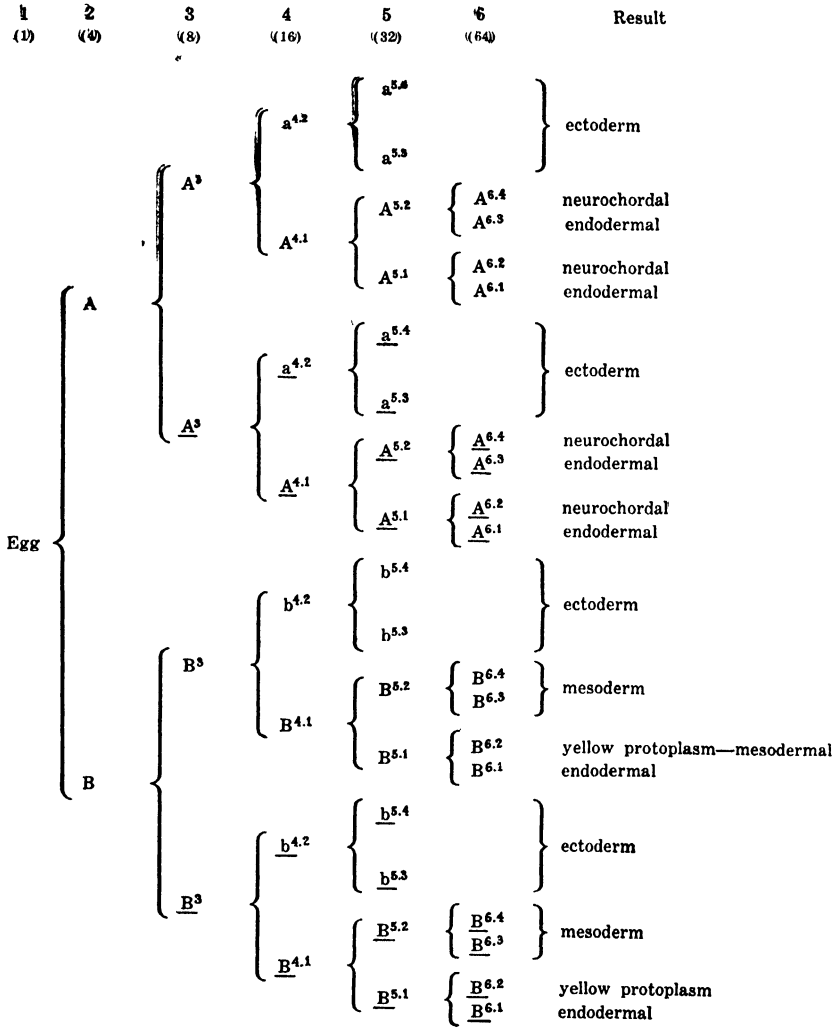
$A^{7.2}$, $A^{7.1}$, $B^{7.2}$, $B^{7.1}$, are pure endoderm cells; $A^{6.2}$, $A^{6.4}$, are cells of neurochorda anlage; $B^{6.2}$, $B^{6.3}$, $B^{6.4}$, mesoderm crescent; $A^{7.6}$, $A^{7.7}$, go to form the notochord; $A^{7.8}$, $A^{7.4}$, form hinder part of neural plate. $A^{7.5}$ contains gray yolk (endoderm), but it contains material which will be segregated as mesenchyme. $B^{7.4}$, is muscle forming; $B^{7.3}$ mesenchyme. In each case the symmetrical mates of the left side have a similar fate.

and $B^{5.2}$ lie behind one another. $B^{5.2}$ and half of $B^{5.1}$ are the material from the yellow crescent. The left half is similar.

From here on all the cells of the animal half produce only ectoderm cells. The cells of the vegetative half are more diversely differentiated. $B^{6.2}$ and $\underline{B}^{6.2}$ consist only of yellow protoplasm and are purely mesodermal; $A^{6.3}$, $A^{6.1}$, and $B^{6.1}$ with their mates of the right side are almost purely endodermal. The neurochordal cells $A^{6.2}$ and $A^{6.4}$ lie next to them anteriorly, and the crescentic mesoderm anlage, $B^{6.2}$, $B^{6.3}$, and $B^{6.4}$, behind.

From this it will be seen without going into further details that the cleavage of the ascidians is a very good example of the determinative type. Thus organ-forming regions are definitely localized during the maturation and fertilization processes and by the process of cleavage

are segregated into definite cells from which arise the organs of the later embryo in the characteristic ascidian manner.



CELL LINEAGE OF CYNTHIA FROM DESCRIPTION OF CONKLIN

3. Vertebrates

The cleavage of the vertebrates is not usually of a determinative character as is that of the ascidians, and there are many variations in the characters it presents throughout the different groups. In general, however, they may all be referred to the bilateral type or derived from it, although the very considerable amounts of yolk present in the telo-

lecithal eggs bring about important modifications in the modes of cleavage. This account leaves out of consideration the cleavage of mammals which is modified in an extreme manner in spite of the lack of yolk. Several groups of vertebrates have discoidal cleavage; this type,

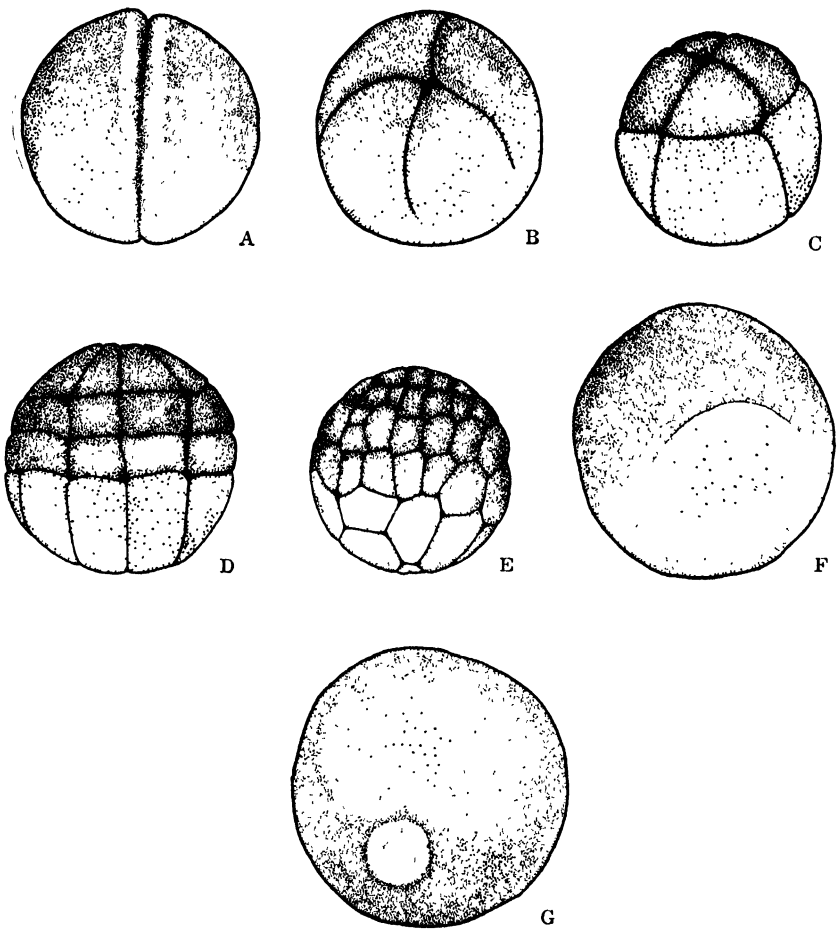


FIG. 22. Cleavage of the frog egg. A, B, C, D, 2-, 4-, 8-, 32-cell stages; E, later cleavage stage; F, dorsal lip of blastopore; G, circular blastopore.

however, is joined to the holoblastic egg types by many transitional stages and types.

All amphibia have total, unequal cleavage in correlation with the telolecithal type of egg structure which characterizes this group. The egg of the frog has been studied by many investigators and may be taken as an example of holoblastic cleavage in the vertebrates. A dark

brown or blackish pigment distinguishes the animal from the vegetative portions of the egg. About the vegetative pole extending perhaps one-third upwards is a white region which contains the material that will later go to form the endoderm.

In some form of amphibians, as of other groups, it is impossible to relate the cleavage planes to the later axes of symmetry of the embryo or larva. In other forms the bilateral arrangement of the blastomeres is unmistakable.

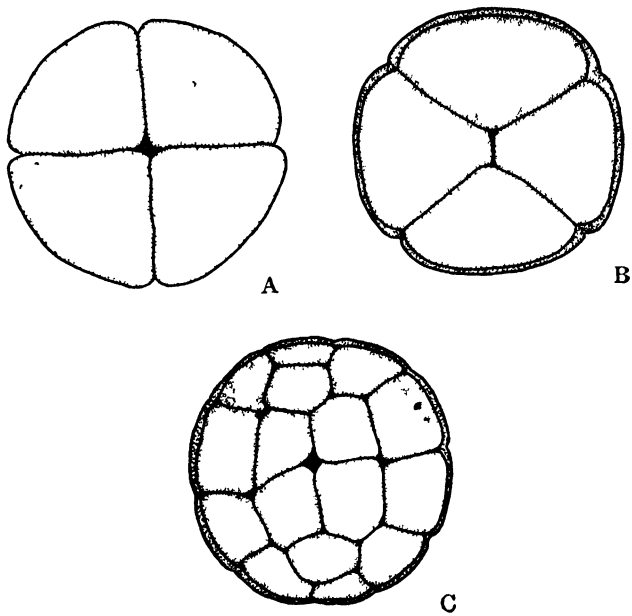


FIG 23 Cleavage of the frog egg seen from the animal or pigmented pole A, B, C, 4-, 8-, 32-cell stages

In the frog there is considerable variation as to the relations of the cleavage planes. Brachet found in 48 per cent of the cases which he observed that the first cleavage plane and the median plane coincided; in another 20 per cent the deviation was not more than 20 degrees. However, the bilaterality of the egg may often be shown to be independent of the cleavage figures. In certain amphibian eggs it has been shown that there is present in both fertilized and unfertilized eggs a bilateral arrangement of part of the egg substance, the so-called "gray crescent," which appears at the edge of the white part of the egg against the dark portion, recalling the condition in ascidians.

The first cleavage divides the egg meridionally, the formation of the

furrow beginning at the animal pole and extending to the vegetal pole. The second is similar to the first but at right angles to it. The third cleavage is latitudinal and the blastomeres resulting form two circlets, one of four large cells and the other of four smaller. The fourth cleavages are meridional again. In later cleavages the yolk in the vegetative region causes a retardation in the rate of division so that the animal cells become much more numerous. In the fifth and sixth cleavages only the small

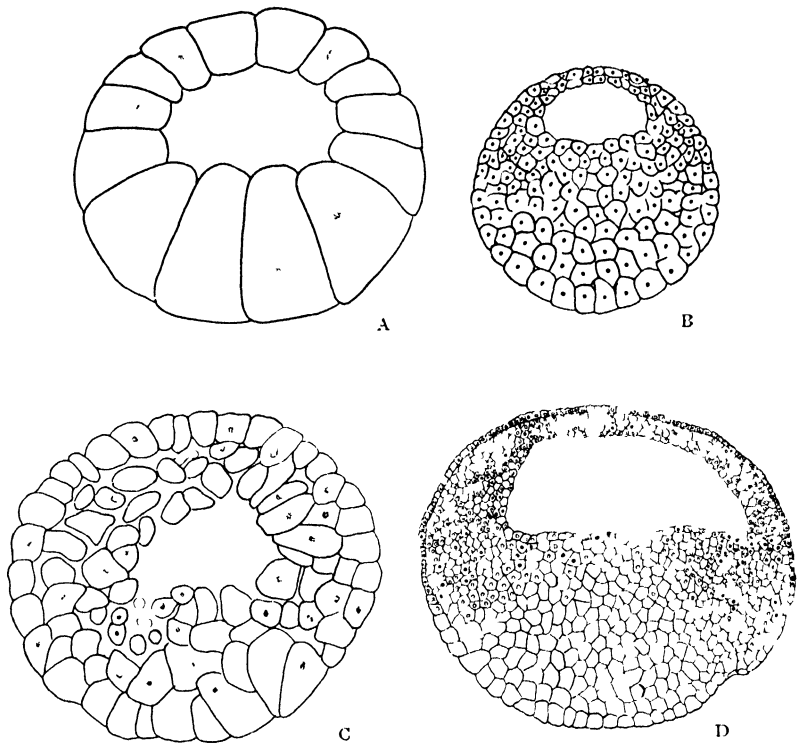


FIG. 24. Sections through blastulae up to the beginning of gastrulation in the frog's egg. (B, C, D, after Ziegler.) These show the character of the ectoderm and endoderm cells, the nature of the blastocoele, and the first sign of gastrular invagination.

upper circlets of cells are divided and even in these disturbances in the pattern of the blastomeres shortly appear. No longer is the wreath-like arrangement of the cells maintained and they become relatively smaller and much more numerous as compared with the large yolk-laden cells of the vegetal region.

The cleavage cavity which may be discerned in the 8-cell stage is smaller than that of amphioxus or the ascidian and is pushed toward the animal pole by the larger yolk cells. It gradually becomes more

extended laterally, but in places it is reduced to a mere slit as development proceeds. When the fully formed blastula is reached in the frog, a difference, which is characteristic between vertebrates and invertebrates, becomes apparent in that the wall consists of many cells. In the invertebrates the ectoderm and later the endoderm consist of a single layer of columnar or cuboidal epithelium, but in the vertebrates these germ layers are of several cells in thickness and many of the cells do not lie in contact with either the outer or inner surface.

4. Nematodes

The cleavage of nematodes may be illustrated by reference to the development of *Ascaris* as worked out by Boveri; a number of other investigators have worked on other forms and have shown that they are in agreement with the *Ascaris* type.

The egg is small and practically yolk-free, and no evidences of differentiation are visible in the uncleaved egg, nor of relation between cleavage planes and polar bodies, but as soon as the 4-cell condition is reached the orientation is complete. In addition, however, to the differentiation which is expressed in the orientation of the 4-cell stage, another mark of differentiation is evident in the nuclear processes. This is the chromatin diminution which occurs in the cells which are somatic in character, and is lacking in the prospective germ cell line, that is, in P_1, P_2, P_3, P_4, and G and G_1.

The first cleavage plane results in the formation of two cells somewhat unequal. Of these the larger is designated as S_1 or AB and the smaller as P_1. S_1 is shown by its subsequent development to contain only material which is to be distributed to somatic cells, while the P_1 contains materials which will contribute to both soma and germ plasm. The next cleavage planes are not parallel to each other, for the spindle of the AB cell lies parallel to the first division plane, but that of the P_1 cell is perpendicular to it. Thus there arises a T-shaped figure, the top being formed by the cells A and B and the stem at first by P_1, which divides more slowly than the S_1 cell, and later by the derivatives, cells S_2 and P_2. Subsequently the P_2 cell shifts posteriorly so that it comes to lie in contact with both S_2 and B, forming a rhomboidal figure.

Now the bilateral character of the embryo becomes apparent. The cells A and B are dorsal, A being anterior; S_2 and P_2 are ventral, S_2 being anterior. The dorsal cells now divide somewhat ahead of the ventral by a plane of cleavage which lies in the median line; A thus divides into a on the right side and α on the left, and B into b and β. The embryo for a brief time, therefore, is in a 6-celled condition, but the division of the two ventral cells follows shortly, the planes of divi-

sion lying perpendicular to the median plane. The 8-cell stage, therefore, consists of a plate of four dorsal ectomeres (two right and two left) and

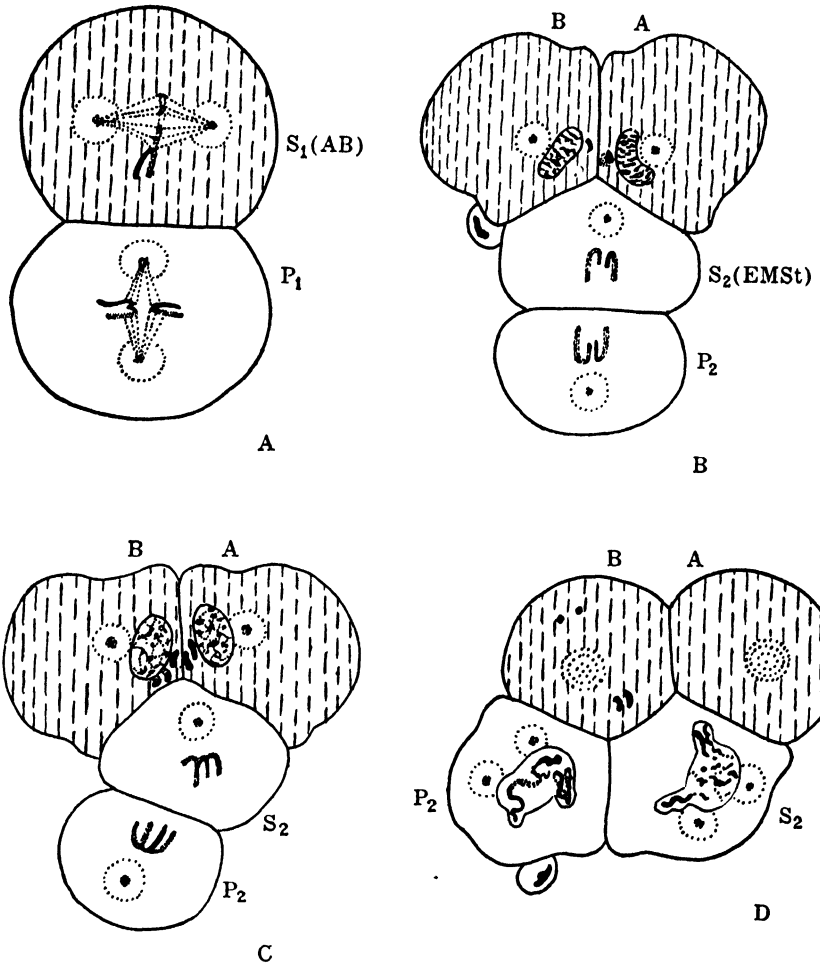


FIG. 25. Cleavage of *Ascaris megalocephala*. (Redrawn from Korschelt and Heider after Boveri.)

A, 2-cell stage with spindles for the third cleavage; S_1 which equals AB is the first somatic cell and P_1 is the blastomere from which the germ line will be segregated (stem cell); B, C, D, 4-celled stages showing in B the characteristic T-form resulting from the spindle directions of the second cleavage in C the P_2 cell swinging around to form the characteristic rhomboidal figure of the 4-cell stage. P_2 is the stem cell.

a row of four ventral cells slightly curved. S_2 (*EMSt*) divides into *MSt* and *E*, the former being the anlage of mesoderm and stomodaeum and the latter that of the primitive endoderm. P_2 divides into S_3, an ecto-

mesoderm cell, and P_3, a continuation of the germ line. In this 8-cell stage the cleavage cavity makes its first appearance.

While the cleavage processes are taking place in *Ascaris* as described they are accompanied by nuclear changes of a character which has occasioned much study and discussion. In certain of the cells there occurs a process known as chromatin diminution by means of which

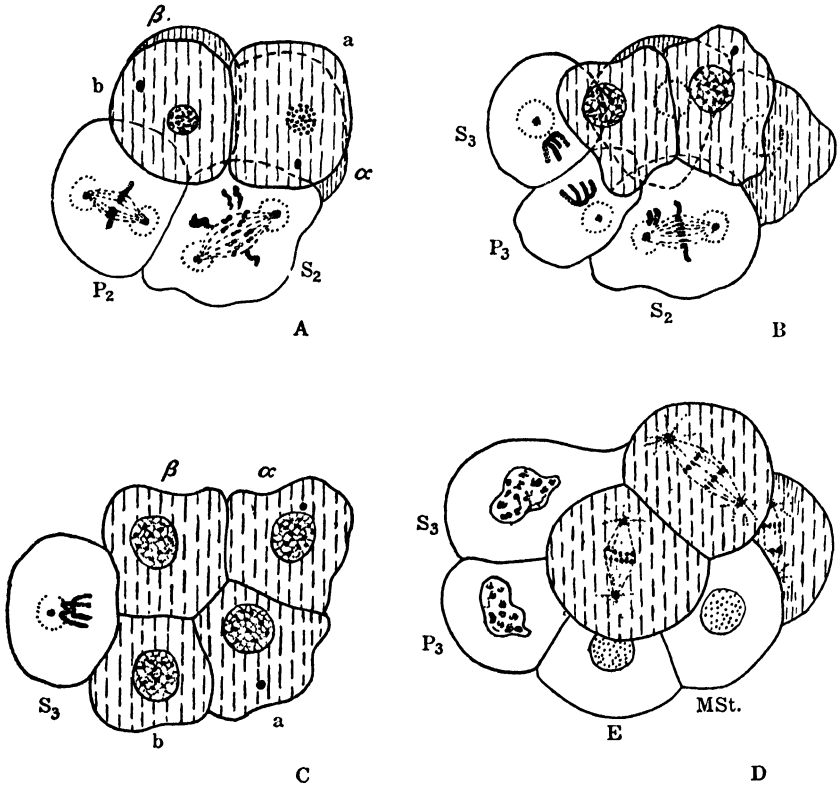
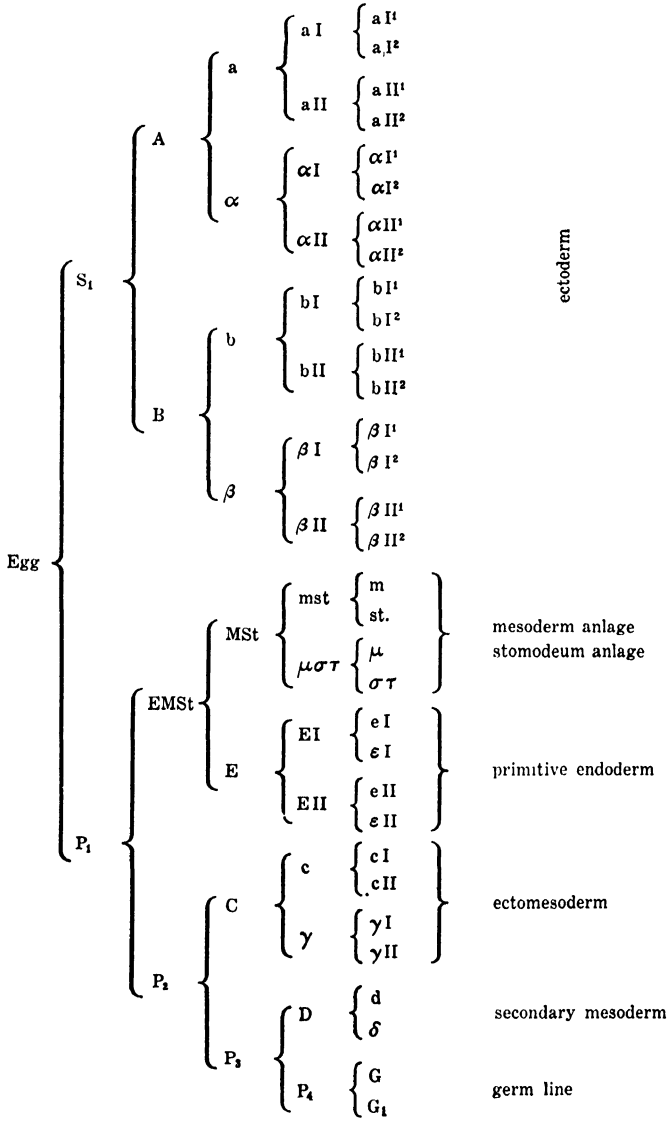


FIG. 26. Continuation of Fig. 25.

A, 6-cell stage. Blastomeres A and B divided into *a* and *a* and *b* and *β*; B, 7-cell stage P_2 having divided into P_3 and S_3. A and B are seen from the right side; C, seen from the dorsal side; D, 8-cell stage. S_2 having divided into E and MSt.

the thickened ends of the chromosomes are left behind in the cytoplasm as the telophases of the cleavage divisions take place. Chromatin diminution never occurs in those blastomeres which are antecedent to the germ cells of the later stages; but every blastomere which will produce only somatic cells goes through the process of chromatin diminution once. In the 2-cell stage S_1 undergoes chromatin diminution while P_1 does not. In the 4-cell stage S_2 repeats the process but P_2 does not. In

the 8-cell stage again P_3 retains its entire chromatin complement while its sister cell S_3 shows the differential loss. This setting aside of the germ



CELL LINEAGE OF ASCARIS FROM DESCRIPTIONS OF ZUR STRASSEN AND BOVERI

line continues for five cell generations, at the end of which time no further somatic cells are produced from this line and the cells rest from further division until the organism has reached a considerable degree

of maturity. The distinction which is made possible by following through the chromatin diminution between germ plasm and soma in these early blastomeres of *Ascaris* has been of great theoretical significance and often referred to as evidence in support of the theory of germinal continuity. In this connection it is further discussed in Part Two, Chapter I. The process will be more clearly understood by referring to that discussion and also to the table showing the cleavage of *Ascaris* which appears herewith.

5. Rotifers

The remaining example of the bilateral type of cleavage is that of the rotifers. This example is not in many respects characteristic of the type and its development is not fully understood. It is to a certain extent intermediate between the bilateral and the spiral types. The rotifers have no cleavage cavity or gastrula cavity. (That is, they have stereoblastulae and stereogastrulae.) The resemblance between the developmental conditions of annelids and molluscs and of the rotifers is perhaps not so surprising in view of the fact that all these groups pass through larval stages which are morphologically trochophores.

Although the character of the cleavage of rotifers is sufficiently bilateral to be placed with other forms of this type, the peculiarities are such that it seems unnecessary to follow out the details for these forms.

IV. SPIRAL CLEAVAGE

Spiral cleavage is characteristic of polyclads, nemerteans, polychaetes and many molluscs. Eggs which cleave spirally are with few exceptions well filled with yolk and therefore segment unequally. Frequently the inequality is so great that only a small cap of cells comprising the ectoderm lies on the large endodermal cells with a consequent more or less complete reduction of the cleavage cavity. This condition also results in a much modified type of gastrulation from that which is characteristic of holoblastic eggs.

Nevertheless it is not difficult to derive the spiral type of cleavage from the equal radial type. The first two cleavages are meridional and about at right angles to each other, thus dividing the egg into four nearly equal blastomeres. In the 4-cell stage blastomeres *A* and *C* (see nomenclature below) are nearer the animal pole than *B* and *D* and form with each other what is known as a polar furrow, as do *B* and *D* at the vegetal pole. But the polar furrows at the two poles are perpendicular to each other. The departure from the radial type becomes clearly distinguished in the third cleavage, owing to an oblique shift in spindle direction so that when viewed from the animal pole the upper end of

the spindle is turned either to the right (which is the usual case) or to the left. Bearing in mind the fact that the yolk is massed at the vegetal end of each blastomere and the pure cytoplasm at the other ends, and the general rule (Sachs-Hertwig laws) that a spindle axis tends to lie in the center of the cytoplasmic mass, one sees that an unequal cleavage of each of the four blastomeres will occur. They are divided into small upper cells or micromeres, and large ones in the lower hemisphere or macromeres, and the upper quartette of cells thus arising alternates in

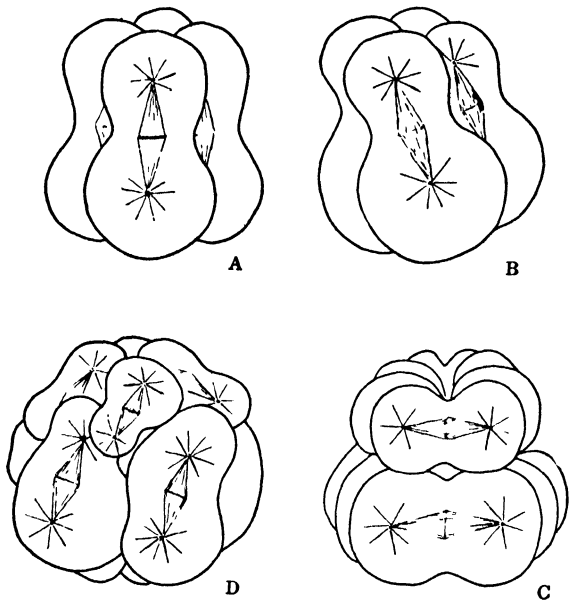


FIG. 27. Comparative diagrams of radial and spiral cleavage. (Redrawn from Korschelt and Heider.)

A, radial type; B, spiral type in third cleavage; C, D, radial and the spiral type respectively in fourth cleavage.

position with the lower (Fig. 30 *c*, *e*). The upper quartette of cells, called ectomeres, appears to be given off spirally from the lower endomeres, although of course speaking strictly it is not correct to think of these cells in this manner, for both quartettes are composed merely of the daughter cells of the preceding 4-cell stage. This third cleavage is said to be a dexiotropic or right-handed division since the cells of the ectomere quartette are turned 45 degrees from those of the macromeres in a right spiral.

The fourth cleavage spindles are now formed in accordance with the general tendency expressed by the so-called alternation rule that suc-

cessive cleavage planes tend to intersect each other at right angles. The spindles, therefore, take up such positions that the next divisions are laeo-

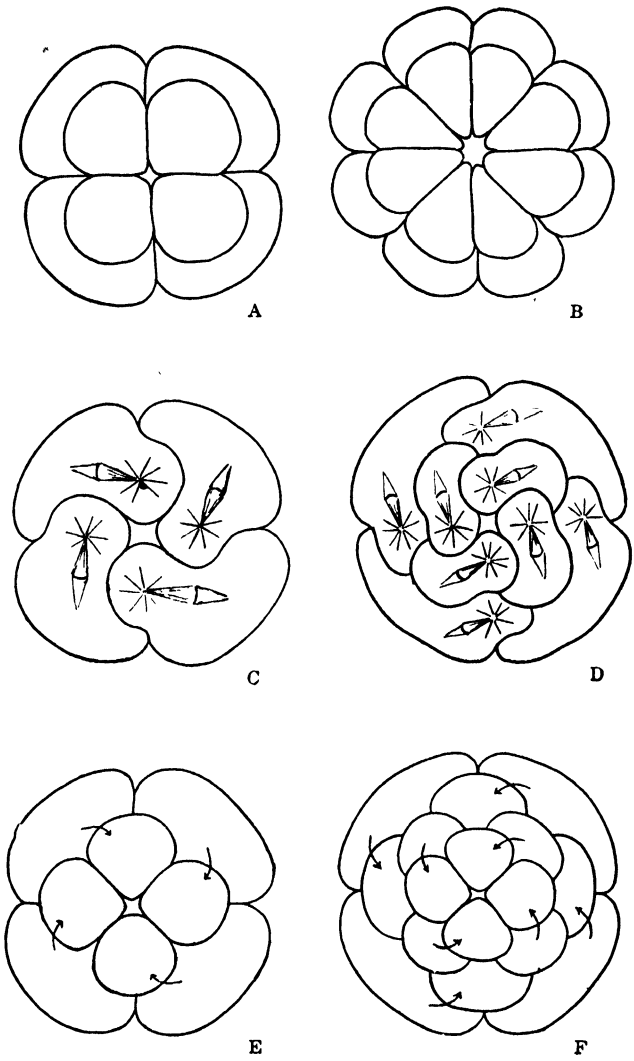


FIG. 28. Continuation of Fig. 27. Polar views.
A, B, 8- and 16-cell stages radial type; C, D, third and fourth cleavages, spiral type; E, F, 8- and 16-cell stages spiral type.

tropic, that is in the direction of left-handed spirals. This alternation continues in regular order until four quartettes have been produced, and meanwhile each of the cells divides according to the same general laws of

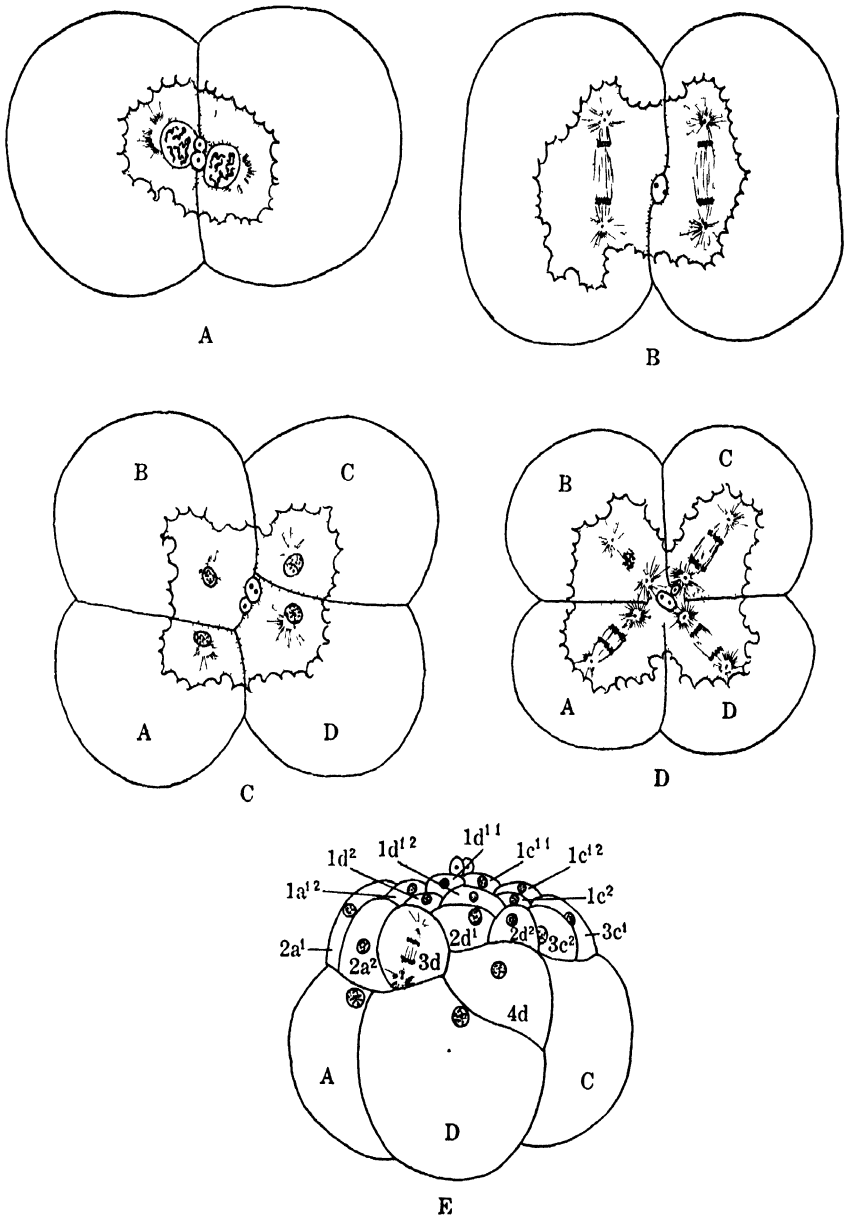


FIG. 29. Cleavage of *Crepidula*. (Redrawn from Conklin.)

A, resting stage after the first cleavage; blastomeres flattening against each other and nuclei, asters, and protoplasmic area rotated dexiotropically; B, anaphase of the second cleavage. The shift which will result in the formation of a polar furrow just beneath the polar bodies is already making its appearance, C, completion of the second cleavage Polar furrow well formed; D, third cleavage; E, side view of egg in 29-cell stage. The relation of the mesentoblast cell 4d is shown to the macromere D.

cleavage. Since the blastomeres of spirally cleaving eggs are always unequal, differences occurring even within a quartette as well as between different quartettes, it follows that the cleavages cannot long remain entirely regular, for differentiation is rapidly taking place.

Owing to the fact that spiral cleavage is one of the most markedly determinative in character, this form presents an unusually good example for the study of cell lineage. To bring out the relationships between cells it is found to be necessary to have a definite *terminology* and *nomenclature* for the blastomeres of eggs of this type. The system adopted by Conklin for expressing the relationships in the case of *Crepidula* is now in general use. Certain special cells should be given special names, but in general the system adopted should show immediately the derivation of each cell by its name. For this purpose Conklin modified the system previously introduced by Wilson to describe the cell lineage of *Nereis*.

In the nomenclature used in *Crepidula* Conklin designated the quartettes of cells by coefficients: "e.g., the first quartette of micromeres and all their derivatives are designated by the coefficient 1 (1*a*, 1*d*, $1a^{1.2}$, $1c^{2.2}$, etc.), the second quartette and its progeny by the coefficient 2 (2*a*, 2*d*, $2c^{3.1}$, etc.), the third quartette by the coefficient 3 (3*a*, 3*d*, etc.), and the fourth quartette by 4 (4*a*, 4*d*, etc.)." It is desirable to emphasize in this manner "the differences between the quartettes of micromeres because in general their histories are very different," and because the distinctions afford the basis for tracing the cell lineage in the more advanced stages. The coefficients designating the quartettes are retained in all subsequent stages and the further cell lineage indicated by exponents. The cell 1*a* divides to form $1a^1$ and $1a^2$. Then further exponents are added for each new cell generation always retaining the complete designation of the parent cell. The cell $1a^2$ produces $1a^{21}$ and $1a^{22}$; $1a^{21}$ produces $1a^{211}$ and $1a^{212}$, etc. These are the designations used for the micromeres and their descendants.

The second division resulted in the formation of four blastomeres, *A*, *B*, *C*, and *D*. (Capital letters are used to indicate macromeres.) These divided in turn to produce 1*A* and 1*a* in the *A* quadrant of the egg and similarly in the other quadrants. Each successive macromere receives a new coefficient as it divides into a new macromere and a micromere; thus 1*A* produces 2*A* and 2*a*; 2*A* in turn 3*A* and 3*a*. Numerical coefficients are used to designate successive quartettes, while the exponents indicate the products of their division. Cleavage is dexiotropic, or oblique to the right, if the spindle direction is turned clockwise from the axis of the egg, and laeotropic when turned to the left.

By reference to the accompanying table the relationships of the blasto-

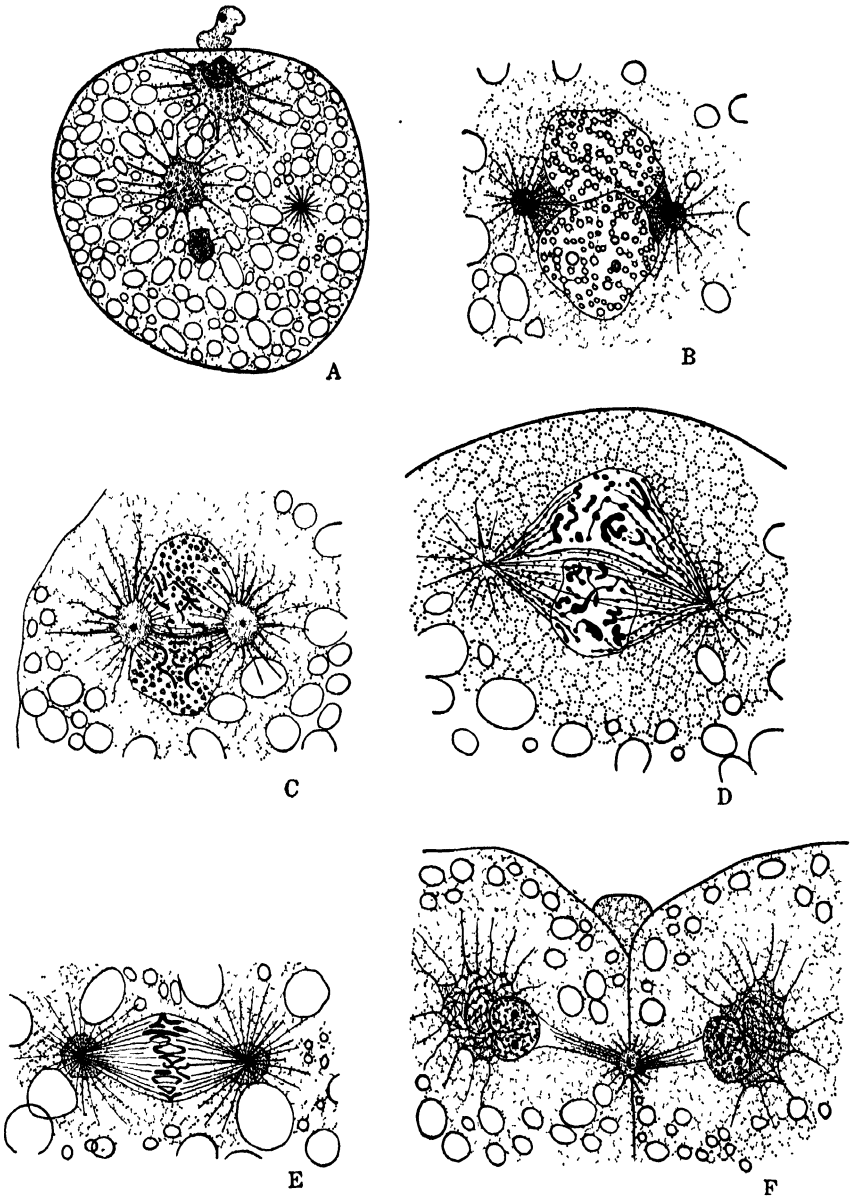


FIG. 30. First cleavage of *Crepidula plana*. (After Conklin.)

A, approach of sperm and egg pronuclei; B, nuclei in contact, asters and centrosomes at their poles, central spindle not yet formed; C, central spindle appearing between the two cleavage centrosomes; chromosomes aggregating on the spindle; other granules dissolving. Sphere substance increased in size and radiating into the cytoplasm; D, prophase of the first cleavage. Chromatin granules assuming their relation to the spindle fibers; E, metaphase of the first cleavage; F, telophase of the first cleavage; spindle axis bending. Nuclei of left cell partially divided indicating origin of upper part from egg pronucleus from female and lower from the male.

meres and their fate will be more clearly understood and the application of the system of nomenclature shown for this case.

In general the 8-cell stage of an egg with spiral cleavage consists of four micromeres, 1*a*, 1*b*, 1*c*, 1*d* about the animal pole, and four macromeres, 1*A*, 1*B*, 1*C*, 1*D*, at the vegetative pole. In most cases the third cleavage is dexiotropic. The 16-cell stage is reached by a aeotropic division and consists of the quartettes of micromeres, $1a^1$,$1b^1$,$1c^1$,$1d^1$, $1a^2$,$1b^2$,$1c^2$,$1d^2$, 2*a*,2*b*,2*c*,2*d*, and one quartette of macromeres, 2*A*,2*B*,2*C*,2*D*. There is an intermediate condition in eggs having spiral cleavage in which there are actually twelve cells only, for the second quartette, 2*a* to 2*d*, is budded off before the division of the first, 1*a* to 1*d*, is completed. There is a similar lack of regularity of division in other stages, some quartettes dividing while others hold back even to the extent that some cells may be an entire cell generation behind others. In the transi-

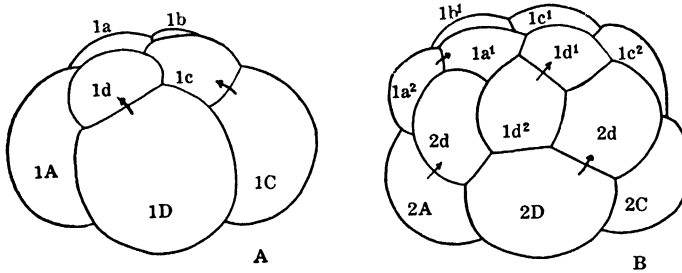


FIG. 31. Cleavage of *Trochus*. (Redrawn from Korschelt and Heider, after Robert.) A, 8-cell stage viewed from the side (the D quadrant will become the dorsal side); B, similar view of 16-cell stage.

tion to the 32-cell stage there is actually no resting stage in the sense that all the cells are at once in that same state of progress.

The division producing the 32-cell stage is usually a dexiotropic one. The cells of the first quartette receive their second exponent, $1a^1$ becoming $1a^{11}$ and $1a^{12}$; those of the second quartette their first, $2a^1$ and $2a^2$; and the four macromeres divide, producing 3*A* and 3*a* (using the *A* quadrant as a sample). There are now sixteen descendants of the first quartette and eight of the second.

The 32-cell stage brings to the spiral type of cleavage a certain degree of completeness, for three successive divisions have produced three quartettes of ectomeres that represent chiefly ectoderm-forming cells, although larval mesenchyme is also produced from some of the derivatives of the second quartette. The four macromeres subsequently produce two more quartettes of micromeres and are in general endoderm-forming cells. An exception is found in the cell 4*d* in the higher forms having spiral cleavage, for it is the parent cell of the mesoderm.

In later stages some blastomeres are found to divide meridionally, and the necessity appears of a new criterion for numbering the products. In such a case the dextral cell is given the exponent 1 and the sinistral 2. The succession of alternating left and right divisions continues to the 64-cell stage. Later on bilateral conditions develop and the embryo takes on the symmetry of the adult.

Spiral cleavage, as already indicated, occurs in several of the great divisions of the animal kingdom. It first appears in the polyclads. Our knowledge of the cleavage of this group is based especially upon the

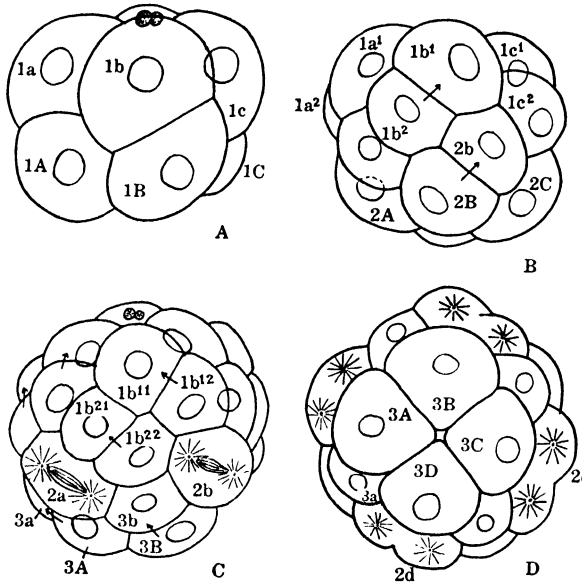


FIG. 32. Cleavage of *Cerebratulus marginatus*. (Redrawn from Korschelt and Heider, after Zeleny.)

A, 8-cell stage; B, 16-cell stage; C, 28-cell stage from side view; D, 28-cell stage from vegetative pole.

study of *Discocoelis* by Lang, of *Leptoplana* by Wilson and of *Planocera* by Surface. Cleavage in these forms follows the general scheme as previously given for all of its main features. There are variations due to differences in the size of the blastomeres and other such factors, but the general plan as described is entirely applicable here. There are certain features in the development of these forms which suggest a relationship to the cleavage of the other flatworms, but there are a great many points of agreement between the method of cleavage of polyclads on one side and that of the annelids and molluscs on the other, and it has been held by the most careful investigators that a true homology exists with respect

to the early development of these forms. The points on which the agreement is observed are sufficiently important that they seem to indicate more than a mere adaptive coincidence on the part of the previous types of the two groups. The formation of three ectomere quartettes which alternate in dexiotropic and laeotropic formations, the agreement in the relationships of the cells of the quartette $1a^2$–$1d^2$ with the primary trochoblasts of the annelids and molluscs, and complete homology in the development of the mesoderm and also in the formation of the ectodermal structures near the animal pole, all seem important in bringing the polyclads into line with the annelids and molluscs.

The nemerteans also conform to the general method of spiral cleavage, and indeed they are almost diagrammatic in the regularity which they show. The studies of Coe on *Micrura*, of C. B. Wilson on *Cerebratulus lacteus*, and of Zeleny on *Cerebratulus marginalis* are sufficient to establish the important features of cleavage in this type. The work on these forms as well as upon others of this group is so similar to that described for other cases that it does not seem necessary to consider it in detail although it is important and of excellent character.

Spiral cleavage is especially developed among the polychaetous annelids. Of special importance are the studies of E. B. Wilson on *Nereis*, of Child on *Arenicola*, as well as the observations of Mead, Treadwell, Nelson, Torrey, and Gerould on other forms of polychaetes. The later stages have been made more clear by the work of Woltereck on *Polygordius*. The cleavage of *Arenicola*, which leads to the development of the trochophore larvae in its fundamental features, corresponds to that of the general type already described. The cleavage axis running from animal to vegetative pole corresponds to the chief axis of the trochophore larva, and although the first cleavages present slight differences in order and in the angles they make with the egg axis the result is the same as in the general type, for by the time of the 8- and 16-cell stages the normal relationships have been returned although there are certain marks of size differentiation among the various blastomeres. The differences which exist between the cell lineage of *Arenicola* and that of the type form are minor differences only. There are, however, some special features about the cleavage of the annelids which deserve mention. In the animal half of the egg there presently develop certain configurations which are especially useful in homologizing the blastomeres of these forms with those of the very similarly cleaving eggs of the gastropod molluscs. The blastomeres about the animal pole arrange themselves to form what is known as the cross of the annelids, and this becomes the chief characteristic of the embryo for the stages immediately following the 64-cell condition. As will be seen by consulting the accompanying

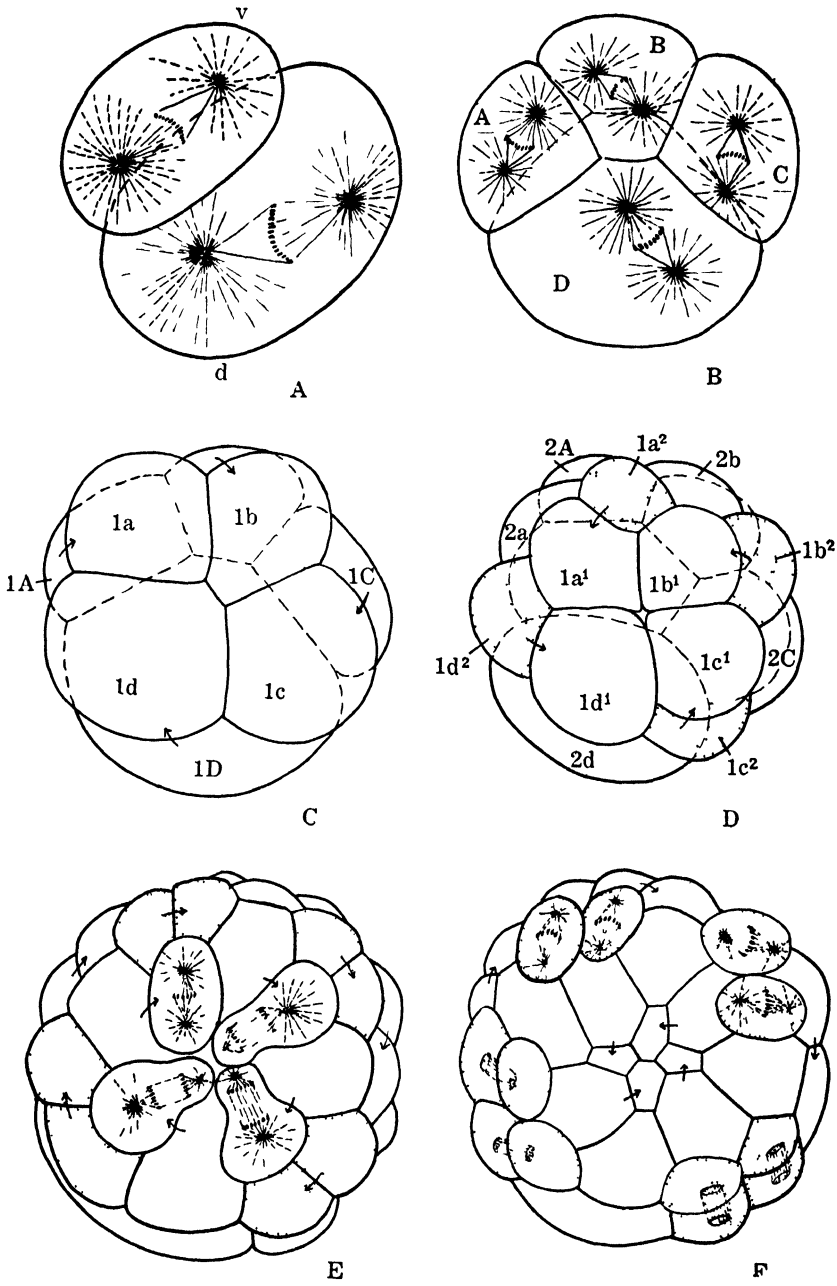


FIG 33 Cleavage stage of *Arenicola cristata* (Redrawn from Korschelt and Heider, after Child)

A, 2-cell stage, B, 4-cell stage C, 8-cell stage D, 16-cell stage Primary trochoblasts are stippled, E, the apical stem cells $1a^{11}$–$1d^{11}$ are dividing to produce in F the apical rosette cells $1a^{111}$–$1d^{111}$ and the stem cells of the cross $1a^{112}$–$1d^{112}$ The primary trochoblasts are $1a^{21}$–$1d^{21}$ and $1a^{22}$–$1d^{22}$

figure there are in each quadrant apical rosette cells, stem cells of the cross and intermediate cells. These cells when the four quadrants are considered together form a very definite picture of a cross, and not only are the cells themselves homologous with the similar condition found in the gastropods but there is a decided similarity in their arrangement, as will be seen by comparing Fig. 33 for *Arenicola* with the similar figure of *Crepidula* (Fig. 29).

The first appearance of the mesoderm in *Arenicola* is from the cell 4*d* which has come to be known as cell *M*. Here again the homology between the annelid and gastropod types is quite complete. The study of cell lineage in the annelids was carried on so thoroughly and the results were so beautifully worked out that there are many other features in the development of this group that call forth the admiration of the observer to the precision of the developmental mechanism. This is determinative cleavage at its best and the specialist in this field of embryology finds a great deal here to interest him.

The cleavage of the Oligochaeta and the Hirudinea with certain modifications shows the general agreement with the type just discussed. These differences are correlated with the differences in the mode of existence between the water-living polychaetes and the other more specialized classes of annelids. In one of these forms, *Clepsine*, a leech, the first study of cell lineage was made by Whitman in 1878.

As already indicated, the most important studies of the spiral type of cleavage have been made in various molluscan eggs. Here there is a wealth of observation and the literature contains many excellent accounts of the cell lineage of these forms. Only a very few can be mentioned including the following. For the Amphineura there are the observations of Metcalf on *Chiton* and Heath on *Ischnochiton*; for the Lamellibranchia those of Lillie on *Unio*. The gastropod eggs have been especially studied as is indicated by the following investigations on the three subdivisions of this class. Conklin on *Crepidula*, Robert on *Trochus* are especially important contributions to our knowledge of the development of the Prosobranchia, as is the work of Heymons on *Umbrella*, Casteel on *Fiona* for the Opisthobranchia, and Holmes on *Planorbis* and Wierzejski on *Physa* for the Pulmonata. Many other forms have been studied, but these are examples of the various conditions found here. It may be noted that the cleavage of *Planorbis* and of *Physa* may be readily followed in the laboratory, at least during very early stages, and they present perhaps the best opportunities for a student to observe this type of development.

Spiral cleavage in the molluscs conforms to the type and indeed might serve as the type, as may be seen from the table summarizing the devel-

opment of *Crepidula*. The importance of the homologies existing between the blastomeres of the annelids and of the molluscs have already been pointed out and need not again be referred to. Certain other interesting features, however, should be noted. One of these is the presence of the yolk lobe described by a number of investigators in heavily yolk-laden mollusc eggs. A similar structure has been described also in some annelids. Here there appears at the vegetative pole in each of the first few cleavages a lobe more or less clearly marked but never completely cut off from one of the blastomeres. It is known as the yolk lobe and is of varying size in different animals. Before each succeeding cleavage it unites again with its blastomere, only to present itself in the interim before the next cleavage. Finally it becomes completely united with the cell *D* and development proceeds. Experiments have been performed in which this lobe has been cut off with the result that the larvae lack the ectoderm of the posterior portion of the body. Another interesting phenomenon observed in some cases of spiral cleavage concerns the relation between the symmetry of the adult and that of the embryo. Crampton pointed out as a result of studying the cleavage of *Physa* that the reversed spiral of the shell of this snail is not a matter of adult life only but is so definitely impressed upon the nature of the species that the direction of the cleavage planes from the third division on is exactly reversed from what is seen in eggs in which there is a shell coiled in a right-handed spiral.

The later stages in the cell lineage of spirally cleaving eggs lead us to a consideration of the methods of gastrulation and of mesoderm formation and as such seem more properly to be considered in the chapters which deal with these matters.

Bibliographic Note

Among the more important accounts of the subjects contained in this chapter are the following: Echinoderms: Korschelt and Heider, Selenka, Boveri, Plough, Morgan (Experimental Embryology); Ctenophores: Ziegler, Metchnikoff; Amphioxus: Cerfontaine, Hatschek; Ascidians: Conklin, Chabry, Castle, Driesch; Frog: Brachet, Morgan; Ascaris: Boveri; Spiral types: Conklin, Holmes, Wilson, Child, Lillie, Robert, Coe, Lang, Surface, Whitman, Metcalf, Casteel, Wierzejski, Crampton. These works are cited in full in the bibliography on page 406.

CHAPTER V

MEROBLASTIC TYPES OF CLEAVAGE

I. SUPERFICIAL CLEAVAGE

Superficial cleavage is found to occur most extensively among the arthropod classes and indeed by many it is supposed to be coextensive with this phylum of animals. There are, however, some other cases which may be properly spoken of as superficial cleavage. Among these latter are the eggs of some coelenterates which are heavily laden with yolk, for example, *Renilla*, hydrocorals and *Alcyonium*. In addition it has been described for the holothurean, *Cucumaria glacialis*.

Furthermore, it is by no means true that all arthropods undergo superficial cleavage. Among the Crustacea there are many cases of total cleavage in holoblastic eggs, and at the other extreme of the phylum the scorpions have discoidal cleavage. Examples of holoblastic cleavage may be chosen from *Branchipus* (Brauer, 1892), *Artemia*, *Lucifer*, some parasitic copepods, the cirripeds and most free-living copepods. These examples include cases in which cleavage is very nearly equal and in others, as the cirripeds, distinctly unequal. One illuminating study has been made on the cleavage of the barnacle *Lepas*, which suggests relationship to the annelid type and even has certain characteristics of the rotifers. In view of the generally assumed fact that the arthropods are derived from annelid ancestry, the case of *Lepas* as studied by Bigelow seems to have unusual importance.

With the exceptions of the forms mentioned the arthropods have superficial cleavage. It will be observed that the diversity of this phylum is so great that many different conditions of cleavage are found here and even among the cases of superficial cleavage there will be seen to be a great variety. To such an extent is this true that we cannot describe as typical of superficially cleaving eggs any one particular form, and, although we may classify superficial cleavage into subdivisions, these are by no means separated from each other sharply but gradations exist between them. Superficial cleavage is characteristic of eggs which are centrolecithal in character; eggs of the centrolecithal type are derived from forms having total and in most cases equal cleavage, while on the other hand they lead to an extreme type of superficial cleavage which is so different from the usual arthropod egg as to

be classified with eggs having discoidal cleavage rather than superficial. Reference is made to the eggs of the scorpion which are without question derived from the extreme superficial type but which as a result of parallel convergence resemble most clearly the discoidal type of vertebrates. In centrolecithal eggs the cleavage nucleus is usually at the center of the egg and is surrounded by the mass of yolk spherules and fat droplets. Penetrating this mass in every part is a network of cytoplasm which is continuous with the central cytoplasmic portion around the nucleus and also with a very thin superficial cytoplasmic layer on the outside of the egg. This latter layer is in some cases so delicate as to be difficult to make out, whereas in other cases it is clearly visible. It is to be regarded as characteristic of the centrolecithal egg, however. The shape of these eggs varies from spherical or elliptical to a greatly elongated, almost cylindrical type, but they are seldom "egg shaped" in the sense that they have one blunt and one pointed end. There is practically no trace of an axial structure which is characteristic of so many other egg types.

As a first type of superficial cleavage we may take up one of the insects, for in this form it is especially clearly developed. The well-known figures of the cleavage of *Hydrophilus* suggest the early course of this type of development. The cleavage nucleus lying at the center of the egg in the midst of its plasma island divides into 2, 4, 8, 16 nuclei, each surrounded by a small portion of cytoplasm and each somewhat separated from the others in the form of amoeboid or star-shaped areas. Actually all are in communication through the plasma network that penetrates the yolk mass. At this stage and for some time later the egg actually is a syncytium, for there are no definite cell boundaries marked off around any nucleated areas. It has been the practice on the part of some writers to speak of these areas as cleavage cells, although they are not cells in the sense of other types of cleaving eggs. After a few divisions these cleavage cells come to form a layer within the yolk which by one continued division tends to approach the periphery of the egg. At length they unite with the superficial cytoplasmic layer in *Hydrophilus* near the middle of the egg first and at the ends only some time later. Then cytoplasmic boundaries become visible and the cleavage is truly superficial, although of course only partial, since the divisions do not extend through the yolk mass.

In insect eggs it frequently occurs that some of the cleavage cells do not take part in the formation of the blastoderm but remain in the yolk while the others pass to the surface. They take on special functions for the assimilation of the yolk substance and become known as the *vitellophages*. In some cases incomplete cell boundaries seem to be formed

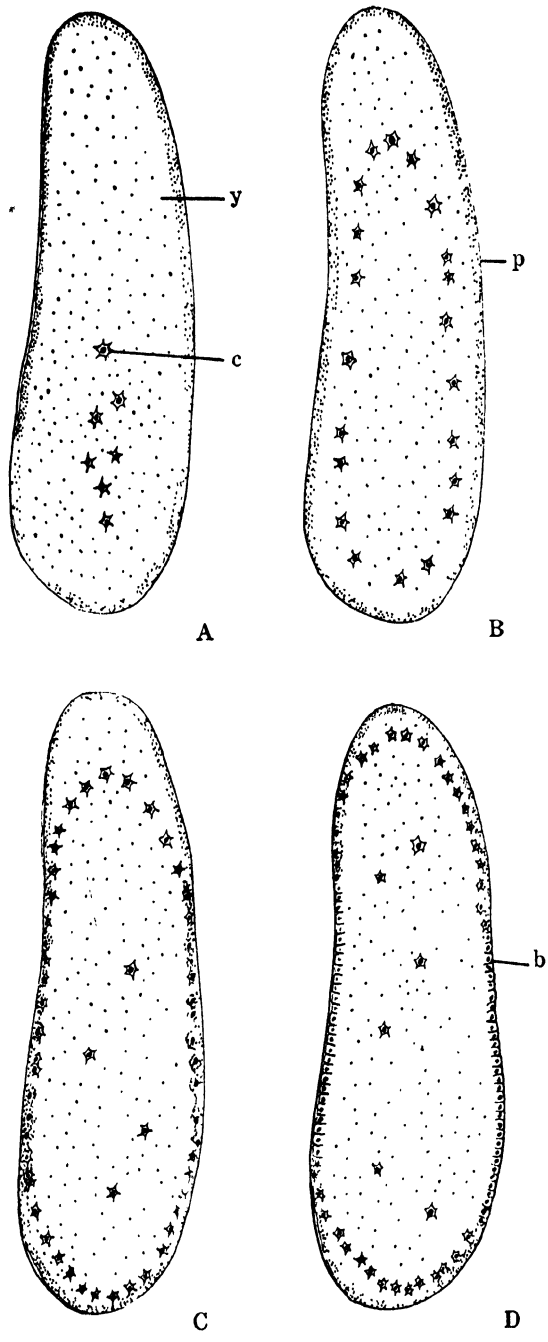


FIG. 34. Blastoderm formation in the water beetle *Hydrophilus*. (Redrawn from Korschelt and Heider, after Heider.)

b, blastoderm cells; c, "cleavage cells"; p, peripheral layer of protoplasm; y, yolk.

around these vitellophages, giving rise to the so-called secondary yolk cleavage. The vitellophages are to be regarded as endoderm, and for that reason some investigators have described superficial cleavage as leading directly to the formation of a gastrula-like stage since two kinds of cells, ectoderm and endoderm, are formed about the same time. This view has not been widely adopted.

Superficial cleavage usually results in the formation of blastomeres of equal size and cleavage is indeterminative.* Inequalities appear very early in some forms, however, and there are many variations from the forms of the cleavage as already given for *Hydrophilus*. These variations seem at first sight to correspond in a general fashion to the systematic groups of the arthropods, for the crustacean eggs are distinguishable from the spiders and from the insects, by certain characteristic features; but these differences do not lend themselves to accuracy of statement nor form a satisfactory basis for classification. It is wiser therefore to classify the superficial cleavage under the following heads: Group 1. Cleavage at first total but later superficial. Group 2. Cleavage purely superficial. Both of these groups in their turn may be divided into two subdivisions in one of which the blastoderm forms on all sides simultaneously and in the other on the ventral side precociously.

1. Cleavage at First Total but Later Superficial

This type of cleavage, which is typical of many crustaceans, begins with the division of the plasma island into 2, 4, and perhaps 8 blastomeres about which all cell boundaries are cut off at once. Cleavage during this period of the development and for some successive cell divisions thereafter is total and approximately equal. Presently there comes a time when the furrows at the surface are unable to cut entirely through the egg, or if they do the yolk masses at the center must fuse together and cleavage becomes superficial with a cellular region sharply separated from the yolk mass. The blastula consists of a layer of superficial cells of equal size and an inner yolk mass replacing the cleavage cavity. In many cases there is a distinction between the central-lying yolk mass and the yolk which formed the inner end of the blastomere. They were left incomplete by the failure of the furrows to penetrate to the center of the egg. These inner ends are spoken of as the yolk pyra-

* Nevertheless, experiments on the egg of the house fly, *Musca domestica*, made by Reith (1925) show for this animal (and make it probable for others having this type of cleavage) that the egg is highly determinative. The embryo is fore-shadowed in the cytoplasm of the egg and the regions are clearly organized in relation to their future destiny. Add to this the very definite nuclear determination which the experiments on the genetics of *Drosophila* have demonstrated, and it is clear that the eggs of Diptera can by no means be called indeterminative.

mids (sometimes as Rathke's yolk pyramids). The significance of these structures is not entirely clear but their appearance is characteristic. This type of cleavage easily suggests a transition from the holoblastic eggs of other Crustacea to the form in which cleavage is purely superficial. This type of cleavage, as already pointed out, occurs in two subdivisions:

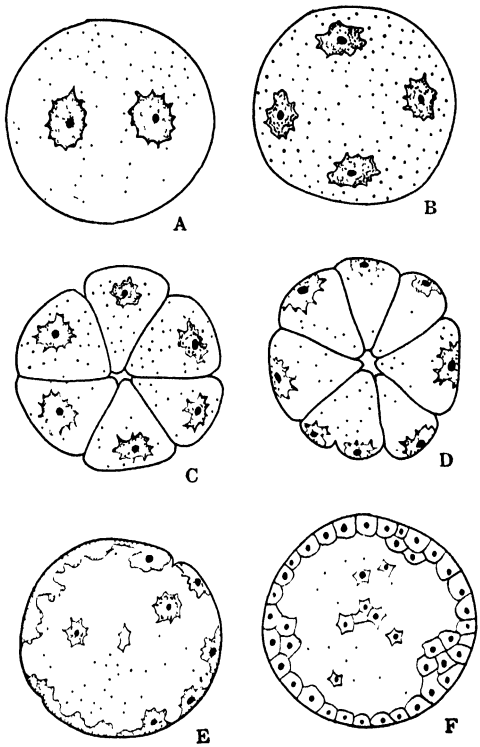


FIG. 35. Cleavage of *Macrotoma vulgaris*. (Redrawn from Korschelt and Heider, after Uzel.)

A, two cleavage nuclei; B, four cleavage nuclei; C, section through 16-cell stage with blastomeres completely separated from one another; D, similar section through 32-cell stage. E, F, later stages in the formation of the blastoderm, the yolk mass in the center having fused.

a. Eggs in which the superficial cleavage leads to blastoderm formation simultaneously all over the egg. To this subdivision belong the eggs of those free-living copepods which are not holoblastic, some parasitic copepods, the ostracods, spiders, and some isolated forms in various groups of the arthropods.

b. Eggs in which the blastoderm develops early on the ventral side, a condition which foretells the position of the forming germ streak. The

decapods in part come under this subdivision as do some of the amphipods as well as other scattering forms.

2. Eggs with Purely Superficial Cleavage

Eggs which come under this subdivision carry still farther those tendencies which are beginning to be expressed in the previous group.

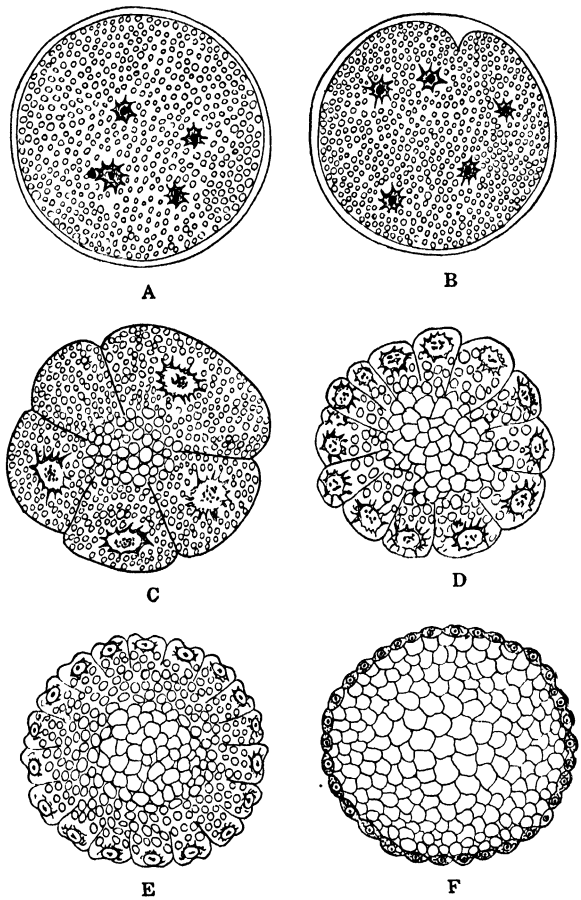


FIG. 36. Cleavage of the crab *Dromia*. (Redrawn from Korschelt and Heider, after Cano.) Successive stages showing "cleavage cells," the yolk pyramids, and the blastoderm.

The cytoplasmic portion of the egg is quite unable to control the yolk mass and therefore the cleavage cannot be complete. The central-lying nucleus with its surrounding plasmic layer divides several times but no cell boundaries are formed by the separate areas. The only indication of division now to be seen is the occasional presence of superficial furrows

which do not penetrate far into the yolk. As the cleavage nuclei increase they approach the surface and furrows become more pronounced. The result is a blastoderm with the cell boundaries cutting in to the yolk although not completely delimiting definite cell bodies from it until a later stage when the blastoderm takes on the form of the definite layer. Cleavage of this type occurs among the Crustacea commonly, although it is much more widespread in the Malacostraca than in the Entomostraca. In some of the latter, however, this condition has been described. In the Malacostraca it is the most common form, occurring in the Decapoda (with the exception of those previously mentioned), *Nebalia,* Cumaceae, the Schizopoda, and Isopoda. It is found also in some of the mites, in nearly all insects, in the Myriapoda and in the Onychophora. With a distribution so widespread as this it is obvious that many variations will be found, although in general the description of *Hydrophilus* as already given is quite applicable. There are cases in which the superficial plasma layer is so thin as to be almost absent and other cases in which it is very definitely marked and these varying conditions have an effect upon the appearance of the blastoderm. There is also variation in the extent to which the blastoderm is formed over the surface of the egg. This latter feature is the basis for the further classification of these eggs into two types as in the previous group.

a. Eggs which have simultaneous formations of the blastoderm all over the egg. Most of the cases of purely superficial cleavage come here, specifically the Cladocera, most decapods, the myriapods, most insects and many other scattered forms.

b. Eggs with precocious development of the blastoderm on the ventral side. Here belong *Nebalia,* the schizopods, the Cumaceae, the isopods, and some decapods, including the lobster. The restriction of the blastoderm to a small portion of the surface of the egg is so marked in some of these forms that it was formerly the practice of some investigators to regard these as cases of discoidal cleavage. We do not at the present time, however, so regard them. Nevertheless this subdivision of superficial cleavage clearly leads to the conditions found in the scorpions as an extreme case, and these latter forms are commonly included with the cephalopods and meroblastic vertebrates under the head of discoidal cleavage.

II. DISCOIDAL CLEAVAGE

Discoidal cleavage is the type generally found among the meroblastic vertebrates, and it occurs also in the ascidian group, the pyrosomes, and in two separate invertebrate classes, the cephalopods and the scorpions. This distribution suggests what is found to be the case, namely that

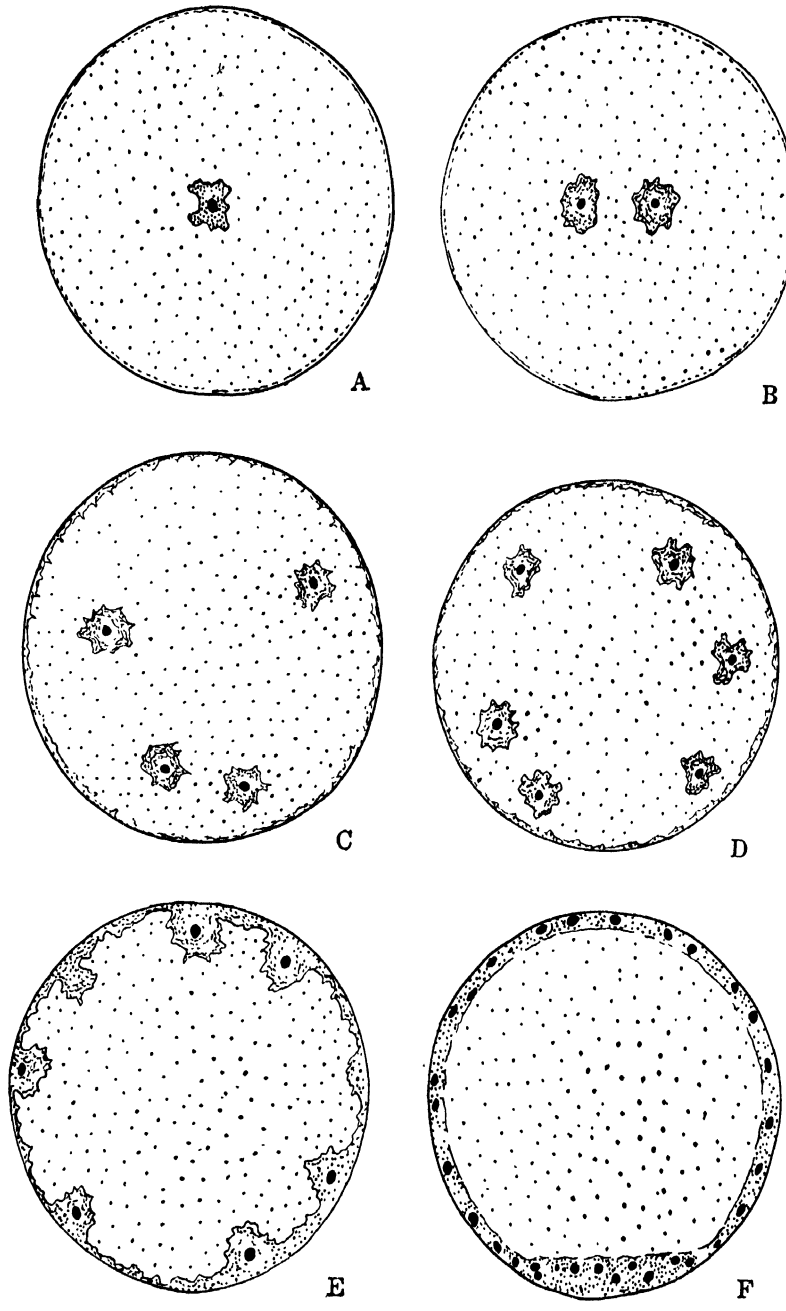


FIG. 37. Cleavage of *Campodia staphylinus*. (Redrawn from Korschelt and Heider, after Uzel.)

Sections showing cleavage and formation of the blastoderm. In F the thickening on the ventral side which will give rise to the blastoderm is to be noted.

discoidal cleavage is derived from simpler, unlike, and unrelated cleavage forms. It may be assumed that the vertebrate type is derived from an extreme case of telolecithal egg in which there is sufficient yolk present to prevent the cleavage furrows from cutting entirely through the egg. This results in a certain degree of opposition between the animal or protoplasmic portion of the egg as contrasted with the vegetative or yolk region. Similar relationships obtain for the other groups having discoidal cleavage except the scorpions in which the discoidal type is derived, as may be inferred from the preceding discussion (page 68) from the extreme case of purely superficial cleavage. Only a small portion of the egg surface is involved in the formation of the embryo body in the superficial forms mentioned.

It is evident that not much in common is to be expected in the elemental features of these two characteristic modes of discoidal cleavage.

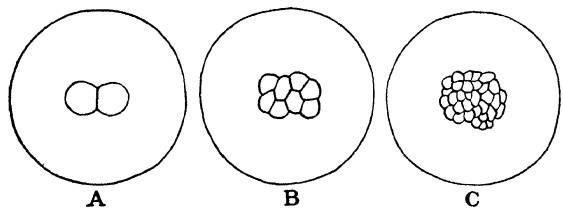


FIG. 38. Germ disc of the scorpion *Euscorpius carpathicus*. Polar views. (Redrawn from Korschelt and Heider, after Brauer.)

It was formerly said that discoidal cleavage occurs also in some crustaceans but it seems wiser to regard these as within the limits of superficial cleavage than as examples of the true discoidal type. Because of the separate origin of these cleavage types it will be convenient to begin this discussion with the cleavage of the scorpion.

1. Scorpions

Only one important piece of investigation, namely the study of the cleavage of *Euscorpius*, by Brauer, is available upon which to base our knowledge of this type. The egg is richly yolk laden and the protoplasmic portion is limited to a disc-shaped area which corresponds to the point of attachment within the egg follicle. With the exception of perhaps a very thin filament over the surface of the egg no other protoplasmic portion can be demonstrated. Segmentation is limited to the protoplasmic disc which divides with fair regularity. About the third or fourth cleavage there is oftentimes what seems superficially to be a bilateral arrangement of the blastomeres, but the appearance is only superficial for the cleavages from here on become quite irregular. The

result of cleavage is a round or oval blastodisc consisting of at first but a single layer of cells. The single-celled condition does not remain long because a number of cells wander down below the surface from a definitely recognizable white speck. This speck is important in determining the plane of symmetry and the hinder end of the embryo. The first cells which wander in are transformed into yolk cells and although they form an irregular layer they take no further part in the development of the embryo. Very shortly after the migration of the yolk cells a group of cells becomes differentiated as a result of ingrowth from the same region and is said to give rise to all the genital cells. True endoderm

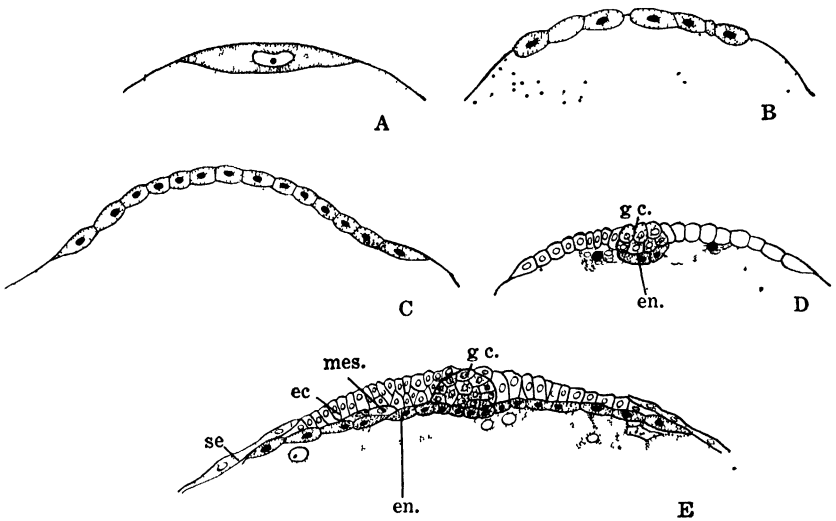


FIG 39 Sections of stages similar to Fig 38, and of two later stages.

A, immature germinal vesicle, B, early cleavage, C, blastoderm formed, D, beginning of germ layer formation showing segregation of the germ cell g c and of the endoderm, en E, later stage in germ layer formation ec ectoderm, mes mesoderm, se serosa

cells also appear in like manner at the same time. Brauer holds that the endoderm cells do not contribute to the formation of yolk cells nor do the yolk cells take part in the formation of the endoderm.

Mesoderm arises from a division of ectoderm cells near those which are to produce genital cells. The subsequent development of the blastodisc shows the formation of a segmented germ streak which gradually develops into the typical body form of the scorpion and which grows finally to include entirely the yolk in the middle region of the gut. The forming embryo is suggestive of certain stages in the later development of eggs having superficial cleavage but it is likewise very similar in a number of particulars to the conditions found in vertebrate embryos

which develop by discoidal cleavage. There are clearly produced in these different groups similarities which are purely embryonic and therefore transitory; they have no evolutionary significance.

2. Cephalopods

The cleavage of cephalopod eggs differs in many particulars from that of other molluscs and also from the forms within the groups having discoidal cleavage. The differences seem to be in keeping with the peculiarities of egg structure, which are pronounced.

The eggs are laid in large numbers, usually together, and the mass may assume various forms. There is always a protective covering which is the means of attaching the mass of spawn. In *Sepia* the eggs are dis-

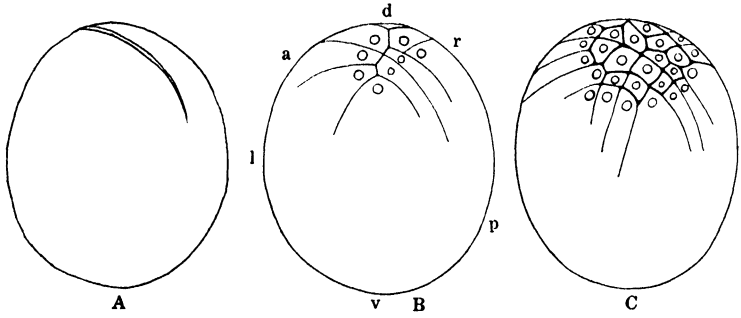


FIG. 40. Eggs of the squid *Loligo pealii*. (Redrawn from Korschelt and Heider, after Watase.)
d. dorsal, v. ventral, a. anterior, p. posterior, r. right, l. left.

crete and each is in its own capsule. In *Loligo* many are laid together in a gelatinous tube and numerous tubes are attached in one mass, known to the sailors as "dead men's fingers." Fertilization follows after various peculiar types of copulation in which spermatozoa are transferred in spermatophores; fertilization is internal in some groups, and external in others, of which the squids mentioned are examples.

The eggs themselves are relatively large for marine eggs and all contain very much yolk. The development of the squids, particularly of *Sepia* and of *Loligo*, are best known and will be used from here on as examples of this form of development. *Sepia* eggs have the size of ordinary peas, whereas those of *Loligo* are much smaller, but even they are larger than the eggs of most marine molluscs.

Cephalopod eggs have been studied by numerous investigators, but our knowledge of the cleavage depends chiefly upon the work of Vialleton on *Sepia officinalis* and of Watase on *Loligo pealii*. The eggs of *Loligo*, at the time they are ready for cleavage, are somewhat oblong in shape,

one end being more pointed than the other. The egg consists of a mass of fine granules of food yolk surrounded entirely by a thin protoplasmic layer. This layer is thicker at the pointed end of the egg where the germ disc will form. Thus this egg represents an extreme telolecithal type and is a perfect example of meroblastic cleavage.

The uncleaved egg from surface study alone gives evidence of bilateral symmetry which bears a definite relation to the subsequent history of the embryo, and it is possible to distinguish an anterior border from a posterior as well as right from left sides of the organism. The germ disc end of the egg corresponds to the dorsal side of the embryo and the

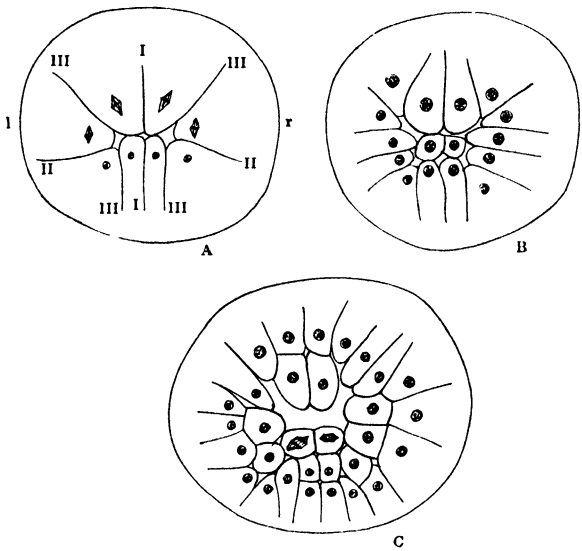


FIG. 41. Germ disc of the squid *Sepia officinalis*. (Redrawn from Korschelt and Heider, after Vialleton.)
I to III, indicates direction of successive cleavage planes.

yolk pole to the ventral side. On the anterior side the germ disc extends farther down toward the equator than upon the opposite side, but it is symmetrical with respect to right and left. The animal pole of the egg is slightly eccentric to the center of the germ disc, being nearer its posterior edge. Here in *Loligo* may be seen three polar bodies in the perivitelline fold inside the chorion. The egg is surrounded by chorion which is secreted by the follicular epithelium and is often very tough. The sperm enters through a micropyle in this chorion.

The early cleavages are limited to the germ disc. The first furrow corresponds to the median plane of the future embryo, dividing the egg into right and left halves. In the center of the blastodisc it cuts in

deeply, dividing the entire protoplasmic cap at this point, but toward the edges of the disc it becomes shallower until it is a mere groove and finally disappears entirely. The successive cleavages behave in a similar manner. Thus cleavage is incomplete even with respect to the protoplasmic layer. The second furrow is at right angles to the first, and like it, fades away into the thin protoplasm. The later furrows which extend outward likewise fade away and the cells thus formed are incompletely separated peripherally. This gives rise to the distinction made by Vialleton of the products of cleavage into blastomeres and blastocones,

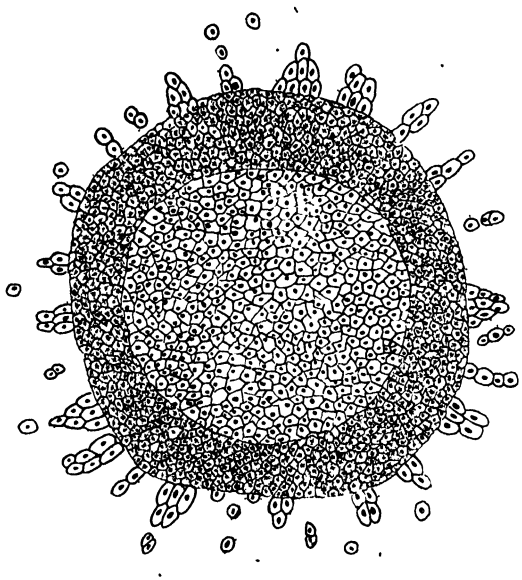


FIG 42 Older germ disc of *Sepia officinalis* from the beginning of germ layer formation The darker cells indicate the region where the disc is of several cells in thickness Later stage than FIG 41

the term blastomere being applied only to those cells which are completely cut off, while the peripheral ones which have no outer boundary are spoken of as blastocones.

In the preceding types of cleavage we have seen that the cleavage cells have usually received special designations. In the present case, on the other hand, it has been found more convenient to indicate a distinction between the various cleavage cells by numbering the furrows which separate them, the first furrow lying in the median plane and indicated by the polar body numbered I. The second furrow, II, is at right angles to it. The third furrow likewise is meridional but it divides

the two anterior blastomeres and blastocones equally and runs obliquely to the previous furrows. The two hinder blastocones are divided very unequally, for furrow III is almost parallel to the median plane and cuts off two very narrow segments. In the fourth division the inner ends of these narrow segments are cut off to form the first two blastomeres by the transverse plane. The remaining six cells are again divided in a direction which is correctly spoken of as meridional, although the various planes are not entirely regular and do not converge at the center

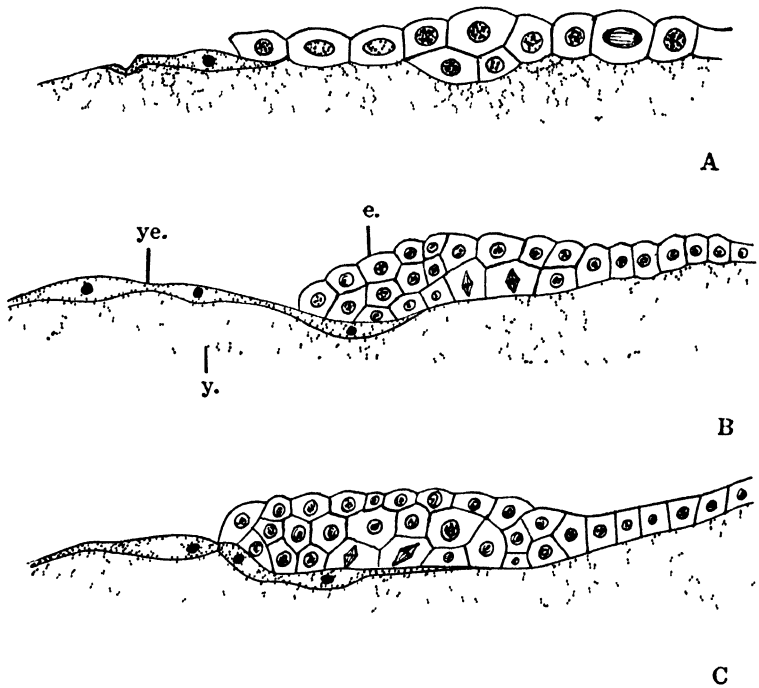


FIG. 43. Sections through the edge of the germ disc in successive stages. B, corresponds to Fig. 42. y, yolk, y e, yolk epithelium, e, embryonic cells.

of the disc. By this time the bilaterality which existed in the uncleaved egg has become very apparent, each half of the egg consisting of seven blastocones and one blastomere. The 32-cell stage is reached partly by meridional and partly by transverse furrows. Numbering the blastocones of each half of the egg from the anterior side backwards as one to six, the following divisions occur. Number one divides transversally, producing a blastomere and a blastocone. Numbers two and three divide meridionally, each producing two blastocones. Numbers four and five each cut off a blastomere by a transverse division, and number six divides meridionally. The posterior narrow pair of blastocones again

divide transversally, cutting off blastomeres, and the two first blastomeres likewise divide. The 32-cell stage thus consists of fourteen blastomeres and eighteen blastocones symmetrically arranged. Thus the center of the disc comes to be filled up with a compact layer of blastomeres radiating from which are the blastocones.

Subsequent cleavages bring about a decrease in the size of the blastomeres and are responsible for their regular disposition. The germ disc thus comes to consist of a one-layered plate of polygonal cells with peripheral blastocones passing over into the mass of formative yolk. Gradually these peripheral cells detach themselves from the germ disc and scatter about the surface of the food yolk. It is claimed that they even wander beneath the cells of the germ disc and gradually form a layer which spreads over the entire yolk. The solid blastodisc at length becomes many-layered and by its extension grows over the layer of yolk cells derived from the blastocones.

The relationship of the primary germ layers of the cephalopods is complicated, and the manner in which the embryo body is formed is not properly a subject for detailed discussion here. The reader is referred to the descriptions of these processes in more extensive works.

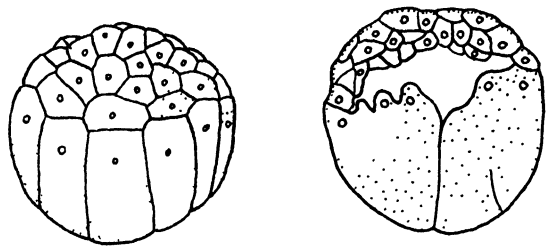
3. Vertebrates

The most characteristic and best-known cases of discoidal cleavage occur among the chordates. There is a single example among the lower chordates, that of the pyrosomes, which has some resemblance to the discoidal type as found in teleost eggs. In pyrosome eggs, however, certain cells around the edge of the disc recall the blastocones of the cephalopods.

Among the vertebrates proper discoidal cleavage occurs widely in scattered groups. It is found in the myxinoids, the elasmobranchs, teleosts, Gymnophiona, all reptiles and birds, in the monotremes, and it has been recorded for some marsupial eggs. In the opossum, however, according to the accurate and clear description of Hartman, cleavage is certainly not discoidal, nor is that of *Dasyurus* as figured by Hill. Thus it occurs in five classes of vertebrates, and possibly in the sixth; in some of these it is the characteristic method and in others it is quite exceptional. This distribution points out, as has been noted previously, that there is no taxonomic or phylogenetic significance in the classification of cleavage types. An inspection of the list as given shows discoidal cleavage alongside of the holoblastic type in many of these groups. The myxinoids are discoidal while the lampreys have holoblastic cleavage in the class Cyclostomata. Among the fishes, elasmobranchs and teleosts are extremely discoidal, but the dipnoid and the ganoid

fishes are holoblastic in type. The typical amphibian egg is holoblastic, but the Gymnophiona show discoidal cleavage. Instead of having taxonomic significance, the distribution of discoidal cleavage is related entirely to the amount of yolk present in the egg.

The relationship between the holoblastic and meroblastic types of cleavage will be best understood by recalling the cleavage of the frog egg (Figs. 22 and 26). From the condition found in the frog egg we may pass by a consideration of the cleavage of *Acipenser*, the sturgeon, *Amia*, the bowfin, and *Lepidosteus*, the gar pike, to those found in the teleost egg. That is, we pass from the telolecithal egg of the amphibian or lamprey by increasing degrees of separation of yolk and protoplasmic portions to the meroblastic type of the modern teleost.



A B

FIG. 44. A, cleavage stages of the sturgeon *Acipenser ruthenus*, B, section through same. (Redrawn from Ziegler, after Whitman and Eycleshymer.)

The cleavages of the lamprey and of the frog show remarkable similarity. In both there is a large proportion of yolk, but it is still sufficiently separated into spherules for the cytoplasm to cut entirely through it. Cleavage is total and unequal, the first two furrows being meridional and separating the eggs into four blastomeres. The third furrow is latitudinal. In *Petromyzon* the fourth furrows mark the beginning of irregularities in cleavages whereby the cells of the animal half cleave more rapidly than the vegetative half, some of the furrows not being completed until very late.

In the sturgeon, according to the description of Dean, the first cleavages separate the blastomeres but do not penetrate deeply into the yolk. The egg in the late segmentation stages shows a protoplasmic cap of small cells of irregular size and outline. Horizontal cleavages have also occurred and meridional cleavages have separated off marginal cells in an irregular manner so that the surface of the yolk half of the egg becomes subdivided into many-sized polygonal cells. While the protoplasmic cap of small cells constitutes a blastodisc, it does not include all the protoplasmic portion of the egg, as in the case of the true discoidal

types. The cells adjacent to it are connected with the yolk. The blastodisc proper, however, is separated from the yolk by the cleavage cavity, the floor of which consists at first of a few irregular yolk-bearing cells. There is no sharp distinction between these cells and the yolk which they are doûbtless helping to elaborate into nutritive supplies for the growing blastodisc. Obviously the blastula here, which consists of a blastodisc grading into shallow cells covering the yolk mass and separated by the segmentation cavity from the yolk-laden cells beneath it, has points of marked similarity with the telolecithal type of the lamprey.

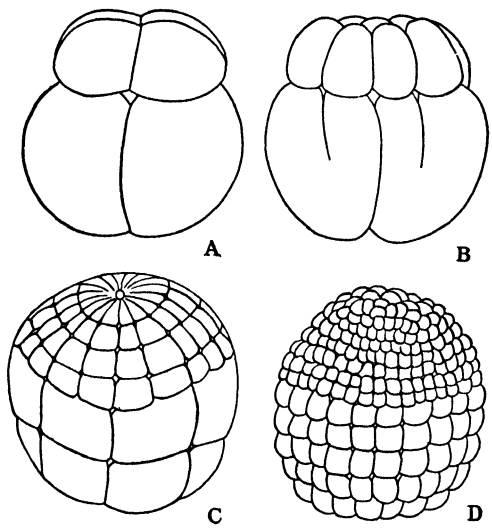


FIG. 45. A, B, cleavage stages of *Petromyzon fluviatilis*; C, D, cleavage stages of *Petromyzon planeri*. (Redrawn from Ziegler, from Hatschek.)

The inner yolk-containing cells of the latter are not represented in the sturgeon, in the yolk of which no cell boundaries are to be distinguished although the outer polygonal cells of the yolk hemisphere possess dividing nuclei. The formation of the body of the embryo is more distinctly limited to the blastodisc in the case of the sturgeon.

The next step toward the meroblastic type is seen in *Amia*. Here the cleavage furrows begin much as in the previous cases but the furrows are more retarded in spreading over the egg surfaces. While at first the meridional furrows are making their way toward the opposite pole, four vertical furrows appear near the apical pole and extend downward. The next cleavage is the meridional one, cutting off thus a group of eight micromeres. At the next division these then divide into a superficial and a deeper segment and the macromeres divide by vertical furrows. A new latitudinal furrow cuts off additional micromeres and

there is thus formed an apical disc limited to one end of the egg and much larger cell areas over the yolk portion; in the latter portion some of the developing furrows never become entirely completed. In *Lepidosteus* the protoplasmic cap is still more sharply distinguished. The early furrows spreading downward over the egg's surface never reach much

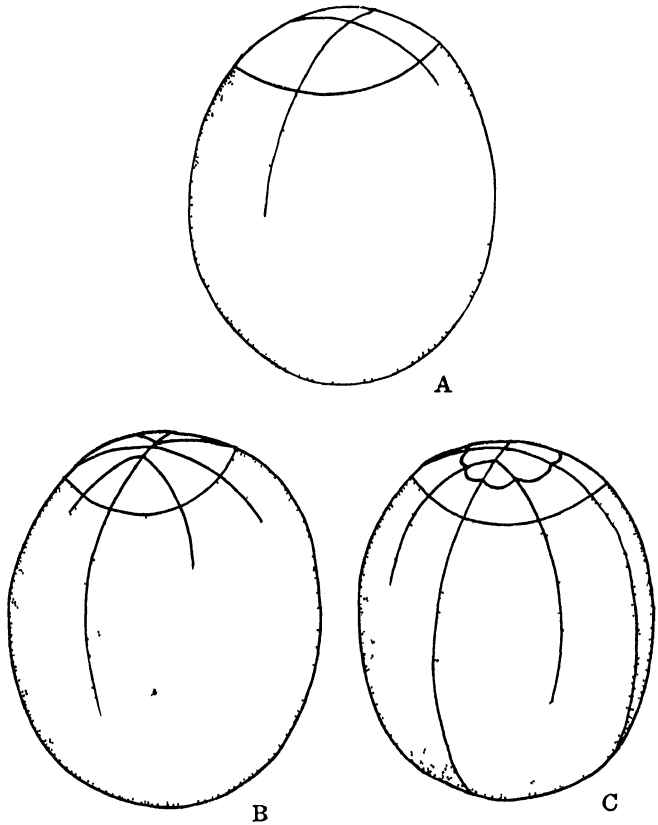


FIG 46 Cleavage of *Amia calva* (Redrawn from Ziegler, after Whitman and Eycleshymer)

beyond the equator so that the yolk hemisphere does not normally segment at all, that is, it more nearly attains the meroblastic condition. The furrows which extend from the blastoderm over the yolk mass seem in later stages to disappear, and the protoplasmic layer in connection with the yolk contains numerous nuclei forming the so-called syncytium. These transitional stages bring us to the true discoidal cleavage as shown, for example, in teleosts.

Eggs in which the distinction between the yolk and protoplasm is

very sharply apparent have meroblastic cleavage, in the cases under consideration here, of the discoidal type. The first group of the vertebrates in which discoidal cleavage occurs is the Myxinoidae. Of the myxinoids the development of *Bdellostoma* is best known. It has been studied by a number of investigators, notably by Doflein and by Dean. The egg is quite large, being in the neighborhood of an inch long and shaped something like a banana. It is well filled with dense yolk and the germinal disc is limited to one end. Its cleavage is very similar to that of the bony fishes which are shortly to be described. The first two furrows are approximately meridional, but irregularities begin with the direction of the third furrow and very shortly the arrangement of the furrows is that of a network enclosing the blastomeres. The outer cells are in continuation with the yolk and form a layer known as periblast. The divisions continue and the blastoderm comes to consist of several layers forming a cap covering the animal pole of the egg and gradually growing farther back over the egg. No blastocoele has been observed. The further development of the blastoderm is not entirely symmetrical, for the cells become more numerous on one side and on this side a separation of the thickened edge into two layers begins which represents the process of gastrulation in that the endoderm is separated off and with it mesoderm. A gastrula cavity is not present. Thus there is no definite end to the processes of cleavage and blastula formation after which gastrulation may be said to have begun, for the proliferation which permits the separation of the germ layers is entirely continuous with the cleavage processes. This type of development is seen to be totally different from that of the lampreys, which is so much like that of the amphibians as to require no special description.

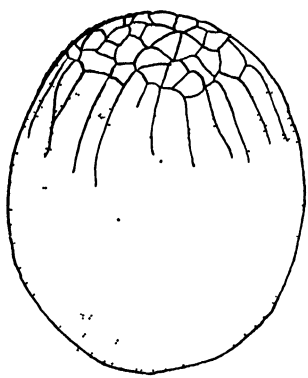


FIG 47 Egg of the gar pike *Lepidosteus osseus* in cleavage (Redrawn from Ziegler, after Eycleshymer)

So far as known all selachian eggs are extremely meroblastic and have discoidal cleavage. If a member of this group of fishes should be found with small holoblastic eggs, it would be a matter of considerable interest from the standpoint of the gradual adaptations which accomplish those modifications that are secondary to yolk accumulation. Dean has found a condition in the shark, *Cestracion*, which suggests the approach of cleavage to the total, unequal type as described for *Amia* and *Lepidosteus*. Among the selachians are found genera which are viviparous as well as others which are oviparous. The former include, among others,

the following genera: *Hexanchus, Notodanus, Acanthias, Galeus, Squalus, Mustelus, Carcharias,* and *Torpedo.* The egg-laying selachians include *Scyllium, Pristiurus, Cestracion,* and *Raja.* The oviparous species produce eggs enclosed in a horny characteristic shell oftentimes equipped with long processes by which they are attached to the water plants. The egg itself is spherical or ellipsoidal and consists of a germ disc lying upon a very large yolk mass. The yolk is of the consistency of a rather

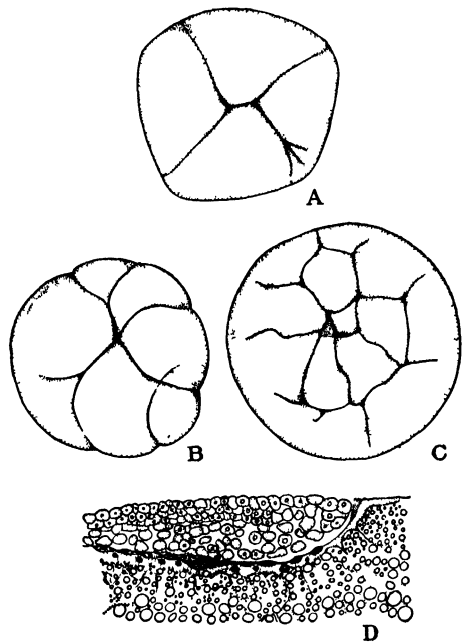


FIG. 48. Cleavage stages of *Bdellostoma stouti.* (Redrawn from Ziegler, after Dean.) A, B, C, surface views; D, section through edge of blastoderm showing dorsal lip.

ropy liquid, pale yellowish with perhaps a greenish cast. Our knowledge of the early development of the selachian goes back chiefly to Ruckert.

In a number of selachians the fertilization process is completed and the fusion nucleus undergoes two mitoses before any cytoplasmic division is to be seen, although there are exceptions to this condition. The first furrow then makes its appearance as a slight groove across the disc and a second follows more or less at right angles to it. It is an interesting deviation from the expected sequence of events that oftentimes the second furrow corresponds to the first nuclear division. The divisions continue, and by the time the 16-cell stage has been reached the furrows separating the blastomeres form a network over the surface of the egg.

