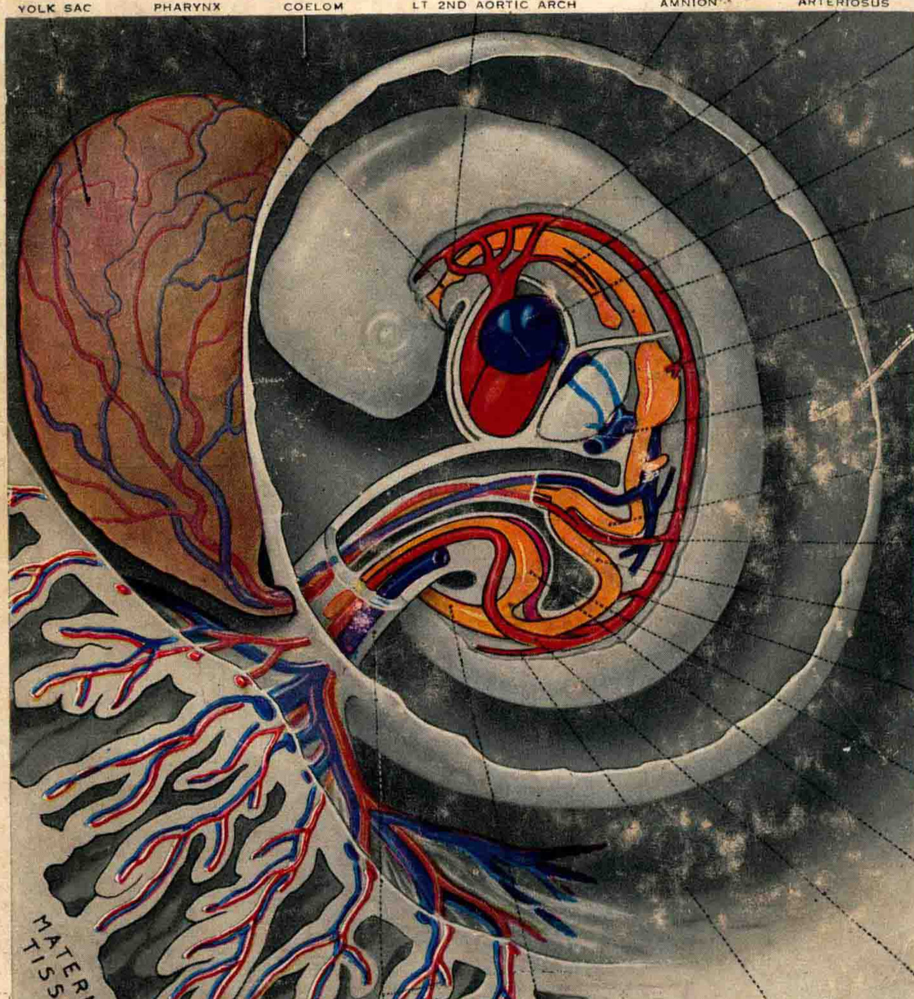
HUMAN EMBRYOLOGY

(Prenatal Development of Form and Function)

W. J. HAMILTON, M.D., D.Sc., F.R.C.O.G., F.R.S.E.
Professor of Anatomy in the University of London at Charing Cross Hospital Medical School.

J. D. BOYD, M.A., M.D., D.Sc.
Professor of Anatomy in the University of Cambridge and Fellow of Clare College, Cambridge.

H. W. MOSSMAN, M.S., Ph.D.
Professor of Anatomy in the University of Wisconsin



HEFFER, CAMBRIDGE

HUMAN EMBRYOLOGY

(PRENATAL DEVELOPMENT OF FORM AND FUNCTION)

by

W. J. HAMILTON, M.D., D.Sc., F.R.C.O.G., F.R.S.E.
Professor of Anatomy in the University of London at Charing Cross Hospital Medical School.

J. D. BOYD, M.A., M.D., D.Sc.
Professor of Anatomy in the University of Cambridge and Fellow of Clare College, Cambridge.

and

H. W. MOSSMAN, M.S., Ph.D.
Professor of Anatomy in the University of Wisconsin.

Illustrations *by* A. K. Maxwell, Esq.