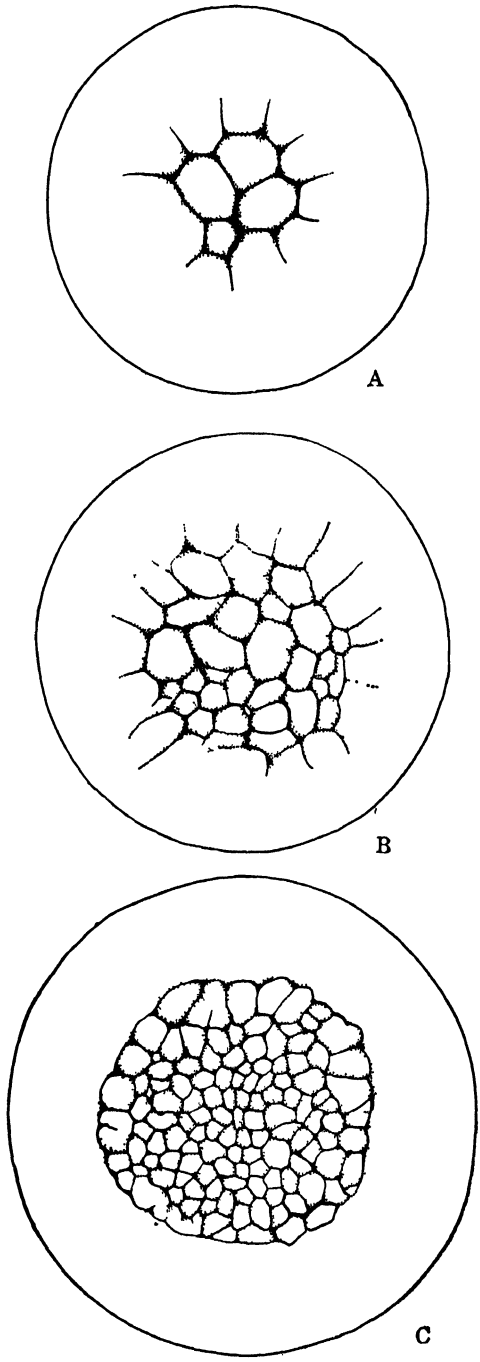


FIG. 49. Cleavage of A, *Torpedo ocellata* (16 cells), B, C, *Scyllium canicula*, the dog shark (64 and 145 cells respectively). (Redrawn from Ziegler, after Ruchert)

The direction of the cleavage spindles in the fifth division is such that part of the cells are cut off under the others and of the 32 blastomeres, one sees at most only 20 to 24. In each case the peripheral divisions do

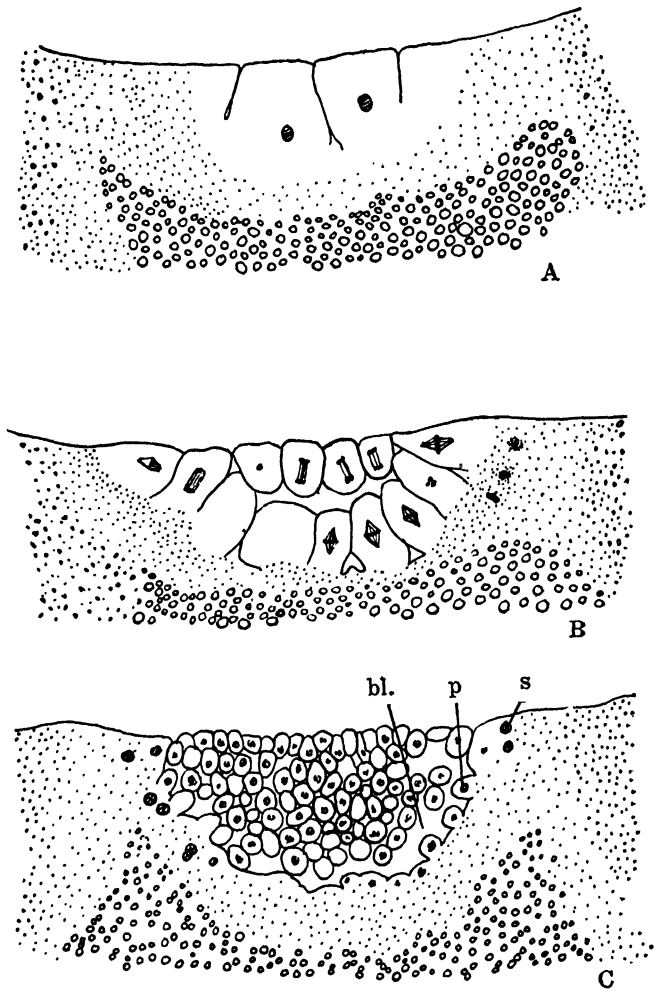


FIG. 50. Sections through 16- 64-cell stages and through blastoderm at the end of the cleavage period. (Redrawn from Ziegler, after Ruchert.)

In Fig. C the posterior is toward the right, and the blastoderm, cleavage cavity, bl., periblast cells, p., and supernumerary spermatozoa, s., are shown.

not entirely cut off the corresponding cells but some remain in connection with the yolk by means of the continuous protoplasm which surrounds the blastodisc. The progress of cleavage now serves always to increase the number of free blastomeres and the cells below the surface likewise

become more numerous. For example, in a *Torpedo*, in which the blastodisc consists of 128 cells, it is said that about 70 of these are at the surface. In more advanced stages of cleavage the blastocoele appears at first as a space beneath the growing disc. This completes the formation of the blastula.

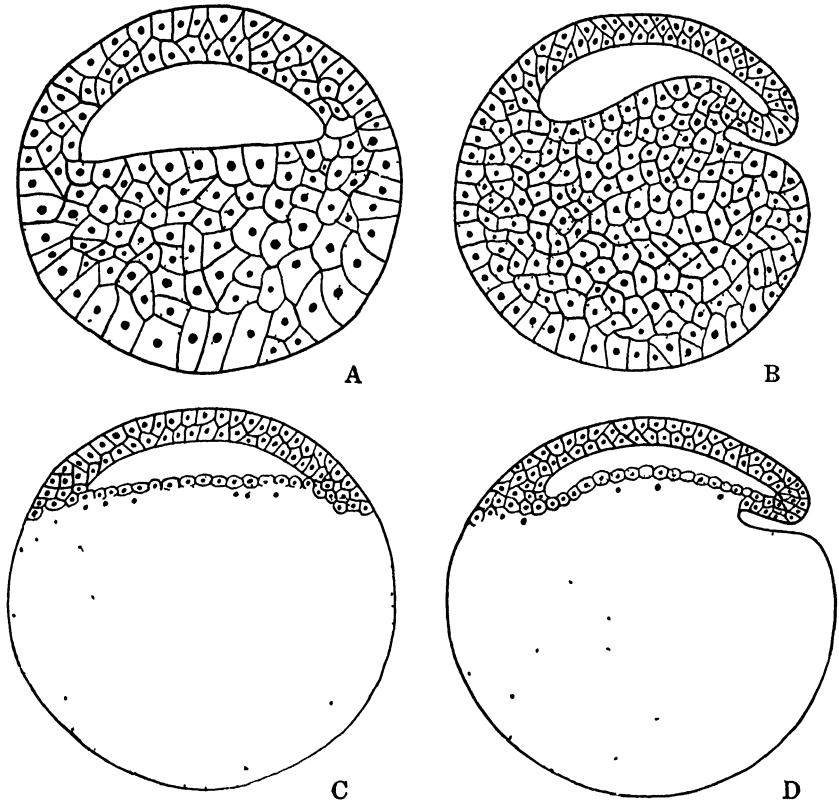


FIG 51 Comparative diagrams of holoblastic and discoidal eggs (Redrawn from Ziegler, after Boaz)

A, B, blastula and gastrula of the amphibian, C, D, blastula and gastrula of selachian

During the formation of the blastula there are to be observed around its edge, on the floor of the blastula cavity, nuclei scattered here and there singly or in groups. These differ in appearance from the nuclei of the blastomeres, in that the chromatin is arranged in thicker masses and stains more darkly. They may be spoken of as yolk nuclei. The yolk nuclei are of double origin. The peripheral blastomeres which are in communication by means of a common circular band of protoplasm produce some of these yolk or periblast nuclei. The remaining yolk

nuclei are derived from supernumerary spermatozoa which enter the egg at the time of fertilization. These yolk nuclei constitute a syncytium, a layer of protoplasm with scattered nuclei which surrounds the edge of the blastoderm and is continuous with the yolk. They are concerned with the nutritive changes involved in utilization of the yolk as food material. They disappear in the later stages and take no further part in the formation of the embryo proper.

The homology of the discoidal type of cleavage as shown by the selachian and the holoblastic as shown by the amphibian is made clear by the accompanying diagrams from Boas. The blastula stages of the two show that the blastoderm of the selachian type is comparable to the small-celled animal half of the amphibian egg, a comparison which suggests the reason why the ectoderm of the amphibian is many-celled in thickness. In the amphibian egg the floor of the blastocoele is a mass of large yolk-filled cells; in selachians it is a layer of sub-blastocoele endoderm covering the yolk sphere and the scattered periblast nuclei. The linings or the floor coverings of the blastocoeles in the two cases are homologous, and periblast plus yolk cells of the selachians is homologous to the large, yolk-filled endoderm cells of the amphibian. In the gastrula stage this homology is emphasized by the fact that the gastrular invagination takes place at a point which is the morphological equivalent of the hinder edge of the selachian blastoderm where also the gastrulation will occur in this form.

These comparisons serve to point out that there is much less of a distinction between the cleavage types of holoblastic and meroblastic eggs than seems to be the case upon superficial observation.

The cleavage of the teleosts has been described for a great many forms and there is in general a fairly close agreement in the details. The eggs show the most complete segregation of protoplasm and yolk of all the vertebrates. Among the teleosts of the present day it is the rule that very large numbers of eggs are produced, and in line with this fact the eggs are uniformly small, varying in size from that of a small pea to that of a pinhead. There are a few exceptions to this statement among some fresh-water fishes, and these, together with the sharp segregation of yolk and protoplasm, suggest that in ancestral forms the yolk may have been very much larger in quantity and the egg more comparable in size to that of a selachian or of a bird. In most selachians the yolk is spherical and the protoplasmic layer which before fertilization shows but very slight localization accumulates at one place as a distinct elevation, after the entrance of the sperm. During polar body formation and cleavage, the protoplasmic portion is sharply distinguished from the yolk, but with the growth of the blastodisc the separation becomes less

marked and at length the germ layers completely enclose the yolk mass. The yolk itself is at first in the form of spherules, but at the time of fertilization these run together to form a single spherical mass of glassy transparency. In many eggs, oil droplets are to be noted within the yolk which are presumed to regulate the specific gravity of the egg during

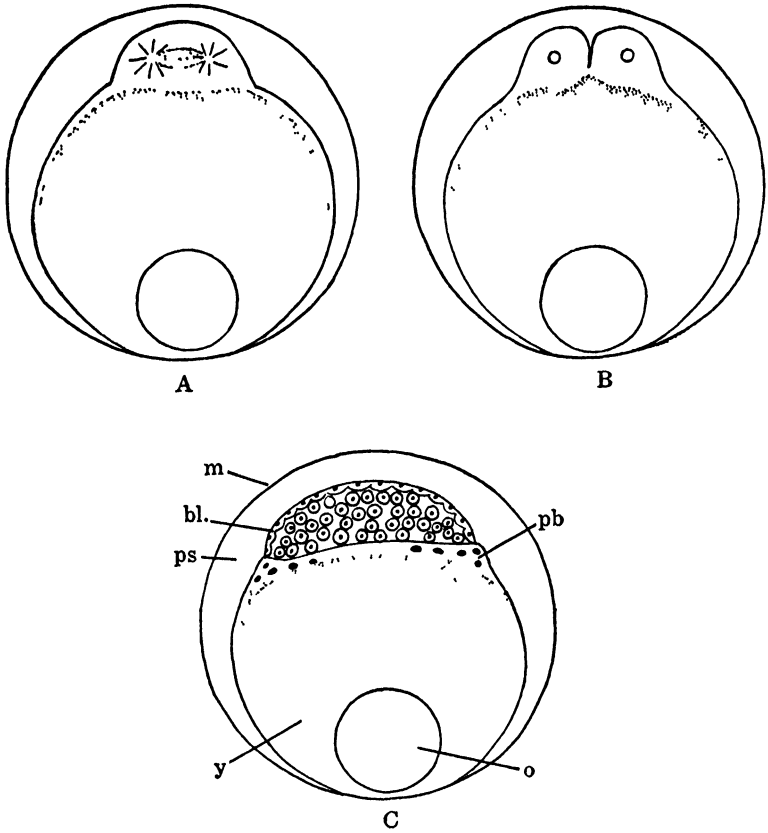


FIG. 52. Diagram of teleost cleavage. (After Ziegler)
m., egg membrane, bl, blastodisc, p s., perivitelline space, y., yolk; o., oil drople pb., periblast.

development. In the great majority of marine fishes the eggs float at the surface of the water during their early stages.

Except for the fact that the planes of cleavage are much more regular, the segmentation of the germinal disc of the teleost egg does not differ in principle from that of the other types having discoidal cleavage. The first two furrows are meridional, the first one appearing in the shorter diameter of the ellipse, the form assumed by the blastodisc with the beginning of cleavage. The third and fourth furrows are vertical, becom-

ing arranged so that they are practically parallel to the first and second. Thus, it is the rule that in the 16-cell stage the teleost egg consists of four rows of four cells each. In section it is to be noted that the first furrows do not extend to the yolk, but a continuous basal layer of protoplasm is left between the cleaving blastoderm and the yolk. The blastocoele appears at the end of the fourth cleavage by the third and

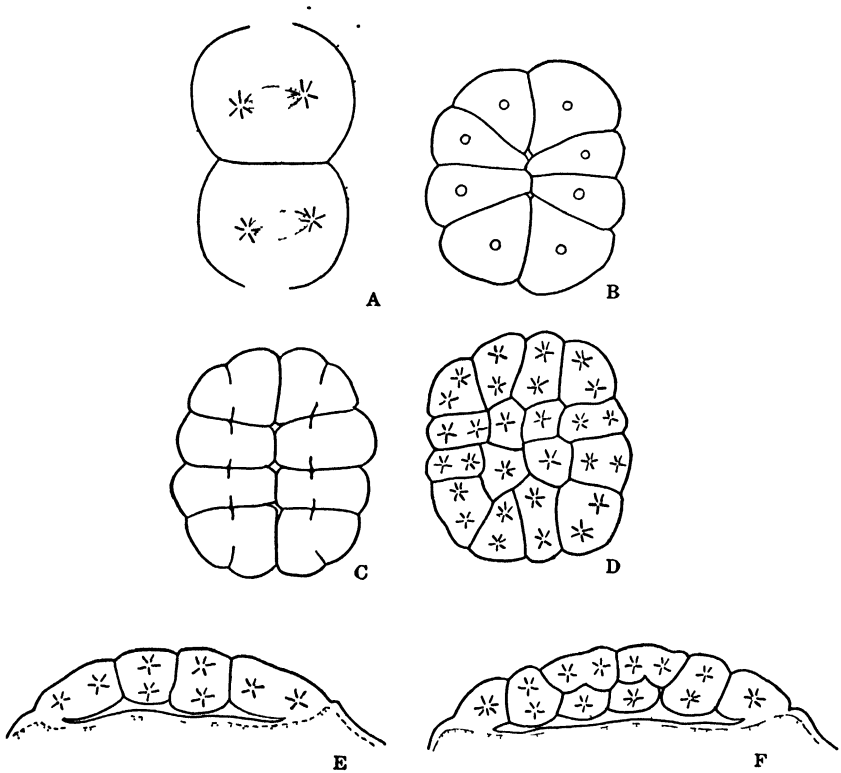


FIG. 53 Cleavage and formation of the blastoderm of the sea bass, *Serranus strarius* (Redrawn from Ziegler, after H B Wilson)
A, B, C, D, are surface views, E, F, sections

fourth furrows curving around in their deeper portions to meet the preceding division planes which were perpendicular to the surface. Thus the 32-cell stage consists of a layer of superficial cells and a deeper-lying layer.

The next division results in the blastoderm becoming three-layered, and in the fourth another layer is added, while at the same time the superficial cells also become more numerous. In subsequent stages it is seen that the cells of the basal portion will divide less rapidly but with

this exception up to the twelfth division the nuclei divide regularly. The basal, uncleaved protoplasm gives rise to the yolk syncytium, the nuclei dividing repeatedly without cell boundaries being cut off about them. The peripheral layer of protoplasm, the periblast, enclosing the yolk mass, is continuous with this basal yolk syncytium. As development progresses, the nuclei of this layer divide regularly and assume appearances which are characteristic of cells whose chief function is concerned with digestion of the yolk. Multiple mitoses are observed, nuclei become numerous, are strangely lobed, and present appearances which are unlike

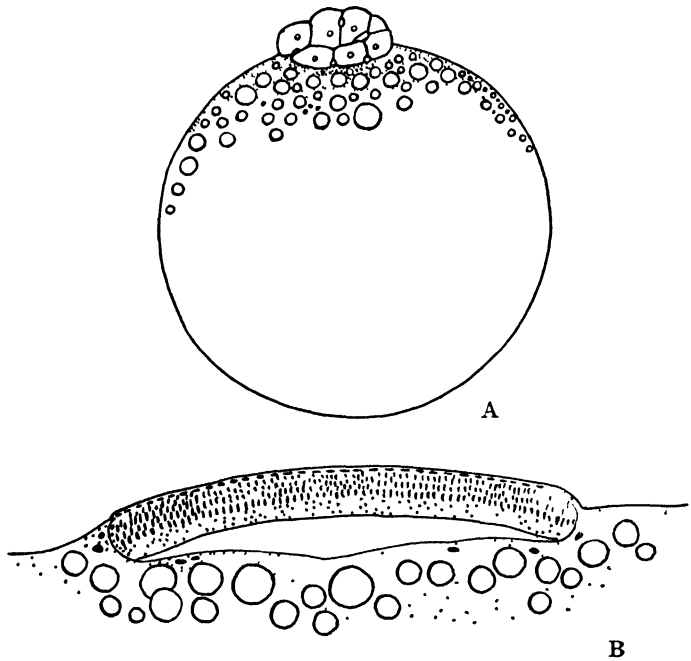


FIG. 54. Cleavage of the salmon, *Salmo salar*. (After Ziegler.)
A, 16-cell stage; B, blastula.

the nuclei of normal cells but are understood when it is realized that their function requires immensely increased surface area. The cleavage cavity forms between the blastoderm and the periblast. The edges of the blastodisc thicken and gastrulation begins at a spot upon one edge of the blastoderm. The subsequent stages involve a gradual extension of the blastoderm over the entire yolk mass, steps which are concerned with the processes of gastrulation and subsequent development.

The cleavage of the eggs of Gymnophiona are among those which show the discoidal type. The earliest stages have not been observed, but the egg and the later cleavage stages are so similar to those of birds and

reptiles, particularly of the former, that it seems unnecessary to devote space to that here. The student of general embryology is interested in them for the contrast which they present to other types of amphibian development.

The eggs of birds and reptiles are very much alike in all fundamental respects and resemble also those of the elasmobranchs. The amount of yolk present is large and the protoplasm and yolk are distinctly separated from each other, although the segregation is less sharp. The germinal disc is more intimately connected with the yolk and at its edges

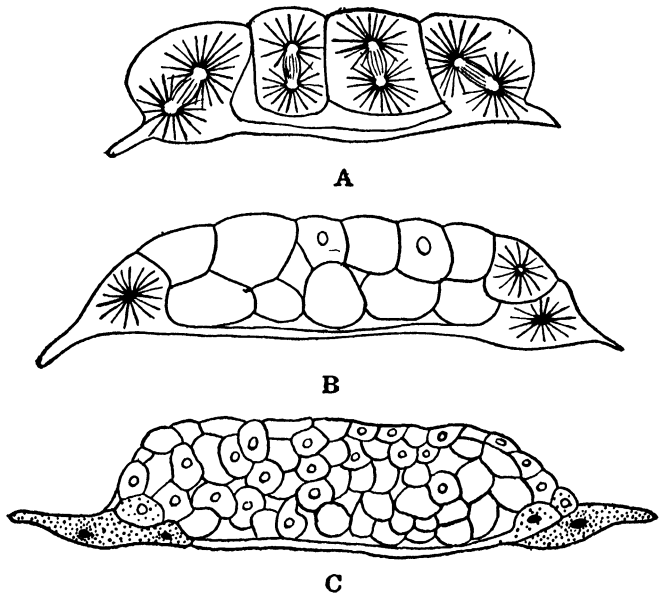


FIG. 55. Cross sections through the germ disc of the teleost, *Ctenolabrus*. (Redrawn from Korschelt and Heider, after Agassiz.)

gradually passes over into yolk in distinction to the sharp line of separation noted in the teleosts. The eggs of birds have been studied by many investigators and the early stages are quite thoroughly understood. The general procedure is similar to that of elasmobranchs, but the early furrows are more regular, the first two being meridional and at right angles to each other. They are then followed by a pair of vertical furrows. The irregularities are many, however, and in certain species they occur earlier than in others. In the early cleavage of the chick, for example, Patterson found that the third cleavage furrows may not be clearly recognizable. Accessory sperms enter the eggs of birds, and the phenomenon known as accessory cleavage is due to an attempt at segmentation on the part of the yolk in which these accessory sperm nuclei

come to lie. The attempt is transitory, however, and subsequently the partial furrows disappear and the nuclei degenerate. The first cleavages result in the formation of the central cells which are completely cut off from the unsegmented protoplasm, and of marginal cells which are still connected with the surrounding protoplasm. From the marginal cells new central cells are constantly cut off and new marginal cells arise peripherally. With the formation of the central cells, due to horizontal cleavages, a space is formed between them and the underlying protoplasm. This space becomes filled with fluid and is the segmentation cavity. In general the processes already described for the formation of discoidal cleavage in the lower vertebrates also apply to that in reptiles

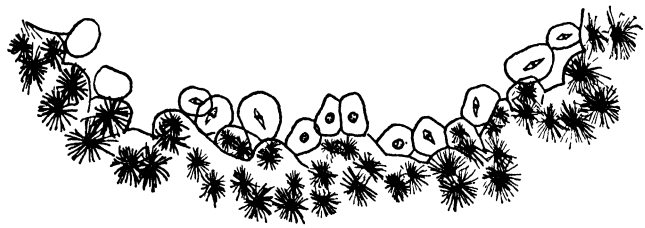


FIG. 56. Periblast cells showing many mitoses from a cleavage stage of *Belone*. (Redrawn from Ziegler, after Kopsch.)

and birds. For more detailed descriptions the reader is referred to such comprehensive accounts as those of the chick which are easily available.

III. IRREGULAR CLEAVAGE

In bringing to a close the sections dealing with cleavage it is necessary to mention certain irregular cleavages which do not fall in any of the classes previously discussed, or at least are so modified that their relationships have not yet been certainly made out. The cleavages of some sponges and of some coelenterates, though undoubtedly fundamentally radial, present variations from the normal type that are so great as to be understood only with difficulty. The cleavages of the trematodes and cestodes are among the most irregular of all forms. The cleavages of brachiopods and of bryozoans, although total and equal, do not follow the usual pattern. In most of the cleavage classes discussed an occasional irregular case is also to be found. Perhaps further work will bring many of these into line with the more regular forms.

BIBLIOGRAPHIC NOTE

Among the more important accounts of the subjects contained in this chapter are the following: Superficial: Brauer, Korschelt and Heider, Bigelow, Heider on *Hydrophilus*, Cano; Cephalopods: Watase, Vialleton; Vertebrates: Dean, Whitman and Eycleshymer, H. V. Wilson, Agassiz, Kopsch, Patterson. These works are cited in full in the bibliography on page 406.

CHAPTER VI

TYPES OF BLASTULAE

It is customary to say that cleavage continues from the fertilization of the cell to the formation of the blastula. We usually think of a blastula as a hollow spherical embryo consisting of a single layer of cells, and indeed the most typical blastulae are so constructed. Actually, however, there are many different forms of blastulae, of which the hollow, one-layered type is but a single one, and these different types of blastulae are now to be considered.

The different forms of blastulae occur as the end stage of many varied cleavage processes, as has been seen in the foregoing sections. The cleavage types are largely traceable to the amount and distribution of yolk present in the egg, a factor which differs widely in the several groups of animals. For the same reasons it is to be expected that the different cleavage types will lead to different kinds of blastulae, and this expectation is borne out. There are seven types of blastulae as follows: coeloblastula, stereoblastula, morula, placula, amphiblastula, superficial blastula, and discoblastula.

A. Coeloblastula

The simplest type of blastula is that known as a coeloblastula, the kind having a cavity at the center. Coeloblastulae may be further subdivided, depending upon the size of the segmentation cavity and upon the relative sizes of the cells. They include both equal and unequal blastulae, distinguished as to whether the cells are of uniform size throughout the embryo, and each of these is again of two distinct subtypes, depending upon the size of the blastocoele cavity. If the altitude of the cells is low so that they resemble a sort of cuboidal epithelium, then necessarily the cavity is very large, and we have the simplest type, the equal coeloblastula—the one with which we are most familiar, since it occurs in amphioxus. If, on the other hand, the altitudes of the cells are greater than their breadth, the cavity is correspondingly reduced; there is still a blastocoele but it is very tiny and is surrounded by a very thick cell wall (consisting nevertheless of only a single layer of cells). This occurs in *Sagitta*, a member of a group closely related to nematodes.

Usually in the equal coeloblastulae the cells are about the same size

and in about the same condition. Very evidently the equal coeloblastula is a type in which there is very little differentiation among the cells of the embryo as the cells are in approximately the same condition and of about the same size. Those at the vegetative pole usually show a slight size difference because of their content of yolk, but these cells do not have much yolk and the distinction is not very great.

Equal coeloblastulae occur in the following groups but do not include all members of these divisions. They are found in coelenterates, in nematodes, *Sagitta*, echinoderms, *Phoronis*, brachiopods, *Balanoglossus*, and amphioxus. It will be seen that these forms have equal cleavage which is mostly radial or bilateral cleavage. (The spiral type, however, does not entirely lack the coeloblastula, and there are a few annelids

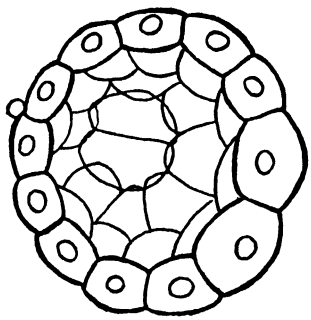


FIG 57 Blastula of amphioxus. (After Cerfontaine)

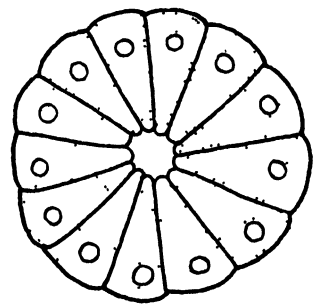


FIG 58 Coeloblastula of *Sagitta* (Redrawn from Korschelt and Heider, after Hertwig)

which rather closely approach the coeloblastula type.) Gastrula formation in types having coeloblastulae is usually by invagination; however, the method to be discussed presently, polar ingrowth, sometimes applies to this type.

The unequal type of coeloblastula is derived from the equal, with increased difference in size of the blastomeres at the animal and vegetal poles. Those at the animal pole are shorter than those at the vegetal pole. The blastocoele is not often very large and, owing to the larger size of the vegetative cells, it is pushed into the animal hemisphere. Eggs of this kind are characterized by a more sharp differentiation between yolk cells which are to form endoderm and animal cells which are to form ectoderm. The eggs are mostly spherical, sometimes a little elongated, and because of the difference in yolk content variations appear, just as in the equal type of coeloblastulae; among them are variations in the thickness of the wall, and in the size of the cleavage cavity.

The unequal coeloblastula is widespread in eggs which cleave spirally.

Therefore we should expect to find it in the polyclads, annelids, molluscs, Bryozoa (Fig. 59) and among the holoblastic eggs of vertebrates (Fig. 26). The blastulae of vertebrates can readily be distinguished from the other types, for in them we always find a great many cells in the wall. In the vertebrates, epithelium is differentiated into many layers of cells. It is as if this differentiation that is characteristic of adult vertebrates makes itself felt even in the cleavage stages, so that the wall of the blastula consists of many cells, and each germ layer, ectoderm and endoderm, is several cells thick. This characteristic is clearly seen in the amphibian type, the frog and salamander, with such modifications as the difference in yolk would involve. Unequal coeloblastulae are derived from unequal

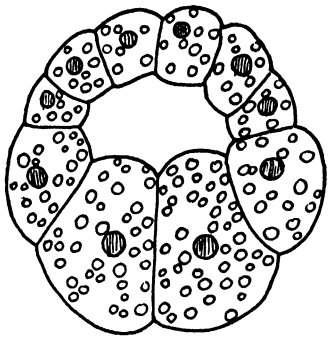


FIG 59 Unequal coeloblastula of *Flustrella hispida* (Redrawn from Korschelt and Heider, after Pace)

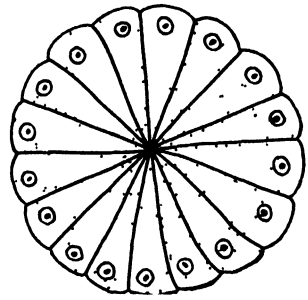


FIG 60 Equal stereoblastula of *Lucernaria* (Redrawn from Korschelt and Heider, after Bergh)

total cleavage. Gastrulation is commonly through invagination, although polar ingrowth and epiboly also are found in these eggs.

B. STEREOBLASTULA

The stereoblastula (the term means a solid blastula) is obviously derived from the coeloblastula, and may be equal or unequal, depending upon whether it is produced from the equal or unequal type of coeloblastula. If the cleavage cavity in the type of equal coeloblastula with small blastocoele continues to be reduced until it vanishes, we have the equal type of stereoblastula such as is found in *Lucernaria*. With increase in volume of cells and decrease in size of blastocoele we get a stereoblastula.

More common than this, however, is the unequal type of stereoblastula such as we find in *Crepidula*. The unequal stereoblastula is derived through unequal cleavage and may occur either in the spiral types or the more extreme of the bilateral types. Gastrulation regularly occurs in this

type by epiboly. The spiral types lead easily to this type of blastula in which by continued growth the ectoderm cells will surround the endoderm cells and so form a gastrula by epiboly.

C. Morula

A second type of blastula in which there is no blastocoele is the morula. It was formerly customary to include this stage in almost all the types; that is, it was said that the morula followed the 16-cell stage, and in the strictly radial type there is some reason for that description, but we do not speak now of a morula stage. However, in some types there are formed blastulae which can best be described as morulae or solid blastulae. In *Clava* we have an example of the solid blastula which illustrates the difference from the stereoblastula type, for inside the morula there are cells, whereas in the stereoblastula there were no cells which did not

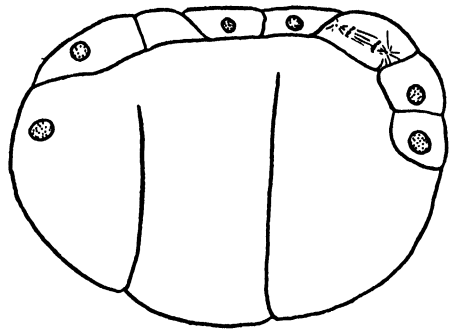


Fig. 61. Unequal stereoblastula of *Crepidula*. (After Conklin.)

have a boundary at the surface of the egg. Superficially the two are quite alike, but actually there is an important distinguishing feature. The morula is produced by a characteristic shifting of the spindle position during cleavage. In all the other cases that we have dealt with the spindle may be said to occupy a paratangential position; the result is that the new cleavage plane divides the cell in such a way that part of it touches the surface and part the center of the egg. However, the morula comes about by a shifting of the spindle to a radial rather than a paratangential position, and the result is that some of the daughter cells touch the surface and others are only in the interior of the egg. So the morula, although superficially similar to the stereoblastula, is really a distinct and different type. Morulae occur chiefly in coelenterates.

D. Placula

The placula presents another kind of modification which has been produced from the equal coeloblastula. Let us suppose that, instead of

the cavity vanishing, the egg is compressed from the animal to the vegetative pole. The result would be a flattened blastula consisting of two plates of cells. This is the placula and is found especially well developed in the ascidians and to a less extent in the nematodes. The edge of the placula corresponds to the equator of the coeloblastula, the upper layer of cells to the animal hemisphere, and the lower layer to the vegetative hemisphere.

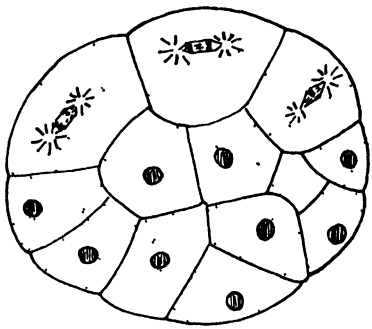


FIG. 62 Morula of *Clava squamata* (Redrawn from Korschelt and Heider, after Harm)

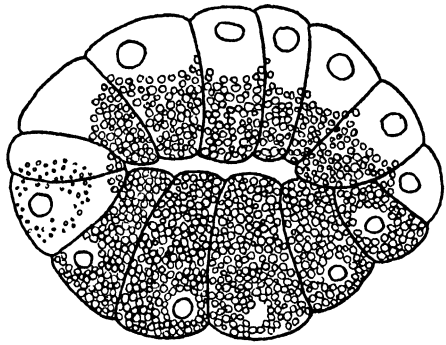


FIG 63 Placula of *Cynthia partita* (After Conklin)

Gastrulation takes place by two different methods, epiboly and the sinking in of the flattened plates; that is, it is accomplished by a combination of epiboly and invagination.

E. AMPHIBLASTULA

The amphiblastula of the sponge is of somewhat different type. This structure was first described by Haeckel for the free-swimming larval stage of calcareous sponges, and hence does not strictly correspond to other blastulae which we have described. This larval form, however, is usually spoken of as an amphiblastula. The cells at the animal half are very sharply distinguished by their small size and ciliated covering from those in the vegetative part of the egg. This type is not particularly important although it is necessary to include it.

F. SUPERFICIAL BLASTULA

The superficial blastula, or periblastula, is characteristic of those forms having superficial cleavage, an example of which is the crab, *Dromia* (Figs. 36*f* and 37*d*). The result of superficial cleavage is the formation of a layer of cells around the inner yolk mass. There is no blastocoele and it is usual to say that the blastocoele is occupied by the

yolk mass. But the case is not quite so simple. Actually it will be recalled that the superficial cleavage resulted in the formation of blastomeres which at first were continuous with the yolk at their inner end. These blastomeres presently are completely cut off, a protoplasmic blastomere being separated from the yolk-laden inner mass. Their inner ends, or the outer part of the yolk mass, therefore, is not homologous to the cavity. It is only that very small part of the mass which was never segmented at all which is homologous to the cavity.

Reference has already been made to the rôle of the yolk cells, the vitellophages within the yolk mass Some of the nuclei during cleavage remain behind and are presently to be described as yolk cells for they

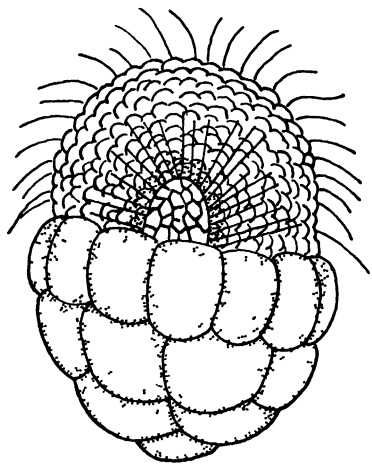


FIG 64. Amphiblastula of *Sycandra raphanus* (Redrawn from Korschelt and Heider, after Schulze)

digest the yolk. In this process the yolk becomes divided into areas around these nuclei, a process of division which is often spoken of as the yolk cleavage. But by some these nuclei are regarded as endoderm. If this latter view is adopted, there would be no such thing as a superficial blastula because the embryo would consist mostly of two cell layers, the outer being ectoderm, and these internal yolk cells endoderm. This designation of these cells as endoderm has not, however, come into general use. Rather we say that the superficial is a true blastula.

G. DISCOBLASTULA

Finally we have the discoblastula, the end stage of the eggs having discoidal cleavage. It is derived from the unequal coeloblastula (Fig. 52) upon the assumption that the yolk becomes so abundant and so dense as

to hinder quite completely the separation of the cells in the vegetative half of the egg. Recalling that discoidal cleavage occurs both in the vertebrates and invertebrates we should say at once that discoblastulae are of two sorts, the vertebrate type (Fig. 55) and the invertebrate (Fig. 44); the latter consists of the single layer of cells which is found in all invertebrates having discoidal cleavage in contrast to the former which is of many cells. Usually in all types having discoidal cleavage there are some cells, perhaps around the edge of the disc or perhaps elsewhere, which are continuous with the yolk. These cells, however, usually do not take an active part in the formation of the body of the embryo. In the vertebrates these cells are designated as the periblast or the yolk sac endoderm. In the cephalopods they are the blastocones. Gastrulation is usually by the limited type of invagination, invagination taking place in one segment of the disc only.

These are the different types of blastulae. They are obviously related by the differing types of cleavages and they lead us to the different types of gastrulation which is the next step in the development of the animal's body.

Bibliographic Note

Among the more important accounts of the subjects contained in this chapter are the following: Korschelt and Heider, Minchin, Maas, Hertwig, Wilson, Conklin, Schultze, Brauer, Whitman and Eycleshymer, O. Herting, Ziegler, Brooks and Rittenhaus. These works are cited in full in the bibliography on page 406.

CHAPTER VII

ENDODERM FORMATION

I. METHODS OF GASTRULATION

After the formation of the blastula, which in its simplest condition is a spherical mass (usually hollow) of more or less undifferentiated cells, the next stage in the development of the embryo is the formation of a gastrula. The gastrula develops from the blastula by the differentiation of two layers of cells, an outer or ectodermal layer, and an inner or endodermal layer. From the ectoderm develops the nervous system and the outer parts of the future animal, and from the endoderm comes the lining of the alimentary canal.

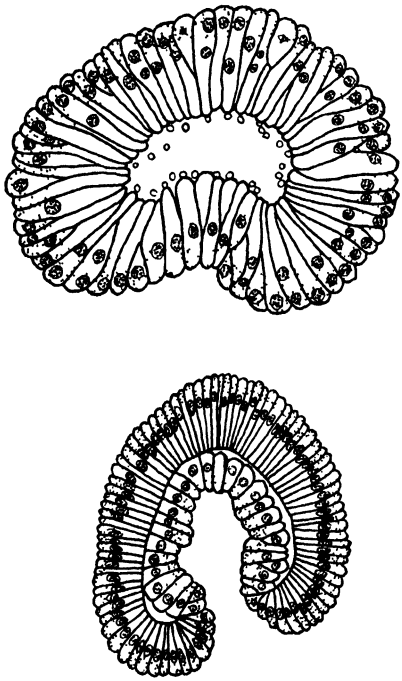


FIG 65 Two stages in the gastrulation of *Terebratulina septentrionalis* (After Conklin.)

There are several different types of gastrula formation, depending partly on the type of blastula preceding the gastrula. We shall consider each of these types and describe its method of formation.

A. EMBOLIC GASTRULATION

The best-known type of gastrula is the embolic or invaginate gastrula. Gastrulae of this kind occur in embryos with total cleavage, resulting in cells of either equal or slightly unequal size. The blastula resembles a hollow sphere whose wall, except in vertebrates, has a thickness of one cell. A blastocoele cavity is often, though not always, present inside the sphere, but sometimes it is quite restricted, as in *Terebratulina*, a brachiopod which has a thick-walled type of coeloblastula. The cells of the blastula are often wedge shaped, with the wedge narrowing inwardly.

In one part, the vegetative half, the cells are typically larger than elsewhere, and sometimes markedly so.

The first sign of gastrulation is a slight flattening of the vegetative region. This flattening gradually deepens and the resulting gastrula has the appearance of a rubber ball which has been pushed in at one point by pressure with a finger. The inpushing of the cells at the vegetative pole continues until this invaginated layer lies close to the cells of the outer wall. In this way a two-layered gastrula is formed and two cell layers are differentiated. The outer or ectodermal layer covers the gastrula and is continuous with the inner or endodermal layer at the

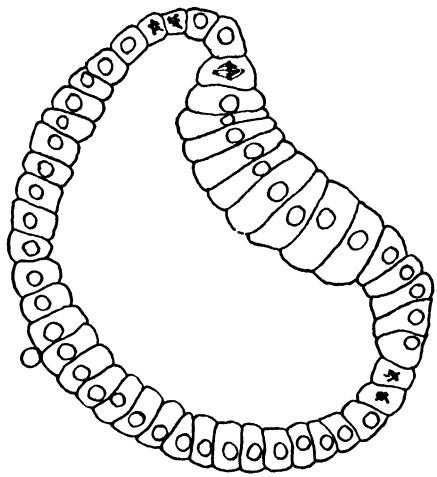


FIG. 66. Gastrula of amphioxus. (After Cerfontaine.) Oriented with respect to the future embryonic axis.

point of invagination. Between the two layers there remains for a while a cavity which is the remnant of the segmentation cavity or blastocoele. The blind cavity, which communicates with the outside through a single blastoporal opening and which is formed by the inturned cells as the lip of the depression gradually narrows, is the primitive gut cavity, or the archenteron.

It will be seen at once that the conditions in an invaginate gastrula closely resemble those of the adult coelenterate which has two cell layers and whose gut has a single opening to the outside. However, it is to be noted that in only a few coelenterates are the gastrulae formed by invagination, and that the same result in these forms is attained in another way.

The mechanism which causes invagination has been attributed by different workers to different causes. Rhumbler regarded it as an active

attempt at migration by the cells of the vegetative half, a process which in the last analysis is to be attributed to chemotactic influences. If this explanation is correct, then the migration of the endodermal cells must be connected with a change in surface tension at the boundary between the endoderm and the blastocoele content, especially in the region of the cells which undergo invagination.

Some of the causes which play a part in the invagination may be the following: (*a*) Differences in the rate of growth between the ectodermal and endodermal portions of the blastoderm by which a lateral pressure is exerted on the endodermal plate. (*b*) Resistance of the closely pressed egg membrane, so that the surface increase of the blastoderm is possible only internally. This factor is probably not often effective, for in many cases the egg membrane appears pushed away by the perivitelline fluid and the blastocoele is not an empty cavity but is filled with fluid which would have to be displaced. (*c*) The constantly decreasing fluid of the blastocoele which perhaps exerts a sucking effect on the endodermal plate. We must at the present time regard this factor as of doubtful occurrence and significance.

The size of that part of the old segmentation cavity or blastocoele which remains after gastrulation may be very small, as in amphioxus, or it may be quite extensive, as in the echinoderms in whose embryos the primitive gut is relatively small. The fate of the fluid or gelatinous content of the blastocoele is uncertain; some authors think that it is resorbed by the endoderm cells. If this process could be satisfactorily demonstrated it would be regarded as a causal factor in gastrulation.

A monaxial structure, with the axis from the middle of the blastopore opening to the opposite pole, is characteristic of the early gastrula. In many forms the gastrula becomes later bilaterally symmetrical as the blastopore becomes narrowed and the gut laterally compressed. In most coelenterates the monaxial structure remains.

A special type of gastrulation occurs in *Lumbricus* and the ascidians. Here the blastula is a placula having a two-layered, plate-like structure. Gastrulation is brought about by the hemispherical inbending of the placula which then rounds out so that a complete sphere is formed. In some cases there is an active increase by epiboly in the ectodermal cells preceding invagination. This may be one of the causes for the invagination in placulae, but on the other hand the process may be due to the same causes that lie back of typical invagination.

Invagination occurs in the following forms: some actinians, some syphopolyps; *Sagitta, Pedicellina,* brachiopods; *Phoronis,* nemerteans; echinoderms; some arthropods, as crayfish, *Palimonetes*; some insects; many annelids, as *Polygordius, Podarke, Eupomotus*; some molluscs, as

Chiton, Dentalium, Dondersia; lamellibranchs, as *Cyclas, Pisidium, Unio, Ostrea*; gasteropods, as *Paludina, Planorbis*; *Balanoglossus*, amphioxus; the vertebrates in general.

It will be observed that this type of gastrulation is typical of primitive forms. Hence it is most striking that very few coelenterates belong to this group. Invagination with a preceding placula includes the following: some nematodes, some ascidians, some Lumbricidae. Many forms are transitional between the type of pure invagination and the placula type.

B. Epibolic Gastrulation

In the invaginate or embolic gastrula just described, the blastomeres were approximately equal in size. In many other embryos, however,

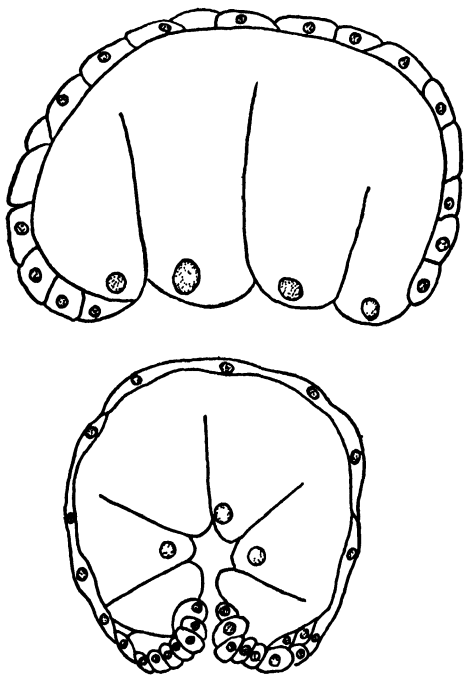


Fig. 67. Gastrulation by epiboly in *Crepidula*. (After Conklin.)

there is a great difference in size between the small cells at the upper or animal pole and the large cells at the lower or vegetative pole. The small cells are called micromeres, and the large ones which contain much yolk and are few in number are called the macromeres. In the blastulae of embryos which have undergone this kind of cleavage, the cleavage cavity is small or absent.

It is obvious that there is no room for invagination of endoderm cells

in embryos with practically no cleavage cavity. Gastrulation, which cannot be embolic, occurs in the following way. There is a multiplication of the small cells at the animal pole and an overgrowth or migration of these cells down over the larger cells at the vegetative pole. These large yolk-filled cells seem to take no active part in the process. The free edge of the growing cap is called the blastopore, and the protruding yolk-filled cells, the yolk plug. When the overgrowth by the small cells is complete, the blastopore usually closes and the large cells are enclosed. The small, overgrowing cells are ectodermal and the large yolk cells endodermal. A gut cavity is not present at first but is formed later by a splitting and solution of the yolk in the solid endoderm cells. The cause of epiboly, or gastrulation of this kind, has usually been ascribed to the activity of the micromeres, but there is no evidence of a lack of activity on the part of the large, inwardly migrating macromeres.

The part of the gut so formed is known as the mesenteron, for it is the middle portion of the entire tract. The anterior end, or stomodaeum, and the posterior end, or proctodaeum, are formed by ingrowth of the ectodermal cells which later unite with the mesenteron so that there is a continuous lumen through the gut.

There are many transitions between epibolic and embolic gastrulation. If in the embolic gastrula the endoderm cells are larger and the lumen of the invagination correspondingly smaller, then the gastrulation approaches the epibolic type.

Epiboly is very widespread and occurs in all cases where the eggs are sharply telolecithal and where there are, as a result, size differences of the cells at the animal and vegetative poles.

Epibolic gastrulation occurs in ctenophores (Fig. 14B), rotifers, turbellarians, some molluscs, some annelids, and many crustaceans including many parasitic copepods.

C. Polar Ingression

1. Restricted Unipolar Ingression

In some cases of gastrulation there is neither a sharp invagination nor an epibolic overgrowth. In these cases there is a total, unequal cleavage, resulting in an embryo with a micromere cap resting on a few large yolk-laden cells. The micromeres continue to multiply, and a blastula is formed of cells of very unequal size; in it a well-developed blastocoele is present. The large cells, never many, the common anlage of mesoderm and endoderm, reach the interior not by invagination or epiboly, but through their own active inward migration.

The mechanism by which the large cells get into the interior is little

understood, and the term polar ingression is not adequately applied. This method of gastrulation is often not sharply separated from either of the preceding types.

The following forms have gastrulation by restricted unipolar ingression: some holoblastic Crustacea (some schizopods and Cladocera), *Ascaris megalocephala* and other nematodes, and some molluscs (*Patella*).

2. Many-Celled Unipolar Ingression

In the ciliated blastulae of the coelenterates occurs a second form of gastrulation by polar ingrowth. In it there is an elongated, oval or sausage-shaped, free-swimming stage (called a planula) which is really a blastula with a large blastocoele and with thickened cells at the posterior pole. Here there takes place the migration of cells to a position within the blastula which is responsible for a second type of unipolar ingression. This migration extends from the pole over the nearby portion of the embryo. The cells which first migrated into the blastocoele lie isolated in the jelly contents but, later, when migration takes place *en masse*, a plug of cells turns inward from the posterior pole and gradually fills the blastocoele. The migration of cells inward is not limited to the vegetative pole, but reaches to neighboring parts. There is no interruption in the continuity of cells at the hinder pole, and no gap in the outer superficial cell layer. At the end stage of the gastrulation, there is an embryo with an outer superficial epithelial layer and with a solid cell mass filling the interior of the larva. This stage is variously called a stereogastrula or a parenchymella.

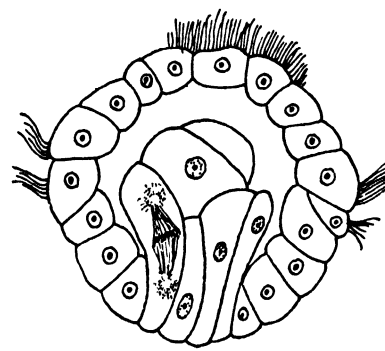


FIG. 68. Endoderm formation in the gastropod *Patella* by unipolar ingression. (Redrawn from Korschelt and Heider, after Patten.)

The formation of the gut cavity follows later by dehiscence. A gradually widening split occurs in the interior of the endoderm cell mass and later breaks through to the outside, forming the mouth. During this process, the endodermal cells become arranged in a layer.

It will be seen that there is a close relation between gastrulation by invagination and gastrulation by polar ingression. Both methods might be regarded as modifications of one process. In the invagination process a closed epithelial layer is shifted by infolding to the interior, and in the ingrowth process the cells of the vegetative half pass inward as a solid cell mass, later taking on an epithelial character, and develop-

ing a lumen. Metchnikoff concluded that the polar ingression was brought about by a simple migration of cells without differential division.

There are transitional types between gastrulation by invagination and gastrulation by polar ingrowth. In many forms, as in *Aurelia aurita*

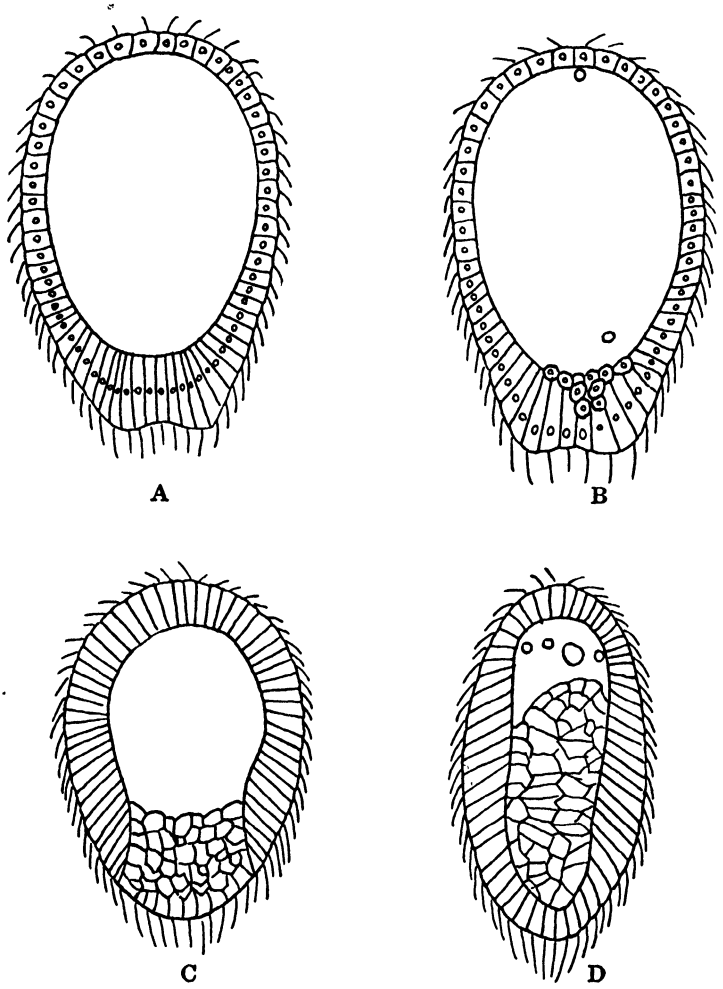


FIG. 69. Endoderm formation by unipolar ingression in the planula of *Aequoria* according to Claus. (Redrawn from Korschelt and Heider.)

and *Eupomotus*, there are invaginate gastrulae in which the gut lumen is reduced to a very narrow split. If we think of the originally solid invaginated mass as broken up into cellular parts, we have a picture of unipolar ingression.

Examples of unipolar ingression are found in the following forms: hydroids which have free medusae and whose eggs produce ciliated swimming blastulae (*Tima* and *Obelia*), some anthomedusae, some scyphomedusae, and some leptomedusae. This type of gastrulation is widespread in arthropods.

3. Multipolar Ingression (Apolar Ingression)

A process similar in many respects to the two types of polar ingrowth just described and perhaps related to those types is that of multipolar ingression. It differs from the preceding only in the inward movement of a small number of cells from several places instead of a single one on the surface of the blastula. It might be spoken of as apolar ingrowth as distinguished from that which proceeds from a single restricted portion of the blastula. However, since the processes are so similar to those already described in this section it seems desirable to assign this type to the same division even if it presents some departures from the other cases placed here.

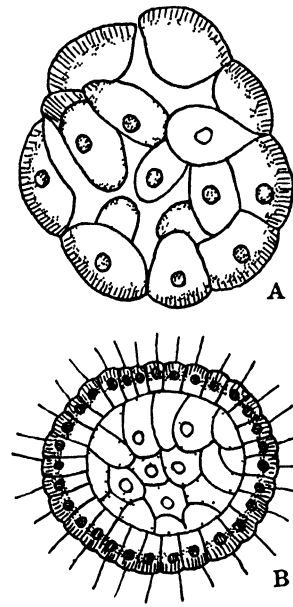


FIG. 70. Multipolar ingression in *Aegenopsis mediterranea*. (Redrawn from Korschelt and Heider, after Metchnikoff.)

Multipolar ingression occurs in coeloblastulae when cells, few in number at first, pass inside the embryo from various places on the surface. The blastocoele is in this way filled up with endoderm. The resulting stage is a solidly built mass of cells like a morula. It may be called a pseudo-morula, differing from the typical morula in that there is no differentiation of cells in the latter whereas here the endodermal cells, now a solid mass, have already arisen by an inward movement of some of the outer cells. The gut cavity appears later inside the endoderm. Cases of this sort of endodermal formation seem to be rare. The best investigated case is that of *Aeginopsis* (Metchnikoff).

D. DELAMINATION

In the cases already studied, there was a separation of the inner or endodermal layer from the vegetative pole, or from the vegetative half of the embryo. There is, however, another type of endodermal formation, common in coelenterates, in which there is no polar localiza-

tion of endoderm-forming cells, for in these the endoderm comes from diffused parts of the embryo. The endoderm may arise in one of the following ways: by migration into the coeloblastula from visible spots in the embryo, by cutting off from the cell wall of the coeloblastula by division, or, when the end stage of cleavage is a solid embryo, by histological differentiation of an epithelial outer layer from the parenchymous inner mass. There are many types which belong here. It will be observed that multipolar ingrowth might from some points of view be included under this heading. The term delamination has been used to bring together processes of different kinds and is no longer a definite term of definite meaning. Many of the forms intergrade with each other through transitional stages. It is best to consider, under the head of delamination, all cases of endoderm formation which are not derived from invagination, epiboly, or polar ingression (including multipolar ingression).

Delamination occurs in those forms whose embryos are set free late in development either from spore sacs or from eggs with membranous and gelatinous coverings. Many forms classified here belong to the intergrading or mixed type of endoderm formation.

1. Coeloblastic or Mixed Delamination

When, in a coeloblastula, paratangential division planes which are differential in character cut off single cells into the interior of the blastocoele and these subsequently become arranged into a layer, coeloblastic delamination may be said to occur. These inner cells constitute the endoderm and at length fill the blastocoele. By dehiscence within them the gut subsequently arises. In many cases of this mixed delamination, there is a combination of the origin of endoderm by cell division and by multipolar migration. Many of the hydroids, of which *Hydra* is the best example, show this mixed delamination. *Geryonia*, the actinians, and some syphomedusae have coeloblastic delamination.

2. Morula Delamination

In morula delamination, also called secondary delamination, cleavage results in the formation of an embryo without a blastocoele, that is, in one solid from the first. Early in cleavage there occur both radial and paratangential division planes with no histological differentiation resulting between the inner and outer cells. An embryo arising in this manner is a true morula in which a cleavage cavity never develops. Haeckel thought that the morula, the stage of solid aggregates of blastomeres, always preceded the blastocoele. But it is now well known that a

blastocoele cavity appears in some forms as early as the 4-cell stage, and that true morulae, as the above, are formed only in certain cases. In multipolar ingrowth and in coeloblastic delamination, the pseudo-

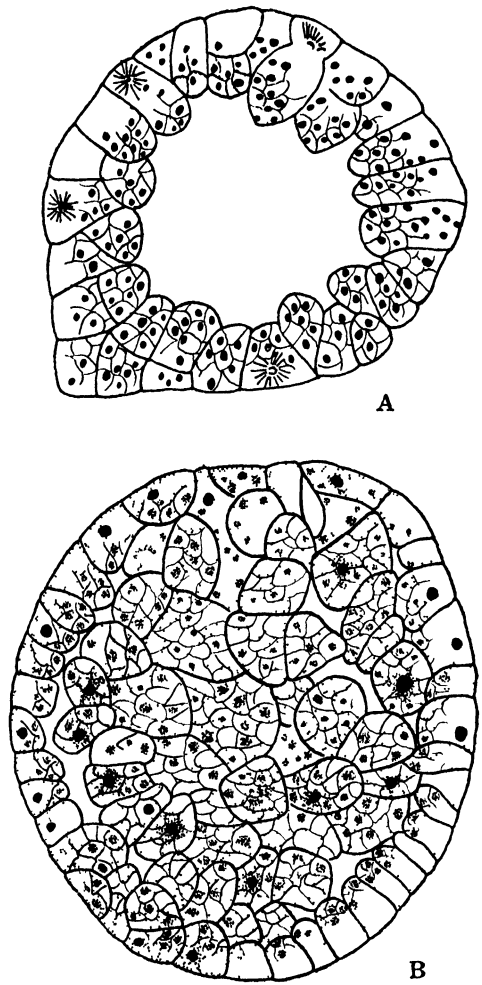


FIG 71. *Hydra* embryo (Redrawn from Korschelt and Heider, after Brauer)
A, showing early migration of some endoderm cells inward and the origin of others by cell division, B, a later stage in which the endoderm fills up the cavity

morula follows the blastocoele stage instead of preceding it, differing from Haeckel's description of a typical embryo.

In morula delamination, differentiation of layers follows the solid stage of undifferentiated cells. In *Clava* (Fig. 62) the cells at the surface

take on a prismatic character and lose their polyhedral shape. An undulating line of separation is first formed between the outer or ectodermal cells and the inner or future endoderm cells. The basement membrane is later differentiated, and the inner cells connected with it become epithelial. The cells adjoining them lose their yolk content and take positions among the outer endodermal cells. The innermost cells of the yolk mass finally liquefy and are resorbed, thus giving rise to the gastrula cavity. The formation of a definite endoderm is accomplished, and the planulae, now ciliated, swarm out.

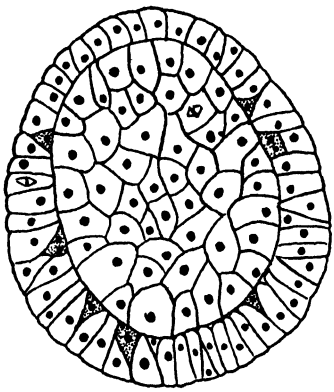


FIG. 72. Endoderm formation of *Clava squamata* showing the formation of the outer and inner germ layers by differentiation. (Redrawn from Korschelt and Heider, after Harm.)

Morula delamination occurs also in Trachymedusae, Siphonophora, in alcyonians, Stauromedusae, and Cubomedusae.

3. Syncytial Delamination

Syncytial delamination occurs in those coelenterates whose embryos show a similarity to the superficial cleavage of arthropods. In these embryos, only a few cell boundaries may be seen, and there is a solid syncytial inner mass, instead of a morula stage. The ectoderm becomes separated from the inner mass, which in part forms the endoderm and in part gradually is used up.

In *Turritopsis* there is at first total cleavage, but in later stages the blastomeres flow together and form a syncytium. In this case the syncytial nuclei at the surface multiply, cell boundaries cut in and a delicate basement membrane is formed. Differentiation of the endoderm comes later, when its cell boundaries begin to appear. The primitive endoderm cells are irregularly disposed, but later, when a split forms inside, representing the gut cavity, they are definitely formed into an epithelial layer.

In the hydrocorals, according to Hickson, there is no separation into blastomeres and the embryo is a syncytium from the first, recalling the cleavage of insects. Nuclear fragmentation of the first cleavage nucleus has been described in these forms as the source of the nuclei of the plasma islands, which arise, according to this account, from regenerating chromatin particles. About the plasma islands cell boundaries appear, and later the ectoderm becomes differentiated as a layer and development proceeds.

There is considerable doubt as to whether all the cases classified as

syncytial belong here, as different authors give different accounts for the same material and describe many doubtful processes. Some of their

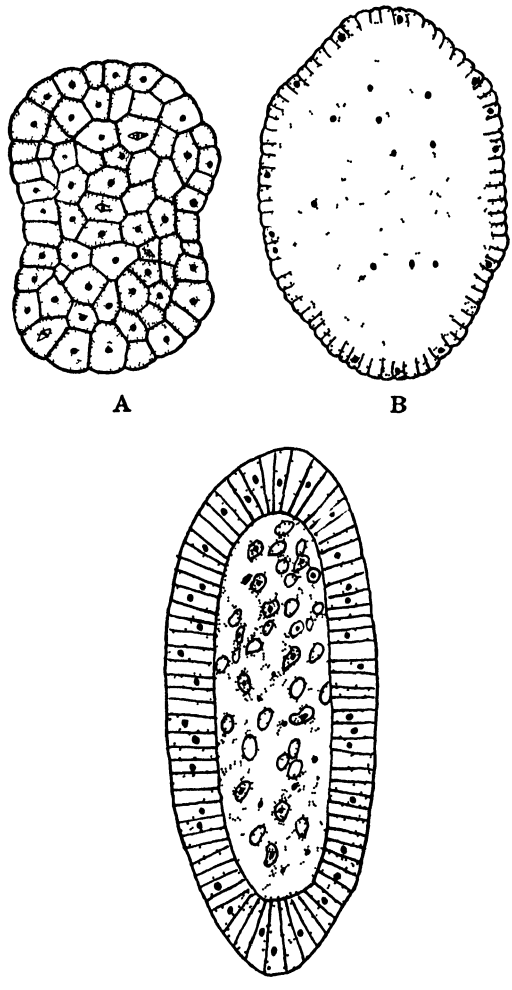


FIG 73. Development of *Turritopsis*. (Redrawn from Korschelt and Heider, after Brooks and Rittenhouse)

A, morula, B, fusion of blastomeres, C, young planula with definitely formed ectoderm.

material may be improperly fixed, and a reinvestigation may furnish more complete knowledge of their development.

Many hydroids probably belong in this group which develops by syncytial delamination.

II. THE DISCOGASTRULA

A. Vertebrates

All the forms of gastrulae studied so far have eggs in which there is not a great quantity of yolk. In those forms, however, which have a discoblastula, a mass of yolk occupies the greater part of the egg and

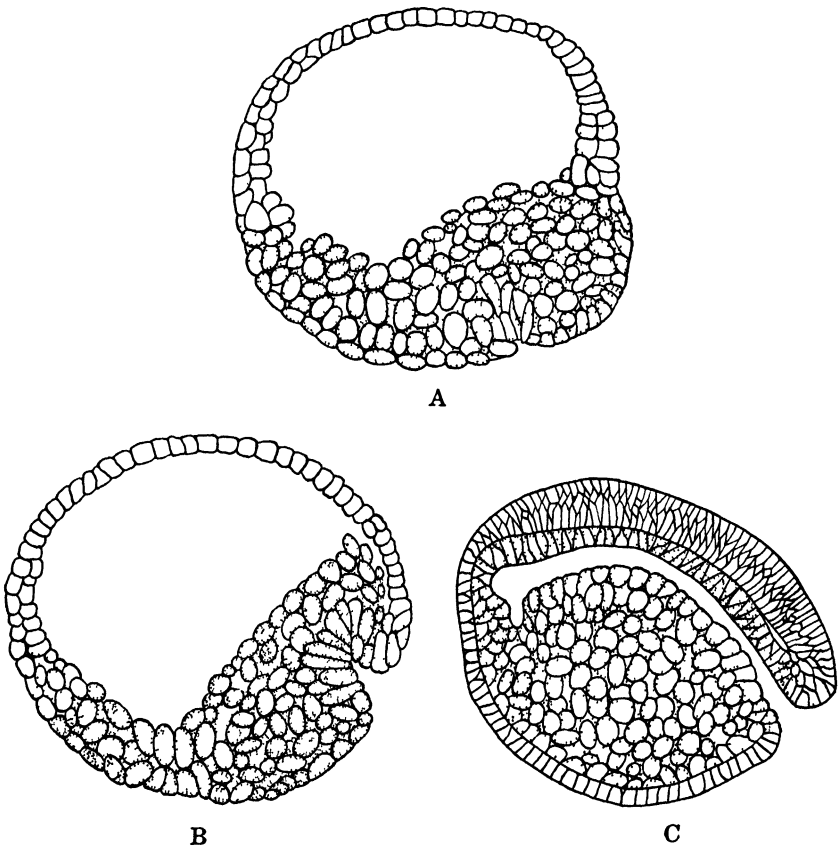


Fig 74. Gastrulation in *Petromyzon fluviatilis* (Redrawn from Ziegler, after Goette) A, B, beginnings of gastrulation, C, formation of an anlage of the medullary tube.

the protoplasmic part is limited to a small disc at one end. The inert yolk is incapable of division and the protoplasmic disc alone undergoes segmentation. Gastrulation usually takes place by the inturning of the edge of the disc and the spreading out of this inturned layer between the protoplasmic disc and the yolk mass. This inturned layer is the endoderm and the two-layered embryo thus formed is a discogastrula.

The discogastrula lies on top of the yolk and spreads around it. The edge of the embryo is the blastopore.

In order to understand this process of gastrulation thus briefly described it will be necessary to take up at greater length the gastru-

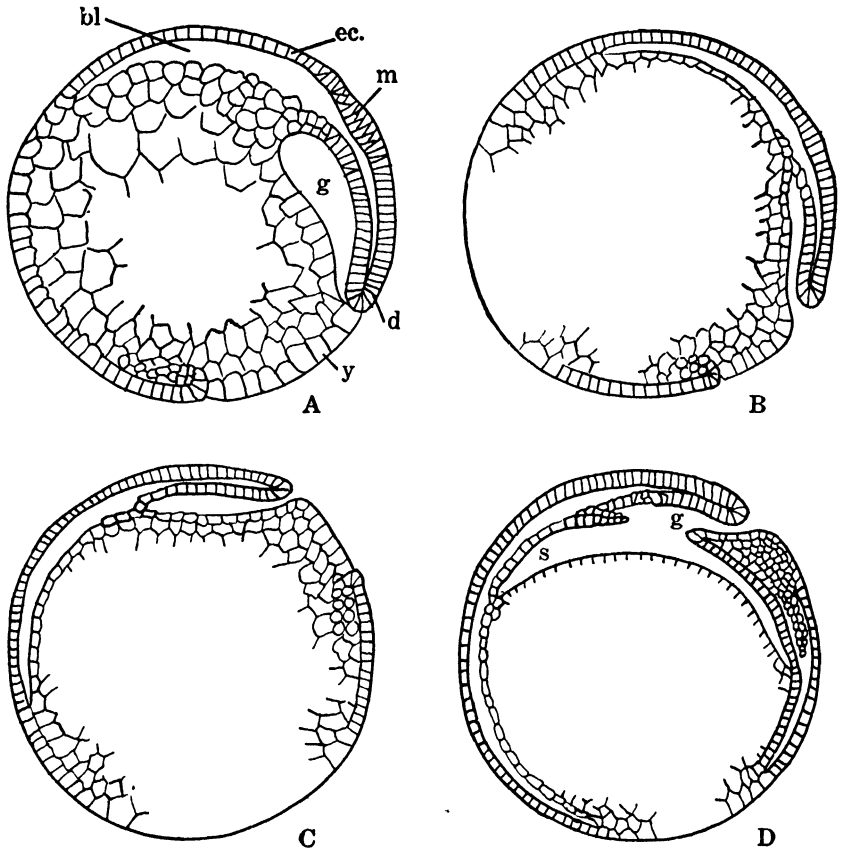


FIG. 75. Series of diagrams showing gastrulation. (After Ziegler.)

A, B, sections through embryos of an amphibian or a dipnoan in the stage of the circular blastopore; C, gastrula of a hypothetical intermediate between Amphibia and Amniota; D, gastrula of a reptile. y., yolk plug; ec., ectoderm; bl., blastocoele; g., gastrocoele; m., medullary plate; d., dorsal lip of the blastopore; s., subgerminal cavity.

lation of the frog (Figs. 22, 51, and 74–76); although its egg has total, unequal cleavage and gastrulation is therefore chiefly embolic, the study of its egg enables us to interpret much better the conditions in a true discogastrula. In the lower or vegetative part of the frog's egg, the white portion, there is an accumulation of yolk material; the animal half or dark colored part of the egg, however, is not entirely devoid of it.

Because of the small amount of yolk the animal half divides more rapidly than does the vegetative half. Cleavage thus results in an embryo with large and small blastomeres, the larger ones serving to force the cleavage cavity nearer the animal pole. Between the pigmented and the lighter portions of the egg is the gray crescent. The bilateral arrangement of the egg substances is shown in some amphibia, but the appearance of the so-called gray crescent which is similar to that of the ascidians cannot always be made out.

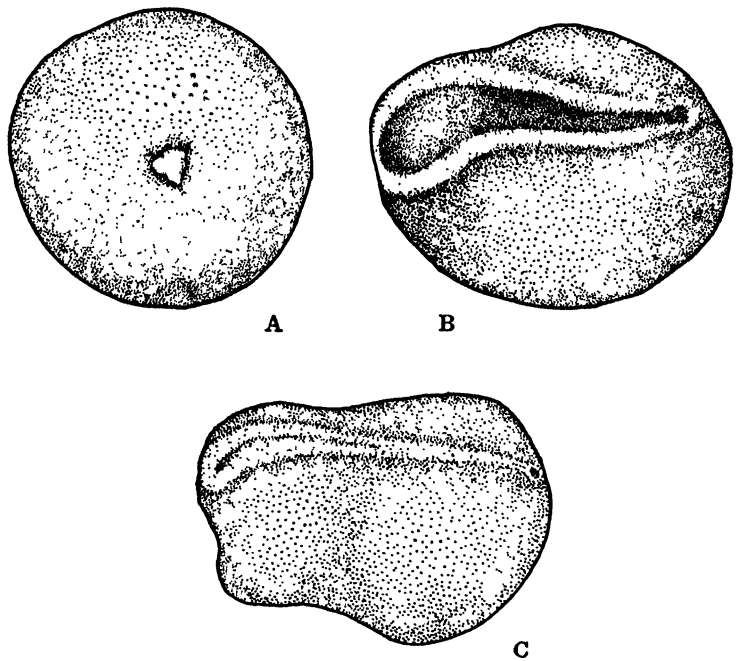


FIG. 76. Development of embryo of *Rana* showing closure of blastopore, formation of neural folds, and development of body form.

Invagination begins as a slit-like groove at the border of the gray crescent somewhat below the equatorial region. The large amount of yolk in the vegetative region prevents an equal invagination. The slit-like invagination is the dorsal lip of the blastopore. The invagination gradually extends to each side of the gastrula and the groove becomes crescent shaped.

Previous to the invagination a change in the distribution of the black pigment has occurred, for the small black cells have started to move down over the white cells and the black pigment extends below the equator. It will be seen at once that overgrowth, or epiboly, as well as invagination, plays a part in gastrulation of the frog's egg.

The crescent groove follows the border of the pigment zone as the latter gradually extends over the yolk and represents the dorsal side of the egg. The ventral lip of the blastopore appears later, when invagination takes place there, and the blastopore then becomes circular. The dorsal lip advances over the yolk more rapidly than does the ventral, probably on account of the greater amount of yolk in the latter region. The gullet, formed by invagination, is of a circular, slit-like character, and the yolk mass extends as a plug in the primitive gut lumen. As the

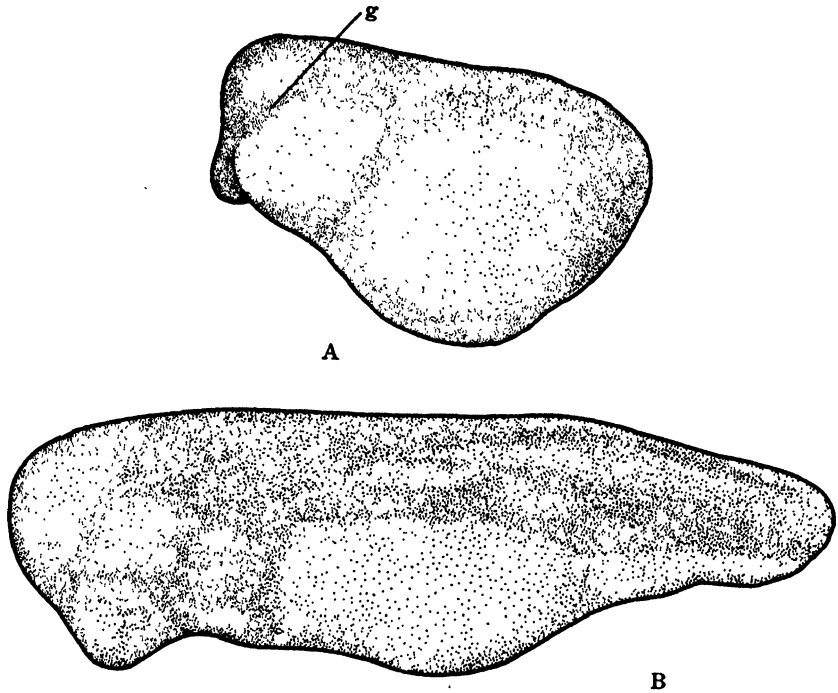


FIG 77. Embryos of *Rana* A at time of first indication of gill slit, B, just before appearance of external gills at time of hatching

ring-shaped blastopore grows together, owing to the greater downgrowth of the dorsal lip, it draws down on the yolk plug and finally becomes a small median, slit-like furrow. This process of the growing together of the edges of the blastopore is concrescence We may then regard concrescence as a third factor in the gastrulation of the frog.

At first the dorsal, and later the ventral, blastopore lips become differentiated with distinct ectodermal and endodermal layers. The cells of the yolk mass, which have extended as a plug in the blastopore, become pushed within as the blastopore grows together. We might thus

consider this kind as a solid cell ingrowth, perhaps a fourth factor in gastrulation.

Four factors may be said to enter into the gastrulation of the frog's egg: invagination, epiboly, concrescence, and a solid cell ingrowth.

a. Selachians. We may now contrast the selachian egg with that of the frog. In the former, a protoplasmic disc rests on the top of the yolk mass, and there is thus a sharp division between protoplasm and yolk, instead of a gradual transition as in the frog. The disc-shaped protoplasmic mass of the selachian becomes furrowed by cleavage planes, but the yolk mass remains unsegmented. That part of the endodermal mass of the frog which we have called the yolk plug must be represented in the selachian by the uncleaved yolk mass. Although the yolk does not cleave, in it are to be found scattered nuclei of various origins, but these nuclei appear to take no further part in development. Some of the scattered nuclei may be metamorphosed sperm nuclei, for polyspermy exists in the selachians. A part of the nuclei originate from the peripheral cleavage

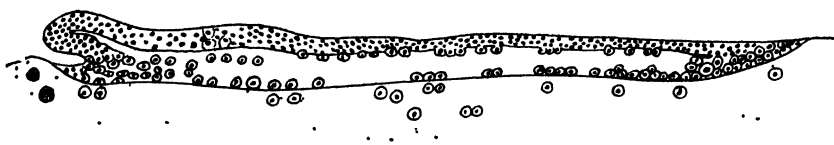


FIG. 78. Median section through the gastrula of *Torpedo* (After Ziegler)

cells and are called the periblast cells. These periblast nuclei are comparable to the cephalopod blastocones; that is, they are blastomeres not cut off from the yolk mass by cleavage.

In the selachians the cell mass of the germ disc forms a many-layered embryo which lies in a depression in the surface of the yolk. The cells of this mass are at first round, indifferent cells, not arranged in an epithelial layer. Between the cell mass and the surface of the depression a space known as the germ cavity appears which may be regarded as the cleavage cavity. It does not extend equally under the embryo, but is pushed toward one side which corresponds to the hinder end of the future embryo.

The first change in the germ disc of the selachian is the formation of an epithelial layer from the superficial cells which become flattened and broadened. This layer begins to form at the hinder part of the embryo where the cleavage cavity is larger and the germ disc thinner and spreads from this region over the whole germ disc.

Gastrulation begins as a depression at the hinder edge of the germ disc where the cells begin to turn under. Thus is formed the primitive gut cavity, lying between the inturned cells and the surface of the yolk

which has previously become covered with so-called periblast. The invaginating cells are formed from the round cells of the blastoderm, spreading as an iris-like ingrowth from the edge of the germ disc and of the growing epithelial endoderm. The invaginated gastrula cavity is at first crescent shaped, comparable to that of amphibians, but with an axial cavity in addition to the peripheral cavities of the crescent. The edge of the entire germ disc may be thought of as a blastopore.

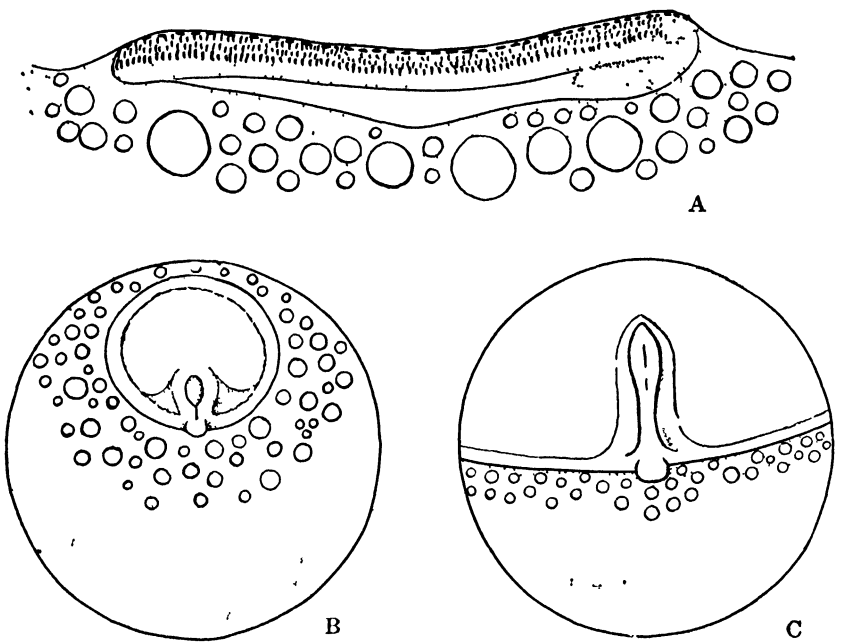


FIG 79 Gastrulation of the salmon, *Salmo salar*; B, growth of the blastoderm of the salmon, C, embryonal shield stage, the germ ring covering one-half of the yolk (After Ziegler)

The anterior edge of the blastopore becomes extremely active and begins to grow down over the yolk. Finally it grows entirely over the yolk, passing over the vegetative pole, and reappearing behind the embryo as the ventral lip of the blastopore. The yolk thus becomes entirely covered and the blastopore eventually closes.

b. Teleosts. In the teleosts the process of gastrulation is essentially similar to that in the selachians. The formation of the blastopore begins at the posterior edge of the germ disc where invagination brings about the origin of the primitive gut. There is a slight invagination at the lateral and anterior edges also, and the yolk is finally enclosed by the blastoderm, the anterior margin becoming the ventral lip of the blasto-

pore. In the elasmobranchs, teleosts, and also in the myxinoids, there are two periods in the closure of the blastopore. During the first period the overgrowth is practically limited to the dorsal lip, and the material for the formation of the body of the embryo is produced. During the second, the lateral and anterior margins of the blastoderm gradually cover the yolk. Thus only the dorsal lip takes part in the formation of the future embryo, while the lateral and ventral lips are entirely extra-embryonic.

c. Reptiles and Birds. In all the vertebrates thus far described, the Anamnia, the blastoporic lip appears at the edge of the blastoderm. In the reptiles and birds, which have embryonic membranes and hence belong to the Amniota, the blastoporic lip lies wholly within the blastoderm. The archenteric cavity is well developed only in primitive forms and is usually represented only by the neurenteric canal. In most cases

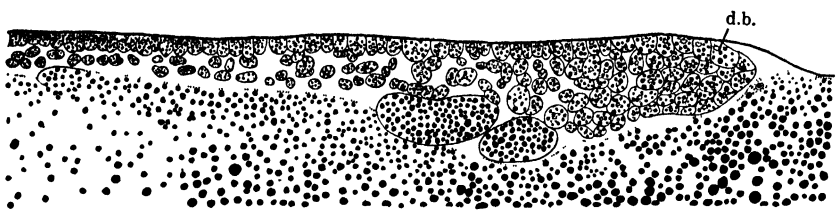


FIG. 80. Gastrula of pigeon. (After Patterson.)
Thirty-six hours after fertilization. Section through the posterior portion of the blastodisc showing the blastopore and its dorsal lip, d.b.

the upper layer or future ectoderm alone gives rise to the blastopore and archenteron.

In the chick the blastoderm or germ disc lies on top of the yolk mass, as in the fish. The endoderm is formed by an inturning of the germ disc at the posterior margin and by ingrowth from the inturned portion. At the posterior part of the germ disc the primitive streak is formed by a linear thickening of the upper layer of the blastoderm. The endodermal layer in the chick gradually grows around the yolk and the yolk sac is thus formed. The ectoderm does not directly cover the yolk sac, however, as here conditions are complicated by the presence of embryonic membranes, the amnion, which encloses the embryo, the allantois, and the chorion.

In the chick the primitive streak represents the fused lips of the margin of invagination and therefore is to be considered as the closing blastopore. In the primitive streak the germ layers are fused, the differentiated part of the embryo being formed in front of it, the anus at the posterior end, and the neurenteric canal at the anterior end. We must

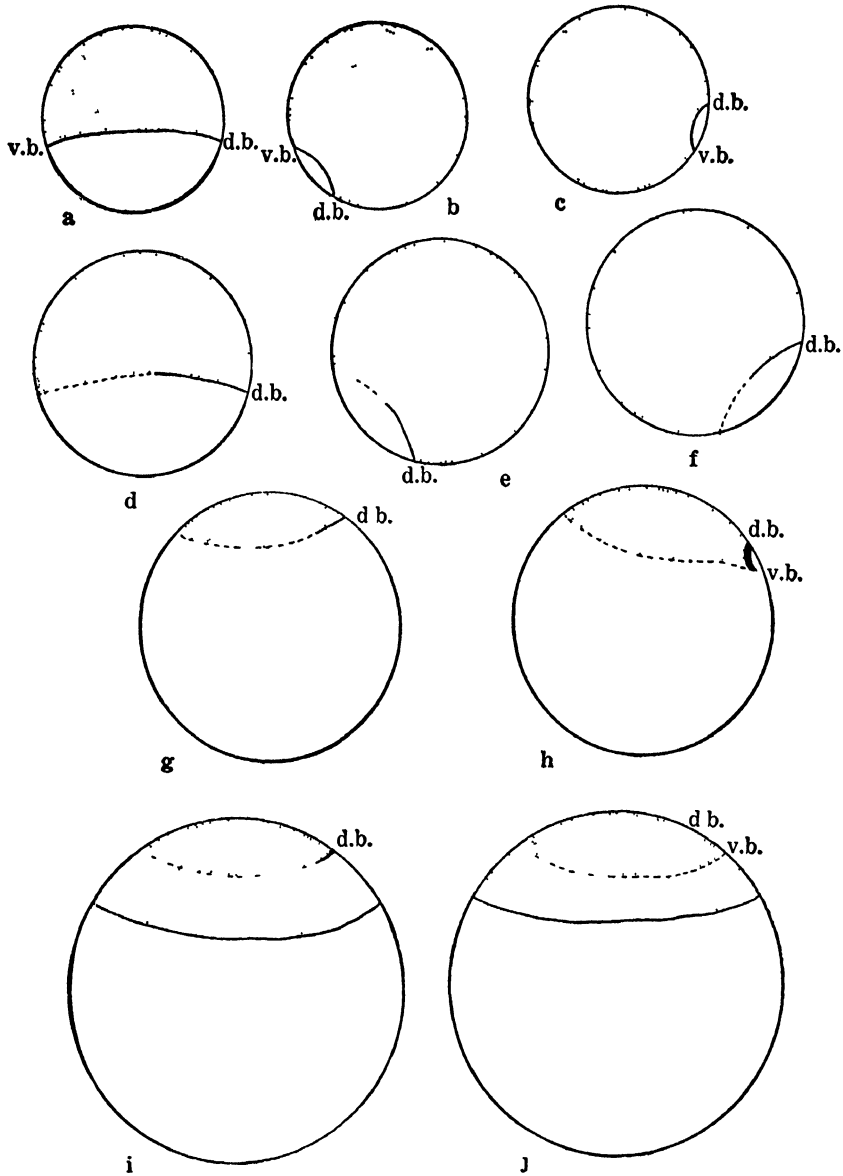


FIG 81 Diagram to illustrate difference between the blastopores of Anamnia and Amniota (After Jenkinson)

a–c Closure of blastopore in frog or similar form Stippled region is the blastoderm. The edge of the blastoderm represents the edges of the blastopore d–f Ventral lip of blastopore is absent in such a form as *Lepidosiren* The heavy solid line represents the portion which forms the lips of the blastopore g, h Only a small part at the posterior end becomes the blastopore as in the Gymnophiona The yolk remains uncovered i, j The condition of the blastopore in Amniota The embryonic shield is darkly stippled and is equivalent to the entire blastoderm of the Anamnia The amniote blastoderm extends further as indicated by the light stippling

therefore consider the primitive streak of the bird as an elongated blastopore since the relations are similar to those found, for example, in the frog.

In the reptiles there is a so-called embryonic shield at the posterior end of the blastoderm. This is a circular or oval area of columnar cells resting upon a lower layer and surrounded by a region of flattened cells.

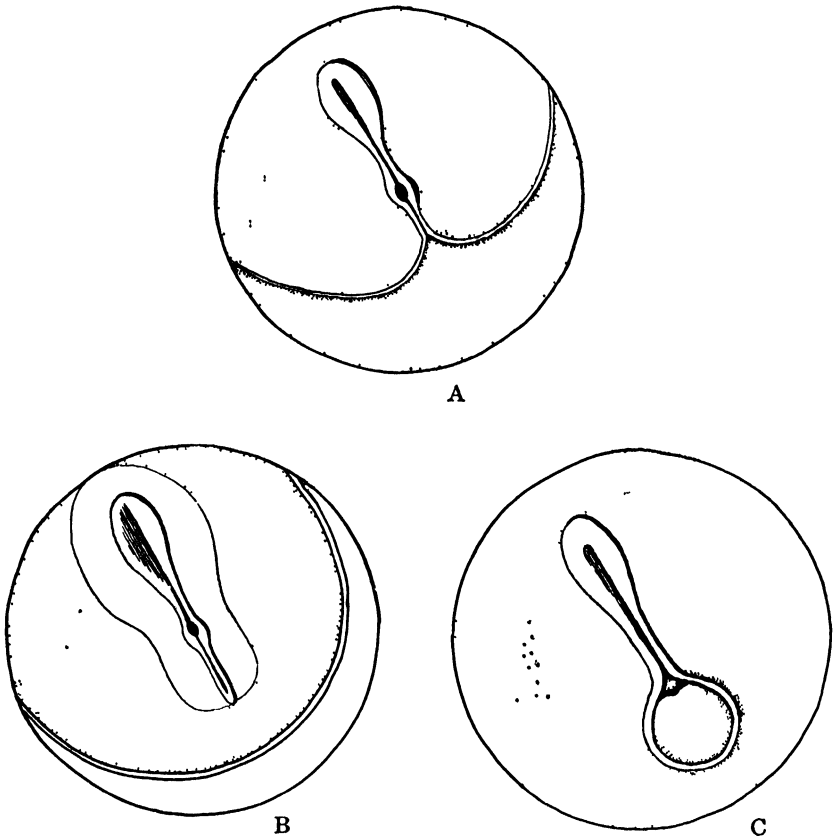


FIG 82 Balfour's diagrams showing homology between blastopore and the primitive streak (After Ziegler)

At the posterior margin of the embryonic shield the two layers come together A depression in the primitive plate appears here which is the beginning of the archenteron, and its anterior margin is the dorsal lip of the blastopore Lateral lips which are formed later turn back and fuse, in some cases forming a vertical or even actual ventral lip, although cases occur in which there is no ventral lip at all.

A mass of primitive cells surrounded by the dorsal and lateral lips

corresponds to the yolk plug. It will be seen that cells which are the morphological equivalents of the yolk cells of the Amphibia are thus to be found in the reptilian blastoderm. Here, as in the birds, the blastopore arises inside the blastoderm and not from its edge as in the Anamnia. The comparison of the Anamnia and Amniota can best be understood by observing the development of the gymnophionan egg. Here the blastoderm is an oval layer of columnar cells resting upon a partly segmented yolk. Only a part of the edge of the blastoderm at its posterior end is converted into a blastoporic lip which is at first dorsal. Later the lateral lips turn back around a small part of the yolk, and then join to form the ventral lip. The anterior part of the blastoderm thus plays no part in the formation of the blastopore. In this form the yolk remains uncovered. We have seen that, in other Anamnia, the ventral lip develops from the anterior edge of the blastoderm and the yolk is necessarily covered where the blastopore closes.

The embryonic shield of the Amniota must be represented, in the Gymnophiona, by the blastoderm, and the marginal zone of the amniotes, together with the lower layer with which it is connected at the primitive plate, must be represented by the yolk cells or nucleated yolk. It seems natural to suppose that in the transition from forms like the Gymnophiona to the higher vertebrates, as the yolk material in the egg increased in amount, segmentation has become restricted, not to the blastoderm alone as in the fishes, but to the blastoderm and those adjacent and subjacent cells which in the Gymnophiona are partly segmented from the yolk.

These points may make it clear why the blastopore of the amniote is formed inside the blastoderm but at the edge of the embryonic shield which is really equivalent to the anamnian blastopore.

B. Scorpions

The discoidal type of gastrulation is not limited to the vertebrates alone, for the scorpion and the cephalopod eggs are also of this type. Gastrulation is of course dependent on the structure of the egg, and an egg with a protoplasmic disc resting on a large yolk mass will belong to this type, regardless of whether it is an invertebrate or a vertebrate egg.

The scorpion egg (Fig. 39) is rich in yolk and the cleavage of the egg is discoidal. In young stages there is found a small, one-layered cap on the yolk. The blastoderm spreads gradually from this point and advances over the yolk. Scorpion eggs, since they have cleavage of this type, are comparable to eggs in which there is superficial cleavage with early formation of the blastodisc on one portion of the egg only. The scorpion condition is probably an extreme case and is to be traced back to the

type which is widespread among the arachnids. The scorpion egg, therefore, represents a modification of the type common to its group (as is also indicated by its viviparous mode of development).

The endoderm arises by the differentiation of the cells of the germ disc which lie next to the yolk and form a regular epithelium. The irregular cells which remain between the outer layer or ectoderm and the inner or endodermal layer become arranged into two symmetrical mesodermal bands.

It will be seen at once that in the scorpions, as in the myxinoids, the endoderm does not develop from the edge of the germ disc and thus the edge of the germ disc cannot be regarded as the blastopore.

C. Cephalopods

The cephalopod eggs are unusually rich in yolk and consequently they are considerably larger than are the eggs of other molluscs. There is a considerable difference in egg size throughout the group, but the yolk takes up by far the most of the egg. The entire egg is covered with a thin layer of protoplasm, which becomes thicker to form a disc at the upper or future animal pole. The germ disc thus formed is sharply marked off from the yolk mass.

The cephalopod egg is bilateral even before cleavage sets in. At the part of the germ disc which is to become the anterior end of the embryo, the disc extends farther down toward the equator. Since the cleavage furrows pass into the thin layer of protoplasm surrounding the yolk, the first cleavage cells and later the outer cells of the disc, the blastocones, are not cut off from the peripheral protoplasm. The germ disc spreads out and increases in size, at first chiefly at the expense of the formative yolk with which the blastocones are connected. The germ disc, one cell in thickness and covering only a small part of the egg, becomes thickened at the periphery as it spreads. When the cleavage cells have increased to a considerable number the peripheral blastocones become detached from the germ disc. These cells, according to Vialleton, wander beneath the germ disc and become arranged into a layer which spreads over the food yolk, forming a yolk epithelium. The exact origin of the yolk epithelium seems still to be in doubt. Some say it originates from the nuclei within the yolk, others from cells of the germ disc itself. The origin of the layer need not be of very special concern to us at this time, for the yolk epithelium, though it surrounds the yolk mass, does not become the endoderm.

While the yolk epithelium is forming and the edge of the germ disc is becoming thickened, the outer cells of the disc increase rapidly (Fig. 44). This outer layer extends gradually over the whole egg (and thus

covers the growing yolk epithelium) and forms the ectoderm. These two layers, the ectoderm and the yolk epithelium, are soon followed by a third or middle layer, which also covers the yolk. There are thus two definite regions in the egg at this time, the germ disc which forms the embryonic rudiment, and the yolk sac composed of three layers.

The function of the yolk epithelium is to form an envelope for the yolk and to make it available to the embryo. In later stages this envelope surrounds the yolk as in the earlier development. There gradually forms an outer yolk sac of part of the yolk not covered by the developing embryo, and an inner yolk sac of that part which is embraced by the embryo's body; the latter remains always connected to the outer yolk sac, but the connection becomes restricted to a narrow duct. The external yolk sac evidently passes on its contents to the internal sac and then the nutritive material is conducted to the embryo from the inner yolk sac by aid of the yolk epithelium. This seems the most logical method of absorption, for there are no known vessels in the external sac.

The extension of the germ disc over the yolk varies greatly in different cephalopods. In *Sepia* the germ disc is very small and the yolk sac very large. In forms like *Loligo* and *Octopus* the external yolk sac is more reduced and the embryo contains the greater part of the yolk. Grenacher studied a form in which there was scarcely any external yolk sac and at an early stage the small yolk mass was enclosed by the embryonic rudiment.

We have seen how the yolk sac and germ disc are formed and how their outer layers become ectodermal. We have still to describe the method of formation of the endoderm. About the time when the first rudiments of organs appear externally in the embryo, there may be seen, next to the yolk, an epithelial plate of only a few cells. It is the first indication of the enteron. It soon increases in size and becomes sac like, finally separating from the yolk. The yolk epithelium, previously lacking at this point, now grows under the enteron. The origin of the endodermal plate thus described is probably from the thickened peripheral mass at the edge of the germ disc which evidently represents meso-endoderm. The whole process is thus to be regarded as a much modified invagination, and the edge of the germ disc is the blastopore filled by the large yolk plug, which also fills the whole enteric cavity.

Bibliographic Note

Among the more important accounts of the subjects contained in this chapter are the following: Korschelt and Heider, Conklin, Selys-Longchamps, Patten, Claus, Lankester. These works are cited in full in the bibliography on page 406.

CHAPTER VIII

MESODERM FORMATION

Mesoderm is the layer between the outer epidermis and the midgut epithelium. It produces the muscles, coelome, and the structures belonging to the coelome, all the body and intestinal musculature, the gonads, the kidneys (except the Malpighian vessels) and the anlagen of connective tissue and blood vessels.

The concept mesoderm lacks a genetic unity throughout the animal kingdom and is really a collective name, since the mesoderm has a number of different origins within the same group. In spite of the lack of genetic unity of mesoderm, the anlagen of mesodermal structures have a common origin in single great groups of the metazoa. There is a definite homology in the mesoderm of all forms with a spiral type of cleavage, as turbellarians, nemertines, molluscs, annelids, and also the arthropods. The mesoderm of all these forms is endodermal, arising from the endoderm, and therefore the anlagen of the mesoderm bands are in genetic unity. It is also true that the ectomesoderm or mesoderm arising from the ectoderm of many forms is homologous. It is furthermore probable that the enterocoeles of echinoderms, Enteropneusta, and chordates are of common origin.

I. ECTOMESODERM

We will now consider the different kinds of mesoderm formation. Since the mesoderm is preceded by the ectoderm and endoderm there are only these two possible origins for the third layer. We will consider first the ectomesoderm.

Ectomesoderm arises from the primary ectoderm and usually produces mesenchymatous muscle cells and connective tissue. This kind of mesoderm occurs in the flatworms, rotifers, nematodes, annelids, and molluscs. Although the coelenterates are usually considered as two layered, there is often present a third layer, mesogloea, which, though not cellular, may be considered as ectomesodermal. In sponges, the dermal layer contains both ectoderm and mesoderm (using the term in a doubtful sense) and consists of (*a*) outer epithelium, (*b*) canal system except the flagellated chambers which are not included here, (*c*) connective tissue, and (*d*) sex cells.

In coelenterates a distinction can hardly be made between ectoderm and mesoderm. Although hydroids have only two layers, still the interstitial cells which are at the bases of the epithelial cells and which later form the gonads may be considered as forerunners of the mesoderm.

In the Actinozoa, the distinction between ectoderm and endoderm is more sharply defined than in hydroids. In the Alcyonaria, there is often connective tissue with skeletal structure, and this layer is ectodermal. The ectoderm becomes many layered, and a jelly substance is secreted between the cells of the deeper layers which become separated from each other. Thus two layers are formed, an outer epithelium and an inner gelatinous layer which may be thought of as representing ectomesoderm.

In the ctenophores the mesoderm becomes more highly differentiated histologically. A definite mesodermal layer is here present and this layer is now known to be ectomesodermal in origin. Kowalevsky and Chun found that the mesoderm originates from ectoderm in the region of the gut. (See Fig. 14*B*.) This is of particular interest because in forms with the spiral type of cleavage the larval mesoblast originates in relation to the blastomeres which produce the stomodæal invagination. In the polyclads, according to Surface, the ectomesoderm produces chiefly musculature of the pharynx.

Since the work on cell lineage, the development of annelids and molluscs is more definitely understood, and we now know that the part of the mesoderm which is to become the mesenchyme of the trochophore arises from the ectoderm. In all forms with spiral type of cleavage, the first three quartettes form the ectoderm. Wilson has shown for polyclads that the mesoderm cells are formed by division of the cells of the second quartette, and that the third quartette probably also shares in the formation of mesoblast. The mesoderm formed in this way is the larval mesoblast, the adult mesoderm arising from cell 4*d* as in molluscs and annelids and therefore being endodermal in origin.

The relations in other flatworms, nemerteans and nematodes, is not clear. In rotifers the entire mesoderm appears to be larval mesoblast and some of this has been shown to be ectodermal. There are no mesoderm bands and the gonads come from primary endoderm and may represent the mesoderm bands of related forms.

In a number of the annelids the ectomesoderm arises definitely from three cells of the third quartette, 3*a*, 3*c*, 3*d*. The anlage is thus asymmetrical, being paired on the dorsal side, while on the ventral only the left forms it. In some molluscs the ectomesoderm arises exclusively from the third quartette. In *Trochus* (Fig. 33, 34, Table p. 56) it arises from 3*c* and 3*d* from the dorsal cells of the quartette. In other forms the second

quartette also contributes. Part of the body musculature of the annelids is also known to be of ectodermal origin. The presence of ectomesoderm in arthropods is not clearly shown.

Ectomesoderm produces connective tissue and larval musculature of the trochophore larva. For annelids and molluscs it seems to be a provisional tissue degenerating upon metamorphosis. It is doubtful whether the ectomesoderm produces excretory organs, but the kidneys of some molluscs are known to be of ectodermal origin.

We have seen that ectomesoderm occurs in many coelenterates and also in many groups which are near the trochophore type; these may be collectively called Prostomia. Endomesoderm appears to be lacking in the Molluscoidea. It seems striking that the entire group called Deuterostomia (composed of Enteropneusta, Echinodermata, and Chordata) shows scarcely any ectomesoderm. Thus we recognize two great stems of the animal kingdom, in so far as mesoderm formation is concerned.

II. ENDOMESODERM

We will now consider the formation of the endomesoderm, which includes all the mesodermal structures in the embryo that come from endoderm. Endomesoderm may arise as bands of cells, as hollow coelomic pouches or through the coming together of mesenchyme cells. We shall consider each of these cases separately under the following heads: (*a*) Teloblastic mesoderm band formation. In these cases paired mesoderm bands take their origin from two primitive mesoderm cells as in annelids and molluscs. (*b*) Secondary or derived mesoderm band formation. Those cases belong here in which paired mesoderm bands are laid down without primitive mesoderm cells. They are often modifications of the first type, since they occur among forms derived from annelids and molluscs. Examples are arthropods and cephalopods. (*c*) Enterocoele formation. The formation of coelomic sacs by outfolding from the primitive gut characterizes this type. (*d*) Coelome formation by solid ingrowth. Obviously this case is closely related to the foregoing type. (*e*) Mesenchymatous coelome formation. The coelome is formed through the coming together of mesenchyme cells.

It might seem natural to consider mesenchyme structures as different from mesoderm. Mesenchyme is, however, not a genetically uniform structure, but only a histological conception. It may arise by migration from various places of origin: (*a*) from the coelome walls, (*b*) from the primitive gut, (*c*) from the blastoderm of the coeloblastula, or (*d*) from the reduction of the mesoderm band, as in molluscs. Mesenchyme cells may come together and form a coelome as in the collar and body coelome

of some tornaria. The multiplicity of the origin of mesenchyme shows that there is no unity in the term.

1. Teloblastic Mesoderm Band Formation

In cases of teloblastic band formation, a pair of originally solid mesoderm bands is formed by the budding of two terminally lying primitive mesoderm cells. This type of mesoderm formation occurs in forms with the spiral type of cleavage.

The two primitive mesoderm cells arise always from the 4*d* and thus must be considered as primary endoderm, since the ectoderm is complete with the third quartette and whatever comes from blastomeres arising

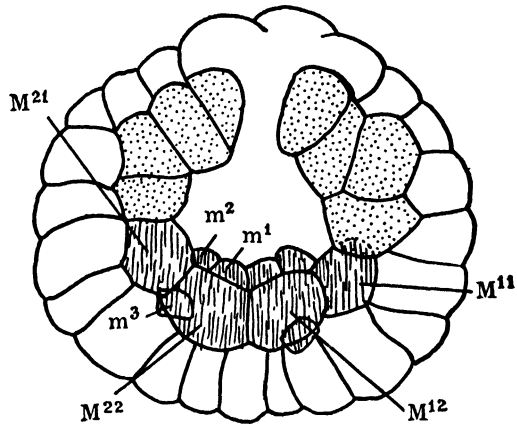


FIG. 83. Mesoderm of the snail, *Physa*. (Redrawn from Korschelt and Heider, after Wierzejski.)

The four larger cells M^{21}, M^{22}, M^{11}, and M^{12} and three pairs of micromeres m^1, m^2, m^3, give rise to the endomesoderm.

later is to be regarded as endodermal. The two primitive mesodermal cells are more nearly like endoderm than ectoderm in appearance, size, and yolk content. This intimate relation of 4*d* to primary endoderm is shown by the fact that, besides the primitive mesoderm, 4*d* often produces other cells which contribute to the formation of the midgut.

In most forms the third quartette is given off dexiotropically and 4*d* by laeotropic division. The next division of cell 4*d*, now called *M*, is again dexiotropic or simply bilateral and the two daughter cells are symmetrical. Cell *M*, originally in the region of the endoderm plate, early sinks inside the remnant of the cleavage cavity and cells M^1 and M^2 become symmetrically arranged. By these first divisions, small cells, enteroblasts which take part in midgut formation, are formed. An equal division of M^1 and M^2 takes place and thus four large dorsal cells are

formed, the two median ones of which, M^{12} and M^{22}, become the primitive cells of the mesoderm bands. The two other large cells in some forms (pulmonates) have been said to give rise to the primitive kidney of the trochophore. A series of divisions of primitive mesoderm cells now follows and two rows of small cells are formed. These become surrounded by the ectodermal products of the first quartettes.

In many yolk-poor eggs, as in oligochaetes, mesoderm bands are formed very early before the beginning of gastrulation, and lie in the blastocoele at the blastula stage. The mesoderm bands are at first dorsal and produce cells ventrally. With changes of body form they go through changes of position. The bands are given off horizontally and become lengthened parallel to the prototroch. (See p. 127.) Since the

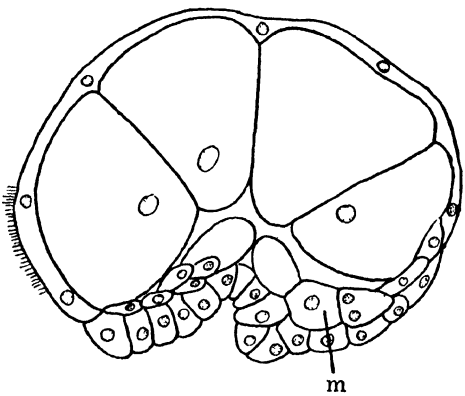


FIG. 84. Embryo of *Crepidula* at the time of the closure of the blastopore and the formation of the stomodaeum. In addition mesodermal cells (m) and the gut cavity are to be seen. (After Conklin.)

middle of the embryo is taken up by the gut they must bend, so both become curved and form a right and a left curve lying in a horizontal plane. When later the blastopore closes and pushes ventrally, the bands shift and lengthen ventrally. The original large mesodermal cells soon divide into smaller ones.

In annelids, the mesoderm bands break up later into segments which hollow out and become coelomic sacs. From their walls develop the trunk and gut musculature, gonads, and nephridia. Ectodermal cells, however, often take part in the formation of the nephridia.

In molluscs, mesoderm bands do not elongate as in annelids. Early in development they are lost in the mesenchyme and, according to one author, produce only connective tissue and musculature while the anlagen of the definitive kidney, pericardium, and gonads are from the ectoderm. Subsequent authors indicate that, in some molluscs, kidney,

gonads, and pericardium come from the mesoderm, bringing the later development of mesoderm bands of molluscs more nearly in line with that of the annelids.

2. Secondary or Derived Mesoderm Band Formation

In the arthropods, as in the annelids, we find a pair of mesoderm bands. At first solid and sometimes single layered, these bands later

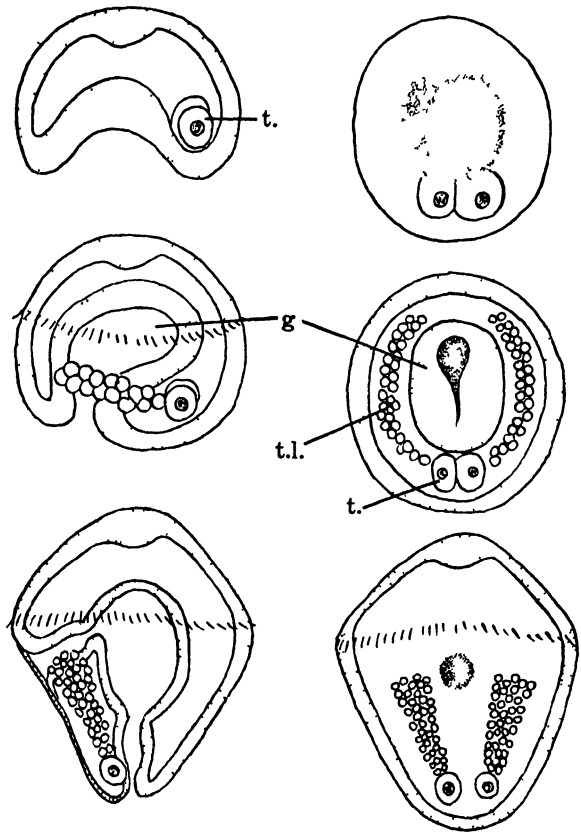


FIG 85 Diagrams of the development of Trochophora showing the shift in position of the mesoderm bands (After Korschelt and Heider)
g, gut, t, teloblast cells, t b, teloblastic mesoderm bands

become segmented and coelomic cavities develop in them. We cannot doubt the homology of the mesoderm bands of arthropods and annelids, but the method of formation of these bands is very different. In the few-celled embryo of the annelids, as we have seen, the combined anlage of the entomesoderm sinks into the cleavage cavity as two cells, the

paired mesoderm cells, and from them mesodermal bands develop. These bands arise through teloblastic growth, by successive cell proliferation from the sides of the original mesoderm cells. In the arthropods, on the other hand, the anlage of the mesoderm bands is many layered from the beginning. From the moment that they may be recognized as separate cell groups no original mesodermal cells are observed and no teloblastic cells increase. Gastrulation in most of the arthropods is in the form of a many-celled solid ingrowth. The ingrowing cells form the primitive entoderm which spreads itself under the ectoderm as a so-called under layer since it pushes up to the opposite side of the central yolk mass. Now there is a division into an endodermal cell mass and into paired mesoderm bands. These latter spread out and divide into segmented sections.

In some few arthropods there has been observed a special place of ingrowth for the mesoderm. In the crustaceans, *Astacus* and *Asellus*, this special place is the region of the forward blastopore lip; in *Peripatus*, at the hinder blastopore region. Certain crustacea deviate from the general scheme given for mesoderm formation in the annelids, because their cleavage is more nearly total. In these forms (*e.g.*, *Branchipus*) there are paired mesoderm cells or cells which because of their position and fate may be compared to paired mesoderm cells. They are similar to the annelid type.

A teloblastic method of mesoderm growth is found in the isopods. Here there is at the hinder end a cross row of eight teloblasts which through division produce the mesoderm of the metanauplius germ band. There is here only a distant relation to the annelid type as the conditions are entirely different.

In the cephalopods there is no trace of the spiral type of cleavage and cleavage is typically discoidal. The growth at the edge of the germ disc leads to the formation of the endoderm. Later a cell ingrowth in the hinder part of the germ disc takes place and this produces mesoderm and gonad cells. Thus we see that the arthropods and cephalopods are sharply contrasted to the annelids, for in many arthropods and in the cephalopods the mesoderm originates from groups of cells rather than from a single mesodermal cell anlage.

A consideration of the mesoderm formation of the Enteropneusta will lead us naturally to the other chordates. The Enteropneusta form an ancestral group through which the echinoderms are brought into connection with the chordates. In this small group different types of coelome formation may be observed, the type of simple enterocoele formation and the type of coelome formation by the coming together of single

mesenchyme cells. Since all these types of mesoderm formation belong in such a small genetic group of animals, they evidently must belong together. Let us consider first the coelome of *Balanoglossus*. Here there are, corresponding to the three body regions, three coelomic cavities; the head, unpaired but showing by its two pores and incomplete mesentery a double nature, the collar, and the body coelomes. The latter coelome consists of paired spaces separated by a median mesentery. The head coelome is known to be formed by the cutting off of a vesicle at the forward end of the primitive gut, the end opposite the blastopore. The water vesicle of the tornaria larva is the anlage of the head coelome.

There are several descriptions of the method of formation of collar and body coelome. In those forms of *Balanoglossus* which have no tornaria, but develop directly, two pairs of gut diverticula become the collar and body coelomes. In another form these two pairs of sacs are cut off behind from the first-formed coelomic vesicle, the water vesicle. Here in a form without tornaria there is a similarity to the formation of the enterocoele in most echinoderms which also form an originally forward vesicle through the fusion of three pairs of coelomic sacs, the forward enterocoele, the hydrocoele, and the hinder enterocoele.

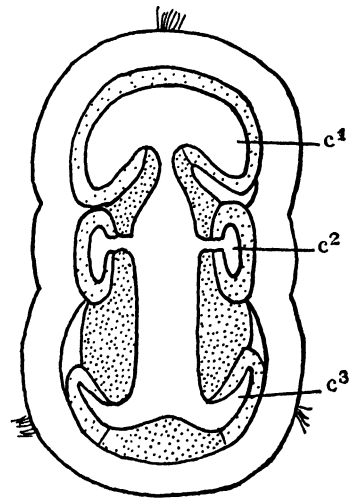


FIG. 86. Diagram of the coelome formation of *Balanoglossus Kowalevskii*. (Redrawn from Korschelt and Heider, after Bateson.)

In the New England tornaria the collar coelome is formed by paired cell proliferations at the side of the stomach, the trunk coelome by solid evaginations of the intestine. In the tornaria from the Bahamas, on the other hand, these coelomic spaces are formed at a considerable distance from the gut by aggregations of single mesenchyme cells. In one form they arise from the wall of the acorn coelome.

There are thus, in this one group of Enteropneusta, three types of coelome formation: (*a*) through diverticula of the gut, (*b*) through cell proliferation, (*c*) through aggregations of mesenchyme cells.

The second type is easily derived from the first, as solid cell proliferation is one step beyond a diverticulum. The third type differs, but may be considered to fall into line if we think of the cells of a solid proliferation as having lost their connection and become scattered mesenchyme cells.

3. Enterocoele Formation

In the echinoderms and also in amphioxus the coelome is formed by outpocketings from the alimentary canal, that is, by the formation of an enterocoele. We must first consider the mesoderm formation of echinoderms.

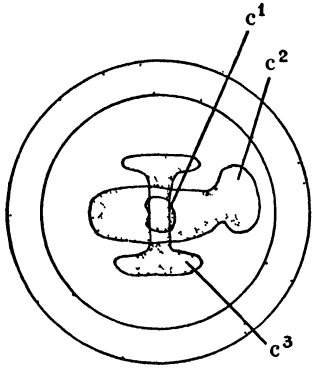


FIG 87 Diagram of the coelome formation in *Comatula* (Redrawn from Korschelt and Heider, after Seeliger)

In echinoderms, mesoderm formation is in general like that of Enteropneusta, by an evagination of the coelomic sac from the primitive gut. At the same time mesenchyme cells migrate into the blastocoele. Mesenchyme formation may begin very early, even before the beginning of gastrulation, or may not begin until later. Following this process there occurs in echinoids and in *Comatula*, at the place which will become the top of the primitive gut, a cell migration into the gelatinous blastocoele. By the time gastrulation is completed, cell migration from the top of the gut has already been going on for some time.

There are two types of coelome formation in the echinoderms. *Comatula* is an example of one type. In this form the hydrocoele develops

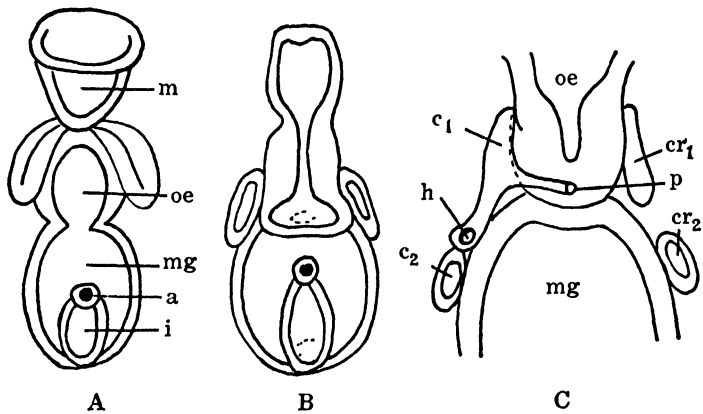


FIG 88 Metamorphosis of the coelome sacs in the echinoids (A, B, redrawn from Korschelt and Heider after Theel, C, redrawn from Korschelt and Heider after Bury)

a, anus, c_1, left anterior enterocoele, c_2, left posterior enterocoele, cr_1, right anterior enterocoele, cr_2, right posterior enterocoele, h, left hydrocoele anlage, i, intestine, mg, stomach, m, ectodermal mouth invagination, oe, oesophagus.

as an outgrowth of the forward part of the gut and the enterocoele as outgrowths from the posterior part of the gut. The hydrocoele is the anlage of the ambulacral system.

The other type of coelome formation in the echinoderms is illustrated by the echinoids. In these forms coelomic spaces arise from the division of an unpaired coelomic pouch given off at the top of the primitive gut. This vesicle soon divides into right and left halves, each of which divides again producing anterior and posterior right and left enterocoeles. The anlage of the hydrocoele or ambulacral system develops from the left forward enterocoele sac.

In chaetognaths and the brachiopods also we find enterocoele formation. Amphioxus must also be considered since its mesoderm is formed as an enterocoele, paired diverticula originating on the dorsal side of the embryo and later surrounding the gut. Hatschek thought that there were primitive mesoderm cells in amphioxus, and such cells are figured in many text-books, but they do not exist. The enterocoelous diverticula of the alimentary canal occur shortly after gastrulation when the embryo is lengthening. The notochord forms on the dorsal side of the alimentary canal by folding and is cut off lengthwise. At the sides of the notochord are the two grooves at the anterior end of which paired evaginations occur. These evaginations are the anlagen of the segments and increase in number as new ones are formed behind. They become completely separated from the gut and form paired coelomic sacs which are the primitive segments of the embryo.

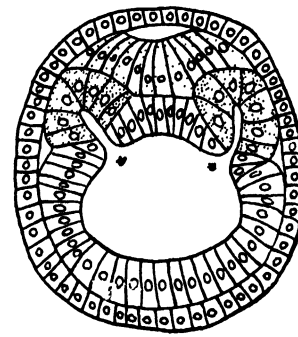


FIG. 89. Cross section of an amphioxus embryo showing coelomic cavities at* in connection with the enteron. (Redrawn from Korschelt and Heider, after Hatschek.)

4. Coelome Formation by Solid Ingrowth

In many cases the coelomic sacs are formed by solid outgrowth from the gut. As already mentioned, the collar and body cavities of the New England tornaria originate by outgrowths from the gut. There are similar cases among the echinoderms, for in *Ophiothrix fragilis* the two coelome anlagen have at first no lumen, and also among the brachiopods a similar condition prevails. In the tunicates likewise mesoderm originates as a solid outgrowth from the primitive gut.

The mesoderm formation of vertebrates may also be considered to belong to this type. In the embryo of *Triton* there is at each side of the notochord a small region of the gut from which coelomic sacs take their origin. These sacs appear as two many-layered cell masses which push in at the sides between the ectoderm and the entoderm. A split occurs in each mass and thus is begun the separation between the outer somatic

and inner splanchnic layers of the mesodermal sacs. The vertebrates do not show the division of the mesoderm into primitive segments from the first, as does amphioxus, but the segmentation appears later in the form of ring-shaped invaginations. Under the dorsal lip of the blastopore the invagination is deep and elsewhere it is hollow. The mesoderm formed near the dorsal lip is called the gastral mesoderm, and the mesoderm formed all around the blastopore itself is called the peristomial mesoderm.

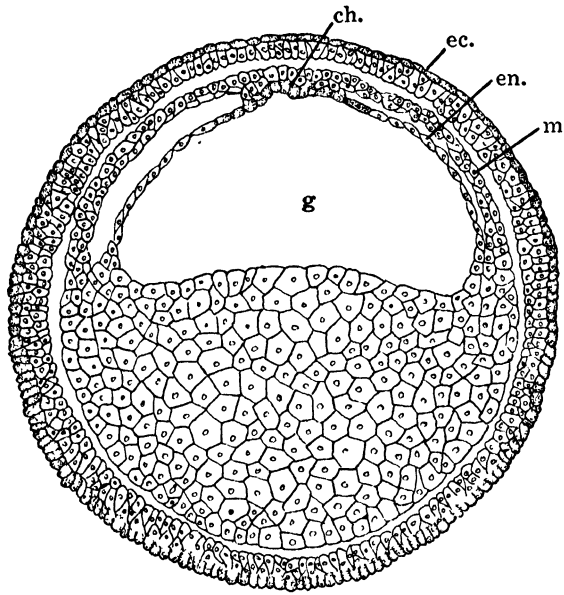


FIG. 90. Gastrula of *Rana fusca*. (Redrawn from Ziegler, after Schwink.)
ch, chorda anlage; ec, ectoderm; en, endoderm, m, mesoderm, g, gastral cavity.

In the frog there is a trace of the outpocketing of the endoderm to form the mesoderm sacs, for a groove occurs on each side of the notochord so that the notochord endoderm is separated from the lateral endoderm.

In selachians mesoderm formation occurs around the entire disc edge. At the anterior region and on the forward parts of the lateral edges the mesoderm under the blastocoele becomes split and later the blood islands form here. In the more posterior region of the embryo the mesoderm is formed by ingrowth of cells from mesoderm forming grooves of the endoderm. One of these grooves is adjacent to the notochordal region and the other is peripheral. From these grooves groups of cells grow into the peripheral blastocoele.

5. Mesenchymatous Coelome Formation

The origin of coelomic sacs by combination of originally free or independent cells seems to occur. It has already been stated that Morgan found this method of mesoderm formation to occur in the tornaria from the Bahamas.

In general the mesoderm formation of *Phoronis*, one of the Molluscoidea, is from scattered mesenchyme. *Phoronis* has an invaginate gastrula. At this stage single endodermal cells migrate into the blastocoele and become mesenchyme. This migration appears to be especially great in the region of the edge of the blastopore. The mesenchyme cells which are at first scattered in the blastocoele early show an inclination to lie on the inner surface of the ectoderm, and later on the gut endoderm. Thus are formed the somatopleure and the splanchnopleure.

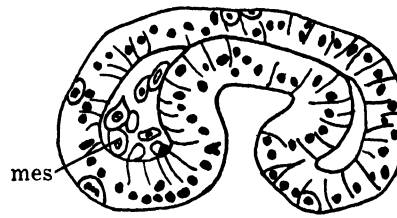


FIG. 91. Oblique section through gastrula of *Phoronis sabatieri*. (Redrawn from Korschelt and Heider, after Selys-Longchamps.) Showing mesenchyme cells (mes.).

It is doubtful whether or not such a method of mesoderm formation is primitive. We might infer that such is the case since Morgan's tornaria from the Bahamas shows this kind of mesoderm formation and other Enteropneusta do not. It may be, however, that a secondary change has occurred in the matter of mesoderm formation.

III. THEORIES OF THE ORIGIN OF THE COELOME

A discussion of the origin of the mesoderm involves also a consideration of the origin of the coelome. We might define the coelome as a body cavity lined by peritoneum from which arise the gonads. The tissue of the coelome, as of all other mesoderm, must necessarily arise from the ectoderm or endoderm. We have already discussed the double origin of the mesoderm, but have not yet considered the origin of the coelomic cavity itself.

The theories of the origin of the coelome are of course hypothetical. There are three main theories, the enterocoele theory, the gonocoele theory, and the nephrocoele theory. The enterocoele theory was first suggested by Leuckart in 1848 in one of his first papers on the coelenterates. According to this theory, the coelome of higher forms is represented by the radial canals and gastric pouches of medusae. The gastric cavity of coelenterates thus corresponds to both the gastric cavity and coelome of worms and higher forms. This original idea was suggested

long before Kowalevsky and Metchnikoff found that the coelome of forms such as *Sagitta*, brachiopods, echinoderms, and amphioxus really develops from gut pouches. The enterocoele or gut-pouch theory was taken up with enthusiasm by many, including Balfour and Lang. Lang developed the theory, illustrating it by *Gunda*. He regarded the metamerism of higher animals as resulting from the separation of paired gastric pouches. Each diverticulum of the gut had perhaps originally the task of producing genital products as well as the excretory function. As the diverticula separated from the gut, new structures must be formed to bear the functions of gonoducts or nephridia. Since the ancestry of the vertebrates is so problematical, this theory as applied to them must be largely conjectural. Lang finally gave up this theory for another.

This theory may be true as applied to the enterocoelous or vertebrate series of animals, but is evidently not true in the old sense as applied to the teloblastic series of animals where the coelome is derived from a pair of pole cells.

Hatschek is responsible for the gonocoele theory of coelome formation, suggesting it in 1876. According to this theory, the pole cells of the teloblasts are primordial germ cells, and the germ band, in which the coelome develops, is homologous to a gonad. Lang and others took up this theory and assumed that the original ancestral type has essentially the same morphology as a rotifer or flatworm. They suggested that the original gonad cavity was still connected to the outside by the gonoduct. Along with the extensions of the gonad cavity, a corresponding reduction in the parenchyma took place and the walls of the gonad became partly sterile, muscles developing in them. It is thus clear why the gonoduct does not connect directly with the gonad, for since the walls of the gonad are partly sterile, the eggs or sperm fall into the coelome and are taken up by the gonoducts. This theory explains also the reduction of the spaces in the flat worm occupied by the circulatory fluid. These spaces are entirely outside the body cavity and from them are formed the blood vessels. In some annelids there is a sinus-like blood system in at least part of the body and this is regarded by Lang as a primitive condition.

The nephrocoele theory is due chiefly to Ziegler, who regards the coelome as primarily and originally an organ of excretion. This organ consisted of a vesicle, the nephrocoele, and its duct. By its expansion a modification of the coelome is produced.

These three views are not as sharply distinguished from each other as might perhaps seem, since cases may be cited in which the coelome functions in such a manner as to suggest all of them.

Bibliographic Note

Among the more important accounts of the subjects contained in this chapter are the following: Korschelt and Heider. See also note at end of Chapter VII, and Hatschek, Wierzejski, Bateson. These works are cited in full in the bibliography on page 406.

TYPES OF INVERTEBRATE LARVAE

Phylum	Larger Group	Larva	Type Genus
Porifera		AMPHIBLASTULA (Pseudogastrula)	Sycandra raphanus
			Leucosolenia variabilis
		Parenchymula	Clathrina blanca
Coelenterata	Hydrozoa	Actinula	Tubularia
		PLANULA	Most Hydrozoa
			Many Actinozoa
	Scyphozoa	SCYPHISTOMA (Hydratuba)	Aurelia
		Scyphula	
		Ephyra	
		Strobila	
	Actinozoa		Actinia urticinia
		Arachnactes	In family Cereanthidae
		Zoanthella, Zoanthina	Zoanthidae
Ctenophora		Cydippid larvae	Order Cydippidae
Platyhelminthes	Turbellaria		
	Polyclads	MÜLLER'S Larva	Planocera
	Trematodes	Sporocyst, Miricidium, Redia, Cercaria	
	Cestodes	Cysticercus	
	Nemertinea	PILIDIUM	Cerebratulus
		Larva of Desor	Lineus
Annelida	Chaetopoda	TROCHOPHORE	
		Mitraria	
	Gephyrea	Trochophore	Phascolosoma
			Sipunculus
Molluscoidea	Phoronida	Actinotrocha	Phoronis
	Polyzoa	Cyphonautes (Closely related to trochophore)	Membranopora
	Brachiopoda	(Related to trochophore)	Terebratulina

Phylum	Larger Group	Larva	Type Genus
Trochelminthes	Rotifera	(Supposed to be persistent trochophore, but early development is not in accord with this view)	
Chaetognatha		(Fairly direct)	Sagitta
Arthropoda	Crustacea	NAUPLIUS	A few schizopods A few decapods
		Metanauplius, Protozoaea, Zoaea, Calyptopsis, Copepodid, Metazoaea, Mysis, Phyllosoma, Megalops, Cypris	
		Erichthoidina Erichthus (Pseudozoaea)	Stomotopod
		Alima	
	Insecta	Larva, pupa, caterpillar (eruciform)	
		Campodeiform	
Mollusca	Gastropoda	Ctenophore stage	Patella
		TROCHOPHORE	Dreissensia
	Lamellibranchia	VELIGER	Unio, Dreissensia
		Glochidium	
Echinodermata	Asteroidea	BIPINNARIA	Asterias
	Ophiuroidea	Ophiopluteus (Pluteus)	Ophiothrix
	Echinoidea	Echinopluteus (Pluteus)	Echinus
	Holothuroidea	Auricularia	Synapta
	Crinoidea	Pentacrinoid	Antedon
Protochordata	Enteropneusta	Tornaria	Balanoglossus
	Tunicata	Tadpole	
		oozoid Cyathozoid Ascidiozooid Trochozooid Phorozooid	Salpa

CHAPTER IX

TYPES OF INVERTEBRATE LARVAE

The development of the eggs of the many invertebrate animals shows an amazing diversity in the methods by which the adult is reached after the period of germ-layer formation has been passed through. In some groups most complicated life histories involving adaptations to environments that are totally different from that in which the cleaving egg found itself are present, while in other groups development is simple and direct. Of these many types of invertebrate larvae it is possible to describe only a few in this chapter. No attempt is made here to describe the cases of direct development as they occur in the various phyla, but it is desired to explain how certain important invertebrate larval types undergo their metamorphoses and reach the adult condition. In the table showing the occurrence of the various types of invertebrate larvae those forms which are selected for description are indicated by small capitals, while the others mentioned are of less general significance and are for reference.

I. THE PORIFERA

The Amphiblastula

The free-swimming larva which occurs in all families of the Porifera is known as an amphiblastula. Its development has been investigated in a number of forms, among the best known of which is *Sycandra raphanus* (see Fig. 64) studied by Schultze in 1875. Other important studies are the series of papers of Maas on various sponges, and the studies of Minchin on *Leucosolenia*.

The eggs are fertilized in the supporting jelly of the sponge wall, a position which they occupy when ripe and where the early stages of development are passed. (In *Cliona*, the boring sponge, the early stages of development take place externally.) In a cavity near a flagellated chamber which has a definite cellular lining the cleavage stages are undergone, forming an embryo of characteristic appearance known as an amphiblastula. At length the embryo emerges into the flagellated chamber and thence passes to the outside to become free swimming. After twenty-four to forty-eight hours of swimming about, it gradually finds lodgment and attachment to a substratum, a process which is

spoken of as fixation, and which involves a complete and quite sudden metamorphosis. In different genera of sponges the events do not all take place in exactly the same order nor do the larvae reach the same degree of advancement at the time of emergence or of fixation. The essential principles of development are probably not dissimilar, however.

The free-swimming larva is at first an ovoid blastula in which there are cells of two obviously different sorts. The large anterior portion of the embryo is composed of flagellated cells and the posterior portion of larger, non-flagellated, granular cells. Originally open at both ends, the blastula soon grows so that the pointed end is closed and rounded out and the cells become columnar in form and flagellated. The opening at the other end is surrounded by the granular cells which upon its closure take the form of a flat layer, some of whose cells proliferate and presently push into the cavity. It is supposed that the flattening is due to pressure against the unyielding mass of spicules, while the columnar cells produce a swelling into the space of the flagellated chamber adjacent. This is the condition in which the embryos are extruded to form free-swimming larvae.

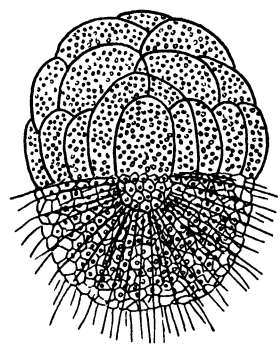


FIG 92. Amphiblastula of *Sycandra*. (After Schultze.)

In *Leucosolenia*, Minchin has described the columnar cells each as differentiated into two regions, a refractile portion at the internal end and a sharply marked-off granular part at the outer end. In living embryos the two portions are so distinct as to give superficially the appearance of an inner layer of refractile cells covered by an outer granular layer of cells; they are, however, merely parts of the same layer. Between the columnar and the posterior granular cells of this form is a zone of intermediate cells which are flagellated and entirely granular. The flagellated cells are much more numerous but much smaller than the granular cells. As development proceeds, the number of granular cells increases at the expense of the flagellated ones.

The larvae of *Leucosolenia* are transparent so that a mass of yellowish-brown pigment at the center may be easily seen. This is shown by sections to have the form of a tube open in front and behind, enclosing a lens-like body of gelatinous character which fills the central, blastocoelic space. The central cells are so arranged that Minchin regarded this structure as a premature, light-perceiving organ of larval significance only.

The free-swimming stage of the amphiblastulae is not of long duration.

They remain at the surface for perhaps a day, then sink to the bottom and after another twelve hours of swimming there they are ready for fixation. Fixation takes place at the anterior pole, the granular cells growing about and enclosing the flagellated cells in the process of metamorphosis. This process involves in some genera also the active invagination of the flagellated cells. At this stage only the two kinds of cells may be distinguished, the central cells appearing to be thrown out with the pigment in metamorphosis (Minchin). The outer cells become the dermal or covering layer, the inner the gastral tissues.

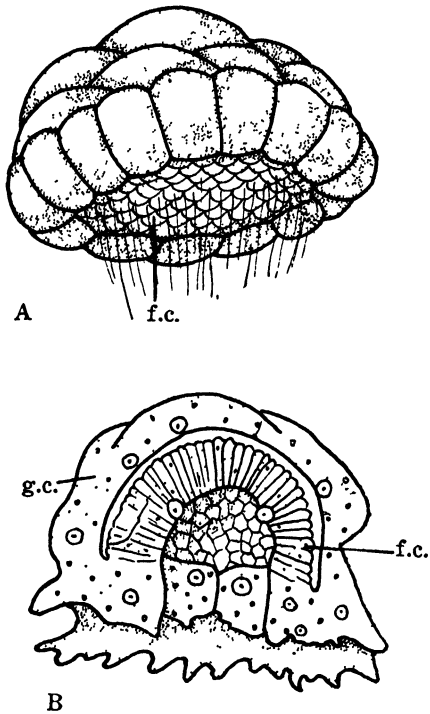


FIG. 93. Fixation of the larva of *Sycandra raphanus*. (After Schultze)

A, flagellated cells retreating into the interior; B, cup-shaped larva attached by amoeboid processes of the outer granular layer, f.c., flagellated cells; g.c., granular cells.

Postlarval changes in both layers now set in by which the adult condition is reached. The dermal layer differentiates and two kinds of cells result, one superficial, the other migrating beneath the former. They differ also in the type of spicules which they are to secrete, the superficial producing the monaxones and the other the triradiate spicules. The gastral cells take on a radial arrangement, a cavity appears in the center of the organism which presently (in five or six days) develops an opening to the outside, the osculum, and becomes more complicated. The cells themselves elongate and assume the typical collared appearance of choanocytes. The development of the pores and canals is a matter of slow growth, involving the formation of pouches and the rearrangement of cells, the secretion of spicules and gelatinous intercellular substance.

Other types of larvae have been described for sponges but they may all be regarded as modifications of the amphiblastula type just described, as was shown by Maas (1898). Some sponges also reproduce by formation of gemmules, but this is a process of budding and no free-swimming larvae are developed.

II. THE COELENTERATA

The larvae of the coelenterates exhibit a considerable variation in appearance in conformity to the wide range of adult conditions to be met with in the phylum. Like the adults, the embryos have, in spite of dissimilarities of form, a fundamentally common type of structure. Numerous experiments into developmental possibilities seem to have been tried out by the coelenterates with many diverse results, considering the paucity of material and restricted structural limitations. The structural simplicity is visible throughout the general similarities that may be discovered in the different kinds of embryos.

1. The Planula

The typical coelenterate swimming larva is a planula. It occurs in nearly all Hydrozoa (*Tubularia* is an exception) and in very many

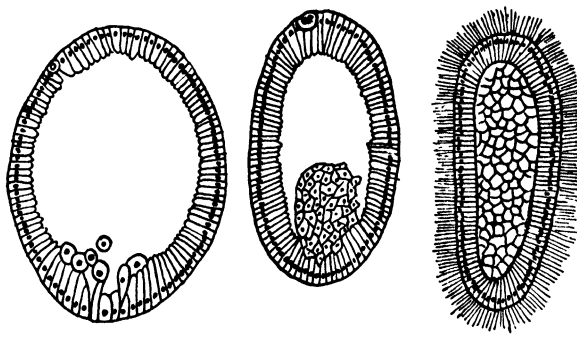


FIG. 94. Development of the planulae of *Clytia* showing the formation of endoderm and the character of the ciliated planula. (After Metschnikoff.)

Actinozoa and Scyphozoa. Because of its simple character and its widespread occurrence, an evolutionary significance as the ancestral coelenterate is suggested. It is the most important larval stage of this phylum.

The term planula was formerly applied to a blastula stage, but the usage is now obsolete. The blastula develops by the migration of cells inward, and by cell division into a two-layered, ciliated, swimming larva. The outer layer is in the form of a ciliated, columnar ectoderm; the inner is usually, in the Hydrozoa, a solid endoderm mass formed by many-celled, unipolar ingressions. There are numerous cases in both Actinozoa and Scyphozoa in which the endoderm is hollow. The gastral cavity does not appear in the hydroids until about the time of attachment of the swimming larvae. In *Pennaria* the hydranths are not covered by a hydrotheca; each has two circles of tentacles, one about the

oral end and another at the aboral end. At the time of maturity a row of medusa buds appears around the middle of each zooid which gradually enlarges and differentiates into the somewhat reduced medusae characteristic of the genus. These produce the germ cells and do not become free until the sex products are shed. Sexes are separate. After fertilization inside the bell of the medusa, the eggs are immediately shed. The medusae are then free, and after a few hours of feeble swimming, they die. In this genus the medusa is the less important of the two generations. The fertilized egg as described by Hargitt goes through a very irregular cleavage, forming an irregular cell mass which differentiates into a solid planula of two layers, the outer ciliated. After perhaps twelve hours of swimming about, the planula begins to settle down and at length attaches to grow into a new hydroid colony.

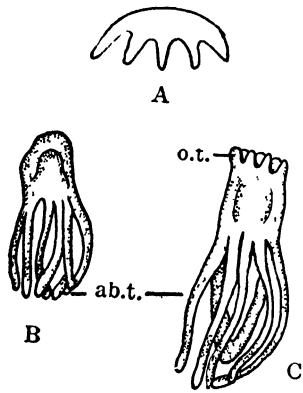


FIG. 95. External features of the various stages of development of the embryo of *Tubularia indivisa*. (Redrawn from Allmen.)

A, Planula beginning to bud off aboral tentacles. B, C, Actinulae. ab.t., aboral tentacles; o.t., oral tentacles.

2. The Actinula

In some forms, notably *Tubularia*, an intermediate larval form, the actinula, intervenes between the planula and the attached stage. It is neither fixed nor swimming, but creeping, and is formed by the appearance upon the planula of blunt protuberances, the tentacle buds, which gradually grow longer. First appear the rudiments of the aboral tentacles directed toward the future aboral end. The gastral cavity now is evident with thinned walls at one point where the mouth opening will presently break through about the time when the buds of the oral tentacles appear. The actinula now escapes and creeps about on the bottom, oral end downwards. It later tips over, attaches by the aboral end, elongates rapidly and gives rise to the buds which are to form the different zooids of the colony. Thus the actinula larva is important as the ancestor hypothecated by Brooks for the origin of the diverse kinds of coelenterates.

3. Origin of Coelenterate Larvae

The grouping of the various members of this phylum depends upon the presence and degree of development of two primary structural forms, the polyp and the medusa. In the Hydrozoa both are present; in the Actinozoa the polyp form is especially developed, and in the Scyphozoa

the medusa is the structural type. Within the Hydrozoa it is possible, as is commonly done, to form a series with *Cunina* and *Liriope* at one end,

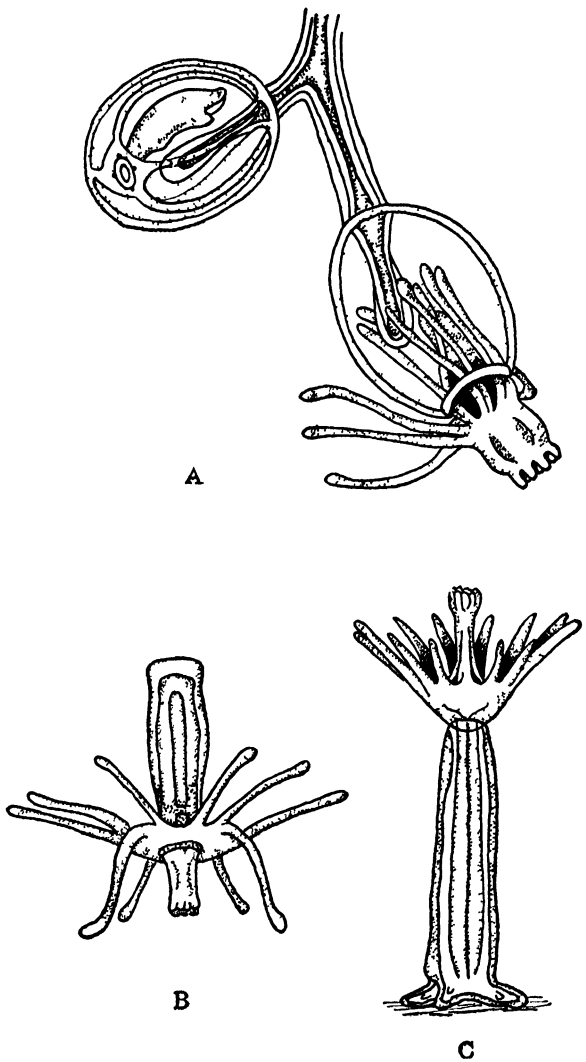


FIG 96 A, gonophores of *Tubularia indivisa* containing embryos B, creeping Actinula larvae, C, attached form of *Tubularia indivisa* (Redrawn from Allman)

in which the hydranth generation is quite lacking, and *Hydra* at the other end, in which there is no medusa. These forms are as follows:

Cunina, Liriope (medusa only)
Gonionemus (very rudimentary hydranth alternating with medusa generation)

Obelia, Bougainvillea (medusa and hydranth alternating and of equal importance)
Pennaria (medusa fixed during sex cell stage, and of reduced importance)
Gonothryca
Tubularia
Clava, Hydractinia
Campanularia, female
(In these four cases the fixed medusa undergoes more and more reduction in structure and importance.)
Eudendrium, Campanularia, male (remnant of medusa bud)
Hydra (no medusa, hydranth only)

It is evident for reasons that need not be gone into here that the evolution of this series cannot have been gone through from *Hydra* toward *Cunina*. On the other hand to assume the medusa to be the more primitive form of the two types is hardly in keeping with obvious structural features, and for this reason it is not possible to regard this series as having begun with a form similar to *Cunina* and to have been evolved in the reverse order. Rather some such suggestion as that made long ago by Brooks (1885) seems better adapted to the facts. According to this suggestion the most primitive type is neither a medusa nor a hydranth, but some small generalized form like the actinula. From this it may be supposed that four, perhaps five, series of forms have been derived. First there is the hydroid colony like *Obelia* in which both medusae and hydranths are present in alternating fashion. The remainder of the series from *Obelia* through *Pennaria* to *Eudendrium* and possibly to *Hydra* may be derived from this hydroid by the gradual degeneration of the medusa. A second series radiating from the actinula-like ancestor produced *Cunina* and *Aeginopsis* in which no hydranth is present. Perhaps a third produced hydra-like forms at the other end with no medusae. The Scyphozoa and the Actinozoa are the fourth and fifth series respectively which owe their origin to this primitive form of coelenterate, the actinula-like form.

Of this form the planula is the larval type (Fig. 94). It is the free-swimming larva common to all coelenterate groups. In some, the Hydrozoa and many Actinozoa, it is a solid mass of endoderm with an ectodermal covering. In others, the Scyphozoa and some Actinozoa, the endoderm is a definite layer with a gastral cavity. The latter is held by some workers to be the more primitive of the two, for it is argued that as the organisms were evolved an animal with a solid internal mass of cells would be quite unable to support itself. An embryonic type developing from stored yolk material would find no such difficulty, and so the solid form of the planula was thought to be secondary.

The general plan of coelenterate embryology is now clear. The fertilized egg develops gradually into a planula which swims about for a time and, it may be after intervening larval stages have been passed

through, settles down, attaches, and elongates into a hydra-like form representing the hydranth generation. This may grow into a hydroid colony or into the coral polyp or actinian, or go through further development processes to produce asexually a medusa which in turn produces new germ cells.

4. Actinozoan Development

In the Actinozoa the development of the sea anemones is very similar to that of the Scyphozoa up to a certain point. The planula is reached in the usual manner. The early stages of their development may be undergone in the gastral cavity of the mother in some cases, shedding of the embryos in the gastrula stage or thereabouts occurring through the mouth, or the eggs may be discharged from the mouth and fertilized among the tentacles or free in the sea water. After the free-swimming period the attachment at the broader end takes place. At the other end a narrow pit forms as the beginning of the stomodaeum; it deepens and its lower end breaks through, connecting the gastral cavity with the outside. The subsequent history is largely one of differentiation especially of the mesenteries. Some special forms of larvae occur in various subdivisions of this group but the general features are similar.

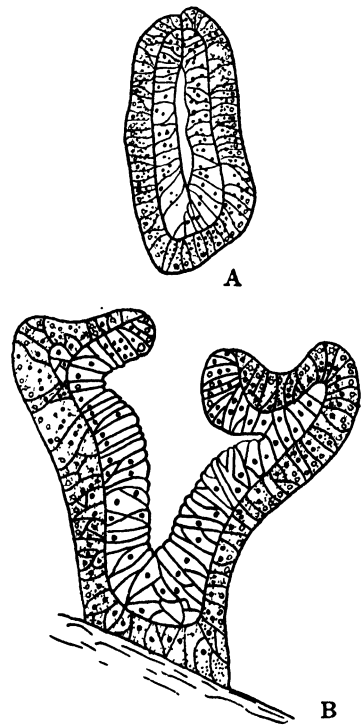


FIG. 97. A, free-swimming planula of *Aurelia aurita*; B, section through scyphistoma having four tentacles. (Redrawn from Hein.)

5. Scyphozoan Larvae

In the Scyphozoa the development of *Aurelia* may serve as a convenient type. The eggs are shed into the digestive cavity, pass out through the mouth after fertilization to pockets on the inside of the oral arms where they undergo those processes which lead to the planula stages. Then they emerge and swim about freely. Histological differentiation continues, and the larvae are ready for attachment in four or five days. After attachment the larva elongates and broadens out, the mouth opening becomes widened and four tentacle buds appear between which the taenolae, endodermal ridges projecting into the gastral

cavity, develop. This larva with its flattened, broadly open, oral disc is spoken of variously as a scyphistoma, scyphula, or hydra-tuba. Secondary tentacles develop between the original four and then continue to form until the appropriate number is reached. After further differentiation, the process sets in which distinguishes scyphozoan development from other types. It is the cutting off of its oral disc by means of a constriction around the scyphistoma to form an ephyra larva. This process is known as strobilization and in a well-fed scyphistoma may take place repeatedly until a dozen or fifteen ephyrae are formed from the one larva.

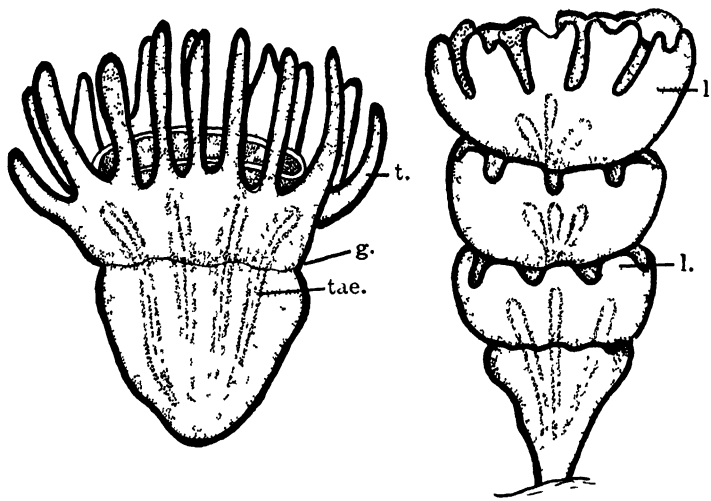


FIG. 98. Strobilization of *Aurelia aurita* (Redrawn from Claus.)
t, tentacles of strobila; tae, taeniolae; g, first transverse groove; l, lobes of ephyra.

The more usual condition is for each scyphistoma to produce two or three ephyrae. The constrictions appear progressively later as they pass down the larva, and in the well-fed individuals the appearance of a pile of saucers is readily suggested.

The ephyra now continues rapidly its development and differentiation leading to the form of the adult jellyfish. The scyphistoma tentacles are resorbed and in their place the characteristic lobes of the ephyra appear. The oral cone lengthens into the manubrium and other changes occur which result in the metamorphosis of the ephyra into the jellyfish.

III. THE PLATYHELMINTHES

Development in the flatworms presents many diverse aspects. Some eggs undergo no metamorphosis but pass directly into the adult condi-

tion. Others reach their final development only after passing through the most complicated life histories, involving different hosts, totally different environmental conditions, and totally different types of organization. Since it is not the purpose of this chapter to follow through life histories, but merely to show the relations of types of larvae to embryos and adults to which they are related, only meager reference can be made to the group as a whole. One well-known form is Muller's larva, the

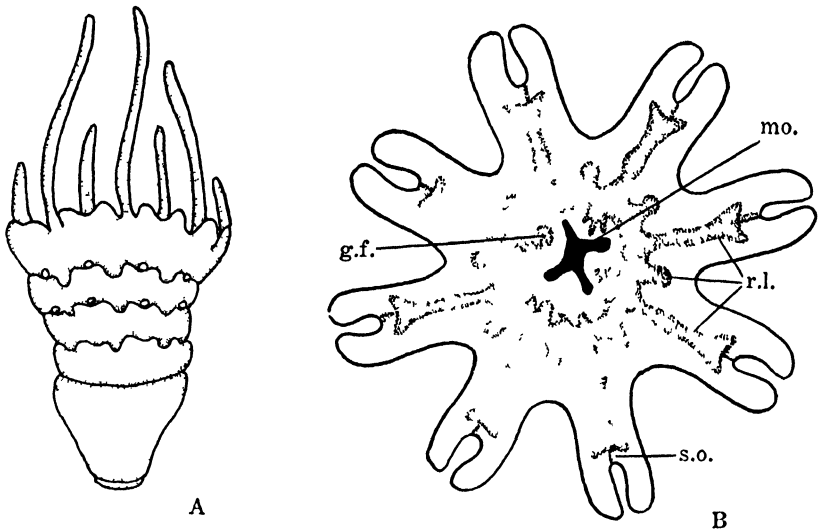


FIG. 99. A, strobilized scyphistoma of *Aurelia aurita*. B, ephyra larva of *Aurelia aurita* after liberation. (Redrawn from Claus.)

g.f., gastric filaments; mo., mouth; r.l., various radial lobes of ring-like gut; s.o., marginal sense organs.

free-swimming larva of the polyclad Turbellaria named after its discoverer, Johannes Muller.

1. Müller's Larva

The development of the polyclads is known chiefly from an older study by Lang on *Yungia* and a later and more detailed investigation by Surface on the embryology of *Planocera inquilina*. The cleavage is of the spiral type as found in such groups as gastropods and annelids. The cell lineage is similar in the early stages and the localization of formative substances surprisingly like that in the higher forms; the similarity is especially surprising when it is remembered that the Turbellaria agree closely with the ctenophores in many points of structure. Gastrulation is by the method characteristic of eggs with spiral

cleavage, namely epiboly. An oval embryo with ciliated ectoderm and endoderm results. The stomodaeum opens into a rather broad gastral sac, in the wall of which are only small endoderm cells in place of the large endomeres of an earlier stage. Mesoderm, ectomesoderm, and ganglion cells are also present in the embryo of *Planocera* when it is ready for escape from the capsule. Eight ciliated lobes appear at this time just below the equator; these are the characteristic features of Müller's larva.

Lang followed through the metamorphosis of *Yungia* in detail. The position of the mouth shifts from the pole to the ventral side of the

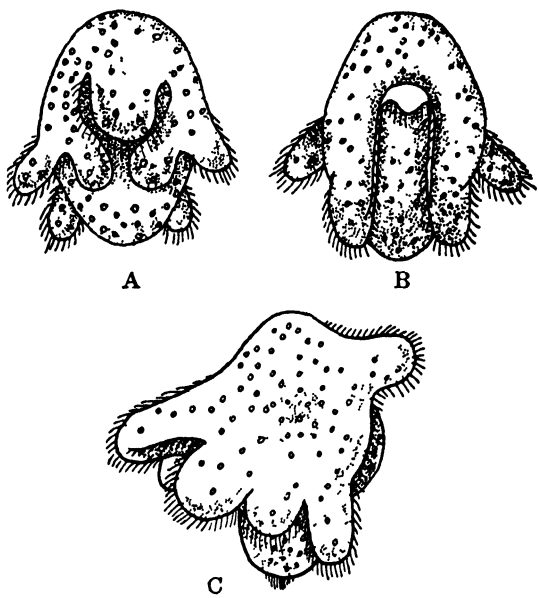


FIG 100 A, B, C, dorsal, ventral, and lateral views of free-swimming larva of *Yungia*. (After Lang)

elongating larva. Symmetry is lost because of the inequality in the rate of growth of the two sides of the larva. Of the eight lobes one is dorsal, one ventral, and the others make up three pairs laterally placed, and all are connected by a continuous ciliated band. Growth in length with the gradual reduction of the lobes until the usual form of a flatworm is reached brings about the external changes. Among the internal changes involved are the development of eye-spots, the development of the parenchyma (which fills in the interior of the adult, and of which some cells differentiate into muscle cells), and the differentiation of the brain and genital organs. The most marked changes, however, are those

concerned with the development of the protusible pharynx. There is an invagination of the larval stomodaeum, in which the pharynx arises as a ridge-like thickening. The pharynx itself grows in length and the stomodaeum becomes everted to form the sheath of the pharynx.

Surface records that the early cleavages as well as the maturation divisions require about one hour each. By forty-eight hours small ectodermal cells cover the entire embryo, which become ciliated, and during the third day rotation is set up in the capsule. Eye-spots appear about the fourth day. By the end of the fifth day the ciliated lobes appear, and

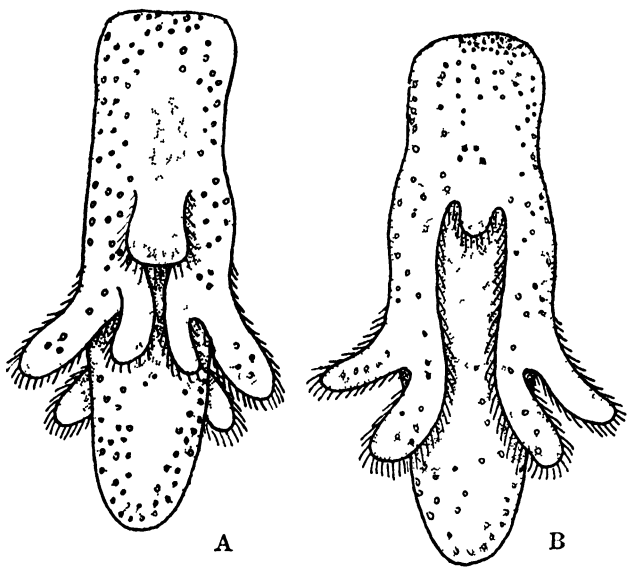


FIG. 101. A, B, dorsal and ventral views of larvae of *Yungia aurantiaca* in process of metamorphosis. (After Lang.)

on the sixth the larva breaks from the egg membranes to become free-swimming. It swims for some time, eventually settling to the bottom and assuming the shape and habits of a typical polyclad.

Some of the turbellarian larvae, owing to disproportional development of some lobes, resemble fairly closely the pilidia of the nemerteans. There is resemblance between this larva in turn and the trochophore which Balfour thought was derived from it. Obviously, therefore, larval forms of this group are of importance in working out the ancestral history of invertebrate types, and by some the history is thought to run from the ctenophores through the turbellarian forms to the pilidium and trochophore.

2. Trematode Larvae

Trematodes are commonly divided into Monogenea and Digenea, depending upon whether one or more than one host is necessary for their development. Since only one host is necessary in the Monogenea, development is direct or nearly so, whereas in the Digenea there are often several intervening stages. In the sheep liver-fluke, which may be chosen as an example, the egg undergoes the early part of its development in a chitinous shell while it is passing through the alimentary canal of the

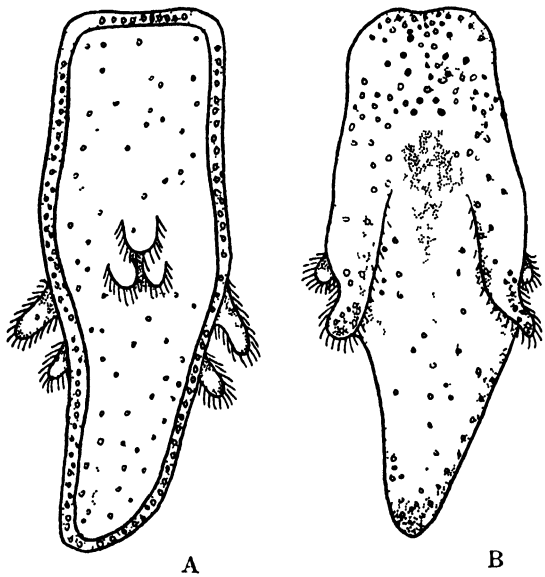


FIG 102 A B, same as above but older

sheep. After it has been passed from the body of the host it at length escapes from the shell as a ciliated larva, the miracidium. This larva swims about in water or moves over damp vegetation until it comes in contact with a pond snail or perishes. It is said to be sensitive to the presence of the snails and to move toward them actively. At this time the larva is in many respects similar to a rhabdocoele turbellarian without gonads. It has nearly the shape of a slender cone with two eye-spots near the head end (the broader end). There is a brain, a pair of flame cells representing the excretory system, and an imperfect intestine. The rest of the body is filled with germ cells. After the miracidium has penetrated the body of the snail it loses its ciliated ectoderm, the enteron degenerates, and it elongates into a sporocyst, which is thus

merely a transformed miracidium. Within the sporocyst, germ balls* are budded off and each begins a development similar to total cleavage to form a blastosphere which in its turn is converted into a gastrula. This passes then into the larval type known as the redia. The redia is usually looked upon as parthenogenetically produced, and the alternation of generations thus involved differs from that seen in coelenterates in that here a sexually developed sporocyst gives rise parthenogenetically to the redia. In the winter the rediae produce other rediae in a similar manner, but in summer they develop into another type of larvae, the cercaria. The rediae differ externally from miracidia in the absence of cilia, of eye-spots, and in the presence of a pharynx and a simple sac-like intestine, and their general shape is more cylindrical with a collar or circular ridge near the anterior end and short locomotor processes near the posterior. The cercariae have in an undeveloped condition all the organs which are found in the fluke and some other structures including a tail which are of brief duration and last only during larval life. The cercaria leaves the snail, encysts upon a blade of grass, and must be eaten by a sheep to continue its development. Its final metamorphosis consists in the loss of its tail, the rapid growth of the organs already present, and the development of its reproductive organs.

Variations from this case occur; in some forms stages are omitted and the cercaria may develop without a tail. The complication of two or more hosts and an intermediate larva which reproduces paedogenetically recalls the cases of polyembryony recorded in numbers of groups throughout the animal kingdom.

3. Cestode Larvae

Cestode larvae and life histories are only a little less complicated and involved than are those of the trematodes. The eggs of cestodes, like those of the trematodes, are surrounded in the uterus by a thin egg-shell. In some forms there are also included "yolk cells" which contribute food to the developing embryo. Cleavage is irregular in type, although characteristic features are to be recognized. An outer layer of cells is formed from micromeres which separate from the larger cells early in cleavage. In most cases this layer of superficial cells gives rise to a chitinous coat which is thrown off ultimately. Only the central mass of cells which is enclosed by the superficial layer takes part in the formation of the embryo. This mass of cells develops a series of six hooks and becomes the hexacanth embryo. The development of the hexacanth

* There is reason to doubt whether these germ balls are true germ cells involving a reduction of chromosomes in their development. If these are not germ cells then the rediae are asexually produced and a true alternation of generations exists.

embryo (onchosphere or proscolex) and the formation of the chitinoid coat with its inclusions of yolk material take place within the uterus of the posterior proglottids, and in this condition the embryo is passed to the outside; it must await its appropriate intermediate host for further development.

If it is ingested by the proper animal (often along with the entire proglottid in which it was produced) the digestive juices soften the coat of the embryo, which is set free and at once begins boring its way through the intestinal wall and passes into liver, mesentery, peritoneum, brain, or other organ. Here it encysts but continues its development. If the intermediate host is an invertebrate the next stage is a cysticercoid; if a vertebrate, a cysticercus. The difference is chiefly in the large bladder or caudal vesicle of the latter. The cysticercoid has typically three parts: scolex (the tapeworm head), a body, and a caudal vesicle, the latter forming an enclosure for the other developing parts inside it. In the final host the scolex is pushed out, attaches with its hooks to the wall of the alimentary tract and begins to grow.

The cysticercus is attained from the hexacanth embryo in the following manner. The hexacanth embryo, having reached its place of encystment, enlarges markedly and its interior becomes filled with fluid or with loose spongy tissue. For this reason it is called the bladder worm stage. A small invagination into one side appears which deepens and develops into the scolex, but in an inverted condition. An elevation at its bottom becomes the rostellum, and the suckers appear on the inside of the lateral walls near their base. Metamorphosis of such a larval form takes place after transference to the final host by the eversion of the scolex and very active growth in the region just behind it to form proglottids.

In a few instances instead of a single scolex many are produced by the cysticercus by budding from its interior. This is the case in *Taenia coenurus*, which produces as invaginations from its wall many scolices that are later to be everted. In *Taenia echinococcus* this polyembryonic development may go even farther, the ingrowths from the wall becoming separated off to drop into the cavity as secondary bladders. Each of these goes on actively producing new crops of scolices.

IV. THE NEMERTINEA

Of the four groups of the nemertean worms only one so far is known which develops by metamorphosis. In this (Heteronemertini, Schizonemertini) there are two families and the characteristic and important larval form is the pilidium. In one of the families, however, there occurs a creeping form which is really a pilidium modified for creeping in keep-

ing with the similar habit of the adults. This is known as the larva of Desor and is that of the genus *Lineus*. The nemerteans, while showing a distinct advance over forms already considered in acquiring a second opening to the alimentary tract, the anus, exhibit many primitive features which relate them to the flatworms and also to the ctenophores. The pilidium itself also is prophetic in certain of its features of the trochophore larvae of higher groups. *Cerebratulus* has been most studied in America as a type of nemertean development.

The Pilidium

The eggs of *Cerebratulus* cleave spirally and form blastulae and gastrulae in the manner typical for such eggs. The free-swimming pilidium is a helmet-shaped larva with a spike of long cilia arising from the apical plate of cells. The cilia elsewhere are uniform in size and distribution except at the margins where a ciliary wreath may be seen. This is the locomotor organ of the embryo, the prototroch. The sides of the umbrella-like portion are prolonged downward into hanging lobes or lappets. The pilidium is produced from the symmetrical gastrula by the unequal development of certain parts so that the blastopore opening becomes oval in shape and the invaginated enteron grows in length and bends over to one side, the bent sac-like portion becoming the stomach and the open funnel the oesophagus.

From two large cells which pass into the blastocoele the mesenchyme is produced. It consists of a mass of stellate cells whose amoeboid movements may be seen through the transparent outer covering. The pseudopodia which are put out enable the cells to attach and some are converted into muscle fibres. The pilidium is completed in this condition and swims about for a considerable period, perhaps two weeks, feeding at the surface of the sea upon microscopic organisms.

The metamorphosis of the pilidium has proven elusive since it is not yet possible to rear the animals under laboratory conditions and only fragmentary information has been gleaned from the material collected from the sea. We have to do here with the first of those transformations, numerously observed in the invertebrate groups, in which only part of the larval structures are included in the adult animal. In this case the larval ectoderm, prototroch, lappets, and apical sense organ, as well as the fused outer parts of the amniotic invaginations about to be described, form a double bell-shaped structure which is left behind like a cap after the little worm is differentiated and has constricted itself away. This larval rudiment swims about for a time, and then, unable to feed and exhausted, it dies.

The formation of the worm's body from the larval pilidium begins with four ectodermal invaginations which appear on the flattened lower surface. These amniotic invaginations form, in their deeper portions, the imaginal discs which contribute largely to the definitive body of the worm. They are distinguished as right and left anterior, in front of the

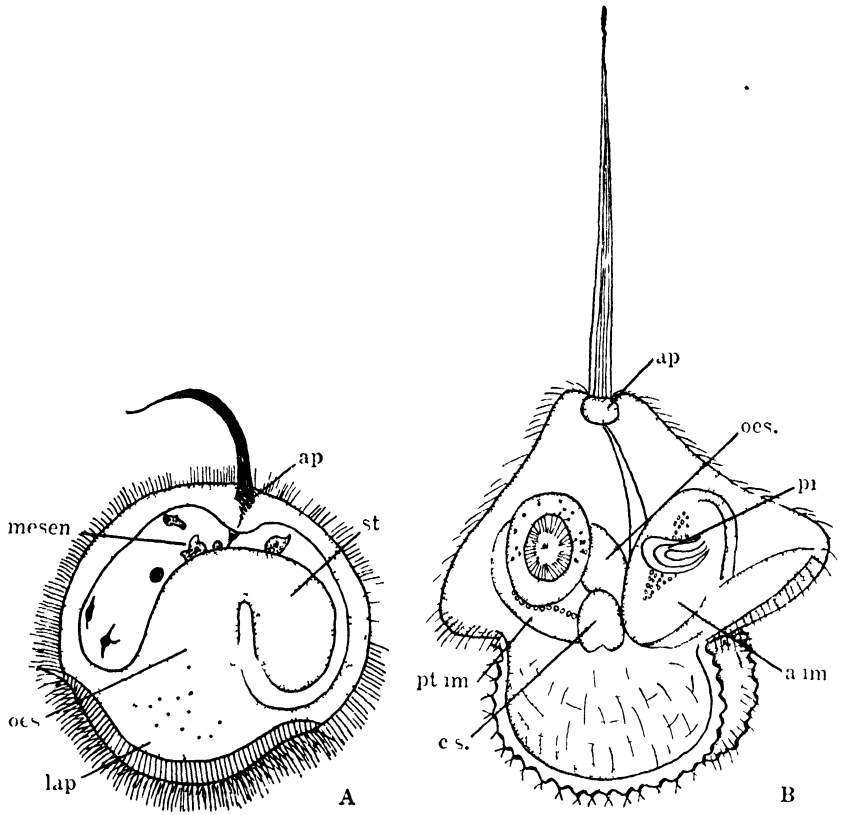


FIG 103 A, pilidium larva of *Cerebratulus lactus* (Redrawn from MacBride, after C B Wilson) lap, lappet, oes, oesophagus, st, stomach, mesen, mesenchyme cells a p, apical plate B, a pilidium just before metamorphosis (Redrawn from MacBride, after Metchnikoff) a p, apical plate, a im, anterior imaginal disc oes, oesophagus, pr, anlage of proboscis; pt. im., posterior imaginal disc

mouth, and right and left posterior behind it. They continue to deepen and broaden, growing up over the already formed alimentary canal until they finally meet and coalesce. Their outer walls form a temporary larval covering known as the amnion, a delicate envelope separating the body of the worm from the covering of the larva, and the inner fused portion is the skin of the future worm. The intestine of the pilidium is

thus included in the body of the new worm and becomes the endodermal portion of the latter.

Rudiments of the other organs of the worm are developing meanwhile. The proboscis develops as an ectodermal invagination projecting into a mesodermal mass which is to become the proboscis sheath. The brain

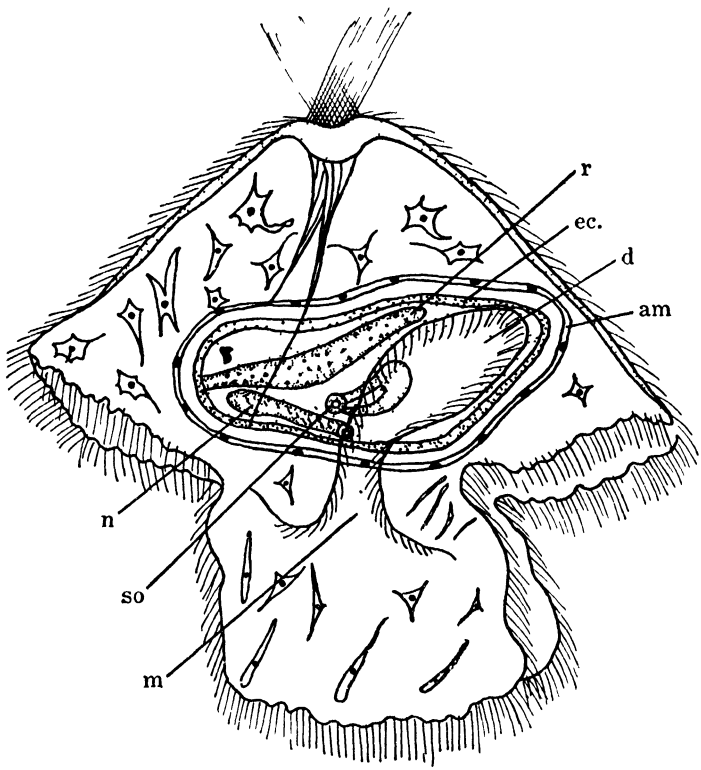


FIG 104 Fully developed pilidium with young nemertean within its amniotic sac (Redrawn from Korschelt and Heider, after Butschli)

am, amnion d, intestine of pilidium larva surrounded by worm, ec, ectoderm of worm, m, mouth of pilidium, n, beginning of nervous system, r, proboscis so excretory organ

is of compound origin, arising as an ectodermal proliferation from the anterior imaginal discs. The formation of the anus and certain other organs has not been followed through because of the lack of material. However, from the endodermal portion of the pilidium and the inner part of the amniotic invaginations comes the material from which all the later structures are derived. The outer part of the fused invaginations, such as the lining of a cap of which the larval walls are the chief

parts, is cast off, and the young animal which it covered is the young worm ready for life upon the sea bottom.

V. THE ANNELIDA AND MOLLUSCA

1. The Trochophore Larva

The trochophore larva is typical of annelids and molluscs. Other smaller groups, among which are the Polyzoa, Sipunculoidea, and Echiuroidea, also have trochophore larvae, and still other larval forms show relationships to the trochophore which are more or less close. In some larvae simple modifications are present and in others the relationships are harder to trace. It is unquestionably the most important invertebrate type of larva, viewed from a phylogenetic standpoint.

The trochophore larva in its simple condition is ovoid and possesses certain characteristic features, most conspicuous of which is the prototroch, a ciliated band about the equator. The prototroch is the chief organ of locomotion and participates in getting food. A group of cells known from its position as the apical organ functions as a sense organ, and from these sensory cells apical cilia grow out as a tuft. In some cases a metatroch, a secondary belt of cilia, is present posterior to the prototroch and a second tuft of cilia known as a telotroch is present at the pole opposite to the apical tuft. Departures from the typical arrangement of cilia occur in various species. Larvae with the primary ciliated band only are spoken of as monotrochal. In some, called atrochal, the prototroch is not developed, but cilia are present all over; in others, without a prototroch, a metatroch only is developed. Polytrochal larvae also occur, but these are later stages in which secondary bands of cilia are formed about the developing post-trochal region.

Internally a trochophore has a complete digestive tract with mouth, large, bulb-like stomach, short intestine, and an anus. The mouth of annelids is the old blastopore or an opening developed at the same place as the blastopore after a very temporary closure. The intestine meets an ectodermal invagination, the proctodaeum, with which it fuses, and an opening breaks through completing the alimentary tract. An archinephridium is present, running from the oesophageal region diagonally to the end of the intestine. Each contains a flame cell, or solenocyte, with a cavity and a tuft of cilia, and an excretory tube leads from it. In some trochophores there is an eye-spot containing red pigment cells; it is sensitive to light. The mesoblast cells, or teloblasts, derived from 4*d*, are present (or their descendants) and later elongate into two strings of cells, the mesoderm bands.

A typical trochophore as described is derived from the gastrula by some simple changes, the chief of which is the shift of the blastopore from the vegetative pole to the lateral position. In this process several factors take part. One is the increase in rate of development and in the actual number of cells of the dorsal posterior cells, particularly the descendants of the 3*d* cell. This inequality of growth tends to push the opening sideways. In some forms the blastopore also becomes oval in shape, then constricts in the middle, and the portion in the *a-b* quadrants is pushed anteriorly by the growing cells of the *d* quadrant. Of these two openings the anterior one becomes the mouth and the posterior one after temporary closure reopens to become the anus. This procedure is made much of by some authorities who reason that in this manner must have come the separation of the original ingestive opening into two, one of which is ingestive and the other egestive.

In the fully grown trochophore larvae of all the groups in which it occurs are found the points of structure as described. Indeed, the homologies between the various groups are complete even to the details of trochophore development. When one considers the remarkable array of organisms which in spite of total absence of agreement in adult features have this type of larval structure, he must find the fact of their common method of origin very surprising and significant. Nemerteans, rotifers, annelids, molluses (except cephalopods), and bryozoa, form a series which certainly presents a complete gamut of adult variation, yet all come from larval forms with features that are in general the same. It would, however, be even more surprising if there were not minor differences to be found in the trochophores. There are variations in the relation of the mesoderm mother cells to the gut wall, of the persistence of the protonephridia, the presence of the anal vesicle, of a prototroch, of the gastropod shell gland, and in other special organs which are the peculiarities of particular groups. It is not these differences, however, which deserve emphasis, but the common features of development which find expression in the widespread trochophore type.

2. The Annelid Trochophore

The metamorphosis of the trochophore into an adult annelid may next be considered. It consists chiefly in the growth in length of the post-trochal region and in the differentiation of certain elements found there. The essential features of the metamorphosis are (*a*) the elongation of the post-trochal region to form the body of the worm (post-cephalic portion) and its consequent segmentation; (*b*) the apparent reduction (which is not real, but simply relative) of the trochal and preoral regions

and their resulting changes to form the head of the worm; and (*c*) the disappearance of the ciliated bands. The processes are of course accomplished gradually, there being no moment at which the trochophore ends and the adult begins. The post-trochal region is one of active growth, differentiation, and cell multiplication. As an example of the metamorphosis of the annelid trochophore, *Polygordius* has been often studied,

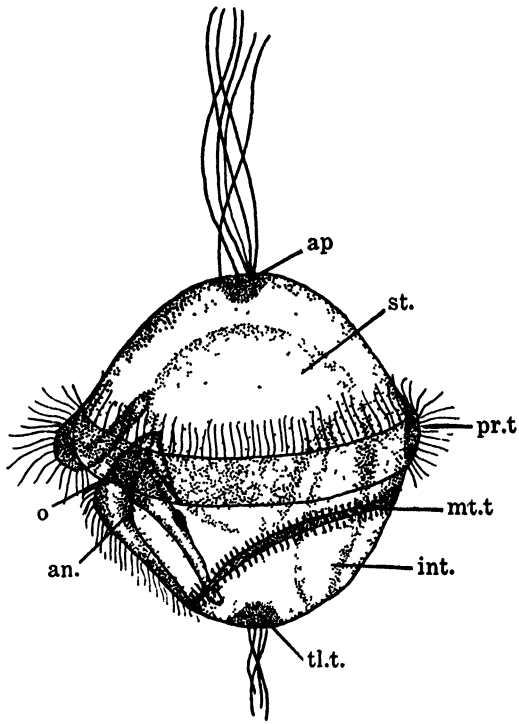


FIG. 105 Trochophore of *Polygordius*. (After Woltereck.) An., archinephridium; a.p., apical plate; int., intestine; mt.t., metatroch; o., mouth; pr.t., prototroch; st., stomach; tl.t., telotroch.

and no other is probably better known. It has been figured by Hatschek, Agassiz, and others, but is best known from the work of Woltereck.

The elongation of the post-trochal region in *Polygordius* involves especially the growth and division of the cells of the intestinal walls, the multiplication of ectoderm cells and the growth and differentiation of the cells of the mesoderm bands. The descendants of the 4*d* cell form the mesoderm bands, symmetrically placed on the two sides of the intestine. The pole cells from which they came are known as teloblasts. The bands multiply not only in length but in thickness and in each a series of cavities

gradually appears. These are the mesoblastic somites, and they are marked off from each other by constrictions which are traced on the surface by transverse grooves in the ectodermal covering. Their cavities grow progressively, and as they expand they fill up the surrounding space which was the remnant of the blastocoele. Ultimately they meet above and below the intestine, their walls fuse and break through, making a continuous pouch in each segment instead of a pair. Evidently in the growing worm, segments are not all in the same degree of advancement.

The origin of the excretory organs shows two stages in development in the annelids. The archinephridium by the addition of other cells from the third quartette and by increase in number of its own cells becomes a protonephridium, a rather complicated structure whose basis is flame cells. The origin of the permanent nephridia has been the cause of controversy among embryologists, particularly as to their derivation from ectoderm or mesoderm. These posterior or permanent nephridia or metanephridia grow from strings of cells lying in the wall of the embryo and running posteriorly. These break up into loops and a lumen appears which later becomes the tubular nephridium. In *Nereis*, Wilson described these as of ectodermal origin except for the funnel-shaped opening which he said is mesodermal. Others consider that the entire end is mesodermal and secondarily attached to the ectoderm. Still others think the entire structure is ectodermal. Although the details in *Polygordius* are not fully known, Woltereck holds to the ectodermal origin.

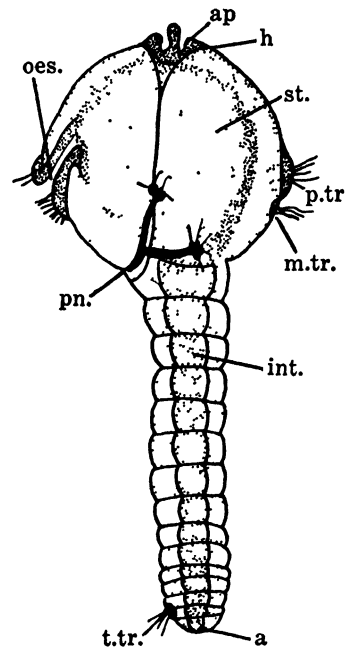


FIG. 106. Older trochophore of *Polygordius appendiculatus* (After Woltereck)

a, anus, h, posterior border of the head anlage pn, protonephridia oes, oesophagus

The nervous system begins with the mass of nerve cells that lies under the apical tuft. This is the cerebral ganglion and from it radiate eight nerves to the cells of the prototroch. Two, the lateral nerves, continue across the prototroch around the oesophagus to form the ventral nerve cords.

These are the beginnings of the more important organ systems of the transformed trochophore. For the details of the metamorphosis and for

the conditions as found in special forms reference should be made to the monographs and special articles dealing with these matters.

3. The Molluscan Trochophore

The metamorphosis of the molluscan trochophore is more complicated and involves the interposition of another larval stage, the veliger. While in the trochophore the characteristic larval organs are present, in the veliger there are also the rudimentary stages of the adult organs. There are three characteristic organs of this stage: the velum, the shell gland,

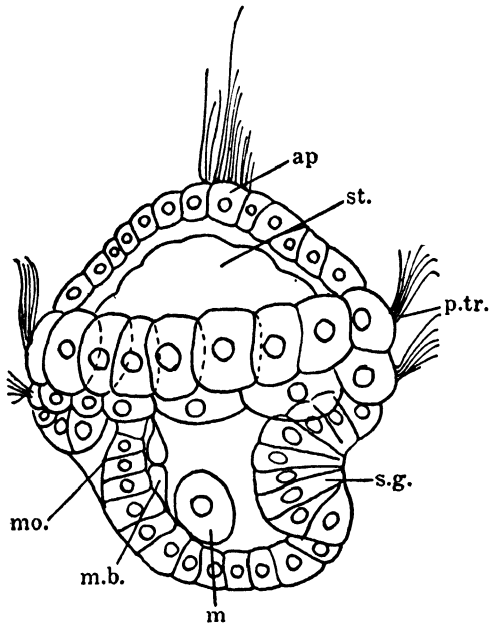


FIG. 107. Young trochophore of *Patella coerula*. (After Wilson.)
a.p., apical plate; m., mesodermal cell; m.b., beginning of mesodermal band; mo., mouth; s.g., shell gland; st., stomach.

and the foot. The velum is derived from the prototroch of the trochophore and is in the form of a bilobed, much enlarged projection abundantly provided with cilia. It serves as the locomotor organ of the veliger and disappears with metamorphosis. The shell gland is at first a simple invagination opposite the mouth which may open out to form a flattened disc or actually evert. Its cells secrete a thin, horny cuticle which is the shell of the trochophore. In the case of the bivalve molluscs the rapid extension of this area forms the mantle and calcareous as well as horny material is secreted, thus forming the two valves of the shell

connected by a region known as the hinge. The variations in the shape of the mantle lobes and the shell secreted by it and in the length and proportions of the hinge correspond to the various systematic groups. The foot in the earliest stages is a thickening of the ectoderm cells just ventral to the mouth. In the cavity of the slight hollow forming behind this bulging foot are mesenchyme cells which contribute to the differentiation of the organ. Other organs make their appearance in the developing veliger, including a larval kidney which disappears as the foot grows out, the muscles, the ganglia, gills, coelome, liver, and secondary structures as crystalline sac and otocyst. The details of their development are beyond the scope of the present account.

The metamorphosis of the veliger into an adult-like form is not a process of long duration. The appearance of the organism is surprisingly

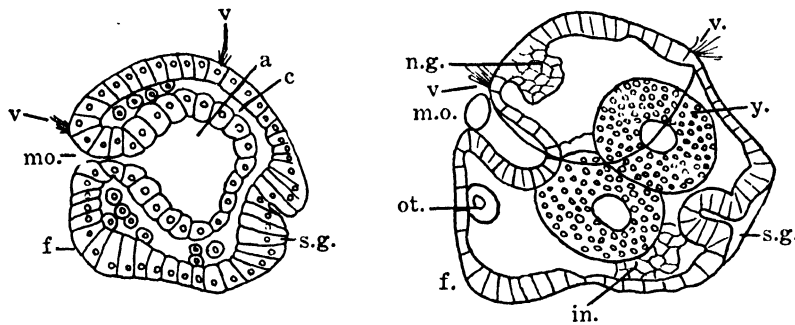


FIG. 108. A, embryo of a heteropod. (Redrawn from Balfour after Fol.) a., archenteron; c., body cavity; f., foot; mo., mouth; s.g., shell gland; v., velum. B, young veliger of *Pleurobranchidium*. (Redrawn from Balfour after Lancaster.) in., loop of intestine; y., residual yolk spheres; n.g., nerve ganglion; ot., otocyst.

changed largely because of the sloughing off of the cells which formed the velum; this process and some others coincident with it allow the organism to assume the external shape of a small adult. The larval muscles disintegrate, the anterior region with the mouth shrinks, drawing closer together the mouth and anterior adductor and advancing the front end of the foot. As a result the position of the developing gills is changed and the intestine is shifted and straightened. Although now the organism has the recognizable form of an adult, it is of course very immature and numerous internal changes have yet to be undergone. Labial palps and gill filaments in the bivalves, adult kidneys, pericardium, the final relations of the mantle cavity, development of the gonads and their ducts, all these and other minor processes of differentiation are still to be undergone before the final condition is reached.

VI. THE CRUSTACEA

Nauplius Larva

O. F. Müller long ago studied some forms of copepods which we now know are larval, but he thought them adults and gave them the name *Nauplius*. In this way originated the term that now represents a stage common to all Crustacea. In the lower forms it is usually a free-swimming larva and in many of the higher ones it is simply a stage passed through in the egg, but it is represented in the development of all Crustacea

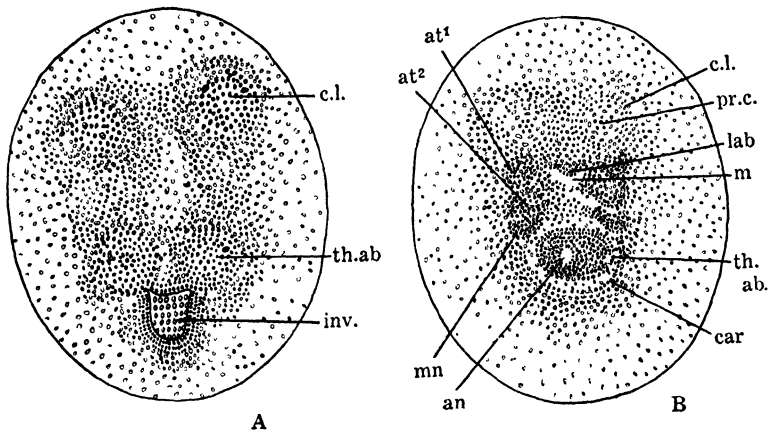


FIG. 109. A, ventral view of gastrula stage of *Astacus fluviatilis*. (Redrawn from MacBride, after Reichenbach.) c. l., cephalic lobe; th. ab., thoracic abdominal thickening.

B, nauplius stage of the same. an., anus; at¹, rudiment of first antenna; at², rudiment of second antenna; car., ridge making the first trace of the carapace; c.l., cephalic lobe; lab., labrium; m., mouth; mn., rudiment of mandible; pr. c., protocerebrum; th. ab., rudiment of thorax and abdomen.

Crustacean eggs show a great range of developmental types, and the various stages show no uniformity throughout the different groups. Cleavage may be superficial or it may be total and nearly equal. A cleavage cavity is usually lacking because of the large amount of yolk, but some species have it in more or less restricted condition. Gastrulation is usually by epiboly and ingression, but here again conditions vary. Cross fertilization is usual, but there are cases of self-fertilization, and parthenogenetic development may alternate with normal fertilization. There are some cases in which only parthenogenesis has so far been discovered. But in all cases a nauplius stage is at length reached.

The form named nauplius by Müller actually had four pairs of appendages, whereas the stage now known as the nauplius has only three

pairs. It was Claus in 1858 who named the three-appendage stage which is of such widespread occurrence throughout the Crustacea. A nauplius

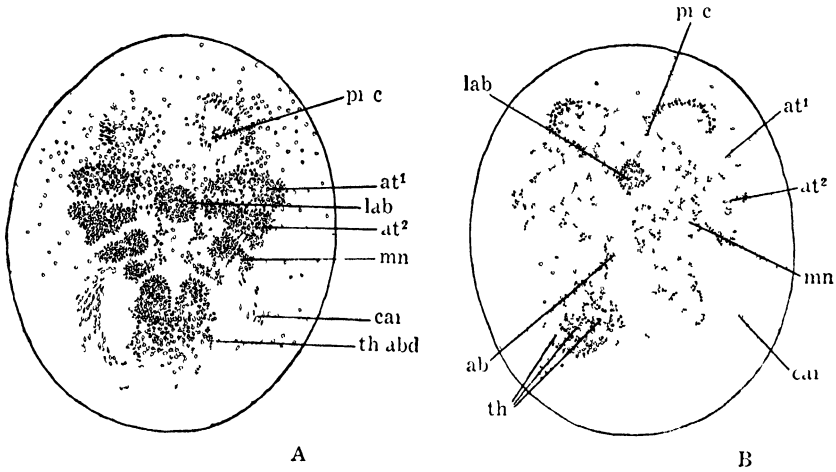


FIG 110 Continuation of Fig 109 In A the rudiments of maxillæ have appeared and the caudal fork is visible In B thoracic appendages are seen and the abdomen in segmenting

larva, therefore, has three pairs of appendages, first and second antennal, and mandibular. But the degree of development toward independent existence in the nauplius stage is by no means uniform in the different Crustacea. There is in fact a series of larval stages in the Crustacea, the most important of which are nauplius, metanauplius, protozoaca, zoaea, mysis, all leading to the adult. There are, also, others less generally important, but more specialized for particular groups. Of those mentioned a completed series occurs in the higher crustaceans only, in some forms of which all are represented. For every stage listed there is some group of higher crustaceans in which the larva hatches from the egg at the corresponding time. That is, hatching, or the breaking out of the free-living form from the egg membrane, is a variable process and some certain species may be

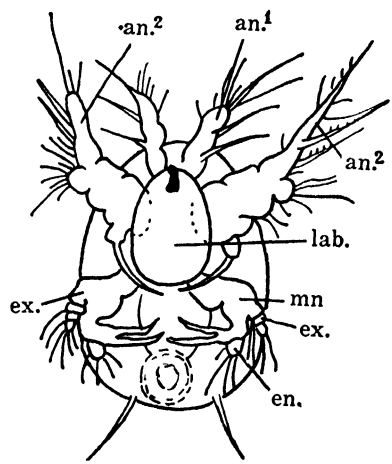


FIG 111 Nauplius of *Cyclops* (After Claus)

an¹, first antenna; an², second antenna, man, mandibular appendage, lab, labrium, ex, exopodite, en, endopodite

found to illustrate each stage as a newly hatched larva. Evidently those which hatch in the more advanced stages (for example, the decapods) do not show the nauplius in a full functioning condition as do those in which the nauplius is a free-swimming larva, as in the barnacles. In the

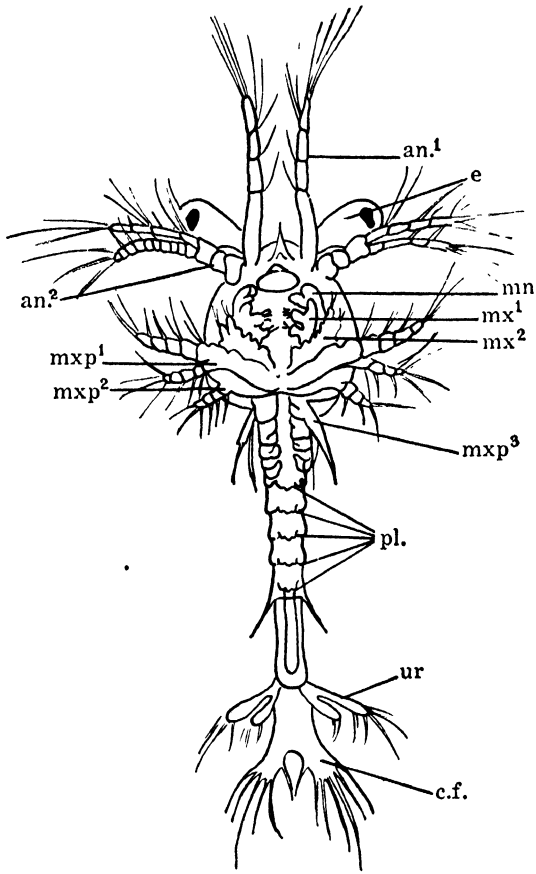


FIG. 112. Zoaea of *Penaeus*, ventral view. (After Claus.)

an1, first antenna; an2, second antenna; e., eye stalk; mn., mandible; mx1, mx2, first and second maxilla; mxp1, mxp2, mxp3, first, second, and third maxillipeds; pl., beginning of pleopods; ur, uropod; c.f., caudal fin.

crayfish, for example, the nauplius condition is shown by three pairs of mere thickenings or buds instead of developed appendages and there are in the embryo in addition only two cephalic lobes, two thoracico-abdominal rudiments, and the endodermal rudiment; this is far from a free-swimming embryo.

The nauplius of *Cyclops* is an elliptical form with no external sign of

segmentation, but with an enlarged labrum, openings of the alimentary tract, a single median eye, and the three pairs of appendages. The first antennal appendage shows no sign of a biramous character, but both the second antennal and the mandibular have at least partially developed both exopod and endopod. Examples of forms hatching in this condition are the copepods as well as *Penaeus*, and *Balanus*. In *Balanus* this stage is preceded by one in which three primary segments are recognizable. These become subdivided into eight lobes, which are the labrum, right and left first antennal from the first primary segment, the right and left second antennal from the second segment, and the right and left mandibular and the telson from the third. Future growth takes place between the mandibular lobes and the telson. From this stage the next changes lead to the metanauplius.

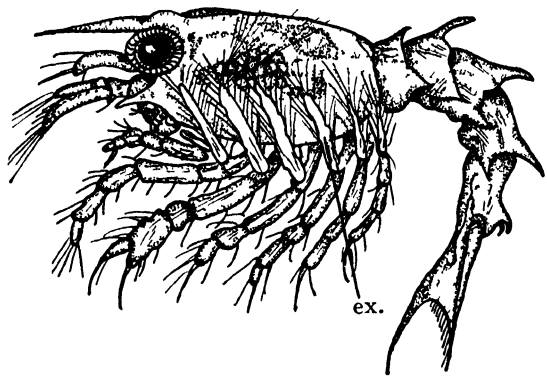


FIG 113 Mysis larva of the lobster, *Homarus americanus*, lateral view. (After Herrick) Exopodites of the walking legs are to be noted (ex.).

The metanauplius condition is reached at the end of the first molt in those forms which hatch as nauplii, but occurs before hatching in such forms as *Branchipus* and *Lucifer*. Two additional pairs of appendages are present, the first and second maxillae, and segmentation is beginning.

In the protozoaea, illustrated by a newly hatched *Squilla*, the first and second maxillipeds are present as well as the appendages of the earlier types, the abdomen is partially segmented, but not the thorax, and the compound eyes are making their appearance.

The zoaea, the stage in which the crab hatches, possesses third maxillipeds, some of the thoracic appendages, the number varying with different forms, compound eyes, an abdomen completely segmented, but the thorax showing as yet incomplete indications.

The mysis stage, characteristic of the genus of that name, has all

five pairs of walking legs with exopods, six pairs of pleopods (although in some they are hardly developed at hatching), and segmentation is complete. The lobster also hatches in an advanced mysis stage.

Finally in the fresh-water shrimp, *Crangon*, the condition of the adult is reached so far as external features are concerned at the time of hatching.

Of the less important larval types of Crustacea, no mention will be made here.

VII. THE INSECTA

Among the insects development may involve the formation of very complex larvae and more or less complete metamorphosis, or may be direct. The structure and cleavage of typical insect eggs have already been described in the section on superficial cleavage. The cleavage of the centrolecithal egg results in the formation of a blastoderm which may be of uniform thickness in development or may have early shown a differentiation on the ventral side of an aggregation of cells which will become the primitive streak.

Even if the blastoderm is at first of uniform thickness, it gradually becomes thicker in the ventral region by the multiplication of the cells, and thus in this case too the primitive streak or germ band is formed. Mesoderm is formed by one of three methods. (1) Along the medial line of the germ band an invaginating groove is presently to be seen in many embryos of which those of the Coleoptera may be taken as an example. From the material of this invaginating groove comes the inner layer from which is derived the mesoderm and endoderm. (2) In certain other insects the formation of the mesoderm may be accomplished without the formation of a tube but rather from a middle plate which remains nearly flat and is overgrown at the edges by the lateral folds. It occurs in certain Lepidoptera and other forms.

(3) Still another type of mesoderm formation takes on the character of a proliferation and invagination of cells from a median ventral blastoderm region. This is characteristic of the Orthoptera. At the edges of the germ band the blastoderm begins a folding process and at length the two folds meet over the band in the middle region. Their union produces two membranes, the outer one, known simply as a serosa, and the inner one, the amnion. The germ band continues its own growth and the marks of segmentation become apparent shortly as transparent grooves. An anterior pocket growing dorsally from the ventral germ band is the beginning of the stomodaeum and the foregut, and a similar one at the posterior end forms the proctodaeum. A pair of large pro-cephalic lobes at the anterior end of the germ band develop in time into the lateral

eyes, and antennae and mouth parts appear as rudiments in proper serial fashion. The appendages likewise are outpocketings of the ectodermal germ bands. It is evident of course that the variety of structures found among the insects means great variability in details by which these steps are accomplished. The general body form of the insect is

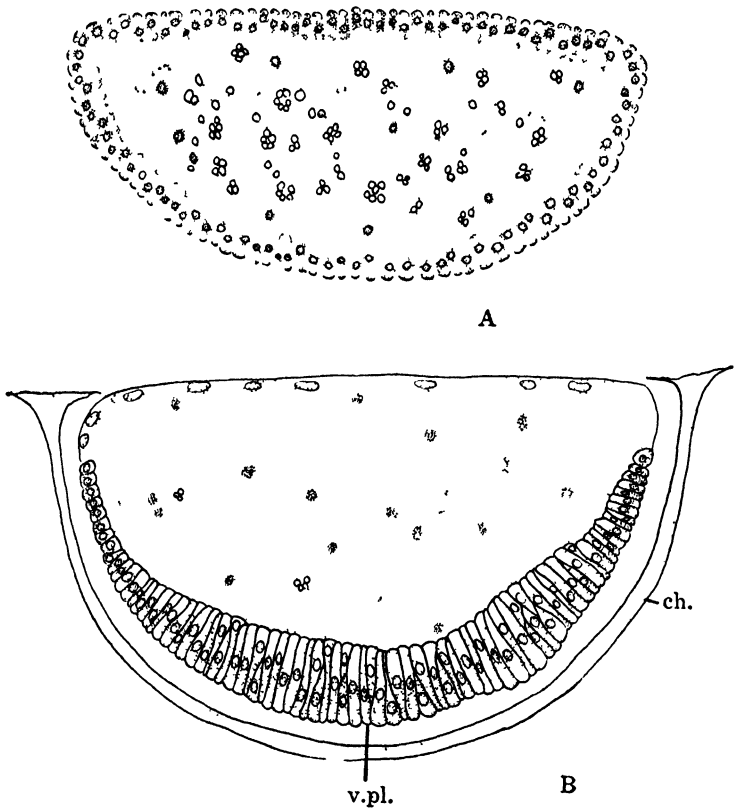


FIG 114 Developing eggs of *Bruchus quadrimaculatus* (After Brauer)

A, transverse section, showing completed blastoderm Dorsal side of egg toward top of page B, section of egg a little older than in A through anterior region Cells of ventral blastoderm are crowded, but dorsal blastoderm is thin and flattened ch , chorion, v pl ventral plate.

completed by the growth of the margins of the germ band dorsally until they finally close over and form a dorsal wall. While this has been going on the progress and development of the internal structures has likewise been considerable and the embryo continues its development until finally ready to hatch into a larva.

During the larval stage the insect feeds, sometimes voraciously, storing up nutritive material for its subsequent development. In the more

specialized insects this development is very complicated involving a complete metamorphosis with entire changes of form and often of function as well. The groups of insects which undergo this indirect type of development or complete metamorphosis are spoken of as the Holometabola and include the Coleoptera, Strepsiptera, Neuroptera, Mecoptera, Trichoptera, Lepidoptera, Diptera, Siphonaptera, and Hymenoptera. The less specialized groups comprise the Heterometabola in which

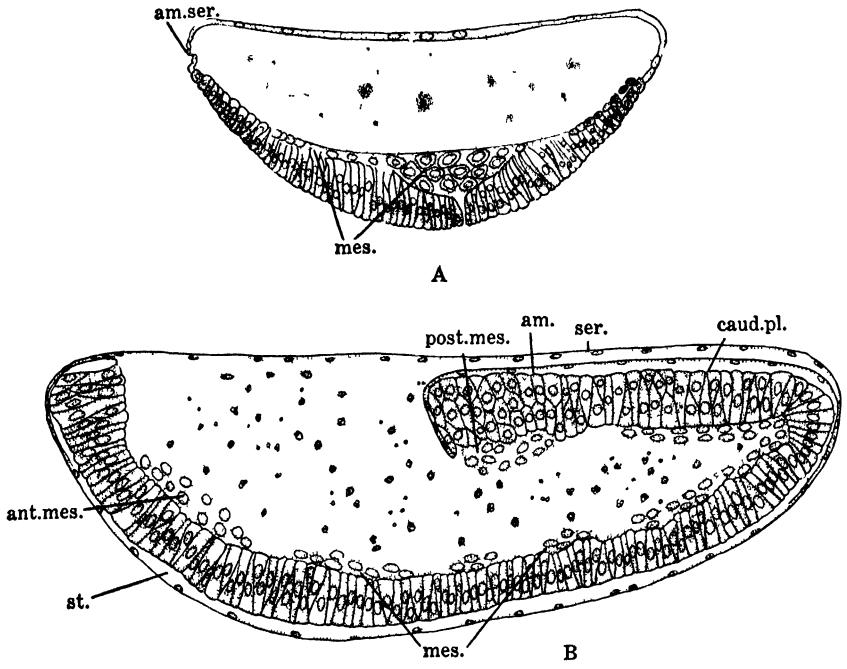


FIG. 115. Continuation of 114.

A, Transverse section of egg after closure of gastral invagination. mes., mesoderm; am. ser., amnio-serosal fold. B, Longitudinal section of an embryo of 32 hours. Am , amnion, ant. mes., anterior mesenteron anlage; caud. pl., caudal plate, mes., mesoderm; post. mes., posterior mesoderm anlage; ser., serosa; st., stomodaeum.

development is direct and metamorphosis incomplete. These forms lack a true pupal period. They include Orthoptera, Dermaptera, Platyptera, Placoptera, Ephemeridae, Odonata, Thysanoptera, and Hemiptera. Since a distinction between larvae and pupae is lacking it is common to use the term nymph to apply to the young insects after hatching and several molts or ecdyses may be gone through among these forms. The terms, stages or stadia, are used to designate the intervals between the molts; and the term, instar, is frequently used to designate the insect at any particular stage. The Thysanura and the Colembola develop

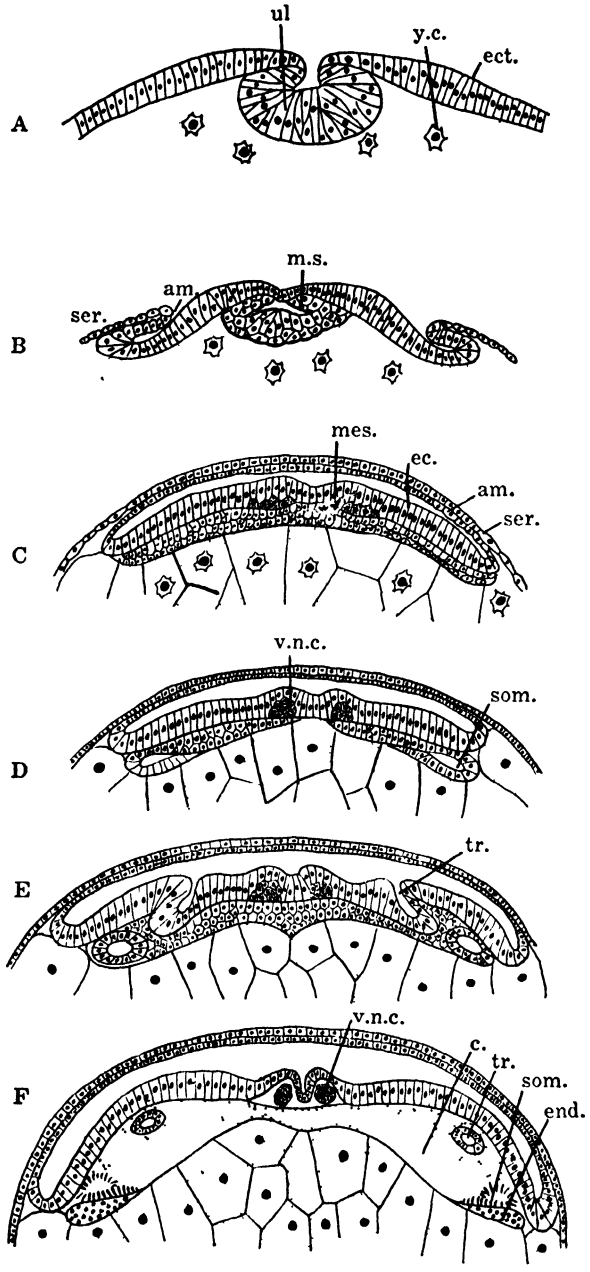


FIG. 116. Successive stages through the embryonic area of *Hydrophilus*. (From Korschelt and Heider, after Heider.)

A, Formation of invaginated groove which is to produce the under layer B, Origin of amnion. C, Amnion completed over embryonic area Yolk has undergone cleavage D, Origin of somites. E, Formation of trachae F, Formation of coelome

Am., amnion; c, coelome, ect, ectoderm, end, endoderm, mes, mesoderm, m s, first split of mesoderm, ser, serosa; som, somite, tr, trachea, u l, under layer, v n c, ventral

without any metamorphosis and hence are often spoken of as the Ametabola.

Upon hatching, the larvae emerge with certain characteristics depending upon the group to which they belong; one is usually able to classify the insect into its order by mere reference to the type of larva. Common

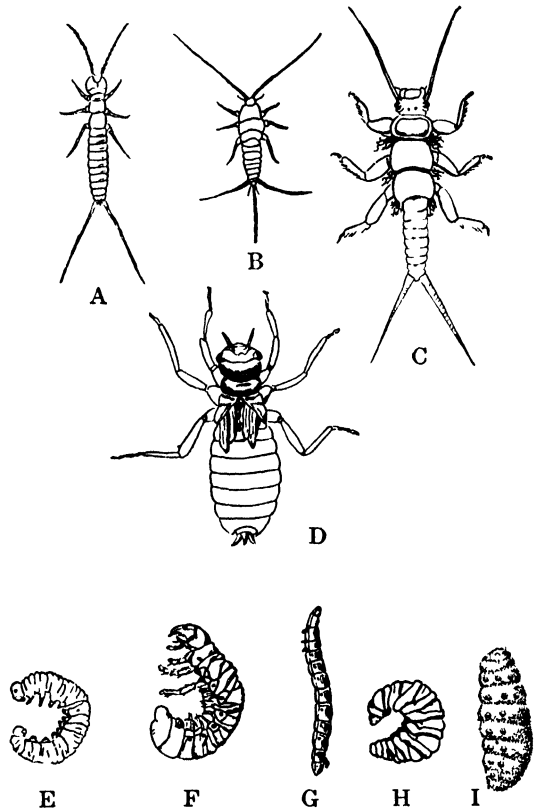


FIG. 117. Types of larvae. (From Folsom.)

A, B, Thysanura; C, thysanuriform nymph; E–I, eruciform larvae; A, *Campodea*; B, *Lepisma;* C, perlid nymph (Placoptera); D, *Libellula* (Odonata); E, *Tenthredopsis* (Hymenoptera); F, *Lachnosterna* (Coleoptera); G, *Melanotus* (Coleoptera); H, *Bombus* (Hymenoptera); I, *Hypoderma* (Diptera).

names are given to the larvae, such as the caterpillar of the Lepidoptera, the grub of the Coleoptera, and the maggot of the Diptera. The types of larvae are known to entomologists under several terms but they are fundamentally of two groups, the campodeiform and the eruciform. The campodeiform are also spoken of as the thysanuriform in reference to their generalized structure. They are flattened, have a long body, often

with long legs and antennae, hard body plates, caudal cerci with well-developed mandibles and active habits. These are characteristics of the adult thysanurans, especially of the genus *Campodea*, but in the insects which undergo metamorphosis they are of transitory importance only. The eruciform larva is illustrated by the caterpillar or maggot with the cylindrical body which may grow to become more or less spindle shaped, weak integument, and with mouth parts, antennae, legs, and caudal cerci reduced, even to complete disappearance; correspondingly it leads

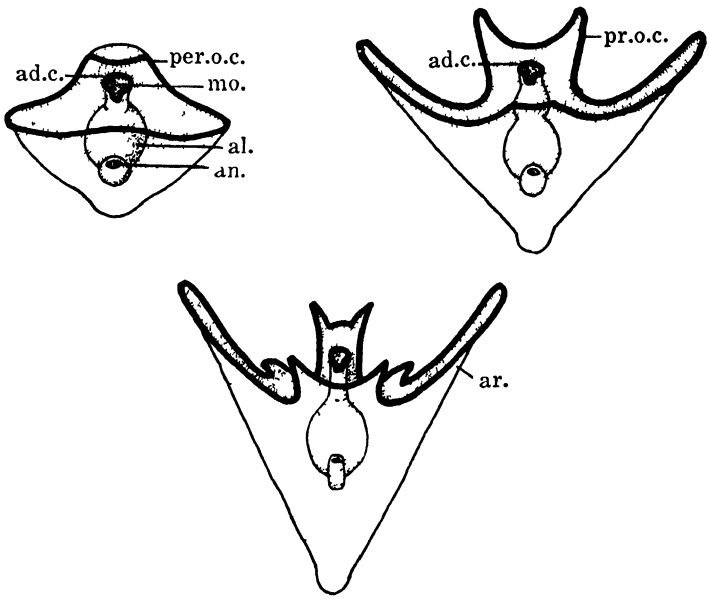


FIG 118 Successive stages in the development of a pluteus of the Ophiuroidea and Echinoidea (After Leuckart)

ad c, adoral ciliated band, al, alimentary canal, an, anus, ar, arms, mo, mouth, per o c, perioral ciliated band

a very inactive life. Between these two types transitional forms are easily recognized, for instance, in the genus *Mantispa* of the Neuroptera the larva which hatches from the egg is distinctly campodeiform and active but it enters the egg sac of a spider and becomes sedentary, loses use of its legs and completely changes its form, that is, becomes an eruciform larva. For the changes which occur during the growth of the larvae, the frequency of the molts, and the great variety of adaptive structures that are assumed in the various larvae of the insects, the reader must refer to treatises on entomology. Likewise the formation of the pupa is a subject which varies too much in detail to be discussed in this work.