"*And surely we are all out of the computation of our age, and every man is some months elder than he bethinks him; for we live, move, have a being, and are subject to the actions of the elements, and the malice of diseases, in that other World, the truest Microcosm, the Womb of our Mother.*"

Sir Thomas Browne's *Religio Medici*, 1642

CAMBRIDGE
W. HEFFER & SONS LIMITED

First Published	- - - - - - -	1945
Reprinted	- - - - - - - - -	1946
Reprinted	- - - - - - - - -	1947
Second Edition	- - - - - - -	1952
Reprinted	- - - - - - - - -	1956
Reprinted	- - - - - - - - -	1957
Third Edition	- - - - - - -	1962

Printed in Great Britain at the Works of

W. HEFFER AND SONS LTD., CAMBRIDGE, ENGLAND

To the memory of

THOMAS HASTIE BRYCE

and

GEORGE LINIUS STREETER

to whom the first edition of this work was

dedicated

FROM THE PREFACE TO THE FIRST EDITION

This book is a presentation of the subject of human embryology in the light of the advances which have been made in it during the past twenty years. An attempt has been made to fit the development history of the embryo into the background of the physiological changes in the maternal organism and to correlate the development of embryonic function with that of its form. We have also tried to introduce the concepts of development which have been established by the work of experimental embryologists (Chapters I and VIII). These concepts are of great theoretical, and, we believe, practical importance but, unfortunately, they do not readily lend themselves to elementary exposition. Further, as they are based almost exclusively on the results of experiments on animals below the mammals it is extremely difficult to present them without assuming a knowledge of comparative Embryology. A brief survey of comparative vertebrate development is, therefore, given in the last chapter, and if the reader finds difficulty with Chapter VIII, it is recommended that he studies Chapter XVI before proceeding with the attempt at mastering the concepts of determination and the organizer. Special attention is paid in Chapters V and XVI to placentation and the embryonic membranes, as a knowledge of these aspects of embryology is of special importance in the study of the problems of embryonic and foetal nutrition and of the prenatal relationship between the mother and the child.

References to embryological literature are full, not, however, with the intention that the student shall consult all, or even more than a very few of them. We feel that direct reference to the original literature is a habit that the student should acquire early and that an extensive bibliography gives an opportunity to track down original observations on any aspect of a biological problem which may particularly interest the reader. For this reason titles of papers have been deliberately given in full even at the expense of lengthening the text. It is felt that the bibliographies may also be of service to more senior students.

A sound knowledge of embryology cannot be obtained solely from a textbook. We recommend students, therefore, to obtain access to serial sections through mammalian or, if possible, human embryos and to study them carefully. Many difficulties in organogenesis can quickly be resolved by reference to such series and the drawing of a number of representative sections provides a most excellent discipline for acquiring a knowledge of the basic structure of the mammalian body.

London
October, 1944.

W. J. H.
J. D. B.

PREFACE TO THE THIRD EDITION

For this edition the whole text has been extensively revised and many chapters have been recast and completely rewritten. In Chapter I an account of cell division has been included, and in Chapter II the description of chromosome behaviour during maturation of the ovum and sperm has been brought into line with recent investigations. Chapter IV is now based almost entirely on human material. In view of the advances made since the last edition it has been found necessary to recast the Chapter on the development of the human placenta. Many modifications have also been necessary in the Chapter dealing with abnormal development. Another Chapter that has called for extensive modification is that on the urogenital system; and more especially that part of it which is concerned with differentiation of sex. The Chapter on comparative embryology has been extensively revised and, it is hoped, clarified.

Unfortunately, as a consequence of the necessary inclusion of new information, there has been some increase in the size of the book. Much of this increase has been due to the addition of seventy-seven new illustrations; many other figures have been redrawn.

W. J. H.
J. D. B.

March, 1962.

ACKNOWLEDGMENTS

WE wish to acknowledge our indebtedness to the Carnegie Institution of Washington for the valuable help we have received from the numerous publications on embryology by members of the Staff and others associated with them. We would thank especially Dr G. W. Corner for original photographs of human embryos at different stages of development. To Dr A. T. Hertig and Dr J. Rock we are also indebted for photographs of early human embryos.