The internal changes which accompany the metamorphosis of insects are only incompletely understood. In those forms in which metamorphosis is incomplete certain organs are of larval significance only and their tissues must give way and provide the materials for other structures characteristic of the imago. This destruction of larval tissue, or

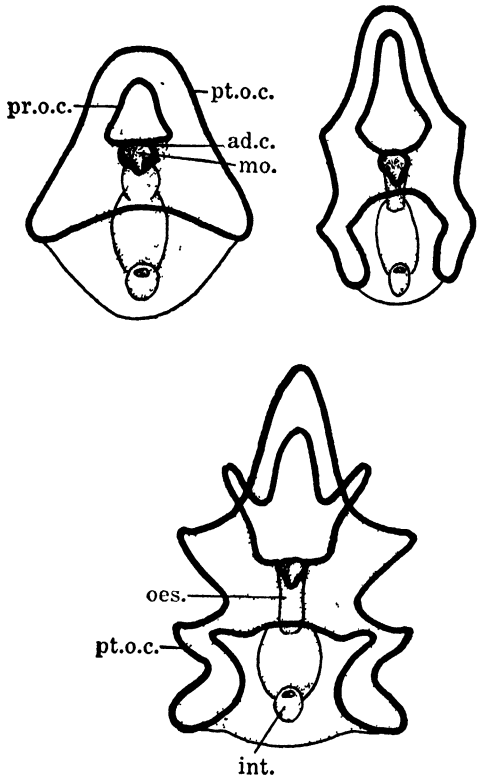


FIG. 119. Successive stages in the development of a bipinnaria of the asteroids. (After Leuckart.)

ad. c., adoral ciliated band, int., intestine, mo., mouth, oes, oesophagus; pr. o. c., preoral ciliated band; pt. o.c., post-oral ciliated band.

histolysis, takes place commonly during the pupal period through the activity of phagocytes and is followed by a period of the construction of imaginal tissues or histogenesis. At the end of this constructive period the animal is ready for the final metamorphosis which converts it into an active adult or imago.

VIII. THE ECHINODERMATA

In the phylum Echinodermata are to be found some of the most remarkable forms of larvae of any group in the animal kingdom, and

in this group metamorphosis has a peculiar and unusual significance. The larval stages are preparatory for the changes which are to adapt the adult forms to a great variety of living conditions, and they bridge the gap from a rather simple ancestral form to the diverse conditions found among the five classes of living echinoderms. One has only to consider the different types of adults found among the asteroids, echinoids, ophiuroids, the holothurians and the crinoids to appreciate the problem which confronts the simple type of ancestral larval form. Such extensive metamorphoses as are found here have for their special function the adapting of the organism to the very changed conditions in which it finds itself. Since the larval condition is retained to the last possible moment in development, although internal changes of the most

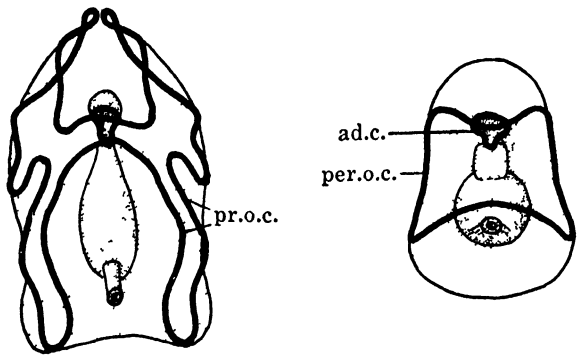


FIG. 120. Successive stages in the development of an auricularia of the holothuroids (After Leuckart)
Letters as in 118 and 119.

profound character are taking place, metamorphosis is of a type sometimes spoken of as cataclysmal.

The gastrula of all the echinoderms develops into a form similar to what was probably the hypothetical ancestor of all the classes of the group; it is known as a dipleurula. From this dipleurula it is conceived that the echinopluteus of the sea-urchin, the ophiopluteus of the brittle-star, the bipinnaria of the starfish, and the auricularia of the holothurian are derived. Of these four larval forms the first two are more closely related to each other than to the other group, in which also affinities are discoverable to the larvae of the crinoids. It is not feasible to undertake here a description of the development of the larvae and their metamorphoses in all these cases. For particular reasons and also because it is the form most commonly studied, we choose to follow through the development of the starfish larva, the bipinnaria, from its origin from the gastrula to its metamorphosis.

1. The Bipinnaria

It is characteristic of echinoderm larvae that there should be a bilateral symmetry. Two important trends are noticeable as the transition from larva to adult takes place, namely the great development of the left side of the larva with the corresponding decrease of the right side, and the complete change of symmetry to a radial type whose axis is at right angles to the former larval axis. The egg of the starfish goes through a rapid and regular process of radial cleavage resulting in a blastula of about equal-sized cells. The cells become ciliated uniformly, the embryo bursts the egg membrane and, rising to the surface, begins its larval life. Gastrulation has in the meantime been going on, but the formation of mesenchyme is not completed until after the free-swimming stage is reached. The larva elongates, becomes somewhat flattened on the ventral side, the blastopore is metamorphosed into the anus, and the larval mouth breaks through anteriorly. The cilia disappear except for certain specialized ciliated bands which take on a shape characteristic of particular species. Gradually there appear as outgrowths certain special processes which as they grow carry out the ciliated bands to their borders and give the larva its bipinnate form to which is due the name "bipinnaria" given it by Sars.

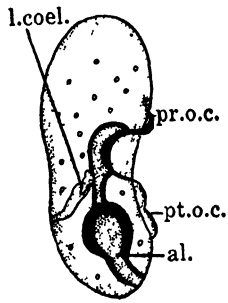


FIG. 121. Lateral view of young larvae of *Asterias* (After Agassiz.)

al., alimentary tract, mo., mouth; pr. o. c., pre-oral ciliated band; pt.o.c., post-oral ciliated band.

The earlier investigators were inclined to the opinion that the starfish arose as a bud on the left side of the larva. It is now realized that this appearance is superficial only and that the larger part of the larval body is involved in the production of the adult. The earliest trace of the adult body appears as a five-lobed structure in the left posterior portion of the larva. By the growth and differentiation of this structure and the loss of the larval mouth and oesophagus and the formation of the new opening, the metamorphosis into the little starfish is accomplished. In the case of the starfish a temporary fixed stage which is not found in all types of echinoderm is passed through. During this stage certain other larval arms and processes are developed and the larva is spoken of as a brachiolaria.

It is now desirable to follow in more detail the changes which have been thus briefly sketched.

2. The Dipleurula

All echinoderm larvae agree, while showing much variation from each other on other points, in the possession of the following features: they are bilaterally symmetrical, have a locomotor organ in the form of a curved and bent longitudinal ciliated band with preoral and anal loops. They possess a V-shaped adoral ciliated band; the alimentary canal

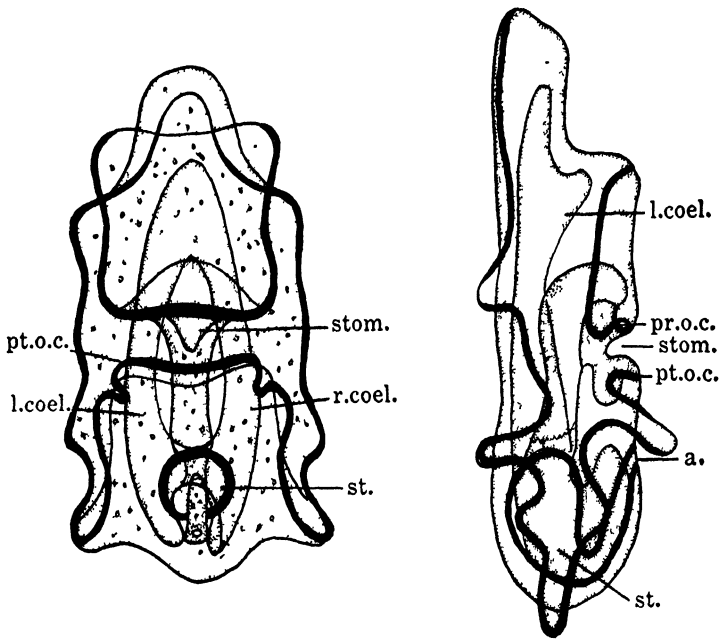


FIG 122 A, Bipinnaria of *Asterias* (Frontal view) B, Side view of an older bipinnaria. Median brachiolarian process beginning to grow out at b (After Agassiz)

a, anus, a d a, anterior dorsal arm, ad c, adoral ciliated band, int, intestine, L coel, left coelome, m d a, median dorsal arm, mo, mouth, oes, oesophagus, pt d a, postero-dorsal arm, pt l a, postero-lateral arm, pt o a, post-oral arm, pt o c, post-oral band of cilia, pr a, pre-oral arm, pr o c, pre-oral band of cilia, r coel, right coelomic sac, st, stomach

consists of the typical four parts, namely, the mouth parts, oesophagus, enlarged stomach and intestine. The coelome is budded off from the apex of the archenteron as a pouch which divides into two lateral pockets and has communication with the exterior by a ciliated canal opening on the dorsal surface to the left of the median line. This canal is the stone canal. Since these features are common to all the different groups of echinoderms it is believed that they must have been the characteristics of an ancestral type from which the different classes of

the echinoderm existing today have been derived. This hypothetical ancestor is known as a dipleurula. To this larva the characteristic features of the ophiuroids most faithfully conform, and of all echinoderm types it is the most nearly bilaterally symmetrical, for which reason, along with others, it is probably to be regarded as the most primitive. In addition to the features ascribed to the hypothetical dipleurula, it is assumed to possess right and left coelomes more or less divided into three parts, anterior, middle, and posterior. However, a departure from the characteristic dipleurulan condition is seen in *Asterias* in that the coelome is divided into only two parts (unless the stone canal and its connections are to be regarded as the homologues of the anterior coelome of the dipleurula and the anterior coelome of the starfish is homologous to the middle portion of the dipleurulan structure). The middle part of

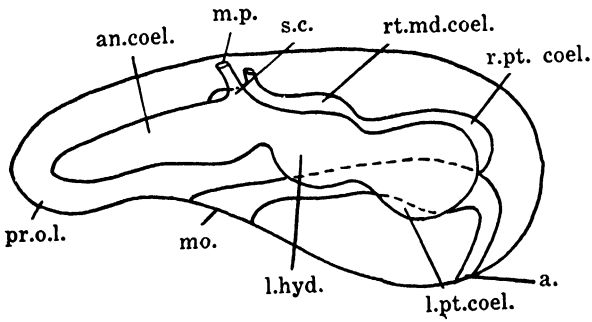


FIG. 123. Diagrammatic reconstruction of ancestral dipleurula.

a., anus; an. coel., anterior coelome; l. hyd., left hydrocoele; l.pt. coel., left posterior coelome; m.p., madroporic pore; rt. md. coel., right middle coelome; r.pt. coel., right posterior coelome; s.c., stone canal.

the body of dipleurula has lobe-like processes extending outwards and covered with ciliated epithelium into which the middle coelome extends. These processes permit a comparison with the lip of the brachiopods and so-called lophophore which bears ciliated tentacles. In both cases the organs function to direct food-bearing currents of water to the mouth. Resemblances have also been pointed out between dipleurula and a simple ctenophore plan of structure, and upon the basis of these suppositions it has been concluded by some that there once existed a class of simple marine animals one member of which was of the general character of a dipleurula and another the ctenophore-like ancestor of the annelids and molluscs. These speculations seem also to give a basis for consideration of the similarities that exist between the trochophore type of larva and those of the echinoderm and at the same time of the very great differences that are to be seen in the early development of these types.

As an example of the development of an echinoderm, although it is hardly to be regarded as a type of the other classes, the embryology of *Asterias* is now to be traced in detail.

3. Metamorphosis of *Asterias*

By the end of the first day after hatching the free-swimming blastula has been converted into a gastrula which is distinguished from the usual type of invaginate gastrula in that the archenteron occupies only a small portion of the inner space, leaving a large blastocoele still present. Into this space are early budded mesenchyme cells which send out processes and make a network in a gelatinous fluid filling the blastocoele. The gradual lengthening of the gastrula to take on the form of a cylinder with round ends, the differentiation of the ciliated apical plate and especially of the longitudinal ciliated band, involving a thickening of the epithelial cells where it is to form, with a general increase in the number of cilia on them and a corresponding decrease of the cilia elsewhere on the larval body, are the next characteristic changes through which the young larva passes (Fig. 122). In the meantime the coelomic sacs are budded off from the apex of the archenteron as in the case of dipleurula. The gut grows forward, becomes constricted into the intestine, stomach, and oesophagus, and the oesophagus turns forward and ventrally to meet an invagination from the ectoderm, the stomodaeum, with which it fuses. The anus is the blastopore of the gastrula stage. With the formation of the arms the bipinnaria attains the completion of its outer features. The structure of these arms varies in the different species. In *Asterias vulgaris*, there are borne by the preoral band two larval arms called the preoral and a median dorsal one, while in *A. glacialis*, *A. rubens*, and *A. berylinus* (Fig. 122), European species, there are also on this preoral band a median ventral arm and a median dorsal one which are directed forward; there are also in both cases a pair of antero-dorsals, and a pair of postero-dorsals, a pair of postero-laterals, and a pair of post-oral arms. When the time for metamorphosis has been reached and the larva begins to take on its temporarily attached condition there grows out from its anterior part a series of processes known as brachiolas, a median and two

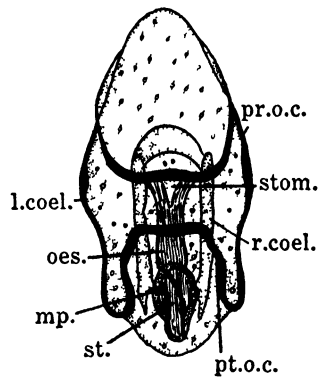


FIG 124 Larva of *Asterias* six days old, from dorsal surface (After Agassiz)

l coel, left coelomic sac, m p (left), persistent madreporic pore, r coel, right coelomic sac, pr o c, pre-oral ciliated band, pt o c, post-oral ciliated band, stom, stomodaeum, st, stomach, oes, oesophagus.

dorsals, and the larva is termed a brachiolaria. Into these arms extensions of the coelome push out and in this particular they differ from the arms of the bipinnaria. These arms as listed include all the lobe-like outgrowths which are found in other starfish larvae.

The Coelomé. The development of the structures connected with the coelome form the most important contribution to the starfish body. After a period of slow enlargement near the oesophagus where they originated, the coelomic sacs begin a remarkable series of transformations. The right sac in some of the larvae grows to form a canal leading to the outside through the right madroporic pore, which soon closes up and is lost; and in other larvae it is never found. The left coelomic pouch, however, always makes a short vertical growth to the dorsal surface, forming the pore canal and uniting with an ectodermal depression to form the primary madroporic pore. Through the beating of its cilia water is passed into the coelome. These structures of the left side are significant for the future of the larvae, for it is the left side which will develop to the greater part of the adult starfish, while the right contributes only a minimum. Nevertheless, ancestrally the larva was symmetrical and the two sides originally produced similar organs.

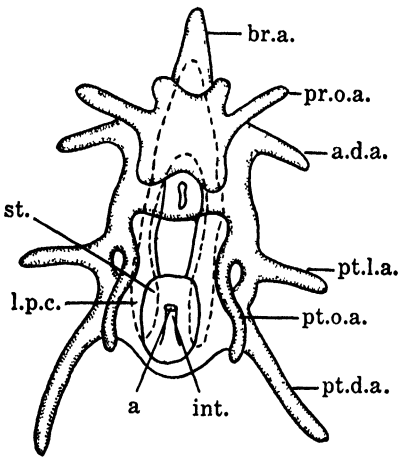


FIG. 125. Frontal view of a Brachiolaria (somewhat after Agassiz).

a., anus; a.d.a., anterior dorsal arm; br.a., anterior median brachiolar arm; int., intestine; l.p.c., left posterior coelome; pt.l.a., posterior lateral arm; pt.o.a., post-oral arm; pr.o.a., pre-oral arm; pt.d.a., posterior dorsal arm; st., stomach.

The sacs now grow in length and the right and left fuse together in the pre-oral lobe, although not elsewhere. A partial constriction of the left sac just behind the madroporic pore divides it into the left anterior and posterior coelomes. The hinder part of the left anterior coelome swells out to form a rather circular five-lobed outgrowth. This is the beginning of the water vascular system and is known as the hydrocoele. In some forms (*Asterina*) a right hydrocoele and the right anterior and posterior coelomes are also formed, but this is not the case in *Asterias*. A madroporic vesicle is formed near the median line from the right anterior coelome. Its origin and position have been variously described, but the best opinion now seems to derive it from the right coelome. These developments complete the structure of the free-swimming bi-

pinnaria. It is now ready to settle down, become attached, grow into the brachiolaria and begin the metamorphosis which will produce the adult starfish. Between the bases of the brachiolar arms a region of fixation appears by means of which the larva becomes attached (Fig. 126*B*). It is a thickening of the ectoderm, which becomes glandular, enabling it to fix the little animal to the substratum. The attachment takes place near the anterior end of the larva and gives to that portion

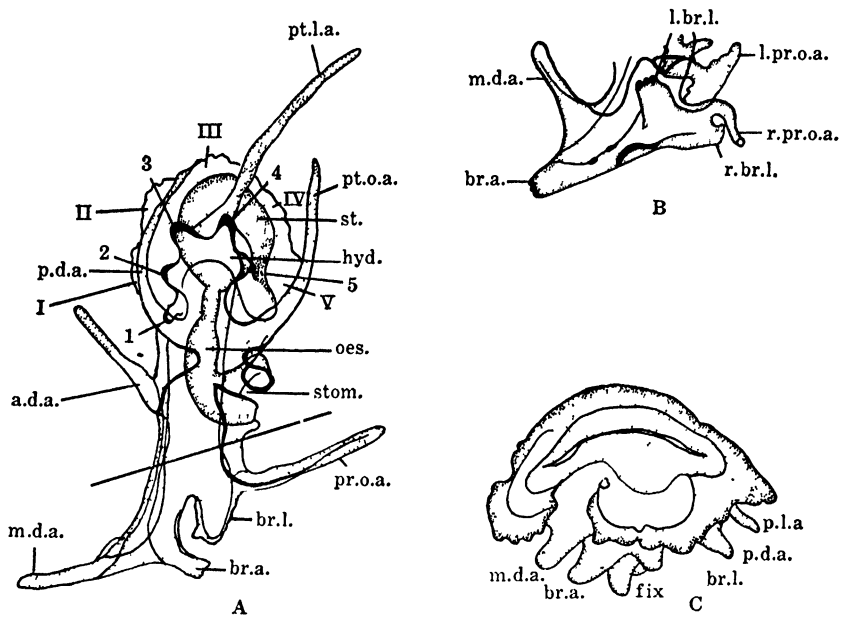


FIG. 126. Lateral views of brachiolaria of *Asterias vulgaris* in process of fixation. (Modified from Goto.)

A, Left side of earliest stage, temporary fixation by the brachiolarian arms; B, part of older brachiolaria corresponding to the base of A and showing the permanent fixation by means of the fixing disc, the pre-oral lobe having shrunk. 1–5, lobes of hydrocoele growing out to corresponding enlargements of the body wall, I–V, the anlagen of the arms. br.l., lateral brachiolar arm; fix, disc by which the larva attaches itself; l.pr.o.a., left pre-oral arm; m.d.a., median dorsal arm; oes., oesophagus; p.d.a., posterior dorsal arm; p.l.a., posterior lateral arm; r.pr.o.a., right pre-oral arm; stom., stomodaeum.

of the body the function of a stalk for the enlarging posterior portion or disc. As development continues the stalk shortens and the disc grows. In the latter are the right and left coelomes, the hydrocoele, stomach, and intestine. In the former are the anterior coelomes, oesophagus, mouth, and madroporic vesicle.

While the larva is becoming attached and constrictions which divide both right and left coelomes into anterior and posterior parts are becom-

ing more evident, the left posterior sends a process over to the right side around the gut. This at length fuses with the right anterior coelome, and as the left and right anteriors were already joined in the pre-oral lobe the entire complex presently becomes metamorphosed into the single anterior coelome. The right posterior coelome, however, is entirely cut off from the right anterior and forms the epigastric coelome of the adult. During the time that the left anterior and posterior coelomes were divided from each other by a septum formed by their opposed portions, there appears a groove on the anterior side which is to form the stone canal, while one on the posterior side is the first sign of the perioral coelome. The stone canal becomes connected with the madroporic pore canal and runs down to the hydrocoele. The perioral coelome becomes a crescentic tube about a bud of the stomach which represents the adult stomach. The further development of the alimentary tract is as follows: The stomodaeum gradually disappears, having first become disconnected from the midgut. The oesophagus itself gradually shortens and atrophies as does the intestine, the larval anus having closed. It is stated, however, that a remnant of the intestine persists and from it the rectum of the adult arises. From the left side of the larval stomach the bud referred to above grows to form the cardiac stomach of the adult which may be everted for the purposes of food getting. As it grows it is surrounded by the perioral coelome. At a point on the left side of the larva, which consequently is to be known as the oral side of the adult, the growing bud makes connections with the ectoderm and an opening appears forming the mouth. The rectum and adult anus do not appear until the body form of the young starfish is practically complete.

Final Metamorphosis. Since the left side of the larva becomes the oral side the right side is the future aboral and the first signs of the body form are seen as five thickened lobe-like elevations of the ectoderm which are the rudiments of the arms and indicate the position of the aboral disc. With the growth of the disc portion, the coelomes become displaced backward in the larva to occupy what will be their position in the new body region. The hydrocoele becomes entirely separated from the anterior coelome. It sends out lobes which are the rudiments of the radial canals of the water vascular system and from which tube feet and outer parts of the system are later formed. The stalk portion, becoming less and less both in size and in importance owing to changes in the shape of its cells and to phagocytic action on some of the others, is reduced until it is included in the disc area and is covered by the oral disc. Only a small projection from the oral disc holds the larva fast to the substratum and at length it is broken as the little starfish completes its body form and wrenches itself free. The further changes which the

organ system undergoes are gradually accomplished during the growth of the young animal.

IX ENTEROPNEUSTA

Tornaria of Balanoglossus. The modes of development in *Balanoglossus* and its related genera present a great deal of variation. In the genus

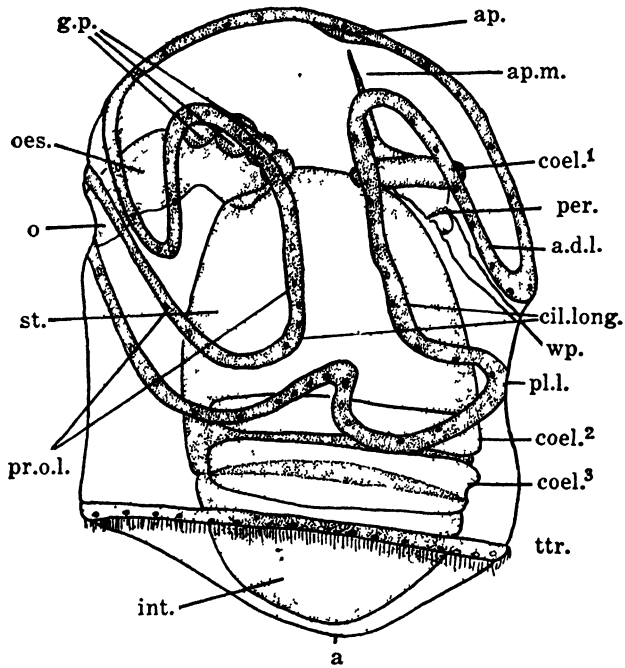


FIG 127 New England tornaria from left side (After Morgan)

a, anus, a d l, anterior dorsal loop of the longitudinal ciliated band ap, apical plate; ap m, apical muscle band, cil long longitudinal ciliated band coel 1-3 first, second, third, pairs of coelomic cavities g p, gill pouches int, intestine o mouth oes, oesophagus per, pericardial sac, pl l, posterior lateral arm of the longitudinal ciliated band, pr o l, pre-oral loop of the longitudinal ciliated band, st, stomach ttr telotroch, wp, water-pore.

Dolichoglossus there is a large yolky egg which by a much-abridged series of stages reaches the adult without the intervention of any independent larval form. In several of the species of *Balanoglossus* a type of development which is probably more primitive is found, involving the presence of a special larva known as the tornaria. Originally when this larva was first found in towings it was assumed to belong to some genus of the Asteroidea, but the works of Bateson, Morgan, Ritter, and Heider have left no doubt as to its relations to *Balanoglossus*. Strangely enough

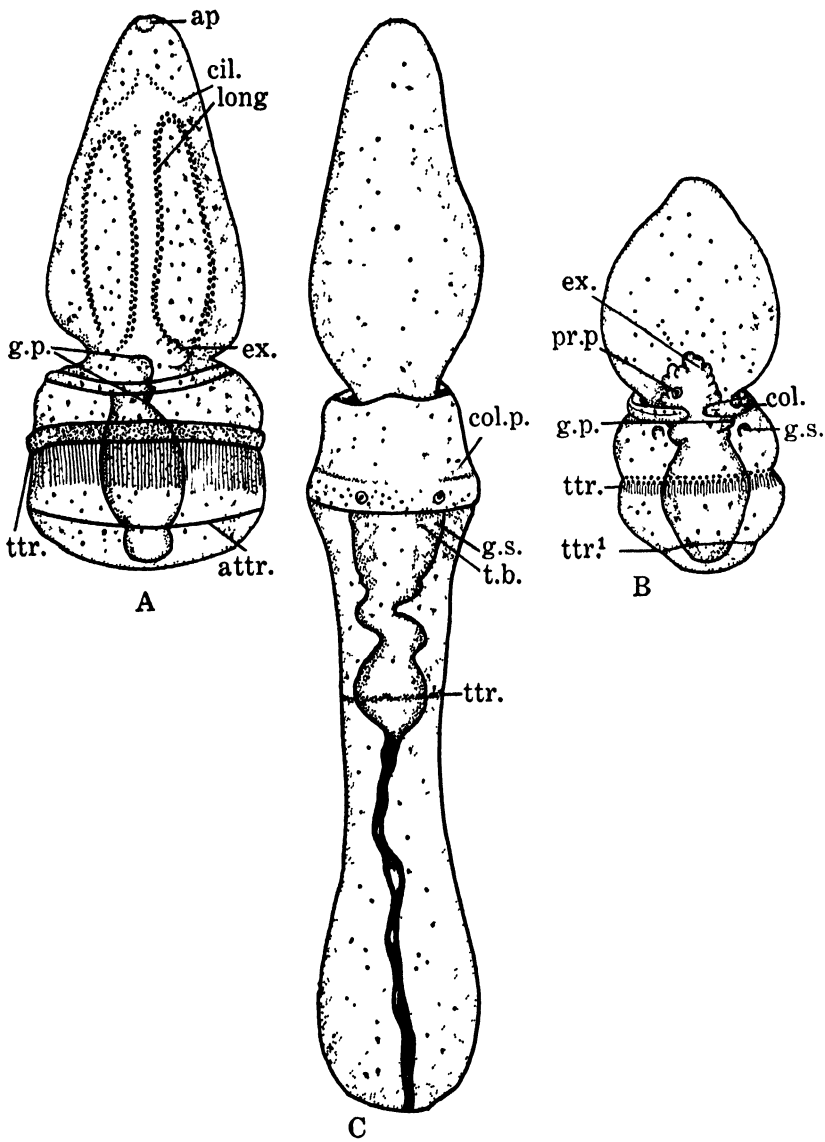


FIG. 128. Metamorphosis of tornaria from Bahamas. (After Morgan.)

A, just before metamorphosis; B, during metamorphosis; C, young balanoglossid worm with three pairs of gill slits. ap , apical plate, cil. long., degenerating longitudinal ciliated band; col., collar region, col.p , collar-pore; ex., excretory tissue in posterior wall of proboscis coelom, g p., endodermic gill pouch, g.s., gill-slit; pr.p., proboscis-pore; t b , tongue-bar dividing the gill-slit; ttr., telotroch; attr., accessory telotroch characteristic of the Bahamas larva.

not all of the types of tornaria which have been described agree in all points, and several different methods for example of mesoderm formation and development of the coelomic cavities have been recorded for this larva. The work of Heider, although the most recent, is the only one which deals with the early stages. He was able to follow through the cleavage and development of the larva up to the tornaria stage, but could keep his specimens alive only eight days. Morgan's account from species taken at Woods Hole begins about where Heider's specimens died so a fairly complete history is available. The structure of the tornaria larva superficially resembles that of the young bipinnaria and it is possible to apply the designations used on the bipinnaria to the longitudinal ciliated band and the posterior transverse ciliated band. This latter is also known as a telotroch, corresponding roughly to the same structure of the annelid trochophore. From the anterior end of the growing archenteron a vesicle is budded off which becomes the anterior coelome or the coelome of the proboscis of the adult worm. From the posterior wall of the anterior coelome are developed rudiments of the pericardium and the peculiar dorsal heart of the *Balanoglossus*, as well as the rudiments of the head kidney. The more posterior part of the archenteron proliferates two groups of cells which are the beginnings of the middle or collar coelome and the posterior or trunk coelome. At a level in front of the collar coelome a constriction appears which marks the separation of the proboscis from the collar region of the adult. These two posterior coelomes are formed by different methods in the tornaria taken by Morgan at Woods Hole and those later studied by him in the Bahamas. In the former a solid outgrowth of cells from the gut wall is the rudiment of the coelome. In the latter the wall of the body cavity is formed by the aggregation of scattered mesenchyme cells, a procedure which is rarely witnessed in the animal kingdom. At the time of the constriction dividing the proboscis region from that posterior to it a dorsal strip of ectoderm remains unconstricted and becomes depressed beneath the surface in the form of a groove. This is the neural groove which is folded off and forms a neural tube in the fashion characteristic for vertebrate embryos. Below it from the anterior part of the oesophagus a median dorsal pouch grows

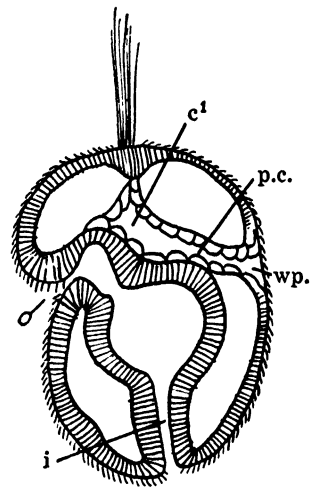


FIG. 129. Larva of *Balanoglossus clavigerus*. (After Heider.)
c, anterior coelome; p.c., pore canal; w.p., water pore; o., mouth; i., intestine.

out to form the notochord. Gill pouches develop as several pairs of pockets from the sides of the oesophagus. The further development of the larva consists largely in the formation of the modifications of the coelome, the formation of the collar pores and of the genital organs. The metamorphosis is brought about by the development of a fold in the region between the collar and trunk coelomes and the general growth in length of the trunk portion of the animal. No such complications of development and metamorphosis are found here as characterize the echinoderms, for the adult itself is of comparatively simple structure. Since the aim of this discussion is merely to show how the embryo is transformed into a young worm similar to the adult, it seems unnecessary to follow through further the details of the development of this very interesting animal.

Bibliographic Note

Among the more important accounts of the subjects contained in this chapter are the following: Schultze, Minchin, Maas, Metchnikoff, Götte, MacBride, Brooks, Appellöf, Korschelt and Heider, Salensky, Lang, Surface, Woltereck, Balfour, Claus, Grave, Bury, Field, Bateson, Morgan, Ritter, Heider on *Balanoglossus*. These works are cited in full in the bibliography on page 406.

CHAPTER X

FORMATION OF THE MAMMALIAN EMBRYO

[NOTE: In developing a knowledge of comparative embryology especially as it applies to the vertebrate groups the most logical procedure at this stage (in which we have considered various invertebrate larval forms) would be the detailed study of various vertebrate embryos. It would be proper to consider in detail the embryology of amphioxus, the shark, a teleost fish, the frog, and the chick, as is so commonly done in courses dealing with this subject, and of course there are other forms also which might be chosen.]

So much material is available upon these various forms that it has seemed wiser not to devote space to a discussion of the embryology of any of them. Most textbooks of vertebrate embryology discuss at least some of these, and both brief and detailed expositions of the subject are easily available in English. Indeed the choice of forms differs a great deal with instructors so that it would perhaps be necessary to include all of them to satisfy the selections that would be made in different institutions. In our own case it is our practice to ask the student to obtain a copy of Patten's "Embryology of the Chick" and to study the chick intensively in the laboratory. Because of the good accounts which are so generally available and because it is felt that nothing could be added in the present volume that is not easily available in other accounts, it has been decided not to offer any special discussion of the groups mentioned.

On the other hand, the mammals present many embryological variations from the other vertebrate groups, the understanding of which is by no means so easy to obtain as the preceding cases. In the discussions in the chapters on cleavage, gastrulation, etc., it has often been said that the conditions throughout the animal kingdom are as stated except for the mammals. For these reasons it has seemed best to include here an account of the formation of the mammalian embryo and to leave other groups and the later history of the mammalian embryo for more detailed works.

Considered from the point of view of the embryological differences which are manifested in the class Mammalia, three distinct groups are found: the monotremes, the marsupials, and the higher mammals. In the monotremes, eggs containing a large amount of yolk are laid after the fashion of the reptilian groups, and the special features of the embryology of these forms hark back to the type of development as found in the reptiles more than they forecast conditions of the higher mammals. A detailed discussion of the monotreme seems to be unnecessary.

I. MONOTREMES

The monotreme egg is the largest of all mammalian eggs since it contains yolk varying in diameter from 2.5 to 4.0 mm. The accounts as given for *Echidna* and *Ornithorhynchus* do not all agree in the exact size

but variations are within the limits indicated. The egg is developed within a follicle consisting of only one or two layers of cells and when it is passed to the outside receives a covering of albumen and a shell. In *Ornithorhynchus* the shell is said to be calcified.

Owing to the presence of a large amount of yolk, segmentation is discoidal, forming a blastoderm upon an unsegmented yolk mass. Semon failed to find yolk nuclei here as is the usual case in eggs with discoidal cleavage. The first two furrows are meridional while the third is parallel to the first and at right angles to the second. A many-layered blastoderm is formed having the shape of a biconvex lens, the deeper-arched side of which is imbedded in the yolk.

The chief departure of the monotreme egg from other discoidal eggs comes in the rapid closure of the blastopore which takes place relatively early. The edges meet in a spot where yolk and upper and lower cell layers are continuous with each other, a condition which has given rise to some misconceptions as to the homologues of these structures with those of lower forms. The blastopore itself is the elongated primitive groove.

II. THE MARSUPIALS

The embryology of marsupials has been the subject of study during the last fifty years on the part of several investigators. The work of Caldwell and Selenka in 1887 represents the first attempt at a study of the early stages of development, the subject of the former study being the Australian marsupial, *Phascolarctus*, and of the latter the North American opossum, *Didelphys virginianus*. In 1910 Hill's study of the Australian native cat, *Dasyurus viverrinus*, appeared, and this study in connection with the work of Hartman in 1916 and 1919 on the opossum gives us our best understanding of the embryology of this group. Other workers have been Minot, and Spurgeon and Brooks on the opossum, but the two mentioned are the chief source of our information.

The ovarian egg of *Dasyurus*, the largest mammalian egg known outside of the monotremes, measures 0.28 mm. according to Hill, while that of the opossum varies from 0.14 to 0.16 mm. The egg of the former is scantily supplied with albumen, whereas around the latter albumen is laid down in delicate concentric lamellae. The eggs of marsupials are richly supplied with yolk compared to other mammalian eggs but a strange phenomenon occurs by which yolk is extruded from the cells themselves into the space surrounding the blastomeres. In *Dasyurus* (Fig. 130), according to Hill, extrusion takes place before cleavage begins in the form of a yolk body at the vegetative pole, but in *Didelphys* the yolk

is passed out during the first few cleavages and at no time forms a concentrated mass. It is thrown out from both ends or from all sides in greater or less amount during the early divisions. The greatest amount seems to be given off between 2- and 4-cell stages and since the yolk is distributed around the periphery of the egg no one point is the center of the extrusion. The orientation of the eggs likewise differs in these two genera. According to Hill the accumulation of the yolk marks the vegetative pole. The first three cleavages of *Dasyurus* are meridional but the fourth is horizontal and divides the eight blastomeres into an upper ring of eight small lighter-staining cells and a lower ring of larger more darkly staining blastomeres. The upper cells are regarded by Hill as formative or embryonic and the lower ones as non-formative giving rise to the outer layers of the embryos which are spoken of as trophoblastic. The embryonic portion is derived from the small cells of the 16-cell stage and therefore from the vegetative half of the egg as marked by the earlier extrusion of the yolk body. The larger cells from the animal region of the egg produce the trophoblastic portion.

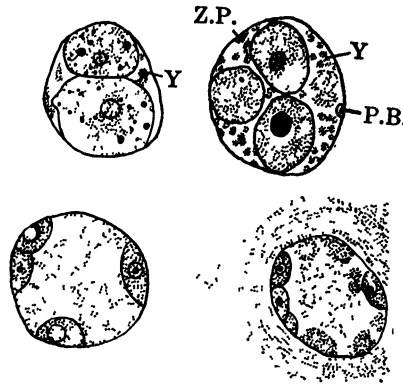


FIG 130 Cleavage stages in the development of the opossum *Didelphys virginianus* (After Hartman)

A, section through 2-cell stage, B, section through 4-cell stage P B, polar body, Y, yolk masses, Z P, zona pellucida, C, section through stage with 17 cells, D, section through stage with 28 cells.

In *Didelphys* no such orientation can be found. Here the first cleavage divides the egg into two blastomeres without any indication of polarity or of qualitative differentiation. The second cleavage plane is in general about at right angles to the first, but a shift takes place by which two opposite blastomeres come into contact with each other as is typical of many eutherian eggs. Thus no section can be cut through the centers of all four blastomeres.

From the 4-cell stage on, the cleavage of the opossum egg is irregular, it being possible to find stages of 6, 8, 10, 12, 15, cells and others. The space between the blastomeres represents a blastocyst cavity and even in the early stages the blastomeres tend to migrate to the wall of the "ovum" and arrange themselves in contact with it. Here they flatten and their outer surfaces take on the curvatures of the surrounding albumen layer. Thus the yolk is left within the cavity formed by the dividing cells which are uniform in size and structure throughout. The

embryo is now spoken of as a blastocyst which is regarded as completed by the 32-cell stage.

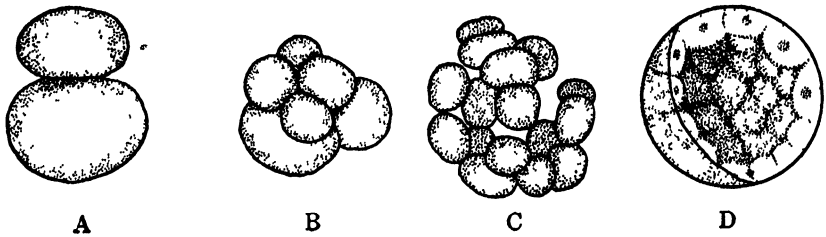


FIG. 131. Drawings from models of the opossum egg. (After Hartman.)

A, 2-cell condition; B, 6-cell stage showing polarity and the fate of the two blastomeres of the preceding stage; C, 16-cell stage; D, a blastocyst of 40 to 50 cells in which no evidence of polarity is seen, although it will shortly be reestablished with reappearance of the endoderm.

The blastocyst of *Dasyurus* is formed in a different manner. Here the cells of each ring multiply rapidly but continue to occupy their horizontal positions. Upon reaching the periphery they too become applied to the inner surface of the shell, the formative cells at one pole and the non-formative at the other. This constitutes a blastocyst of one layer of cells, a unilaminar blastocyst. Here too the yolk remains within the cavity. Presently the cells of the formative ectoderm become differentiated and a little later endoderm formation begins. Individual cells (called endodermal mother cells) migrate in an amoeboid manner below the surface and there unite to form a second layer, the endoderm. This layer continues to proliferate and finally closes up to form a complete lining for the entire blastocyst, the primitive endoderm. This description of the formation of the endoderm by Hill seems to concern an entirely different method from that found elsewhere among the mammals.

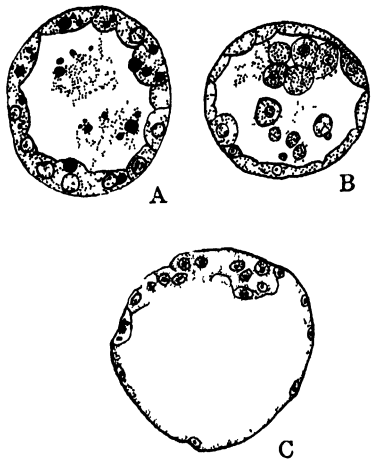


FIG. 132. Sections through the blastocyst of the opossum. (After Hartman.)

A, section through the blastocyst of 70 cells but without endoderm, B, section through blastocyst of 82 cells showing six of the ten endoderm mother cells; C, little later blastocyst showing differentiation of the embryonic area and trophoblastic ectoderm.

Hartman also describes endoderm mother cells which detach themselves from their place in the blastocyst wall and behave in the manner

closely resembling that described for *Dasyurus*. The important difference here lies in the impossibility of distinguishing formative and non-formative cells among the early blastomeres and of relating such cells to the trophoblast and to the ectoderm of the unilaminar blastocyst.

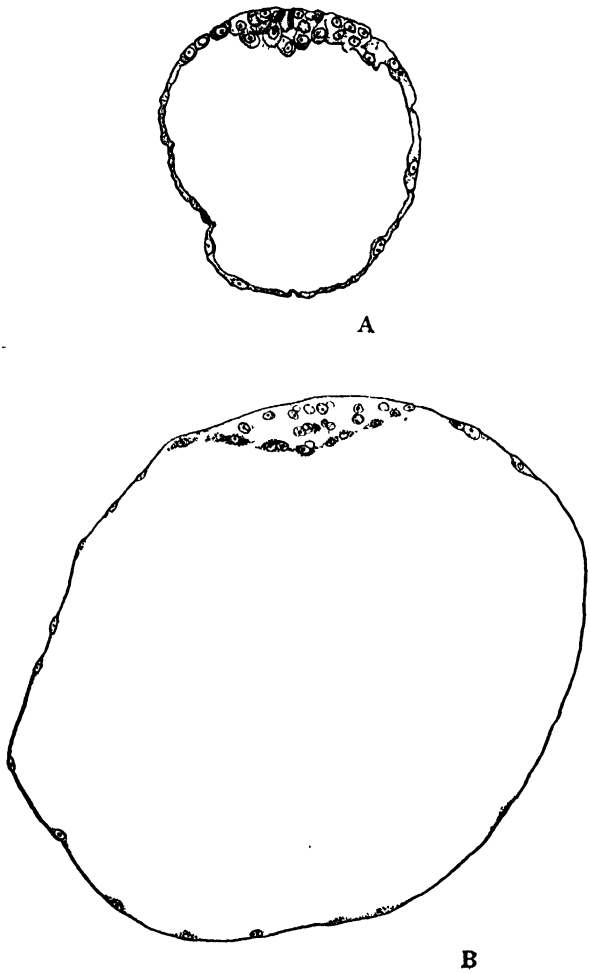


FIG. 133. Continuation of Fig. 125. A, B, showing progressive differentiation of the endoderm and trophoblast.

Clearly there is no way by which the endoderm mother cells can be traced back in the opossum to the early cleavage stages and, as Hartman remarked, cleavage is entirely indeterminative.

While the cleaving ovum of *Dasyurus* shows a greater degree of determination and much more marked polarity than is the case in *Didelphys*,

Hartman is in agreement with Hill on the general facts as to marsupial development. Even in the opossum egg a polarity of a sort is shown in the fact that the cells in one hemisphere divide more rapidly than those in the other and it may be assumed that the embryonic area is formed by the cells of the more rapidly dividing group and the trophoblast from those of the other. In discussing the formation of the embryo of Eutheria it will appear that the "inner cell mass" of the latter is homologous with the embryonic area of the marsupials and the trophoblast of both groups show a complete homology, a view in which both Hartman and Hill join. In the Eutheria, however, it is held that the trophoblast comes from one of the two blastomeres of the 2-cell stage and the inner cell mass from the other. Clearly the fate of the two blastomeres is different in these two groups for in the marsupials one half of each blastomere goes into the production of the embryonic area and the other half into the trophoblast.

Endoderm Formation

The embryonic area having become localized, a number of small cells undergo modification and become the endodermal mother cells previously referred to. This process begins to take place in blastocysts containing 50 to 60 cells. The cells increase in size, project into the blastocyst cavity, and undergo other recognizable changes. Presently they free themselves from the wall of the blastocyst and by putting out extended tips come in contact with each other so that a layer of endoderm is formed. Sometimes several divisions take place on the part of these cells before they have detached from the wall. The process of true endoderm formation in the opossum and in *Dasyurus* as well is thus one of the multiplication and migration of cells differentiated as endodermal mother cells from one pole of the blastocyst wall and in no case does it include more than one half of the entire blastocyst, the other half being made up of trophoblastic cells. It will prove interesting to compare this process with that of the placental mammals.

Hartman holds that the opossum egg is intermediate between that of *Dasyurus* and the placental mammals in its type of development. In size, in absence of polar differentiation in the unsegmented egg, in the crossed arrangement of blastomeres in the 4-cell stage, in indeterminate cleavage, and in early proliferation of endoderm it approaches the conditions of the Eutheria. But in the lack of a morula stage, of an inner cell mass as such (although the embryonic area is homologous with it), and in the method of endoderm formation it resembles *Dasyurus*. The large size of the latter as compared to other mammals, and the localization of the yolk mass in one portion of the egg (not far removed from

the telolecithal condition), remind one of the derivation of these forms from a reptilian ancestry.

The blastocyst of the opossum during its later stages undergoes some important changes, most of which are concerned with its growth and enlargement. Its growth takes place by means of spreading and attenuation as well as by the more rapid multiplication of the trophoblastic

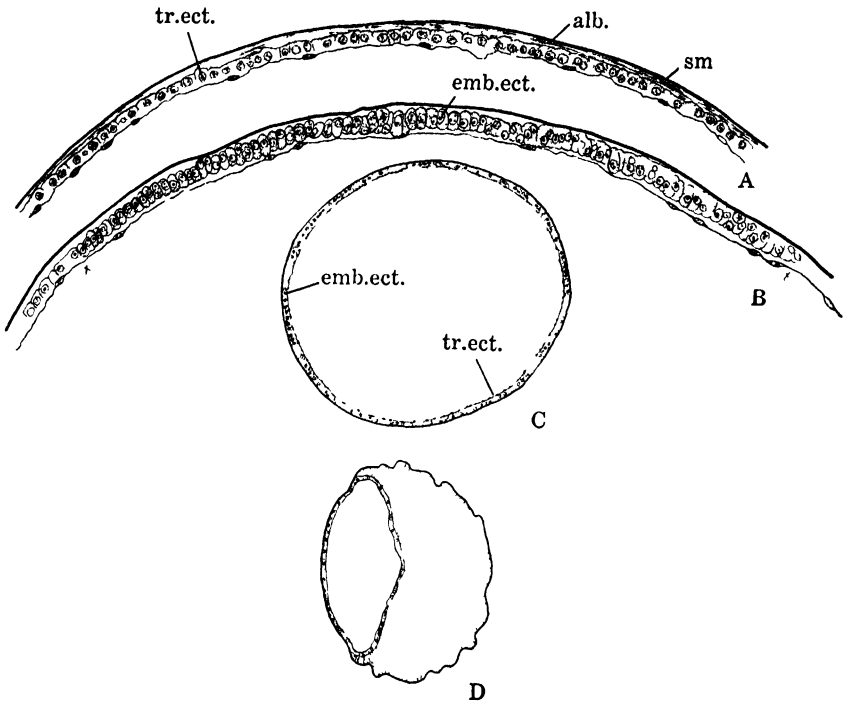


FIG. 134. Later blastocysts of the opossum. (After Hartman.)

A, just completed bilaminar blastocyst. B, a later blastocyst nearly ready for the appearance of the mesoderm; C, D, sections showing details of the trophoblast and of the embryonic ectoderm respectively.

emb. ect., embryonic ectoderm; tr. ect., trophoblastic ectoderm; s.m., shell membrane; alb., albumen.

cells. Finally a maximum size of 1.0 to 1.5 mm. is reached and the trophoblastic region has come to occupy from four-fifths to five-sixths of the entire surface of the blastocyst. That is, the increase in size concerns the trophoblastic region mainly, the embryonic area growing very slowly if at all.

The cells of the embryonic area become much crowded and the endoderm attains a depth of three or four cells without taking on an epithelial character. The cells of necessity are irregular in size and shape. Then largely because of the migration of the endoderm cells they begin

to spread. While this migration is active on the part of the cells, no amoeboid movement has been detected by Hartman. With this process the blastocyst becomes biconvex in form, the flattening occurring in the direction of the egg axis with the formative area pressed against the shell membrane. The continuous growth of the trophoblastic area finally brings it also in contact with the shell membrane, thus effecting for the blastocyst a return to the spherical shape and completing the formation of the blastoderm.

Meantime the endoderm continues its lateral migration until it has reached the trophoblastic pole of the egg, that is, until it has reached the point opposite its own place of origin, and thus a closed endodermal sac is formed within the ectoderm. During all this time the ectoderm has remained as a single layer of quite flat cells. Now, however, these gradually thicken and become cuboidal. With this condition reached, the formation of the blastocyst of the opossum is completed. From the formative area, the embryo proper is developed in a manner quite comparable to that of the placental mammals; this phase of its embryology is not taken up here as a separate subject for discussion, since the formation of the embryo in the placental mammals is the subject matter of the next division of this chapter.

III. HIGHER MAMMALS

1. General Discussion

The third distinct embryological group of mammals is made up of the Eutheria or Placentalia. The eggs differ from all others in a number of characteristics, the first of which is the size, for all are microscopic. They range from 0.07 mm. in the mouse to 0.145 mm. in the dog and 0.13 to 0.14 mm. in man. (These figures are from Hartman's calculations.) Even at this small size, however, the eggs are large in comparison to other cells of the body, for here as in other animals there is an accumulation of yolk within the egg. In the older works it was commonly said that the eggs of mammals are alecithal or yolkless. As already pointed out in an earlier chapter this is untrue, for although it is small in amount in the eggs of Eutheria some yolk is present in every sort of egg.

The term isolecithal or homolecithal previously used in this work for such eggs as these is better applied here than alecithal and some writers use also the terms microlecithal, or small-yolked, to distinguish these eggs from the more usual and larger-yolked types which are spoken of as megalecithal. Although more sparsely distributed than in other eggs, spherical yolk granules and often fat globules as well are to be found in the eggs of mammals.

The lack of yolk in the eggs of placental mammals is one of the two major factors which are responsible for a very extensive modification found in the early embryology of these forms. Derived as they are from forms that are highly laden with yolk with an extremely discoidal type of development, it is not to be expected that the reduction in yolk would mean for the Placentalia a return to the simple methods of development which are found in the holoblastic regularly cleaving forms, as for example the echinoderms and amphioxus. The presence in all forms of Placentalia of a yolk sac, which is derived through the discoidal ancestry, is an evidence, although it contains no yolk, of the lack of simplicity in the development of these forms. The marsupials we saw constituted a step toward the reduction in yolk, for there the yolk was eliminated during the first cleavages. Not even the elimination takes place among the higher mammals for so little is present that it is easily used up during the early divisions. The lack of a yolk-filled hemisphere in the uncleaved mammalian egg very obviously means a most striking modification of the type of development, for in the discoidal monotreme only the animal half of the egg participates in the formation of the embryo. The fate of the yolkless vegetative half in the formation of the trophoblastic portion of the egg has already been foretold in the formation of the blastodisc of the marsupials.

The second major factor in bringing about the modifications which are characteristic of higher mammals is also correlated with the yolkless condition. It is the development of an entirely different mechanism for supplying nourishment to the embryo and foetus before birth and takes the form of a placenta. The segmenting ovum becomes related to the uterine wall in a most intimate manner. This process is called placentation.

As a result of the factors mentioned the mammalian egg develops through a blastocyst stage. Clearly it is difficult to work out the homology between this type of embryo formation and that of the forms described in previous chapters. Indeed some writers on mammalian embryology have positively denied the homologies that would be implied if we use the terms descriptive of the embryology of earlier forms for the conditions in the mammals. Such terms as formation of germ layers and primitive streak, etc., may lead to confusion and even contradiction

2. Development up to the Blastocyst

The ovarian egg of mammals exhibits certain characteristic features. It is formed within a follicle which consists morphologically of the same sort of germ cells as itself, but after one of them differentiates in an early embryological stage to become the ovum the remainder develop

only into follicle cells. The ovary of the mammal at the time of its birth is said to contain in an undifferentiated condition all of the oogonia which are to be developed during its later life.* That is, the multipli-

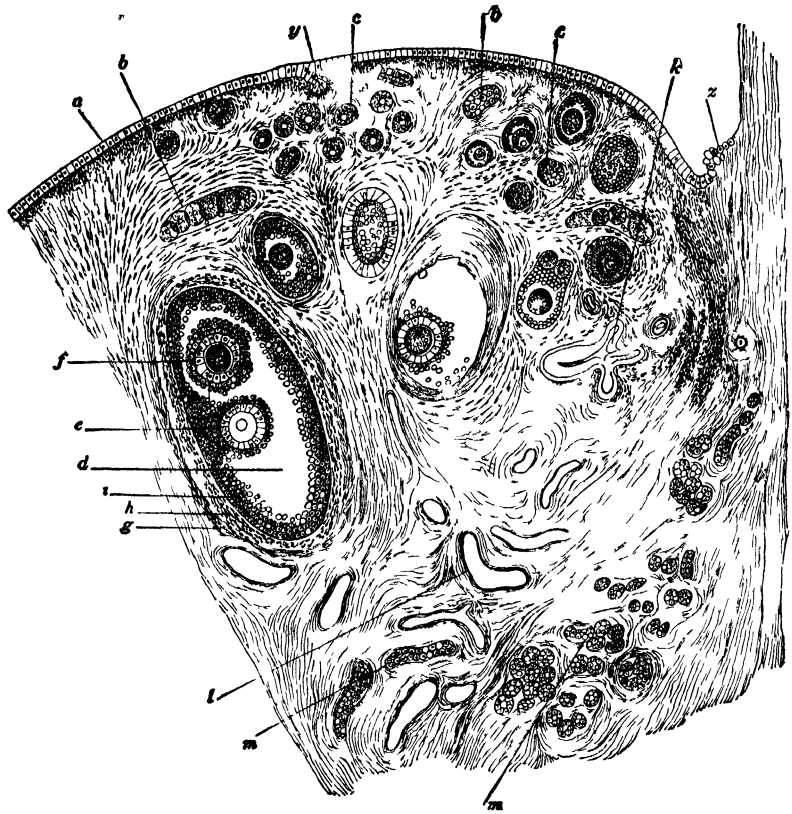


FIG 135. Section through ovary of a dog (From Kellicott, after Waldeyer)

a, 'Germinal epithelium', b egg tubes c, small ovarian follicles, d, older ovarian follicles, e, ovum surrounded by discus proligerus, f, second ovum in follicle with e (Only rarely are two ova thus found in a single follicle) g, outer capsule of the follicle, h, inner capsule of the follicle, i, membrana granulosa, k, collapsed, degenerating follicle, l, blood vessels, m, sections through tubes of the parovarium, y, involuted portion of superficial epithelium, z, transition to peritoneal epithelium.

cation period is past at the time of birth. Many of these cells are utilized only in the production of the follicle cells which surround each developing ovum. They multiply, become columnar, and form a follicular epithelium. On one side only of the follicle a definite accumulation of cells

* In a recent paper, Hargitt (Jour Morphology and Physiology, Vol 50, 1930) makes the claim that in the ovaries of some mammals the production of germ cells is continuous

known as the *discus proligerus* surrounds the ovum while on the other side a cavity is left by the separation of the cells of the follicle from each other as the structure enlarges. This cavity becomes gradually larger, and is filled with a liquid, the *liquor folliculi.*

The fully grown follicle is known as a Graafian follicle. The egg itself passes through its growth period in this follicle and becomes surrounded by definitely recognizable layers. The presence of the vitelline membrane, derived from the ovum, is a matter of dispute in various mam-

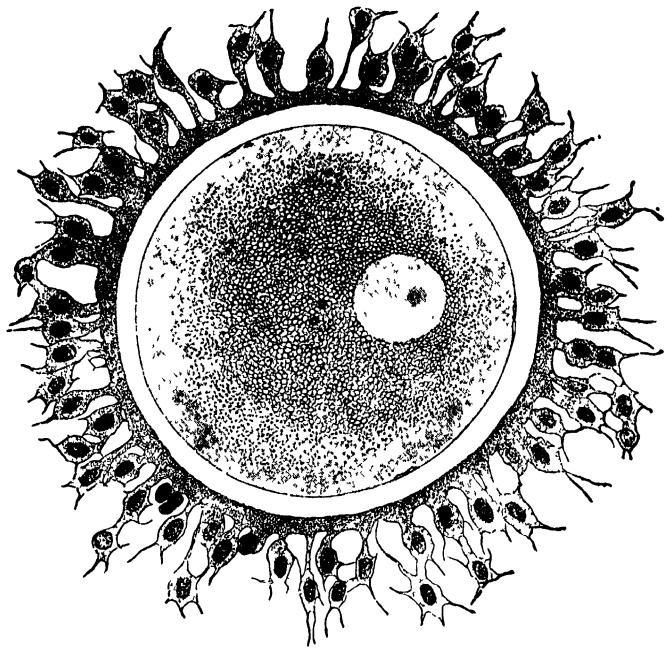


FIG. 136. Fully grown human oöcyte just removed from the ovary. (From Kellicott, after Waldeyer.)
Zona pellucida and follicular epithelium (corona radiata) outside the oöcyte. Nucleus in germinal vesicle stage.

mals. The secondary membrane derived from the follicle is present as a *zona pellucida.* Often this membrane is so perforated by tiny canals as to give the appearance of radiation in the membranes and hence is spoken of as the *zona radiata.* In some forms this is surrounded by a layer of regularly arranged follicle cells spoken of as the *corona radiata.*

The beginning of maturation occurs at the end of the growth period when the egg is ready to escape from the ovary. The first polar body is given off before the ovum has escaped. In the mouse this process is said to take place one-half hour before ovulation. Then the Graafian

follicle is ruptured, the ovum escapes into the periovarian space, and is carried into the fimbriated ostium of the oviduct which is in close proximity. The second polar spindle has already been formed and the second polar body is given off after fertilization in the upper part of the oviduct. Related to the process of ovulation are changes in the follicle, which is converted into a *corpus luteum*, as well as physiological changes of great importance to the organism. However, the history of the embryo, which is the subject of this discussion, does not require the further consideration of these changes connected with the ovary itself, although they are of greatest physiological importance. The cleavage stages occur slowly as the egg passes down the oviduct. Twenty-four to forty-eight hours elapse before the completion of the first and second cleavages in the mouse. In the rabbit fourteen or fifteen hours are occupied by the first cleavage and a correspondingly longer time elapses before the ovum reaches the uterus. Eighty hours are required in the mouse for this passage through the oviduct, four days in the rabbit, eight to ten days in the dog and in certain ungulates it is said that the ovum remains over the winter in the oviduct. Cleavage is total and at first nearly equal, but rapidly becomes very irregular. Figures are available for various animals showing cleavage stages consisting of almost any number of cells up to sixteen or twenty. Evidently it is impossible to determine for these eggs the orientation, polarity, and such facts of promorphology as have been shown to apply to eggs of lower forms. Cleavage results in a mass of cells spoken of commonly as a morula. The cells at the outside become differentiated to form a subzonal layer lying next to the *zona pellucida* while those within the mass constitute the inner cell mass. The inner cell mass is in contact with the subzonal layer on one side but elsewhere a cavity is formed between it and the outer cells. The subzonal cells are also spoken of as a trophoblast or simply as the wall of the blastodisc vesicle, and their relation to the trophoblast of the marsupials is clear from the description already given for those forms.

3. The Blastocyst

The homology of the inner cell mass with the embryonic or formative area of the marsupial as shown by both Hartman and Hill has also been mentioned. The view held by some that the trophoblast of the Eutheria comes from one of the two blastomeres of the 2-cell stage while the inner cell mass is derived from the other is based on evidence from some forms only and probably cannot be maintained with certainty for all mammalian types owing to the difficulties in determining the orientation. The cavity of the blastodermic vesicle or blastocyst becomes filled

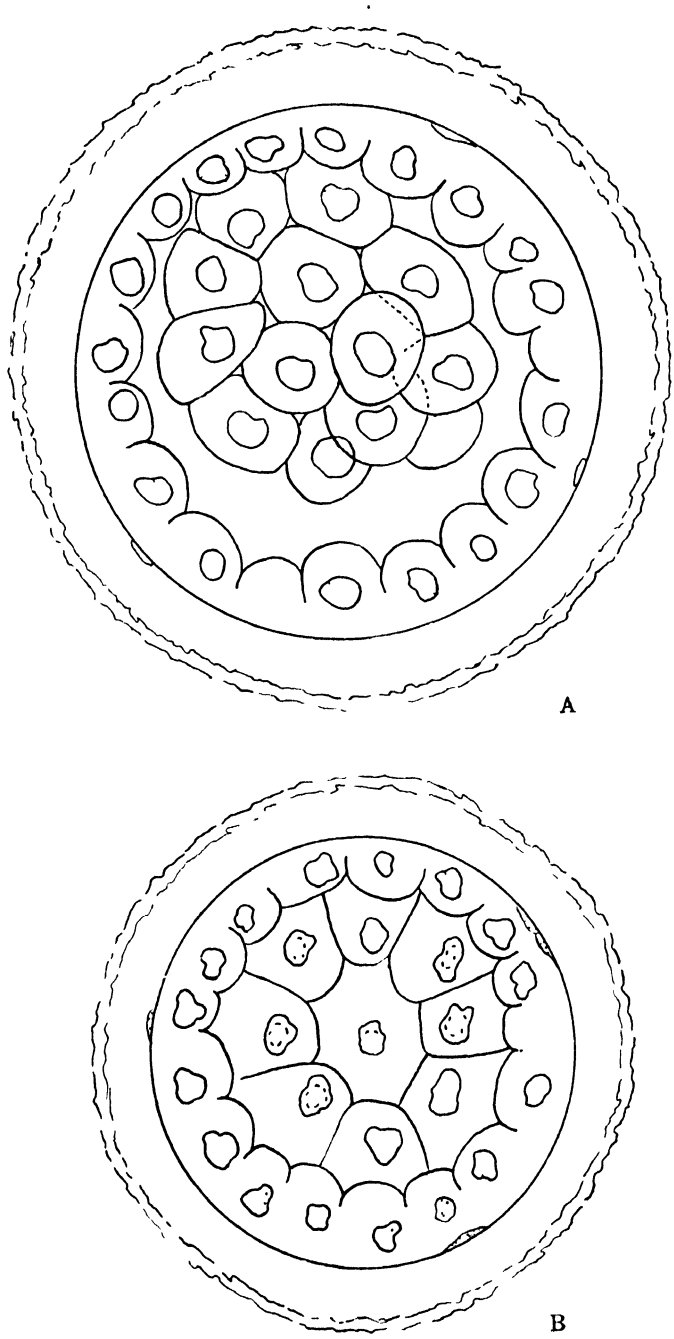


FIG. 137. Developing eggs of the rabbit. (After Van Beneden.)

A, so-called metagastrula stage preparatory to the formation of the blastocyst; B, beginning of gastrula cavity, the formation of the inner cell mass.

with a fluid which is supposed by some to represent the yolk of lower forms. The subsequent history of the blastocyst varies in different groups.

There are three important stages in connection with the early history of the formation of the embryo. The first is the growth of the blastodermic vesicle during which it takes on the form typical of the particular animal discussed. The second is the formation of the embryo body proper; this stage involves the subsequent history of the surrounding layers of cells. The third is the implantation of the blastocyst in the uterine wall and the formation of the placenta; this stage scarcely comes within the scope of this discussion and can be considered only in passing.

The growth of the blastocyst takes place in a manner similar to that described for the marsupial, the trophoblastic cells becoming extended and flattened and the cells increasing rapidly in number. Cell division at this stage takes place much more rapidly than in the earlier cleavage stages, for in three days after the embryo has reached the uterus in the rabbit it is ready for implantation. In other cases a somewhat longer period is necessary. The size of the vesicle at the end of its growth like the size of the mammals themselves is variable, as is also its shape. It is spherical in the mouse, ovoid in the rabbit, and in the ungulates is long and tapering, reaching a length of 20 cm. in the sheep of twelve days although the diameter remains nearly constant, a couple of millimeters. This growth, as in the opossum, is limited to the trophoblast, the inner cell mass remaining very small and restricted.

Up to the conclusion of the growth of the blastocyst no distinction as to ectoderm and endoderm has been possible among its cells. Formation of the endoderm now begins as described for the opossum from the differentiation of those cells of the inner cell mass which border the cavity of the blastocyst. Here these cells multiply rapidly and at the same time migrate so that they come to form a layer over the entire inner surface of the trophoblast which in this manner becomes two-layered and may be spoken of as a gastrula. Obviously this method of endoderm formation is more closely related to that of eggs cleaving discoidally than to that of the holoblastic types. In some mammals, notably the primates, the endodermal layer remains much smaller than the blastocyst itself, thus forming a double-layered vesicle whose outer layer is separated from the inner by reason of its more rapid growth. Thus of the inner cell mass the layer bordering the cavity becomes embryonic endoderm and the remainder is spoken of as the embryonic ectoderm. From this mass is to be differentiated the body proper of the embryo. It is formative in the strict sense, for all of the embryo is derived from it. The cavity of the blastocyst with its endodermal lining is now spoken of as the yolk sac and, although it contains no yolk, is reminiscent of the

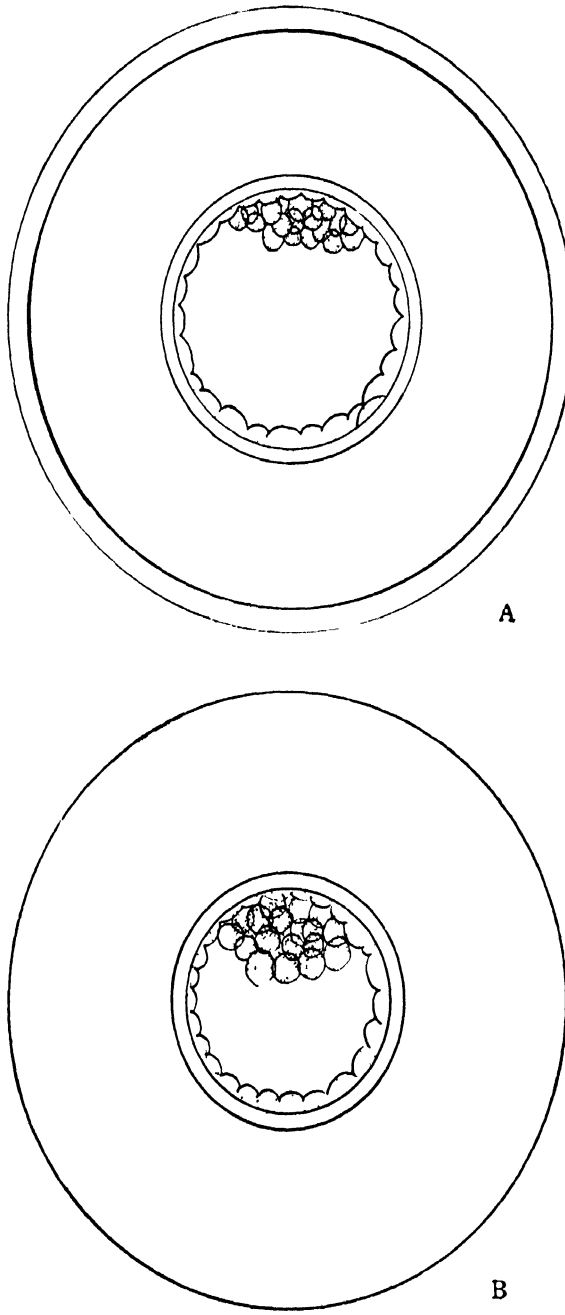


FIG. 138. Continuation of 130. A, B, growth stages in the formation of the blastula cavity and the transformation of the inner cell mass into a plate.

large-yolked reptilian forms. The inner cell mass minus the yolk sac endoderm which, though once part of it, has migrated away is now restricted to embryonic endoderm and embryonic ectoderm above it; by this time it should be spoken of as the embryonic knob. It is the history of the embryonic knob which especially concerns us.

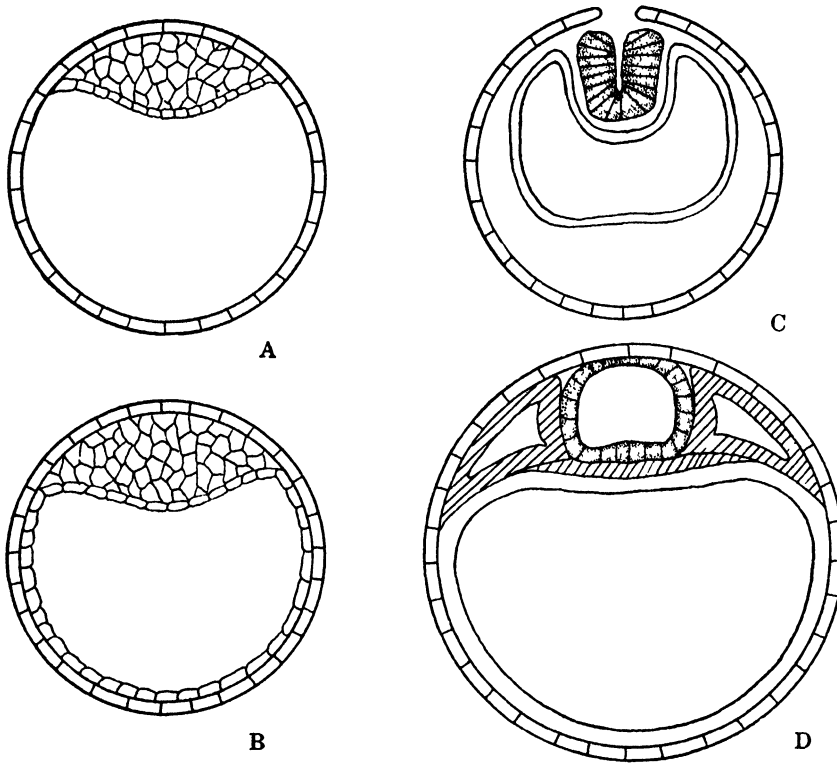


FIG. 139. A, B, diagram showing the early formation of the blastocyst and the relation of the ectoderm, endoderm, and trophoblast; C, diagram of the development of the insectivore *Tupaija* from Hubrecht's observations showing the development of the cavity of the ectoderm which opens out to form the embryonic shield; D, development of hedgehog type, also after Hubrecht's observations. (Modified from Keibel.)

At about the time when the embryonic knob is distinctly recognizable, implantation takes place. That is, the blastocyst becomes attached by means of the trophoblast to the wall of the uterus and the placenta begins to form. Of implantation there are three types.

(*a*) Central, found in Carnivora and Ungulata, in some rodents including the rabbit in which it was first described and in the lower primates. In this type the blastodermic vesicle attaches directly to the uterine wall, projecting as it grows into the cavity of the uterus.

(*b*) Eccentric, as in the mouse and Insectivora where a uterine fold forms in which the vesicle lies and is later enclosed as the edges of the fold come together.

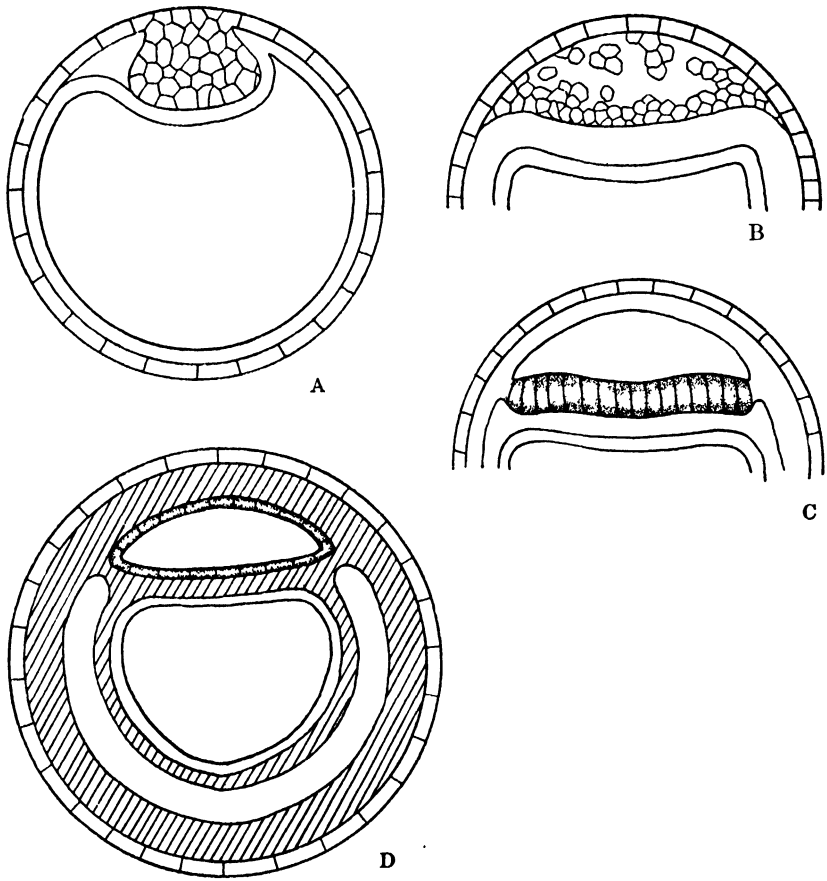


FIG. 140. Series of diagrams to show development with entypy of the germ. A, the relations of the embryonic knob, the endoderm, and the trophoblast; B, later stage showing the cells of the embryonic knob arranging themselves to form an embryonic shield with the amniotic cavity and Rauber's layer above it; C, later stage in its development. D, Still later stage showing inversion of germ layers. (Modified from Hubrecht's observations on the hedgehog.)

(*c*) Interstitial, as in the guinea pig, most rodents, and in man. Here the vesicle makes its way into the mucous lining of the uterus and after becoming embedded therein is entirely covered over by the mucosa.

The time relations of the development of early stages in the mammals seems worthy of note, for a very much greater amount of time in

relation to the entire embryonic period is consumed by these earlier stages in the mammals than is the case in the Sauropsida or in other lower forms. Even the cleavage stages take place much more slowly. It has been suggested that the delay in the development of the embryonic shield (see below) until after implantation has been accomplished

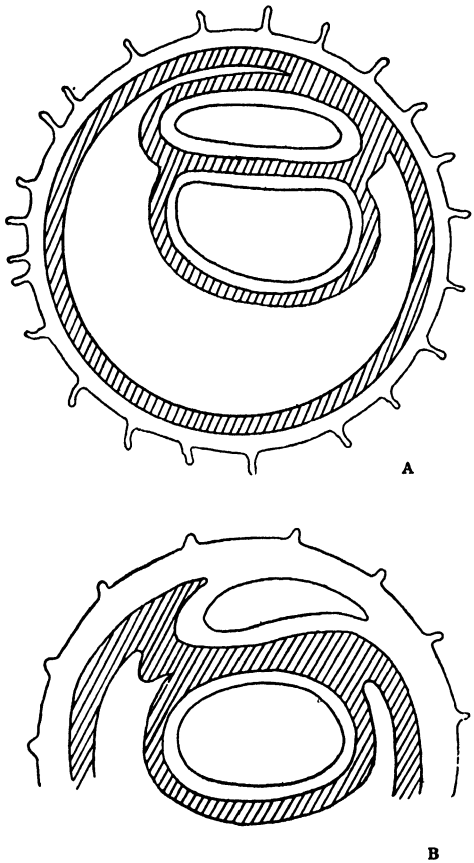


FIG. 141. A, B, diagram showing the rapid enlargement of the extraembryonic coelome and the chorionic vesicle. (B, modified from Bailey and Miller.)

is due to the very meager nutrition of the embryo during the period in which it is free in the uterus. This view is perhaps borne out by the sudden rapid development which takes place immediately after implantation has been accomplished. Yet it is hard to understand why the cleavages should be slowed down to such an extent as we find to be the case if the factor of nutrition is the only one involved, for during the cleavage stages at least a small amount of yolk is always available.

In the meantime the embryonic knob has continued its development and mesoderm has been formed. As the extraembryonic mesoderm grows, it pushes in between the trophoblast and the yolk sac endoderm and the entire extraembryonic wall takes part in the formation of the so-called chorion, the serosa. Sometimes the trophoblast is spoken of as the chorionic ectoderm. The formation of the mesoderm in the mammal is a less conspicuous process than in some of the lower forms. It takes place entirely by delamination and migration of cells which are usually from the ectoderm. In some forms it is stated that the mesoderm is related to the endoderm but certainly the usual derivation is from the embryonic ectoderm. It will be noted that this separation of the mesodermal layer takes place before the formation of a primitive streak or any other elements of the body proper.

4. The Amnion

We may now return to the behavior of the embryonic knob as such. From this structure are derived not only the embryonic shield, which corresponds in a general way to the blastodermic disc of Sauropsida, but also the amnion and the structures connected with it. It is necessary to consider the formation of the amnion before taking up the origin of the embryonic shield. Mammals show two separate methods of the formation of the amnion depending apparently upon the relations of the trophoblast to the embryonic knob. In some forms the trophoblast comes to be interrupted over the embryonic knob which thereupon develops in a manner that is entirely different from the cases in which the trophoblast is continuous. In the latter case the trophoblast cells are said to constitute Rauber's layer.

The details of the formation of the amnion under these two conditions vary in the different orders of the mammals and cannot be gone into here. The important features only can be pointed out. Taking up first the case in which the trophoblast is interrupted above the embryonic knob, we find two different methods by which the amnion is established although its subsequent history is quite alike in both cases. In the one which is illustrated by the insectivore *Tupaija* studied by Hubrecht, the knob develops a cavity which opens to the outside as a groove. The edges of this groove make contact with the trophoblast adjoining them and later fold over to form the amnion itself. The bottom portion of the groove gradually flattens out and it is this area which becomes the embryonic shield and from which the body of the animal develops. In the other case as illustrated by the rabbit, the cells of the knob take on the form of a flat plate without the formation of a groove, but their edges are connected with the trophoblast and fold upward to form the amnion

as in the preceding case. There is here suggested a certain relation to the method of formation of the body of the embryo and the amnion in those forms having typical discoidal cleavage such as the birds and reptiles. The subsequent development of the embryonic shield in these forms also recalls that of the sauropsidan blastoderm. (Figs. 139 and 140.)

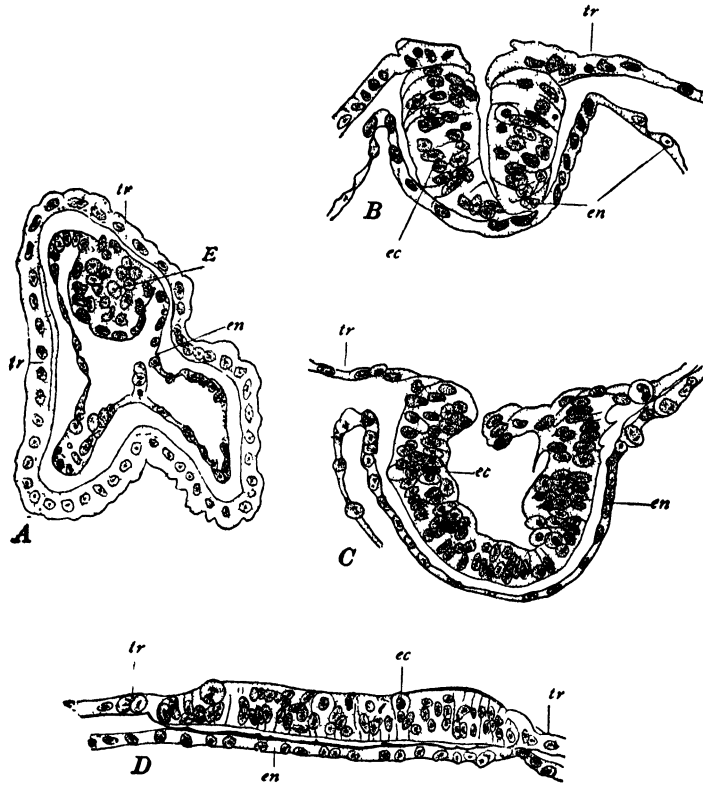


FIG. 142.—Sections through four stages in the early development of the insectivore *Tupaia javanica*. (From Kellicott, after Hubrecht.)

A, blastodermic vesicle completely closed, endoderm still continuous with the embryonic ectoderm. B, C, embryonic ectoderm split and folding out upon the surface of the vesicle, pushing away the trophoblast cells: D, embryonic ectoderm forming a flat disc on the surface of the blastodermic vesicle.

E, inner cell mass ("ectodermal shield"); ec., embryonic ectoderm; en., endoderm; tr., trophoblast.

The second method of formation of the amnion differs in many particulars from the first. The trophoblastic layer entirely covers the embryonic knob, a condition which is known to mammalian embryologists as entypy of the germ, and because of it the so-called inversion of the germ layers is brought about. It will be recalled that the embryonic

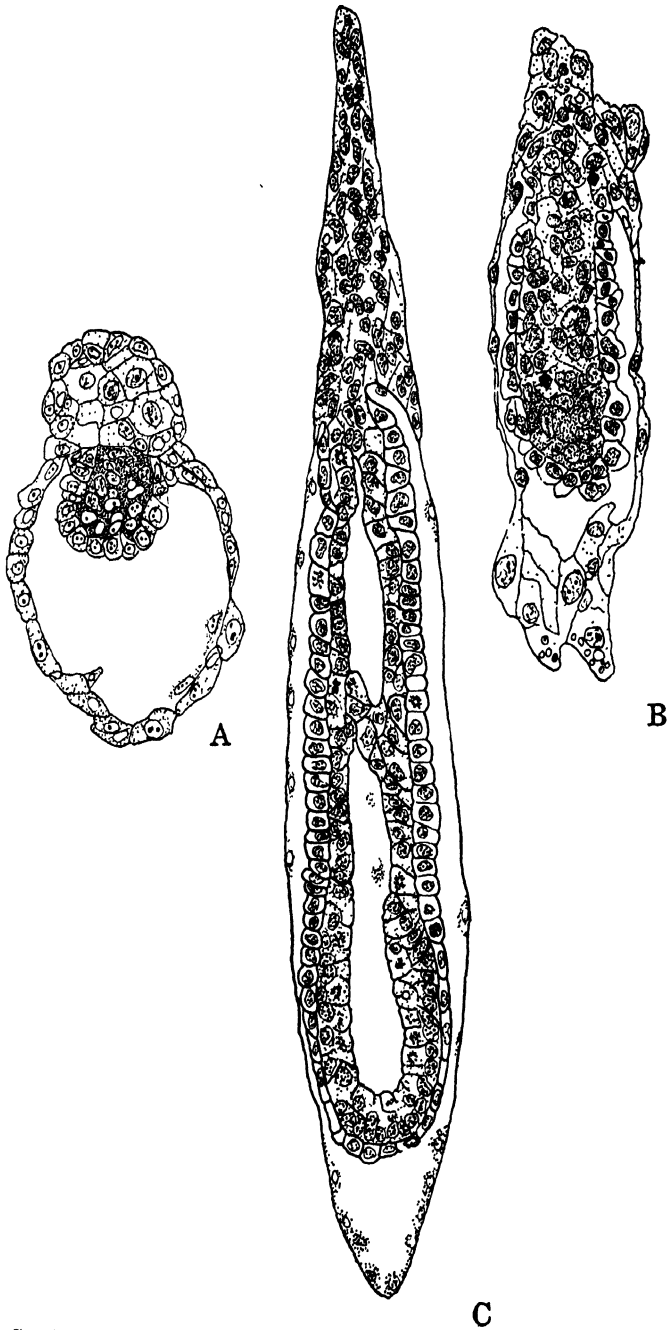


FIG. 143. Sections through the blastodermic vesicle and blastocyst of the albino rat. (After Huber.)

A, stage of 6 days 14 hours; B, 7 days; C, egg cylinder stage of 8 days.

knob may be likened to a hanging drop projecting within the cavity of the vesicle and that the lining of the cavity is endoderm, although this actually covers the outer or convex surface of the knob. Within the ectodermal portion of the knob where the cells are loosely arranged spaces now appear which gradually coalesce and they form the amniotic cavity. The ectodermal cells forming the roof of the cavity become the ectodermal layer of the amnion. The floor of the cavity on the other hand becomes the embryonic shield. The inner layer of the shield is thus ectodermal while the layer which for a time is actually the outer is endodermal. (Fig. 143.)

In some cases, of which the guinea pig and the mouse are examples, the embryonic knob moves gradually to a position well down inside the blastocyst. This process carries with it the lengthening of the endodermal layer on the outside. Earlier students of these forms removed the outer layers of the blastocyst and came upon the embryonic knob which they properly regarded as the formative area. Because of the removal of the outer layers, however, they completely misinterpreted the method of development and were surprised to discover this covering layer to be of endoderm. Hence they spoke of the inversion of the germ layers in these forms and the phrase is still retained in the literature of mammalian embryology, although of course it has no real significance as will be seen from the future development of the body form.

In some forms it happens that the trophoblast layer covering the embryonic knob becomes thickened forming a trophoblastic knob above the embryonic knob proper. This is the *Träger* of various writers on mammalian embryology. This may enlarge and in the forms just referred to accompanies the embryonic knob in its growth into the cavity of the vesicle. Within this trophoblastic knob a second cavity may occur called the false amniotic cavity. It has no relation to the true amnion, a completely closed vesicle, and presently disappears without further significance. In certain cases other spaces also develop as in the case of the interamniotic cavity between the cavities of the true and false amnions in the guinea pig. The false cavity may reach a relatively large size in the early stages while the true amniotic cavity remains quite small. Later on the relations are reversed.

5. Embryonic Shield

Regardless of the methods of formation of the amnion there arises in the development of all higher mammals a plate of cells from the lower cells of the embryonic knob in connection with the endoderm which lies just beneath it. This plate of cells is the embryonic shield and from its subsequent development the embryo proper is derived. Of

the entire blastocyst and all the structures connected with it, it is the only true embryonic portion.

In many respects the embryonic shield of the mammal is comparable in its developmental processes to the blastoderm of the chick or other discoidal type. Its development has been studied in a great variety of mammals including the bat, dog, mouse, rabbit, guinea pig, mole, hedgehog, pig, and of course man.

As one looks down upon the shield after the trophoblast has been removed from above, the similarity to the chick is so striking that a very brief description will be sufficient to make clear the main point in development. It consists of a layer of embryonic ectoderm three or four

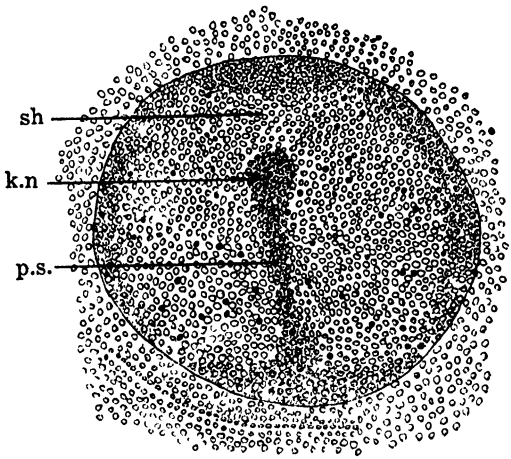


FIG. 144. Surface view of the embryonic shield of a dog, 13 to 15 days. (After Kellicott.) sh., embryonal shield; k.n., Henson's node; p.s., primitive streak.

cells thick under which is a single-celled layer of endoderm. This stage is reached during the seventh day in the rabbit and about the fourteenth in the dog. Then there occurs in the middle region of the disc a thickening and condensation of the cell layers to form Henson's node or the primitive knot, and extending backward from this is a rather broad line which disappears in the thicker cells of the posterior margin of the shield. Henson's node and this broad line are of course recognizable as the primitive streak, and shortly extending along its middle the primitive groove is to be discerned. In front of the node the mesoderm can be easily made out in sections appearing as a sheet between the ectoderm and the endoderm. This sheet early separates and, as has already been stated, its extension into the extraembryonic area participates in the formation of the serosa. It is supposed that the mesoderm takes

its origin from the region of Henson's node. From the primitive streak stage on, the arrangement of the layers and parts of the blastoderm are not different in any fundamental particulars from that already noted for the Sauropsida. This is to be expected from the relations of the yolk sac, as already remarked, and shows that the conditions found in the mammals represent a modification of the type of development where a large mass of yolk is present. The development of the body cavity, embryonic and extraembryonic, of the splanchnic and somatic layers of the mesoderm, appearance of the head fold, and the other characteristic features of the sauropsidan type of development have their counterparts here, and as it is not the purpose of this chapter to discuss mammalian embryology after the body form has been established, it seems unnecessary to go into these matters. For a discussion of them the reader is referred to the many available accounts particularly of human embryology.

Bibliographic Note

Among the more important accounts of the subjects contained in this chapter are the following: Caldwell, Gatenby; Hill, Hartman; Assheton, Jenkinson, W. Heape, Heuser and Streeter, Huber, Hubrecht, Patterson, Minot, B. M. Patten, Van Beneden, Wilson and Hill. These works are cited in full in the bibliography on page 406.

CHAPTER XI

EGG AND EMBRYONIC MEMBRANES

I. EGG MEMBRANES

The eggs of all animals are covered with one or more membranes and in these coverings there is a wide range of variation due largely to the environment and to the rate of development of the eggs. Those which are surrounded by water develop rapidly and are produced in great numbers, as are eggs of marine invertebrates, and are scantily provided with coverings. Those which take a long time for development, as birds' eggs, or those which are exposed to unfavorable conditions, as insect eggs, have more and thicker egg membranes.

Egg membranes are classified with reference to their origin. *Primary* egg membranes are formed by the egg itself, *secondary* by the follicle cells around the egg, and *tertiary* by the uterus or oviduct. All three types of membrane are sometimes found covering one egg, as in one of the cockroaches, *Periplaneta.*

If only one egg covering occurs, this can be secondary or tertiary, for example the egg of *Distoma* which is covered by a secretion from the oviduct. Only in exceptional cases are the egg membranes cellular as the zona radiata of *Taenia*, the spindle-like egg-shell of *Echinorhynchus*, the chitinous indented shell of *Hydra*, and the layers covering the ascidian egg. These membranes are really embryonic membranes. In the case of *Hydra* there are two membranes which are formed after cleavage from the ectodermal cells of the developing embryo.

The eggs of some animals have been described as naked. Although they may possess none of the three membranes described above as primary, secondary, or tertiary, still they are not actually naked. We now know that all eggs possess a cortical layer, the outer part of which has been called a plasma membrane. This structure is really a delicate surface layer of cytoplasm which cannot be distinguished from that which underlies it but which is physically different from it. The presence of the layer has been demonstrated by micro-dissection experiments (Chambers and others) which show that the surface of the egg is firm and somewhat elastic, offering resistance to mechanical injury. Plasmolysis experiments have shown that this layer is a semi-permeable

membrane and that it plays an important rôle in regulating the exchange which takes place between the cell and its surroundings.

In the animal kingdom as a whole there is an astonishing variability in the manner in which egg coverings appear. In sponges, the development of the egg within the adult body makes membranes unnecessary. They are lacking also in Hydrozoa, Siphonophora, and Anthozoa, although some coelenterates do have them. In some of the lamellibranchs, as *Dreissensia*, no membranes are present, and the eggs of some others, as *Mytilus*, have them in the beginning of their development and later

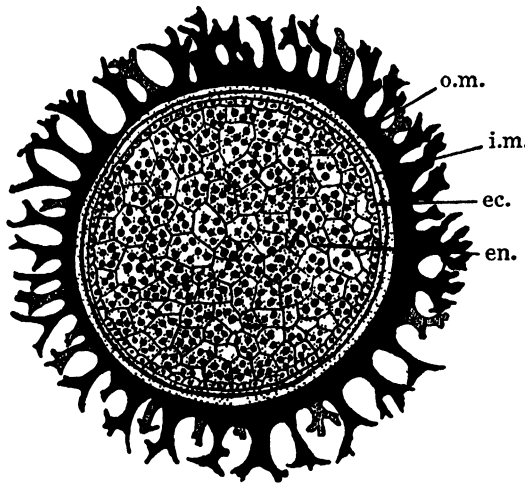


FIG 145 Cross section of egg of *Hydra grisea* (Redrawn from Korschelt and Heider, after Ehrenberg and Brauer)

ec, ectoderm, en, endoderm with yolk granules, i m, inner membrane, o m, outer membrane

throw them off. In some animals, as echinoderms, the eggs are without membranes when shed into the water but the fertilization membrane is formed as soon as the sperm enters the egg.

A. Primary Membranes

Vitelline Membrane. In many and perhaps in all eggs, there is a vitelline or fertilization membrane. This membrane is the most constant of the primary membranes, though it is often difficult to see before fertilization. Early workers had thought that the fertilization membrane was formed at the moment of fertilization, for, in many eggs, as for example in those of echinoderms, it separates from the egg immediately after fertilization. The presence of this membrane, often some distance from the egg, is an index of fertilization. Its origin is not clearly known.

It has been thought by some to represent a precipitation reaction, by others to be merely the outer layer of the egg which has been lifted away, or to be a substance produced at the time of fertilization. The separation of the vitelline membrane may be due to several causes, as follows: the egg itself may lose water and shrink, the membrane may swell, or the substance between the egg and membrane may absorb water. The space so formed between the egg and its fertilization membrane is the perivitelline space. (See Fig. 7) Its size varies considerably in different species.

In some eggs the vitelline membrane is apparently lacking and the egg is covered directly by a chorion or secondary membrane as in the cephalopods. In many eggs it is present before the sperm touches the egg, though the evidence that all eggs possess it is not conclusive. There are many vertebrates in which it has not been recognized, although for some representatives of nearly all classes of vertebrates it has been described. In these animals it is frequently termed the zona pellucida or, if it is traversed by pores, the zona radiata. It is possible that the vitelline membrane of the ripe vertebrate egg may be the result of the fusion of the primary and secondary membranes of an earlier stage.

Amphioxus is described as having a thin vitelline membrane formed near the end of the growth period. When the egg is shed into the water, another membrane, the perivitelline, is formed inside the first, a space intervening between the two. But immediately upon the entrance of the sperm, the

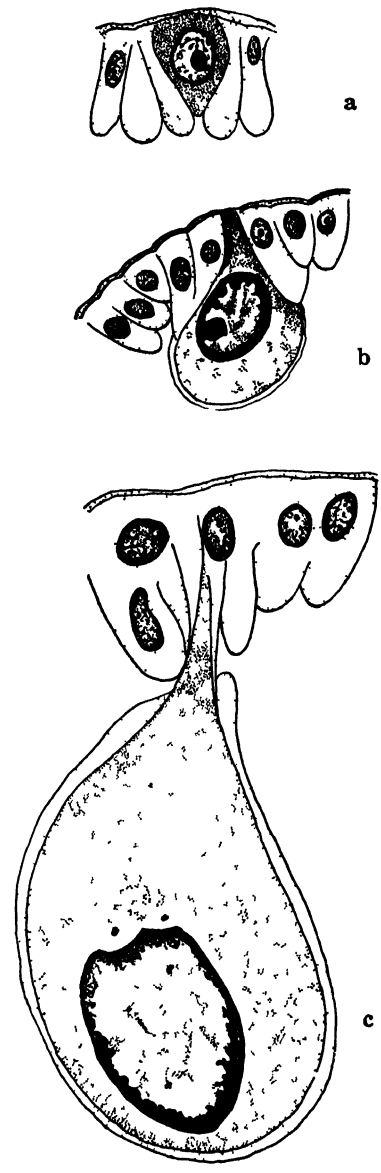


FIG 146 Successive stages in the development of the egg and vitelline membrane of *Cyclas* (After Stauffacher.)

inner membrane hardens and the two fuse. Thus is formed the fertilization membrane which is separated from the egg by the perivitelline space.

The vitelline membrane is usually structureless, transparent, and devoid of canals. It is found throughout all the animal phyla, although, it may be, not universally. Often it forms very quickly at the moment of fertilization as just mentioned, but there are cases in which it appears more gradually. In the latter instance, as in *Cyclas*, the attachment of the egg to the ovarian wall remains as the micropyle. A micropyle is not always present in the vitelline membrane, for sometimes it is unnecessary, since in some eggs fertilization may take place before its formation and in others it is porous and easily penetrable by the sperm. There are, however, some invertebrates, as cephalopods, in which the vitelline membrane is lacking.

The true vitelline membrane is a part of the egg itself, but it proves difficult in some eggs to determine whether the membrane which has been designated the vitelline is really primary or secondary. Confusion also seems to exist regarding the origin of the zona radiata, the membrane which lies next to the vitelline membrane. In some eggs, as in selachians and in *Petromyzon*, the zona radiata lies inside it, and in others, as in the teleosts, the zona radiata lies outside it. This membrane derives its name from the radiations which are seen in cross section and which in the vertebrates at least are due to fine canals.

To classify properly the vitelline membrane and zona radiata of a particular form as belonging to the primary membranes we must know their origin, and this may be very difficult to ascertain. A review of the text-books will show that these names have been given to diverse coverings in many cases without any knowledge of their origin. Hence it will be clear that the membranes as so named are not homologous in all forms. The question is not of great importance, but is considered only because of confusion in the terminology.

It is obvious that the egg itself, if not surrounded by follicle cells, must secrete its own membrane. However, if follicle cells are present they take a greater or less part in forming the coverings of the egg.

Zona Radiata. The zona radiata occurs in some echinoderms, worms, and molluscs, but there is much doubt as to its origin in these forms. In some cases it is evident that the membrane is a product of the egg itself.

Lillie has shown that the zona radiata in the *Nereis* egg is not a membrane at all but merely the outer layer of the egg protoplasm, and that it consists of closely crowded alveoli. "The zona radiata," he says, "is in fact a coarse emulsion or foam structure. The jelly is formed by the extrusion or the diffusion of the alveolar contents of the cortical

layer through the vitelline membrane." The zona radiata of *Nereis* thus secretes the jelly which surrounds the egg. The structures which seem to be radiations are the collapsed walls of the alveoli. The so-called zona radiata of *Nereis* is thus not homologous to the membrane of that name in other forms.

The zona radiata has a wide distribution among vertebrate eggs and is found in all classes. It is in many cases doubtful whether this layer is formed from the outer part of the egg or from the follicle epithelium. In cases where there are two or more egg coverings it probably arises from the egg itself. When the zona radiata arises later than the egg membrane outside it, it must be formed from the egg, as Eigenmann has shown for *Fundulus*.

The purpose of the zona radiata in many vertebrates is clear. Through the delicate canals of this layer, protoplasmic processes of the follicle cells penetrate. Nutrient substances from these cells are passed through the processes to the egg. The purpose of the zona radiata is thus that of a nourishing membrane. In the vertebrates it is of considerable thickness during the development of the egg. After the growth of the egg the layer often diminishes greatly in size.

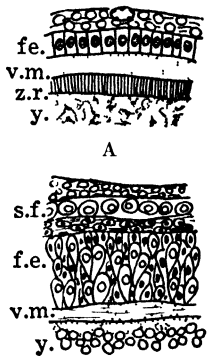


FIG 147. A portion of the egg follicle and surface layer of the egg of *Scyllium canicula*. (Redrawn from Korschelt and Heider, after Balfour.)

f.e., follicular epithelium; s.f., secondary follicular epithelium; v.m., vitelline membrane; y., yolk; z.r., zona radiata.

In the shark's egg (Balfour, Giacomina) the zona radiata develops inside the vitelline membrane which is here a true primary membrane, being formed before the follicle cells themselves. The zona in this form is a provisional egg covering for the development of the egg in the ovary. When the egg development is complete, it becomes a thin membrane.

Contrasted with the provisional zona radiata of the selachians is the permanent layer of that name of cyclostomes, teleosts, and ganoids. In these fishes the zona radiata is often divided into two layers or at least there are two radially striped layers which have the same origin. In some of the fishes as *Perca* there is a gelatinous layer outside of it through which run cell processes that connect it with the follicle cells. This and other similar structures frequently present on the surface of the teleost egg would seem to be chorionic membranes. However, Eigenmann has shown that in *Fundulus* they are derived from the egg itself.

Although the origin of the zona radiata from the egg has been determined for the selachians and for *Fundulus*, more work must be done

to ascertain what part the follicle cells may have in the formation of this layer in other fishes.

In the bony fishes, since the egg membrane, which is often sticky, is present before fertilization, the micropyle must be formed in a different manner from that mentioned above for many of the invertebrates. Eigenmann has described its formation in *Pygosteus* in which each micropyle is due to a long process of one of the follicle cells that reaches to the egg and passes through the zona radiata.

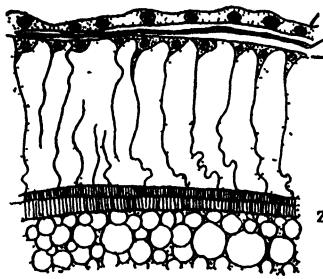


FIG 148 Section through the follicle and outer layers of the egg of *Perca fluvatilis* (After Korschelt and Heider)

f c, follicular epithelium, g, gelatinous layer, ov, ovarial epithelium; th f, theca folliculi, y, yolk, z r, zona radiata.

The eggs of amphibians and reptiles have a vitelline membrane which later forms a zona radiata. The formation of this layer has been clearly described by Retzius for one of the lizards, *Lacerta viridis*. In this form the early follicle is one-layered. In it cells of two sizes may be distinguished, large cells, the centers of which are filled with round nuclei, and smaller cells with oval nuclei. The egg itself has a delicate surface layer, the first anlage of the zona radiata. This membrane, as in the selachians and *Fundulus*, is formed from the egg itself. During the growth of the egg the zona radiata grows thicker, the large follicle cells become pear shaped and send processes to the egg. Some of the cells remain round and lodge between the stalks of the pear-shaped cells. The small cells take up a more distal position, and meanwhile the processes of the larger ones become branched to penetrate the plasma of the egg. The follicle now thickens and the zona takes on its characteristic radiate structure. Finally the follicle cells diminish in size and the follicle itself is thinner. The nuclei of the large cells lose much of their chromatin as the cells are stretched more closely over the egg. The zona radiata, in which two layers can now be distinguished, is likewise thinner. The nourishing function of the follicle is thus clear, for it is evident that substances are passed to the egg through the long processes of the follicle cells which penetrate the zona radiata. It is seldom that the ormation of the membrane is as clear as in this form. (Fig. 150.)

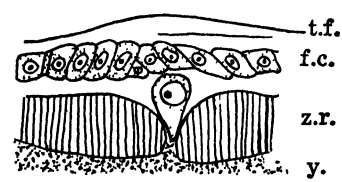


FIG 149 Micropyle formation in the teleost *Pygosteus pungitius* (After Eigenmann)

f c, follicular epithelium, t f, theca folliculi, y, yolk, z.r, zona radiata.

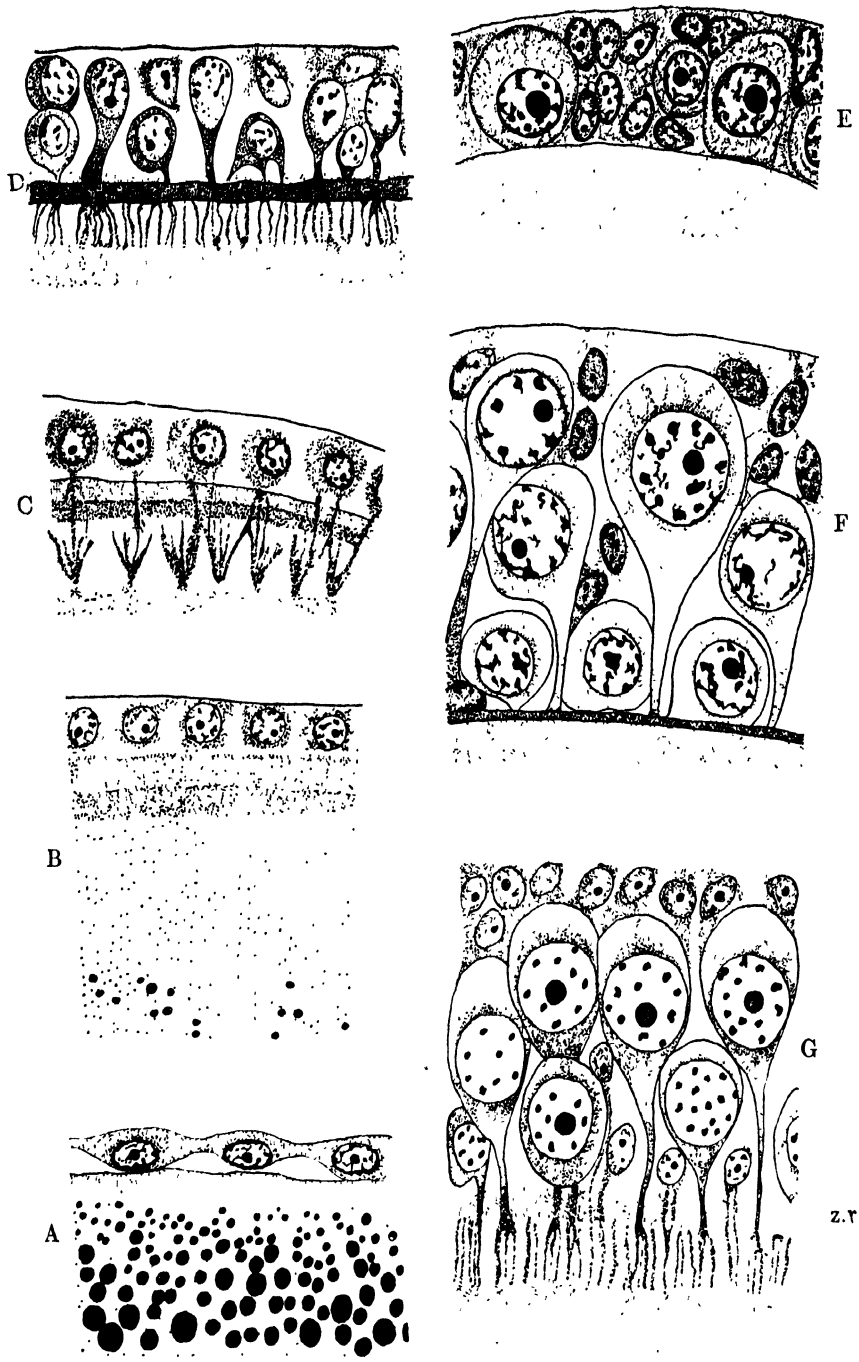


FIG. 150. The origin of the egg membranes of *Lacerta viridis*. (Redrawn from Buchner, after Retzius.) For description, see text.

In the bird's egg the primordial follicle contains the ovum surrounded by a one-celled layer of cubical follicle cells, the granulosa. The zona radiata is later formed between the ovum and the follicle cells and there has been considerable discussion as to its origin. Bartelmez thinks that it is formed from the follicle cells; at any rate it has a function similar to that described for the reptiles. The follicular cells are connected to the ovum by delicate strands of protoplasm which pass through the zona radiata. Nutriment diffuses through these protoplasmic bridges, there being no evidence of the passage of solid food particles.

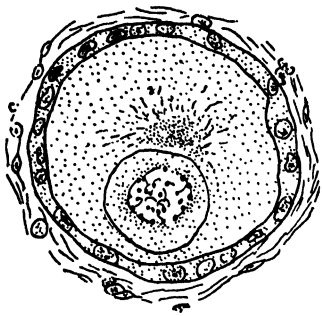


FIG. 151. Primordial egg follicle from ovary of hen. (Redrawn from Lillie, after Holl.)

In the bird the primary vitelline membrane formed immediately

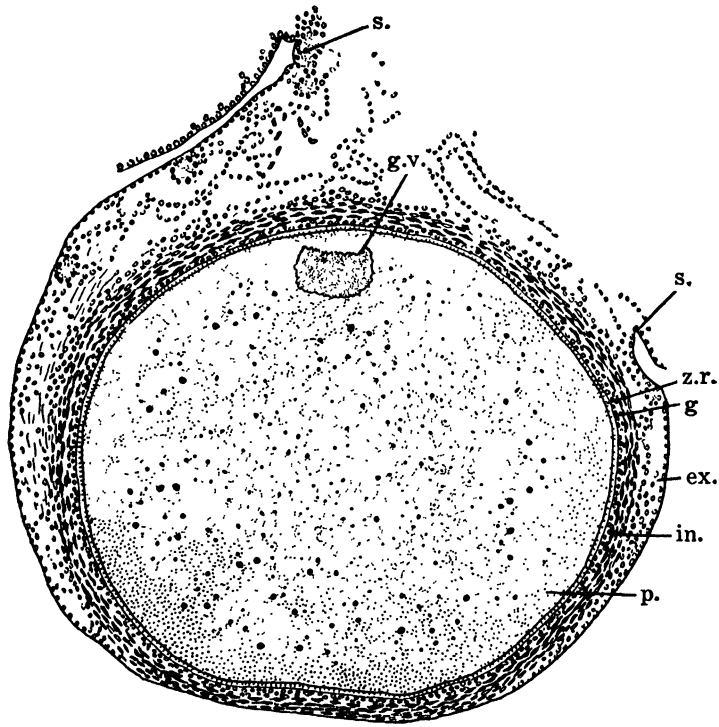


FIG. 152. Section through an ovarian egg and follicle of the egg of a pigeon. (Redrawn from Lillie.)

ex., theca externa; g., granulosa; g.v., germinal vesicle; in., theca interna; p., peripheral protoplasm; s–s, stalk of the follicle; z.r., zona radiata.

over the egg as in other animals is a structureless membrane usually considered as from the egg itself, though some workers have thought it to be a product of the follicle cells. It is very difficult to differentiate the vitelline membrane from the zona radiata and the former may be in part the anlage of the latter, as in the case of *Lacerta* which we have just described.

In mammals, the origin of the vitelline membrane is uncertain. Some authors say that there is no true vitelline membrane; the transparent zona pellucida next the egg is thus referred to by others. However, if

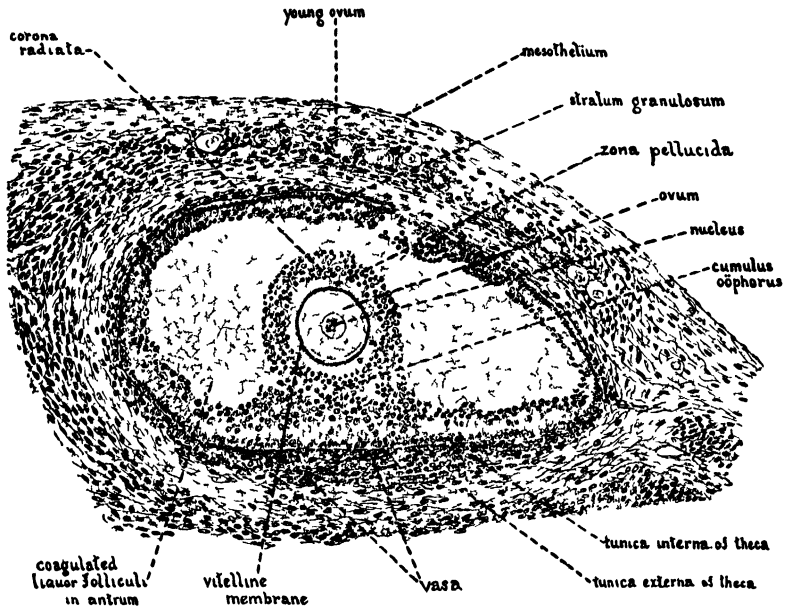


FIG 153 An ovarian follicle approaching maturity (From Patten)

this layer is secreted by the follicle cells, as some workers believe, it is of course a secondary membrane. The zona pellucida may be fairly thick, and in the mole, pig, rabbit, and sheep it takes on a radiate appearance and hence becomes a zona radiata.

The term zona radiata is unfortunate in the mammals, for outside this structure is a cellular layer of radially elongated follicle cells called the corona radiata and confusion is apt to exist between these two.

B. Secondary Membranes

Secondary membranes are formed by the follicle cells which surround the ovum and are therefore the product of maternal tissue. These membranes, which are truly chorionic in nature, may take on a variety of

forms, depending upon the activity of the follicle cells. The distribution of the chorion is more restricted than that of the vitelline membrane, but some eggs which lack the latter possess a chorion. It may arise as a cuticular secretion of the follicle cells forming a delicate membrane which later thickens to assume the character of a chitinous-like shell, as in the insect egg. On the other hand, it may be formed by direct transformation of the follicle cells themselves. Its substance is, according to Tichomirow, chemically like keratin and much richer in nitrogen than is chitin.

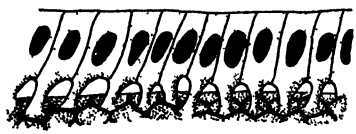


FIG 154 A portion of the follicular epithelium with the chorion of *Dectícus bicolor* (Redrawn from Korschelt and Heider)

Both primary and secondary membranes are often present before fertilization takes place, and since they are usually impenetrable to the sperm they are perforated by one or more micropyles situated at or near one of the poles of the egg. In nemerteans and in most molluscs the micropyle is near the lower pole, but in cephalopods and also in echinoderms, insects, and fishes, it is near the upper pole. In the Orthoptera it may be either at the lower pole or at the side of the egg, and in *Oedipoda* there is a circle of 30 to 40 micropyles at the lower pole.

The *chorion* of insect eggs is very different in different species. In the simplest cases it is smooth, one-layered, and structureless. Its surface may have elevations or may be divided into polygonal areas so that it looks like an epithelium. In certain forms, as in the locust, the follicle cells send out processes to these polygonal areas of the chorion around which a shell is secreted. After formation of the shell, the cell processes are withdrawn.

Porous canals through which the egg obtains air often penetrate the chorion. In *Ranatra* and *Nepa*, water scorpions, in which the eggs are sunken into plant stalks, the chorion is drawn out at the end of the egg into delicate processes which serve to aereate the egg.

FIG 155 An egg of *Nepa cinerea* possessing seven processes which serve as air tubes (Redrawn from Korschelt and Heider)

The micropyle is formed in a characteristic manner in insects as has been described in the locust *Meconema varians*. One of the follicle cells sends a long conical process down to the egg. This process remains during the secretion of the chorion and then is drawn back into the follicle. The chorionic pores referred to are formed in the same way. Also the micropyle of the bony fishes is formed in a

similar manner, but as has been earlier mentioned, the membrane perforated in this case is a zona radiata and not a chorion. (See Fig. 191.)

In *Octopus* the chorion is drawn out into a stalk which becomes twisted and serves to anchor the eggs. Among the chitons there are different types of chorions. Some of the eggs have a knobbed surface and some are covered with needle-like processes. Many actinian eggs also have sharp processes.

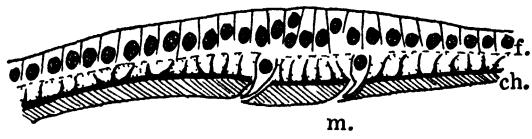


FIG. 156. The egg membranes of *Meconema varians*. (From Korschelt and Heider.) ch., chorion; f., follicular epithelium; m., micropyle.

The myxinoids have a characteristic shell. At each pole of the egg are attached stiff anchoring filaments which end in lobed umbrella-like expansions. At the germinal pole of the egg the shell is marked by a furrow-like ring so that a lid is formed which the animal pushes off at the time of hatching. There is considerable disagreement about the

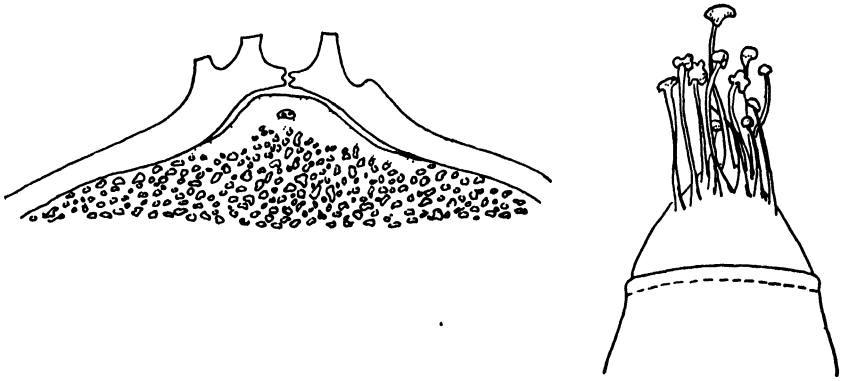


FIG. 157. The upper part of the egg of *Bdellostoma stouti*. (Redrawn from Ziegler, after Doflein.)

A, section showing nucleus and above it the micropyle between the bases of the processes. B, Operculum and processes.

origin of this shell. Cunningham says that it is the product of the egg itself, and Doflein derives it from the follicle cells. The membrane is usually considered as a true chorion.

In *Petromyzon* the inner egg membrane is covered with a mucous layer which causes the egg to hang onto stones. This membrane is formed through transformation of the follicle cells themselves. In some

of the ganoids also there is a similar adhesive membrane formed from the follicle cells. Under this layer are two membranes, the chorion and the vitelline membrane.

The follicle cells of the ascidians show a special type of modification. Five envelopes are formed by these cells and thus we have a unique example of an egg covered by five secondary membranes. When the ascidian egg is still young it is surrounded by a primary follicular epithelium derived from undifferentiated ovarian cells. Beneath this epithelium is a band of structureless membrane. These flat epithelial cells multiply and become cubical. Some of them find their way inside the epithelium and form an inner layer called the test-cell layer while certain of them wander into the egg. The cells of this layer do not give rise to the test of the adult ascidian as was formerly supposed to be the case. As the egg matures, these test cells degenerate and later are found in a gelatinous mass over the egg.

In the solitary ascidians the follicle layer of the egg takes on a special character. These cells, instead of remaining cubical, increase in size and become highly vacuolated, resembling a foam structure. They also develop processes which support the egg and enable it to float.

In addition to the follicular membrane and the test-cell layer, two other membranes are formed around the ascidian egg. Beneath the two layers already described, the follicle cells secrete a structureless membrane called the chorion. Still another membrane is formed between the basement membrane and the follicle cells. This is the external pavement epithelium and it may be regarded as another layer of follicle cells. There are thus five membranes over the ripe ascidian egg. Next the egg is the gelatinous layer of test cells, and over this are the following layers, the chorion, the foam-like follicle epithelium, the external pavement epithelium, and the basal, follicle membrane. Since all these membranes are derived from the follicle cells they are secondary membranes.

In the Dipnoi the primary membranes are covered by the gelatinous tertiary membranes, and secondary membranes are absent. In the frog's egg there is a thin but tough membrane around the vitelline membrane and this is secreted by the follicle cells. Over this is the jelly formed by the oviduct, so the frog egg has all three types of membranes. In the urodeles this secondary membrane appears to be lacking. In reptiles and birds the chorion seems to be entirely lacking, for the egg white and shell are tertiary membranes.

From the descriptions just given it will be seen that the chorion has a variety of forms and that its special characteristics are adaptations to the needs of the different species.

Comparable to the chorion in a certain sense is the mammalian

follicle which is here described in some detail. Although of course it cannot be considered as a membrane, still it is formed by the follicle cells. As described in an earlier chapter, the mammalian ovum is highly specialized because of the intra-uterine development of the embryo. There is only a very small amount of yolk present in the egg as contrasted with the large amount in the eggs of birds and reptiles. Secondary and tertiary membranes are entirely lacking, but the Graafian follicle

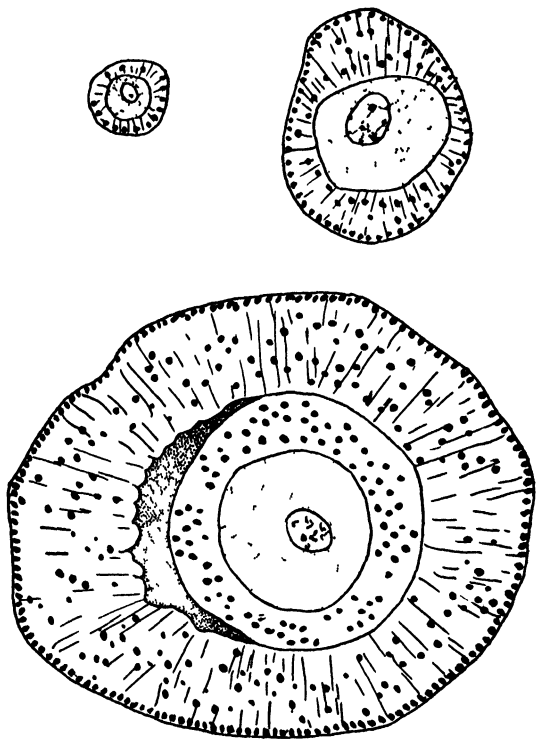


FIG 158 The developing ovum and follicle cells of the cat (Redrawn from Buchner) See text for description.

which surrounds the egg is a product of the follicle cells. (Fig. 153.) In earlier times mammals had eggs similar to those of reptiles and birds, for the monotremes, the simplest mammals, possess an egg with shell and egg albumen. In the entire animal kingdom the Graafian follicle is probably the most specialized structure produced as a modification of the follicle cells. In the middle of the follicle is a cavity filled with liquid, known as the liquor folliculi. The ovum itself, surrounded by the zona pellucida and the zona radiata, is attached to the wall of the Graafian follicle by a group of cells known as the discus proligerus. The cells of the discus

are continued into the cells of the follicle wall known as the membrana granulosa, which consists of many layers. Outside the membrana granulosa the ovarian connective tissue is differentiated into two investing layers, the theca folliculi. The outer of these is densely fibrous, and the inner cellular, penetrated by small blood vessels. Buchner describes the growth of the Graafian follicle in the cat (Fig. 158), where it begins as a simple follicle in which the ovum is surrounded by a cellular layer, the granulosa. The cells about the ovum multiply rapidly, and part of them lose their connection with the upper surface of the egg to wander out to form a second loose ring around it. The rings separate sharply from each other and each becomes many-layered, while a cavity known as the antrum appears between them. This cavity becomes filled with a liquid which is secreted into it and quickly enlarges. The outer ring of follicle cells is crowded to the periphery and forms the stratum granulosum. The ovum itself at first bulges slightly into the cavity of the follicle, and later as its surrounding cells are undercut protrudes farther into it, its attachment to the follicle being now a delicate stalk of cells, the discus proligerus. When the egg is ready to escape, this narrow stalk is broken, and the follicle ruptures, the egg passes through the tube into the uterus. The follicle now undergoes a transformation and becomes the corpus luteum. Sometimes the follicle cells immediately around the egg are found clinging to it after its discharge.

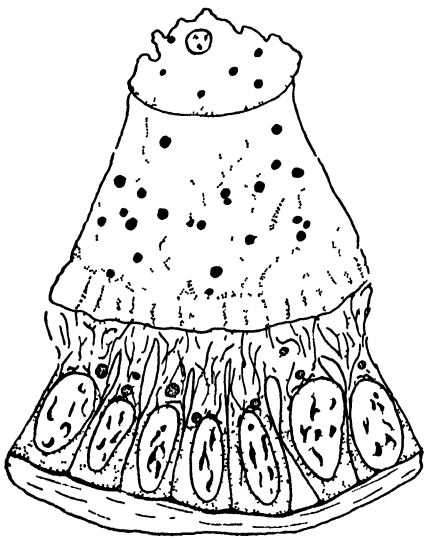


FIG. 159. Follicle and portion of cytoplasm and nucleus of ovum of a rabbit. (Redrawn from Buchner.)

The mammalian ovum and its surrounding follicle are thus seen to be highly specialized. The presence of only a very small amount of yolk and the preparation for the attachment of the ovum to the uterus are all adjustments to the intra-uterine development of the embryo. The rôle of the follicle cells as a nourishing membrane has been definitely determined in the dog by experiment. The follicle cells are filled with mitochondria. When lecithin is injected into a dog these mitochondria increase in number. However, if the dog is allowed to become hungry, the mitochondria decrease. They seem to act as store houses for the

developing egg. The identification of lecithin or of a similar substance has been observed by Russo in the protoplasmic bridges which penetrate the zona radiata.

C. Tertiary Membranes

Tertiary membranes, unlike primary and secondary, are formed after the egg has left the ovary. They are secreted by the oviduct or uterus or by special glands connected with them. Tertiary membranes are of various kinds, gelatinous, horny and chitinous, or cocoon-like. The type of membrane shows adaptive correlation to the needs of the developing embryo. The gelatinous type of covering is frequently seen in fishes and in amphibians and also in many invertebrates, as *Geryonia*, and *Sagitta*. Chitinous shells cover the eggs of the phylopods, *Apus* and *Branchipus*, and a horny capsule covers the shark's egg. Under the tertiary membranes there may be a primary or secondary membrane or both for in some eggs all three types are present.

In the cephalopods the tertiary membranes show different modifications. The egg of *Sepia* and its surrounding albumen is covered by a black leathery capsule, but in *Loligo* the eggs, each in a firm envelope, are arranged in a gelatinous mass known as "dead men's fingers." The flatworms have cocoons which are formed in the ovary. There are various kinds. *Branchiobdella* has a stalked capsule containing one egg. The rhabdocoele turbellarian cocoon contains several eggs. In this capsule and in that of the triclad *Dendrocoelum* there are a great number of yolk cells included beside the egg and these serve to nourish it. The trematode capsule contains one egg and is either stalked or provided with a lid or both may be present. The polyclad capsule is similar to that of the trematode but there are several eggs in one cell.

Cocoons are sometimes formed by special skin glands. Thus in the lumbricids and in the Hirudinea the cocoon which has a ring-like form is secreted by a special skin gland and drawn off over the anterior end of the worm. In it is deposited albumen for the nourishment of the embryo.

In the gastropods the cocoon is large though the eggs themselves are small, for they are surrounded by albumen. In *Limax*, *Helix*, and *Paludina* there is only one egg in a cocoon. In some gastropods only one egg is deposited but in other cases there are a number, all but one of which are utilized to contribute to the growth of the embryo formed from that one. (This is the phenomenon called by some "cannibalism.") Some insects also have cocoons which are tertiary membranes as contrasted with the larval cocoon formed for the pupa of the butterfly. *Periplaneta* has a cocoon which is carried by the female attached to the

posterior end of the abdomen. In this cocoon the eggs are laid each enclosed in the vitelline membrane and the chorion. *Hydrophilus*, the water beetle, has a round cocoon in the form of a web. The female places the cocoon on the under side of a leaf to which it is attached by a stalk. There are forty-five to fifty eggs in palisade-like arrangement within the cocoon nourished from the substance of the web.

The selachians, when not viviparous, have tough egg membranes. The albumen and the horny capsule are deposited about the egg while it is in the oviduct. The capsule differs in different species. In the sharks its four corners are drawn out into fibers which permit the egg to cling to water plants. Occasionally several eggs are found in one capsule.

Water-living animals frequently have eggs with gelatinous coverings. They are laid singly in the urodeles and in *Ceratodus*. In many other animals, however, the eggs are laid in gelatinous masses of varying sizes which may be irregular (cephalopods and frogs), plate-like (some polyclads, gastropods and insects), or rope-like (some nemerteans and annelids).

A gelatinous covering over the egg is typical of the amphibia. In many of the amphibia there are special modifications of these tertiary envelopes. An interesting example of this kind is that of a tropical anuran. At the time of egg laying the oviducal secretion which surrounds the eggs is beaten up by the hind legs of the parents into a fine foam containing many air bubbles. This foam is sometimes placed on the surface of the pond and floats about or it may also be placed in a wet hollow on the ground. Some of the frogs deposit the eggs in a mass of jelly on one or more leaves. Sometimes the single leaf is bent by the copulating frogs into a funnel which retains its shape by means of the adhesive jelly around the eggs. Some of the Dipnoi (*Ceradotus* and *Lepidosiren*) have a gelatinous tertiary envelope similar to that of the amphibians. In *Lepidosiren* this layer may be very thin or very well developed.

In birds there are a series of tertiary membranes. The hen's egg may be described as typical. Immediately around the yolk is deposited a spirally wound layer of egg albumen, the edges of which are twisted into the chalazae, stretching from the yolk to the poles of the egg. About this layer is deposited another layer of egg albumen and over the entire mass the shell is formed. The shell membrane is double and the two layers are separated at the blunt end of the egg to form an air chamber. The shell membrane lies immediately below the shell, which is porous and brittle and impregnated by calcium salts, even to the extent of 98 per cent of the substance. The tertiary membranes of birds are in general like those of the hen's egg, the differences which occur being due to

size, shape, and color of the shell. The egg that is relatively largest is that of *Apteryx*, for in this species it weighs one-fourth of the body weight of the parent.

The eggs of reptiles are much like those of birds, but the chalazae are lacking. In some forms, as in the lizards and snakes, the shells are more like parchment, little calcareous matter being deposited. The egg-shells of crocodiles and of some turtles are hard like those of birds.

Tertiary membranes are entirely lacking in the mammals with the exception of the primitive monotremes. The egg of *Echidna* is much like a bird's egg but the egg-shell is formed of keratin and not of calcareous salts. The mammals have adapted themselves to a terrestrial life by the development of a series of embryonic membranes, as the birds and reptiles have done with egg membranes, but in the higher group it has been found necessary to forsake entirely the devices which were adequate for the lower forms. The membranes of the embryos are considered in the second part of this chapter.

II. EMBRYONIC MEMBRANES

A. Invertebrates

In addition to the membranes which properly are classed with the egg itself the animal kingdom possesses numerous cases of accessory membranes which are developed by the embryo. These are in the nature of cellular sheets which by various invaginations, foldings, outpushings, etc., cover the embryo or parts of it. They are especially well developed in the vertebrates and to that group most of the attention is paid by embryologists. Nevertheless, among the invertebrates there are a good many illustrations of the development of different embryonic membranes. Most common are the amnion and the membrane which is formed in connection with it, the serosa. But there is also an invertebrate yolk sac, surrounded by a yolk membrane, and even a placenta occurs in a prevertebrate group, the ascidians of the family Salpidae. Of course this latter structure is very rudimentary. It is really nothing but a tubercle or knob of rather specialized maternal tissue which becomes richly vascular and to which the zooid already budded off attaches itself for nourishment.

Of the invertebrates proper certain molluscs show the development of the yolk sac. The amnion occurs more or less developed in the pilidium larva of the nemerteans, in the developing *Polygordius*, in the echinopluteus when it is undergoing a metamorphosis, in the larva of insects and of the scorpions. Some of these groups also show the possession of a serosa in connection with the amnion.

The Yolk Sac

The true yolk sac can occur only in forms having an extreme telolecithal type of egg structure. Among the invertebrates the cephalopods

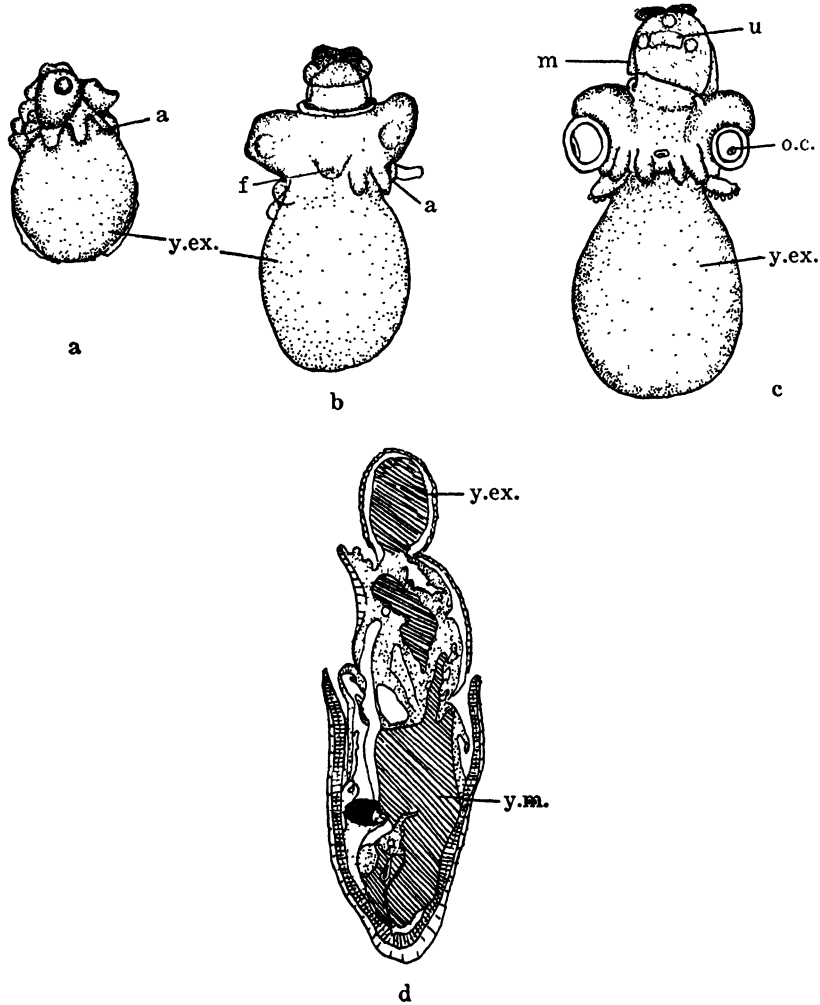


FIG. 160. Various stages in the development of a squid, showing the relations of the yolk sac. (Modified from Harman and Gardiner.)

B and C, dorsal views; D, sagittal section. a., arm rudiment; f., funnel; m., mouth; o.c., optic cup; y.ex., external yolk; y.in., internal yolk.

are the only forms which show a well-developed yolk sac. As is described in Chapter V, the cleavage of the protoplasmic cap of the cephalopod

egg results in the formation of a series of blastoccnes arranged about a germinal area consisting of blastomeres. These blastocones separate entirely from the blastoderm and rapidly extend themselves over the surface of the yolk underneath the growing blastoderm. They constitute a yolk membrane enclosing the large mass of yolk. Over them the blastoderm gradually extends and when the embryo proper is formed it becomes marked off by a groove from the yolk sac, as it may now be properly called. The yolk sac takes on the appearance of an appendage which it maintains until the development of the embryo is far advanced. Actually the yolk mass becomes divided into two portions, one of which is contained within the body of the embryo and constitutes the internal yolk sac in contrast with the external one which has just been mentioned. The yolk masses of the two are joined at all times, however. The further growth of the embryo results in proportionate decrease in the size of the external yolk sac which is gradually included within the body of the young animal. Morphologically this structure represents a precocious median development of the mass of tissue which produces the arms of the squid and is comparable to the developing foot of other molluscs. Strictly speaking it is not merely an embryonic appendage, for all of it is finally withdrawn into the body of the growing animal. A somewhat similar ventral protrusion from the foot of the snail, *Helix*, was described by Lankester and while not a true yolk sac it is indicative of the tendency so markedly illustrated by the squid.

The Amnion

The first structure in the animal kingdom which may be designated as an amnion is seen in the metamorphosis of the pilidium larva of the nemerteans which is described on page 153 (Figs. 103 and 104). On the flattened under surface of the helmet-like pilidium, four amniotic invaginations form. They consist of ciliated ectoderm and other deeper parts and are known as the imaginal discs. Their continued inward growth upward and over the alimentary tract finally results in their fusion. The inner parts, or the imaginal discs, form the outer covering of the little worms while the remaining portions of the coalesced invagination make up the amnion which is a temporary covering entirely lost with metamorphosis.

The second appearance of the amnion is seen in the metamorphosis of the annelid, *Polygordius*. This structure superficially resembles that of the pilidium, for here also four invaginations appear arising from a circular groove which is known as the amniotic fold. It serves as a covering for the folded portion of the worm which remains in this con-

dition until the time of metamorphosis when, by severe contraction, the worm becomes straightened out and the amnion has no further function.

An amniotic invagination is described in the metamorphosis of the echinopluteus of the sea-urchin. It occurs on the left side of the larva as a pit which deepens and broadens until it comes in contact with the wall of the hydrocoele. The tissue which lies between the amniotic in-

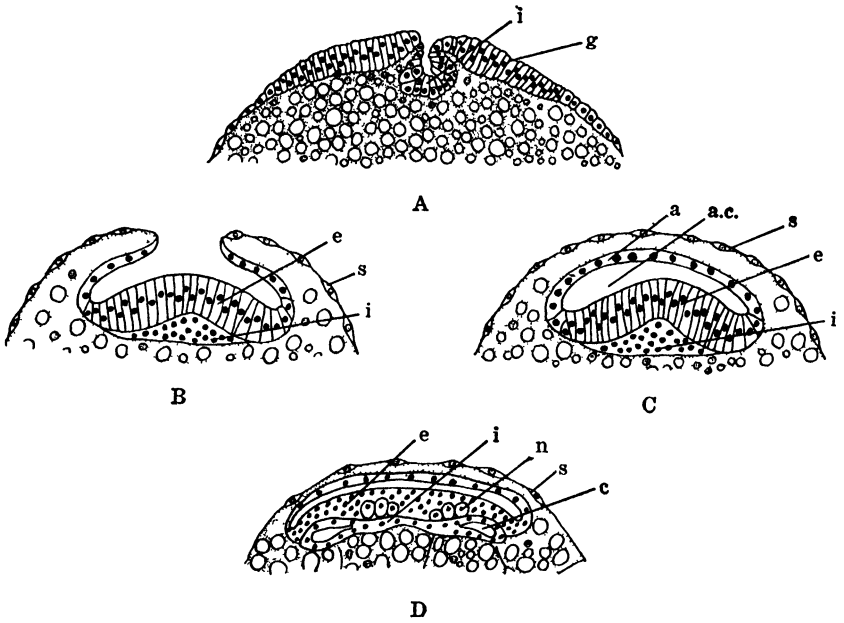


FIG. 161. Transverse sections of embryos of *Clytia*. (Redrawn from Folsom after Lecaillon.)

A, through germ band at gastrulation; B, through germ layers and amniotic folds; C, stage later than B; D, through germ layers. a., amnion; a.c., amniotic cavity; c., coelome sac; e., ectoderm; i., inner layer; g., germ band; n., neuroblasts (primitive nervous cells); s., serosa.

vagination and the hydrocoele undergoes important changes and is involved in the formation of the so-called echinus rudiment. Into the amniotic cavity this rudiment and especially the tube feet develop.

In the scorpion, according to Brauer, an amnion is formed shortly after the two-layered stage shown in Fig. 39. At the edge of the blastoderm a sheet of cells arises and grows backward and presently a second layer is formed over the first. The outer is the serosa and the inner the amnion. Underneath these the embryo develops.

The amnion of invertebrates reaches its highest development among the insects, and of these the Coleoptera show it most clearly. There are

really two types of development with respect to the relations of formative area and of yolk shown in the insects. In the one type, called by some "overgrown," the germ band retains its original position in the blastoderm and the folds of the amnion and serosa arise at its sides and

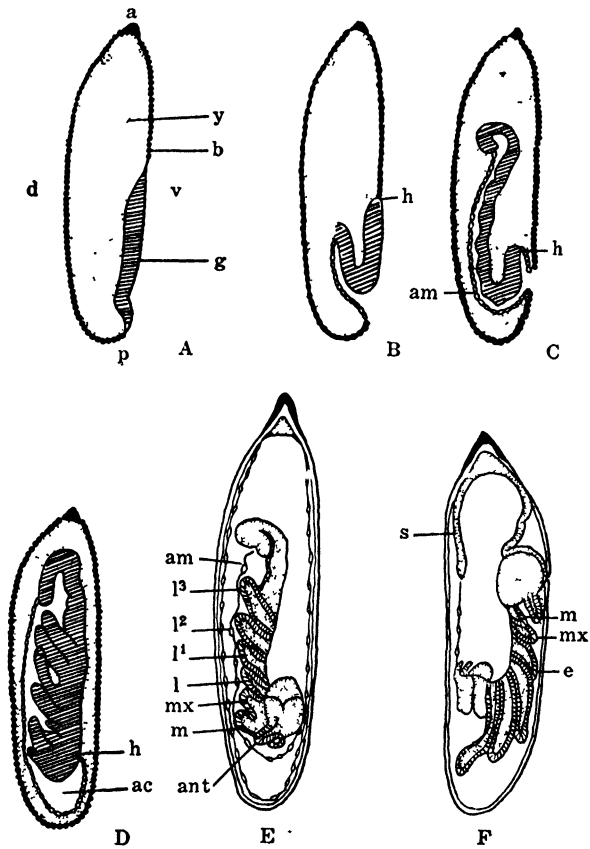


FIG. 162. Diagrammatic sagittal sections of *Calopteryx*. (From Folsom, after Brandt.)

A-D, illustrate invagination of germ band; E-F, illustrate revolution of embryo. a., anterior pole; a.c., amniotic cavity; am., amnion; ant., antenna; b., blastoderm, d., dorsal; g., germ band; h., head end of germ band; l., labrium, l^1-l^3, thoracic legs; m., mandible; mx., maxilla; p., posterior pole; s., serosa; v., ventral; y., yolk.

grow over it. This occurs in the Coleoptera, and it is for this reason that the formation of the amnion is most clearly shown there. In the other type, called "invaginated," the germ band invaginates at one end of the egg, and is finally carried into a cavity formed within the yolk mass; after the closure of the opening of the invagination the formative portion develops entirely within the egg. Due to this invagination the ventral

surface of the developing embryo comes to face the dorsal side of the egg and a subsequent process of revolution is necessary before the embryo at length resumes its original orientation. This type occurs in the Odonata. The relations of the folds of the amnion are shown in Fig. 116, which illustrates the development of *Hydrophilus*, and also in Fig. 161, which shows the formation of the amnion in the beetle *Clytra*. Folds of the blastoderm arise at the side of the germ band and continue their growth until they meet above it and fuse. The membrane thus formed directly above the germ band is the amnion, and the outer one, the serosa. The formation of the amnion and serosa in *Calopteryx* is shown in Fig. 162.

Students of the embryology of the insects are not entirely in agreement in regard to the morphological relations of the embryonic membranes found throughout the group and several types of classification have been proposed. It hardly seems necessary to consider the various classifications or the points upon which they have been based. Serosa and amnion occur together in the Coleoptera, Lepidoptera, Diptera, Dermoptera, Isoptera, and Odonata. An amnion only is found in *Xenos*, a strepsipteran parasite, and even it is vestigial in such forms as *Musca* and *Drosophila*. A serosa only is formed in a few of the Hymenoptera including the honey bee, wasps, and ants.

B. Vertebrates Except Mammals

Embryonic membranes are especially well developed in the vertebrates where they serve for protection, nutrition, and respiration. These are the yolk sac, amnion, serosa or chorion, allantois, and its derivative, the placenta. Since the name "chorion" is also correctly used for the secondary membrane secreted by the follicle cells, it is confusing to use this term for an embryonic membrane of different origin. We shall therefore always designate this embryonic membrane as serosa although it is quite customary among mammalian embryologists to use "chorion" in both senses.

For an introduction to the nature of the embryonic membranes of the vertebrates and of the parts they play, the conditions as found in the chick may be taken as a type. Of course these conditions apply particularly to the Sauropsida; however, they may be looked upon as providing a point of departure for the entire study. They have often been described, so a brief reference here is all that is necessary. The first membrane to appear in the chick is the yolk sac. As the blastoderm grows over the yolk its lower edge consists after gastrulation of primary ectoderm. At an early stage the endoderm and later the mesoderm may be seen back of the edge gradually making their way over the yolk. The

mesoderm divides into two layers, and the investments of the yolk are first the splanchnopleure (endoderm and mesoderm), then the extra-

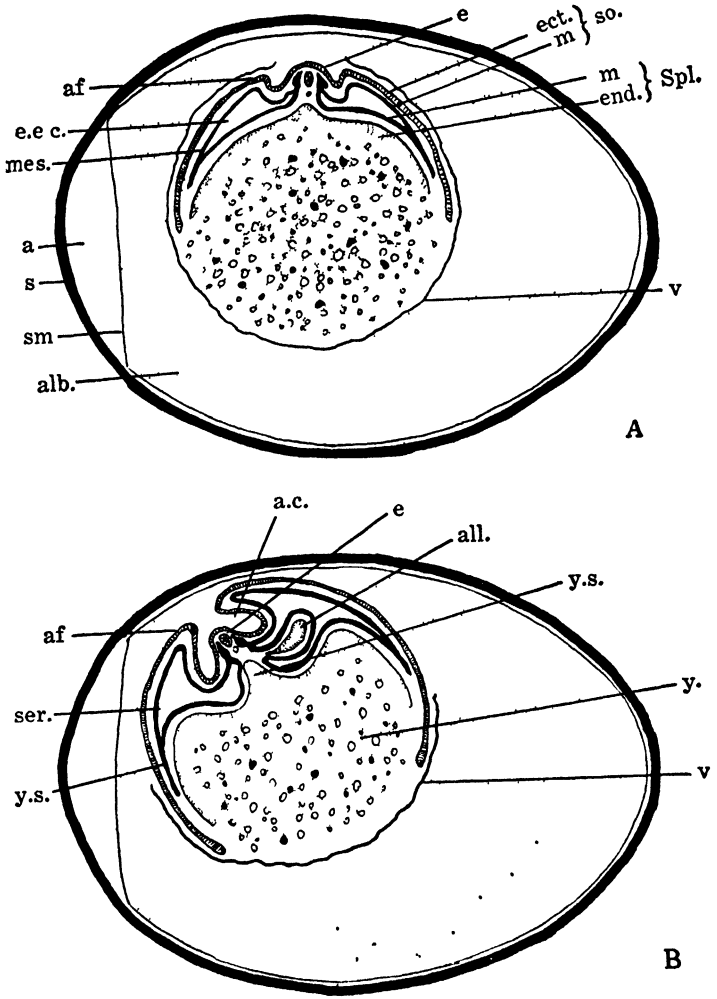


FIG 163 Diagrams of the extraembryonic membranes of the chick (From Duval's Atlas)

A, 2-day embryo, B, 3-day embryo a, air space, af, amniotic fold, a c, amniotic cavity, all, allantois, alb, albumen, e, embryo e e c, extraembryonic coelome, ect, ectoderm, end, endoderm, m, mesoderm, ser, serosa; s, shell, sm, shell membrane, so, somatopleure, sp, splanchnopleure, v, vitelline membrane, y, yolk, y s, yolk sac

embryonic coelome, and last the somatopleure (mesoderm and ectoderm). The anterior and posterior parts of the gut are the first to develop

a cellular floor. The midgut remains open to the yolk for some time. As the embryo becomes constricted off from the yolk the two ends of the gut enlarge further, and the extent of the opening of the midgut is decreased. Finally, the latter is connected to the yolk only by a narrow

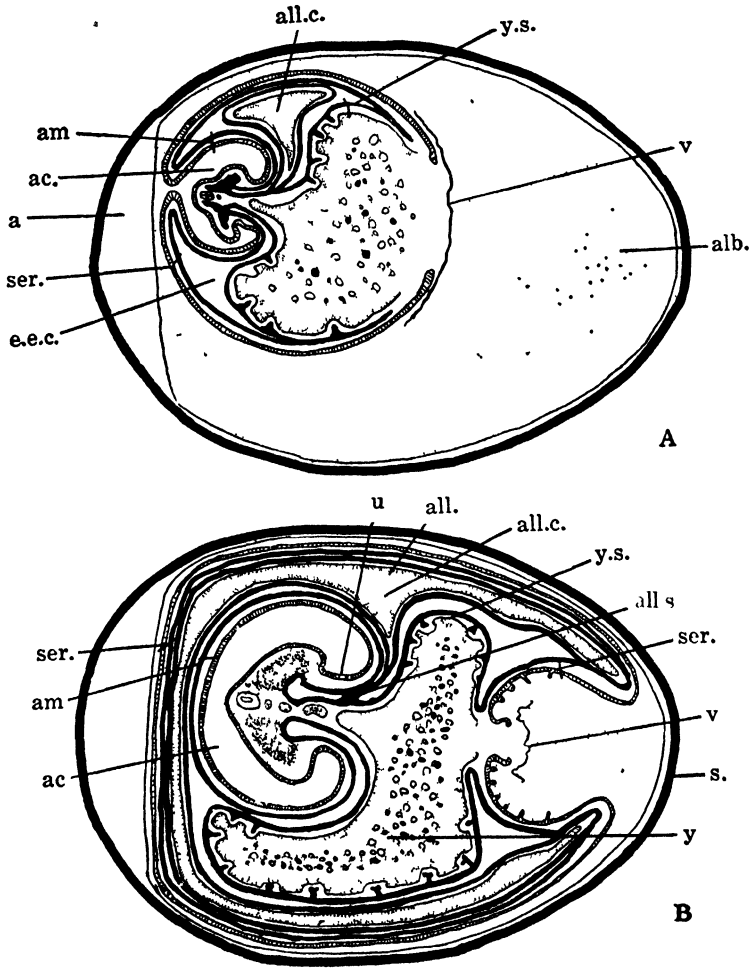


FIG 164 Continuation of Fig 163
A, 5-day embryo, B, 14-day embryo
am , amnion, all c , allantoic cavity, all s , allantoic stalk, u , umbilical stalk

neck called the yolk stalk. At the yolk stalk the inner and the extra-embryonic splanchnopleure are continuous. The absorption of yolk material through the yolk stalk probably does not occur directly but is brought about by the vitelline circulation. Deep yolk sac septa which are folds of the splanchnopleure grow down into the yolk, and glandular

absorbing cells aid in its digestion and in passing it to the vitelline circulation. The yolk mass decreases toward the end of the incubation period, and finally the entire structure is drawn into the body.

During the early growth of the blastoderm over the yolk the amnion and the serosa are being formed. The amnion arises by folds of the extraembryonic somatopleure near the anterior region of the embryo. First to form is the head fold of ectoderm and endoderm, for the mesoderm has not yet reached this region, and this is joined by lateral folds of somatopleure so that the entire region is covered. Later a similar tail fold covers that region and is continued forward until it meets the anterior lateral folds. The fusion of these folds encloses the embryo in an ectodermal cavity, the amniotic cavity. The union of its two folds has caused the somatopleure to double back on itself and thus to enclose the embryo in two membranes of similar nature although of inverted order. The inner membrane or amnion consists of ectoderm continuous with that over the embryo and of somatic mesoderm. The outer membrane or serosa consists of somatic mesoderm and of ectoderm continuous with the outer covering of the yolk. Between the amnion and the serosa is the extraembryonic coelome which is connected with the body cavity of the embryo. The serosa is merely a continuation of the somatopleure which has made its way nearly around the yolk. It also covers the allantois which later develops within the extraembryonic coelome. Thus it comes to line the shell and covers the embryo and the other three membranes.

The allantois arises as a diverticulum from the hind gut within the body of the embryo. As development proceeds it extends out into the extraembryonic coelome, its connection with the gut being designated as the allantoic stalk. Since it arises from the gut, it has necessarily a lining of endoderm and an outer layer of mesoderm, that is, it is an extension of the splanchnopleure. It grows very rapidly and unites with the serosa so that there is now a double layer of mesoderm in its outer wall, splanchnic on the inside and somatic from the serosa on the outside, and over all is the ectoderm of the serosa. The rapid growth of the allantois carries it first over the amnion, with which it fuses, and then over the yolk sac. Finally, pushing the serosa before it, it extends even over the albumen, which has become dense from loss of water, forming an albumen sac which is lined by the ectoderm of the serosa. The allantois has a rich blood supply in a network lying close to the porous shell, through which the exchange of gases is made easy. The chief function of the allantois is respiratory but it also serves as a receptacle for the excretory wastes from the kidney of the embryo. Shortly before the chick hatches, its beak breaks through into the air space of

the egg, pulmonary respiration begins, and the circulation of the allantois becomes slower and slower, until at hatching time the dried allantois remains inside the shell.

With conditions as found in the chick as a starting point, the development of the various vertebrate membranes may be appropriately taken up.

The Yolk Sac

The yolk sac is the only accessory structure which may be looked upon as of general occurrence among the vertebrates. It will be recalled that all vertebrate eggs show a distinction between yolk pole and animal

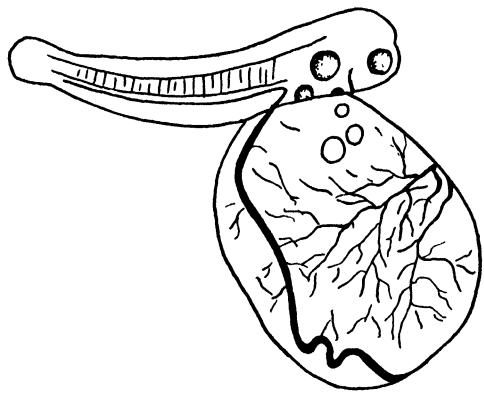


FIG. 165. Embryo of toad fish showing large yolk sac attached.

pole. (See Chapters IV and V.) The eggs are markedly telolecithal whether the cleavage is holoblastic or meroblastic. The relation between discoidal and holoblastic eggs is discussed and illustrated earlier (Fig. 51) where it is shown that the condition of the large yolk-laden endoderm cells of the bilateral type is gradually converted into the yolk mass of the discoidal type. The latter is incapable of segmentation, yet the homology between them is complete. The yolk-laden endoderm cells which make up the ventral portion of the enteron and serve as a source of food supply for the growing embryo may be regarded as the antecedent of the yolk sac of the meroblastic vertebrates. They do not constitute a true yolk sac, but the mass of cells is the equivalent of an internal one and corresponds in a general way to the outer sac of fishes, reptiles, birds, and mammals. For all of these it is an important nutritive structure.

In myxinoids, elasmobranchs, and teleosts, in all of which the eggs are extremely telolecithal, a well-developed yolk sac is characteristic.

Before the blastoderm spreads over the yolk the embryo has formed and has become folded off. The yolk sac, therefore, is formed by the extraembryonic blastoderm which grows over the yolk and it is attached to the embryonic body by a stalk, narrow in elasmobranchs but wide in teleosts. The yolk sac has an area vasculosa that is rich in blood vessels by which the food material of the yolk is brought to the embryo.

Although it is an outgrowth of the gut the connection of the yolk sac with that structure may be interrupted early in development as is found among the teleosts (for example the salmonids), in which case the absorption of the yolk is brought about exclusively by the vitelline vessels. In addition to its nutritive function, the yolk sac often acts as a respiratory organ. It functions in this manner in the lower vertebrates where an exchange of gases takes place between the blood vessels of the yolk sac and the surrounding water and also in the early stages of higher vertebrates where the exchange takes place between its blood vessels and the medium surrounding the egg. Even in mammals the yolk sac may have a respiratory function. In the monotremes it still retains its yolk and hence its nutritive function. In the placental mammals, however, it is devoid of yolk but may still be functional in early stages when it is quite vascular and helps to absorb nourishment from the maternal tissues.

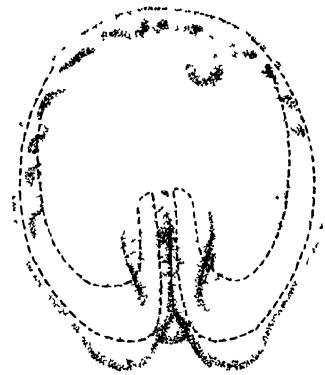


FIG 166 Embryo of *Torpedo* showing boundaries of the mesoderm as it grows peripherally from the posterior part of the embryo (After Ziegler.)

Structurally the yolk sac is developed in about the same manner throughout the lower classes of vertebrates. A distinction is to be observed in the appearance of the mesoderm of the elasmobranchs, as *Torpedo*, and in teleosts, as the salmon. In the former the mesoderm is an undivided layer which grows out peripherally from the posterior portion of the embryo and then anteriorly about the edge of the blastodisc. In the latter case the mesoderm splits into somatic and splanchnic layers which grow laterally from the embryonic area and carry the body cavity as the extraembryonic coelome out over the yolk.

The Amnion

In the Sauropsida (reptiles and birds), we have the first appearance of the amnion, serosa, and allantois. This group and the mammals make up the Amniota, which possess the amnion and are contrasted with the Anamnia which lack it. Since most of these latter lay their eggs in a

fluid medium, usually water, the developing embryos are not subjected to injury through violent motion or through changes in temperature or in humidity, and thus no special covering of the embryo is necessary. All the higher vertebrates either lay their eggs in a shell or hold them in the mother's body during the embryonic period and some kind of protection is needed to prevent the embryo from drying or from pressure or stress that might reach it. Therefore these embryos are protected by two membranes, the amnion and the serosa. When fully formed the amnion has always an inner layer of ectoderm and an outer layer of mesoderm.

Of the Amniota the reptiles are the most primitive. It is interesting to note that in them the amnion develops earlier than in the birds at a time before the head has become prominent and before the mesoderm

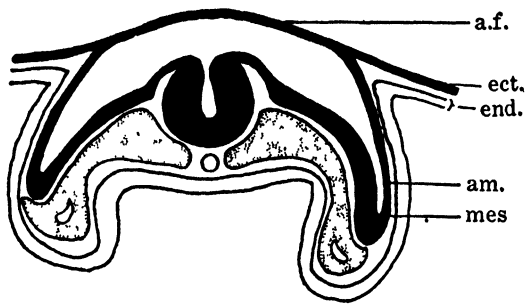


FIG 167 Diagrammatic transverse section through a Chelonian embryo (*Clemmys*). (Redrawn from Kerr, based on a figure of Mitsukuri.)

has split into its two layers. In the Chelonia the amnion is at first formed of ectoderm and endoderm, the mid-dorsal part being of solid ectoderm. Later after the mesoderm has split it is drawn up into the amniotic folds and the endoderm takes no further part in the formation of the amnion. This is different from the condition in the chick in which the amnion is formed from the somatopleure except in the proamnion region where there is no mesoderm. (A proamnion region is characteristic of reptiles in general.) The broad ectodermal band in the roof of the amnion in the Chelonia has its counterpart in a small area known as the sero-amniotic isthmus in the amniotic roof of the chick. This latter may be considered as the remains of the broader layer of the reptiles.

The amniotic fold in the Chelonia begins at the head end and proceeds backward until the whole embryo is covered. By the backward growth of this flap there is formed a long tunnel-like cavity covering the embryo and extending posterior to it. Later this posterior extension becomes partly obliterated and the cavity is closed. This again is quite

different from the formation of the amnion in birds in which a head fold, lateral fold, and a tail fold take part. In some reptiles, however, as the chameleons, amnion formation is similar to that of birds, from lateral folds around the embryo, and is not due to the elongation of one an-

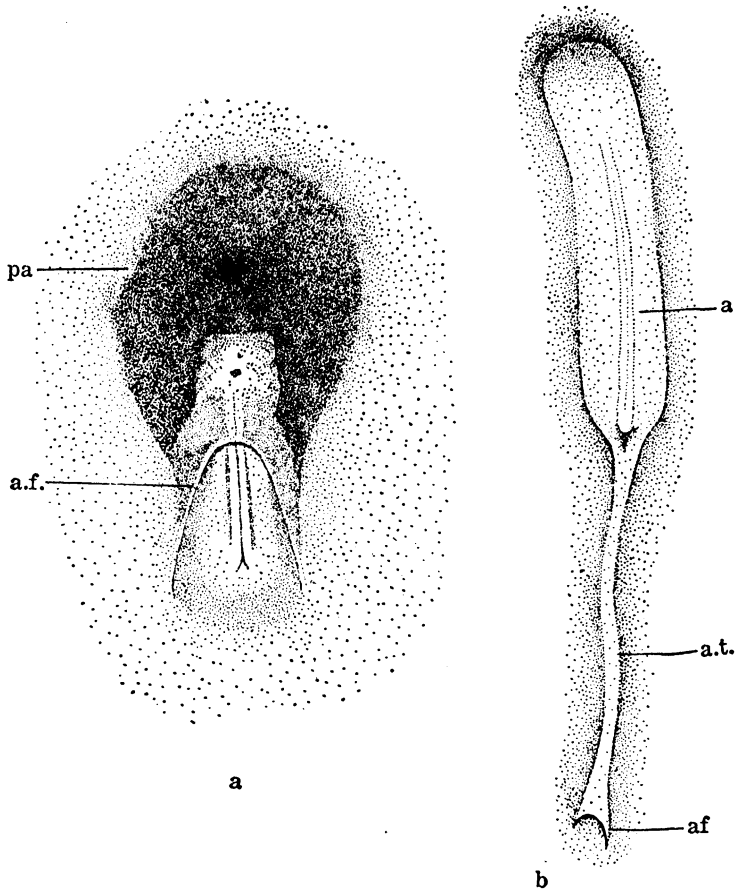


FIG 168 A and B Chelonian blastoderms showing stages in the formation of the amnion A, *Chelydra*, B, *Clemmys* (Redrawn from Kerr after Mitsukuri)
a, amnion showing neural groove beneath it, a f, edge of amniotic fold, a t, amniotic tunnel.

terior fold. Since the amnion appears earlier in the reptiles than in birds, and its cavity is formed before the embryo is marked off from the yolk, there is no need for the amniotic folds to grow upward as in the chick.

The development of the amnion in the birds as a group offers nothing different in principle from the type already described, that is, from the

conditions in the chick. The amnion of the mammals presents new features, however. Their character has already been discussed in the chapter on the formation of the mammalian embryo, Chapter X, to which the student is referred, and the chief facts regarding it are again mentioned a little later in this chapter under the head of the membranes of the mammalian embryo.

The Allantois

The allantois appears first in reptiles and is developed in all amniota. It always originates as an outgrowth of the hind gut and has therefore an endodermal lining and an outer layer of mesoderm. In amphibians it is represented by the urinary bladder. In reptiles the outgrowth has been described as solid, but it has also been maintained that the contracted space in which it develops causes it to be collapsed and so to take on the appearance of a solid structure. In birds it has a three-fold function. It increases in size, probably on account of the large amount of excretory wastes collected during the embryonic life in the shell. Its large size and its position next to the air space in the end of the egg and just inside the shell are correlated with its new use as a respiratory organ. Also, pushing the serosa before it, it forms a double-layered sac over the albumen. This sac is thus lined with ectoderm. Since it absorbs the albumen, the allantois thus has a nutritive function, although of course this is of less importance than the similar function of the yolk sac.

The allantois of mammals acquires no further function. It aids the trophoblast in the formation of the placenta which is for the mammal an embryonic organ of nutrition, respiration, and the removal of excretion. It thus becomes the most important embryonic membrane of mammals, for through the participation of its blood vessels it has the chief part in the formation of the placenta.

Viviparity among the vertebrates is especially related to the functions of the allantois and the adaptations which it makes, but other embryonic membranes are also employed in bringing about this mode of caring for the embryo. Viviparity occurs in all the vertebrate groups, beginning with the fishes, except the ganoids, lung fishes, and birds. It is possible that it may have been characteristic of some fossil ganoids, but probably the birds because of their habit of flight have never been able to develop this method of caring for their young. There are many parallel methods of relating the embryo to the maternal tissues, and functional placentae are not confined to the mammals. In all forms below the mammals the embryo is chiefly nourished by the yolk sac even though other structural modifications supply additional sources of food. Development within the body of the mother always serves the same function, protection in

the struggle for existence. Among the elasmobranchs there are several genera in which there is intra-uterine development. In *Acanthias* the typical egg membranes are formed but the horny egg-shell is thin and disappears in later stages. The uterine wall contributes to the nourishment of the embryo by supplying the albuminous fluid which is enclosed in the egg-shell. In this form the lining of the uterus during later stages of development is thrown into folds which are brought into contact with the yolk sac. In *Torpedo* the folds are prolonged in trophonemata, nutritive threads which reach the pharynx of the embryo through its spiracles. In *Mustelus laevis* the folds of the uterus fit into grooves in

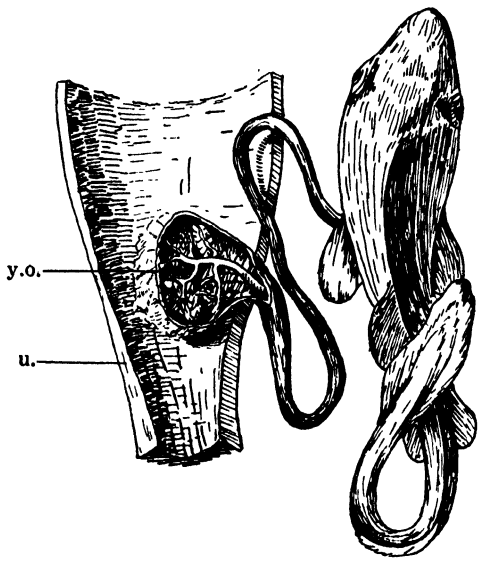


FIG. 169. Yolk sac placenta of *Mustelus laevis*. (Redrawn from Corning after Joh. Müller.)

u., wall of uterus; y.o., yolk sac.

the yolk sac and, as both are highly vascular, they form a functional yolk sac placenta. A placenta used in this broad sense means any structure which brings about, by means of an abundant vascular supply, an intimate relation between maternal and embryonic tissue, for the purpose of conveying nourishment from the one to the other. Here the yolk sac functions in this manner, but in the Amniota it is usually the allantois.

The teleosts include a number of viviparous forms. In some the eggs are developed in the ovary itself where vascular processes on the surface contain the eggs and act as placentae. In others the embryos develop in the enlarged oviduct or uterus. Sometimes (Scorpaenidae) the maternal body merely holds the embryo which obtains its food supply entirely

from the yolk. In other cases (*Anableps*) the yolk sac sends out villi to the uterine wall.

Viviparity is uncommon among the amphibia. In one of the salamanders (*Salamandra atra*), however, development takes place in the oviduct but usually only one of the eggs reaches maturity for the others break up and are absorbed by the outer gills of the developing embryo. The embryo has additional nourishment from the blood which escapes from the uterine wall.

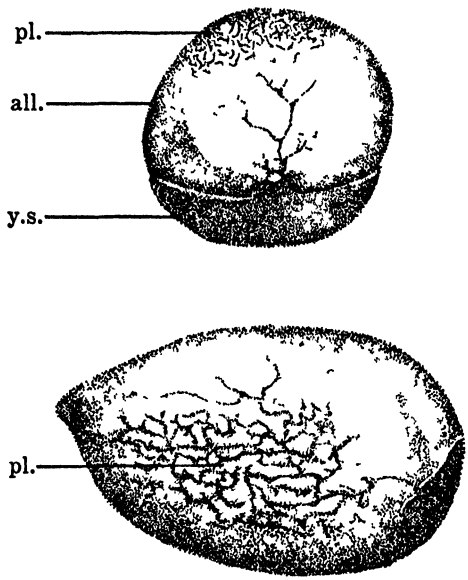


FIG 170 Embryo of *Chalcides tridactylus* (Redrawn from Kerr after Giacomini)
A, 7-mm embryo, B, 15-16-mm embryo seen from apical pole all, allantois, pl, allantoic placenta (foetal portion), y s, yolk sac

The reptiles include a number of viviparous forms. In some, the blind worm and the viper, there is a thin shell and the embryo is simply held in the uterus, the lining of which remains unchanged. In the lizard, *Chalcides ocellatus*, the shell breaks and finally disappears, the uterine wall becomes vascularized, and is thrown into folds which fit other folds on the yolk sac. Both the allantois and the uterus adjoining it are highly vascularized but the placenta-like folds develop only on the yolk sac. A type of placenta more nearly approaching that of the mammals is found in still another lizard, *Chalcides tridactylus*. The condition of its allantois and yolk sac may be regarded as transitional from that typical of the Sauropsida to that of the mammals, for both function as placenta and projections from both vascularized sacs interlock with

similar projections from the uterus. But of the two the allantoic placenta is the more highly developed. Thus the true allantoic placentae are not limited to the mammals but appear also among the reptiles.

Evidently the problem of relating the embryo to the maternal tissues has been solved in similar ways, although employing different structures in the different groups of the vertebrates. In the mammals the wall of the uterus is highly developed as a special adaptation of the maternal tissue to accomplish this function. In other forms the oviduct sometimes takes care of the need so far as the mother is concerned, and even the ovary may serve in this capacity. On the part of the embryo, the yolk sac of lower forms often carries on the function, while in the higher forms it is taken over by the allantois.

C. Mammals

Of the embryonic membranes of mammals the greatest interest is in connection with the allantois and the placenta which it helps to form. The yolk sac and amnion are also present, however, and show features which are new. In the entire early development of mammals there is a rather general lack of structural homology with that of other classes of vertebrates, and although similar results are reached convergently so far as the structure of the embryonic membranes is concerned, their methods of origin in some forms is very different.

There are two great *types of development* found in the mammals. The monotremes are unlike all the others in that their eggs are large and yolk-laden and their cleavage discoidal. The animals are oviparous, the development of the embryo taking place within the egg-shell, as in the case of the Sauropsida. Cleavage, germ-layer formation, and the development of the embryonic membranes is comparable to these processes in that group. The membranes are of the same kind and are probably formed in the same manner.

The marsupials are the lowest mammals which have intra-uterine development. The young are born in an immature condition, and are placed in the abdominal pouch where they become attached to the teats of the mammary glands. Here the immature period is completed. Since at birth the young are so immature, the allantois, which in the higher vertebrates functions as a medium for obtaining nourishment from the uterus, is usually small. The yolk sac contains no yolk, though it is large and well developed. Its wall opposite the embryo contains only endoderm, and the extraembryonic coelome is absent in this part of the blastoderm. During uterine life there is usually a yolk sac placenta.

The marsupials include a number of forms which taken together make up a series similar to that described for the lower vertebrates and illus-

trate the change from a yolk sac placenta to an allantoic placenta. The first of the series is the opossum, *Didelphys*, in which the yolk sac has a nutritive function. This membrane makes connection by means of folds

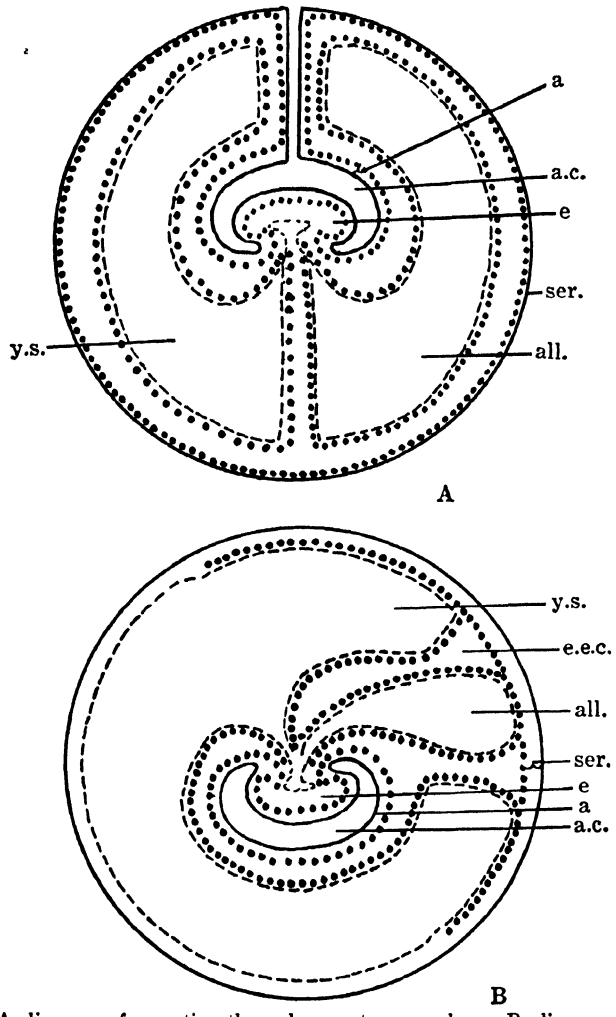


FIG. 171. A, diagram of a section through monotreme embryo; B, diagram of a section through marsupial embryo. Ectoderm, solid line; mesoderm, dotted line; endoderm, dashed line.

a., amnion; a.c., amniotic cavity; all., allantois; e., embryo; e.e.c., extraembryonic coelome; ser., serosa; y.s., yolk sac.

with the uterine wall. The latter secretes a nourishing fluid which is absorbed by the trophoblast and the endoderm of the yolk sac, and thus is transported to the embryo. In *Dasyurus* we have a further step

in the series. The allantois is still small and the yolk sac large. The trophoblast forms a syncytium with pseudopodial processes which attack the uterine wall and engulf uterine blood vessels. The nourishment is passed on to the yolk sac which may be correctly called a yolk sac placenta, for the connection between the uterine wall and the yolk sac is very close. *Parameles* illustrates the last step in the series. In this form the yolk sac is large and probably has some function in absorbing nourishment. The allantois, however, grows larger, develops a richer blood supply, and reaches the trophoblast. The uterine wall, at the place where the trophoblast touches it, develops a syncytium and becomes vascular. The trophoblast disappears and the blood vessels of the allantois and the uterus come into close contact. There are no true villi as in the higher mammals. This is the first case we have met of a true allantoic placenta and the only one known for the marsupials. It differs from all others in that the syncytium is entirely of uterine origin and does not develop from the trophoblast as it does in those mammals having a discoidal, deciduate placenta.

The development of the eggs of the true placental mammals involves the formation of a blastocyst with differentiated trophoblast and inner cell mass as is described in Chapter X. The embryo resembles a hollow vesicle with a drop hanging from one spot on its inner surface. The inner cell mass is the formative or embryonic portion. The trophoblast is non-formative and produces only ectoderm of the serosa. After this stage the inner cell mass or embryonic knob differentiates into two portions, an upper ectodermal layer from which will be derived the ectoderm and amnion of the embryo and a lower endodermal layer from which will be derived the enteric canal, the yolk sac, and the allantois. The embryo proper at this stage is called the embryonic shield and consists of a layer of ectoderm covering a layer of endoderm. (See Fig. 144.)

Jenkinson has offered an interesting suggestion about the significance of the early development of the mammal. He compares the blastocyst stage with the later stage of the reptile or bird in which the yolk sac has become completely covered and the serosa contains the embryo in the amnion with its yolk sac and allantois attached. In the mammals also there is a stage of a closed sac, the blastocyst, which contains in the inner cell mass all the material for the embryo and its membranes. Jenkinson regards this as a precocious separation of the materials of the mammalian embryo due to the loss of the yolk on the part of the ovum.

The Yolk Sac

The yolk sac has the same layers as that of the lower forms, but in the placental mammals it arises in a different way and is functional

only in early stages, if at all. The endodermal layer is formed from the innermost layer of the inner cell mass. These cells proliferate and spread around the inside of the trophoblast until they form a closed sac just as would have happened had a central yolk mass been present and they had grown around it. Sometimes the cavity is very small and the yolk sac correspondingly restricted, as in man, when it forms a very small vesicle owing to its slow growth. In other cases the endoderm is quite closely applied to the inner side of the trophoblast and a large yolk sac is thus formed. In no case, however, does it contain yolk. It is therefore an insignificant structure and the function which it serves in the lower

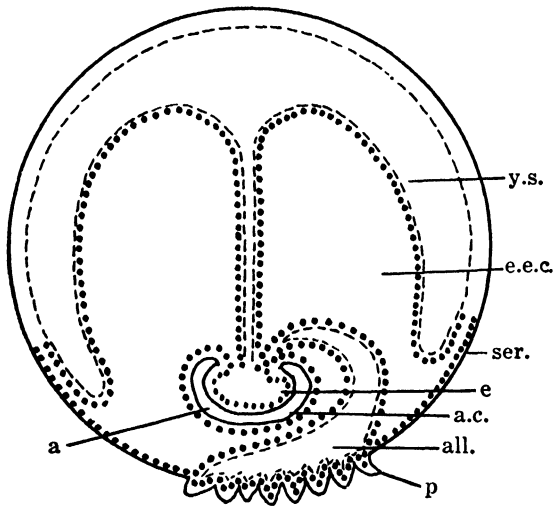


FIG. 172. Diagram of a section of rodent embryo. p., placenta; other letters as in Fig. 171.

forms is presently taken over by the allantois. The completion of the yolk sac comes only when in later stages the mesoderm is formed between the endoderm and the trophoblast.

The formation of the mesoderm begins in the embryonic area and some time elapses after its separation into its two layers before it grows out into the space between the endoderm and the trophoblast of the vesicle; but at length it pushes out from the embryonic area and grows between these two layers. An early split in this mesodermal layer completes the yolk sac which thus consists of endoderm and splanchnic mesoderm. The vitelline circulation develops to provide the usual vascular supply.

In many of the higher forms it is merely a vestige of the membrane through which the embryos of simpler forms obtain their food. In some cases, however, it retains something of its earlier function in that in

early stages it absorbs nutriment from the uterus. In these cases it becomes a yolk sac placenta. When this occurs, as in the ungulates, carnivores, and rodents, the yolk sac is large and its blood supply is rich and comparable to that of the chick. In marsupials, in which the uterine development is of short duration, the yolk sac placenta often functions until birth.

In rodents the yolk sac is always large and invagination of its upper wall by the embryo gives apparent inversion of the germ layers. This may take place early in development as in the mouse and guinea pig, or

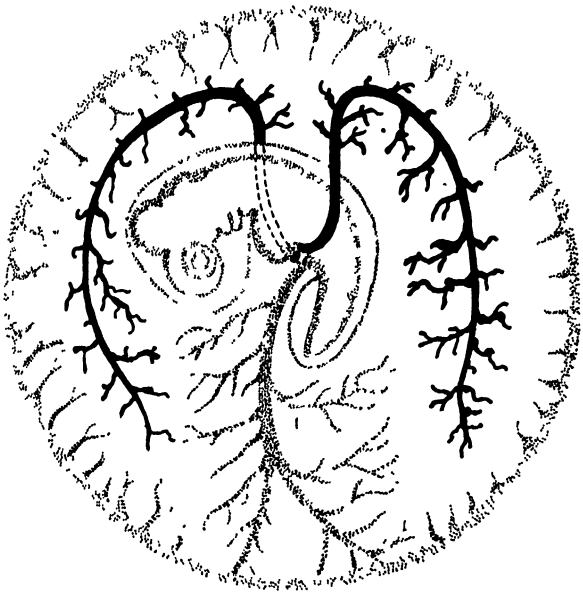


FIG. 173. Area vasculosa of the yolk sac of the rabbit (After Jenkinson.)

later as in the rabbit and squirrel. In some cases the lower wall of the yolk sac is never formed. In others it disappears along with the trophoblast, and the cavity of the yolk sac is in communication with the cavity of the uterus. The upper wall of the yolk sac absorbs the material secreted by the uterus and its supply of blood vessels carries this to the embryo. Thus the yolk sac acts as an accessory placenta.

In the rabbit the yolk sac, which contains a nourishing fluid, is very large and in early stages practically fills the blastodermic vesicle. A rich supply of blood vessels develops in the side of the yolk sac which is toward the embryo. The opposite side contains no mesoderm and fuses with the trophoblast. Later the yolk sac shrivels and since its outer wall is fused with the trophoblast it remains as a mushroom-shaped

membrane. The two walls of the shrunken vesicle thus come close together and later they fuse. Since the inner wall of the yolk sac was highly vascularized its blood vessels continue to supply a large part of the blastoderm. The rest of the blastoderm is fed by blood vessels of the allantois which help form the placenta. The yolk sac of the rabbit is probably a more important nutritive organ than the allantois. The vitelline artery ends in the sinus terminalis and the blood is returned to the heart by the vitelline veins.

In the Insectivora the yolk sac is often large in early stages and its blood vessels may begin to form a yolk sac placenta as in *Tupaija*. In

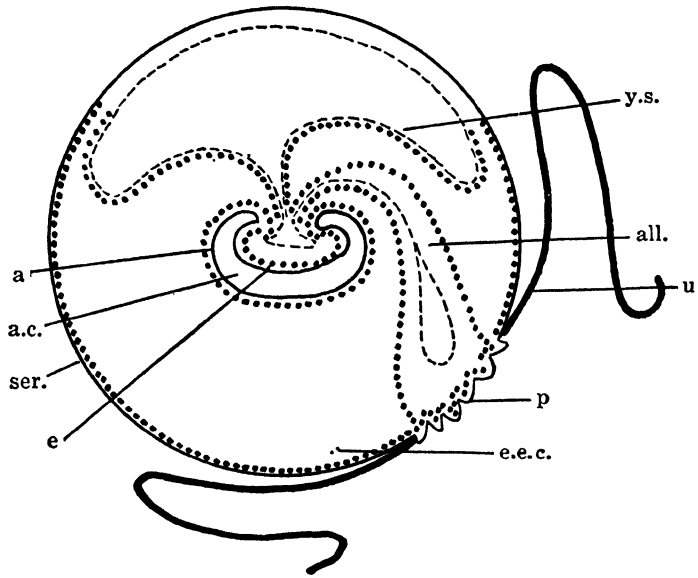


FIG. 174. Diagram of a section of embryo of insectivore. u., wall of uterus; other letters as in Fig. 171.

some insectivores the mesoderm never covers the lower wall of the yolk sac (*Sorex* and *Talpa*). This organ is unimportant in later stages.

The yolk sac circulation in most mammals is of importance in early stages only, while the allantois is being formed. Later, when the allantois has established its relation to the uterine wall, there is a retrogression of the yolk sac. This is true of man, in which the yolk sac becomes a solid strand of cells extending out into the body stalk or umbilical cord. In some forms, as the insectivores, the yolk sac remains until birth.

The Amnion

The formation of the amnion is due to the development of the upper portion of the inner cell mass. It is accomplished by one of the two

methods described in Chapter X. In the one case, there may be a fold or a series of folds of the extraembryonic somatopleure similar to those of the chick, as in most ungulates and some insectivores. In the other case, the cavity may arise *in situ* in the embryonic knob or between it and the trophoblast, and thus may never open into any other cavity, as in the mouse, guinea pig, bat, other insectivores, and many primates, including man.

The Allantois

The allantois arises as a diverticulum from the gut in the same manner as in the chick. In the rabbit this diverticulum extends out into the extraembryonic coelome and soon comes into relation with the trophoblast. In many rodents, as the mouse and guinea pig, it is often small and its endoderm may not grow beyond the body of the embryo. In *Tarsius* and in man the vesicle never extends freely into the extraembryonic coelome but is enclosed as a vestigial structure in the body stalk. This latter is an enlargement of the primitive connection between the embryo and the trophoblast, a connection which is never lost. It is regarded as the equivalent of the allantoic stalk of other forms.

The allantois of lower mammals assumes the function of nutrition but retains its cavity which may serve as a receptacle for urinary wastes. The size of the cavity is progressively reduced until in some forms it disappears completely with the inner endodermal layer and only its mesodermal portion remains. This part becomes highly vascularized and, together with the trophoblast, forms the embryonic part of the placenta, a structure which comes into relation with the circulatory system of the mother and so takes care of the functions of respiration, nutrition, and excretion. Thus the mesodermal portion of the allantois accomplishes the function which in lower forms the entire structure serves.

The Placenta

A placenta is any organ which brings about an intimate relation and vascular connection between maternal and foetal tissues. The formation of a yolk sac placenta has been described for some of the fishes and reptiles and is again found among the marsupial mammals. Among the higher mammals, however, it is always the allantois which brings about this relation between the embryo and the mother. In these forms, the actual contact is made by the trophoblast, as part or all of it comes into relation with the uterine walls. It is the allantoic vessels, however, which, through contact with the trophoblast, furnish the foetal part of the placental circulation.

The placenta is necessarily a compound structure. Its embryonic

parts are trophoblast (sometimes lined with mesoderm) and the allantois with its blood vessels. Its maternal part is the vascular uterine wall. The foetal and maternal parts may be merely in apposition or they may be in such close relation that they are actually fused. In both cases an intimate physiological relation results. The blood vessels of the allantois (umbilical arteries and veins) are brought close to those of the uterus, making possible an exchange of substance by diffusion. The embryo is thus able to obtain oxygen and food materials and also to dispose of its waste products. There is never any direct connection between foetal and maternal blood vessels.

The placentae of mammals are of two general types, deciduate and non-deciduate. The non-deciduate placenta is sometimes called a semi-placenta. The name deciduate was given to indicate the loss, at birth, of the lining of the uterus. Although this loss is not significant in all so-called deciduate forms, the name has still been retained. The following brief classification of types of placentae of the Placentalia is partly based on the conception of Jenkinson. The types are closely related to the method of implantation of the ovum in the uterine wall. Of implantation there are three types, central, eccentric, and interstitial, as mentioned in Chapter X. Implantation is central if the embryo becomes attached superficially to the uterine wall; eccentric if it is enclosed by folds of the uterus; or it is interstitial if the embryo burrows down into the uterine wall. The classes of placentae are as follows:

The non-deciduate type is that in which the surface of the trophoblast develops villi which fit into crypts or depressions in the uterine wall. In the simplest cases, the uterine epithelium persists in these crypts and at birth the villi are drawn out without damage to the uterus. In other cases, the uterine epithelium is destroyed and there is a closer union between foetal and maternal tissues. This type of placenta is characteristic of the ungulates, but is also found in the Cetacea, Edentata, Sirenia, and Lemuroidea except *Tarsius*.

The second type is the deciduate in which the uterine epithelium is always destroyed by the trophoblast. Foetal and maternal blood are in close relation. There are two subdivisions of this type as follows:

(*a*) Zonary deciduate placenta. The maternal blood circulates in capillaries of the uterus. The placenta, which is zonary or band shaped, is composed of foetal and maternal tissue in very close relation. At birth part of the wall of the uterus is destroyed and there is a true decidua. This type of placenta is characteristic of the Carnivora. (*b*) Discoidal deciduate placenta. The trophoblast destroys not only the uterine epithelium but also the subepithelial tissue. The maternal blood circulates in lacunae or spaces in the trophoblastic wall. These spaces

represent maternal blood vessels which have been engulfed by the trophoblast and they are the chief part of the placenta contributed by uterus. Blood is the principal maternal tissue lost at birth, and the deciduae are thin and more or less degenerate. The portion of the trophoblast which is involved in the placenta is disc-shaped. Placentae of this type are found among rodents, insectivores, bats, in *Tarsius*, monkeys, and man. These three types of placentae require a more detailed description.

Non-deciduate Placenta. The non-deciduate placenta may be illustrated by that of the ungulates. In these forms the allantois enlarges rapidly and occupies the length of the trophoblast. The surface of the trophoblast forms villi into which extend the mesoderm and blood vessels of the allantois. These may be single or branched. They may be scattered over the surface of the trophoblast (diffuse placenta, as pig);

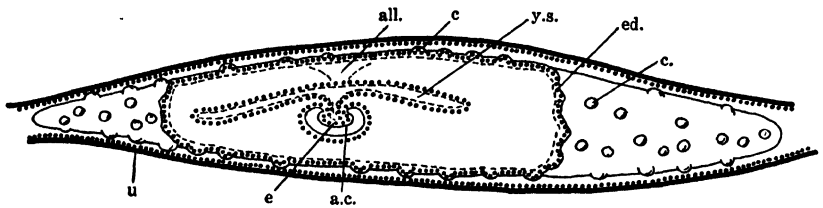


FIG. 175. Diagram of embryo of ungulate contained in uterus. Wall of serosa has been cut, showing embryo in section. c., cotyledon; ed., edge of cut in serosa.

they may be situated in groups or cotyledons (cotyledonary placenta) when they are also branched (ruminants), or there may be a combination of the two types, both cotyledons and simple villi being interspersed (intermediate placenta). Also the cotyledons may be grouped in a band around the trophoblast.

The trophoblastic villi fit into crypts or corresponding depressions in the uterine wall. When the villi are in groups or cotyledons, the corresponding groups of crypts on the maternal side are called maternal cotyledons. These latter are in some cases stalked and the foetal cotyledons are then concave.

In the diffuse placenta, the uterine epithelium persists as the lining of the crypts and at birth the villi are withdrawn with no injury to the uterus. In the cotyledonous placenta the uterine epithelium, except that of the glands, degenerates and there is a much closer union of maternal and foetal tissues. Consequently the two portions of a placenta of this type separate with some difficulty.

In the diffuse placenta the uterine and trophoblastic epithelia are in contact, but in the cotyledonary placenta the contact of the tropho-

blastic epithelium is with a highly modified and vascular subepithelial connective tissue. In the latter case, however, there is no true fusion of foetal and maternal tissue as is true of the deciduate placentae. The maternal connective tissue and trophoblastic villi are both rich in capillaries. Nourishment is obtained by the trophoblast in two ways. Both the villi and the trophoblastic cells at their base absorb nourishment ("uterine milk") from the maternal tissues, and extravasted blood and

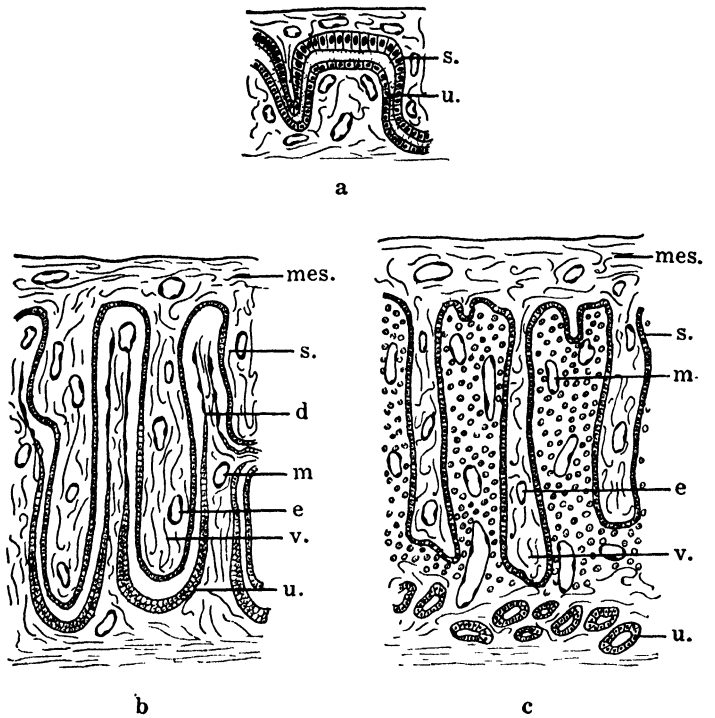


FIG. 176. Cross sections of placentae. A, pig; B, cow; C, cat. (Modified from Grosser.) d., degenerating uterine epithelium; e., embryonic capillary; m., maternal capillary; mes., mesoderm of serosa; s., epithelium of serosa; u., uterine epithelium; v., villus.

cell débris from the crypt walls are ingested by pseudopodia from the cells of the trophoblast. Absorption and respiration also are brought about by diffusion between the maternal and foetal capillaries. The blood in these vessels is separated by very thin layers of tissue only. The placental layer consists, on the foetal side, of the capillary walls, connective tissue, and trophoblast, and on the maternal side of connective tissue, capillary walls and, in the diffuse placentae, of uterine epithelium also. Thus diffusion between foetal and maternal blood vessels is easily carried on.

In the allantoic fluid of ungulates are found bodies of queer shapes called hippomanes. These are formed as accumulations of coagulable material from the uterine milk and become saturated with calcium oxalate crystals. They push, pocket-like, into the trophoblastic wall between the cotyledons and later are pinched off into the cavity of the allantois, surrounded by trophoblast and allantoic wall.

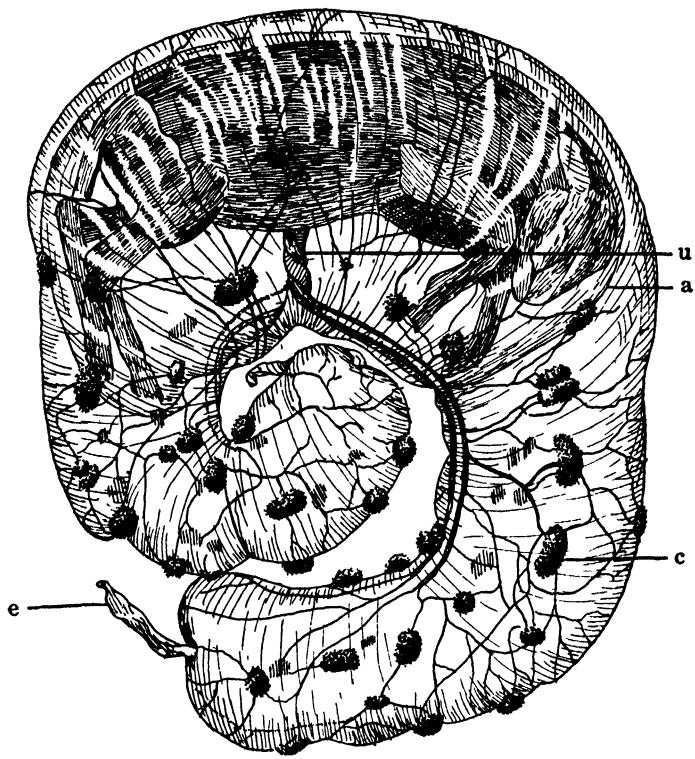


FIG. 177. Embryo of sheep enclosed in embryonic membranes and showing cotyledonous placenta characteristic of many ungulates. (Redrawn from Corning after O. Schultze.) a., amnion; c., cotyledon; e., atrophied end of blastodermic vesicle; u., umbilical cord.

Deciduate Placenta. In deciduate placentae the union of maternal and foetal tissues is closer than in placentae of the non-deciduate type. In deciduate placentae, part or all of the trophoblast, called the trophoderm, becomes specialized and, by the aid of enzymes, erodes the uterine wall. At birth the lining of the uterus is removed as part of the placenta. In the zonary deciduate type, this lining or decidua is in close union with the foetal blood vessels, so that maternal and foetal tissues are removed together. In the discoidal deciduate type, however, the

maternal blood, which circulates in spaces in the trophoblast, is the chief tissue lost from the uterus, for the deciduae are thin and degenerate.

The placenta of the *zonary deciduate* type occurs only in the Carnivora. This placenta may in some respects be considered as intermediate between the non-deciduate and the discoidal deciduate types. In the Carnivora, the trophoblast destroys the uterine epithelium (but not the subepithelium) and eats its way into the wall of the uterus. Here trophoblastic villi invade the connective tissue of the uterus and come in contact with the maternal capillaries which are much richer than in the nondeciduate placenta. Foetal capillaries grow into the villi and foetal and maternal blood are thus brought close together. This placenta is therefore formed by fusion of foetal and maternal tissues. The trophoblast, connective tissue, and the capillaries of the allantois contribute the embryonic part, and the capillaries and connective tissue of the uterus contribute the maternal part. The maternal blood is separated from the foetal blood only by these structures. The placenta is at first thin, but becomes thicker owing to the elongation and branching of the villi and the growth of the connective tissue. When birth takes place the blood vessels and connective tissue of the uterus are removed along with the foetal vessels and the connective tissue. It is possible that this placenta may have originated from the non-deciduate type by a closer association of the maternal and foetal tissues after the erosion of the uterine wall.

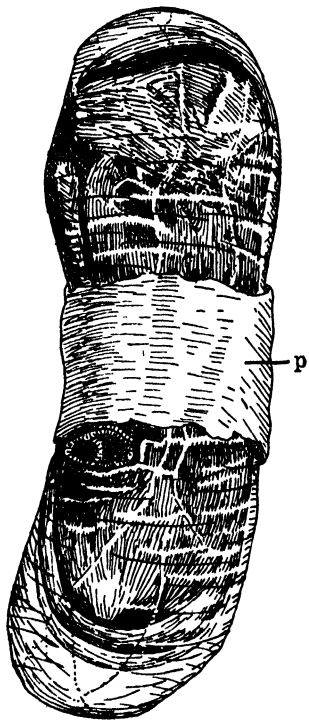


FIG. 178. Embryo of dog showing band-shaped placenta characteristic of the carnivores. (Redrawn from Corning.)

The placenta of the *discoidal deciduate type* differs from the carnivorous placenta in that the cavities in which the maternal blood circulates are not maternal capillaries but broad lacunar spaces which have formed in the walls of the trophoblast itself. The tissue of the placenta is almost entirely of foetal origin. The placenta of the mouse may be taken as typical of this type. In this form the trophoblast erodes the uterine epithelium and attaches itself to the subepithelial tissues of the uterus. It then develops lacunar spaces in its wall. Later after the allantois has developed and has reached the somatopleure its capillaries

grow in between the lacunae of the trophoblast. Further growth of the placenta is brought about by the thickening of this wall. Maternal blood from ruptured vessels of the eroded uterine wall circulates in the lacunae. The maternal and foetal blood, however, are separated from each other only by the walls of the trophoblastic lacunae, a small amount of connective tissue, and the walls of the capillaries themselves. As these lacunae are in such close relation to the allantoic capillaries, diffusion is easily possible between them. The placenta of the mouse is typical of the deciduate placentae of the discoidal type. Since maternal blood

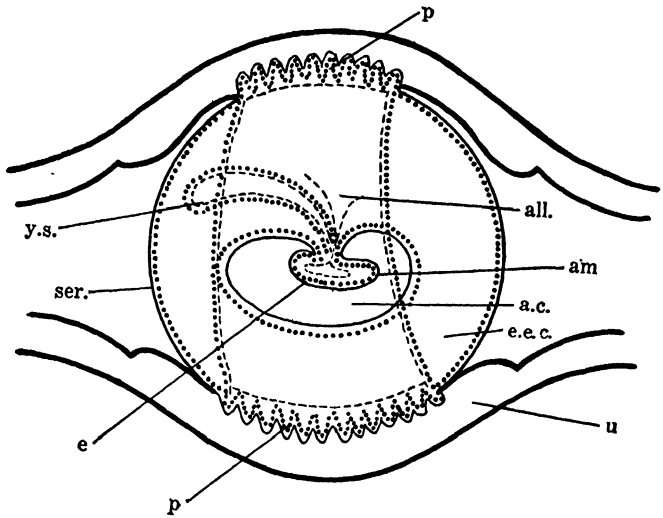


FIG. 179. Diagram of embryo of carnivore enclosed in uterus. Band-shaped placenta is represented as if transparent, showing embryo in section below. Letters as in Fig. 171.

circulates in lacunar spaces in the trophoblast one can readily understand why the uterus loses only blood and a slight amount of connective tissue at birth.

The discoidal deciduate placenta of the Anthropoidea is similar to that of the rodents.

When a human trophoblast reaches the uterus it bores its way into the wall and implantation is thus interstitial. The trophoblast becomes differentiated into two layers, plasmotrophoblast, which is a plasmodial covering without cell walls but containing many nuclei, and an inner cytotrophoblast or layer of Langhans, which consists of distinct cubical cells. The plasmotrophoblast at its place of attachment to the uterus erodes the uterine epithelium and dissolves the wall. The outer trophoblast has a spongy character and the spaces become filled with maternal

blood from the eroded uterine wall. Later the spongy network is transformed into villi which are surrounded by the trophoblastic spaces that now have the character of lacunae. These villi are at first solid, with the syncytial layer on the outside and cells within except in late stages when the cellular layer disappears. Some of the villi branch freely into the lacunar spaces, but others anchor the trophoblast to the uterine wall. The lacunae are lined everywhere, even on the maternal side, by a syncytial layer. Later the mesoderm of the serosa wanders into the villi which are now branched and which become vascularized by the allantoic blood vessels. These so-called villi are very different from the

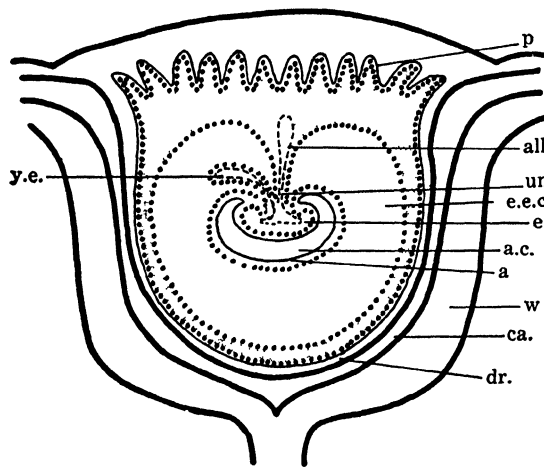


FIG. 180. Diagram of human embryo *in utero*.
d.r., decidua reflexa; c.u., cavity of uterus, um., umbilical cord.

villi of ungulates, which are projections from the wall of the trophoblast. They are not villi at all in this sense but are more like the foetal capillaries of rodents which are covered by the trophoblast into which they have pushed. They are merely the irregular walls of the lacunae of the trophoblast.

The villi are at first over the whole surface of the trophoblast (diffuse placenta), but later, those nearest to the cavity of the uterus degenerate so that here the surface is smooth. The villi of the other side remain giving rise to a discoidal-shaped placenta.

The uterine covering over the trophoblast is known as the decidua reflexa or decidua capsularis. The part of the uterus where the embryo is attached is known as the decidua basalis or serotina, and the opposite wall the decidua vera. In man the decidua capsularis comes in contact with the decidua vera in the fifth month and the uterine cavity is obliterated. Later the capsularis becomes non-vascular and gradually

disappears so that the trophoblast is in contact with the decidua vera. This in turn, becomes degenerate, as only the inner portion of the uterine lining remains. The decidua basalis, on the other hand, takes an important part in the formation of the placenta. The uterine capillaries increase and maternal blood from this region supplies the placenta. In late stages, the basalis is reduced to a very thin membrane.

When birth takes place the amnion is ruptured, the amniotic fluid escapes and contractions of the uterus expel the embryo, the break taking place across the degenerate deciduae. Later, there is discharged an afterbirth, which consists of the placenta with the thin layer of decidua basalis and the attached membrane composed of fused amnion, serosa, and deciduae capsularis and vera. It is thus evident that the

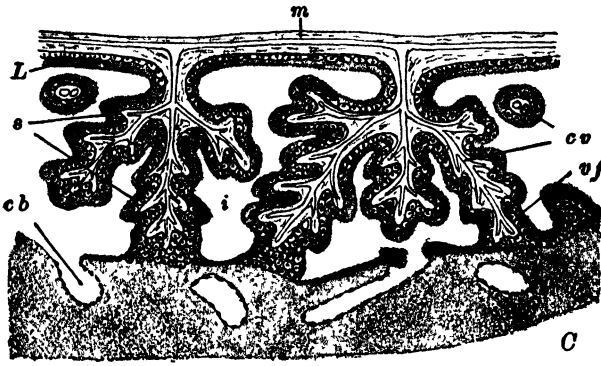


FIG. 181. Diagram of villi of human placenta. (From Kellicott, after Bryce.)

c.b., capillary of the decidua basalis; c.v., capillary of a villus; i, inter-villous cavity filled with maternal blood; L., Langhan's cells (cytotrophoblast); m., mesoderm of the serosa; s., syncitial layer (plasmotrophoblast); v.f., fixation or anchoring villus.

only maternal tissue which is lost is uterine blood and a small amount of connective tissue representing the thin layer of the degenerate lining of the uterus. The largest part of the placenta is of foetal origin, but maternal blood accompanies it.

In some of the monkeys, the placenta is double. There is, of course, only one umbilical cord, but the two placentae are connected by blood vessels. In the orang and gibbon there is a single placenta. The lower monkeys have no decidua reflexa.

The following outline lists the characteristics of the embryonic membranes and placentae in the different orders of mammals. In many cases the embryology of only a few forms has been investigated. It is probable that further investigation may show that some animals do not follow in all respects the usual types found in their orders. Indeed, it may be found that some show quite aberrant types, as is true of the lemur, *Tarsius*.