We wish to thank the following for permission to reproduce illustrations:—(*a*) The Wistar Institute of Anatomy and Biology for illustrations from the *American Journal of Anatomy*. (*b*) *The American Journal of Obstetrics and Gynecology* for Fig. 29. (*c*) *Surgery, Gynecology and Obstetrics* for Fig. 24A. We also wish to express our indebtedness to the *Journal of Anatomy* for permission to reproduce a number of illustrations from the paper by two of us (W.J.H. and J.D.B.) on the human placenta.

In the present edition we have to thank Professor A. Pomfret Kilner for permission to reproduce Fig. 209; Mr P. J. Blaxland, F.R.C.S., for permission to reproduce Fig. 335; Professor Ian Aird, F.R.C.S., for permission to reproduce the photograph of the Kano twins (Fig. 134); Mr W. P. Greening, F.R.C.S., for permission to reproduce Fig. 232; and Dr E. W. Dempsey for permission to reproduce the figures of the electron photomicrographs of the human placenta (Figs. 106 and 107). Acknowledgments for all these permissions are made in the appropriate legends.

All the new illustrations are from the skilled draughtsmanship of Mr A. K. Maxwell and we have to thank him for his skill and patience.

We record our indebtedness to Dr Jean R. W. Ross and to Dr T. W. Glenister for their help in reading the manuscript and proofs and for their constructive criticism. We wish to express our thanks to Mr E. J. Park, F.I.S.T., Senior Technician in the Anatomy Department, Charing Cross Hospital Medical School, for the care he has taken in "pasting up" the annotations of the new illustrations. He has also given us much help in the checking of the references and in proof reading; he has also prepared the index.

We have been fortunate in the cordial relationship which has existed between us and Messrs W. Heffer & Sons Ltd., the Printers and Publishers. Mr R. G. Heffer has always been willing to meet and satisfy our many demands for more illustrations. Finally, we wish to thank Mr H. G. Newman, works director of Messrs W. Heffer & Sons Ltd., for his patience, resourcefulness and enthusiasm.

CONTENTS

CHAPTER I

INTRODUCTORY CONCEPTS

REPRODUCTION

MEMBERS of all multicellular animal species (Metazoa) have a more or less limited life span. Consequently, if a species is to survive a mechanism must exist for the successive production of new generations of that species. This process is called reproduction. In most metazoan species, including the vertebrates, reproduction is effected by a complicated process involving the presence of two sexes, male and female, and the production by each of these sexes of specialized sex cells called *gametes*. The organs which produce the gametes are known as the *gonads*, or primary sex organs; those of the male are the *testes*, those of the female, the *ovaries*. The male gametes are named *spermatozoa*, the female gametes *ova*. The union of a spermatozoon with an ovum is called fertilization and results in the formation of a single cell, a *zygote*. The relationship between the members of one generation of an animal species and those of another is said to be an *hereditary* or a *genetic* one. It is obvious that a zygote has an hereditary relationship, by way of the gametes from the fusion of which it resulted, with a male and a female parental organism.

In addition to the primary sex organs each sex is usually characterized by the presence of *accessory sex organs* which transmit the gametes from the gonads. In the male the accessory sex organs may include an intromittent organ, the *penis*, which enables the sperms to be deposited in the female genital tract. In the female, the accessory sex organs frequently include a special receptacle for the sperms, the *vagina*, and a "brood chamber," the *uterus*, for the reception and incubation of the zygote. The male and female sexes are usually further distinguished by the presence of yet other differences, both physical and mental, such as mammary development, hair distribution and patterns of behaviour, not directly concerned with reproduction. These are known as secondary sexual characters.

DEVELOPMENT AND EMBRYOLOGY

The zygote which has been formed by the fusion of a male and female gamete is a single-celled organism. After a longer or shorter period this unicellular organism will become progressively transformed by the processes of cell division, cell migration, growth and differentiation into a multicellular mature member of its species. The term development is used to describe these progressive changes. After a period of maturity, of variable duration depending on the species, retrogressive changes leading to senility, and eventually death, occur.

Consequently, in the earlier stages of development structural changes occur and organs appear before the necessity or possibility for their functional activity. Thus they have a future or *prospective* rather than an actual or *immediate* value in the life of the developing organism. This can be expressed by saying that structure in the embryo is frequently *antecedent* to function. Although it is obvious that no definite limit in this total developmental process can be fixed as the end of a strictly embryonic period, still, in general, the processes discussed in this book include almost all of those antecedent to function and most others up to the establishment of the basic functional patterns characteristic of the human foetus during the last six months of prenatal life. In other words, embryology is usually concerned with an organism from the zygote stage up to the anatomical establishment of the definitive organ systems, and often into their early functional period. An exception to the latter would, of course, be the reproductive system which matures functionally relatively late.

The term *ontogeny* is used to describe the complete life period of an individual organism. *Embryology* is the study of the earlier stages of development and, in the true mammals, including Man, is restricted to the developmental processes occurring before birth, i.e., in the *prenatal* period of life. It must be stressed, however, that there are no essential differences between prenatal and *postnatal* development; the former is more rapid and results in more striking changes in the shape and proportions of the organism, but the basic mechanisms are very similar if not identical in both periods. Indeed in many lower organisms (see Child, 1941, for details) the ability to revert under certain conditions to the embryonic type of development is retained throughout life and the phenomena of repair and regeneration, which are present to some degree in the adults of even the highest animal types, present fundamental similarities to embryonic processes. Ageing in most metazoa, however, has a marked retarding effect on developmental reparative changes.

Developmental changes can be contrasted with the day by day non-progressive and, in general, much more rapid physiological changes, such as respiratory, circulatory, digestive and nervous activities, which are directly essential for the maintenance of life. When the earlier embryonic stages are considered this contrast is particularly striking, for developmental changes are then at a maximum and the organ systems of the body are not yet established to perform the physiological activities peculiar to them in later life. Such earlier stages may be referred to as the pre-functional period of development, the later stages constituting the functional period. It must be emphasized, however, that at all stages of development the embryo is a living organism capable of maintaining itself as such. As it grows and differentiates, new mechanisms are continually coming into activity so that the organism may cope with changing internal and external conditions, for embryos not only grow and differentiate but also live, and the requisite physiological functions must be exercised during these developmental alterations. If embryos are to maintain themselves, their structure must be such that for each developmental period an adequate physiological performance is assured. Equipped with this structural organization an embryo might live indefinitely at any particular stage if no changes in itself or in its environment rendered that level of organization inadequate. But changes in the embryo do occur as a function of time and as the requirements for existence are progressively modified. The new needs are met by the development of new devices which one after another are discarded or remodelled when the needs change and pass. "Thus one meets with a series of increasingly complex ephemeral organs and structural arrangements characterizing the periods of development that space off the anabasis of the embryo from the microscopic one-celled egg up to the large, highly specialized fetus of later stages" (Streeter, 1942).

SUBDIVISIONS OF EMBRYOLOGY

In its evolution as a science embryology has passed through several stages.* It was at first, and for the greater length of its history, purely *descriptive*, but as detailed knowledge of the development of related types became established a science of *comparative* embryology arose. This, in turn, was succeeded by the attempt to introduce an analytical embryology based on experimental methods. This *experimental* embryology, which was first properly established by Roux (1888) as *Entwicklungsmechanik* (developmental mechanics), has widened the scope of the science, so that now the investigation of the causal mechanisms of development has been added to the descriptive and comparative approaches. Observational embryology can merely record the sequence of developmental events, only experiment can acquaint us with the forces involved and their possible modes of action. During the past fifty years the experimental approach, using lower forms, has resulted in the elucidation of many fundamental problems of development but, unfortunately, the possibilities of using similar experimental procedures on embryos of the higher, and particularly viviparous, types are still very restricted and, of course, in Man are non-existent.

Human embryology is, strictly speaking, still in the descriptive stage. However, the application of the concepts of comparative and experimental embryology has added greatly to the rational interpretation of the processes of human development.

* For the history of embryology consult Russell (1916, 1930); Nordenskiöld (1929); Cole (1930); Needham (1959); Meyer (1936, 1939, 1956); and Adelmann (1942).