EMBRYONIC MEMBRANES OF MAMMALS

	Yolk Sac	Amnion	Allantois	Placenta	Remarks
Monotremata	Like bird, contains yolk on left side of embryo	Has sero-amniotic connection and is probably formed as in Sauropsida	Well-developed, on right side of embryo	None	Oviparous eggs with hard shell Young fed milky secretion from primitive abdominal mammary glands
Marsupialia	Large well-developed, but contains no yolk No extra-embryonic coelome in blastoderm opposite embryo During uterine life there is yolk sac placenta with exception of *Perameles*	Probably formed by folds	Small, since young are born immature	Found only in *Perameles* Trophoblast not involved in its production, uterine wall develops vascular syncytium	Series of forms through *Didelphys*, *Dasyurus*, and *Perameles* showing transfer of placental function from yolk sac and allantois
Artiodactyla	Small functional only in early stages, when it is vascular	Inner cell mass moves upward pushing trophoblast away The folds from edge of embryonic shield form amnion	Large, occupying most of uterus Goat embryo 2 inches long has allantois 2 feet long Hippomanes in allantoic fluid	Non-deciduate Most ruminants cotyledonary Pig diffuse unbranched villi Deer hippopotamus camel, giraffe intermediate	Central implantation
Perissiodactyla	As in artiodactyls	As in artiodactyls	As in artiodactyls	Non-deciduate placenta Horse branched diffused villi	
Cetacea		Large amnion of *Orca* obliterates extraembryonic cavity		Non-deciduate placenta with diffuse, branched villi	Membranes incompletely described
Sirenia			In *Halicore* is large, as in artiodactyls Hippomanes in allantoic fluid	Non-deciduate with branched diffuse villi arranged in band around trophoblast (Really zonary non-deciduate)	
Proboscidea		Amnion obliterates extra-embryonic coelome in *Elephas*		Zonary placenta with diffuse villi Not classified yet as to deciduate or non-deciduate but villi seem to be like those of artiodactyls	Embryology not sufficiently investigated

Carnivora	Large yolk sac probably functional in early stages	Formed from series of folds	Extremely large Grown between yolk sac and trophoblast completely lining serosa	Zonary deciduate	Central implantation
Edentata	Disappears	Large amniotic cavity obliterating extraembryonic coelome	Small	May be zonary oval bell-shaped or diffuse *Manis* non-deciduate with simple diffuse villi *Tatusia* modified discoidal deciduate	Little known about membranes of edentates Central implantation in *Tatusia*
Rodentia	Large Invagination of upper wall gives apparent inversion of germ layers Upper wall acts as accessory placenta That of rabbits is more important nutritive organ than allantois	Rabbit amniotic posterior folds formed after embryonic shield has taken superficial place on blastoderm Mouse, rat guinea pig closed sac inside inner cell mass	Often small and endoderm does not grow out beyond body as in mouse and guinea pig	Discoidal deciduate	Implantation is central in rabbit eccentric in mouse, interstitial in guinea pig and gopher
Chiroptera	Functional only in early stage Replaced by allantois	*Vespertilio* confluence of irregular spaces in embryonic knob forms amnion	Replaces yolk sac	Discoidal deciduate saucer shaped or bell shaped	
Insectivora	Large Yolk sac placenta formed in *Tupaia* Mesoderm restricted to upper wall in *Talpa* and *Sorex* Persists until birth	Hedgehog forms a closed cavity in embryonic knob *Talpa Sorex Tupaia* formed by folds	Grows out prominently dwarfing yolk sac	Discoidal deciduate usually concave In hedgehog is decidua reflexa like that of man but formed differently In mole a contradeciduate condition (allantoic capillaries separated at birth from placental trophoblast which is absorbed by uterine walls)	Implantation eccentric (in some forms)
Primates Lemuroidea	Small yolk sac early disappears (*Tarsius* small vascular)	*Tarsius* formed by folds	Very large fills extraembryonic space *Tarsius* small outgrowth of gut remains in base of umbilical cord	Non-deciduate diffuse (*Tarsius* discoidal deciduate)	Implantation central
Anthropoidea	Small yolk sac with cavity and vascular in early stages	Amniotic cavity formed in inner cell mass	Small contained in stalk of umbilical cord	Deciduate, discoidal	Implantation interstitial

APPENDIX TO PART ONE

THE EMBRYOLOGICAL CONDITIONS OF ANIMALS FOUND IN THE VARIOUS PHYLA

✱ = Occurrence in the Phylum
× = Occurrence in Smaller Divisions of Phyla

	Egg Structure			Germ Formation		Types of Cleavage						Types of Blastulae								Types of Gastrulation								Mesoderm Formation						Larval Forms
	Homolecithal	Telolecithal	Centrolecithal	Holoblastic	Meroblastic	Radial	Disymmetrical	Bilateral	Spiral	Superficial	Discoidal	Coeloblastula		Stereoblastula	Morula	Placula	Amphiblastula	Superficial	Discoidal	Embolic	Epibolic	Unipolar	Polar Ingression		Multipolar	Delamination			Ectomesoderm	Teloblastic	Endomesoderm				
												Equal	Unequal										Many-celled			Coeloblastic	Morula	Syncytial			Secondary	Enterocoele	Solid ingrowth	Mesenchyme	
Porifera	✱	✱		✱		✱						✱		✱						✱									✱						Amphiblastula and Parenchymula
Coelenterata	✱		✱	✱		✱						✱		✱	✱					✱	✱	✱	✱		✱	✱		✱	✱						Actinula, Planula, Scyphostoma, etc.
Ctenophora	×			×			✱						×							×														
Hydrozoa	×		×	×	×	×				×				×	×							×	×		×									
Scyphozoa	×			×											×																			
Anthozoa	×		×	×	×	×				×									×	×						×	×	×						
Platyhelminthes		✱		✱					✱				✱							✱	✱								✱	✱					Muller's, Sporocyst, Miricidium, Cysticercus, Pilidium, Redia, Cercaria, Larva of Desor
Turbellaria		×		×					×				×	×							×								×					
Trematoda																																		
Nemertinea		×		×					×											×									×	×				
Nemathelminthes		✱		✱				✱				✱				✱					✱								✱						
Nematoda		×		×				×				×				×					×	×							×						
Trochelminthes		✱		✱				✱													✱								✱						Supposed to be trochophore
Rotifera		×		×				×													×								×					
Molluscoidea		✱		✱				✱				✱								✱			✱						✱					✱	Actinotrocha, Cyphonautes
Phoronida												×								×			×						×					×	
Brachiopoda				×								×	×							×									×		×	×			
Bryozoa																																		
Chaetognatha		✱		✱				✱				✱								✱												✱			Direct development
Sagitta		×		×								×								×												×			Direct development

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33

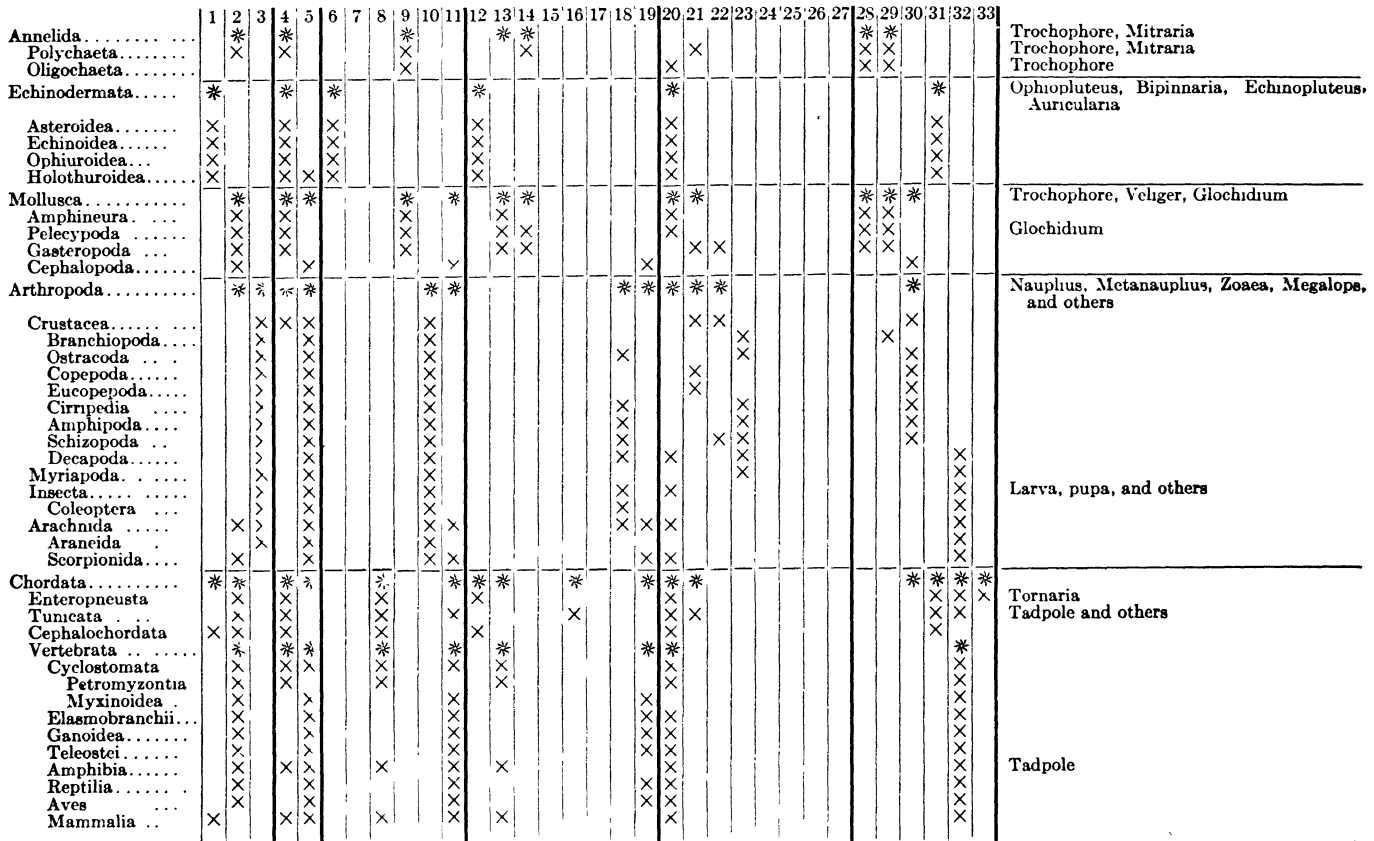

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33
Annelida
Polychaeta
Oligochaeta
Trochophore, Mitraria
Trochophore, Mitraria
Trochophore
Echinodermata
Asteroidea
Echinoidea
Ophiuroidea
Holothuroidea
Ophiopluteus, Bipinnaria, Echinopluteus, Auricularia
Mollusca
Amphineura
Pelecypoda
Gasteropoda
Cephalopoda
Trochophore, Veliger, Glochidium
Glochidium
Arthropoda
Crustacea
Branchiopoda
Ostracoda
Copepoda
Eucopepoda
Cirripedia
Amphipoda
Schizopoda
Decapoda
Myriapoda
Insecta
Coleoptera
Arachnida
Araneida
Scorpionida
Nauplius, Metanauplius, Zoaea, Megalops, and others
Larva, pupa, and others
Chordata
Enteropneusta
Tunicata
Cephalochordata
Vertebrata
Cyclostomata
Petromyzontia
Myxinoidea
Elasmobranchii
Ganoidea
Teleostei
Amphibia
Reptilia
Aves
Mammalia
Tornaria
Tadpole and others
Tadpole

TABLE OF ANIMAL CLASSIFICATION INCLUDING EXAMPLES OF EMBRYOLOGICAL SIGNIFICANCE

(NOTE: This table does not include the forms listed in certain chapters in Part Two, in which the systematic position is given in the text.)

Phylum Porifera
- Class 1. Calcarea
  - Order 1. Homocoela
    - *Leucosolenia*
  - Order 2. Heterocoela
    - *Grantia, Sycandra*
- Class 2. Hexactinellida
  - *Euplectella*
- Class 3. Demospongia
  - Order 1. Tetraxonida
    - *Geodia, Cliona*
  - Order 2. Monaxonida
    - *Spongella*
  - Order 3. Keratosa
    - *Euspongia*

Phylum Coelenterata
- Type 1. Cnidaria
  - Class 1. Hydrozoa
    - Order 1. Anthomedusae (Tubularia)
      - *Hydra, Hydractinea, Pennaria, Bougainvillia, Clava, Clavellina, Turritopsis, Eudendrium, Tubularia, Margelis, Nemopsis, Sarsia, Podocoryne, Protohydra, Microhydra, Polypodium*
    - Order 2. Leptomedusae
      - *Obelia, Campanularia, Plumularia, Sertularia, Clytia, Corymorpha Monocaudis, Gonothyrca, Tima, Aequoria*
    - Order 3. Trachymedusae
      - *Campanella, Geryonia, Trachynema, Persa, Liriope, Aeginopsis, Gonionemus, Haleremita*
    - Order 4. Narcomedusae
      - *Cunina, Cunocantha, Aegina*
    - Order 5. Hydrocorallinae
      - *Millepora, Stylaster*
    - Order 6. Siphonophora
      - *Physophora, Nanomia, Diphyes, Physalia, Porpita, Velella, Halistemma*
  - Class 2. Scyphozoa
    - Order 1. Stauromedusae
      - *Tessara, Lucernaria*
    - Order 2. Peromedusae
      - *Pericolpa, Periphylla*

Order 3. Cubomedusae
Charybdea
Order 4. Discomedusae
Aurelia, Pelagia, Cassiopea, Cyanea, Ulmaria, Stomolophus, Polyclonia
Class 3. Anthozoa (Actinozoa)
Subclass 1. Alcyoniaria
Order 1. Stolonifera (Tubiporidae)
Tubipora
Order 2. Alcyonacea
Alcyonium
Order 3. Gorgonacea
Corallium
Order 4. Pennatulacea
Pennatula, Renilla
Subclass 2. Zoantharia (Hexacoralla)
Order 1. Edwardsiidae
Edwardsia
Order 2. Actinaria
Metridium, Halcampa, Paranemonia, Bunodes, Sagartia, Tealia, Bicidium, Epizoanthus
Order 3. Madreporaria
Sclerophylla, Oculina, Astrangia, Astrea, Faira, Fungia, Madrepora, Porites, Astroides, Meandrina
Order 4. Antipatharia
Antipathes, Gerardia
Order 5. Zoanthidea
Order 6. Cerianthidea
Cerianthus
Type 2. Ctenophora
Class 1. Tentaculata
Callianira, Pleurobrachia, Hormiphora, Mnemiopsis, Bolina, Cestus
Class 2. Nuda
Beröe, Idyia
Phylum Platyhelminthes
Subphylum 1. Platoda
Class 1. Turbellaria
Order 1. Rhabdocoelida
Microstoma, Vortex, Monosceles, Monops, Stenostoma
Order 2. Tricladida
Polyscelis, Planaria, Dendrocoelum, Bipalium, Bdelloura, Gunda, Polychaerus, Syncoelidium, Phagocata
Order 3. Polycladida
Leptoplana, Stylochus, Thysanozoon, Discocoelis, Planocera, Yungia
Class 2. Trematoda
Order 1. Monogenea (Polystomea, Heterocotylea)

Polystomum, Sphyranura, Epibdella, Gyrodactylus, Diplozoon, Tristoma, Microcotyle

Order 2. Digenea (Distomeae)

Distomum (Fasciolaria), Monostomum, Bilharzia, Clonorchis, Paragonimus

Class 3. Cestoda

Caryophyllaeus, Archigetes, Ligula, Tetrarhynchus, Echinobothrium, Acanthobothrium, Bothriocephalus, Taenia, Anoplocephala, Moniezia, Dipylidium

Subphylum 2. Nemertinea

Class 1. Nemertea

Order 1. Protonemertini

Carinella

Order 2. Mesonemertini

Cephalothrix

Order 3. Metanemertini

Geonemertes, Tetrastemma, Amphiporus, Malacobdella, Nectonemertes

Order 4. Heteronemertini (Schizonemertini)

Lineus, Micrura, Cerebratulus, Zygeupolia

Phylum Nemathelminthes

Class 1. Nematoda

Ascaris, Ancylostoma, Trichinella, Filaria

Class 2. Gordiacea

Gordius

Class 3. Acanthocephala

Echinorhynchus

Phylum Trochelminthes

Class 1. Rotifera

Philodina, Hydatina

Phylum Molluscoidea

Subphylum 1. Podaxonia

Class 1. Phoronida

Phoronis, Echiurus, Bonellia

Class 2. Gephyrea

Sipunculus

Subphylum 2. Polyzoa

Class 1. Entoprocta

Urnatella, Pedicellina, Loxosoma

Class 2. Ectoprocta

Order 1. Gymnolaemata

Gemmellaria, Bugula, Flustra, Flustrella, Eschara, Crisia, Tubulipora, Alcyonidium, Valkeria, Paludicella

Order 2. Phylactolaemata

Plumatella, Pectinatella, Cristatella

Subphylum 3. Brachiopoda

Terebratula, Terebratulina, Lingula, Waldheimia, Rhynchonella

Supplementary group of uncertain position, showing some relations to Enteropneusta:

Pterobranchia

Cephalodiscus, Rhabdopleura

Phylum Chaetognatha

Sagitta, Spadella

Phylum Annelida

Class 1. Archiannelida

Polygordius

Class 2. Chaetopoda

Subclass 1. Polychaeta

Order 1. Phanerocephala

Nereis, Podarke, Aphrodite, Lepidonotus, Autolytus, Polynöe, Diopatra, Nothria, Eunice, Syllis, Trypanosyllis

Order 2. Cryptocephala

Eupomatus, Hydroides, Spiroides, Arenicola, Amphitrite, Terebella, Sabella

Subclass 2. Oligochaeta

Order 1. Microdrili (Limicola)

Tubifex, Dero, Nais, Sparganophilus, Lumbriculus, Ctenodrilus, Branchiobdella

Order 2. Macrodrili (Terricola)

Lumbricus, Allolobophora, Diplocardia

Class 3. Hirudinea

Order 1. Gnathobdellidae

Hirudo, Macrobdella

Order 2. Rhynchobdellidae

Clepsine, Pontobdella, Piscicola

Phylum Echinodermata

Class 1. Asteroidea

Asterias, Asterina, Astropecten, Heliaster, Pythonaster, Asteriscus, Culcita, Hippasteria, Ctenodiscus

Class 2. Ophiuroidea

Ophiura, Ophiothrix, Ophiopholis, Amphiura, Astrophyton

Class 3. Echinoidea

Cidaris, Arbacia, Toxopneustes, Strongylocentrotus, Coelopleurus, Spatangus, Echinocardium, Brissus, Echinarachnius, Clypeaster, Mellita

Class 4. Holothuroidea

Holothuria, Cucumaria, Psolus, Thyone, Caudina, Molpadia, Synapta

Class 5. Crinoidea

Rhizocrinus, Pentacrinus, Comatula, Antedon

Phylum Mollusca

Class 1. Amphineura

Order 1. Placophora (Chitonida)

Amicula, Trachydermon, Chiton, Ischnochiton, Cryptochiton

Order 2. Aplacophora (Solonogastres)
Proneomenia, Conchoderma, Chaetoderma, Dondersia
Class 2. Scaphopoda
Dentalium
Class 3. Pelecypoda (Lamellibranchiata, Acephala)
Order 1. Protobranchia
Nucula, Leda, Yoldia, Solenomya
Order 2. Filibranchia
Arca, Mytilus, Modiola, Anomia, Trigonia
Order 3. Pseudolamellibranchia
Pecten, Ostrea, Meleagrina, Lima, Pinna
Order 4. Eulamellibranchia
Anodonta, Unio, Pisidium, Cyclas, Cardium, Astarte, Mya, Pholas, Teredo, Aspergillum, Venus, Mactra, Tellina, Solen, Dreissensia
Order 5. Septibranchia
Silenia, Cuspidaria, Poromya
Class 4. Gasteropoda
Order 1. Prosobranchia
Suborder 1. Aspidobranchia
Acmaea, Patella, Haliotis, Margarita, Trochus
Suborder 2. Pectinibranchia
Littorina, Strombus, Sycotypus, Busycon (Fulgur), Crepidula, Urosalpinx, Murex, Paludina, Carinaria, Pleurobranchidium
Order 2. Opisthobranchia
Suborder 1. Tectibranchia
Bulla, Philine, Aplysia, Haminea, Umbrella
Suborder 2. Pteropoda
Cymbiliopsis, Hyalaea, Clione
Suborder 3. Nudibranchia
Doris, Asolidia, Fiona, Staurodoris
Order 3. Pulmonata
Helix, Bulimus, Limax, Lymnaea, Physa, Planorbis
Class 5. Cephalopoda
Order 1. Tetrabranchia
Nautilus
Order 2. Dibranchia
Suborder 1. Decapoda
Spirula, Ommastrephes, Architeuthis, Loligo, Sepia
Suborder 2. Octopoda
Octopus, Allopasus
Phylum Arthropoda
Class 1. Crustacea
Subclass 1. Entomostraca
Order 1. Phyllopoda
Suborder 1. Branchiopoda
Branchipus, Artemia, Apus

Suborder 2. Cladocera
Daphnia, Simocephalus, Leptodora, Polyphemus
Order 2. Ostracoda
Cypris
Order 3. Copepoda
Suborder 1. Eucopepoda
Cyclops, Lernaea
Suborder 2. Branchiura
Argulus
Order 4. Cirrepedia
Suborder 1. Eucirripedia
Balanus, Lepas
Suborder 2. Rhizocephala
Sacculina
Subclass 2. Malacostraca
Superorder 1. Leptostraca
Qrder 1. Phyllocarida
Nebalia
Superorder 2. Arthrostraca
Order 1. Amphipoda
Gammarus, Caprella
Order 2. Isopoda
Asellus, Porcellio
Superorder 3. Thoracostraca
Order 1. Cumacea
Diastylis
Order 2. Stomatopoda
Squilla
Order 3. Schizopoda
Mysis
Order 4. Decapoda
Suborder 1. Macrura
Palaemonetes, Crangon, Penaeus, Lucifer, Astacus, Homarus
Suborder 2. Brachyura
Dromia, Cancer
Class 2. Onychophora
Peripatus
Class 3. Myriapoda
Order 1. Pauropoda
Pauropus
Order 2. Diplopoda
Julus
Order 3. Chilopoda
Lithobius, Scutigera, Scolopendra
Order 4. Symphyla
Scutigerella

Class 4. Insecta

Macrotoma, a thysanuran; *Hydrophilus*, the water beetle; *Miastor*, the fly; *Leptinotarsa*, the potato beetle; *Copidosoma*, parasitic hymenoptera

Class 5. Arachnida

Order 1. Araneida

Epeira

Order 2. Scorpionidea

Buthus, Euscorpius

Order 3. Phalangidea

Phalangium

Order 4. Acarina

Lepas, Ixodes

Order 5. Pedipalpi

Tarantula

Order 6. Palpigradi

Kaenenia

Order 7. Solpugida

Eremobates

Order 8. Pseudoscorpionida

Chelifer

Order 9. Xiphosura

Limulus

Order 10. Eurypterida

Eurypterus

Supplementary Arachnid groups of doubtful relationship

Pantapoda, Tardigrada, Pentastomidea (Linguatulina)

Phylum Chordata

Subphylum 1. Enteropneusta

Balanoglossus, Dolichoglossus, Harrimania

Subphylum 2. Tunicata

Cynthia, Ciona, Molgula, Appendicularia, Distaplia, Botryllus, Salpa, Pyrosoma, Doliolum

Subphylum 3. Cephalochordata

Amphioxus, Branchiostoma

Subphylum 4. Vertebrata

Series 1. Anamnia

Class 1. Cyclostomata

Order 1. Myxinoidea

Myxine, Bdellostoma

Order 2. Petromyzontia

Petromyzon, Lampetra

Class 2. Pisces

Subclass 1. Elasmobranchii

Superorder 1. Selachii

Squalus, Mustelus, Galeus, Raia, Acanthias, Scyllium, Carcharias, Cestracion, Hexanchus, Notodanus, Pristiurus, Torpedo

Superorder 2. Holocephali
Chimaera
Subclass 2. Ganoidea
Amia, Acipenser, Lepisosteus (Lepidosteus)
Subclass 3. Teleostei
Serranus, Ctenolabrus, Belone, Salmo, Fundulus, Perca
Subclass 4. Dipnoi
Ceratodus, Protopterus, Lepidosiren
Class 3. Amphibia
Subclass 1. Stegocephalia
Subclass 2. Lissamphibia
Order 1. Apoda (Gymnophiona)
Dermophis, Coecilia
Order 2. Urodela
Fam. 1. Proteidae
Necturus, Proteus, Typhlomolge
Fam. 2. Sirenidae
Siren, Pseudobranchus
Fam. 3. Amphiumidae
Amphiuma, Cryptobranchus
Fam. 4. Salamandridae
Ambystoma (Amblystoma, Siredon), Diemyctylus, Salamandra, Triton, Plethodon, Eurycea (Spelerpes), Desmognathus
Order 3. Anura
Bufo, Rana, Pseudacris (Chorophilus), Hyla, Pipa, Alytes
Series 2. Amniota
Division 1. Sauropsida
Class 4. Reptilia
Order 1. Rhynchocephalia
Sphenodon
Order 2. Squamata
Suborder 1. Sauria (Lacertilia)
Gecko, Draco, Anolis, Lacerta, Varanus, Iguana
Suborder 2. Serpentis (Ophidia)
Python, Boa, Thamnophis, Hydrophis, Elaps, Vipera
Order 3. Loricata (Crocodilia)
Alligator, Crocodilus, Gavialis
Order 4. Testudinata (Chelonia)
Testudo, Chrysemys, Clemmys, Chelone
Class 5. Birds
Division 2. Mammalia
Subclass 1. Prototheria
Order 1. Monotremata
Echidna, Ornithorhynchus
Subclass 2. Eutheria
Division 1. Didelphia

Order 1. Marsupialia
Didelphys, Perameles, Dasyurus, Macropus, Phascolomys, Phascolarctus
Division 2. Monodelphia (Placentalia)
Section A. Unguiculata (Clawed Mammals)
Order 1. Insectivora
Sorex, Talpa, Erinaceus, Tupaija
Order 2. Dermoptera
Galeopithecus
Order 3. Chiroptera
Vespertilio, Pteropus, Desmodus, Myotis
Order 4. Carnivora
Suborder 1. Fissipedia
Canis, Hyaena, Procyon, Felis
Suborder 2. Pinnipedia
Odobalnus
Order 5. Rodentia
Lepus, Sciurus, Mus, Cavia
Order 6. Edentata
Bradypus, Dasypus, Tatusia
Order 7. Pholidota (Lepidota)
Manis
Order 8. Tubulidentata
Orycteropus
Section B. Primates
Order 9. Primates
Suborder 1. Lemuroidea
Lemur, Tarsius
Suborder 2. Anthropoidea
Cebus, Ateles, Simia, Gorilla, Cynocephalus, Homo
Section C. Ungulata (Hoofed Mammals)
Order 10. Artiodactyla
Bos, Camelus, Sus, Dicotyles, Hippopotamus, Cervus
Order 11. Perissodactyla
Equus, Tapirus, Rhinoceros
Order 12. Proboscidea
Elephas, Loxodonta
Order 13. Sirenia
Manatus, Halicore
Order 14. Hyracoidea
Procavia (Hyrax)
Section D. Cetacea
Order 15. Odontoceti
Dolphin, Delphinus, Phocaena, Grampus
Order 16. Mystacoceti
Balaena

PART TWO

EMBRYOLOGICAL PROBLEMS

CHAPTER I

THE ORIGIN AND DEVELOPMENT OF GERM CELLS

A problem of very considerable embryological interest is that of the origin and subsequent history of the germ cells. This is a matter of great theoretical interest because of its bearing on the Weismannian doctrine of the continuity of the germ plasm, and one which has been the cause of much controversy, at least as regards certain of its aspects. There are really three important phases of the problem; the place of origin of the germ cells, the path of their migration to the definitive gonads, and the subsequent history after reaching the germ glands.

A. Vertebrates

In vertebrates the gonads arise from the genital ridges which develop on the dorsal side of the body cavity between the mesentery and the beginning kidney. This ridge is covered by the epithelium of the coelome or peritoneum, which is known as "germinal epithelium." From the differentiation of this germinal epithelium in the four-day chick embryo Waldeyer (1870) first described the beginning of the sex cells or germ cells. Waldeyer's view was accepted by the workers of his time as an adequate explanation of the origin of the germ cells and has been given as the only explanation in all of the text-books until quite recently. It is, moreover, supported by the observations of a school of investigators of the present day. It is, of course, true that with the formation of the gonad the germ cells are to be found in that organ and they appear to have arisen from its epithelial covering.

In 1880 Nussbaum, as a result of his observations on the development of the trout and the frog, reached the conclusion that the germ cells were of blastomeric origin, being segregated in the early stages of development in a region outside that which will see the formation of the embryonic body. They actively migrate or else are carried into the body proper and into the germ gland by the growth forces which operate to give the organism its body form. Since Nussbaum's time the evidence has steadily accumulated that in many vertebrates, as in well-known cases among the invertebrates, the germ cells are early set apart from the somatic cells and develop independently from them. Their history may be traced backward from their appearance in the germ gland to

very early stages and the path of their migration clearly made out in many cases. Many careful investigations (Hoffmann, 1892; Eigenmann, 1892; Beard, 1900; Woods, 1902; Allen, 1906, 1907, 1911; Dodds, 1910; Swift, 1914, 1915, 1916; Jordan, 1917; Okkelberg, 1921; Richards and Thompson, 1921; Swingle, 1926; Hahn, 1927) have failed to reveal any conditions not in accord with Nussbaum's theory. It has been demonstrated that the primordial germ cells arise widely scattered in the

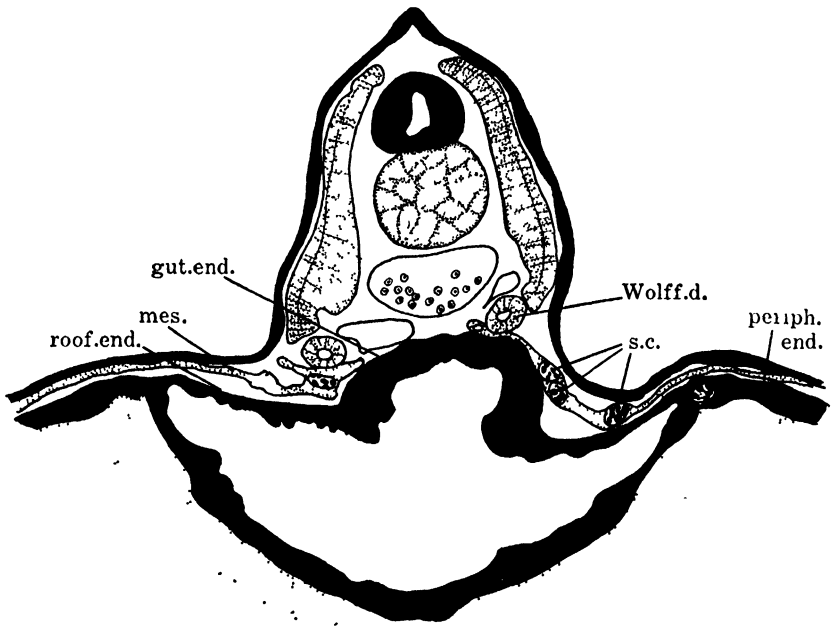


Fig. 182. Transverse section through a 4 mm. larva of the bowfin *Amia calva*. (After Allen.)

Gut end., gut endoderm; roof end., roof endoderm; periph end., peripheral endoderm; Wolff d., Wolffian duct; s c., sex cells; lat mes., lateral mesoderm.

endoderm or in the splanchnic mesoblast in the extraembryonic area. From this point of origin they pass with progressive development to the region of the body axis. This movement has been accounted for in three ways; it is usually thought that the cells actively migrate in an amoeboid manner, passing up the mesentery and into the region of the gonad. Swift found that the primordial germ cells of the chick are carried in the blood stream, and in *Fundulus* it has been shown that the forces which are responsible for the formation of the embryo body carry the germ cells to their place. The movement of the cells of the germ ring by confluence and the other normal processes which lead to

the formation of the axial region of the body brings about the translocation of the germ cells as well as the somatic cells. Along the path of migration there is no difficulty in recognizing the germ cells for they have clearly marked characteristics. On these points there is rather general agreement.

The subsequent history of these cells appears to be an uncertain problem—one much involved at the present time in controversy. Some investigators claim that some of these cells never find their way into the gonad, but undergo degeneration in various parts of the body. Some (Firket, von Berenberg-Gossler, Hargitt, and others) claim also that the primordial cells which may have arrived in the gonad by

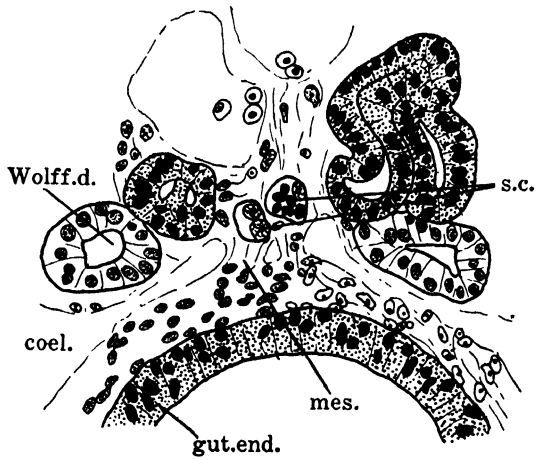


FIG. 183. Transverse section through the hind gut of 9.1-mm. larva of *Amia calva*. (After Allen.)

coel., coelomic cavity; gut end., gut endoderm; mes., mesentery; s.c., sex cells; Wolff.d., Wolffian duct.

migration in the manner indicated do not actually give rise to the definitive germ cells, for they find degeneration of these cells along with proliferation of a second group of germ cells derived directly from the genital epithelium. These latter, or even in certain cases a third proliferation, are the source of the definitive germ cells. The primordial germ cells in these cases may pass far through their cycle before degenerating, as shown by Kingery for the mouse and Swingle for the tadpole, in both of which the maturation cycle is practically completed before the degeneration occurs.

In a recent account Swingle has reviewed the progress of the investigations on the Anura. He has shown from embryological studies that there are in various parts of the United States "local races which differ

markedly in regard to the time of occurrence and character of the developmental processes involved in the formation of the definitive testes." He is convinced that the sex cells appear first in the endoderm, and give origin to those cells which produce ripe sex products. Every stage from the origin of the primordial sex cells to the fully developed spermatozoa has now been traced in the bull frog, and no doubt now remains as to the continuity of the germ line in this form. On the other hand Hargitt and others have claimed that the germ cells arise in urodeles from differentiated mesoderm, the peritoneum. Swingle suggests that the discrepancy is due to an origin and segregation earlier than their appearance in the peritoneum, and that after their segregation they wait in the layer where they are later seen for an appropriate time for development. This is in agreement with his finding that local races differ in the time of development of the sex cells. In the urodeles they are incorporated in the mesoderm in an early stage whereas in the Anura they are in the endoderm, but it is not necessary to assume their origin in either layer because of the fact that later they appear in the one or the other.

It is to be noted that none of the writers who find degeneration have satisfactorily proven that all the primordial germ cells degenerate or that none of them are left to produce definitive germ cells. It is also noteworthy that recently certain evidence has been found which indicates that in an ordinary course of events the definitive germ cells are derived from the primordial cells. However, owing to the power of regulation which animals possess under the stress of unusual and harmful circumstances which bring about the degeneration of the primordial cells, secondary sex cells may be proliferated from the germinal epithelium and produce the definite sperms or ova. At the present time the safest interpretation seems to be that as a rule the primordial germ cells thus early segregated in the vertebrates give rise to the definitive germ cells, but that in exceptional cases, where the regular events are interrupted and the primordial cells so disturbed as to cause their degeneration, the organism responds by proliferating from the germinal epithelium new cells which take their place. It must never be forgotten that the chief characteristic of protoplasm, especially embryonic protoplasm, is its plasticity, its power of regulation and of adaptation to new conditions. "Life is a constant adjustment of internal and external conditions."

Although the matter is still involved in controversy, some important theoretical conclusions have already resulted from the investigations. First of all, it is an obvious fact of major importance that heredity is a matter of germ cells, and that the germ cells contain organized material

through which the continuity from generation to generation is carried on. The germ cell mechanism is the mechanism of inheritance. Not only is there continuity of germ cells but of the organized system (germ plasm) which they convey. Secondly, the distinction between germ cells and somatic cells is often more or less artificial and has in many cases been emphasized unduly. Weismann himself spoke with less certainty about the continuity of germ plasm than many of his followers have done, although he without doubt was expressing a fundamental truth. Yet there still remained a theoretical question which his formulation of his doctrine did not fully state, namely, the possibility of the soma contributing to or becoming part of the germ plasm. Various views on this point have already been given.

It is the author's opinion that with the possible exception of the mammals no group has as yet presented evidence of positive character that contradicts the view that germ cells as well as germinal material are distinct from the somatic from a very early period. In the mammals the situation is not yet clear. It is worthy of note that the cases in which uncertainty or confusion exists are exactly those which have proven least satisfactory for cytological and accurate embryological studies. When favorable material is available there is general conformity to the expectations of those who would emphasize the importance of the germ plasm. These include cases of determinative cleavage where the germ-cell cycle has been followed in case after case, and also those which have yielded clear figures for chromosome studies.

If in addition the recent view be accepted that the chromatin of all cells represents the germ plasm and the cytoplasm the soma, as is now held by numbers of students, the history of the germ cells becomes a study of the specialization of chromatin and nuclei and that of the soma one of cytoplasmic specialization. The general problem of the germ-cell cycle is thus given its proper place as one of embryology, where it is of great interest, and it is no longer one of theoretical significance to geneticists.

B. The Invertebrates

In the invertebrates there are many forms in which no such uncertainty prevails as in the case of the vertebrates. There are two chief methods by which germ path is recognizable: *i.e.*, chromatin diminution and germ-cell determinants. There are of course many cases in which nothing is known of the development of the germ cells, but there are in several groups of the invertebrates cases in which the germ path (Keimbahn) has been worked out in practical completeness. In the coelenterates primordial germ cells were found by Weismann, Kleinen-

berg, and others in a wandering condition early in the development of hydroids. The details of Weismann's observations have been disputed by Hargitt, but his main contention is borne out that the germ cells are recognizable long before the sexual individuals appear. The case

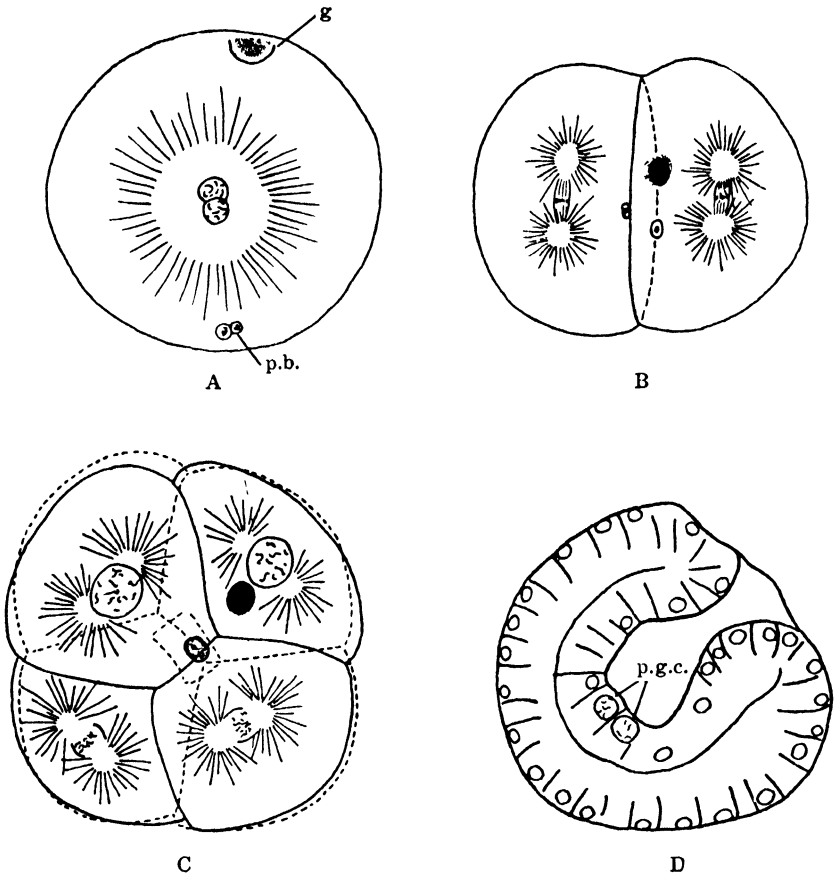


FIG. 184. The development of the germ cells of *Sagitta*. (After Elpatiewsky.)

A, fertilized egg showing the "germ cell determinant," B, 2-cell stage, germ cell determinant in one blastomere only; C, 8-cell stage; D, gastrula with two primordial germ cells.

g., germ cell determinant; p.b., polar bodies; p.g.c., primordial germ cells.

which has been of greatest interest from the standpoint of the history of the germ cells is that of *Ascaris megalocephala* as brilliantly worked out in great detail by Boveri. In *Ascaris* (see Chapter IV, Section 3) the first cleavage division results in two blastomeres, one of which is purely a somatic cell while the other is a stem cell. The history of each

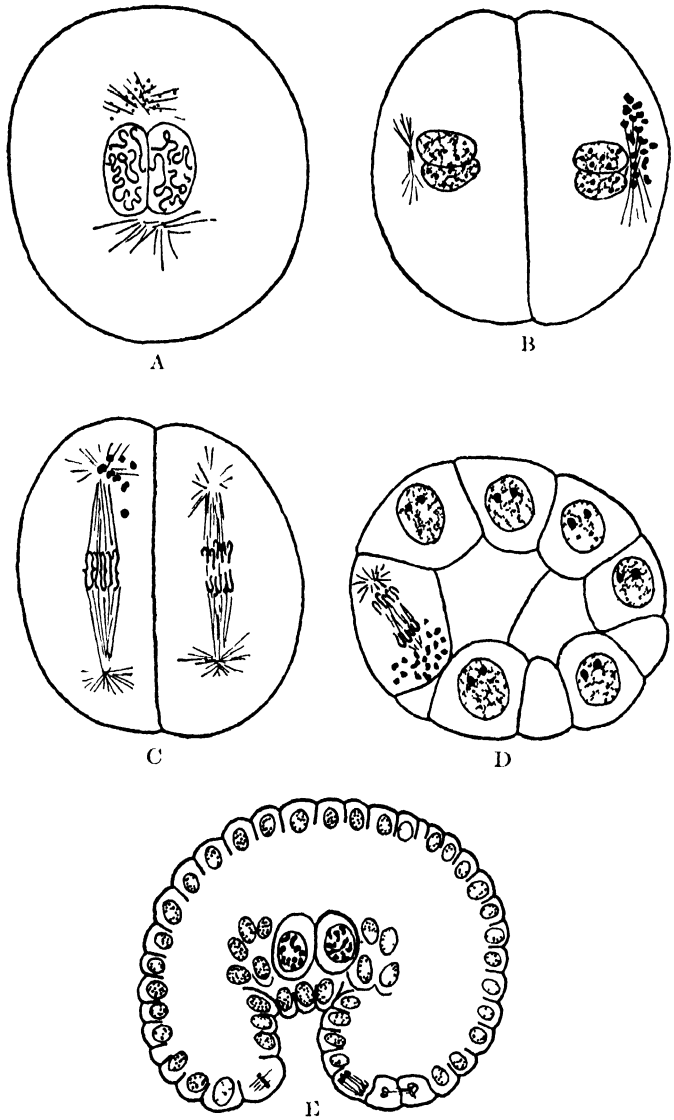


FIG 185 Early stages in the development of *Cyclops fuscus* (After Amma)

A, first cleavage nucleus, B, daughter nuclei reconstructed with the ectosomes or germ cell determinants in one blastomere only, C, second cleavage division with the germ cell determinants again limited to one blastomere, D, 16-cell stage All the blastomeres in the resting condition except the stem cell, E, gastrula in which two primordial germ cells are to be distinguished from the endoderm cells.

of these cells has been fully described by Boveri. They are distinguishable by means of a process known as chromatin diminution, part of the chromatin (the thick ends of the chromosomes) of the somatic cell being cast from the nucleus into the cytoplasm, because of which process the

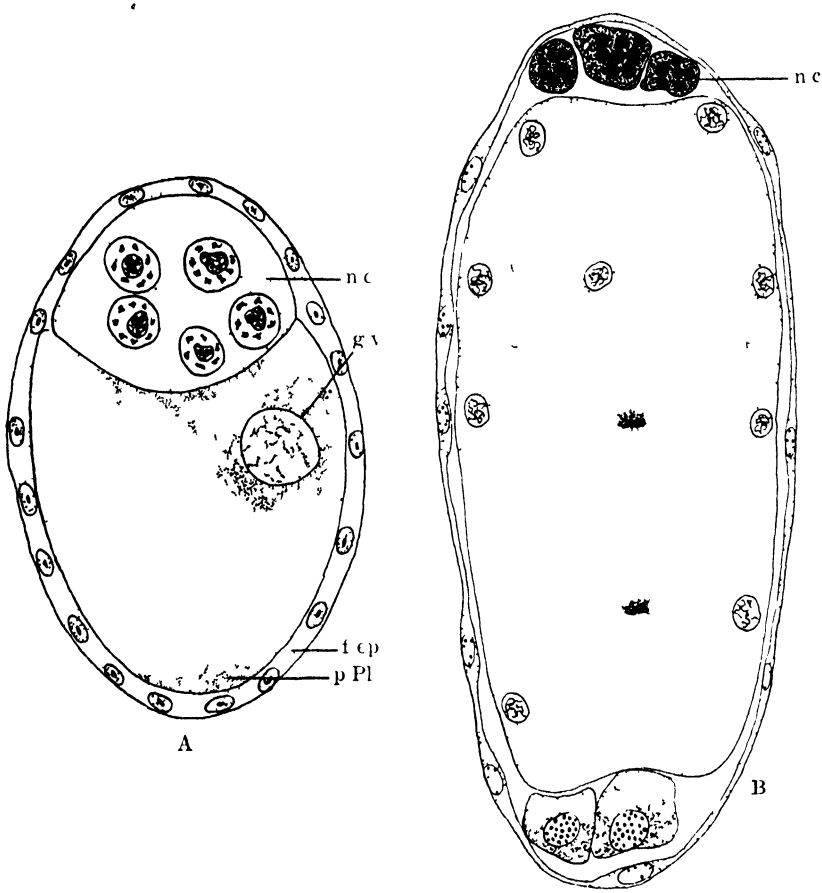


FIG. 186 A, longitudinal section through an oocyte of *Miastor americana* (After Hegner) n c, nurse chamber, g v, germinal vesicle, p Pl, pole plasm, f ep, follicular epithelium B, later stage of same in which somatic cells are beginning to form and blastoderm and the two primary germ cells containing the pole plasm are already cut off

somatic nuclei in subsequent cell generations are both lighter in color and smaller than the germ cells. All the chromatin of the stem cell is retained in this second division, but of the daughter blastomeres produced from it, one undergoes the diminution process while the other divides directly in the regular manner and becomes again a stem cell. Of the products of its division the same relation holds, for the process

is repeated four times. Thus, at the end of the fourth cleavage there are fifteen somatic cells (which will give rise only to somatic cells ordinarily) and one cell which is the primoridal germ cell and the ancestor of all the germ cells. At the next division this divides again

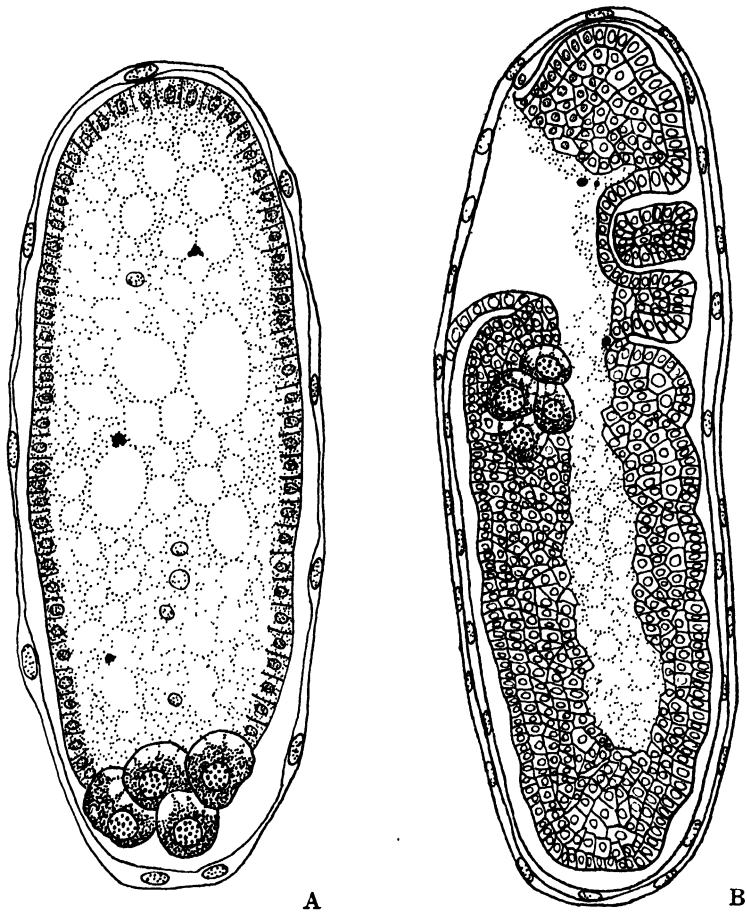


FIG. 187. Continuation of Fig. 186. A, similar stage of eight germ cells, blastoderm complete; B, sagittal section through embryo with eight germ cells near the end of the tail fold.

and the two cells thus produced give rise later on in the interior of the embryo to all its sex cells.

In *Sagitta*, Elpatiewsky recognized in the fertilized egg a structure, called a "germ-cell determinant," which does not divide with the egg. (Fig. 184.) The blastomere containing it becomes a stem cell. In the fifth division the stem cell produces a somatic cell and a primordial germ cell.

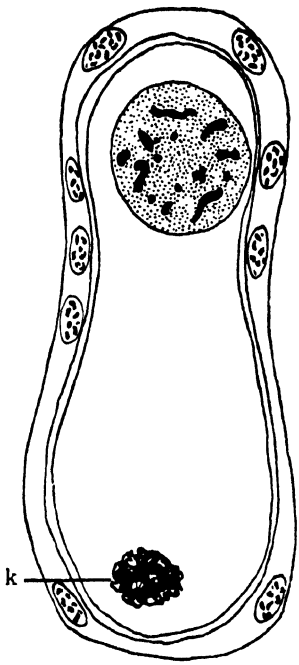


FIG. 188. Longitudinal section through ovarian egg of *Copidosoma* almost ready to be laid. (After Hegner.)

The germ cell determinant (k) lies near the posterior end of the egg.

In an early gastrula four cells have resulted from two divisions of the primordial cell. The subsequent divisions of the four are responsible for the formation of both male and female germ cells (for the animal is hermaphroditic). It is said that the two anterior give rise to the ovary and the two posterior to the testes.

Among the arthropods there are many cases of the early segregation of germ cells, and their recognition is possible either through cytoplasmic inclusions (germ-cell determinants) or by distinctive characteristics, particularly chromatin diminution. In the Entomostraca are two outstanding cases, *Moina* (Grobben) and *Cyclops* (Haecker, Amma). Here the stem cells are distinguished from the first cleavage onwards. In the unsegmented egg of *Cyclops* (Fig. 185) certain granules (ectosomes) are to be found accumulating at one pole of the spindle as the cleavage mitosis advances. These granules are thus segregated into one of the blastomeres which becomes the stem cell. This process continues through the fourth division and results in the segregation of the primordial germ cells.

In the insects a germ path is recognizable in several orders. In the Diptera (*Miastor*, Kahle, 1908, Hegner, 1914, *Chironomus*, Hasper, 1911, and in other cases) the germ cells appear at the posterior end of the egg as pole cells and can be traced into the larval gonads. Here polar granules, germ-cell determinants, or pole plasm, as the mass is variously called, are present in the uncleaved egg and enter the pole cells. In addition to this in *Miastor* (Figs. 186, 187) the pole cells are provided with two nuclei from the third cleavage which have not undergone chromatin diminution as distinguished from the other six. In the Hymenoptera (Fig. 188) an "oösome" is present in

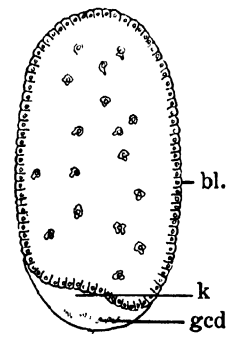


FIG. 189. Longitudinal section through the egg of *Leptinotarsa decimlineata*. (After Hegner.) The posterior end was killed with a hot needle just after the egg was laid. The egg was then allowed to develop for 24 hours. bl., blastoderm; g.c.d., germ cell determinants; k., portion of the egg killed.

the unsegmented egg which in the 4-cell stage becomes associated at the posterior end with the forming primordial germ cells. Finally, in the chrysomelid beetles (Fig. 189), Hegner found a "polar disc" composed of germ-cell determinants which participate in the formation of the primordial germ cells. This relationship was made more certain by an experiment which he performed. With a hot needle he killed the posterior end of the egg, but allowed the remainder of the egg to develop. A blastoderm was formed which was normal in every particular (up to a certain stage) except that it was entirely lacking in germ cells.

These cases prove conclusively that among many invertebrates we may regard the early segregation of the germ cells as satisfactorily established. In addition to these there are many cases of cell lineage in which there is no question but that the germ cells arise from blastomeres segregated at a quite early stage. It is to be noted also that those cases are among the best known of all animals, the material being abundant and most favorable for the study of entire life cycles.

CHAPTER II

GERM-LAYER THEORY

It will be recalled that the old issue between preformation and epigenesis finally resulted in the overthrow of the cruder type of preformation which held that development was simply an unfolding of parts already existing in egg or sperm. As better understanding of the facts of development was obtained it became clear that, although preformation did not occur, there were definite signs of organization within the egg. The developing organs could be traced farther and farther back until it was evident that in general they came from what might be called three plates or layers of tissue lying one over the other. These layers give rise by various processes of folding, outpocketing, unequal growth, etc., to the fundaments of the future organs. It was further obvious that there is a great similarity in regard to this method of origins from these layers in different kinds of embryos. These facts were known in whole or in part to many of the early embryologists, including von Baer (1828), and others, even back to the time of Wolff (1768). Of course the proper interpretation of cells and cell structure in the animal kingdom had not as yet been made and it had proven impossible to explain the origin of the layers in terms of any other lower units of organization. It is not surprising, therefore, that these early embryologists should have looked upon the formation and subsequent development of the germ layers as the most critical processes of embryonic life.

The germ-layer theory, which is distinctly an attack on the problem of the organization of the embryo, has been one of the most fruitful of all biological speculations. After the true nature of cells and their methods of reproduction and growth came to be understood, the theory acquired new significance and, in the hands of Oscar and Richard Hertwig, had attained the status of a full-fledged theory of development by the early eighties. Through a great variety of forms, blastulae and gastrulae were identified, their methods of origin understood, and the remarkably uniform character of all the processes involved in germ-layer formation and in the laying down of the fundaments of the organs was made out. Exceptions occur; indeed, some of them are of great importance, but considered as a whole there is striking uniformity in

the relationships of the layers to each other and to the subsequently developing organs.

The essential point of the theory is that the three layers are homologous throughout the animal kingdom above the Porifera. In the coelenterates, as in the gastrulae, there are present only ectoderm and endoderm. Since the layers are homologous, the organs which arise from them are also homologous wherever they are found.

In all metazoa there normally develops following the blastula, a stage called the gastrula, of which the two component layers are known as primary germ layers, the outer being the primary ectoderm (epiblast, ectoblast), and the inner the primary endoderm (hypoblast or endoblast). The primary ectoderm in etenophores, turbellarians, rotifers, annelids, and molluscs contributes with the primary endoderm to the formation of the middle germ layer, the mesoderm (mesoblast) giving rise to the distinction of ectomesoderm and endomesoderm respectively. From the outer layer also come: (*a*) the covering tissues with all their modifications and appendages including hair, horns, nails, scales, skin, glands, (*b*) the nervous system and sensory epithelium, and (*c*) in many cases the stomodaeum and proctodaeum (extreme anterior and posterior regions of the alimentary canal).

The primary endoderm, in addition to endomesoderm, produces the lining of the midgut and of all the organs which are derived from it, as pancreas, liver, etc. There is also exceptionally endoderm participation, for example, in the formation of a part of the nervous system in some coelentrates, but in some other cases, as in formation of blood cells and endothelium of blood vessels, the evidence is not yet clear as to whether it takes part.

The mesoderm may come, as already indicated, from the two primary germ layers, but except as mentioned above, only the endoderm seems to be involved in its production. There are several methods of mesoderm formation. Isolated endoderm cells may push into a space between the two layers at the same time with the secretion of a jelly-like substance, thus giving rise to a filling-in layer known as mesenchyme from which certain organs or parts of organs take their origin. In a second method the typical epithelial character of the primary germ layers is preserved and by outfoldings from the walls of the archenteron (primitive gut), which becomes entirely disconnected from it, lateral coelomic pouches are formed. This type of mesoderm is known as mesothelium. There are also other processes by which coelomic walls may in special cases be produced. The inner or splanchnic wall of the coelomic pouch unites with the endoderm to form the splanchnopleure, and the outer or somatic layer forms with the ectoderm the somatopleure. In some

animals the middle germ layer is entirely mesenchymous; in others, entirely mesothelial; and in many, both types are present. Either one may develop first; in the echinoderms the mesenchyme arises before the mesothelium and in the vertebrates after it. The mesoderm gives rise to muscles, blood, skeletal and connective tissues, the excretory organs (at least in part) and usually to the sexual organs, but not the sex cells.

The surprising uniformity with which these relationships are held throughout the higher groups of animals has been responsible for the great importance of the germ-layer theory as outlined by the Hertwigs. Probably no other theory or working hypothesis, with the exception of Darwin's own contributions, has been so fruitful as a stimulus for constructive zoological work. The germ-layer theory has to its credit much of the most important embryological accomplishment. As a working hypothesis it is of the greatest importance.

As an analysis of the problem of organization of the embryo, less can be said for the germ-layer theory. Researches of later years with careful technique have shown that the exceptions which formerly were overlooked are in many cases difficult to harmonize with the theory and, indeed, often operate decidedly to limit its usefulness.

Among the objections that have been cited are the following: inverse relationships of the layers found in the sponges; the fact that the outer layers of the cestodes are in part or *in toto* thrown off leaving the covering tissues doubtfully related to the ectoderm; the varying derivation (depending upon the viewpoint of the observer) of the notochord and mesenchymous connective cells in different chordate groups; the composite nature of some organs, for example the nephridia, from more than one germ layer; the identification of the mesoderm as the 4*d* cell, not a layer at all, in the individuals with spiral cleavage; and especially the facts of budding, differentiation, and regeneration. Some of these objections have proven difficult to answer although this fact should not make us underestimate the value of this conception as a whole. However, it is now known that there are many evidences of organization appearing long before germ layers are formed. The promorphology of the ovum in the eggs with determinative cleavage and the possibility of tracing out the cell lineage during cleavage and organ formation in embryos of this class distinctly minimize the importance of the germ layers. Back of this the organization of the chromosomes and their part in shaping development as is now known from studies of cytology and genetics again detract from the value of the theory.

We have now come to view the germ layers as representing a stage in development just as we regard the blastula or gastrula as progressive

steps. It is an essential stage in most cases through which the course of development must pass, but in special cases adaptive modifications have arisen. Homologies exist between blastomeres—sometimes, as in the 4*d* cell and the "cross" of the annelids and molluscs, of very striking character; they also exist between germ layers, between organs and between organ systems. All are important, perhaps equally so. But the germ layers are probably best regarded as temporary embryonic organs which play their part and give rise to subsequent stages of development rather than as units of organization which determine the future course of development.

CHAPTER III

THE RECAPITULATION THEORY

The corner-stone of the science of embryology as it developed during the last half of the nineteenth century was the recapitulation doctrine. This doctrine is also called the biogenetic law, or in Haeckel's term, the fundamental law of biogenesis. To it was due much laborious and painstaking research, and the interpretations based upon it have had far-reaching significance. Yet during the last decade critical studies have dethroned this doctrine so that as a "law" it is now of value only historically. As a tendency, however, recapitulation is still a useful conception for it unquestionably expresses a partial truth, a fact to which is due the long-continued interest in the doctrine and the stimulating effect it has had upon embryological studies.

Recapitulation refers to the parallel which exists between the history of the race, in its largest sense, and the development which an individual organism belonging to that race goes through. "Ontogeny recapitulates phylogeny." The life cycle of an individual is a brief summary of racial history. The animal kingdom presents innumerable instances which illustrate this principle. The development of the frog tadpole, through stages showing successively external gills, hind legs, front legs appearing later, and the disappearing tail and gills, closely parallels the taxonomic series of legless apoda and the salamanders. This series begins with the lower urodeles which have external gills and weak legs, then comes *Amphiuma* with small posterior legs, the Salamandridae in which external gills are lost, the legs are about equally developed, and the tail is about equal to the body in length, and finally the Anura which have no gills or tail and the hind legs are the better developed. (Fig. 190.)

The development of the decapod Crustacea, in which the larvae pass through stages comparable to those characteristic of some other orders of the Malacostraca (Fig. 191), is an often-cited case to illustrate the biogenetic law. Another illustration involves the ascidians (Fig. 192) which superficially resemble shapeless jelly-like masses, and whose relationships are therefore difficult to decipher. A study of their embryology revealed the fact that they pass through a tadpole stage with dorsal nerve chord, notochord, etc., all recognizable as vertebrate characters, but when they settle down and become sessile these structures

gradually undergo degeneration and the creature loses all recognizable signs of its former organization. An analogous situation obtains in the

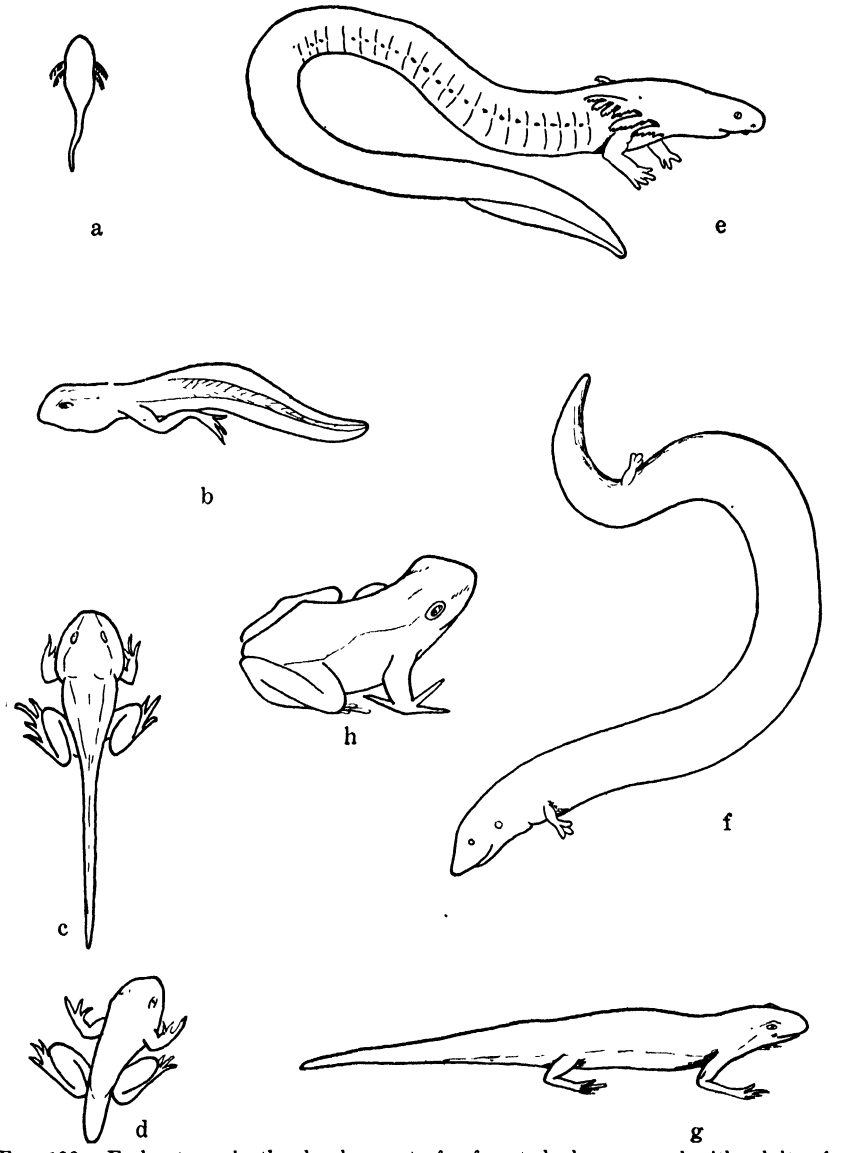


FIG. 190. Early stages in the development of a frog tadpole compared with adults of *Siren* (*e*), *Amphiuma* (*f*), *Desmognathus* (*g*), and an adult frog (*h*).

Cirripedia or barnacles. Barnacles have heavy shells, are sessile, and in general are quite unlike the ordinary Crustacea to which their develop-

ment clearly shows them to be allied. A second suborder of the Cirripedia doubtless presents the most extreme case of modification to be found in the animal kingdom. This is Rhizocephala to which the parasitic *Sacculina* belongs. (Fig. 193.) During its larval stages *Sacculina* is a free-swimming form having about the usual external features of a young branchiopod crustacean. It attaches itself to the abdomen of a crab, sends root-like branches into the body by means of which it obtains nourishment, and completes its development to the adult stage. The

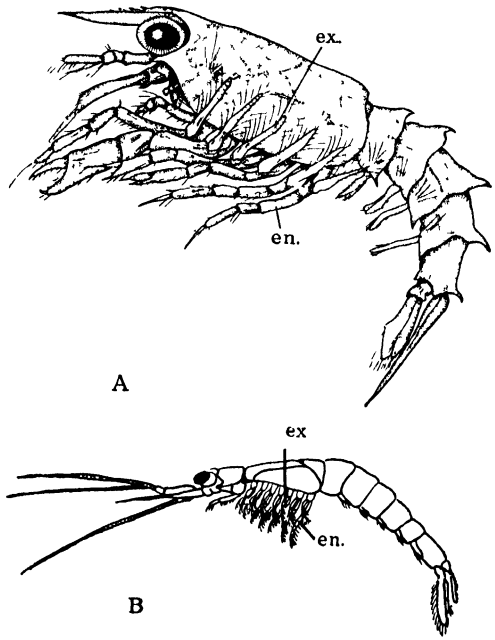


FIG. 191 A, A young lobster at the time of the third molt (after Herrick), and B, An adult *Mysis* (after Verrill).
ex., exopodite, en., endopodite.

adult is so degenerate that it has no crustacean characteristics but is merely a tumor-like sac on the abdomen of the crab in which even the usual internal organs are scarcely present, the body being chiefly filled with the gonads and their products. Only its life cycle reveals its relation to the other Cirripedia. The anatomical relationships by means of which its phylogenetic position is determined are made clear by a study of its embryological development.

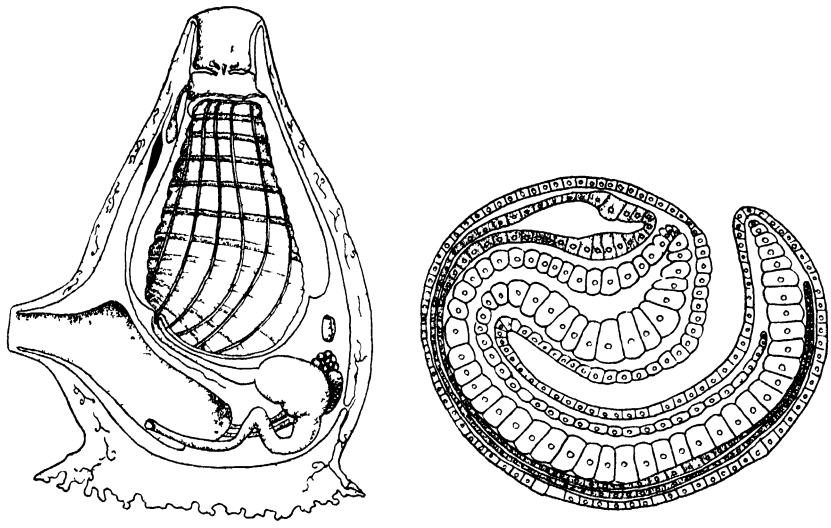
Vaguely foreshadowed by the writings of Meckel and others of the period, the recapitulation principle is first directly hinted at in the writings of von Baer. Indeed, by some von Baer's laws have been con-

fused with the recapitulation theory. These laws are, however, more properly regarded as alternative to certain phases of recapitulation. They were formulated by von Baer in 1828 as follows:

"1. The more general features of a large division of animals arise in the embryo earlier than the special features.

"2. From the most general features of structure arise those that are less general, and so on until the most specific features arise.

"3. The embryo of a definite species tends away from the specific forms of other species, instead of passing through them.



A B

FIG. 192. An adult ascidian (A) compared with a larval form of the same type (B). (After Delage and Herouard.)

"4. Fundamentally, therefore, the embryo of any higher species is never like the lower species, but only its embryo"—(Lillie).

It will be noted that these laws antedate the cell doctrine and the publication of the "Origin of Species," and that the simultaneously enunciated germ-layer theory represented the last word in embryological knowledge of the time in the matter of early structural organization. The advances made possible by the cell doctrine resulted in the enunciation by Fritz Muller in 1863 of the recapitulation doctrine as such. Haeckel developed the theory and applied it in his famous blastea-gastrea hypothesis.

Haeckel's hypothesis lays stress on the importance of the universal occurrence in the embryology of animals of the single-celled egg, the

blastula, and the gastrula, and supposes that the phylogenetic development has taken a similar course. The protozoa represent the first step and are comparable to the egg stage in individual development. No animal is known which exactly corresponds to the blastula or the gastrula, although there are protozoan colonies quite like the former in some respects and simple coelenterates have features in common with the latter. Haeckel, therefore, supposed that there must have been stages corresponding to each of these forms which had now ceased to exist, and to these hypothetical forms he gave the names "*Blastea*" and "*Gastrea.*"

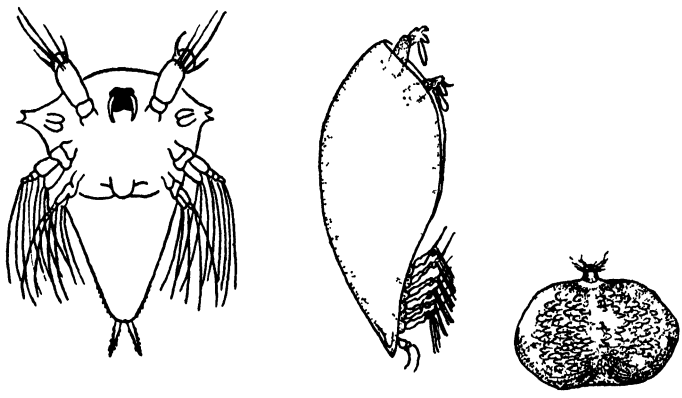


FIG. 193. A nauplius (A) and a Cypris larva (B) compared with an adult (C) *Sacculina carcini.* (After Delage.)

Haeckel also pointed out the significance of certain characters as indicating phylogenetic history whereas others are to be regarded as embryological adaptations of importance to the individual organism, but not to the race. Characters of the first class are spoken of as palingenetic; of the second, as cenogenetic. A chick embryo, for instance, has a good many characters which recall fish or amphibian conditions, but it could not exist in the environment where these forms dwell, and is different in numbers of other characteristics including the important one that it will develop not into a fish or amphibian, but a bird. It cannot, therefore, in its development represent with entire correctness the ancestral forms. Some of its characters are those which adapt it to development in a shell at a temperature of 37° C. and enable it to live in the peculiarities of its own environment. These are new or cenogenetic characters. Other features, as the aortic arches, gill slits, brain

vesicles, and others, are supposed to be of ancestral significance and are, therefore, palingenetic.

A critical consideration of many cases which may be cited, however, shows that the facts do not all easily fit into the biogenetic interpretation. When one attempts to classify clear cases such as the shell tooth on the one hand or the aortic arches on the other as adaptive or ancestral little difficulty is experienced, but the problem of applying the same classification to a doubtful case becomes much more complex, and as a rule the results cannot be certainly relied upon. Indeed, recognition of a particular character as palingenetic or cenogenetic may be quite an impossible task.

Other difficulties may be cited which require modification of the strict biogenetic way of looking at the animal kingdom. For example, there is a general tendency which becomes more marked the higher one goes in the animal kingdom to shorten and condense the ancestral phases of an organism's development in favor of the specializations which relate it to its own environment. This tendency, in addition to complete changes of developmental conditions (for example, the long placental life of mammals, or the abundance of yolk in bird eggs), may result in the entire omission of certain stages (as in the case of embryos where gills do not develop in connection with gill arches).

This abbreviation or contraction of development is called by some writers tachygenesis. In the brief period of embryonic development, if recapitulation occurs even in the most sketchy fashion, an immense contraction of stages must be thought to take place so that the necessary abbreviation may be accomplished. To this shortening of the developmental time, or tachygenesis, there are two aspects, the actual quickening of the rate of development and the fusion and omission of certain stages. The one aspect may occur without the other in special cases as in the response of the developmental rate to rise in temperature, where the rate is hastened without fusion or omission of stages.

Heterochrony also presents an argument against the recapitulation doctrine, as was pointed out by Keibel, Mehnert, and others. By heterochrony is meant the disturbances, which now are known to be of quite common occurrence, in the time of appearance of structures in a known sequence of stages.

These modifications in the rates of development bring about results which cannot be interpreted either as of phyletic or adaptive significance. Stockard has lately pointed out the importance of the arrest of development, even over a small part of the germ ring, whereby various types of monsters are produced. Evidently, here are structural modifications not to be related to recapitulation. Furthermore, the normal develop-

ment of the organ systems of the embryos of different classes does not preserve the same synchrony as may be noted by comparing tables of the development, let us say, of the chick and pig (Keibel, *Normentafeln*). In addition, there are extreme cases in which the actual order of development is the reverse of what would be demanded by recapitulation, as, for example, in those cases in which the joints become rounded off before movement is acquired. Another illustration is the formation of the tongue and the teeth. There is no question that in the phylogenetic series teeth made their appearance before tongues, but in the embryological development of mammals the teeth are much later developed than the tongue. Ontogeny not only fails to recapitulate phylogeny in this case, but completely misrepresents the facts.

In a recent discussion De Beer has summarized the possibilities of variation which heterochrony offers in comparing the appearance of structures in the individual with that in the ancestral forms. This summary with no further elaboration is sufficient to make clear that some of the possible variations not only do not fit in with the conception of recapitulation but are actually opposed to it. The summary is as follows:*

"A character which is present or makes its appearance in the young stage of an ancestral animal may in the ontogeny of a descendant appear:

"A. In the young stage only, producing youthful adaptations or caenogenesis,† not affecting phylogeny.

"B. In the young and adult stage, producing a substitution of the new adult condition for the old, resulting in a progressive *deviation* in the ontogeny of the descendant from that of the ancestor.

"C. In the adult, by a relative retardation of the development of the bodily structures as compared with the reproductive organs, resulting in paedogenesis and neoteny.

"A character which is present in the young and adult stage of an ancestral animal may in the ontogeny of a descendant appear:

"D. In the young stage only, resulting in the reduction of the character to a vestige.

"A character which is present or makes its appearance in the adult stage of an ancestor may in the ontogeny of a descendant appear:

"E. In the adult stage, resulting in those differences which distinguish individuals, varieties, and races: adult *variation*.

* Reprinted from De Beer's "Embryology and Evolution," by permission of Oxford University Press.

† The same as cenogenesis.

"F. In the late adult stage, *i.e.*, too late, resulting in the reduction of the character to a vestige by *retardation*.

"G. In the same stage, which is no longer adult, the new adult stage being relatively delayed, resulting in overstepping the previous ontogenies or *hypermorphosis*.

"H. In the young stage, producing precocious appearance of the ancestral character and *acceleration*.

"Cases B and C which produce phylogenetic effects by introducing youthful characters into the line of adults may be combined under the term *paedomorphosis*. Cases E, G, and H which produce phylogenetic effects by modifying characters which were already present in the line of adults may conveniently be included under the term *gerontomorphosis*."

In many forms the problem is further complicated by the prolongation of certain stages while internal processes are going on. If the gradual internal development of a caterpillar were accompanied by gradual conversion of the biting type of mouth parts to the sucking type, the insect would starve, for during the transition the mouth parts would be adapted to neither the larval nor the adult method of nutrition. By the prolongation of the biting stage until the internal parts are ready for the metamorphosis, the animal's relation to its surroundings are unchanged until the internal conditions are right when a sudden metamorphosis brings about the necessary adjustment to a new environment without loss to the insect. These facts are not in keeping with the biogenetic law as usually interpreted.

Experimental embryology and genetics have not been without their bearing upon the doctrine of recapitulation. It is now possible to show that external factors reacting with the internal, inherited constitution of the organism can produce such effects upon the offspring that its entire ontogeny including the adult is modified. As stated elsewhere, Stockard has shown, for example, that developmental arrests will produce many types of modifications in *Fundulus*, including two heads, trunks, or even two individuals where only one would have otherwise been formed, and conversely that the age-old tendency of the egg to produce two eyes on the fishes' head can be changed by the simple addition of a little magnesium chloride. The internal, inherited control of development is thus capable of modification. And Muller has shown that by exposure to X-rays the developing *Drosophila* can be induced to react in such a manner that entirely new mutations, that is, self-perpetuating strains, can be produced, even at will. Thus is phylogeny deceived by ontogeny. For these reacting ontogenies have produced an entirely new series of adults which can undoubtedly become an-

cestral to others later to appear. Now phylogeny means to most thinkers just a series of adult forms having racial significance. Hence it would appear that such experiments as that of Muller have brought us face to face with an exact reversal of the usual causal rôle attributed to phylogeny, for here phylogeny is the direct result of ontogenetic modification and certainly not the controlling cause of it, as it is usually considered to be by the adherents of recapitulation.

From the criticisms which have been given the student can understand why the so-called "fundamental law of biogenesis" has passed from the high position of a natural law, and is little more than a general tendency. It is a tendency, furthermore, which is more valuable in retrospect than as a means of prediction. And since prediction upon the basis of known facts and postulates is the goal of science and the fundamental test of a "natural law," this doctrine does not measure up to scientific expectation and cannot be regarded as a law.

Yet the partial truths which are involved are of sufficient value in view of the widespread occurrence of the tendency represented to warrant a revaluation of the essential idea of the doctrine. We find an attempt of this kind expressed some years ago by Lillie in his work on the embryology of the chick. His views appear to go far in the direction of harmonizing the useful parts of recapitulation and at the same time to avoid some of the contradictions which have been mentioned. He conceived that the entire life history of an organism is as necessary for the definition of species as any other character. Ontogenies are inherited also and are subjected to variation with resulting modification. Ontogenies of closely related species are more nearly alike than those less related. In the evolution of a species those stages of ontogeny latest to have arisen (those found in the adult) are the ones most easily to be modified and hence the embryo as a rule retains the ancestral resemblances the longest. This is not because these have phylogenetic, that is palingenetic, significance, but because the embryo is less susceptible to outside influences and therefore its characters are less the material upon which selection may operate. Embryonic resemblances being more conservative and actually older are nearer the ancestral condition. They are statements of historical facts rather than causes of recapitulation. But because of the chronological sequence of stages in a great many observed cases, the older recapitulationists came to regard each stage as strictly causal to the succeeding ones and thus to look upon the tendency exhibited as a law of nature.

CHAPTER IV

ASEXUAL REPRODUCTION

Asexual reproduction occurs in a great many plants and in many animal groups. Among the invertebrate phyla it is lacking only in the arthropods, molluscs, and nematodes of the more important groups; while among the chordates, the tunicates present many illustrations of this mode of reproduction. It should also be noted in passing that even in certain mammals a process of budding in the embryonic state occurs. This matter is discussed at length in the chapter on polyembryony where it is shown that the blastocyst of the armadillo regularly buds to produce four embryos. This gives asexual reproduction a place even among the highest groups.

In spite of the fact that this method of reproduction manifests itself in many different forms, it is essentially a very simple process. It occurs in organisms (or in portions of organisms) which have retained to a considerable extent their embryonic, undifferentiated character, and is really a mass division, due especially to the cells of some particular area undergoing a proliferation which presently results in the constriction and cutting off of a greater or lesser portion of the animal. If the portions are approximately equal the process is spoken of as fission; if unequal, as budding. These, with sporulation, are the common forms of asexual reproduction, but each shows many variants in the animals in which it occurs.

Sexual reproduction, involving the participation of two individuals, is known as amphigony; asexual, since only a single individual is necessary, is monogony. In the former, germ cells or their equivalents are produced; in the latter, there are no special cells employed for the purpose. The alternation of a sexual with an asexual generation is called metagenesis. The alternation of a biparental sexual generation, that is, a case of amphigony, with a uniparental, a parthenogenetic, generation is heterogony. Sexual reproduction was further called gamocytogony or cytogony by Hartman, and asexual, agamocytogony (also agamogony).

Fission is the division of an organism into two parts which are approximately equal and into whose formation very little new material has gone. The parent organism is lost in the production of the daughters, which therefore can have no living ancestors, and can undergo only

what may be termed accidental death. It occurs when growth has taken place in excess of the needs of the individual. Contrasted to the condition in sexual reproduction, the new organism is at once provided with at least part of the organs which characterize the adult, and differentiation is always well advanced, considering that the organism is seldom high in the scale of animal life. Two categories were distinguished by F. von Wagner for organisms undergoing fission: in the one, paratomy, a special zone, in which the constriction will occur, is prepared before fission begins; in the other, architomy, no preparation is made, constriction taking place more primitively with little bodily reorganization. It should be noted that both of these categories may apply to either longitudinal or cross fission. Under the general head of fission may also be placed those cases of fragmentation, such as occur in oligochaetes and starfish, due to external influences, which may, if conditions are suitable, develop into mature individuals. These cases are also spoken of as autotomy, and sometimes as augmentation.

Budding, although not much more complex as a process than fission, is productive of much more complicated organisms and life cycles. It often is the means by which colonies are formed, and results in marked polymorphism with some of the individuals of the colony being specialized for nutritive and vegetative purposes and others for reproduction. Commonly where a considerable degree of specialization follows the budding processes the life cycle includes a sexual as well as an asexual phase and we have alternation of generations. A bud is a small portion of the parent organism which has begun to grow actively and to proliferate and from which will be derived a new individual having the full degree of differentiation characteristic of the species. There are buds in some triploblastic animals which involve only one of the germ layers, whereas in other animals the buds may include more than one layer. Furthermore budding may occur in embryonic, larval, or mature animals. Abundant illustrations of all these cases may be cited.

There are several types of budding. The most familiar are the external buds such as occur in *Hydra* for example in which a small portion of the parental tissue grows and constricts off to become a new, small hydra. In some of the hydroids, however, the separation is incomplete and the new individual remains as a permanent bud. The so-called "free buds" of certain forms are also of the external type. As buds they break off and become for a time at least free swimming. A second type includes the internal buds among which are the gemmulae of sponges, the statoblasts of the bryozoa, the germ balls of trematodes and others. A group of proliferating cells becomes isolated within the mother organism and in time results in a new organism. A third type involves the formation

of a stolon or a "runner" from which numerous buds arise. This might seem to be really a kind of external budding but it is sufficiently distinct from the usual cases of this kind to warrant special mention. Finally a fourth series of processes should be included under budding, although they are often overlooked; they are the processes known as frustulation and laceration in which small fragments separate off from the parent organism, when unfavorable conditions arise, and form new individuals.

Closely related to asexual reproduction and of much significance to the comparative embryologist are the phenomena of regeneration. Morphogenic processes are involved in the reorganization of portions of the old individuals to produce new ones which do not greatly differ from the preceding. These processes involve the regeneration of the organism or of portions of it. The student of comparative embryology should give careful thought to these processes, for in them are manifested fundamental capacities and characteristics of living protoplasm. Indeed some of these processes take us far into the innate organization and give an insight into the nature of living stuff which the more usual studies fail to offer.

The formation of colonies is closely correlated with the occurrence of asexual reproduction. Colony formation occurs in certain phyla in a striking manner, and it is in these very phyla that asexual reproduction is a dominant method. It may be taken as a general rule (not, however, without exception), that whenever individuals are found organized into colonies, either asexual reproduction, or polyembryony, or parthenogenesis will be found to occur in the same groups. The simple sexual mode of reproduction seems not usually adequate to produce enough individuals to be associated together in a permanent colony, and one of these three accessory modes must be depended upon for the increased task.

Occurrence of Asexual Reproduction

The first examples of asexual reproduction to be found in the animal kingdom are in the protozoa, but the process here is one of single cells and perhaps is thus somewhat outside the general problems of embryology. We may begin our study therefore with the Porifera.

Porifera. In the phylum Porifera, asexual reproduction predominates; here are to be found fission, budding, formation of free buds, and of gemmulae. Since most sponges exist as colonies it is easy to see how the dividing processes have lacked completion resulting in a degree of union that is more or less extensive. Asexual reproduction commonly leads to formation of colonies rather than to independence of organisms. But budding and fission are shown to advantage in *Leucosolenia*. Some of

the individuals indicate fission as their mode of reproduction for the products are equal in size although they remain attached at the basal end. However, the presence of small immature individuals on certain

FIG 194 Budding and fission in *Leucosolenia blanca.* (From Korschelt and Heider)

specimens as well as the mode of colonial formation shows that budding is often the actual method of their origin. When fission does occur it begins as a split at the osculum and progresses toward the base. When

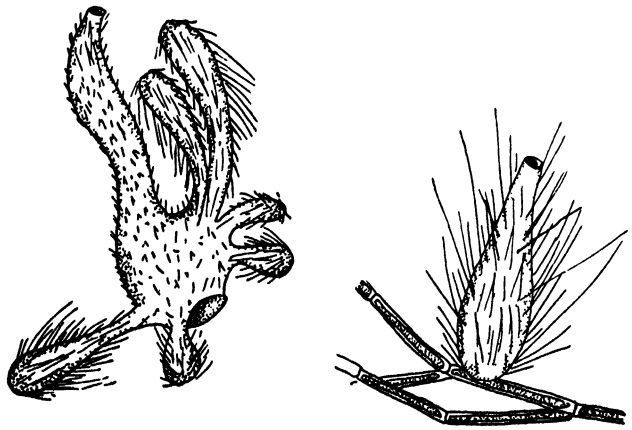


FIG. 195. A, Budding in *Leucosolenia botryoides* B, A bud which has become free and attached to an algal filament. (From Korschelt and Heider, after Vasseur.)

it is not complete the beginning of a colony is seen and these colonies often become quite complex.

In another species of *Leucosolenia* (*botryoides*), Vasseur long ago

found free bud formation. The buds form from an indifferent group of cells, grow irregularly from the parent individual, undergo differentiation to a considerable extent, and at length break off, the ruptured end forming the osculum of the new little sponge; they then settle down to produce young sponges.

The sponges show what is perhaps their highest degree of asexual reproductive activity in the formation of gemmulae, seen to best advantage in the Hexactinellidae. They are derived from parenchymal cells which have wandered into the mesoglea and become separated from their original layers. Aggregates of these "archeocytes" (also called "sorites" by some) take on an oval or rounded shape, become

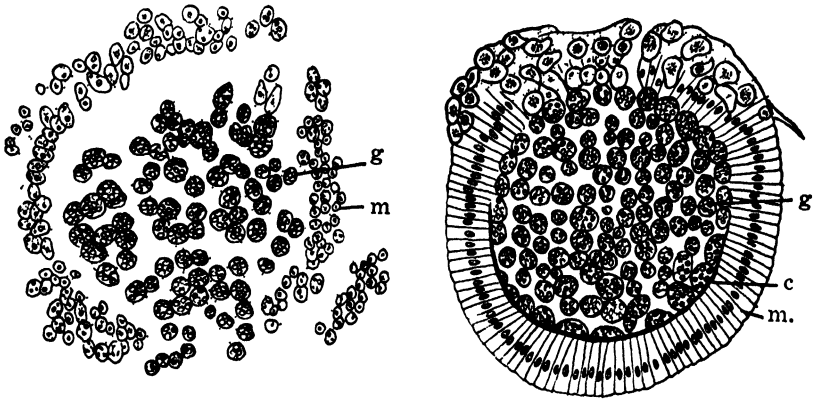


FIG. 196 Gemmule formation in *Ephydatia blembingia* (From Korschelt and Heider, after Evans)

A, an early stage showing the aggregation of the "germ" cells (g) from those which make up the covering membrane (m) B, the cuticular membrane (c) is beginning to form from the outer membrane

surrounded by a special membrane and thus make up a gemmule. During conditions which are not favorable, this structure, which is really an internal bud with a protective covering, tides over the organism until a more suitable time. Thus this form of reproduction is a device which enables these sponges to adapt themselves to changing environments. The parental tissue degenerates and dies after the gemmules are formed. When the gemmules begin to grow, the cells multiply rapidly, those at the surface arrange themselves in a layer, and from the gemmules a larva issues which is strikingly similar to the sexually produced young in form, structure, and ciliation.

Division among sponges is often accidental so far as the organism is concerned, and the animals offer opportunity for experimental fragmentation as well as normal. H. V. Wilson's work and that of J. S.

Huxley give illustrations of the extent to which sponges may be dismembered (in these cases by being squeezed through bolting cloth) and subsequent regeneration serve to produce new sponges. Often cell masses come together, fuse, and grow into a new individual from which a colony is formed. This is called concrescence and may also occur between larvae as they creep about on the bottom.

Coelenterata. By the coelenterates many developmental experiments along the lines of asexual reproduction seem to have been tried as well as those which have already been shown for the various types of embryos, cleavage patterns, and methods of gastrulation. Nearly all types of asexual reproduction are exhibited somewhere in the phylum. In addi-

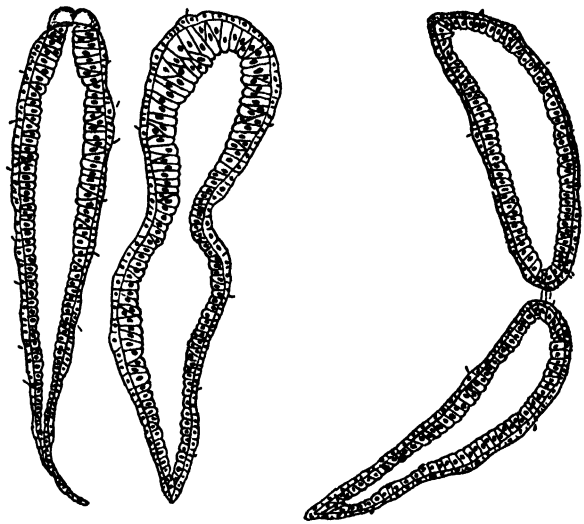


FIG. 197. Transverse fission in *Protohydra*. (From Korschelt and Heider, after Aders.)

tion there is often manifested an extreme polymorphism in the forms which are thus produced, and the details of the manner of their production especially where a compound type of budding is involved are often extremely complicated. Mention here can be made of only a few cases which constitute a very meager outline indeed. Fission, both transverse and longitudinal, budding both larval and adult, and colony formation by the production of permanent buds, metagenesis, stolon formation, fragmentation, frustulation, laceration, and the related, although in its results opposing, process of concrescence, occur in this group.

The Hydrozoa exhibit only a few types of asexual reproduction. Transverse fission occurs in *Protohydra*, according to Aders, and consists in

the simple constriction of the animal around the region of its greatest diameter. The constriction cuts the animal into a proximal and a distal portion. The one develops a new base, the other a new oral region. A similar type of division may occur in *Hydra*; although undoubtedly very rare, this was one of the earliest cases to be described (Trembly, 1744; see also Koelitz, 1908). It is of essentially the same character as in *Protohydra*, the chief points of difference between the two being in the simpler structure and the lack of tentacles on the part of the latter. Longitudinal fission has been described for *Polypodium* only among hydrozoa.

Budding by larval as well as by adult hydroids is not uncommon. An example of budding in the larval condition occurs in *Gonionemus* and in *Haleremita* as described by Schaudinn, Perkins, and others; the details of the life cycle in these forms have been lately worked out by Joseph. The egg hatches into a tiny planula larva. It develops a mouth and creeps about actively while feeding in *Haleremita*, although the planula of *Gonionemus* is sessile. On the sides of these larvae protuberances appear which grow into buds that gradually elongate to become like the planula, then constrict at their bases, and at length separate. The descriptions of the processes differ greatly in detail as described by the different investigators. For example, from one to six buds have been reported by the various students, according to Joseph, near the base of the larva, although Perkins found them about the middle rather than the base. In some cases the endoderm of the bud is said to be solid, in others to contain a cavity derived from the gastrovascular cavity of the mother larva. There are also other differences in the details as given, some of which are doubtless to be attributed to the fact that both European and American forms have been used by the investigators. Lateral buds also have been described as occurring on *Microhydra*, where they may form tiny colonies of three or four polyps.

Among adult hydrozoans budding occurs in both polyps and medusae. In *Hydra* and the colonial hydroids it is of such common occurrence that mere reference to it is sufficient. The bud of *Hydra* consists of a protuberance which grows, develops tentacles, a hypostone, and at length a mouth, after which it is ready for separation from the parent. Both layers of the body wall participate in the formation of the bud, and the gastrovascular cavity is continuous between parent and bud until the time of complete constriction of the latter. Budding is much more common than sexual reproduction. In hydroids the process is essentially the same except that it is usually incomplete, the bud remaining in connection with the parent stalk; in this manner a colony is formed.

A special case of hydroid budding is that which gives rise to the medusa which is morphologically the equivalent of a bud from a polyp. There are cases, to be sure, where the ontogeny is so abridged that the polyp stage is extremely rudimentary or even entirely lacking, unless the planula be regarded as representing it, and the egg develops continuously into the medusa. But in general the medusoid generation is produced by budding from the hydroid generation or the polyp. The main stalk, or hydrocaulus, has attached to it in *Obelia*, modified hydranths, called gonangia, which bud to produce medusae. In *Obelia* these become detached to swim freely. In the Narcomedusae a proliferating stolon buds off medusae which may remain in clusters or may separate off completely. In the siphonophore, *Halistemma*, the planula develops an ectodermal thickening at the aboral pole which develops into the pneumatophore or float. Part of the planula becomes the coenosarcal axis from which spring buds which become several different kinds of individuals. Those near the float are bell-shaped medusae, through whose efforts the colony is enabled to swim. Next come a series of covering scales which seem to be retrogressed medusae; they are protective in function. At various places along the coenosarc are feeding tubes in general similar to a hydranth. The tentacles and feelers also are probably to be looked upon as hydranths. Finally there are the reproductive individuals which resemble certain types of medusae.

It will be seen from a consideration of all these cases that budding in the hydrozoa directly results in the formation of colonies. All hydroids which bud form colonies. Usually the planula develops directly into the first hydranth and the colony is formed by the subsequent branching and budding. This brings about in all except the simplest cases a very considerable degree of polymorphism. There are defensive and sensory individuals and in these the cnidoblasts are well developed. The gonanth or gonangium has for its purpose the function of reproduction, and various modifications of gonangia are to be found throughout the group. Medusae which are free swimming show a greater degree of differentiation than any of the other types of individuals produced in a hydroid colony. There are, however, numbers of medusa forms in which the development is incomplete, and some of them are quite simple in structure. As an accompaniment to the more complex polymorphism which we find in these colonies metagenesis, the alternation of generations, is perhaps shown here to a degree of completeness that scarcely exists elsewhere in the animal kingdom. The sexual functions are transferred to certain individuals while others specialize along the lines of feeding and protecting the colony. Hydroid colonies not uncommonly reproduce by another asexual method, namely stolonization. From the

hydrorhiza of the original polyp cylindrical projections grow out which elongate, creep about on the bottom and may branch or even anastomose. These are stolons or runners upon which new hydranth buds appear to produce new individuals. *Bougainvillia* and *Clavularia* both serve as illustrations of colonies which grow by stolon formation.

In the second class of coelenterates, the Anthozoa, transverse fission occasionally occurs in young animals which have not yet developed sex organs. In *Fungia* as described by Bourne, the development of the larva at a certain stage results in the separation of its distal part and after a complicated series of processes two individuals are produced. Longitudinal fission is common among the Anthozoa. It is a slow process beginning at the oral pole in some cases, although in *Actinia* it may take place simultaneously from the aboral as well. In the adult *Sagartia* and in *Paranemonia* a constriction begins at the pedal disc and passes in the course of twenty-four hours to the oral region. In some others it is much more rapid. Furthermore the constriction of one individual to produce several at the same time may take place. In this case the resulting individuals will be of varying size, and it has been observed that one division may not even be completed before a new one begins. Strange complications thus arise in which the individuals may have several mouths or several systems of septa at the same time owing to this multiple fission. In the Anthozoa irregular longitudinal fission sometimes gives the appearance of budding. True budding, however, is rarely met with in the sea anemones. In the alcyonarians, however, much-branched colonies are formed by budding and the individuals undergo modification and even produce a very considerable polymorphism. The Zoantharia likewise owe their extremely complicated type of development to both fission and budding. In both these latter groups stolon formation and subsequent budding are commonly observed.

One of the most striking forms of asexual reproduction is that manifested by the scyphozoa in the process of strobilization. This has already been described in the chapter on "Types of Invertebrate Larvae." The planula develops into a hydranth-like form called the scyphistoma. By a series of divisions which may be repeated perhaps a dozen times there are constricted off from the scyphistoma, ephyra larvae of which there may be one or several. If there is only a single ephyra strobilization is said to be monodiscal, if several are produced it is polydiscal. The question arises as to the nature of these divisions. The first ephyra is commonly said to be formed by terminal budding and it would seem that if the successive ones are produced only slowly they likewise are terminal buds. However, in certain well-fed scyphistomae the process of strobilization takes place so rapidly that the lower individuals are

already indicated by constrictions before the upper ones have progressed to any considerable degree of independence. It would seem that the distinction between terminal budding and transverse fission in this case is a difficult one to draw. The scyphistoma also for a considerable part of the year produces other scyphistomae by lateral budding in a manner similar to that in *Hydra*. All at length, however, undergo strobilization and produce ephyrae which gradually develop into adult jellyfish. In certain scyphozoans another type of asexual reproduction occurs in the formation of stolons from the original hydranth. They grow out from its base but remain in connection with it and their buds form a colony.

Before leaving the coelenterates the attention should be called to the other methods of asexual reproduction already discussed, namely, fragmentation or laceration and frustulation. In the actinians laceration is rather frequently seen, especially when the conditions of the water become unfavorable. A part of the basal rim begins to spread out, the ectoderm develops quite profusely and endodermal portions grow out into this new area. This then separates from the main body owing to the contractions of the latter and from the pieces so produced new anemones may at length regenerate, although the ability to do so is dependent upon the number of septa present. Frustulation occurs in the hydroids. Occasionally a small bud-like branch is observed to constrict off from the parent colony, to settle down on the bottom in which condition it is called the frustulum, to grow and to produce a hydranth. In hydranths also a process somewhat similar to laceration has been observed, although in this case it is spoken of as fragmentation, for a basal portion of the polyp is cut off and may develop into a young polyp.

A final question in connection with the coelenterates naturally arises as to which condition is the more primitive. There are three possibilities: one, that budding is the primitive type of asexual reproduction; two, that fission is primitive; and three, that both arose independently of each other. There have been interesting discussions of this question and transitions between them have been pointed out. It need only be said here that the evidence is not conclusive for either view.

Platyhelminthes. In the phylum Platyhelminthes the dominant type of asexual reproduction is fission, although there are also cases of budding. It is most common among the Turbellaria, although the polyclads have not been shown to reproduce asexually. Undoubtedly the phenomenon of asexual reproduction in this division of the animal kingdom is closely concerned with the ability which all members of the phylum have for extensive regeneration. In its simplest form, as for example, in the triclads, *Planaria abissima* and *P. alpina*, the reproduc-

tion really consists in the separating of the animal by transverse fission into two portions each of which proceeds to regenerate the missing head or tail. In *Planaria* all the species described show that regeneration may take place at various levels proceeding from the anterior to the posterior. An area of regenerating tissue appears which enlarges and then differentiates. Sometimes a second fission makes its appearance before the complete regeneration following the first, and indeed there are recorded cases of several divisions with the appropriate organs already developing before the first is completed.

Among the rhabdocoels fission is an even more general phenomenon. The small fresh-water *Microstoma* reproduces mainly by fission. Commonly this process occurs before the young have developed sex organs in this form and in *Stentostoma*, and the divisions may take place in such rapid succession that chains of individuals incompletely divided are the result. Division takes place by paratomy very slowly, that is, a zone of division is prepared and the organs of the new portion of the animal are already developed to a considerable extent when constriction occurs. If the constrictions follow each other more rapidly they come under the category of architomy, no special region being prepared in advance. The distinction between paratomy and architomy may perhaps be made clear by saying that the regeneration of the new organs precedes the actual fission in paratomy, no great change in the organization of the animal taking place at the exact moment of division. On the other hand, in architomy, regeneration and subsequent reorganization follow the constriction.

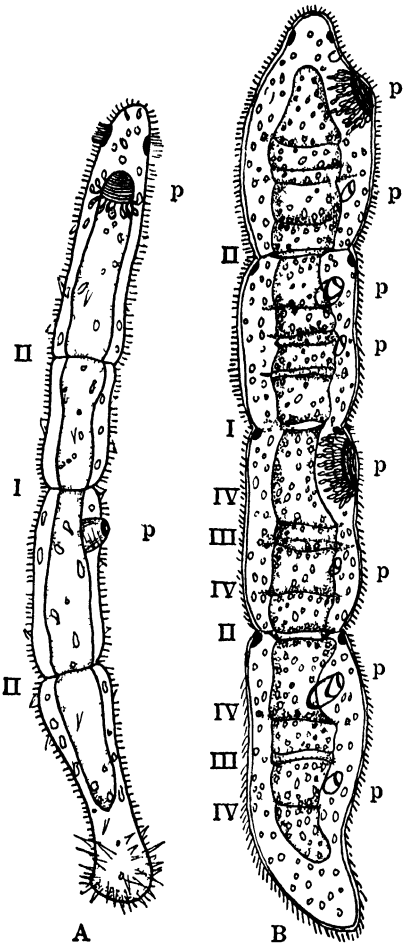


FIG. 198. Successive transverse fissions in (A) *Stenostomum Sieboldi*, and (B) *Microstomum lineare* (From Korschelt and Heider after v. Groff.)

Pharynx is shown for each single individual, and the division planes of the different ranks are numbered

In general, asexual reproduction in the trematodes is rare and certainly never occurs in any individuals which have a complete set of sex organs. The situation with regard to the germ balls which produce the rediae has already been referred to in connection with the chapter on invertebrate larvae and also that on polyembryony. It is still looked upon as an open question as to whether parthenogenesis or asexual reproduction is the method by which the germ ball that forms the new rediae is produced. It has been pointed out that originally they were regarded as asexually produced, more recently as developed in parthenogenic ova, and finally that the cytological study of these supposed ova has not yet shown evidence of a reduction division such as would be necessary to establish surely the fact of their parthenogenic nature. If we are to regard them as asexually produced then this type becomes of much more widespread occurrence among the trematodes than is usually considered.

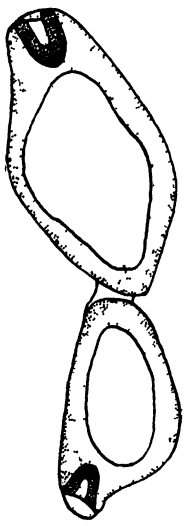


FIG 199 A cysticercus stage (*C longicollis*) of *Taenia crassiceps* with an accessory vesicle attached Scoleces are present in both (From Korschelt and Heider, after Bott.)

Among the cestodes there are two aspects to the question of asexual reproduction. One has to do with the formation of the proglottids from the scolex, the other with multiplication of the cysticercus. The first of these involves a decision as to the fundamental nature of the proglottids. Is the tapeworm to be looked upon as a colony of individuals or as a single one? If the latter view is taken there can of course be no question of asexual reproduction involved. If on the other hand the proglottid is regarded as an individual, since each has a complete genital apparatus and since each has the ability to live at least for some time after the separation from the remainder of the worm, then its method of formation must be looked upon as budding, it being thus produced from the scolex. It is the writer's opinion that the colonial view is not as generally held now as formerly.

There can be no question that in the development of the tapeworm from the cysticercus budding does occur. The egg develops into the onchosphere, the six-hooked embryo, in which condition it escapes from its adult host, and is taken in by the intermediate one. From it develops directly the cysticercus. The scolex of the cysticercus arises from a thickening in the wall of the bladder which becomes depressed into the cavity and later this bud-like structure is everted to form the little worm. It occasionally happens that two or more scoleces are formed by bud-

like thickenings on the wall of the same bladder. This multiple production of scolices is rather rare but occurs regularly as is well known in *Taenia echinococcus* with disastrous results to the host. This is of course reproduction by budding. In some forms the bladders themselves may constrict and produce two vesicles each of which buds into a scolex.

Polyzoa. Since the Bryozoa, or Polyzoa as it is now becoming customary to call them, commonly form colonies, it is to be expected that asexual reproduction will be found in this group, and the expectation is borne out. Three asexual methods, as well as polyembryony, are found here. They are budding, stolonization, and the formation of statoblasts. As a manifestation of the complicated condition which asexual reproduction may reach in a single group, the Bryozoa probably exceed any other branch of the animal kingdom. A student of the subject will find much to interest him in the group and may expect to see an ever-changing variety of detail as he studies the different divisions of it. For

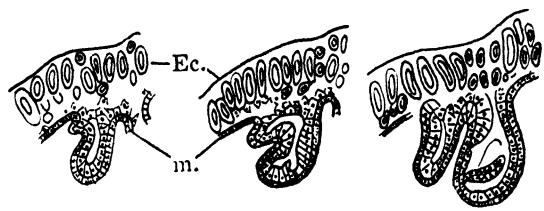


FIG. 200. Buds of *Cristatella mucedo* in median section. (From Korschelt and Heider, after Braem.)
ec., ectoderm; m., mesoderm.

the general student of comparative embryology, however, it seems unnecessary to give more than a very brief consideration to the general types of asexual reproduction as they are developed here. From a consultation of the table of classification on page 260 the student will observe that the phylum Molluscoidea, of which the Polyzoa are a portion, consists of animals very diverse in structure and in their method of development. They range from rather simple colonies to aggregations of organizations which are complex in the extreme. Among the simplest genera is *Cristatella*, coming under the ectoproct order, Phylactolaemata. All the individuals of a colony of this form can be traced to the first one which develops from the larva. This individual produces buds commonly as thickenings of the pharynx on the oral side, both ectoderm and mesoderm participating in the process, although the ectoderm contributes a larger portion to the formation of the new individual. As the bud enlarges it grows out from the parent individual and its inner cell mass undergoes a very considerable differentiation. The intestinal tracts commonly remain in communication at least for

some time. An entire series of buds which in their turn repeat the process is involved in the production of the colony. The development of buds in the Endoprocta closely resembles that just described. The bud is first an enlargement of ectodermal cells which receives migrations of meso-

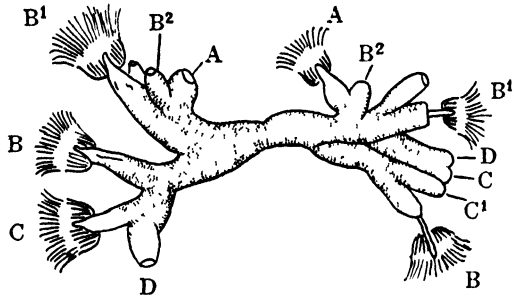


FIG. 201. A piece of *Plumatella fungosa* showing the formation of the primary buds and of those which arise secondarily.

dermal cells as it develops. In this group a stalk makes the bud rather more independent than in the preceding. In some forms, notably the ectoproct groups *Crissia* and *Tubulipora*, budding takes place in the embryonic stages even before any differentiation of the blastomeres begins. The primary embryo produces a great number of buds in such a case and may become a stem and retain the others as secondary embryos or it may be entirely destroyed in their production. Of course the secondary embryos may also reproduce asexually. It is rather rare that the buds should separate naturally from each other, for it is by their uninterrupted relation to each other that the colony is formed. They may branch laterally from the main stalk or may continue in a direct line of the main branch. It is obvious that, in such a highly organized type of animal as this, polymorphism of the individuals is to be expected. Special morphological changes take place so that some of the individuals of the colony become very much changed from the typical form and have very different functions. The Bryozoa always reproduce by sending out stolons which then bud many times. The formation of the stolon itself is like that of the bud but it grows out as a stalk which by repeated incomplete divisions produces a new portion of the colony.

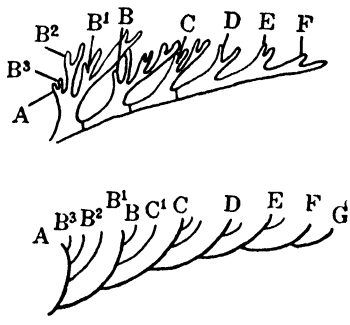


FIG. 202. Diagrammatic representation of the method of branching in *Plumatella fruticosa*. (From Korschelt and Heider, after Braem.)

Some fresh-water forms produce hibernacula which are essentially winter buds, club-like swellings on the stolons which enclose themselves in a cutaneous capsule during the period of severe winter. In the spring their development continues to form new colonies. Accidental subdivision of a colony is of course common, but the multiplication of the colony by fragmentation appears also to occur naturally.

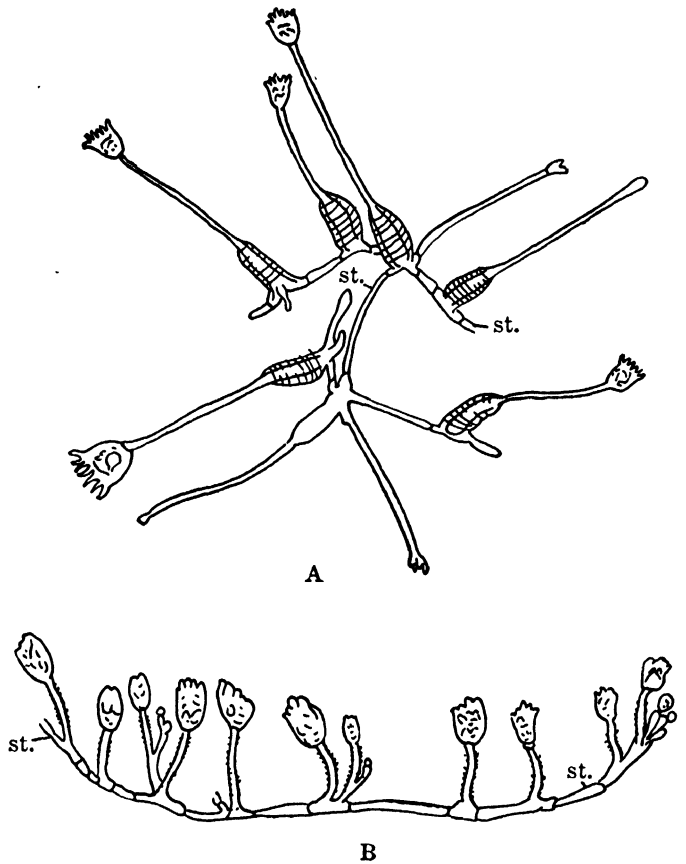


FIG. 203. Production of zooecia as buds from stolons (st.) of *Pedicellina echinata*. (From Korschelt and Heider, after Ehlers.)

A final method of asexual reproduction is the formation of statoblasts. According to an old view a statoblast is supposed to arise from a single cell and upon the basis of this it was thought to be a sort of winter egg. It is now realized that the statoblasts are modified buds which have become changed into reproductive buds. They vary in shape, but are enclosed in a cutaneous capsule and contain several cells. They are

able to withstand extremes of temperature and thus serve to carry on the colony during the cold of the winter.

Annelida. Asexual reproduction is of common occurrence in the two most important groups of the annelids, namely the polychaetes and the

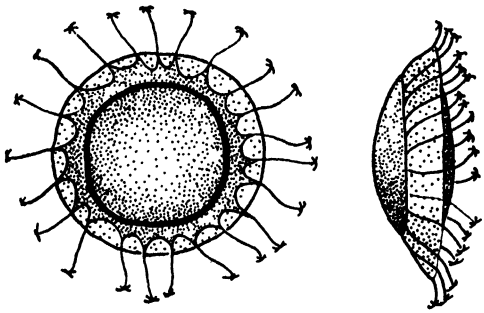


FIG. 204. Statoblasts of *Cristatella mucedo.* (From Korschelt and Heider, after Kraepelin.)

oligochaetes. This is due to the remarkable uniformity in the structure which the annelid body possesses, each segment being very similar to its neighbor. The growth of the annelid worm is accomplished by the

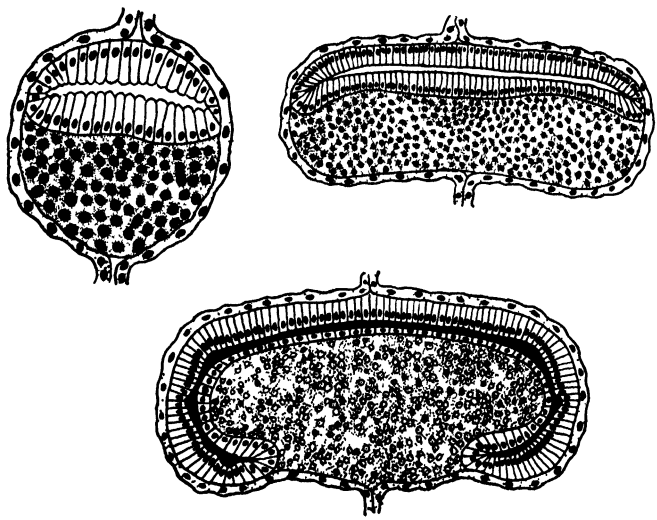


FIG. 205. Developmental stages of the statoblasts of *Cristatella.* (From Korschelt and Heider, after Verworn.)

addition of the somites to the posterior end primarily, although anterior zones of budding are also found. In some instances the formation of new somites takes place more rapidly than their separation from the animal

and thus a chain of individuals is formed. In a simple annelid chain in which the individuals of a series are spoken of as zooids in anticipation

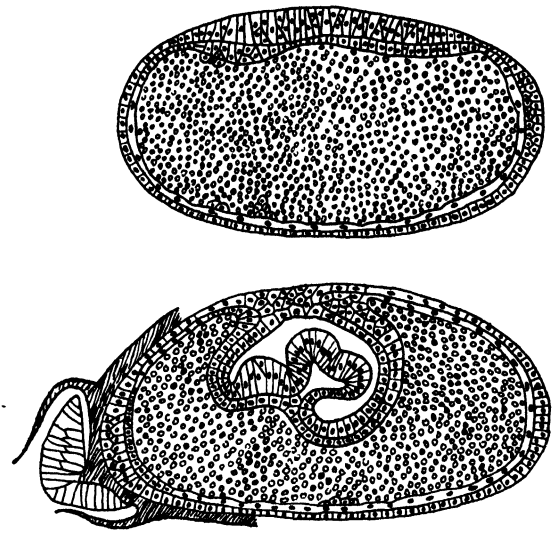


FIG. 206. Germinating statoblasts of *Cristatella*. (From Korschelt and Heider, after Braem.)

A, the "germ disc" stage. B, its invagination preparatory to further growth.

of their future development to individual worms the anterior individual represents the original worm and is obviously the oldest. At its posterior

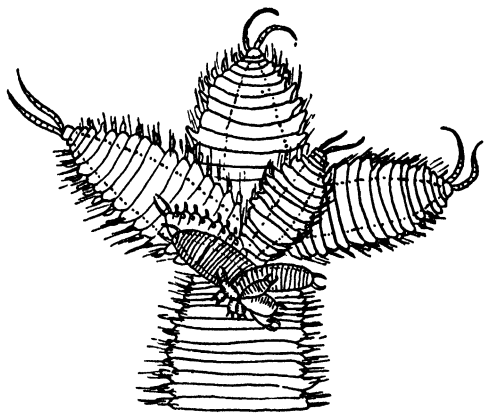


FIG. 207. Posterior end of *Trypanosyllis misakiensis* showing buds of different ranks. (From Korschelt and Heider, after Johnson.)

end is a reproductive zone where the budding off of new individuals takes place. The first individual budded off is of course the most posterior

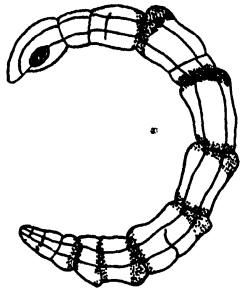


FIG. 208. Fission in *Ctenodrilus serratus*. (From Korschelt and Heider, after v. Kennel.) Stippled areas show zones of division.

of the chain, the others grading in age and size toward the middle of the worm where the budding zone is located. In the more complicated cases, of which the syllids are the extreme example, we may have not only terminal budding but also lateral and even ventral. In one of these forms, *Trypanosyllis misakiensis*, single budding soon produces numerous buds in all directions differing in age but otherwise giving the appearance of a rosette of small zooids. Between these two extremes lie various degrees of complication in the forms produced. In many marine polychaetes a differentiation with respect to reproductive capacity is observed between the different parts of the animal. The anterior portion of the individual is sexless and is spoken of as the atoke. At the time of sexual maturity this slow-moving worm becomes very active, the hinder somites develop gonads, and special bristles and parapodia grow out on them, developing them for rapid and extensive movement. This sexual portion is spoken of as the epitoke, and individuals of this kind were earlier given special systematic descriptions. It is now clear, however, that *Nereis*, for example, passes over into *Heteronereis* or the epitokous stage. In many polychaetes, the epitokous portion separates from the remainder and swims about actively for a certain period at the surface of the sea. This is the swarming which takes place in *Nereis*, and is especially notable in the palolo-worm, *Eunice viridis*, whose swarming in the south Pacific is often observed as a very unusual phenomenon.

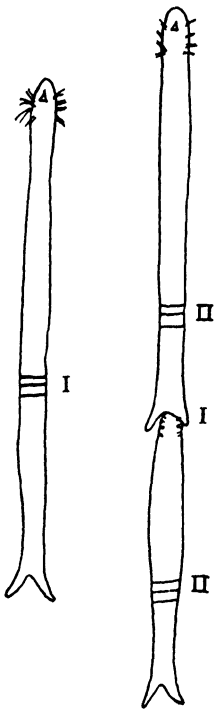


FIG. 209. Diagrams to show the divisions zones in *Dero vaga*. (From Korschelt and Heider, after Galloway.)

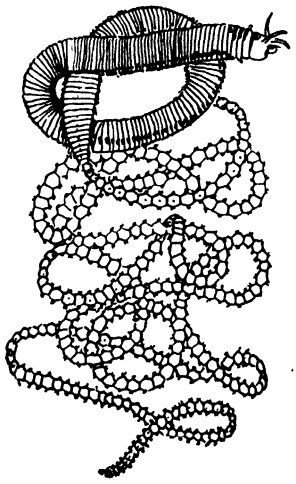


FIG. 210. The palolo-worm, *Eunice viridis*, showing the difference between the anterior atokous part and the posterior epitokous portion. (From Korschelt and Heider, after Woodward.)

A further classification of the asexual methods of reproduction in this group may be based upon the degree of preparation which precedes

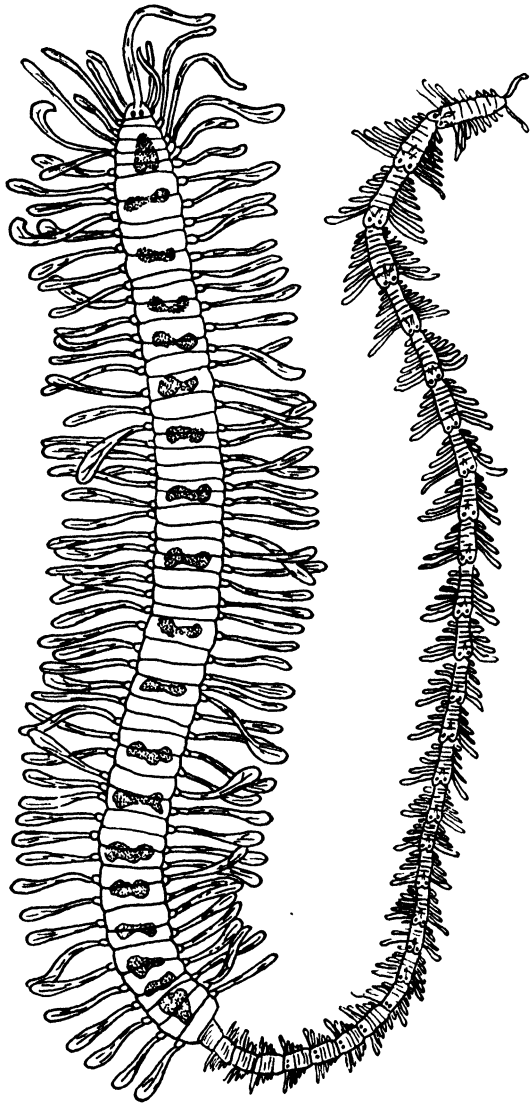


FIG. 211. *Myrianida fasciata* with 29 zooids. (From Korschelt and Heider, after Malaquin.)

the division. In the simpler cases the division of the worm takes place between two segments with no special zone of separation having been developed. Following the division regeneration occurs producing a head

or tail, or in some cases where a portion of the middle of the worm is cut off from both ends both head and tail are regenerated. As has been pointed out this regeneration after division is known as architomy in contradistinction to paratomy where a separation zone is developed before division. Such a zone is really the beginning of an early regeneration and may be present in variable degrees all the way from a simple region of constriction to the formation of a well-developed head and sensory apparatus.

Architomy is illustrated in *Ctenodrilus monostylus* where without much preparation the worm divides into an anterior and posterior part by constriction and the lacking parts are regenerated. Regeneration begins at once, although the new worms continue to creep about actively in spite of the fact that they cannot for a short while take in food. Architomy may be accomplished by autotomous division of the worm. A number of polychaetes and oligochaetes have such great powers of regeneration that they are enabled to separate without external stimulation. *Lumbriculus* is an annelid of this type.

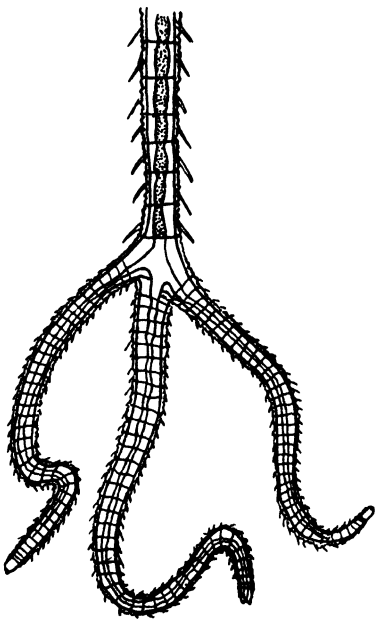


FIG 212. *Tubifex rivulorum* with a primary regenerating portion and two secondary lateral regenerations (From Korschelt and Heider, after C Müller)

Paratomy is illustrated by certain species of *Ctenodrilus* also, especially *C. serratus*, in which the regenerative powers are so great that even single segments may in certain cases produce new worms. Beginning a few segments back of the head, cellular proliferations take place which gradually form thickenings on the anterior side of each segment. They become the head folds and give rise to the organs of the head region in the new zooids. Thus a chain of zooids is formed asexually. Additions of this sort produce what is functionally an alternation of generations. It is scarcely developed with the regularity that characterizes this process in such forms as hydroids. The original individual was developed from the egg. It produces asexually a chain of zooids which separate from each other and when weather conditions become suitable develop further into individuals which reproduce sexually. Of course second and third asexual generations may be produced in the same manner as the first one.

Echinodermata. Among the echinoderms asexual reproduction is described, but it is certainly of rare occurrence and one has difficulty in distinguishing it from an extreme type of regeneration. Spontaneous division of the arms and in some cases the splitting of the disc have been described in the asteroids, ophiuroids, and in the holothurians. Asteroids have been seen to split and separate, beginning at the gullet, and then

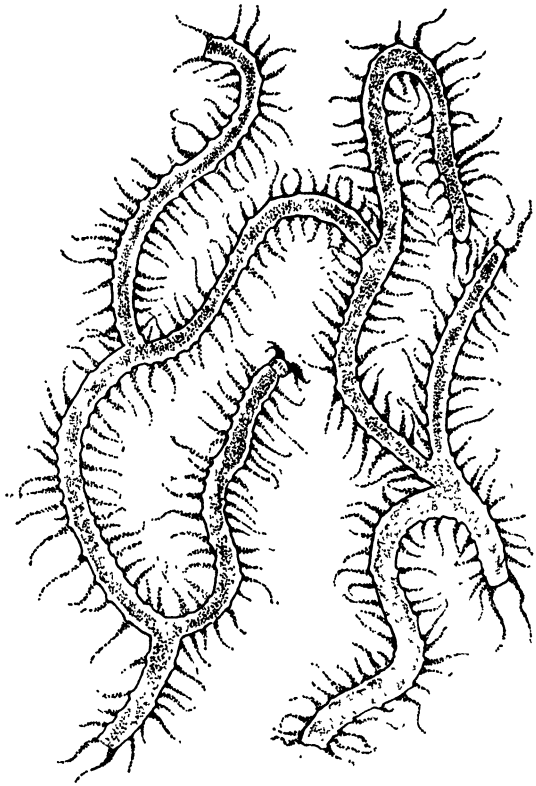


FIG. 213. *Syllis ramosa* showing anterior end of worm and the complex branching. The gut is stippled in the figure. (From Korschelt and Heider, after MacIntosh.)

the missing arms and organs of the disc to become gradually regenerated. It is of course well known that where accidental separation of the parts of an echinoderm is brought about extensive regenerations follow and theoretically there is no great difference between this and a natural type of division followed by regeneration. However, the latter is certainly of earlier occurrence.

Pterobranchia. The two genera *Cephalodiscus* and *Rhabdopleura* which compose the anomalous Pterobranchia both illustrate asexual reproduc-

tion by budding. Their doubtful position showing similarities both to *Balanoglossus* and to the Bryozoa is nevertheless in line with their type of reproduction. *Cephalodiscus* occurs as a single individual from the side of which a stalk grows out. This stalk bears a bud as is shown in the well-known figure often copied from MacIntosh. The bud arises from the apex of the ventral stalk and after a certain age breaks off. The animal lives in a gelatinous coenoecium where a large number of free individuals may be found. The animal possesses practically all the important organs found in *Balanoglossus* and structurally seems related to that form. Its method of asexual reproduction, however, much more strongly resembles that of the Polyzoa. Resemblance to the latter group is rather more striking in *Rhabdopleura* which lives in a tube and reproduces in a manner similar to that of the stoloniferous Bryozoa. The general anatomy, however, closely resembles that of *Cephalodiscus*. In *Rhabdopleura* the individuals derived from the buds remain close together and form small colonies living in branched tubes. They are connected by a muscular cord which passes back to join a common stem or stolon. The cord is the narrowed proximal portion of the body. By its contraction the animal is retracted into a stalk. The stolon is developed as a bud from the original zooid, but the details of the process are not well understood. Part of the stolon loses its formative power and becomes merely a connection between the creeping parts of the colony. The remainder, however, buds, and the buds give rise to branches. In the free-growing part there always appear two buds, an anterior, well-developed one and a posterior much younger.

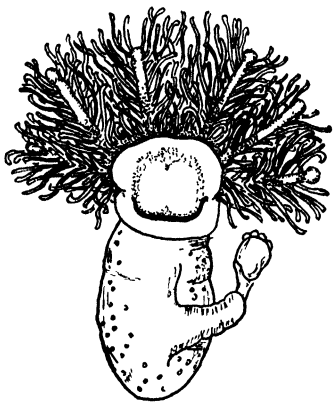


FIG. 214. *Cephalodiscus* with bud. (From MacIntosh, with modifications.)

Tunicata. Among the tunicates asexual reproduction is widespread and occurs as an important means of multiplication in two of the main divisions of this subphylum, the composite ascidians and the salps. The dominant type is budding but the details show various departures from the simple form and even instances of fission are observed within this group. It will perhaps serve our purpose to limit our discussion to the budding in the two cases mentioned, understanding that there are some other minor examples to be met with in this group. The embryology of the ascidians has been described in Part One of this book. The young embryo grows and in many cases begins its process of budding

before it has become attached and begun its metamorphosis. It is customary to speak of the individual which is produced from the egg as an oözooid whereas the individuals which are produced from buds are blastozooids. However, the individuals so called may become modified in various ways and be given other names to indicate functional and morphological differences which later occur, for budding in the tunicates as in some other groups leads to polymorphism and to alternation of generations.

In the compound ascidians there are two main types of budding, palleal and stolonial, differentiated according to the position on the oözooid in which they occur. By palleal budding, which also goes under the name of peribranchial or atrial, or also entero-epicardial, is meant the growth of a bud as an oesophageal or even intestinal outgrowth; it is thus derived near the atrial wall and under the mantle or pallium. It is common for the buds to be formed as lateral evaginations of the body wall in symmetrical parts, but usually only one of them develops. Mesoderm and germ cells migrate from the tissues of the mother into the bud which gradually becomes a complete individual. The processes are the same in the asexually produced blastozooid and in the oözooid which are developed from the egg, but the oözooid undergoes a retardation in development and never reaches sexual maturity whereas the blastozooid develops fully although it is to be noted that both may reproduce asexually as well as sexually. The oözooid is short lived. After it has reached the typical form of the ascidian it undergoes retrogression and its tissues are used to build up new individuals. In the composite ascidians although the buds are independent of each other they yet remain enclosed in a common cellulose mantle, and after a number of generations have been produced the mass takes on the form of buds united into a very definite system built upon the plan of concentric circles of buds. As the system becomes more and more complicated some of the individuals become crowded out of their proper place and so become the center of a new irregular subdivision of buds. In this way a colony is formed.

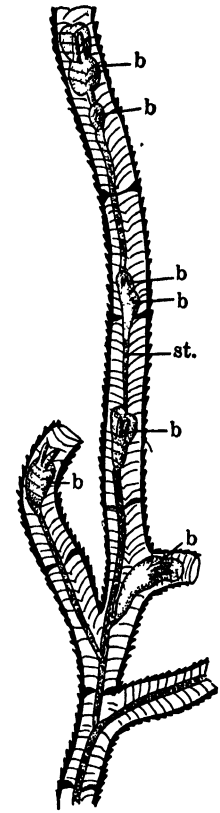


FIG. 215. Budding in *Rhabdopleura normanni*. (From a diagram by Korschelt and Heider.)

b, buds of different ranks from a common stolon (st.).

The place of origin of the buds varies in different genera, a fact which

gives rise to the marked differences in appearance of different colonies of compound ascidians. Thus we have pyloric budding which is also called oesophageal, epicardo-oesophageal, and epicardo-rectal budding. In this type when the blastozooid buds it is really of compound origin, the new process arising from abdominal and thoracic outgrowths, the abdominal bud being an invagination of the oesophagus and the thoracic formed largely from the epicardium. From a diverticulum of the left portion of the epicardium the nervous system is derived. It is thus endodermal in origin in the blastozooid, although in the oözooid it is derived in the regular manner from the ectoderm. Pyloric budding is described chiefly in the didemnids and the diplosomids. Among the modifications of the general type of budding described for the compound ascidians are the cases spoken of as pseudo-stolonial, occurring especially in the distomid *Distaplia* and in the polyclinids. Authorities differ in the manner in which they regard the budding of these forms, some tracing it to the formation of a stolon and others regarding it as more nearly related to the type already described. In *Distaplia* the formation of the "primordial buds" has been described as coming from the epicardium, but Salensky, Julin, Della Valle, and others have described this as a proliferating stolon from the intestine. The first generation of these buds never develops sex products but later generations of buds may reproduce sexually. It may be noted that the epicardium is wrongly named in that it does not participate in the formation of the pericardium and the heart, although it was so described by van Beneden and Julin. Actually it is the endodermal element of each bud. In the polyclinids the budding of the posterior part of the animal resembles horizontal division, for the caudal part of the larva elongates and constricts, forming thorax, abdomen, and post-abdomen; gonads and heart develop in the latter which subsequently segments to form buds. By some this entire caudal portion is regarded as a stolon.

As an example of stolonial budding reference may be made to *Perophora* and to *Clavellina*. In *Clavellina*, after its attachment, root-like processes are sent out from the base giving it a more firm hold. At least one of these processes becomes the proliferating stolon (*stolo prolifer*). The stolon consists of the three germ layers in addition to the cellulose tunic which covers it. From it arise buds as well as side branches. If a particular outgrowth contains an endodermal layer it would develop into a bud. Otherwise it is merely a sterile branch. Buds arise near the growing tip of the stolon, but simultaneously with their development the top continues to grow, so those near the base of the stolon are the more mature. Younger ones sometimes arise as a new generation of buds between the older ones. Stolonial budding takes place only from

the oözooid for the blastozooid can reproduce sexually. It should be noted in regard to the formation of colonies that although the usual condition is the production of an entire colony from a single oözooid, yet small colonies sometimes grow so close together that concrescence results and they fuse into a larger colony.

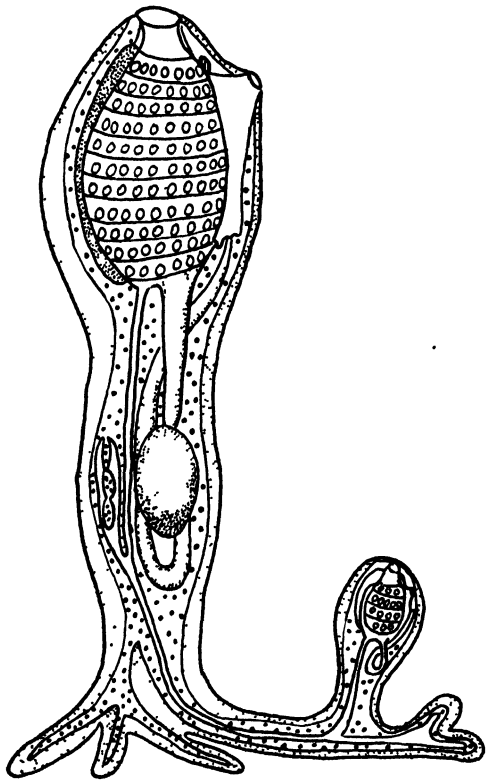


FIG. 216 An adult of *Clavellina* with a stolon and a young individual budded off from it (From Korschelt and Heider, after Seeliger)

In many respects the most interesting case of asexual reproduction in the tunicates is the formation of the *Salpa* chains from a proliferating stolon. When investigators first began to study *Salpa*, solitary individuals were found, and also others which appeared to have a significantly different structure and were connected together like a chain or even a rosette were also occasionally discovered. At length the relation between the two was made out by the poet, Chamisso, who discovered that the solitary individuals can produce the chain salps and that certain individuals of the chains developed sex organs from which the

solitary individuals were again formed. The solitary individual always reproduces asexually but is itself produced from a fertilized egg. From the ventral side of the posterior half of such a solitary individual a bud, which first arose from the pharyngeal endostyle and is therefore in its beginning endodermal, grows out. As it enlarges, the mesoderm and ectoderm surround it, and finally it becomes a projection from the tunic as well. It is a direct continuation of the ectoderm and endoderm of the

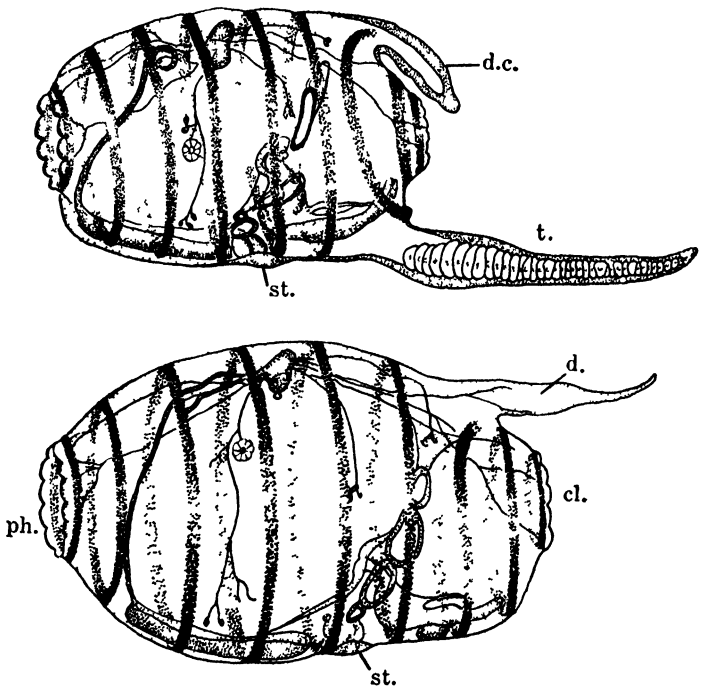


FIG 217. Nearly adult stages of *Doliolum denticulatum* (From Korschelt and Heider, after Neumann.)

cl, cloaca, d c, dorsal horn or cadophore to which buds will be attached, st, ventral stolon from which buds are produced, t, tail with chorda

parent individual and its parts are likewise related to the organs which develop in its buds derived from it. This is the ventral proliferating stolon and the individual producing it is the oözooid developed from the single egg of the gonozooid.

The history of the ventral stolon differs somewhat in *Salpa* and *Doliolum*. Perhaps the latter, as described by Uljanin, is the one more familiar to students. As in the compound ascidians the oözooid produces no sexual organs but a great many buds arise from it in a complicated manner and the chain thus produced exhibits a high degree of poly-

morphism. Indeed, the oözooid itself degenerates until it becomes nothing but a locomotor organ of the rest of the chain. The buds are produced continuously for a considerable time from the ventral stolon. They do not remain in that part of the body, however, but migrate dorsally to a posterior dorsal horn, or cadophore, which is an outgrowth resembling somewhat a stolon but does not of itself bud. Its epithelial cells become specially suited for the attachment and nourishment of the migrating buds which will presently reach it. As a matter of fact, however, there are more buds in the dorsal cadophore than were produced from the ventral stolon, multiplication in the form of a simple division having taken place during the migration. The cadophore continues its growth and more and more buds are attached to it. They arrange themselves in two lateral rows and one dorsal median row. The lateral blastozooids which are attached by a short stalk are nutritive and respiratory. They are apparently unable to reproduce and are morphologically independent of the rest of the colony. The median blastozooids are spoken of as nurse individuals, or phorozooids. They are rounded and have a stalk on the ventral side. It is this stalk which bears the buds that in their turn are to give rise to the sexual forms. Like the lateral blastozooids, the nurses are non-reproductive. The buds which arise from the stalk of the phorozooids are usually spoken of as protogonozooids. From these the sexual blastozooids, that is, the gonozooids, are produced. They are free swimming, undergo a considerable change in their general structure, develop sex organs, and the cycle is completed, for from the fertilized egg comes the new zooid ready to begin the asexual cycle again. Thus there are two distinct asexual generations, the oözooid and the protogonozooid, and some of the blastozooids have also multiplied by simple division in passing to the dorsal cadophore. These two asexual generations alternate with the one sexual generation. The development of all forms of *Salpa* is not understood yet and in some cases it is difficult to interpret the forms that have been found. The life cycle of *Doliolum*, however, illustrates satisfactorily the degree to which asexual reproduction is developed in these forms.

Vertebrata. In forms higher than the tunicates asexual reproduction does not commonly occur. However, the case of the armadillo which is described in detail in the chapter on polyembryony must be mentioned. Here a blastocyst is formed as a result of the cleavage of the egg which produces by budding four individuals. This is, of course, an asexual method of multiplication, and may be of general significance, as is suggested by the production of identical twins in various other forms of mammals.

CHAPTER V

PARTHENOGENESIS

I. NORMAL PARTHENOGENESIS

A. General Discussion

As an embryological problem the phenomenon of parthenogenesis has been of interest since its discovery in 1762 when Bonnet found that the summer generations of aphids reproduce by means of unfertilized eggs. The literature dealing with the subject is a vast one, for parthenogenesis is of wide occurrence in the animal kingdom and is also known among the plants. In spite of the vast amount of research on the problem, however, it has only recently become possible to bring the known cases into any kind of systematic order. The problem also has a great deal of cytological and genetical interest for we are here dealing with uniparental inheritance and it is obvious that the chromosomal mechanism which explains the inheritance of bisexual forms must undergo some modification to account for the appearances in unisexual organisms. While the cytological and genetical aspects of parthenogenesis are closely related to the embryological, we can treat them only briefly and must devote ourselves chiefly to the embryological problems.

Confusion sometimes develops in the mind of the student in relation to parthenogenesis for at first thought one is apt to class all types of uniparental inheritance together and think of parthenogenesis as a modified asexual type. The fallacy of this conclusion is easily seen, however, for parthenogenesis deals with the development of an egg although without the cooperation of a spermatozoon. It is clearly a gametic form of reproduction; its relationships are perhaps more easily understood if we speak consistently of unisexual and bisexual reproduction. It would perhaps be still clearer to use the terms monogametic and digametic, although these are not commonly found in the literature. The critical point in the evidence that we are here dealing with a second type of sexual reproduction is seen in the formation of polar bodies and the behavior of the chromosome during the maturation division. In some cases of parthenogenesis polar bodies are often seen to be formed and in other cases the behavior of the chromosomes in reduction is well known. In addition to this evidence, the sporadic occurrence of

parthenogenesis in diverse classes of the animal kingdom, the occasional occurrence of parthenogenesis along with the bisexual method in the same species of animal, as for example the bee, and the not uncommon alternation of the parthenogenetic mode with typical sexual reproduction in the course of several generations within the same animal all bear out the same conclusion. Furthermore, the fact of artificial parthenogenesis, that is, the activation of an egg which would otherwise develop only by fertilization, shows that we are dealing with what must be a modification of the usual type of bisexual reproduction. Artificial parthenogenesis means the initiation of development on the part of the egg by chemical or physical stimulation artificially induced to take the place of the type of initiation usually to be seen only when the sperm enters the egg. It is really a study in experimental embryology which has been of the greatest value in giving an insight into the true nature of the process of fertilization.

a. Terminology. Throughout the animal kingdom parthenogenesis occurs in a wide variety of types and under many different conditions. Owing to this fact the literature concerning it contains many descriptive terms that are of special application and about which some confusion of meaning has grown up, as has been the case in certain other processes that are familiar but not clearly understood by the majority of students. In some species parthenogenesis is of rare occurrence and the eggs simply possess the power of developing without the intervention of the sperm, although they usually develop after fertilization. Such cases are known as facultative or optional and are of such irregular occurrence in the series as to be of no great embryological significance. Obligatory parthenogenesis occurs regularly in the life cycle of organisms which show it as a constant feature or only at certain intervals. Its importance is manifested by the fact that parthenogenetic eggs are often structurally different from those that are to be fertilized and there are morphological differences between the animals which are produced parthenogenetically and those which are produced bisexually. For instance, the eggs of daphnids which are fertilized usually develop only during the colder seasons of the year; they are known as winter eggs and have a protective shell that is very different from the summer eggs. Oftentimes cases of obligatory parthenogenesis show an alternation of generations although not of an asexual generation with a sexual, but of a unisexual generation or a series of them, with a bisexual generation. Where this type of development is firmly established a series of parthenogenetic generations may be developed to such an extent that it becomes the predominant method of reproduction and sexual genera-

tions appear only at infrequent intervals. Many of the more familiar cases of parthenogenesis are of this type.

Even in the extreme obligatory type it is often possible by experimental means to induce the sexual process, for instance, by a change of the environmental conditions. The rotifer, *Hydatina senta*, as shown by Shull and by Whitney, in old culture infusions will continue parthenogenetically for long periods of time, but if a fresh culture fluid is used bisexual animals soon make their appearance. Conversely, it is possible by maintaining appropriate conditions to postpone or even entirely to suppress the sexual process, as in the case of the rose aphid when cultivated through a series of years in a greenhouse.

In cases where structural differences are to be observed between the parthenogenetic and the bisexually developed eggs, a functional difference also is frequently seen in that the eggs of certain individuals are exclusively male producing, that is arrhenotokous, and of others female producing, or thelytokous. Where both males and females are produced in one brood, parthenogenesis is said to be amphoterotokous. Where the number of parthenogenetic generations which occurs before the sexual animals are produced is indefinite we say that the species has an "open life cycle." Such cases occur in the aphids and in some species of phylloxerans. In others the female which produces the parthenogenetic eggs and hence is known as the stem mother must develop directly from the previous fertilized egg. This is the "closed" type, and it occurs in certain other phylloxerans and in the gall flies.

Considering the embryological aspects we should classify known cases according to the grade or degree of parthenogenesis shown. It is possible to distinguish seven or eight different grades of parthenogenesis as follows: (*a*) Pathological. In the ova of birds it is frequently observed that a few cleavages take place resembling the normal but not leading to the formation of the blastula. Similar phenomena have been observed in some other eggs as well. (*b*) Casual parthenogenesis. In the case of the silk moths under exceptional conditions aspermic development takes place. (*c*) Occasional parthenogenesis. In the ants, bees, and wasps, the development of the egg without fertilization always produces a male. (*d*) Partial parthenogenesis. The queen bee by the copulation on the nuptial flight receives all of the sperm which she will ever use, and afterwards she can fertilize the egg to produce a female, that is, a worker or a queen, or leave it to develop parthenogenetically into a male, since she has control over the process. (*e*) Seasonal parthenogenesis. In the case of *Daphnia* already mentioned and other Entomostraca and also the aphids the summer eggs are parthenogenetic and the winter fertilized. (*f*) Larval parthenogenesis. The case of *Miastor* is described

in the chapter on paedogenesis. Some larvae are produced parthenogenetically and inside of them others through a number of generations, each eating its way out and producing yet others. Finally the last larvae pupate, undergo metamorphosis, and develop bisexual forms. (*g*) Total parthenogenesis. Only parthenogenetic eggs occur through many generations as is especially illustrated by the rotifers.

b. Cytological Relationships. A consideration of the cases of parthenogenesis upon the basis of their cytological conditions leads to the common classification of haploid and diploid parthenogenesis. By haploid parthenogenesis is meant the development without fertilization of an egg which has gone through the process of chromosome reduction. Haploid eggs in general are sexual, and in most cases in which aspermic development is possible the eggs are found to be capable of development either with or without fertilization. Hymenoptera, some of the Hemiptera, and the arachnids often exhibit haploid parthenogenesis. One case, the nematode *Rhabdites* (*R. aberrans* according to Krüger and *R. pellio* according to P. Hertwig), is worthy of note, however. Here the eggs develop parthenogenetically and are of the haploid type but the penetration of the sperm is necessary to initiate the development although it takes no further part and no true fertilization is accomplished. After it has penetrated the cortex of the egg, and thus set going the developmental processes, the sperm degenerates and the further development of the egg is as much parthenogenetic as if no sperm had been involved.

Diploid parthenogenesis is much more frequent, occurring in most of the other groups where parthenogenesis is found at all. Copepods, ostracods, and the Hemiptera-Homoptera have this type characteristically whereas trematodes, nematodes, echinoderms, the phyllopods, the Orthoptera, and the Lepidoptera exhibit it in certain forms. An intermediate condition is found in the rotifers, both diploid and haploid parthenogenesis having been described for these forms. Triploid parthenogenesis is also known but is not common. The best understood case is that of the plant genus *Hieracium* which has been described by Rosenberg. There are two sections of this genus which differ in their cytological behavior. The sexual form of *H. umbulatum* has a diploid chromosome number of 18 whereas the variety *linearifolium* has a diploid number of 27. There are also some in which the diploid number is 36. The parthenogenetic *Archieracium*, which is closely related to this species, shows a chromosome number of 27. Rosenberg explains these facts upon the basis of hybridization. The triploid races, Rosenberg maintains, cannot easily be produced by the usual bisexual processes. Triploid parthenogenesis, however, is of rare occurrence, and the triploidy in

animals which result from fertilization of a diploid egg, if that can occur, certainly produces only an abnormal zygote.

Usually the number of polar bodies which is given off by the animal egg permits the observer to distinguish whether he is dealing with the haploid or diploid mode of development; in the haploid case two polar bodies are produced in the haploid manner, whereas in the diploid a single maturation division produces only one polar body and no reduction of chromosomes can take place. There is one special case of parthenogenesis to which was early ascribed theoretical significance in working out a theory of fertilization. O. Hertwig in 1890 described a process in the maturation of the starfish *Astropecten* which has been many times referred to since, namely, the reunion of the nucleus of the second polar body with that of the egg. A similar condition was reported by Lefevre for the egg of *Thalessema* in which the second polar body is not extruded, the spindle remaining "submerged" in the cytoplasm of the egg. Upon the basis of these observations parthenogenesis was for a long time supposed to be explained as a kind of fertilization by the polar body. It is now known, however, that such cases seldom occur. A somewhat analogous case is that of the wasp *Habrobracon* in which a cytological study shows parthenogenetically produced males to be haploid on account of the fact that the first spermatocyte division is abortive while the second is equational (A. Whiting, 1927). Of course cases in which only one maturation division occurs are not rare.

c. Parthenogenesis and Sex. Much of the work upon parthenogenesis has been due to the bearing of the problem upon sex determination. Many experiments have been made for the purpose of discovering what factors can influence the production of males or females within the parthenogenetic strains. Especially have the rotifers been used for experiments of this kind. Punnett observed various kinds of females as follows: arrhenotokous females or those which produce males; thelytokous females (female producers) of three kinds, namely, those which produce, respectively, a large percentage, a small percentage, and those which produce no arrhenotokous females. In the attempt to influence the sex of the progeny of these forms conflicting results have been obtained. The studies of rotifers have been most extensively carried on by Whitney and by Shull. In general Shull's experiments on the modification of sex have yielded positive results, for he has found a relationship between metabolism and sex determination. When the quantity of food given *Hydatina senta* is small the number of male producers is increased; old food reduces the number. Creatin, ammonium salts, beef extract, and low temperature prevent the formation of male producers. Long-continued parthenogenesis results in a decrease in male production.

Oxygen in the water increases it, although osmotic pressure, alkalinity, and acidity were not found to be effective. Whether the egg is to develop into a male or a female producer is decided during the growth period but not in the early oögonial stages. Sex is determined a generation in advance.

Whitney, on the other hand, has found less evidence that male production is a response to external conditions, although he did find that crowding in *H. senta* permitted the development of fewer male producers in direct proportion to the degree of the crowding. He found, however, that neither oxygen, temperature, nor starvation is a factor. One of his strains when fed continuously upon a diet of *Polytonia* produced nearly all females for twenty-five generations, only three per cent of the daughters being male producing. But a change of diet to *Dunaliella* increased the percentage to 57. When they were fed again upon *Polytonia*, the percentage fell off. The influence of the food was found to be upon the grandmother.

In addition to the work of Shull and of Whitney, Noyes has reported a series of experiments on *Proales decipiens* lasting through 250 generations in which no males appeared although the animals were subjected to various environmental changes including changes of chemical composition, of temperature, and of food. Moro found *Brachionus* to give increased arrhenotokous females upon treatment with chemicals such as ferric chloride (FeCl), whereas constant diet and temperature gave only thelytokous individuals.

Banta has studied the sex production in the parthenogenetic Cladocera, especially in *Moina macrocopa*. His studies with Brown upon sex control showed that experimental crowding brought about male production along with lowered metabolism in the females. The amount of food was not found to affect the results but a change in its character did have a profound effect. Male production was associated with an accumulation of excretory substances. Aeration with oxygen decreased the production of males even in crowded cultures. Similarly carbon dioxide, urea, and ammonium salts as well as other products related to excretion reduced the number of males.

d. Intersexes. Another problem in relation to sex has appeared in some of the parthenogenetic strains of Cladocera. It is the condition of intersexes or, as originally described by Banta, sex intergrades. The intersex condition manifests itself through the secondary sex characteristics in that a typical female has a series of eight secondary sex characters which are identified with her sex, and similarly for a typical male. The eight characters are as follows: (1) body size, the females are the larger at maturity; (2) size and position of the eye, the eyes of the

females being smaller and crowding the margin of the head less than those of the males; (3) outline of the head, the angle of the antero-ventral margins of the head being more acute in the male; (4) absence in the female of the nuchal protuberance; (5) and (6) character of first antennae, the males having swollen basal portions and two lateral stylets; and (7) and (8) the outline and armature of the lateral post-abdominal margins, these being more concave and not serrated in the female. The intersex individuals may have the sex organs of either sex accompanied by from one to several of the secondary sex characters of the other sex. There are also hermaphroditic forms with various combinations of the male and female secondary characters. It is Banta's opinion that these intersex conditions in Cladocera and other parthenogenetically reproducing forms are probably determined by environmental factors.

A relation of hermaphroditism to parthenogenesis appears in certain cases and it has been suggested by Winkler that it is one of transition, hermaphroditism leading over into parthenogenesis. At least the case of *Rhabdites aberrans* as described by Krüger points in that direction. These animals are free-living nematodes found in moist earth and are almost exclusively females, two counts showing four males out of 10,000 females and thirty-two males out of 2026 individuals, respectively. But the females are hermaphroditic and the entrance of sperm into the egg is necessary for the initiation of development, although it takes no further part in the process and the cleavage of the egg is as truly parthenogenetic as if no sperm had penetrated into it. In addition to this relationship, hermaphroditism may be superimposed upon parthenogenesis at least in one family of the Phyllopoda where it appears that *Lepidurus* which under favorable conditions reproduces parthenogenetically "may become hermaphroditic when food is scarce" (Bernard,1896).

e. Geographical Races. Another matter of interest is the occurrence of geographical races which are parthenogenetic. The phyllopod, *Artemia salina,* according to Artom, reproduces differently in different localities. Races which are exclusively parthenogenetic occur in Marseilles, at Capodistria and in certain other Italian localities. Bisexual races occur in Cagliari in Italy and in Utah; and a third class in which both bisexual and parthenogenetic modes occur is found at Odessa in Russia. Vandel described the conditions in the isopod, *Trichoniscus provisorius,* in which there are two distinct races, one bisexual in the usual sense, and the other a female-producing parthenogenetic race. These occupy distinct regions, the latter occurring in northern Europe, but at some points the two may co-exist. These two races are looked upon by Vandel as incipient species. Geographical parthenogenesis has also been observed

in the moth *Solenobia* by Seiler. *Solenobia triquetrella* and *S. pineti* occur in both bisexual and parthenogenetic forms, the latter being permanent and thelytokous. Of *S. triquetrella* the parthenogenetic form is widely distributed in Germany, Austria, and Switzerland, but the bisexual form has been found in a single locality only. In *S. pineti* the bisexual form is common but the parthenogenetic is restricted to a very few localities. Finally *Trialeurodes vaporariorum*, a white-fly has two races, according to Schrader, that are unlike in their parthenogenesis, in that the English race produces females with only three per cent males and the American parthenogenetic females produce males only.

f. Causes of Parthenogenesis. Of the causes of natural parthenogenesis little is known. It would seem that the developmental mechanism of the egg is capable of activation in more than one manner. Usually the entrance of a sperm into the egg sets off the processes which are involved. This initiatory action is capable of imitation by both chemical and physical means as in the cases of artificial parthenogenesis. There are also conditions in which, it would appear, development may go forward automatically, the participation of the sperm being entirely dispensed with. In the case of the normal fertilization of any egg there is no reason for regarding the developmental processes of the egg as brought to a standstill before the sperm enters. Rather they are slowed down to a speed from which they will not recover unless external influences are operative, usually in the form of the sperm. Perhaps monogametic development is possible only where the inhibition is less intense, thus giving automatically normal parthenogenesis. On this hypothesis the failure to induce the development of sperm, by placing them in suitable media as has been reported by Loeb and others, may mean nothing more than that the developmental processes in the sperm are reduced to a still lower speed from which no means thus far tried have been effective to return them to a functional level.

Other suggestions have also been made as to the factors which are responsible for parthenogenesis. Issakowitsch held that in Cladocera the alternation of bisexual and parthenogenetic stages was governed by changes in the nucleo-cytoplasmic ratio. Papanicolau reached similar conclusions. Shull, however, investigated the nucleo-cytoplasmic ratios in the rotifer, *Hydatina senta*, without finding any correlation between it and the mode of reproduction. Although it has been claimed that food, oxygen content, and other similar external factors influence the sex ratios of parthenogenetically produced animals, there is no good evidence that these factors are responsible. Banta thought the formation of an ephippium (winter egg, which develops upon fertilization) to be

due either to unintentional change in the media, in which parthenogenetic daphnids had been developing, or to overcrowding.

Another suggestion (which is so far merely record of an observation rather than a cause) relates to the lagging chromosome of the phylloxerans which is observed to accompany parthenogenesis. Peacock and Harrison working upon lepidopteran crosses reached the conclusion that parthenogenesis is a result of hybridity and later supported this conclusion by a study of the data of other crosses including Nabours' experiments on parthenogenesis in *Apotettix*. Finally Nabours, working upon *Paratettix*, studied genetically two strains one of which was highly parthenogenetic and the other had never so reproduced. He obtained genetic evidence that parthenogenesis is a character which segregates in a Mendelian manner and believes it due to certain genes. Robertson's cytological studies on Nabours' material fall into line with this hypothesis. The entrance of the sperm is necessary for the second polocyte division, and when it is lacking the diploid chromosome number is retained. In this latter case if the specific genes for parthenogenesis are present development is initiated. It is to be noted that Agar working upon *Daphnia* and Whiting upon *Habrobracon* failed to find genetic evidence of the cause of parthenogenesis. But Punnett as long ago as 1906 had suggested the probability that the question is concerned with the zygotic constitution of the egg and thought that it might be a matter of Mendelian segregation. These different hypotheses show the need of much further study of this most elusive subject.

B. The Occurrence of Normal Parthenogenesis

Natural parthenogenesis is of much wider occurrence in the animal kingdom than is generally recognized, and it is not rare among the plants. Examples are found in the following groups of animals: rotifers, nematodes, trematodes, *Dinophilus*, three orders of Crustacea, myriapods, arachnids, and ten orders of insects. It seems desirable to point out some of the important studies in each of these groups, although no attempt can be made toward inclusiveness. For references and a more nearly complete listing of cases the reader should consult Winkler.

a. Rotifera. Among the rotifers, as already described, typical arrhenotokous parthenogenesis occurs in the same groups of animals with thelytokous parthenogenesis. The studies on *Hydatina senta* are among the best known of all studies on the type of reproduction. Three types of females, as previously indicated, are found here, and by experimental means it is possible to change the strains from parthenogenetic to bisexual.

b. Nematoda. For the nematodes the case of *Rhabdites aberrans* as described by Krüger has already been mentioned. The trematodes are commonly described as giving rise to rediae from cells which are segregated at one end of the body of the sporocyst and become an ovary. The new rediae arise from these so-called "ova," which must be thought to develop parthenogenetically if the view of their origin is maintained that these are true eggs. This controversial subject is discussed in other chapters in this work.

c. Annelida. Among the annelids parthenogenesis is suspected in *Dinophilus conklini* from the preponderance of females. In the leech *Hemiclepsis marginata* the early finding of parthenogenesis has failed of confirmation in more recent experiments (Brumpt, 1900).

d. Crustacea. Two orders of the Entomostraca exhibit parthenogenesis and include in their number some cases that have been given a great amount of study, while a third offers a case which is suspected to be parthenogenetic. Among the Phyllopoda examples may be cited from both the Branchiopoda and the Cladocera. Of the former, *Apus*, *Lepidurus*, and *Artemia* are well known. Reference has already been made to *Lepidurus* because of its hermaphroditic relationships. Thelytokous parthenogenesis occurs in these forms. *Artemia salina* likewise has been mentioned in connection with geographic parthenogenesis. On this form the most recent studies are those of Artom (1912). Two polocytes are formed in the development of the egg. Regularly a reduction of chromosomes from 42 to 21 takes place. The parthenogenetic forms are tetraploid and of a type called somatic. There are therefore two strains of *Artemia*, one diploid and bisexual, the other tetraploid and thelytokous parthenogenetic. The following explanation has been upon the basis of the very slight evidence that in the parthenogenetic strain only one polocyte is formed (Brauer) and that without reduction. If the egg and polocyte nuclei, both of which retained their somatic number of 42 chromosomes, should fuse, then an individual with the tetraploid number would be produced. As yet this explanation must be looked upon as purely speculative. Numerous others of the true phyllopods, such as *Branchipus streptocephalus*, have been observed to reproduce parthenogenetically and still others are suspected because of the scarcity or absence of males.

Of the work on the Cladocera, which dates from Weismann's studies in 1879, the general facts are well known. Bisexual generations are followed by parthenogenetic which may continue indefinitely. The various races differ greatly in the number of generations produced within a year and in the frequency of the bisexual forms. Apparently most if not all forms are parthenogenetic at some stage of their life cycles, but

those genera most studied have been *Daphnia, Simocephalus, Moina, Bosmina, Chydorus, Polyphemus,* and *Leptodora,* the first four being best known. The more recent students of the group include Olofsson (1918), Woltereck (1909–1911), Langhans (1911), Kuehn (1908), Chambers (1913), Taylor (1914), Thiebaud (1913), and especially Banta and his co-workers, whose work is published in a series of papers beginning in 1914 and is not yet completed. In all forms the males are rarely seen and in some species are unknown. As already stated much of the work concerns the control of sex and the intersex conditions in these forms. There is good evidence that sex ratios and intersex conditions are both subject to environmental influences.

In the order Ostracoda, parthenogenesis was discovered in 1880 by W. Mueller and by Weismann for a number of genera, it having been shown that virgin females produced eggs that developed into females; that is, produced a thelytokous race. No reduction division was found to occur by Woltereck (1898) or by Schleip (1909) for a considerable number of forms which they studied and parthenogenesis for these animals is therefore of the diploid or somatic type. In 1914 Wohlgemuth studied carefully the reproduction of numerous fresh-water ostracods and thought they fall into two groups, one of a purely bisexual and the other of a purely parthenogenetic type, although there are transitional forms between the two. Other investigators of the Ostracoda have been Menzel (1911), Alm (1916), and Olofsson (1918). Their chief concerns have been to describe the facts of parthenogenesis, the relation of the bisexual generation to the parthenogenetic, the duration of each and the conditions under which they occur. Not much of importance to an embryological account is gained from these forms, although Schleip's work on the various species of *Cypris* offers perhaps the best cytological description of parthenogenesis in any crustacean form. His observation of synapsis not followed by reduction is worthy of note. It may be remarked that some of the studies of ostracods have taken up the question, raised for many Crustacea, as to the possible impairment of vigor of a strain due to long-continued parthenogenesis. Here as elsewhere among the members of the class the general consensus of opinion seems to be that no loss of vigor can be noted.

Mention should be made of the possibility of parthenogenesis in the third order of the Entomostraca, the Rhizocephala. In the genus *Sylon* belonging to that suborder Smith reported his complete failure to find males and so suspected that parthenogenesis is the method of reproduction.

In one order of Malacostraca parthenogenesis has been reported,

namely in the Isopoda. Vandel (1928) has given an account of geographical parthenogenesis, already mentioned in *Trichoniscus*. Two races occupying different localities were described, one bisexual, the other exclusively parthenogenetic. In some places they may exist side by side, but mating does not occur between the two races. Physiological factors tend to keep the two races distinct and to increase the divergence between them. The parthenogenetic race is found to be triploid in its chromosome relations. The eggs of the parthenogenetic female do not undergo synapsis.

For both of the two myriapod groups, the Chilopoda and the Diplopoda, cases of suspected parthenogenesis are recorded. Of the former *Geophilus* and *Lomyctes*, and of the latter *Nopoiulus* and *Polyxenus*, have been studied. All of these include races which either lack males entirely or in which they are to be found in certain localities only. In addition observations were long ago made by Sograff (1882) upon *Geophilus proximus* of which unmated females laid eggs that began to develop.

Among the spiders accounts of parthenogenesis have been reported for nearly a century and it may still be suspected, but Montgomery in 1903 concluded that it is "very rare among spiders, and it is probable that most species do not show it at all." Since Montgomery's finding, it does not appear that any work has been done which successfully establishes parthenogenesis in these animals.

Among the mites there is no doubt that parthenogenesis occurs, for Ewing (1914) described the process in the bisexual *Tetranychus telarius* and showed that the unfertilized eggs give rise to males exclusively. It is arrhenotokous parthenogenesis. Among the ticks, the Brazilian form, *Amblyomma agamum*, has been shown by Aragas (1912) to multiply by thelytokous parthenogenesis.

It has been conjectured by Hennecke (1911) that the tardigrade, *Macrobiotus macronyx*, has an alternation between parthenogenesis and bisexual generations.

c. Insecta. Parthenogenesis is of very general distribution in the insects, having been described in the ten orders. The following table, compiled in part from Winkler, summarizes the occurrence of the phenomenon. For references to most of these cases the reader is referred to Winkler, but a few of especial significance are mentioned following the table.

Aptera

Machilis. Heymons, 1905; doubtful but could find no males.

Forbicina. Verhoeff, 1912; all females, so no doubt parthenogenesis occurs.

Orthoptera

Mantidae

Mantis religiosa, *Sphodromantis*: Przibram, 1909; induced experimental parthenogenesis, not natural.

Phasmidae

Eurycnema. v. Wuelfing, 1899; three generations of parthenogenetic females in Java.
Hanitsch, 1902; two generations of parthenogenetic females.

Bacillus. Dominique, 1896–99; two thelytokous generations of parthenogenesis. Males are rare.
Also Stadelmann, v. Bachr, Cameron, Daiber.

Dixippus. Pantel and de Sinety, 1908. Parthenogenesis established.
Schmitz, 1906. Four generations of thelytokous parthenogenesis.
Hammerschmidt, 1910. Five generations of thelytokous parthenogenesis.
Jeziorski, 1918. Four generations of thelytokous parthenogenesis.

Phasma. Thurau, 1899. Only females.

Monandroptera, *Raphiderus*: Bordoz, 1913. Parthenogenetic.

Leptinia. de Sinety, 1900. Thelytokous parthenogenesis. Pantel and Ucles also found parthenogenesis.

Phyllium. Bordas, 1898. Suspected parthenogenesis.

Locustidae

Saga pedo. Claus-Grobben, 1917. Report parthenogenesis.

Gryllidae

Myrmecophila acervorum. Schimmer, 1909, suspected parthenogenesis.

Tettigidae

Apotettix, *Paratettix*, *Telmatettix*: Nabour and colleagues, 1919–1929. Thelytokous parthenogenesis. Best understood cases.

Coleoptera. Parthenogenesis is of rare occurrence and most of the older accounts are not trustworthy.

Tropiphorus carinatus. Calwer, 1916, reported parthenogenesis.

Otiorrhynchus turca. Ssilantjew, 1906
O. ligustici. Wassiliew, 1909
O. cribricollis. Grandi, 1913
Thelytokous parthenogenesis.

Calandra oryzae. Hinds and Turner, 1911, report parthenogenesis common. Eggs produce both sexes.

Strepsiptera

Stylops, *Xenos*: Brues, 1903; Nassonov, 1910
Elenchus. Muir, 1906
Parthenogenesis probable due to morphology of female genitalia.

Thysanoptera

Most species are bisexual. Many may multiply parthenogenetically. Thelytokous parthenogenesis is established for *Parthenothrips, Anaphothrips, Heliothrips, Taeniothrips,* and *Liothrips;* for a number of others it is probable. Arrhenotokous parthenogenesis is experimentally shown for *Anthothrips verbasci.*

Corrodentia

Ectopsocus. Ribaga, 1904. Probably parthenogenetic.

Hemiptera

Aleurodes. Morrill and Back, 1911. Arrhenotokous parthenogenesis.

Trialeurodes. Schrader, 1920. English race thelytokous. American race arrhenotokous.

Aphididae. Many species of *Aphis* are known to be parthenogenetic and types vary. In north they reproduce bisexually during colder seasons but in south may be exclusively parthenogenetic.

Other genera include *Callipterus, Ceratophis, Macrosiphum, Myzus, Pemphigus, Rhopalosiphum, Schizoneura, Toxoptera; Chermes, Pineus; Phylloxera.*

Coccidae. Many genera of scale insects are described as parthenogenetic although some cases are not known. Included are *Aspidiotus, Ceroplastes, Cryptococcus, Diaspis, Eriopeltis, Lecanium, Lepidosaphes, Orthezia, Parthenolecanium, Pseudococcus, Pulvinaria, Saissetia.*

Lepidoptera

All proven cases belong to family Psychidae or are closely related to it. *Acanthopsyche, Pachythelia, Psyche, Sterrhopteryx, Phalacropteryx, Apterona, Cochlophora,* and *Luffa* are included. The best known is *Solenobia lichenella.* Hoffmann, 1859.

Solenobia triquetrella. Hoffmann, 1859–1869. Usually bisexual. Both bisexual and parthenogenetic modes described. Geographical parthenogenesis. v. Seibold, 1871; Rolph, 1884; Rebel, 1906; Dampf, 1907; Seiler, 1918.

Solenobia pineti

Diptera. Parthenogenesis seldom found in this group.

Chironomus. Godlewski, 1914. Paedogenesis and parthenogenesis intermingled.

Corynoneura. Goetghebeur, 1913. Rarely parthenogenetic.

Metriocnemus. Picado, 1913. Males few in number.

Hymenoptera. Six families show parthenogenesis.

Tenthredinoidea

The following show thelytokous parthenogenesis:

Albia fasciata
Allantus canadensis
Allantus pallipes
Amauronematus puniceus

Amauronematus semilacteus
Caliroa limacena
Cimbex connata
Croesus varus
Empria abdominalis
Empria pulverata
Hemichroa alni
Hemichroa crocea
Nematus erichsoni
Pachynematus obductus
Phyllotoma aceris
Phyllotoma nemorata
Pontania capreae
Pontania viminalis
Pristiphora pallipes
Pristiphora fulvipes
Pteronidea spiraeae
Pteronidea tibialis
Thrimax mixta

The following are probably thelytokous:

Caliroa aethiops
Phyllotoma vagans
Selandria stramineipes
Stronglylogaster lineata

Producing both males and females are:

Ametastegia equiseti
Pseudoclavellaria amerinae

The following are arrhenotokous:

Albia nitens
Allantus cinetus
Allantus viennensis
Ametastigia glabrata
Arge, all species
Caliora annulipes
Cladius
Croesus brischkei
Croesus litipes
Croesus septentrionalis
Lophyrus, all species
Nematus coeruleocarpus
Nematus luteus
Periclista albida
Phymatocera aterrima
Priophorus padi
Pristiphora conjugata
Pristophora crassicornis
Pristiphora geniculata
Pristiphora testacea
Pristiphora alnivora
Pteronidea, 15 species
Trichiocampus viminalis
Trichiocampus lucorum

Cynipoidea

The following genera contain species which are parthenogenetic and in most cases the males are unknown, so they are probably thelytokous:

Andricus
Aulacidea
Ceroptres
Cynips
Drastrophus
Phanacis
Rhodites
Dryophanta
Neuroterus

Ichneumonoidea

Chalcids

The following are arrhenotokous:

Ageniaspis
Anaphoidea
Copidosoma
Encarsia
Encyrtus
Entedon
Litomastix
Melittobia
Micromelus
Microterys
Paracopidosomopsis
Pentarthron
Pteromalus
Schedius
Tropidopria

The following are thelytokous:

Asphelinus	*Coccophagus*	*Tetrastichus*
Aspidiotiphagus	*Oötetrastichus*	*Tripoctenus*

The following contain species that produce both sexes or are uncertain as to sex although parthenogenetic:

Eupelmus	*Isosoma*	*Scutellista*
Habrocytus	*Paniscus*	*Trichogramma*

Braconids: All parthenogenetic species are described as arrhenotokous.

Ichneumonids: All parthenogenetic species produce mixed broods.

Proctrotrupids: *Anagrus, Gonatopus* are thelytokous. *Balus, Paranagrus, Phanurus* produce mixed broods, or first females then later males.

Doubtless many more parasitic hymenoptera are parthenogenetic.

Formicoidea

Arrhenotokous parthenogenesis is very widespread among the ants. Many workers are fertile and lay unfertilized eggs from which males develop. Some hold the view that both males and females may develop from parthenogenetic eggs. A few cases are recorded in which thelytoky is claimed to be established. Certainly it is much less common than arrhenotoky.

Vespoidea

Parthenogenetic eggs of queens and workers develop into males only. Examples are *Polistes gallicus*, v. Seibold, 1871
Vespa germanica, Marchal, 1896

Apoidea

For many bees arrhenotokous parthenogenesis is conclusively established. Haploid males are so produced, and diploid males probably do not occur, that is, males produced from fertilized eggs, although that possibility has not yet been excluded. In a few genera both males and females are produced parthenogenetically but this is not usual. Queens and workers are females produced bisexually.

As is to be seen from the preceding table, a great deal of work has been done upon insect parthenogenesis. It has, however, been to a large extent concerned either with the general life cycle or with the cytological and genetical aspects of the problem. Many studies of very fine character have been made upon gametogenesis and sex determination, and upon the factors involved in the control of sex. Of recent years the problems which are bound up with uniparental inheritance have attracted much attention. It must be admitted that much of this work has not advanced our general embryological knowledge of the insects to any great extent.

Among the many investigations may be mentioned a few to which

the reader is referred if he would pursue further these aspects of the problem. The work of Nabours and his collaborators upon the various forms of grouse locust promises much in the cytological analysis since grasshopper chromosomes are among the most favorable for study. The saw flies have been studied especially by Doncaster and by Peacock and Harrison. Of the aphids and phylloxerans a very careful cytological study was made by Morgan. An earlier study by Tannreuther is a good account of the general embryology of the aphids. Parthenogenesis in the moths is most recently made known to us by the studies of Goldschmidt and of Seiler. Of the Hymenoptera the extensive work done by Patterson on *Paracopidosomopsis* will serve to introduce the student to the relation of parthenogenesis and polyembryony, and the best detailed account of the cytology of the bee is undoubtedly that of Nachtsheim. Other investigations have already been mentioned in connection with other aspects of the general parthenogenesis problems.

f. Plants. Among plants a considerable number of cases of parthenogenesis are already known and it is thought of interest to mention the conditions found there in this discussion although the problems involved are outside the scope of this work. It is difficult without careful study among the plants to distinguish between cases of parthenogenesis and those of the simple vegetative apogamy. Among higher plants haploid parthenogenesis is exceedingly rare, although several cases have been described for the lower forms. Haploid parthenogenesis is also described as generative parthenogenesis by Winkler and as true parthenogenesis by Strasburger. Diploid parthenogenesis is less common among the lower forms but more so among the vascular plants. It is due to the failure of the maturation process to occur to completion. The forms which are known to be parthenogenetic are as follows:

Haploid parthenogenesis: *Spirogyra,* Ernst, 1918; *Vaucheria,* von Wettstein, 1920; *Ectocarpus,* Kylin, 1918; *Fucus,* Overton, 1912 (probably haploid); *Gastrodia,* Kusans, 1915; *Oenothera,* Haberlandt, 1921, 1922; *Datura,* Blakeslee and Belling, 1922, evidence from breeding experiments; *Nicotiana,* Clausen and Mann, 1924.

Diploid parthenogenesis: *Chara crinita,* Ernst, 1918; *Athyrium felix-foemina,* var. *clarissima,* Farmer and Digby, 1907; *Scolopendrium vulgare,* Farmer and Digby, 1907; *Marsilia Drummondii,* Strasburger, 1907; *Allium odorum,* Haberlandt, 1923; *Atamosco texana,* Pace, 1913; *Calycanthus,* Schürhoff, 1923; *Alchemilla,* Murbeck, 1901, Strasburger, 1905, Böös, 1917; *Wikstroemia,* Winkler, 1906; *Eupatorium glandulosum,* Holmgren, 1919; *Erigeron annuus,* Tahara, 1921; *Antennaria alpina,* Juel, 1900; *Chondrilla,* Rosenberg, 1912; *Taraxacum,* Juel, 1904, Osswa, 1913, Sears, 1922; *Archieracium,* Rosenberg, 1917.

II. ARTIFICIAL PARTHENOGENESIS

Of great embryological significance is the phenomenon of artificial or experimental parthenogenesis. It has been the subject of much of the work on experimental embryology of the present century and its chief consideration should be a matter for a treatise on experimental embryology. However, its importance for an understanding of matters which have to do with the normal embryological process is so great that a brief account should be included here. For details and bibliography the reader is referred to the extensive accounts which have been published by Loeb (see his "Artificial Parthenogenesis and Fertilization") and by Morgan in his "Experimental Embryology." Wilson has also critically considered the cytological aspects of the problems concerned with artificial parthenogenesis in "The Cell."

The student should be reminded of the double function which fertilization serves in normal development; the one is hereditary in character in that fertilization provides the mechanism for conveying the contribution of the male to the offspring. The other is strictly developmental in that it sets in motion those processes which have been inhibited or are latent in the egg at the time of maturation. It is with this second aspect of fertilization that experimental or artificial parthenogenesis deals. These experiments have sought to imitate and to interpret the normal processes of development as initiated by fertilization, and because of that a much clearer understanding of the normal process has been reached. The experiments have shown that it is possible to cause the unfertilized egg of a great many animals to develop into larvae largely under the influence of chemical treatment, although physical means have also been used to accomplish the same end less perfectly.

The work on parthenogenesis is chiefly a monument to the insight of Jacques Loeb and his analytical experiments have been of the most importance in solving the many problems involved. However, numerous other students have contributed to the progress of the work both before and since his announcement of the discovery that it was possible to induce eggs to develop artificially. Among the preliminary studies which antedated Loeb's discovery in 1899 may be mentioned the paper of Loeb himself in 1892 in which he studied the effect of addition of sodium chloride to the sea water in which the eggs of *Arbacia* developed; Richard Hertwig's observation (1896) on the effect of treating unfertilized sea-urchin eggs with strychnine; a series of experiments by Morgan (1896, 1899, 1900) on the effect of salt solutions on the segmentation of eggs and the formation of artificial asters; the work of Mead (1896–98) showing that unfertilized eggs of *Chaetopterus* will

form polar bodies in certain solutions in which the amount of sodium chloride is increased. Loeb's papers upon this subject are many, and to him is due the chief credit for the developing of our knowledge of the subject. Among those who early worked upon the problem was Delage, who in a series of papers from 1900 to 1913 added a great deal to the understanding of this difficult subject; he succeeded in rearing through metamorphosis the larvae of both sea-urchins and starfish which had been induced to develop by artificial parthenogenesis. Loeb and Bancroft (1913) brought a parthenogenetic frog through metamorphosis and found that its sex glands contained eggs. Shearer and Lloyd (1913) succeeded even better than Delage in bringing parthenogenetic larvae of *Echinus* through metamorphosis, and more recently (1918) Loeb has brought a considerable number of parthenogenetic tadpoles to the adult stage. It is to be noted, however, that only in the case of frogs has it been possible to imitate in the laboratory the conditions of nature sufficiently to bring animals developed by parthenogenesis to sexual maturity. Since Loeb's work, Bataillon, R. S. and F. R. Lillie, Goldschmidt, Gray, Harvey, Heilbrunn, Herbst, Herlant, and Just are among the many who have made extensive studies into the embryological phases of artificial parthenogenesis.

When one begins to consider the significance of artificial parthenogenesis he is at once led to inquire as to how general the phenomenon is or how generally it may be expected to be possible in the animal kingdom. It may well be that eggs are so constituted as to permit their development without fertilization, at least into the cleavage stages, and that we are merely unable to understand the requirements in each case and to devise the correct procedure. A broad generalization of this character is hardly safe, however, for up to the present time the only forms in which it has been possible to induce experimental parthenogenesis are those in which the experiment is relatively easy to perform. In every case the eggs are of the type which is shed freely into the water. The difficulties of the experimental work which is involved may be the reason for this limitation or it may be that a deeper limitation is operative so that eggs which are adapted for other kinds of development may not be stimulated to cleave artificially. Thus far the following groups have responded to the treatments that have been devised to induce parthenogenesis artificially. By far the most work has been done on the sea-urchins. *Arbacia*, *Strongylocentrotus*, *Toxopneus'es*, and *Paracentrotus* have all shown themselves excellently adapted to experiments of this type and they have been used for much important work, especially the first two mentioned. The sand dollar, *Echinarachnius*, has proved adaptable for similar experiments. Among the starfishes

the eggs of the commoner species of *Asterias* as well as some of the more unusual starfishes, as *Asterina gibbosa*, have been used successfully. It is to be noted, however, that the methods which have proven most successful with the sea-urchin give but very poor results with the starfish, and conversely little success has been obtained by applying the two best methods of inducement for the development of the starfish egg, namely, carbon dioxide and mechanical shaking, to eggs of the sea-urchins. The eggs of the frog, *Rana fusca*, have often been induced to develop by parthenogenesis, and the most successful treatment has been again a mechanical one, namely the simple pricking of the surface of the egg. Various annelids have been studied, especially *Chaetopterus*, *Amphitrite*, *Thalessema*, but the best results in this group have unquestionably been gotten for the eggs of *Nereis*. Here again the means of inducing parthenogenesis is physical rather than chemical, namely, the subjecting of the egg to heat.

A second question arises in attempting an embryological interpretation of the experiments in artificial parthenogenesis, namely, do these experiments produce animals that are fairly normal. As already pointed out Loeb's own studies led him to the belief that "parthenogenetic larvae may be normal and apparently healthy," and indeed, he said, "if the raising of the larvae was not such a tedious process parthenogenetic animals would exist today in large numbers." His methods have certainly produced, both in his own hands and in those of other investigators as well, larvae which resemble the normal in every particular, and Delage, Shearer, and also Fuchs were able to bring parthenogenetic larvae to adulthood. The chromosome relations of the individuals as produced, however, have not been made out with certainty as yet and it is still doubtful whether haploid larvae produce normal adults. Parmenter (1925) has shown that in the larval and adult stages Loeb's parthenogenetic frogs have the diploid chromosome number. The direct evidence is entirely lacking as to whether the adults produced from diploid larvae can themselves produce offspring. It should be said that most investigators are of the opinion that the parthenogenetic larvae which are experimentally produced are not merely normal in appearance but that they really are in every respect what they seem, that is, normal animals.

Loeb developed a theory of parthenogenesis as a result of his experiments, and what he spoke of as his improved method is based upon that theory. According to his view the formation of the egg membrane is the deciding criterion by which the initiation of development may be recognized. Indeed he traced a causal relation between the formation of the egg membrane and the subsequent development of the egg and

attached much more importance to this process than had previously been done. Membrane formation is the deciding condition of development. Not all later workers have agreed with Loeb in this view, but unquestionably his explanations have not been out of harmony with the facts which he observed.

The so-called improved method of Loeb for inducing artificial parthenogenesis consists of two steps. Its details as to concentration and length of exposure must be worked out for the particular species of sea-urchin eggs used and indeed different individuals will respond differently so far as the duration of the exposures are concerned. With regard to these points the method is entirely an empirical one. The procedure as worked out for *Arbacia* eggs is as follows: Unfertilized eggs are placed in a sea water mixture containing 2 cc. of $\frac{N}{10}$ butyric acid to 50 cc. of sea water. Apparently any monobasic fatty acid would serve equally well for this purpose but Loeb found butyric to be satisfactory for the experiments (2.8 cc. of $\frac{N}{10}$ butyric were necessary in the case of *Strongylocentrotus purpuratus* eggs). The duration of the exposure must be brief, from 1½ to 3 mintues. The eggs are then transferred to normal sea water and after 10 or 15 minutes to hypertonic sea water consisting of 8 cc. of 2½ *M* NaCl to 50 cc. of sea water. At a temperature of 23° the eggs must remain in this solution from 17½ to 25 minutes after which they are transferred to normal sea water. The effect of this double treatment has been shown many times and in many different localities. A very large percentage, usually about as great as is obtained from the development of fertilized eggs, go ahead with the cleavage process and develop to larvae; doubtless the inability to rear the larvae is not in any way due to the fact that they were parthenogenetically produced.

The explanation of this double procedure has been a matter of some discussion. Loeb believed that membrane formation sets going certain chemical reactions upon which the future development of the egg depends. These chemical reactions, however, leave the egg in a condition from which it will not itself recover, and unless a second factor is employed in the process it will disintegrate rapidly. These reactions are on the nature of oxidations and are at least to a certain extent cytolytic in nature. The second factor is therefore necessary as a corrective to regulate the extensive oxidization which is produced by the exposure to the first solution. Loeb's conclusion that cytolysis must take place unless corrected by the hypotonic solution has been questioned by some

later experimenters, notably by Just, who has been able to induce the development of normal plutei of *Arbacia* by the use of hypertonic solutions alone. The exact preparations of the salts which are used to render the sea water hypertonic and the durations of the exposure to this mixture must be regulated with a great exactness if successful results are obtained. His optimum solution is made up of 22 parts of $2\frac{1}{2}$ *M* NaCl or KCl plus 78 parts of sea water, although variations from this mixture were also used. Of course great care was used to control the experiment in every way necessary. Eggs subjected to a treatment with this solution form membranes while still in the solution. Subsequently the eggs are returned to sea water and their development proceeds. Upon the basis of these experiments Just holds that the treatment with the fatty acid is not necessary and therefore that the hypertonic sea water is not serving as a corrective to stop the cytolysis induced by the unusual step in Loeb's procedure. He is disposed to relate the activation of the sea-urchin eggs by this treatment to the egg secretion, fertilizin, which is given off by the unfertilized eggs into the sea water and which has been shown to be a necessary intermediary to normal fertilization with sperm. Just suggests that the activating agent which will accomplish experimental parthenogenesis serves to bind the fertilizin produced by the egg and thus to complete the necessary cortical changes which must take place if development is to proceed. The full explanation of the artificially induced changes has perhaps not yet been offered with regard to the experimental facts. However, there is no question that the entire subject has been one of the most successfully studied of all problems of experimental embryology.

CHAPTER VI

PAEDOGENESIS AND NEOTENY

A series of conditions is known that have to do with the early sexual maturity and reproduction of certain animal forms which otherwise retain their youthful characteristics. These are to be taken up under the general heads of paedogenesis and neoteny although not all the cases included come under a strict definition of either of these terms. They occur in a great many widely scattered groups of the animal kingdom and present a great many variations some of them so wide that they are scarcely to be recognized as of related processes. When such diverse conditions appear, it goes without saying that confusion is to be found in the literature dealing with them. Regarding the processes under consideration in this chapter exactly such confusion is found, some writers using the terms loosely and failing to classify with sharpness the cases cited.

The biogenetic law comes into the discussion for the reason that one may regard a particular phenomenon as recapitulating past phylogenetic conditions, or he may look upon the animal showing it as exhibiting neoteny, at least of the characters in question, and perhaps generally. Thus the familiar axolotl which is the sexually mature, unmetamorphosed *Amblystoma* is the usual example of neotenous development, but it is also cited by the proponents of recapitulation as explained by the general progress in development of the *Proteus, Necturus, Amblystoma, Salamandra* series of urodele recapitulation. Most of the cases of this kind, however, are concerned with adult conditions quite as much as with those of immature forms and therefore would seem to be rather outside the scope of the present discussion.

The general subject of neoteny also calls up the problems of heterochrony, by which is meant a disturbance of the synchrony of development. That is, if one might take the normal series of developmental time stages with each organ in its proper relation to the others as a standard, representing perhaps the phylogenetic series, then any departure from this standard would be spoken of as heterochrony. (See also Chapter III, Part Two, for discussion of heterochrony.) Similarly, the sequence of embryonic events when disturbed, as in neotenous development, involves heterochrony. The principle is one with an

interesting history, recalling especially the work of two investigators, Oppel and Keibel, although many others have contributed to it, some independently and others in collaboration with Keibel in the publication of his famous "Normentafeln zur Entwickelungsgeschichte der Wirbeltiere." Oppel began this study comparing the different developmental stages of different animals and arranging them in tabular form to show the comparative progress of the most important organs of vertebrates. He thought himself to have found similar ontogenetic stages and to be able to compare the young stages among themselves as well as the adults among themselves. The recapitulation doctrine found support in his work in that the young stages of higher forms are similar to the older stages of the lower animals. Departures from the series thus established were to be attributed to heterochrony. Keibel as a result of his studies and tables concluded that the time of the appearance of an organ is dependent upon the time at which it will be required to function. Thus the order of appearance of organs in a developmental series is of itself of phylogenetic significance. But Keibel did not recognize the biogenetic law as valid in these cases.

Another early student of heterochrony in vertebrates was Mehnert, who devoted his attention particularly to the relation of the subject to recapitulation. His view on this matter is of interest, for he thought that only the early development of an organ had recapitulatory value in any precise fashion, whereas in later stages the processes are quite schematic. He studied also organs which undergo regression as well as those whose development is only progressive. Heterochrony he found to be due (1) to precocious development of an anlage, (2) to rapidity of growth, (3) to rapidity of histological differentiation, and (4) to abbreviation or omission of intermediate stages. Retardation of these processes as well as their acceleration call forth heterochrony.

Heterochrony is thus seen to deal with parts of organisms rather than the animal as a whole and to show the effects of disturbances of developmental rate and rhythm upon the entire animal only through its effect on its component organs. At first thought paedogenesis might be considered as but very indirectly concerned with a matter such as this, but a reconsideration of the facts easily brings out a very clear relation, for we find paedogenesis to mean merely that, in an animal exhibiting this phenomenon, the organs of the body which have to do with reproduction have been much accelerated in development, while at least some other parts of the body have failed to keep up the pace set by these organs; or in some cases we must think that the reproductive organs and related parts have retained their customary pace while the others have been very considerably inhibited.

During the present century many investigators have contributed to the knowledge of the general subject. It will perhaps serve as an illustration to point out the importance of Stockard's very extensive study on the development of *Fundulus*, particularly with regard to the effect of changes in the rate and to the various means by which abnormalities may be produced, notably by temporary arrests of development.

It is thus clear that the matters which are the subject of this chapter really constitute but special cases of heterochrony. In assigning to these phenomena as a causal factor the disturbance of the speed of development, we bring them all into one category. It is sometimes difficult to decide whether one organ has undergone a retardation of development or whether another is accelerated with respect to the normal and so it has not always proven easy to separate sharply the processes which are involved. By some, especially Giard, Chun, Kollmann, and others, fine distinctions have been drawn, the need for which is not entirely clear if we but relate the different processes to heterochrony.

By paedogenesis is meant sexual reproductive maturity in a pre-adult stage; it is of two types, parthenogenetic and bisexual. As a special form of paedogenesis, Packard has given the term "chrysallogenesis" to a case in which the pupa of *Chironomus* has been found to lay eggs.

By progenesis is meant the permanent retention on the part of the somatic structures of the conditions reached at the time when sexual maturity is attained.

Neotenous organs have youthful characters although the animal possessing them has developed its adult condition. Or, to put it in another way, animals with neotenous organs retain the ancestral larval conditions in the particular structures which show neoteny.

Disogeny is the "sexual maturity of one and the same individual in two different conditions, between which a metamorphosis with retrogression of the sex products occurs" (Chun).

It is true that these definitions savor of dogmatism and of course require much elaboration. It would seem, however, that neoteny and progenesis are not far apart and that paedogenetic animals may easily exhibit disogeny. It will perhaps be best for the purposes of our present discussion if we limit ourselves fairly sharply to the cases which illustrate neoteny and paedogenesis. Of these there are certain classical ones which should receive especial attention. There are only a few of these cases in which the animals as a whole are spoken of as paedogenic or neotenous, but when we come to consider the organs to which the latter conception in particular may be applied, the illustrations abound and important explanations of obscure phenomena have been worked out on this basis.

Of all the cases undoubtedly the axolotl is the best known. As already

stated, *Amblystoma tigrinum* occurs in two forms, the one of which is technically immature, although it is sexually active. This paedogenic form was first described from the lakes about Mexico City and was thought to belong to the genus *Siredon* (*S. lichenoides*). The relation of this form to the well-known salamander was only discovered accidentally in Paris in 1865. Sexual maturity is reached at six months of age and sex products are shed. This is paedogenesis, for the animals have not yet lived long enough for metamorphosis.

However, metamorphosis may never occur. In many high mountain lakes axolotls live their entire life without undergoing the necessary

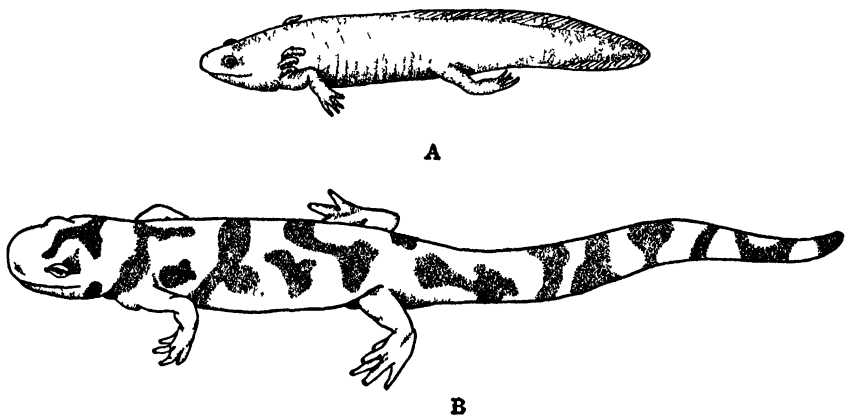


FIG. 218. External views of an axolotl (A) and an *Amblystoma* showing differences of gills, body form, and leg development.

changes to transform them into *Amblystoma*. The reason for this is not clear. If the animal be fed on thyroid, if it be forced gradually to leave the water and adopt a land life, if it be transferred from deep to shallow water, from cold to warm, or if it be brought into the presence of certain chemicals it will undergo metamorphosis promptly, but the causal mechanism is not yet understood. If it remains in the same environment as that in which it became paedogenetically mature, however, it will live its entire life without metamorphosis. This is neoteny, for the larval characters are retained long after the normal time.

The neotenous animal differs in a number of important characters from the type form. It has large external, red gills with gill slits, its tail is long and broad but flattened laterally so that it is adapted for swimming, and the body features are those of a water-living animal. Internal conditions likewise show immaturity, the skull bones, for example, never becoming properly developed. With metamorphosis the animal

undergoes a very extensive transformation. It loses its gills, its legs develop for crawling upon land, and the tail becomes rounded and tapering. It is now a land salamander resorting to the water only for egg laying.

The neotenŷ of axolotl is thus facultative, for with the proper conditions the animal does not remain neotenous but becomes an adult *Amblystoma.* Other urodeles, the perennibranchiates, correspond in their adult structure very nearly to axolotl and may be looked upon as permanently neotenous. *Typhlomolge,* of the underground streams of Europe and Texas, *Proteus, Necturus,* and *Siren,* belong to this group.

Another illustration from the chordates is the tunicate group called Appendicularia or Larvacea. These animals are small tadpole-like creatures averaging about half a centimeter in length and living perhaps one year. They swim in the surface waters of the sea of all parts of the world and the young have been taken in plankton between February and summer, but their development is almost unknown. There is no evidence of reproduction by budding, gemmation, or other modes of asexual type such as are found in the Salpidae, for example, nor is any metamorphosis known. Development is direct and the small appendicularia correspond in a general way to the tailed larvae of the ascidians; that is, they retain the tail portion of the body with its typical chordate characteristics which is lost in the metamorphosis of the more familiar forms such as *Molgula, Ciona, Cynthia,* etc. Hence the group has been named Larvacea and the animals have been looked upon as larval forms which have become sexually mature. They have also been looked upon by some as primitive forms from which other Tunicata have been derived, and, it must be noted, by some they have been thought of as larvae of some adult form which is pelagic. In any case the sex glands are developed and the products shed by the animals as known to us, and we have a clear case of neoteny.

Among the insects a number of cases are known, and some of these are among the most important of all for they illustrate parthenogenetic paedogenesis. The example usually given is *Miastor,* a cecidomyiid fly, but certain species of the genus *Cecidomyia* also show it. The case of *Miastor* has long been known as an illustration of paedogenesis, and is given by Hertwig along with certain other Diptera as an example proving his statement that paedogenesis is parthenogenesis in an immature organism. As is already seen, this statement is much too restricted, for the term paedogenesis is of equal application to cases in which inheritance is biparental.

In *Miastor* paedogenesis occurs normally during the spring, early summer, and autumn, according to Hegner who has been the principal

student of this genus in America. No reproduction takes place during the winter, and the process is interrupted in midsummer by the appearance of male and female adults. The larva of *Miastor* possesses two ovaries in the tenth and eleventh segments. In each are thirty-two oöcytes each with nurse cells and follicular epithelium. After a time one of these oöcytes with its nurse cells and its follicular epithelium is separated off from the rest of the ovary and in a distant part of the body grows and develops at the expense of the tissues of the mother larva. This process is repeated until five to seventeen separated growing oöcytes are thus produced from one mother larva. Then one division takes place, the polar body which is given off divides again and both products degenerate. Parthenogenetic cleavage follows with chromatin diminution as previously described in connection with the history of the germ cells, the pole plasm is segregated and the embryo gradually takes on its characteristic form. No oviducts are present in the mother larva nor is there provision for the escape of the young thus paedogenetically produced. They escape by rupturing the body wall of the mother larva, which is left to die. After the production of a number of generations in this manner, the last larvae pupate and emerge as normal adult males and females.

In the spring of 1869 Grimm found a pupa of *Chironomus* laying eggs. To this form of paedogenesis in the chrysalis Packard gave the name chrysallogenesis, although the differences from other types of paedogenesis are so slight as to make the retention of the term of doubtful necessity. In the autumn other pupa change to flies without laying eggs and these adults are more prolific than the spring pupae were. The process was described as a seasonal phenomenon depending upon temperature.

Paedogenetic reproduction has also been reported for the Tenthredinidae, the saw flies, thus extending this phenomenon to a second order of the insects, the Hymenoptera.

The molluscs show one case of neoteny in the shell-less snail, *Stauro-dorsia verrucosa,* which according to Cuénot reproduces long before true adulthood is reached.

Among the trematodes paedogenesis is found in the most striking form. Two excellent illustrations occur in the suborder Monogena, and conditions in the Digenea offer material for interesting speculation. In the former group the genus *Gyrodactylus* exhibits what is perhaps the nearest approach to the old preformationist theory of "emboitement" to be found in the animal kingdom. The young individual comes to sexual maturity before it is born and produces young in its own uterus.

This process is repeated and as many as four generations have been seen, one within the other.

The second case of this group is that of *Polystoma integerrimum* originally described by Zeller in 1872. The embryo hatches in the water and swims freely. It seeks for a young frog tadpole which it must find within twenty-four hours or die. If one is found it creeps over the surface until it finds the branchial opening, which it quickly enters; it undergoes metamorphosis, and passes down the alimentary canal to the rectum and thence to the urinary bladder. Here it remains for three years to become sexually mature. However, it may happen that the young worm has attacked a very young tadpole which still has external gills. In this case it remains in the gill chamber where nutriment is abundant, grows rapidly, and becomes sexually mature in the short space of five weeks. It does not then pass further along the alimentary tract but dies before the metamorphosis of its host. It differs in its structure as well as its life cycle from the usual form, in that it develops but one male gland instead of several, and it lacks entirely the intromittent organ, vagina, and uterus, or they are developed only to rudimentary vestiges. It is of interest to note that *Polystoma ocellatum* is structurally quite similar to the paedogenetic *P. integerrimum.*

Among the digenetic trematodes the life cycle of the liver-flukes involves questions which are of interest in this connection. The main facts are well known and are referred to in several chapters of this work. In both rediae and cercariae reproduction may take place and daughter rediae, and daughter cercariae may be produced. If it can be shown that these daughter forms are produced from eggs, either parthenogenetically or bisexually, then this is a case of paedogenesis. However, it has lately been shown for some flukes that the germ balls from which the daughter larvae develop are budded off asexually and never undergo any chromosome reduction (F. G. Brooks), so that for these forms at least it cannot be said that paedogenesis occurs in the flukes. It has been the usual view, however, that there is here the production of parthenogenetic ova, and hence paedogenesis.

Of the examples usually recognized, the final one is that of the lobate ctenophore, *Bolina hydatina.* Here the cydippid larvae become sexually mature, producing eggs and sperm. Fertilization follows and the eggs develop in the regular manner. The larval gonads subsequently degenerate, metamorphosis takes place, a new set of gonads appear, the animals again attain sexual maturity, producing eggs and sperm, this time as adults.

These are the classical cases, and they illustrate both neoteny and paedogenesis, both parthenogenetic and bisexual.

In addition to these, experimentally produced delay or acceleration of development are well known. Frogs transferred as larvae to alpine heights where the winters come early have remained in the larval condition over the winter. High temperatures hasten sexual maturity. Brackish water or fresh water will often hasten the maturity of oceanic forms. Hunger in some forms and overfeeding in others result in heterochronic growth. Termites which are fed in a certain manner mature very early while the wings are still undeveloped and eyes have not yet appeared (Grassi). Parasitism may be looked upon as a strong factor accelerating maturity because of the abundant food supply.

It may be observed that there have been omitted many cases of larval budding and other forms of asexual reproduction such as may be so commonly found, for example, among jellyfishes, liver-flukes, bryozoa, and tunicates. These cases, though not a far step from the types discussed in this chapter, are not properly considered here, for paedogenesis and neoteny are matters of sexual reproduction. It is admitted that in some cases, as already shown for the liver-flukes, the distinctions are hard to draw, but for the sake of clarity it is usually thought wiser to adhere to the definitions given.

Thus far we have discussed this subject from the point of view of the entire organism. There still remains the matter of neotenous organs rather than organisms which deserves mention before the subject is closed. This phase of the subject was developed by Garstang and by Bolk and recently discussed by De Beer. There are many instances in the animal kingdom of forms having organs that retain embryonic characters although the organism as a whole has passed on to a new adult condition. Organs of this kind are neotenous. In the succession of somatic stages the organs in question have fallen behind the others, in short they have become distinctly heterochronic. Bolk has discussed the features of man which resemble the structure of embryos of anthropoid apes, assuming that the latter are nearer the ancestral forms of structure. Among the features of man which show resemblance to the embryonic structures of the ancestral types, for which he uses the term foetalization, are the relatively high weight of the brain, the retention of the embryonic cranial flexure with the resulting erect posture as Bolk thinks to have demonstrated, the position of the foramen magnum, the late closure of the skull sutures, the flatness of the face, lack of hair on the body, and others. Bolk's study of cranial flexure and human posture comparing both adult and ancestral conditions is particularly interesting. He finds as a result that the flat face as compared with the elongated muzzle of other mammals is largely responsible for the trend of human evolution with regard to vision and other special senses, the character

of the teeth and of the anterior end of the alimentary canal. And all the characteristics which are distinctly human are clearly neotenous and related to the embryological derivatives from the ancestors.

One other illustration, from those given by De Beer, may be cited to show the part that neoteny may have played in evolution. It is a comparison between the important structural characteristics of adult insects and larval myriapods. The larva of *Iulus* has a head composed of six or seven segments, an elongated segmented body, the first three metameres of which bear pairs of legs whereas posterior segments bear only rudimentary legs at the time of hatching or shortly thereafter (Metchnikoff). These features are quite insect like, in that the insect head has six or seven segments, the thorax three, each bearing a pair of legs, and the abdomen of about ten segments, legless or bearing appendages only as a larva. Insects are known among the lower orders whose structure corresponds more closely with this immature myriapod than does the structure of insects of the very specialized higher orders, and it is suggested by students of these matters that the insect derivation passes through these intermediate forms from neotenous larvae at least not unlike those of the myriapods.

From these cases it would appear that the study of neoteny from the standpoint of embryology offers a productive field of quite a new order for investigations and that the conclusions reached from researches of this kind may profoundly affect our ideas of phylogeny.

CHAPTER VII

POLYEMBRYONY

By polyembryony is meant among zoologists the production of multiple embryos from a single egg. (This definition does not apply to botanical nomenclature.) The number of individuals produced from a single egg ranges from hundreds as in the parasitic Hymenoptera down to two, although in the latter case the condition is usually known as twinning, and not all types of twins are properly thought of as polyembryonic. Polyembryony occurs very widely distributed through the animal kingdom in groups which are totally unrelated to each other. Because of this wide distribution the literature to which reference can be made in this connection is really very extensive and the significance of some of the cases extends beyond the realm of embryology. Of the numerous investigators who have studied the question only a few need be mentioned here, but it may be noted that a reference to their work will serve as an introduction to the much broader literature which is not included in this discussion. Patterson has summarized the knowledge of polyembryony in a paper in Volume II of the *Quarterly Review of Biology* and it is suggested that the student consult this paper in beginning a more extensive study of these problems.

Patterson recognized three types of polyembryony: "(1) experimental polyembryony, or the production of multiple embryos by artificial means; (2) accidental or sporadic polyembryony, or the occasional production of multiple embryos in the species which is typically monembryonic; (3) specific polyembryony, or the habitual production of multiple embryos in a given species." The general student of comparative embryology is interested in the last type much more than in the first two, although cases of the first two throw much light upon many fundamental problems of organization of the egg and embryo and offer a very stimulating field for the experimental embryologist. Many types of eggs whose normal development includes no hint of polyembryony can be induced to produce double monsters and even complete embryos by experimental means.

Cases of occasional or sporadic polyembryony are recorded for coelenterates, cestodes, annelids, echinoderms, arthropods, and vertebrates. Perhaps because the latter group is so much more studied, there

have been more cases reported for the vertebrates than for the others and, as Patterson points out, it is here that the occasional identical twins of the human species should be classified.

Specific polyembryony, to which our attention is drawn as the special problem of the comparative embryologist, is found in the following groups of animals: flatworms, bryozoa belonging to the Cyclostomata, earthworms, parasitic Hymenoptera, and the mammals, especially as illustrated by the armadillo. It is of more widespread distribution, however, than is indicated by these groups, but here are included the more important cases.

An inspection of this list is sufficient to show that polyembryony has no phylogenetic or taxonomic significance, for the groups are too widely separated and too diverse in structure to admit of an interpretation of this kind. Moreover, the structural differences both of adults and embryos in these various groups are so great that it is not possible to look for the causes of the phenomena in any simple embryological process common to all. Doubtless in the final analysis all these processes have a common underlying causal factor which is bound up with the innate protoplasmic organization of the egg substance.

As Patterson has pointed out, it is really not much more strange for an egg to give rise to two embryos than to one. The remarkable fact is that a new organism can be produced from an egg at all, and those qualities of the living substance which make possible its reproduction are basic to the one case no more than to the other. We must recognize that specific polyembryony is as much a characteristic of certain animals as is the formation of a coelome. At a certain stage in development there occurs a series of events which lead perhaps to two buds, or more, from each of which individuals grow. Very little thought is necessary to convince oneself that the directive forces behind this type of budding are not much more astonishing than those which cause, in the other case, the archenteron to bud out to form an enterocoele.

A. The Causes of Polyembryony

Of the explanations of polyembryony, probably the most prevalent is the theory of blastotomy, according to which the blastomeres of the cleaving egg become separated in the 2-, 4-, 8-cell stage or later, and lead entirely independent existences, each arriving at length at the stage of a completely formed embryo. This theory is the basis of the familiar explanation of the origin of identical twins in human beings, that the blastomeres in the 2-cell stage become separated and each gives rise to an embryo. This is a view with few observational data to support it, but it may be presumed to find considerable support in the

experimentally induced development of isolated blastomeres. Numerous experimental devices are well known by which blastomeres can be separated. To mention only two of these devices as examples, it is possible more or less successfully to cut apart the blastomeres with a knife or delicate thread drawn about the egg, or to keep them apart by subjecting the eggs to calcium-free sea water in which the blastomeres do not cohere to one another. In some of these experiments the isolated blastomeres have lived and developed for a time; in others they have produced partial larvae (ascidians etc.); and in others they have developed into whole larvae (amphioxus, *Cerebratulus*). But in forms where direct evidence for blastotomy is possible none has been found which forces one to this view for cases of polyembryony. As will be seen presently, the facts in the armadillo make the explanation based on blastotomy untenable there, and render it highly doubtful elsewhere.

A second explanation takes the form of an assumption that *polyovular follicles*, that is, follicles containing several ova fused together, may provide the mechanism by which polyembryony is accomplished. It is only necessary to say that the evidence for this supposition is entirely insufficient and the theory may now be regarded as abandoned.

In the *budding* theory we have what is perhaps a closer correspondence with the observed facts, although it is more of a descriptive explanation than an attempt at developmental analysis. After cleavage there arise in the blastulae or gastrulae of lower animals or in the blastocyst of mammals certain areas or "growing points" which are essentially buds. If a single one is produced it becomes the apical or head end of a normal embryo and assumes dominance over the remaining parts. If two are formed they are the beginnings of twin embryos. Similarly secondary buds may be formed and four or more embryos result, depending upon the number of buds. The facts observed in the armadillo will be seen to harmonize well with this explanation.

This theory of budding has been criticized rather severely, particularly by Assheton, on the grounds that budding cannot take place unless there is a stock from which the buds may arise and the presence of a stock Assheton claims was not demonstrated in the cases to which the theory was applied. Rather he interprets the described facts as cases of fission. The blastocyst of several mammals, for he was particularly considering the theory as applied to the armadillo, he described as undergoing direct fission into two embryonic rudiments. As a matter of fact the fission hypothesis does not differ from the idea of budding in relation to the facts as described, but is primarily a redefinition of them, for in both cases the embryonic vesicle is regarded as divided into several primordia, each of which is the beginning of a distinct formative area

and consequently will give rise to a separate embryo. Whether the emphasis is placed upon the embryonic mass which is thought to bud or upon the separate areas which arise from it as would be the case in fission does not seem to involve any particular difference in the result nor to carry the explanation of the environmental processes much further toward a final analysis.

As an explanation of the process a physiological interpretation must be called on. Stockard has pointed out that if a *developmental pause* occurs at the critical moment in the formative stages of an embryo, a series of consequences ranging from simple malformation up to the production of double monsters or even two individuals may result. He applies this conception to the explanation of polyembryony, supposing that the reason for the loss of dominance of the first formative region of the embryo and the subsequent ascendency of the primary and secondary buds is connected in some way with the developmental pause. He points out that in the armadillo a period of physiological isolation intervenes at one of the critical moments, making possible the four resultant buds. Certain observed facts bear out this conclusion. In any case within the growing germ the isolation of formative areas occurs and from these formative areas the multiple embryos are produced.

An obvious connection exists between the phenomenon of polyembryony and that of metagenesis which involves at least the alternation of one sexually produced generation with an asexually produced one. It is usually the practice to limit alternation of generations to the lower forms of animal life and to say that it does not occur in higher forms, including the vertebrates. Stockard, however, speaking in terms of the budding hypothesis, has pointed out the fact that the embryonic mass of cells may be looked upon as one generation, namely the one produced from the fertilized egg, and that it is completed when it becomes a stock from which other buds, that is growing points, begin their development. This point of view has its application even to mammals whether only a single organism results or several as in the case of the armadillo. Here the blastocyst would be regarded as the sexually produced individual and the buds which give rise to the four young as those asexually produced from it.

Stockard says, "From a general biological standpoint the adult body of higher animals may be very correctly considered to be derived from a sexually produced embryonic axis, the stock which gives rise by an asexual method of budding to the various special organs. The vertebrate body is thus composed of a group of different zooids, the organs. There are seeing, hearing, excretory zooids, and so on, comparable to the zooids of a siphonophore colony.

"Alternation of generations is here considered a phenomenon, not limited as is generally taught to lower forms, but occurring throughout the animal kingdom."

If the blastocyst is to be looked upon as a sexually produced individual from which arises asexually another or several others of a second generation, it is suggested that in this fact lies the explanation

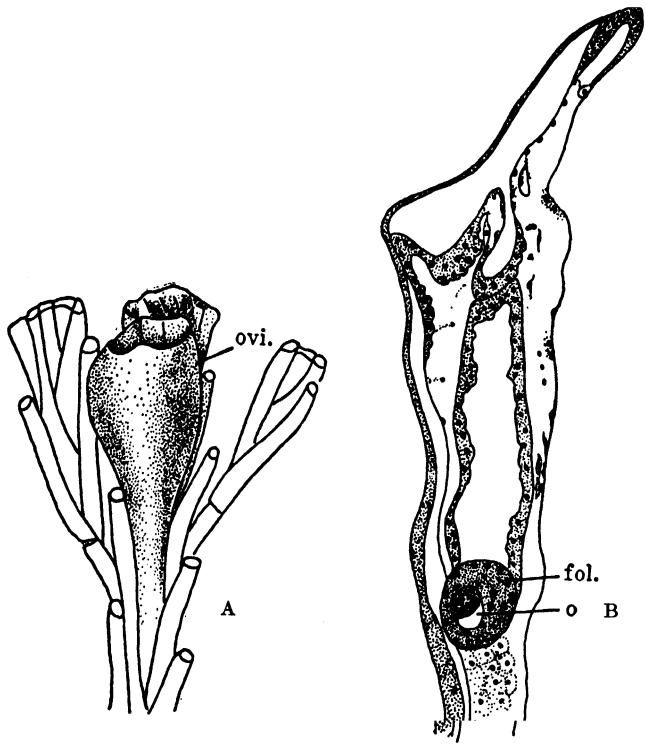


FIG. 219. The development of the ovicell in *Crisia remosa*. (From Patterson, after Harmer.)

A, external view. B, median longitudinal section of young ovicell. fol., follicle formed from polypide-bud; ov., ovary; ovi., ovicell.

of the difficulties that have met every attempt to homologize the early development of the mammals with that of the lower vertebrate classes. These differences have been discussed in Chapter X on the formation of the mammalian embryo; they constitute a problem of major importance to the comparative embryologist.

B. Observed Cases of Polyembryony

We may now devote ourselves to the consideration of the groups of organisms in which specific polyembryony has already been noted to

occur, bearing in mind the different types of explanations that have been offered and attempting to discover how the facts conform to the suggested explanations.

Flatworms. Among the flatworms are many cases which depart from the usual methods of reproduction. Of these some which do not fully fit into the classification have been described from time to time as polyembryony. There is one flatworm in which polyembryony undoubtedly occurs, however; it is the cestode *Taenia echinococcus*, or *Echinococcus coenurus* as it is sometimes known. This small tapeworm produces eggs which develop into the usual hooked embryo, the onchosphere, and these in their turn produce a cyst which becomes the bladder worm or cysticercus. As is usual in the further development of a cysticercus, an invagination from the outside wall of the bladder into its cavity develops a new scolex and neck region which will later become inverted and form the new tapeworm. However, the matter is complicated in this case in that many scolices may be produced within a single bladder by the budding process, and even secondary bladders which produce multiple scolices in their turn are described. Thus the encysted worm may grow to an enormous size to endanger the life of the host and oftentimes bring about its death.

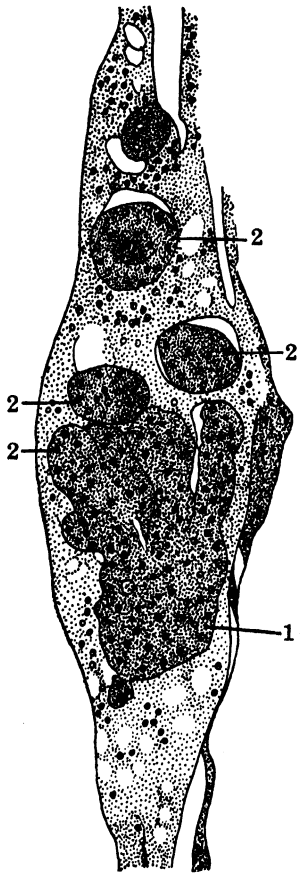


FIG 220. Section through a follicle of *Crisia remosa* showing primary embryo (1) which is producing secondary embryos (2) by budding. (From Patterson, after Harmer.)

Bryozoa. Polyembryony occurs in the cyclostomatous bryozoa belonging to the Gymnolaemata of the Endoprocta. These animals live in colonies made up of zooecia which are in general tubular with densely calcareous walls. The circular orifices of the zooecia give the group their name. At the time of the breeding season a curious specialized zooecium develops which is called the oecium or ovicell. In this is developed the young embryo. Fertilization of a rather unusual sort having been successful, the oecium serves as a brood chamber about the developing embryos and larvae. The primary embryo undergoes a process of budding, and secondary and tertiary buds are

produced all of which may develop into larvae, as many as 150 being on record from a single egg. They develop into ciliated larvae and each is capable of forming a new colony upon escape from the oecium.

Earthworm. In the earthworm of the species *Lumbricus trapezoides* (*Helodrilus caliginosus trapezoides*) polyembryony results in the production of twins, a process which by Kleinenberg was regarded as universal for the species, but by Vejdovsky was held to be abnormal. It is due to a process of fission of the embryo. The cleavage of the egg

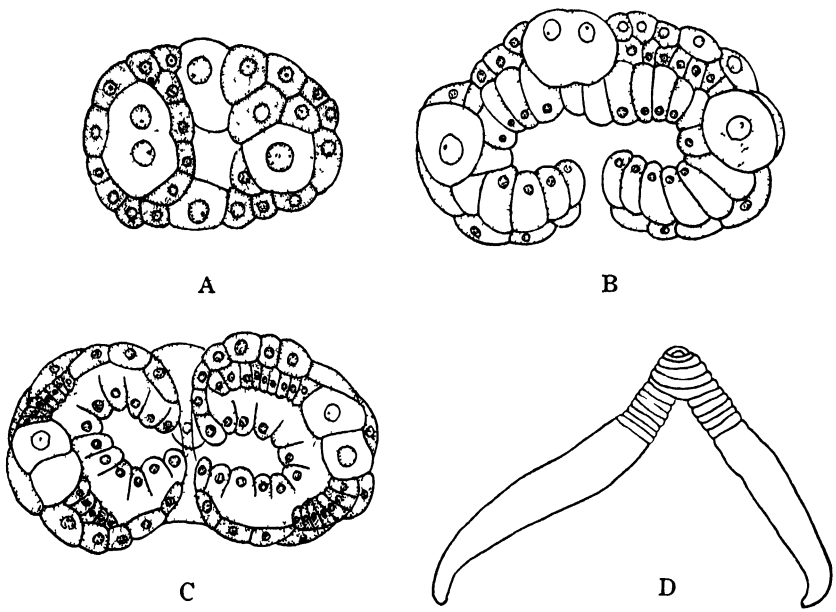


FIG. 221. Twinning in the earthworm, *Lumbricus trapezoides*. (From Patterson, A, B, C, after Kleinenberg.)

A, Section of young twin embryos, the right one being the more developed. B, Double embryo, in section. C, Double embryo about to break apart. D, Type of double monster formed when embryos fail to separate.

is much modified from the typical spiral form of the annelids and indeed is variable. A blastula is formed, the endoderm and mesoblast cells pass into the cavity and the entire mass begins to elongate. Across the equator of the elongating mass a transverse furrow now appears from one side. As it deepens, the embryo is divided into two hemispheres held together by a few ectodermal cells only. Each half is destined to form one of the twins. Differentiation, gastrulation, and the completion of the internal organization go ahead while the worms remain connected. But at length they separate after a series of rotations which breaks them apart, except in a certain proportion of cases which become double

monsters of various degrees of union. Thus from the single egg twin earthworms are produced.

In two other genera of oligochaetes double monsters have been described, and one infers that the twinning phenomena, though rare, may occur in a number of families. These cases were *Tubifex tubifex*, found by Welch, *Tubifex rivulorum* by Penner, and *Sparganophilus eiseni* by Hague.

Parasitic Hymenoptera. In the parasitic wasps we find some of the most important and complex cases of polyembryony in the entire animal kingdom, and certainly here the results of the process are exemplified in the most striking manner. In a single brood hatching from one parasitized caterpillar hundreds of individuals may be seen to issue all developed from one egg. Our knowledge of polyembryony in insects dates from the work of Marchal in 1898 and of Silvestri in 1906. Although a number of important points still remain to be satisfactorily cleared up, a number of investigators have contributed to this subject since then. Among them are Martin (1914), Patterson (1915, 1917, 1921, 1927), Hill (1922, 1923), and Leiby and Hill (1923, 1924), as well as others. The important genera studied include *Litomastix truncatellus*, *Ageniaspis fuscicollis*, several species of *Copidosoma*, *Paracopidosomopsis floridanus*, and three species of *Platygaster*. The most recent study is that of Parker (1931) upon the braconid, *Macrocentrus*. Undoubtedly many other forms of insects show polyembryony, but the ones mentioned include the more important cases studied.

The manner by which a single egg produces multiple embryos in these forms is perhaps best understood by beginning as Patterson has done in the paper earlier referred to with a description of *Platygaster hiemalis*, a parasite of the Hessian fly, which usually produces two individuals from one egg. From this comparatively simple case we have a gradual increase in complexity which at the end of the series is only incompletely understood, but the simpler cases at least are suggestive of the methods by which the more complicated probably develop.

In this species the parasite lays from four to eight eggs either in the egg or young larva of the host. If fertilized, the egg nucleus divides twice, producing two polar body nuclei; these are not passed to the outside of the egg but remain in the cytoplasm where after a time they come together to form a large polar nucleus called the paranucleus. With the fusion of the male and female pronuclei the egg becomes differentiated into two separate regions. The cleavage nucleus and cytoplasm surrounding it become cut off from the remainder and constitute the embryonic region, for only from it comes the material which will go into the formation of the developing embryos. The re-

mainder of the egg containing the paranucleus and its own cytoplasm entirely surrounding the embryonic region functions to absorb and

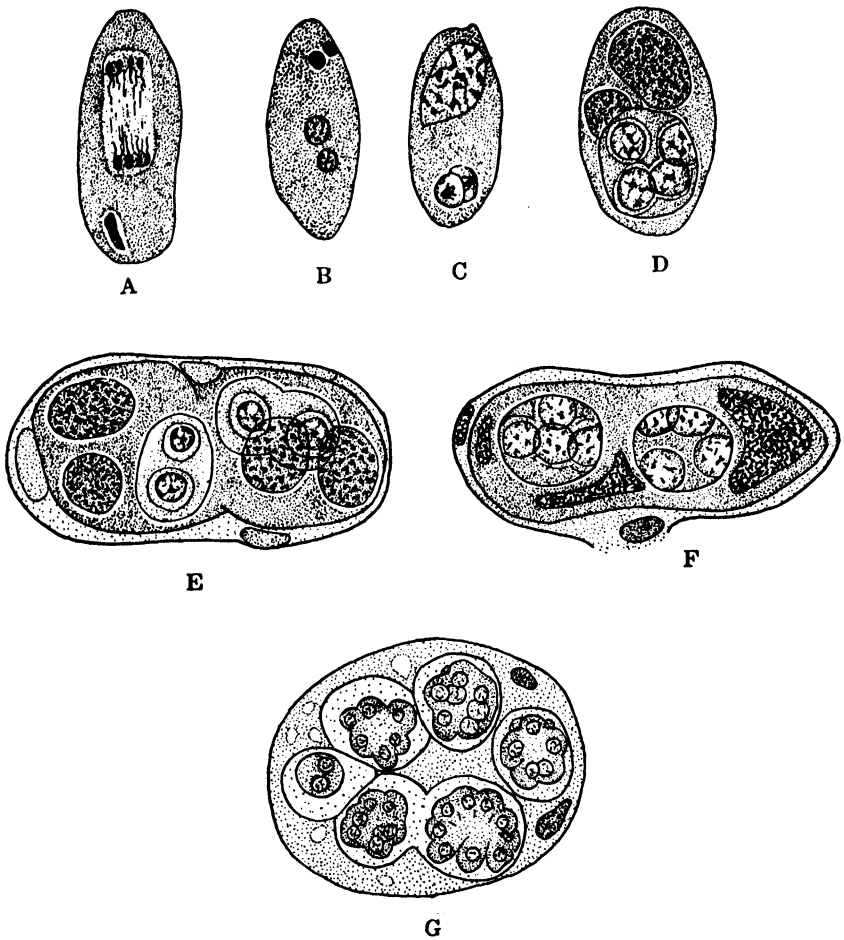


FIG 222. Polyembryonic development of *Platygaster hiemalis* (From Patterson, after Leiby and Hill)

A, Egg of four hours, showing sperm head and first maturation division B, Egg showing two polocytes, and both pronuclei C, Egg with pronuclei in conjunction and polar nucleus D, Embryo, two polar nuclei and a parasitic body showing four nuclei E, Enclosed within a cyst formed from tissue of the host are two embryonic bodies each surrounded by trophamnion F, A later stage in the development of two embryos G, A thirteen-day-old polygerm in section, showing several embryos.

elaborate the tissues of the host for the nourishment of the young embryos and it is therefore spoken of as the trophamnion. In this species, although it is not characteristic of all, the host tissues form a

cyst wall around this developing body which then is spoken of as the parasitic body.

The paranucleus as development proceeds divides amitotically twice and the daughters distribute themselves in the trophamnion surrounding the embryonic region. The zygotic nucleus of the embryonic region divides first into two, then four, embryonic nuclei. Thereupon, the embryonic region becomes constricted and presently separated into two regions, each provided with two nuclei, and the trophamnion with its paranuclei also divides into two corresponding regions. The two halves of the parasitic body thus differentiated remain held together within a single cyst of host tissue, but develop independently. Four, eight, and sixteen nuclei arise from the division of each of these germs, as they are called, and these arrange themselves into the form of a typical spherical blastula, the cell walls being cut off around each nucleus. The remainder of the development is unimportant, for the process characteristic of these blastulae go forward through regular stages. There is produced from each a new individual which hatches in the next year.

In another species of this genus, *P. vernalis*, eight embryos are regularly developed. Here the process has a fundamental similarity to that just described, but the details of development differ. The parasitic body develops a number of embryonic nuclei surrounded by an appropriate amount of cytoplasm and a cell membrane. Each of these becomes a germ for the production of the later embryo and the entire mass is spoken of as a polygerm. It happens that the more complex cases were studied before the development of *Platygaster* was understood and some of the problems that were not clearly worked out would now probably be more easily followed through. It is known now that in *Copidosoma gelechiae* in the formation of the polygerm many primary germs are produced in a manner similar to the eight of *Platygaster vernalis*. These separate germs of the last polygerm stage all divide and give rise to two which develop into separate embryos.

Leiby and Hill believe that in *Paracopidosomopsis*, which is even more complicated, a secondary germ divides to form tertiary ones before the larval differentiation begins.

Armadillo. The final case of polyembryony to be discussed is perhaps the one in which the greatest interest lies. The Texas nine-banded armadillo *Dasypus* (*Tatusia*) *novemcinctus* normally produces four young from a single egg, the quadruplets being identical in respect to sex and to most of their morphological features. The work on the armadillo from the standpoint of polyembryony is of rather recent date, Fernandez concluding in 1909 that it occurs in a South American

species *Dasypus hybridus*, and Newman and Patterson in the same year publishing the beginning of their important studies on the Texas species. Subsequent publications by these authors working independently have made available an account of the development of this form which is practically complete. The processes have been shown to be identical in respect to all important features in the two species which have been studied. The reader is referred to Chapter X for a discussion of the formation of the blastocyst in mammals.

In the armadillo as in other mammals, a monodermic blastocyst is formed which becomes differentiated into a trophoblastic portion and a formative portion, the inner cell mass. Then from the inner cell mass the endoderm is differentiated off, the remainder becoming ectoderm. Up to this point the blastocyst has remained free in the uterine cavity, but now attaches to the uterine mucosa. Attachment takes place directly over the embryonic ectoderm and the *Träger* forms at this point; then the inward growth of the spherical endodermal mass begins which brings about the so-called inversion of the germ layers. Within the ectodermal mass the formation of the ectodermic vesicle marks the beginning of the amniotic cavity. Above it an extraembryonic cavity is formed in the mesoderm. With progressive development the shift of the ectodermal cells takes place, resulting in the formation of the embryonic shield with its thick ectoderm and the true amnion above it. In this stage the first sign of polyembryony makes its appearance, for from opposite sides of the ectodermal vesicle thus formed blunt projections extend laterally. Patterson called these primary buds. By this time that portion of the trophoblast which has not become involved in the attachment to the uterine wall disappears and the yolk sac endoderm is directly exposed in the uterine cavity. The two primary buds now divide each to form two secondary buds, making in all four buds which are rudiments of the four embryos subsequently to arise. Although they arise in this bilateral fashion and develop in the right and left halves of the uterus respectively, they later come to occupy positions that are about equally spaced from each other.

Meanwhile the vesicle grows, and the endoderm of the yolk sac, including those portions with which the embryonic ectoderm of each bud is in contact and which will form the gut endoderm of the embryo, faces the uterine cavity. The anterior ends of all the embryos point toward the apex of the common ectodermal vesicle, that is, toward the original amniotic cavity, and the entire vesicle may now be spoken of as the common amniotic vesicle. The entire structure now grows very rapidly, especially that portion of it which originated from the trophoblastic knob or the *Träger*. This growth finally comes to occupy most

of the space in the fundus of the uterus. The further history is especially concerned with the embryonic buds. Their posterior ends lengthen out with the growth of the vesicle and finally unite at a point opposite the original attachment and from their point of union the umbilicus later arises. The common amniotic vesicle by this growth procedure is left

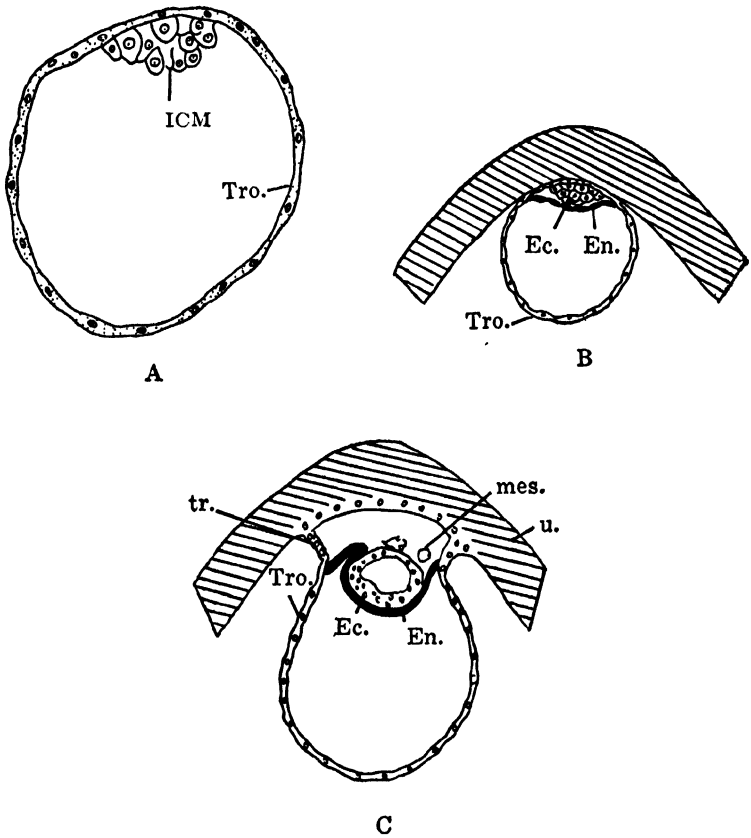


FIG. 223. The development of the blastocyst of the armadillo. (After Patterson.) ec., ectoderm; en., endoderm; icm., inner cell mass; mes., mesoderm; tr., träger; tro., trophoblast; u., uterus.

as a small structure at the lower apex, in contact with each bud, while most of the remaining portion of the cyst mass with its developing embryos is derived from the growth of the *Träger*. The buds meanwhile continue their growth, each forming a primitive streak, which is a substitution for the embryonic shield as described for other mammals.

The further development of the embryos and the subsequent changes which lead to the production of the four foetuses need not be traced

since it is simply a problem of organogenesis and of the steps normally following. The relations which are necessary for the understanding of

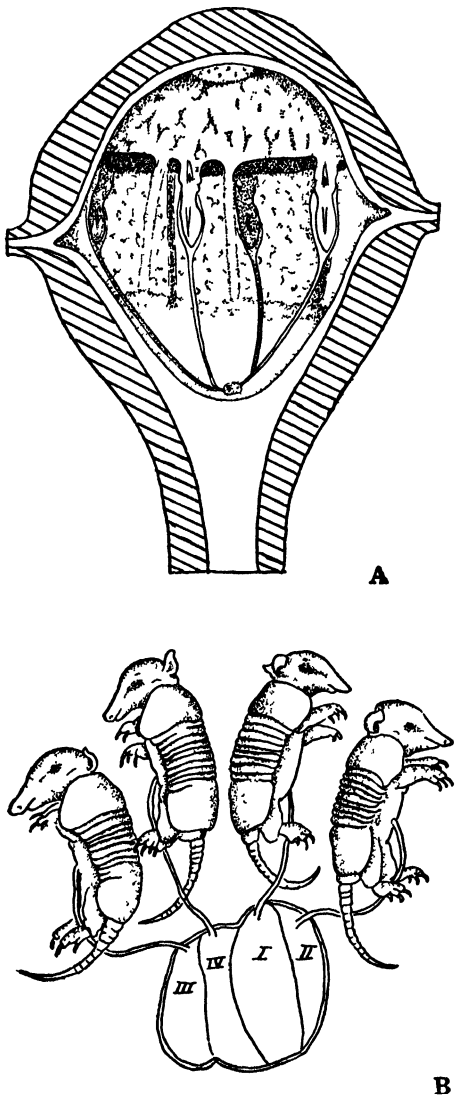


FIG. 224. A, The uterus and entire blastocyst of the armadillo showing paired origin of embryos. B, Half-grown foetuses spread out from uterus wall. (After Patterson.)

the polyembryonic condition all grow out of the budding processes of the embryonic vesicle and are completed with the establishment of the four embryos. There are many interesting problems connected with

the later stages from the standpoint of later embryology and hereditary correlations of the characters of the four offspring as well as from other points of view, but the special problem of polyembryony is explained by these earlier stages.

C. Examples of Doubtful Polyembryony

Two other cases which resemble polyembryony in some respects should be mentioned. As a matter of fact there are some departures in each of them which do not justify their inclusion with this type of development.

Among the trematodes the egg of *Fasciola hepatica*, the sheep fluke, is fertilized in the body of the adult fluke, makes its way down the bile duct and out of the intestine of the sheep and hatches when it rains as a tiny ciliated larva, a miricidium. Entering the intermediate host, the snail, the miricidium becomes a sporocyst and within it parthenogenetic ova appear which develop into rediae, the next larval form. Within the body of the rediae the process may be repeated but at length cercariae are developed which escape from the snail and bring about the reinfestation of the adult host. This case has been called polyembryony by some, but by others it is regarded as paedogenesis, for here the new larvae are said to be produced not asexually but from parthenogenetic ova, a difference in method which, if true, would seem to be fundamental. However, the reservation must be made that if the germ masses from which the new larvae arise are not ova, but are produced asexually (as F. G. Brooks believes, having found no maturation phenomena or other egg-like behavior), then we have to do here merely with asexual reproduction, and not even paedogenesis.

A second case which at first thought suggests polyembryony occurs in the tunicates. Among the Thalaceae as represented by the genus *Salpa* it has long been known that two forms of individuals are to be expected, one a solitary form and the other a colony which is usually found as a chain of individuals. The first of these develops asexually, although produced from a fertilized egg. The second is known to be budded off from a stolon which grows out from the asexual individual, and among the colonial forms some at least are sexual, producing the eggs and sperm which in their turn start the cycle over again. The genus *Doliolum* is rather more complicated than *Salpa* but it seems to illustrate the situation quite well and it is here that the type of development of particular interest to us may be said to occur.

In *Doliolum* an oözoid is developed from the fertilized egg and it reproduces asexually. By some it is regarded as a larval zooid, and certainly it has not yet reached its final character. If it is correct to

regard the oözoid as a larval form, this case is very closely akin to polyembryony. From the ventral posterior part of the oözoid a proliferating stolon is formed as a protrusion of the ectoderm into which the mesoderm penetrates. From the stolon buds arise which break loose in a very immature condition and worm their way upward and dorsally over the surface of the parent. Since the number given off from the ventral stolon is less than the number later found on the dorsal process, it is assumed that the buds must divide *en route*, and some evidence of this process has been found. Arrived at the dorsal side of the oözoid, the buds attach themselves to a posterior process of the test which arises as a middorsal projection (occasionally spoken of incorrectly as a dorsal stolon). These bodies attach themselves in three longitudinal rows to the outgrowth. The two lateral rows of buds are known as trophozooids or gasterozooids and their only function is to nourish the colony. The dorsal row of buds undergoes metamorphosis into several kinds of zooids. Some become phorozooids or nurse zooids, and according to some investigators they produce from a ventral stalk buds which are protogonozooids or primary sex buds. These latter either by budding or fission produce the sexual animals, the gonozooids or blastozooids, in which with further development sex organs are formed and the cycle is completed. Here an alternation of generations occurs in which there are three asexual generations and one sexual generation in one cycle. Whether it is to be regarded as polyembryony depends upon the interpretation of certain stages. It is very complicated in the extreme, and is at least close to true polyembryony.

CHAPTER VIII

THE DETERMINATION PROBLEM

A. Introduction and Statement of Problem

A problem which has run through the entire history of embryology and is yet a live question at the present time concerns the extent to which the egg represents a fixed and definite system whose course is set when its development has begun. This is the determination problem. It first became a live issue during the controversy between the preformationists and the epigenesists of the seventeenth and eighteenth centuries and was even in that crude form an attempt to solve the question of the organization of the egg. The one school held that development was simply an unfolding of an organism already present in an infolded condition; that is, organization was complete and development brought forth nothing new. The other held that the egg was unorganized and that everything in development was new. The history of embryology since these crude beginnings, at least of that part of the science which has concerned itself with the earliest stages in ontogeny, has been an attempt to make clear the manner in which the organization of the egg becomes manifest. Except for historical reasons it is not necessary to trace the course of these studies nor would it be possible except in a much more extensive treatment of the subject than is given here, for the literature is voluminous. It is not even possible to summarize in a short discussion the work which has been done. All that can be attempted is to bring together briefly the lines of work which have been responsible for the present conceptions in regard to determination.

Two sets of facts confront the student who would consider the determination problem. Neither bears directly upon the question at issue, but both serve to limit it. In the first place an egg goes through certain steps with surprising uniformity and produces an individual which is structurally very complex and very much differentiated. It is organized in the highest conceivable degree. In the second place modern genetics has shown that the egg to begin with possesses in the mechanism by which the genes are controlled and distributed a type of organization likewise complex in the extreme; and there is no doubt that this mechanism operates to produce the conditions found in later ontogeny. Yet differentiation is a matter of the cytoplasm of the cells. The repeated

mitoses do not affect in a differential fashion the elements of the nucleus and it is clear that the nucleus contains in the chromatin the non differentiating material, the germ plasm, while the cytoplasmic portion of the cell is the basis of specialization and is indeed the somatoplasm which gives rise to all the differentiations later seen in the course of ontogeny. Between these two sets of facts must lie the third set, little understood and often little appreciated at the present time. Almost nothing can be offered in the way of explanation as to the method by which the gene mechanism produces its fundamental effect upon differentiation. The gene mechanism concerns heredity, that is, by it is explained the similarity existing between generations, but as yet its study has offered little which enables us to understand its relation to the differentiating soma. It was earlier said that heredity was the central problem of biology. Differentiation is certainly a central problem of development, and, let it be said, at the present time one of the most elusive. The questions of differentiation and of determination have dominated a surprisingly large part of the embryological work of the last century.

These two questions are really two aspects of the problem of organization, for differentiation is the manifestation of the operative mechanism of the egg as modified by extrinsic factors. To discover the extreme to which this operative mechanism is already present in the early stages and to which it gives a definite "set" to the differentiating embryo is the aim of the work on determination. If differentiations become visible early and are little modified by extrinsic factors with the result that the course of development is orderly in a high degree, we say that the egg is strongly determinative. But if the egg retains its embryonic plasticity and easily adapts itself to environmental disturbances, that is, is easily modified by extrinsic factors, it is said to be indeterminative or of the regulatory type. If differentiations manifest themselves early, little regulatory capacity is retained by the egg. In other words, the capacity of the egg for regulation is inversely proportional to the progress of differentiation.

It must be borne in mind also in considering the problem of differentiation (and hence of determination) that the chemico-physical organization of the cell limits the conclusions which may be reached. Differentiation manifests itself through colloidal materials. The chemistry of the protoplasmic system is so imperfectly known that whatever conclusions are reached regarding these problems must be subject to revision when a better understanding shall have been attained, for example of the phenomena of gelation which plays a very important part in the physical expression of mitotic phenomena. It has been shown by the studies of

Conklin and of others that the position and size of the spindle are matters of great importance in differentiation. Yet the physico-chemical factors which are involved in spindle behavior have not yet been adequately analyzed. Other problems of physico-chemical nature similarly affect our conclusions. It is not too much to say that in spite of the vast amount of embryological research differentiation and its mechanism is one of the least understood fields of biology.

It has been pointed out that the function of cleavage is to sort out the materials of the egg into cells so that differentiation may progress. The uncleaved egg represents all of the materials present, although not necessarily all that are later to appear. Yet the progressive development of the organism in its usual course at least as indicated by our present knowledge depends upon getting these materials separated from each other. It does not follow, however, that this sorting-out process of cleavage is the cause of the subsequent development. Rather there must be a fundamental underlying mechanism of organization, the manifestations of which are the cleavage phenomena. Primarily determination concerns itself with the manifestations of these underlying methods of organization. It seeks for evidence of pre-localization of areas or substances within the egg or even of an early promorphology which gives visible expression to the underlying mechanism. Some of the questions involved may perhaps be formulated in the following manner: Are there special structures or substances or fundaments which exist in the egg at the very beginning of development and which are independent of each other and of other parts of the egg? If so, how are they formed? Must all appear at the same time, or may some appear later on? Conversely, are the definite structures of the embryo never independent of each other? Are the parts always influenced by the whole of which they are constituents? Or finally, are there some eggs which are determinative in their character and others which are not, or are some parts of the individual embryo determinative and other parts not? Perhaps all these questions may be said to depend most largely for their answers upon this one. At what time and from what sources do differentiations begin in the embryo? It may be noted that the answers to these questions are not the same for all organisms.

B. Classical Theories of the Nature of Development

Historically there have been a number of theories developed in response to these questions. They represent the various forms which the answers of different investigators have taken and must be considered from the standpoint of the information available when they were formulated. One of them was the theory of *organ-forming regions* of

Wilhelm His (1874). This is the doctrine that definitely localized areas perhaps even in the unsegmented egg are the forerunners of the organs and parts of the embryo, that "the germinal disc [of the chick] contains the preformed germs of the organs spread out over a flat surface, and conversely . . . every point of the germinal disc is found again in a later organ." Ray Lankester supported His in his views, saying that it was quite possible for the cell to "contain already formed and individualized, various kinds of physiological molecules. The visible process of segregation is only the sequel of a differentiation already established and not visible." Whitman as a result of his work on *Clepsine* concluded that the embryo is predetermined, even if not predelineated. Rabl and van Beneden likewise were among the early supporters of the view that even in the unsegmented egg an organization of protoplasmic particles which predetermines the development of the embryo is present. In the bald form in which this doctrine was proposed by His it can scarcely be accepted, however, and as a matter of fact His had no great amount of observational and experimental evidence on which to base it. The study of cell lineage offers what of evidence there is in support of this doctrine but even at best it does not force as rigid an interpretation as His placed upon it. Many authors have denied it in its entirety and there are few who do not criticize it.

Another form of answer to the question of determination is the theory of *organ-forming substances*, according to which the materials within the egg rather than the areas which they occupy receive the chief attention. Here again it is obvious that not all materials within the egg, even though they be definitely localized, are formative. Yolk, pigment, oil globules, and other inclusions in the cytoplasm are definitely located in many eggs, yet clearly are not formative. In some instances visibly differentiated substances within the unsegmented egg have been shown to have no formative function. At least they have been displaced by pressure or by centrifugal force, and normal embryos have nevertheless resulted. Yet in other forms the materials of the egg have been traced in a precise manner to the organs of the embryo. Surely a principle of determination is here involved which is too important to deny because of a particular formulation of a doctrine. Perhaps the importance of the visible organ-forming substances has been overemphasized and these are but manifestations of a more fundamental mechanism. Yet in very many eggs the mechanism undoubtedly exists, and later researches have certainly demonstrated the essential correctness of the principle involved in the conception that the egg is not without an organization before cleavage begins which conditions the future course of its development.

As a result of the early work on cell lineage by Whitman, Rabl, van Beneden and others the former view that blastomeres were indifferent both in position and significance was gradually replaced by another to the effect that the cleavage pattern was in the nature of a mosaic, and that a blastomere occupied its particular place because of the material contained in it, that is because of its mosaic character. The views were supported not only by the extensive studies on cell lineage, but also by the experiments of Roux, Chabry, Conklin, Wilson, and others. These experiments are discussed later in this chapter.

The *mosaic theory of development*, if understood in a sense not too rigid to take account of the fundamental plasticity of living protoplasm, certainly expresses one of the underlying truths of embryology. One may, however, raise the question of the manner and the time of origin of the mosaic work and also whether it is so fixed that it is not subject to the regulatory processes of which the organism is capable.

The question of regulation brings up yet another theory of historical significance which we owe to Driesch. His terms prospective significance, prospective potency, his equipotential system, are all well known to those who are familiar with the history of the determination problem. By prospective significance of a cell he meant the actual destiny in the developmental process. By prospective potency he meant the possible fate which the cell might attain. Prospective potency includes the sum of the different developmental possibilities. As the result of numerous experiments, Driesch reached the conclusion which he thought of general application, that the egg is essentially an equipotential system, for the cells of a blastula of a sea-urchin, for example, are of uniform material and might be interchanged like balls in a pile without affecting the result in the development of the embryo; in other words that the fate, the prospective significance, of a particular blastomere is a "function of its position." Driesch's theory was in opposition to the mosaic theory of development according to which the fate of the blastomeres did not depend upon their position alone but upon their organization. It was based upon the conception current at the time when work on cell lineage first rose to an important place. Pflüger had held that the egg and its blastomeres were homogeneous throughout and that cleavage simply multiplied the units out of which differentiations were later to arise. This view was also favored by Oscar Hertwig. Driesch, who although chiefly interested in philosophy, set himself to carry out experiments in development upon which certain philosophical decisions might be based, made this conception the starting point of his theory of development.

Some experimental evidence is at hand upon which to a certain

extent the theory of Driesch may be based, while there is also evidence upon which the mosaic conception may rest. On the one hand certain eggs possess a high degree of regulatory capacity and are hence indeterminative in their cleavage. On the other hand certain other eggs show the marks of differentiation appearing at a very early stage in the development of the egg; these eggs possess but slight regulatory ability and are hence determinative in character. Between the mosaic type and the regulatory type no sharp boundary line can be drawn. Rather there is a gradation from one extreme of the series to the other. If we sum up the results of these researches we may arrange the animal series from the regulatory to the mosaic forms in about this order: amphioxus, teleosts, mammals, nemerteans, urodeles, anura, ctenophores, annelids, molluscs, arthropods, nematodes, and ascidians. But it is to be noted that purely regulatory and purely mosaic types do not occur. In this we are merely emphasizing what has been said before, that the time at which differentiation appears is the real criterion upon which we may recognize the differences between determinative and indeterminative types of eggs. In other words if differentiation sets in early the regulatory capacity of the egg is correspondingly reduced. Conversely, eggs with high regulatory capacity show little evidences of early differentiation.

C. Evidence Bearing on Determination

The present status of opinion in regard to the determination problem has been reached as the result of two different lines of evidence. One of these is the morphological study of normal embryological stages. The other line of evidence embodies the results of many experiments which have sought an insight into the fundamental nature of developmental problems.

1. Morphological Evidence

Of the morphological evidences that some type of determination exists to some extent in all eggs reference should perhaps be made first to the contributions of genetics. The breeding experiments of this century have very clearly shown the existence of hereditary units within the germ cells. Although we do not by any means identify these units with visible structures in the egg cell nor do we know how they express their influence upon the developing organism, yet any theory of particulate inheritance (that is inheritance based upon the presence of organized particles within the germ cell) is, in so far as it is justified, evidence in favor of a morphological basis of determinate development. This evidence bears out the conclusion stated above that, although

the regulatory processes may dominate in certain types of eggs, there are none which are purely regulatory.

The first signs of differentiation in the developing egg have to do with the general features of polarity, symmetry, and pattern of the egg. From these general features more detailed differentiations with respect to polarity, symmetry, location, and pattern of the constituent cells and parts of the embryo arise and later the differentiations of tissues and the beginning of organs. Development is thus progressive, and differentiation, at first relating only to the most general features, gradually passes to specialized details. Since polarity, symmetry, and cleavage pattern are general in character their importance is sometimes overlooked by the student, yet very little thought is required to convince one that they are really basic. Our morphological knowledge with regard to the development of polarity and symmetry has been discussed in the chapters which have to do with the different types of cleavages and reference should be made to those chapters. These observations have been checked by many experiments of the greatest importance and it will perhaps suffice to refer to these experiments under the second line of evidence as listed here.

Morphological studies of the cleavage pattern, as has already been indicated, became the foundation for the mosaic theory of development in the studies at the hands of the students of cell lineage. It early became clear that certain types of cleavage were predominately determinative. Some eggs with bilateral cleavage, certain types of eggs with superficial cleavage, those with spiral cleavage, with disymmetrical, and even an occasional form having radial cleavage, such as *Strongylocentrotus*, were shown at that time to be determinative in their development. Most forms with radial cleavage, some with bilateral, and even occasional examples of eggs with spiral cleavage show the regulatory type, however. For a discussion of these the student is referred to the chapters on cleavage types.

2. Experimental Evidence

The second line of evidence which bears upon the problem of determination is perhaps no more important than the morphological evidence but is certainly not less so and is much more recent. It is obtained from those special experiments* which have to do with this problem.

* As pointed out in the chapter which deals with history of embryology, the present is the period of *experimental embryology*. Much of morphological character remains to be learned, but nevertheless the dominant note in embryological research of the present day is the experimental one. It is now sought to discover those underlying principles which account for the form changes by which the structure of the organism is produced rather than simply to give a descriptive account of those characters.

The first series of experiments of significance for study of determination is concerned with the *localization of the median plane.* Here as with the other experiments to be summarized the student is referred to Morgan's critical discussion as well as to the original papers which describe them. It would be impossible in a work of this size to give even a brief account of the experiments themselves. Reference can only be made to those which seem most significant.

The matter of the localization of the median plane is of importance because it indicates the early determination of symmetry and polarity on the part of the egg. According to some the polarity of the egg may be traced back even into an ovarian condition, and some believe that the type of symmetry is determined in the egg cytoplasm before fertilization. Although the evidence of these relationships seems good in some cases it has not been made clear how much the appearance of symmetry in these early stages is related to the cleavage plane or the symmetry of the embryo. No mechanism for bringing this about has

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Experimental embryology has seemed to cover a wide range of topics and the results of the investigations in this field are scattered throughout many journals. There have been but few attempts to bring this material into the scope of a single volume. The first section of Korschelt and Heider's "Lehrbuch der vergleichenden Entwicklungsgeschichte der wirbellosen Thiere" deals with the experimental results obtained up to that time (1902). In 1909 Jenkinson published his book entitled "Experimental Embryology" and summarized much of the work available at that time. The contribution to experimental embryology most important now, however, is the volume by Morgan entitled "Experimental Embryology" and published in 1927. Morgan has promised a further volume which will deal with such topics as growth, reflex reactions, tropisms of the larvae, the influence of environment upon the embryos, the source of energy of development, etc. It is to be hoped that this volume will appear at no distant date.

In spite of the diversity of topics which have engaged the attention of experimental embryologists it is possible to classify them into a few divisions. (1) The early students of this field were specially interested in the discovery of the effect that external factors have upon the developing embryo. Among these factors are gravitation, mechanical agitation, electricity, light, heat, atmospheric pressure, osmotic pressure, and the chemical composition of the medium in which development takes place. (2) In addition to this the growth problem, the problems centering around fertilization, have occupied much attention during the present century. Something of the nature of these problems is suggested in the section of this book which has to do with artificial parthenogenesis, although this is but one of the many topics that have been studied. (3) Finally a great deal of attention has been given by experimental embryologists to those problems which are concerned with the formative changes of the egg. These factors are internal in their nature and seem to take the experimenter further in his attempts at the analysis of the innate, vital character of the organism than do the other problems enumerated. The experiments which bear on the problem of determination come under this head. Those experiments which have sought light on this problem fall chiefly within a few animal groups, namely: the ctenophores, some hydromedusae, the nematodes, annelids and molluscs, echinoderms, ascidians. It may be remarked that not only are these the forms which seem to have yielded results most capable of analysis but they are also the forms upon which it is easiest to experiment.

been demonstrated. In many cases where a relationship between the first cleavage plane and the planes of symmetry of the later embryo have been made out it is thought that the point of entrance of the spermatozoon and the path traveled by the sperm pronucleus in approaching the egg pronucleus determine the position of the plane. Suggestions have also been made that the pressure of the enveloping membrane of the egg or pressure from the oviduct or other external factors may be related to the position of the plane. Certainly the causal character of such factors has not been demonstrated. It may be said, however, that so long as the possibility remains of a relationship for example at the point of entrance of the spermatozoon it is necessary to assume that the symmetry of the embryo goes back to a predetermined symmetry of the egg.

The question of a relationship of the median plane and the first cleavage plane arises in the history of embryology in relation to the frog egg. As long ago as 1851 Newport had reported that the two coincide, and from that time the question has been often discussed. In the frog egg at the time of cleavage there is already present a bilaterality as shown by the presence of the gray crescent. The real question involved in determining the symmetry relationships is: what in the uncleaved egg causes the material to take the position of the gray crescent, and why does the first cleavage plane cut through the middle of it? Experiments have shown that its position is determined after fertilization and that any meridian of the unfertilized egg may become the median plane. After fertilization the meridians are not equivalent in this respect, however, for usually the crescent forms opposite the point of entrance of the sperm. A long series of experiments can be cited to show that in nearly two-thirds of the cases the first cleavage plane does coincide with the middle of the gray crescent. It is clear, however, that if 30 per cent or more of the eggs fail to show this relationship the mechanism of determination is by no means a fixed one. In other amphibia the experiments of Jordan indicate that the first cleavage plane is at right angles to the axis of the egg of *Diemyctylus*, and Spemann has found for *Triton* that the second cleavage plane coincides with the median plane of the embryo. Of course it is immaterial from the standpoint of determination as to whether it is the first or second plane which shows the relationship. In the teleost fishes according to the work of Morgan and of Clapp no definite relation between the second plane and the median plane of the body can be made out. For the sea-urchin a considerable amount of evidence is available. In *Toxopneustes*, Wilson and Mathews related the appearance of the first cleavage plane to the entrance of the spermatozoon, and Boveri held that it coincided with the median plane of the

embryo. In the eggs of *Echinus*, Driesch found the second plane to correspond to the median plane and Runnström assumed that the first cleavage coincides with the median plane. Recently Von Ubisch has performed a very ingenious experiment by which certain portions of the egg were stained with *intra vitam* stains; in three species of sea-urchins he obtained results which are in agreement. He was able to demonstrate no fixed relation between the planes of symmetry and planes of cleavage, but found some evidence that the first cleavage plane more nearly coincides with the median plane. On the whole for the sea-urchins perhaps this is the most acceptable conclusion. In his study of ascidian embryology Conklin determined that the first cleavage plane is the median plane of the embryo, and that in *Cynthia* its position is indicated before the pronuclei meet. In the nematodes, according to Boveri's work on *Ascaris*, the third cleavage plane of the dorsal cells is the median plane of the embryo. In *Nereis*, Just has found that the second cleavage plane usually corresponds to the median plane of the embryo but the first cleavage plane is determined by the position of the entering spermatozoon. Insect eggs are distinctly bilateral so far as their orientation is concerned, the position of the egg as laid corresponding to the symmetry of the mother's body and definitely indicating the position of the embryo in the egg. For the chick and pigeon Bartelmez has held that bilaterality is present even in the small ovarian eggs.

A second series of experiments related to the problem of determination has sought to discover to what extent localization of germinal areas is present before cleavage, using as a method the *development of egg fragments*. Eggs of *Cerebratulus* were cut along definite planes by Wilson, Zeleny, and Yatsu. Cuts were made both before and after fertilization and development followed without difficulty. The cleavage of the fragments in general corresponded to the type of cleavage of the whole egg but upon a much reduced scale. It was not found to make a difference from which part of the egg the fragment was taken. The experiments show that either factors which determined cleavage were not yet definitely localized at the time of maturation or the egg is capable of very extensive regulation. According to Yatsu if the operation occurred between the formation of the first and second polar bodies the cleavage was entirely regular but if it was after the second division irregularities developed in some of the cases. Wilson experimented with fragments of the egg of *Dentalium*. Here the presence of the yolk lobe complicates the result. If the yolk lobe is entirely present and only the apical portion of the egg cut off, the resulting larva is nearly normal; likewise an entirely symmetrical division of the yolk lobe seems not seriously to interfere with the course of development. If the cuts are made in any other

manner, however, irregularities appear. The ctenophore *Beroë* when cut into fragments in the unsegmented condition produces in some cases partial embryos and in others whole ones depending on whether the cut is symmetrical or oblique. Driesch, Morgan, Yatsu, and Fischel have experimented with this form. With sea-urchin eggs conflicting results have been obtained from experiments with the development of fragments. Taylor and Tennant using accurate methods of cutting with a micro-dissecting machine obtained small pluteae from the developing fragments which were like the normal ones. But Harnley obtained evidence that the materials of the egg were qualitatively different. Finally experiments on the development of parts of *Triton* eggs have been performed by Spemann and Baltzer. Non-nucleated fragments, obtained by separating the egg by means of a hair tied around it, developed but rarely and the nucleated fragments produced dwarf larvae which did not live long enough to undergo metamorphosis although normal in most particulars.

An interesting result of these experiments is the conclusion that the entrance of the sperm into the egg is not of itself sufficient to start development, for, if an egg is cut just after the penetration of the sperm so that the female pronucleus is in one half and the male pronucleus in the other, it is the half containing the sperm pronucleus that develops but not the other. Something else besides the mere initiation of division is accomplished by the entrance of the spermatozoon. The cleavage of egg fragments demonstrates that for many types of eggs the pattern develops along with the mitotic figure.

Another series of experiments has sought to discover to what extent it is possible for a whole embryo to develop from an *isolated blastomere.* In some few eggs it is possible to cut the blastomeres apart at the time when they are most widely separated from each other as the first cleavage is closing. In others it has been found possible to separate them by shaking them in a tube of water, or by squirting them from a pipette. Again eggs of echinoderms permit the easy separation of the blastomeres if they are kept in calcium-free sea water while the first cleavage is taking place. Isolation experiments have been performed on sea-urchins, the hydroid *Clytia flavidula, Cerebratulus,* amphioxus, teleost fishes, *Triton,* and the frog, and in all these cases whole embryos were obtained from the isolated blastomere. On the other hand blastomeres of ctenophores, molluscs, and ascidians when isolated give rise to half embryos only. It would seem that this emphasizes again the distinction between determinative and regulatory eggs.

The question arises as to whether the development of the isolated blastomere is strictly comparable with the results that would be ob-

tained if the material of the missing portion of the egg were present. Experiments have been performed in which the single blastomere has been allowed to develop in contact with the material of its sister cell, which owing to various kinds of injury was prevented from any active participation in the normal result. Experimenting upon the frog, Roux injured one blastomere with a hot needle but did not kill it. He thought that the subsequent development of the uninjured blastomere in contact with the inactive one was in the nature of regulation and that gradually the missing portion of the embryo was restored. This conclusion seems doubtful as there is now good evidence that the resulting embryo is more nearly one-half than a whole. Later studies have shown that, in experiments of this kind, if the plane of the first cleavage goes through the middle of the gray crescent a half embryo results. If the plane is parallel to the gray crescent and the injured blastomere is the one which contains it, nothing recognizable is produced from the opposite one. But if the opposite blastomere is injured the one containing the gray crescent will produce the anterior end of the embryo. Even if the cleavage plane is at other angles the blastomere containing most of the gray crescent will produce an anterior end.

McClendon working on the tree frog, *Chorophylus* (*Pseudacris*), sucked out the injured blastomere to determine whether its presence has any effect. In his experiments the remaining blastomere produced a normal whole embryo of one-half size. Injuries to one blastomere of the egg of *Ascaris* led to the conclusion in the experiments of Stevens, of Boveri, and of Schleip that each individual cell contains the factors which are responsible for its own development; in short, that there is little self-regulation on the part of the blastomeres. The egg of *Cyclops* after the injury to one blastomere has been studied by Fuchs and by Miss Jacobs. The evidence goes to show that for this egg the contact of the injured blastomere with the uninjured one does not materially affect the result of the development of the latter. This results in a partial embryo. In this same connection the experiments of a number of investigators, of whom Hegner and Reith are typical, on the effect of injury to the eggs of insects should be mentioned. Insect eggs apparently may be among the most determinative with which we have to deal, for these experiments indicate that different cytoplasmic areas on the surface of the egg are fixed in their prospective significance even before the cleaving nuclei with their surrounding cytoplasmic islands migrate to the surface and they have no great powers of readjustment. There is indeed a considerable degree of independence in the development of the respective parts. If the injury is not too severe to a certain localized portion of the egg the remainder will go ahead and develop without much

dependence upon the injured portion, but will produce only that part of the embryo which was to be expected. Reith found that, if the posterior end of the egg of the house fly is injured, the parts normally resulting from the anterior end will develop. If the anterior end is the location of the injury, a larva without a head end develops, and in some cases injury to the middle portion of the egg was not so severe but that both anterior and posterior organs developed.

Hegner's experiments on the eggs of the chrysomelid beetles *Calligrapha multipunctata* and *Leptinotarsa decemlineata* are well known. Here the posterior end of the egg is the location of the pole plasm which Hegner found to pass into the germ cells in development. By injuring this region with a hot needle he was able to secure embryos without germ cells. Evidently the cytoplasmic regions in the eggs of these insects are very early set aside to produce definite parts of the embryo. Yet it must not be inferred that the regions in question retain no powers of adjustment. As cleavage progresses the possibilities of readjustment are lessened but are not entirely lacking.

A very important series of experiments carried out in the laboratory of Spemann on the embryos of *Triton* have shown that the future of certain ectodermal areas in the gastrula are much modified by their position. The method by which these experiments were carried out was that of *transplanting a portion of the ectoderm* by means of a micro-pipette from one portion of the body to another and studying its relationship to the development of the neural plate. From this study it appeared that the presence of the endomesoderm beneath the surface of the ectoderm is immediately necessary for the neural plate to be formed. Spemann and his collaborators have shown that the prospective ectoderm is capable of producing entirely different organs upon transplantation. For instance ectoderm from the top of the young blastula or gastrula implanted on the lip of the blastopore and carried to the interior may become notochord, mesoblastic somites, pronephros, or perhaps other organs. Ectoderm taken from a slightly later stage, that is, after the closure of the blastopore, if carried into the mesoderm becomes mesodermal somites; if carried into the endoderm goes to the formation of the archenteron. It should be pointed out that particular organs which differentiate from given substances may through the process of rearrangement be induced in an entirely different direction from that which their normal determination indicated. Throughout the animal kingdom there are many cases which serve to emphasize the difference between the determination of an organ and the actual differentiation which sometimes results from the modification of normal processes. What is shown by such cases, however, is not that the unusual condition

is one of indetermination but that the organism has responded to modifying factors to produce a result different from that which would have occurred had not the unusual factors been present. Determination of an embryo does not always correspond to its differentiation.

A problem which has given rise to a great deal of experimentation in relation to the question of determination is the *influence of pressure* upon cleaving eggs. The work began with the experiments of Pflüger in 1884 in compressing the eggs of frogs between two glass plates. The direction of the first three cleavage planes was found to be at right angles to the plane of compression. When the compression was released, normal embryos developed. Similar experiments have been performed in numerous other eggs with the result that normal development is found to follow the release of pressure on eggs of hydroids, sea-urchins, frogs, and *Cerebratulus*. In eggs of *Nereis*, *Ciona*, and molluscs, abnormal development follows compression. In the first group of eggs it is to be noted that differentiation does not begin until there is relatively a large number of cells present. In the second group signs of differentiation are to be noted very early. This series of experiments was used by O. Hertwig and by Driesch in support of their opposition to the mosaic theory of development. Perhaps the experiments are less crucial than was formerly believed and merely emphasize the distinction previously insisted upon that there are two types of eggs with respect to their capacities for regulation as distinguished from determination.

Finally the literature of experimental embryology contains many records of attempts to bring about the redistribution by *centrifuging* of egg substances, particularly those which are visibly different. These materials as well as the formed materials of the egg yolk, pigment, fat, and other inclusions are often of different specific gravity and hence respond to centrifuging by redistributing themselves in different zones or strata. This transfer of materials throughout the egg takes place as a rule without injury to its living substance. The use of the centrifuge in experimental embryology began with the work of Lyon in 1906, although it had been utilized for the study of the constitution of the cytoplasm by Gurwitsch in 1904. Lyon's paper is a classical one in embryology both because of the introduction of a new method and because of his discovery that the redistribution of the egg constituents does not affect the development of the eggs from the standpoint of determination. Many other investigators have used this method for the study of eggs through a wide range of animal forms. The results of the experiments with the centrifuge have rather uniformly indicated that the visibly stratified substances in the egg are not determinative in the sense that they are organ forming. It would seem that the method,

though productive of results which have great value from other standpoints, has failed to give critical evidence as to the determinative character of development in the eggs to which it has been applied.

As a conclusion to this long catalogue of evidences, the position earlier taken in this chapter may be reiterated. The animal series can be arranged in such a manner as to show a transition from eggs which are highly regulatory to those that are highly determinative in character. No eggs are known which are purely regulatory or purely determinative, but at one end of the series differentiation sets in very late and the regulatory capacity is high. At the other end the marks of differentiation begin even before cleavage and the powers of regulation are correspondingly lessened.

CHAPTER IX

ECOLOGICAL CONTROL OF INVERTEBRATE LARVAL TYPES

Throughout the animal kingdom many diverse environmental relations surround the eggs and young so that many responses might be catalogued. Environmental influences find expression in the places where the eggs are laid, in the amount and kind of food provided for the young during the early development, in the different devices for their protection, in specialized modifications for accomplishing locomotion and other vital activities, and in many cases in the production of forms of larvae which are totally different from the adult organisms. These special types of larvae present a great range of variations from the modes of direct development with which the student of the embryology of vertebrates is familiar. These larval types involve a metamorphosis to the adult form which may be more or less complete and which in such cases brings the growing organism into an environment quite unlike that of its earlier development; metamorphosis is necessary in those forms in which the food and habits of the adult are unsuitable for developing young. If we assume the sea to have been the original home of most primitive stocks, an assumption which is more or less common and seems in line with the fact that very many types of animals develop in a moist atmosphere or liquid medium, we should expect the majority of unusual larvae to be found in the species living in salt water. The transition from salt water to fresh or to the terrestrial forms of life has presented many difficulties to the developing young and as a result those animals which live in fresh water or are terrestrial are more often characterized by direct development than are the salt-water forms.

It may be regarded as an axiom of development that sessile adults have active young. This activity has two obvious results; first, the species secures its dispersal through the migration of the young, and second, it extends the range. The migration is commonly passive but over a period of time serves to extend the range of the species as successfully as if the adults themselves were able to move. To insure dispersal, vast numbers of gametes must be produced, for the chance method of fertilization that usually accompanies such cases results in a failure of very many eggs and sperm to become further activated, and of those eggs which are fertilized only a few can come to maturity. The larval

mortality is very high indeed, for these young are the food of many species as well as the victims of physical forces and of their own inability to find continuously satisfactory conditions for growth. The vast number of eggs produced may be realized by reference to the turbot which produces 9,000,000 in a single season, the cod with 5,000,000, and to the flounder with 1,000,000. In higher animals the reproductive energy is conserved by various means for caring for the young during early life, but in marine forms this is usually not the case. The second consequence of larval activity is to bring the growing forms into an entirely different range. This is illustrated in the case of the lobster, which is a bottom feeder, lurking in the crevices between stones and elsewhere to capture whatever prey may come within its reach, or feeding upon such dead forms as may be found nearby. The larvae, up to the time of the fourth molt, however, swim at the surface of the sea water. In addition to this, they are positively phototropic to light of the intensity of ordinary daylight, but the adults are negative. Thus the pelagic larvae are brought into a range of environment where their food, which consists of plankton, especially copepods, is abundant. Special structural modifications adapt these larvae to their surface pelagic life. They are the better enabled to swim on the surface of the water because of the possession of exopods, the outer branches of the walking legs characteristic of lower crustaceans but not present in the adults of the higher forms. These are retained to the fourth molt, when they are cast off, being no longer useful; the larva then goes to the bottom. Herrick makes note of several changes in structure and instincts which take place at the beginning of the fourth stage, which marks the most surprising leap in the whole history of development. Among these are the following: the primitive swimming branches of the thoracic appendages are lost; the cuticle becomes shell-like, containing more lime; the pigments are denser, the colors brilliant, and the color pattern variable; otocysts are present and orientation is perfect; rotation of the great forceps is complete; the animal, during at least a part of this stage, moves toward the light and swims steadily at the surface with the great claws directed forward and held close together; the preying instinct is more marked; the fighting instinct, the instinct of fear, "feigning," and hiding are all developed by the close of the fourth stage or in the fifth, when the animal goes to the bottom to stay. In many other animals similar gain is accomplished by a specialized form of larval structure. The larva finds itself adapted to securing food which is suitable for it, and structures which have only temporary employment are present and useful. It follows, however, that the gain to the larva would become a loss if the subsequent stages

in the life cycle were not radically changed in such a way as to enable the organism to undertake a new mode of life. In other words, metamorphosis in such cases is a necessity for bringing about those adaptations which fit the organism to live in its permanent environment and there to undergo those further changes which look toward the production of new germ cells.

It is not usual to find metamorphosis in animals which live in fresh water, although there are some outstanding cases in which larvae totally unlike the adult are developed in species inhabiting fresh water. It may, however, be taken as a general rule that development in fresh-water forms with the exception of certain few highly specialized cases is direct. Fresh water presents a great lack of constancy in living conditions as compared with those of the sea. With but few exceptions the bodies of fresh water lack sufficient depth and area to maintain even a low degree of constancy. Fresh water becomes heated much more easily than the sea, it freezes comparatively quickly, its streams are subjected to periods of flood during which the water runs rapidly and scours out the beds or overflows, and when it recedes leaves innumerable forms of life stranded to perish. Every stream has narrows and depths where it runs rapidly and flats where its current is slow, an environment in which the fragile larvae would scarcely be able to maintain themselves, and there are but few animals which go through an independent larval history in fresh water. The lack of suitability of fresh water to larval development without doubt explains why so many groups of marine animals have not been able to gain a foothold in fresh water. A very few sponges, almost no coelenterates, occur in this environment, and many groups of marine fish and ascidians, cephalopods, king crabs, and some of the worm groups have not a single representative in fresh water. Animals that cannot produce eggs which develop into young like the adult are prevented from gaining a foothold by such a changeable medium as fresh water. In addition to the difficulties of fresh-water life already noted, a zone of brackish water between the fresh water and the sea is itself an almost impassable barrier to the entrance of marine forms.

Terrestrial life presents to developing larvae even more dangers than fresh water. To this situation is due the fact that in land forms development is either direct and fairly simple or else specialized with complicated devices for caring for the young during their immature period. The latter condition is illustrated by many forms of insects in which metamorphosis is complete, often involving very complicated life histories. Some of the disadvantageous features of the terrestrial

environment are the following: temperature undergoes a wide range of variation, often passing quickly between the extremes. This condition is quite unknown in the ocean and is much less in fresh water. The very changeableness as well as the extremes of heat and cold are hardships for the developing larvae. The weight of the body is no longer buoyed up by water and must be supported at every moment. This involves consumption of much energy as compared with the almost passive drifting of larvae in aquatic habitats, and renders movement much more difficult and restricted. Food no longer streams by as in the contrasting case, but must be actively sought, is often very much more restricted as to kind, and presents an increased toughness since in the composition of land forms the percentage of water is much less, and the food is necessarily more difficult to find. The glare of daylight and the consequent difficulty of avoiding enemies puts the terrestrial larvae to a disadvantage which forms living in the subdued shadows of the water do not share. Finally the rapid evaporation to which terrestrial forms are subjected requires special devices for protecting the soft exterior of the organism. A heavy shell, mucous glands, and other similar devices show what a serious drain upon the organism requirements of this kind cause.

These considerations account in a large measure for the lack of uniformity in the occurrence of larval types throughout the different groups of the animal kingdom. The particular forms of larvae within a group are of adaptive rather than taxonomic significance.

The general subject of the care of young animals was briefly but instructively discussed by Gamble in "The Animal World" (Holt and Company). Some of the ideas mentioned on the three preceding pages are suggested by his discussion. Gamble sums up the responses of animals to different environmental conditions in which the young must be produced as follows:* "We find isolated examples of the retention of the young by diminution in the size of the family. Speaking generally, marine animals pour their eggs broadcast, and leave their minute larvae to complete their metamorphosis unaided and unsheltered, but in every group there are malthusian species which not only restrict their families in number, but enclose them by protective envelopes. The varied experience of larval life in this curtailed and direct development supplants metamorphosis. The eggs become larger and the young stronger at birth.

"In fresh water the limitation and protection of the family is more generally the rule. Insects and Amphibia are the only large classes

* Quoted by permission of Henry Holt & Co.

which go through their larval life in fresh water. But the protection is usually of the simplest kind and is confined to the earlier stages of development.

"On land the insects form the only class in which the majority still pursues a free larval history. Others develop directly, either growing up into full stature without guidance or protection, or carried and fed by their parents for some time both before and after hatching."

GLOSSARY OF EMBRYOLOGICAL TERMS

A

SOURCE WORDS AND ROOTS FROM FOREIGN LANGUAGES

a, Gr., without
ab, L., away from
acro, fr. Gr. *akros,* highest tip, apex
actin, fr. Gr. *aktes,* ray
ad, L., to or toward, upon
akros, Gr., tip
allos, Gr., other
alter, L., other
amnion, fr. Gr. *amnos,* lamb
amphi, Gr., both
an, Gr., without
ana, L., up
Anlage, Ger., fundament, beginning cell mass
ante, L., before
anthropos, Gr., man
apo, Gr., away
apsis, Gr., an arch
aqua, L., water
archos, fr. Gr. *archaios,* ancient
arrenos, Gr., male
arthros, Gr., joint
askos, Gr., bag, wine skin
astêr, Gr., star
auris, L., ear
autos, Gr., self

basi, L., at the base of
bi, L., two
bios, Gr., life
blastos, Gr., germ
brachium, L., arm
brachy, Gr., short

caecus, L., blind
caeno, fr. Gr. *kainos,* common
cardia, Gr., heart
cauda, L., tail
centro, fr. Gr. *kentron,* center
cephalê, Gr., head
cercoid, fr. Gr. *kerkos,* tail
chiasma, Gr., two crossed lines
chondros, Gr., cartilage (grain of wheat)
chorda, L., a cord or string
chorion, Gr., foetus-membrane
chroma, Gr., color
chyma, Gr., juice, fluid
clast, fr. Gr. *klastos,* broken
cloaca, L., sewer
coelo, fr. Gr. *koelos,* hollow
cope, fr. Gr. *kope,* an oar
corpus, L., body
cortex, L., bark
crescere, L., to increase
cyclos, Gr., circle
cyphon, fr. Gr. *kyphos,* bent, crooked
cyst, fr. Gr. *kystis,* bladder, sac
cytos, Gr., cell or hollow space

de, L., from, away, out of
deferre, L., to carry away
delphys, Gr., uterus
de novo, L. "from a new" or "anew," origin from no known source
dens, L., tooth
derma, Gr., skin
desmos, Gr., fastening, ligament
deuto, fr. Gr. *deuteros,* second, secondary
dexios, Gr., on the right hand
di, Gr., two
dia, Gr., between
diplos, Gr., double
ducere, L., to lead
dura, L., hard

echinos, Gr., hedgehog
ecto, ekt, Gr., the outer, outside
efferre, L., to carry away from
elasmos, Gr., plate, flat, metal plate

embole, Gr., a putting into
enchyma, Gr., something poured in
end, endo, Gr., within, inside
enteros, Gr., inside
ep, epi, Gr., upon
epibole, Gr., a throwing upon
erythros, Gr., red
eu, Gr., well, good
ex, L., out, outside

ferre, L., to carry
filum, L., thread
foetus, L., offspring
foramen, L., an opening

gametê, Gr., wife
gastêr, Gr., stomach
gemma, L., a bud
genesis, Gr., birth
gerere, L., to bear
germinare, L., to sprout
-glea, Gr., gelatinous substance
gonê, Gr., offspring
gynê, Gr., woman

hêmi, L., half
hêpar, Gr., liver
heteros, Gr., different
hippios, Gr., pertaining to a horse
holos, Gr., whole
homoios, Gr., like
homos, Gr., the same
hydra, Gr., water
hyp, hypo, Gr. or L., below
hyper, Gr., over, above

inter, L., between
in toto, L., in entirety
intra, L., within
isos, Gr., equal

karyon, Gr., nut
kata, Gr., down
Keimbahn, Ger., germ track
kinesis, Gr., motion

labium, L., lip
lacrima, L., tear
lacuna, L., space
laios, Gr., left
lamina, L., a thin sheath
larva, L., ghost, mask
latus, L., side
lecithin, fr. Gr., *lekithos,* yolk
leios, Gr., smooth
leukos, Gr., white
lingua, L., tongue
lithos, Gr., a stone
logos, Gr., speech, reasoning
lympha, L., clear water

makros, Gr., large
mamma, L., breast
marsupium, L., pouch
maturus, L., ripe
medius, L., middle
medulla, L., marrow
megos, megalon, Gr., great
meros, Gr., part
mes, meso, Gr., the middle
met, meta, Gr. and L., between or after
metos, Gr., a thread
metron, Gr., a measure
micros, Gr., small
mixis, Gr., mingling
monos, Gr., one
morphê, Gr., form
morula, L., little mulberry
mys, Gr., muscle
myzon, Gr., sucker

natus, L., born
neos, Gr., new, youthful
nephros, Gr., kidney
neuron, Gr., nerve
notos, Gr., back
nucleus, L., kernel

odons, Gr., tooth
-oid, fr. Gr. *eidos,* form, like, in the form of
oikos, Gr., a house
onto, Gr., being
ophis, Gr., a serpent
optikos, Gr., eye
orthos, Gr., straight
ovum, L., egg

paedo, fr. Gr. *pais, paidos,* child
palin, Gr., again
pallium, L., cloak or mantle
para, Gr., beside, near

paren, L. from *parere,* to bear
parthenos, Gr., virgin
penta, Gr., five
peri, Gr. or L., around
petros, Gr., a stone
phag, fr. Gr. *phagein,* to eat
phasis, Gr., appearance
pherein, Gr., to bear
philein, Gr., to love
-phoros, Gr., bearing, producing
photos, Gr., light
phyllon, Gr., leaf
pilidion, Gr., little cap
pinna, L., feather
plakous, Gr., flat cake (in placenta)
planus, L., flat
plasma, Gr., formed, molded
plastos, Gr., formed
plax, Gr., plate, flat
pleura, Gr., a rib, a side
pluteus, L., a shed
pneuma, Gr., breath
poda, Gr., foot
poly, Gr., many
poros, Gr., a passage, pore
post, L., behind, after
pre, L., before
primordium, L., the beginning
pro, Gr. or L., in front, toward
proktos, Gr., anus
protos, Gr., first
pseudês, Gr., false
pteron, Gr., wing
pupa, L., girl, doll
pylê, Gr., gate

quadrans, L., a fourth part

radius, L., spoke of wheel
re- (red-), L., back, again, against
renes, L., kidney
rhis, Gr., nose
rostrum, L., beak

sacculus, L., a little sac
sarx, Gr., flesh
saurus, Gr., a lizard
scleros, Gr., hard
segmentum, L., from *secare,* to cut
semi, L., half
septum, L., fence, wall
sinus, L., curve, hollow bag
soma, Gr., body
sperma, Gr., germ, seed
splanchnon, Gr., one of the viscera
sporos, Gr., a seed, a sowing
stereos, Gr., solid
stoma, Gr., mouth
stratum, L., a layer
stria, L., a furrow
strobilos, Gr., something twisted, as a pine cone
sub., L., under
super or supra, L., above
syn or sym, Gr., or L., together

teleos, Gr., whole
telos, Gr., end
tentare, L., to feel, to handle
tetra, Gr., four
tetrados, Gr., four
thêkê, Gr., a case
thêlê, Gr., a nipple
thelys, Gr., female
tome, fr. Gr. *tomê,* a cutting
tonos, Gr., tension
totus, L., all, whole
Träger, Ger., carrier
trêma, Gr., opening
tri, L., three
trochos, Gr., a wheel
tropê, Gr., a turning
trophê, Gr., nutrition
tunic, L., tunic
-typôs, Gr., impressed form

-ule, L., diminutive
umbilicus, L., navel
ungula, L., hoof
uni, L., one
ur, Ger., primitive
ura, fr. Gr. *oura,* tail

vagina, L., sheath
valent, fr. L. *valere,* to be worth
vas, L., a duct
vegetus, L., enlivened, vigorous, growing
velum, L., a veil
venter, L., belly, stomach

vermis, L., worm
vitellus, L., yolk of egg
vivus, L., alive
volvere, L., to roll
zeugle, zeug, combining form from Gr., yoke, join
zoön, Gr., animal
zygon, Gr., yoke, pair

B

DEFINITIONS OF TERMS USED IN EMBRYOLOGY

Names which are applied to a single structure only, as *notochord*, *mandible*, *ambulacra*, etc., are not usually included in this glossary. Names of systematic groups are also omitted.

Acoelomate. Not possessing a coelome or body cavity.

Acrosome. The apical body at the tip of a spermatozoon. Also called "perforatorium" from its supposed function of penetrating actively into the egg.

Actinotrocha. The larva of *Phoronis*.

Actinula. The larva of many hydrozoa.

Agamocytogony. Asexual reproduction. M. Hartman's term.

Agamogony. The same as agamocytogony.

Alecithal eggs. Eggs without yolk or with very little, and that uniformly distributed. Equivalent to isolecithal or homolecithal.

Alima. A larval stage of certain Crustacea.

Alternation of generations. The same as metagenesis.

Ametabola. A division of generalized insects which do not undergo metamorphosis; the Thysanura and Collembola.

Amitosis. Direct cell division; division without the formation of spireme or chromosomes.

Amnion. A thin membrane enclosing the embryos of some invertebrates and of reptiles, birds, and mammals.

Amphiblastula. The free-swimming larval form in the sponges.

Amphigony. Sexual reproduction, involving participation of two individuals.

Amphoterotoky. The condition in which parthenogenetic organisms of both sexes are produced in a single brood.

Analogy. Similarity of the parts due to function rather than structure.

Anaphase. The stages in mitosis during which the chromosomes pass from the metaphase position at the equator of the spindle to the poles.

Animal pole. The protoplasmic part of the egg from which the polocytes or polar bodies are given off from which the nervous system and sense organs develop.

Arachnactis. The larva of the Cereanthidae.

Archenteron. The primitive gut of the gastrula which later becomes the primitive digestive tract.

Archeocytes. In sponges the wandering parenchyma cells, aggregates of which form gemmules. Also called sorites.

Architomy. Asexual reproduction in which no zone of division is indicated before the division actually begins. Contrasted with paratomy.

Arrhenotoky. The production parthenogenetically of eggs which are exclusively male producing.

Artificial parthenogenesis. The initiation of development in an egg without fertilization.

Ascidiozooid. In *Pyrosoma* buds produced from a stolon which forms on the rudimentary embryo.

Asexual reproduction. Reproduction without the union of germ cells, and consequently without their production, not even reducing divisions occurring in animals which propagate in this manner.

Aster. The star-like radiations which surround the central body during mitosis.

Augmentation. Reproduction by growth above the normal.

Auricularia. The larva of the Holothuroidea.

Atrochal larvae. In annelids ciliated larvae with an apical tuft of long cilia but with no prototroch are spoken of as atrochal.

Axis of cell. A line passing through the nucleus and centrosome of a cell.

Axis of embryo (embryonic axis). The anterior-posterior axis of the embryo, or the line drawn through the uncleaved egg in the position of the future anterior-posterior direction.

Bilateral cleavage. A type of cleavage in which the egg substances are distributed symmetrically with respect to the median plane.

Biparental. Derived from two parents.

Bipinnaria. The larva of the starfishes, so called from the symmetrical arrangement of its arms.

Bisexual. Hermaphroditic. Having organs of both sexes in one individual.

Blastea. The hypothetical ancestral form, corresponding to the blastula in the simplicity of its structure, which Haeckel made use of in his "Blastea-Gastrea" theory.

Blastocoele. The segmentation cavity or the cavity of the blastula.

Blastocone. Cells of the external row about the blastodisc of a cephalopod, the lower ends of which fade into the yolk.

Blastocyst. The hollow sphere formed in mammals by the cleavage of the egg and the migration of the blastomeres; the blastodermic vesicle.

Blastoderm. The layer of formative cells developed about the yolk mass in embryos from meroblastic eggs, particularly vertebrates; it is of only one cell in thickness.

Blastodisc. In eggs with discoidal cleavage the protoplasmic area during cleavage takes the form of a blastodisc.

Blastomere. The cell produced by cleavage of the egg.

Blastopore. The communication between the gastrular cavity and the outside.

Blastotomy. The separation of cleavage cells or portions of them in the early stages of development.

Blastula. The end stages of cleavage. In its simplest condition a hollow sphere of cells.

Brachiolaria. An asteroid larva in a stage of development following the bipinnaria; it has three anterior arms containing diverticula of the anterior coelome.

Bud. An undeveloped branch, usually lateral, of an organism.

Budding. Reproduction in which a small secondary part is produced from the parent organism and gradually grows to become independent.

Calytopsis. The larval form of Euphausiodocea; a zoaea.

Campodeiform larva. An insect larva of generalized form resembling the thysanuran genus *Campodea*. Also called thysanuriform. Has long, flattened body, hard plates, long legs and antennae, caudal cerci, large mandibulate mouth parts.

Cell lineage. The history of the cell divisions from the early cleavages up to the time when the definite fundaments of the organs are laid down.

Cenogenesis (caenogenesis). The developmental processes in an individual which reproduce no old or ancestral characters, but only new and adaptive ones. Opposed to palingenesis.

Centrodesmus. A connection between the centrioles after their division from which the central spindle at least in some cases is said to arise.

Centrolecithal eggs. Eggs in which the yolk, usually of rather large amount, is found in the central region.

Centrosome. The large central bodies formed at the poles of the spindle, consisting of the centriole surrounded by the aster; the center of the divisional activity of the cell.

Cercaria. The tailed larva of the liver-fluke.

Chondriosome. A cytoplasmic body of the character of mitochondria, chondriochonts, etc.

Chorion. The second covering of an egg cell produced by the follicle cells. Also used loosely in other senses. In chick embryo it is equivalent to the outer or false amnion, and in mammalian embryos it is identified as trophoblastic in origin.

Chromatin. That substance in the nucleus which is rendered conspicuous by basic stains and which is the chief constituent of chromosomes.

Chromatin diminution. The elimination, usually in early cleavage, of a portion of the chromatin in the formation of the primordial germ cells.

Chromosomes. Bodies, commonly rod-shaped, seen to best advantage during the metaphase of a dividing cell. The mechanism by which their division is accomplished is the physical basis of inheritance.

Chrysallogenesis. Production of ripe sex products in the pupal stage of *Chironomus*. A special form of paedogenesis.

Cleavage. The cell divisions by which the egg is converted into a multicellular organism, the blastula.

Coelome. The true body cavity lined with mesoderm and lying between the digestive tract and the body wall.

Coenogenesis. *See* Cenogenesis.

Concrescence. (1) The fusion or union of parts of a growing embryo which were already separate. The usual example is the coming together of the lateral halves of the blastoderm in the neighborhood of the blastopore. (2) Also the fusion of masses of sponge cells and their growth into an individual from which a new colony is formed.

Copepodid. A temporary hatched larval stage following the nauplius and metanauplius stages of certain parasitic copepods.

Cydippid. The larval form of higher groups of Ctenophora; named from the most primitive type of ctenophore, the Cydippidea.

Cyphonautes. The larval form of the ectoproct bryozoa.

Cypris. The larval form of certain decapods, named from the genus *Cypris*, an ostracod.

Cysticercoid. The bladder-worm stage of those tapeworms whose intermediate host is an invertebrate.

Cysticercus. A bladder-worm larva of the tapeworms living in solid organs, as muscles, liver, brain, etc., of vertebrate hosts.

Cyte. A germ cell in the stages in the production of a gamete during which maturation

and especially chromosome reduction are accomplished. Oöcyte and spermatocyte of the female and male, respectively.

Cythazoid. One of the larval forms of *Salpa*.

Cytogamy. Conjugation.

Cytogeny. Sexual reproduction by germ cells.

Cytology. The science which devotes itself to the study of cells as individuals.

Cytoplasm. The protoplasm of the cell not including the nucleus.

Dedifferentiation. The return of cells from a condition of specialization to one corresponding to that of their embryonic condition.

Delamination. The formation of a new layer of cells parallel to the old by the division and migration and rearrangement of cells of the primary germ layers.

Desor's larva. A type of larva of certain nemerteans which is really a modified pilidium.

Deuterostomia. A group of phyla which includes all forms in which ectomesoderm is lacking, namely the Echinodermata, Enteropneusta, and Chordata. Contrasted with Prostomia.

Deutoplasm. The food material or yolk within the cytoplasm of an egg.

Dexiotropic. Right handed, or in a clockwise direction.

Differentiation. Process by which originally a simple type of organization becomes structurally or functionally specialized.

Dioeceous. Having the organs of the two sexes in two individuals.

Dipleurula. The hypothetical larval ancestral form of the echinoderms.

Diploblastic. Of two germ layers, ectoderm and endoderm only.

Diploid. Having the unreduced number of chromosomes characteristic of the somatic cells, the gonia, and the fertilized egg.

Discoidal. Having the type of cleavage found in extreme meroblastic eggs in which the cytoplasmic area is limited to a small disc on the yolk surface.

Disogeny. "Sexual maturity of one and the same individual in two different conditions, between which a metamorphosis with retrogression of the sex products occurs" (Chun).

Disymmetrical. Having the type of cleavage found in ctenophores in which two centers of symmetry are developed.

Echinopluteus. The pluteus larva of the echinoids.

Ectoderm (epiblast). The outer germ layer or the formative tissue composing it. Also the derivatives in later development of this layer.

Ectomeres. The micromeres in eggs with spirally cleaving eggs which produce ectodermic structures.

Ectomesoderm. Mesoderm derived from the primary ectoderm.

Ectoplasm. (1) The protoplasmic layer just within the cell wall. (2) The clear protoplasm of the ascidian egg which is derived from the germinal vesicle and collects at the upper pole of the egg, the source of the material of the gray crescent.

Embolic gastrulation. Gastrulation by invagination.

Embryo. The undeveloped organism during the period in which it is nourished only from stored food. Strictly speaking this term is applied to the young organism only while still enclosed in the egg membranes.

Embryonic axis. *See* Axis of embryo.

Embryonic knob. In the mammalian embryo in the cases of entypy of the germ, the thickened trophoblastic cell mass over the embryonic shield which by its enlarge-

ment brings about the so-called inversion of germ layers; the "Träger," also trophoblastic knob.

Embryonic shield. A thickening, more or less shield-like, in the walls of the blastoderm, which will give rise to the body proper.

Endoderm (entoderm, hypoblast). The inner germ layer or the formative tissue composing it. Also the derivatives in later development of this layer.

Endoderm mother cells. Cells in the one-layered mammalian blastocyst which by their inward migration and proliferation produce the primary endoderm.

Endomeres. The large macromeres of spirally cleaving eggs from which the endoderm will arise.

Endomesoderm. Mesoderm derived from primary endoderm.

Enterocoele. A portion of the coelomic cavity which arises by outgrowth from the enteric cavity.

Entoderm. *See* Endoderm.

Ephippium. The shell in which the winter eggs of Cladocera are often contained.

Ephyra. The free-swimming larval form of the Scyphozoa.

Epiblast. *See* Ectoderm.

Epiboly. The process of overgrowth by which gastrulation is accomplished where the endomeres are very large and the micromeres very small.

Epigenesis. The conception that the developing germ cell is structurally homogeneous and undifferentiated, and that its subsequent development is due to the interaction of the protoplasm and the environment. Opposed to preformation.

Erichthoidina. A stage in the development of the larva of stomatopod Crustacea.

Erichthus. A stage in the development of stomatopod crustacean larva.

Eruciform larva. Insect larva of caterpillar or maggot type. Cylindrical, fleshy body, thin integument, reduced mouth parts, antennae, legs, and caudal cerci. Inactive.

Evagination. The unequal growth outward of a surface layer, one of the processes by which the differentiation of organs is produced.

Facultative or Optional. Said of parthenogenesis when the eggs possess the power of developing without the intervention of sperm, but more usually are fertilized.

Fertilization. Broadly considered, the union of the gametes of two sexes. Strictly speaking it has two phases, initiation of development and the union of the egg and sperm pronuclei.

Fetus. *See* Foetus.

Foetus (fetus). The advanced embryo of the mammal.

Formative area. The portion of a blastoderm or blastocyst which gives rise to the body proper of the embryo.

Fragmentation. The breaking into parts which may undergo further growth. Occurs in sponges.

Frustulation. Constriction of small bud-like branches from a hydroid after which it settles down as a frustulum which grows into a new hydranth.

Gamete. The germ cell when ready for fertilization without reference to sex.

Gametocytogony. Sexual reproduction. M. Hartman's term.

Gametogenesis. The process of the development of the mature gamete from a primordial germ cell.

Gamogony. Sexual reproduction.

Gastrea. The hypothetical ancestral form which Haeckel conceived to have the characters of a typical gastrula. *Cf.* blastea.

Gastroblast. Nutritive individual of the tunicate colony.

Gastrula. The two-layered stage following the blastula of the embryo.

Gastrular cavity. The gastrocoele, from which the future enteric cavity is to be developed.

Gemmiparous. Reproducing by means of gemmules.

Gemmule. The reproductive mass of cells asexually derived in the sponges.

Germ balls. Groups of cells in the larval liver-fluke which give rise to the new rediae. By some regarded as parthenogenetic ova, by others as groups of cells asexually budded off.

Germ cell determinants. Cytoplasmic inclusions which are unequally distributed in cleavage but pass into the daughter cells of the germ line.

Germinal epithelium. That portion of the coelomic epithelium which covers the genital ridge and the developing gonads.

Germ layer. A primary formative layer of cells which by subsequent processes of development produces tissues and organs of the embryo.

Germ plasm. The hereditary material, the physical basis of inheritance, specifically localized in the chromatin.

Germ ring. The formative edge of the advancing blastodisc in eggs cleaving discoidally. The material of the embryonic region proper comes largely from this ring.

Germ track. Equivalent to the German Keimbahn. Those cells which are distinguished in the early cleavage stages, and from then on, from whose lineage will come the primordial germ cells.

Glochidium. Larva stage of bivalve molluscs; it commonly lives parasitically in the gills of fishes during part of its life cycle.

Gonangium. An individual without tentacles in a typical hydroid which buds off the medusae asexually.

Gonia. The cells resulting from the division of the primordial germ cells previous to the end of the growth period, spermatogonia for the male and oögonia for the female.

Gonocoele. That portion of the coelome in which the gonads are developed. It is derived from the ventral part of the myocoele.

Haploid. Having the reduced number of chromosomes as found in the matured gametes.

Hermaphrodite. An individual having organs of both sexes.

Hermaphroditic. The same as monoecious.

Heterochrony. Disturbances in the synchrony of development, or in the sequence of the events of ontogeny.

Heterogamy. Production of gametes of different kinds. Opposed to isogamy.

Heterogony. Alternation of generations in which hermaphroditic and bisexual generations are involved.

Heterometabola. Those groups of insects which have incomplete or direct metamorphosis. Included are Orthoptera, Dermaptera, Platyptera, Plecoptera, Ephemerida, Odonata, Thysanoptera, and Hemiptera.

Heterotypic. Having the first meiotic or reduction division as distinguished from the second or homotypic.

Hibernacula. The winter buds of the Bryozoa.

Holoblastic. Having the type of egg structure in which cleavage divides the entire egg.

Holometabola. The more specialized insects in which metamorphosis is complete or

indirect. Includes Coleoptera, Strepsiptera, Neuroptera, Mecoptera, Trichoptera, Lepidoptera, Diptera, Siphonaptera, and Hymenoptera.

Homeotypic. Having the second meiotic or maturation division in which the behavior of the chromosomes is not greatly different from that of somatic mitosis as contrasted with the case in the heterotypic division.

Homolecithal eggs. Equivalent to isolecithal and alecithal eggs; eggs in which the meager amount of yolk present is uniformly distributed.

Homology. The fundamental structural similarity between organs or organisms based upon a common embryological origin.

Hydra-tuba. *See* Scyphistoma.

Hydrocoele. That portion of the coelomic system of an echinoderm from which the chief parts of the water vascular system of the adults arise.

Hypoblast. *See* Endoderm.

Implantation. The process by which the mammalian blastocyst becomes attached to the uterine wall.

Instar. A term used to designate an insect at any particular stage between molts, as: first instar, the insect at time of hatching; second instar, the insect after the first molt.

Interkinesis. The "resting" period intervening between two successive mitoses. The term is now commonly applied to all vegetative nuclei.

Invagination. The unfolding process by which, for example in gastrulation, the primary endoderm is withdrawn into the blastula cavity and becomes enclosed by the primary ectoderm.

Inversion of germ layers. The formation in mammalian embryos due largely to the entypy of the germ of a false amniotic cavity lined with ectoderm and covered by mesoderm. No inversion in a true sense occurs, but a misleading appearance is responsible for the term.

Involution. Technically the rolling in or turning over of cells as over the blastodermal rim into the segmentation cavity. Commonly secondary to epiboly.

Isolecithal. Equivalent to alecithal or homolecithal, indicating in holoblastic eggs the uniform distribution of the yolk granules.

Karyokinesis. Cell division of the indirect type involving primary nuclear activity and the formation of spindle, spiremes, chromosomes, etc. The same as mitosis.

Laceration. Fragmentation due to external unfavorable conditions. Subsequent regeneration normally follows.

Laeotropic. Left handed, or a cleavage plane of the spiral type turned counterclockwise.

Lamella. A thin plate of tissue often connecting adjacent structures.

Lamina. A thin plate, especially of bone or other hard tissue.

Larval stage. An immature but usually active stage in the development of an organism. Since it leads an independent existence a larva often possesses special temporary adaptive characters which may not be of later significance.

Lecithin. One of the lipoid substances commonly found in the egg yolk.

Macromere. One of the larger blastomeres in eggs with unequal cleavage. Also written megamere. Opposed to micromere.

Maturation. The process by which the germ cells or gametes are prepared for fertiliza-

tion. During maturation the reduction of the chromosome number to one-half usually takes place.

Megalecithal egg. An egg of the extreme meroblastic type in which there is a large amount of yolk.

Megalops. The larval stage which follows the zoaea in most crabs.

Megamere. *See* Macromere.

Meroblastic. Having the type of egg structure in which cleavage is only partial owing to the accumulation of yolk in the egg.

Mesenchyme. That part of the mesoderm which is not developed as a surface covering, the mesothelium, or derived from it. Gives rise to connective tissue, blood, bone, etc.

Mesoblast. *See* Mesoderm.

Mesoderm (mesoblast). The middle germ layer or the formative tissue composing it. Also the derivatives in later development of this layer.

Mesoglea. A non-cellular layer of gelatinous consistency between the ectoderm and endoderm of sponges and coelenterates.

Mesomeres. A blastomere of size intermediate between macromeres and micromeres.

Mesoplasm. One of the organ-forming substances, for example in *Cynthia*, where it is formed just below the equator from crescentic material.

Mesothelium. The mesoderm lining the primitive coelome; the epithelium of mesodermal origin.

Metagenesis. Alternation of a sexual and an asexual generation.

Metamerism. The state of being made up of metameres or similar segments (somites) arranged serially.

Metamorphosis. The changes accompanying the transformation of a larva into an adult.

Metanauplius. The larval stage of crustaceans which commonly follows the nauplius, having usually seven pair of appendages.

Metaphase. The middle stage of mitosis during which the chromosomes are arranged about the center of the spindle and are ready for separation.

Metaplasm. Inclusions within the cytoplasm such as yolk, pigment, starch, etc., which are the products of cytoplasmic activity.

Metazoaea. The larva of the decapod crustacea which intervenes between the zoaea and the mysis stages. It shows the first rudiments of the abdominal appendages.

Microlecithal eggs. Eggs containing only a small amount of yolk.

Micromeres. The smaller blastomeres of an egg with unequal cleavage.

Micropyle. The minute opening through the membranes of many eggs through which the sperm enters.

Miracidium. The earliest larval form of a liver-fluke.

Mitosis. Indirect cell division, karyokinesis; opposed to amitosis. It involves the formation of chromatin spiremes which split longitudinally, their conversion into chromosomes, and their separation by means of the spindle apparatus.

Mitraria. An aberrant annelid larva of the trochophore type.

Monoeceous. Having the organs of the two sexes in one individual.

Morula. A type of blastula characterized by the absence of a segmentation cavity. It differs from the stereoblastula in that in it the inner cells do not reach the surface. Found chiefly in coelenterates.

Müller's larva. The larva of many polyclads, having an oval body and eight ciliated processes.

Mysis. The larval stage of certain decapod crustaceans corresponding to the genus *Mysis* in the possession of exopods upon the walking legs.

Nauplius. Primary type of crustacean larva.
Neoteny. The retention of embryonic characters throughout life.
Nephrocoele, Nephrostome. The derivation of the primitive coelome and its cavity from which the nephridia are developed.
Nucleolus. The conspicuous, rather large, round body found during the resting stage of most nuclei, having various functions, but primarily concerned with cell metabolism.
Nucleus. The principal organ of a cell, serving to regulate growth, metabolism and reproduction.

Obligatory. Said of parthenogenesis when it occurs regularly in the life cycle of an organism.
Oecium. An individual among the Byrozoa in which the development of the young embryo takes place, and which is specialized for the purpose. Also called ovicell.
Ontogeny. The entire life cycle of an individual organism, especially the developmental portion of it.
Oöcyte. *See* Cyte.
Oögenesis. The gametogenesis in the female line.
Oögonia. *See* Gonia.
Oösome. The cytoplasmic body developed in the mature ovum of certain forms which, passing into the stem cell, serves as a germ-cell determinant.
Oötid. Mature egg.
Oözooid. In *Salpa* the first zooid produced from the egg; from its stolon is budded off a long series of other zooids that remain in connection with it for a considerable time.
Ophiopluteus. The pluteus larva of the Ophiuroidea.
Optional parthenogenesis. The same as facultative parthenogenesis.
Organ-forming substances. Differentiated materials localized in the eggs of determinative type whose fate as shown by the study of cell lineage is in the formation of definite organs of the embryo.
Organogenesis. The origin and development of the organs and organ systems in the embryo.
Organogeny. *See* Organogenesis.
Ovicel. The same as oecium.
Oviparous. Egg laying.
Ovoviviparous. Retaining the eggs within the oviduct until hatching. The young, however, are not nourished from the body of the mother nor attached to the oviduct.
Ovum. The female gamete ready for fertilization.

Paedogenesis. The production of gametes during embryonic or larval life.
Palingenesis. The reproduction unchanged of old or ancestral characters. Palingenetic characters are those having to do with old or phylogenetic structures. Opposed to cenogenesis.
Paranucleus. The nucleus of the trophamnion developed in the egg of parasitic Hymenoptera, derived from the nuclei of the polar bodies.
Paratomy. Asexual reproduction in which a special zone of division is prepared in

advance of the actual division. More or less regeneration of the new organs precedes fission. Contrasted with architomy.

Parenchyma. Soft cellular tissue, relatively undifferentiated, found in the lower invertebrates.

Parenchymella. Equivalent to amphiblastula, the swimming larva of the sponge.

Parthenogenesis. Development of eggs without fertilization.

Partial cleavage. Incomplete cleavage of extreme telolecithal or meroblastic eggs.

Pentacrinoid. A larva of the crinoids such as *Antedon*. So called from its resemblance to the genus *Pentacrinus*.

Periblast. The cytoplasmic layer with nuclei which surrounds the formative cells of a blastodisc. It aids in digesting yolk and the nuclei often make their way into the yolk.

Perivitelline. About the ovum, as the perivitelline fluid which fills in the space of the same name between the surface of the frog's egg and its jelly.

Phorozooid. A zooid of the free-swimming tunicates which although of the sexual generation does not mature sexually.

Phototropism. The response of an organism either by growth or locomotion to light rays. Phototaxis.

Phyllosoma. The larva of certain decapod crustaceans, as *Palinurus*.

Phylogeny. Racial history of a group of organisms.

Pilidium. The chief type of larva of the heteronemerteans.

Placenta. In higher mammals the embryonic connection between the mother and the young. Furnishes nourishment to the foetus.

Placentation. The formation of a placenta and its attachment to the walls of the uterus.

Placula. A type of blastula in which the animal and vegetative halves are somewhat compressed toward each other.

Planula. The hollow single-layered blastula which is the larval form of many coelenterates.

Pluteus. The free-swimming larva of sea-urchins and ophiuroids.

Polar bodies. *See* Polocytes.

Polar disc. An accumulation of cytoplasmic inclusions at the posterior end of certain arthropod eggs. These inclusions make up the pole plasm which functions as germ-cell determinants.

Polar furrow. The interrupted and bent cross plane at the poles of eggs with spiral cleavage in the 4-cell stage; due to the fact that only two blastomeres meet at either pole instead of all four as in a similar stage of a radially cleaving egg.

Pole plasm. *See* Polar disc.

Polocytes (polar bodies). Two very small cells segmented off from the ovum during naturation. The morphological equivalent to oöcytes but functionless.

Polyembryony. In zoology the development of two or more embryos from one egg. In plants the development of two or more embryos within one embryo sac, whether or not from one zygote.

Polygerm. The masses of embryonic nuclei and surrounding cytoplasm which follow the stage of the "parasitic body" in polyembryonic Hymenoptera and which eventually give rise to the definitive embryos.

Polymorphism. The capacity of an organism to exist in several forms.

Preformation. Opposed to epigenesis. The doctrine that the germ cell contains a young organism in miniature.

Primary ectoderm. The outer germ layer of the gastrula stage.

Primary endoderm. The inner germ layer of the gastrula stage.

Primordial germ cells. The cells set apart during the early development of the embryo from which the definitive germ cells of the future organism are to be derived.

Proctodaeum. The posterior portion of the developing alimentary tract which is invaginated from the outer body wall and hence is lined with ectoderm.

Progenesis. Retention by somatic structures of condition reached by them at the time of sexual maturity in neotenous animals.

Promorphology. The study of the early organization of the egg, particularly with reference to the significance of this organization in the production of later-appearing organs.

Pronucleus. One of the two gametic nuclei within the egg after sperm entrance but before cleavage.

Prophase. The preparatory stages of mitosis during which the formation occurs of spindle, spireme, and chromosomes.

Prospective potency. The total sum of developmental possibilities of which an organ is capable. Driesch's term.

Prospective significance. The actual destiny of a cell or organ in the development processes, regardless of the potentialities. Driesch's term.

Prostomia. A group of phyla in which ectomesoderm is developed at some stage, namely coelenterates, flatworms, rotifers, annelids, molluscs, Molluscoidea.

Protoplasm. The active living substance of the cell, consisting of nucleoplasm and cytoplasm.

Protozoaea. The larva of higher crustaceans which intervenes between the nauplius and the zoaea.

Pseudozoaea. A larval stage in the development of the Stomatopoda.

Pupa. The intermediate quiescent form of metabolic insects following the active larval period.

Quadrant. A fourth part of an embryo with spiral cleavage; all of the embryo which is derived from one of the blastomeres of the four-cell stage.

Radial. Cleavage in which the plane of division may pass through the two poles of the egg in any radius.

Rathke's yolk pyramids. In crustacean eggs having superficial cleavage the inner ends of the blastomeres contain chiefly yolk and fuse in the center of the embryo. These inner ends are spoken of as Rathke's yolk pyramids.

Rauber's layer. The thin layer of trophoblast over the surface of the ectoderm of the embryonic region.

Recapitulation. Repetition in development of an individual organism of its phylogenetic history.

Redia (named after F. Redi). The second larval form of a liver-fluke.

Reduction. The decrease of the chromosome number to the haploid from the diploid in the two maturation mitoses.

Regeneration. The renewal or replacing of lost parts by an organism.

Regulation. The adjustment and readaptation of an organism to changes and accidental modifications of its surroundings or structure.

Sachs law (Sachs-Hertwig laws of cleavage). (1) The nucleus of a blastomere tends to be in the center of the protoplasmic mass. (2) The axis of the mitotic spindle tends to be in the longest axis of the protoplasmic mass.

Scyphistoma (Hydra-tuba). The hydroid generation of the scypho-medusa which subsequently elongates and constricts to form ephyra.

Scyphula. A scyphistoma.

Segmentation. Cleavage of the egg by cell division.

Segmentation cavity. The blastocoele or the cavity of the blastula.

Sexual reproduction. Reproduction involving the union of germ cells, gametes, of two individuals, and thus implying the steps necessary for gametogenesis.

Somatic. Relating to the body or soma of organisms, especially as contrasted with the germ plasm.

Somatic layers. *See* Somatopleure.

Somatopleure. The ectoderm and the outer or parietal layer of mesoderm, the body wall. Contrasted with splanchnopleure.

"Sorite." Same as archeocyte.

Spawn. The egg masses of many aquatic animals as fishes, shell fish, etc., in which many small eggs are contained.

Spermatid. Matured male germ cell which has not undergone metamorphosis into a spermatozoon.

Spermatocyte. *See* Cyte.

Spermatogenesis. The gametogenesis in the male line.

Spermatogonia. *See* Gonia.

Spermatozoon. The male gamete ready for fertilization.

Spindle. The achromatic structure of fibers formed during mitosis upon which the chromosomes arrange themselves.

Spiral cleavage. That type of cleavage in which the direction of the spindle axis is shifted obliquely from the vertical direction with regard to the egg axis. In an eight-cell stage the upper quartette of cells usually appears turned in a right-handed spiral to the lower.

Splanchnic layers. *See* Splanchnopleure.

Splanchnopleure. The visceral layer of the mesoderm and the endoderm taken together. Contrasted with the somatopleure.

Sporocyst. In trematodes the sac-like larval form from whose interior cells are budded off to form the rediae.

Stadia. The intervals between molts or ecdyses in the development of an insect.

Statoblasts. Internal masses of cells which are morphologically buds from which new individuals develop. Occur as gemmules in sponges, germ balls in trematodes, etc.

Stem cell. The cell from which the germ cells of the later organisms are to come.

Stem mother. In a strain of animal reproducing parthenogenetically the stem mother is the product of a fertilized egg but she produces parthenogenetic eggs usually in large numbers.

Stereoblastula. A blastula in which all the cells are in close contact and no blastocoele is formed, as in *Nereis*.

Stereogastrula. A gastrula formed without a cavity by epiboly as in many cases of spirally cleaving eggs.

Stolon. A "runner" or stalk produced by budding of the parent individual. From it new individuals are commonly budded off.

Stomodaeum. The oral part of the developing alimentary tract.

Strobila. The primary larval form of certain Scyphozoa during which the ephyrae are formed as a consequence of the elongation and constriction of the primary larva.

Superficial cleavage. The type of cleavage characteristic of centrolecithal eggs in which after a few preliminary divisions in the center of the egg the nucleated proto-

plasmic areas, the so-called blastomeres, move to the surface and gradually form a superficial layer of cells, the blastoderm.

Synapsis. The pairing of homologous maternal and paternal chromosomes in the maturing germ cell prior to reduction.

Syncytium. An undivided mass of protoplasm containing several nuclei.

Tadpole. The larval form of higher amphibians and of ascidians.

Teloblastic band. A band of cells originally derived from two teloblasts, and producing later the mesoblastic somites.

Telolecithal eggs. Eggs in which the yolk is mainly accumulated in the vegetal hemisphere of the cell which is thus distinguished from the protoplasmic hemisphere.

Telophase. The final stages in a mitotic division involving the reconstruction of the daughter nuclei and the division of the cytoplasmic portion of the cell.

Tentacle plane. In a ctenophore the plane passing through the two tentacles.

Tetrad. A chromosome consisting of four parts owing to the synapsis of the two homologous members of a pair.

Thelytokous eggs. Parthenogenetically developing eggs which are exclusively female producing.

Thysanuriform. The same as campodeiform.

Tornaria. The larval form of those races of the genus *Balanoglossus* which undergo metamorphosis.

Total cleavage. The segmentation of the entire egg including the yolk-filled portion.

Totipotency. The theory that individual blastomeres when isolated are each capable of producing a complete organism.

Träger. The portion of the trophoblast of the mammalian blastocyst which lies directly above the inner cell mass. Within it the false amniotic cavity often develops. Also known as trophoblastic knob.

Triploblastic. Of three germ layers, ectoderm, endoderm, and mesoderm.

Trochophore. A ciliated swimming larva characteristic of flatworms, annelids, molluscs, and some other groups. A trochosphere.

Trochosphere. Same as trochophore.

Trochozooid. One of the lateral zooids budded off of the stolon of the tunicate *Doliolum* which serves to nourish the colony.

Trophamnion. The nutritive layer of cytoplasm and paranuclei in polyembryonic Hymenoptera.

Trophoblast. The portion of the outer ectodermal layer of a mammalian blastocyst which is in contrast to the formative cell of the ectoderm. It contributes no part to the development of the embryo proper, but functions in the attachment of the embryo to the wall of the uterus.

Trophoblastic knob. *See* Träger.

Vegetal pole. The pole opposite the animal pole in the center of the yolk-laden hemisphere of the egg.

Veliger larva. The larva of many gasteropods.

Vitelline membrane. The egg membrane proper; homologous with cell wall of other cells. Often in invertebrates it is not given off until fertilization. So called because in bird eggs it envelopes the yolk mass.

Vitellophages. Cleavage nuclei and their adjacent cytoplasmic areas which are in contact with the yolk of meroblastic eggs and do not take part in the formation of the blastoderm, but function in the assimilation of yolk.

Viviparous. Producing young from eggs which are hatched in the uterus and nourished there.

Yolk lobe or polar lobe. A lower sphere formed in the cleavage of *Dentalium* containing cytoplasm and a certain amount of yolk which is not itself cleaved but is retracted into one of the blastomeres before the next division.

Yolk pyramids. In eggs with superficial cleavage the inner ends of the blastomeres which are yolk-filled and often fused at the center.

Zoaea. An early larva of the higher Crustacea. It precedes the megalops and is characterized by a large cephalothorax bearing spines, large eyes, and antennae and mouth parts adapted for swimming.

Zoanthella. A larval form of the actinian family Zoanthidae.

Zoanthina. A larval form of the actinian family Cereanthidae.

Zooecia. The individual calcified branches of a colony of bryozoans within which is the polypide or soft parts of the animal.

Zygote. Fertilized egg, or embryo after fertilization.

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