VALUE OF EMBRYOLOGY

The value of the study of embryology to the medical student is fivefold :—

(1) From the general biological aspect it gives an understanding of how the different organs and tissues develop from a single cell (the fertilized ovum) into a complex multicellular organism. As the study of the development of structure is linked with that of function, embryology provides a basis for the understanding of the functional activity of the organism during development. Further an appreciation of the causal factors underlying development, about which we have only the tentative beginnings of knowledge, is only possible after normal development has been considered. It must be realized that the study of development in a single complex species (e.g., as in this case, Man) will not always be easily understood. Indeed, in order to obtain a clear understanding and appreciation of developmental processes in Man, it is often necessary to refer to sub-human types since in Man some sequences of development have become shortened to such an extent that the basic primitive steps in the process are difficult to discern, or because sufficient stages of human material are not available for study. This is particularly the case in the early stages of development. Experiments which can be carried out in lower types can not, of course, be performed on the human subject so that the argument from analogy must often be used.

(2) From the vocational aspect the study of development, in a great many instances, gives a rational explanation of the relationships and position of many normal adult structures, e.g., the nerve supply of the diaphragm by cervical nerves, the asymmetry of the veins in the abdominal and thoracic cavities, the nerve supply to the tongue.

(3) Embryology includes not only the development of the embryo but also the development of the membranes which connect the foetus to the mother, i.e., placentation in viviparous vertebrates. A knowledge of the development, relations and properties of these membranes is essential in order to understand obstetrics and as a basis for advances in this subject. Such a knowledge is also obviously necessary for the understanding of the physiological relationship between the foetus and the mother.

(4) Many pathological conditions can only be understood in the light of normal and abnormal development. Until recently the relationship of embryology to medicine was mainly of a theoretical nature. Modern experimental work on the development of lower animal types has thrown light on such problems as growth and regeneration of tissue and the formation of certain tumours. These aspects of embryology are making profound contributions to conceptions of growth and reparative processes and also to the understanding of many pathological phenomena. With the rapid decline in infantile mortality resulting from better environmental conditions, the exploitation of preventive medicine, and the introduction of powerful bacteriocidal substances, the relative percentage of disease and death due to congenital defects has increased. The increase has, indeed, become a pressing human problem. A knowledge of the processes of development is essential for the understanding of such defects and for the study of their causes and possible elimination.

(5) As the student continues his studies through the basic medical sciences and into the clinical subjects embryology will be appreciated more and more as a great correlator of other morphological disciplines such as anatomy, pathology, physical diagnosis and surgery, and even of many physiological aspects of medicine.

DEVELOPMENTAL ADAPTATIONS

Developmental changes occurring in one group of animals do not necessarily occur in other groups. As is described later (Chapter XVI) the ova (eggs) of different groups do not all contain an equivalent amount of stored nutritive material (yolk or *deutoplasm*) and this is correlated with adaptive changes in the method of development. When the amount of yolk is large, as in birds, the embryonic period of development is long enough to allow adult-like characters to appear before hatching. When the amount of yolk is less, as in Amphibia, the young are hatched as larvae (i.e., tadpoles) which lead an active life and derive nutriment from their external environment before assuming, by a process of metamorphosis, the adult form. Again, eggs of many species are shed into water and undergo their development in an aqueous medium, whereas in other types the eggs are laid on dry land, possibly in desert regions, with no access to water other than that with which they are originally endowed by the maternal organism. The latter variety of eggs show adaptations which tend to preserve the scanty water reserves. These include the development of special enclosing shells or membranes and changes in embryonic metabolism such as the excretion of the end products of nitrogenous metabolism as the relatively insoluble uric acid (uricotelic metabolism) and a high resistance to ketosis (Needham, 1942). Yet again, as in the group of true mammals, the egg, with practically no reserves of yolk or of water, is fertilized by the sperm inside the maternal body, and a part of the maternal genital tract, the uterus, becomes modified for the retention and nutrition of the developing individual. This arrangement whereby the eggs develop within the uterus long after their meagre yolk supply is exhausted results in the birth of offspring which are advanced in their development and have been dependent on the maternal organism for a long period. It is called *viviparity* to distinguish it from the condition in which eggs are laid (*oviparity*) and most of the development occurs outside the maternal body. Transitional stages are said to be *ovo-viviparous*. In viviparity a specialized apparatus, the *placenta*, is elaborated by both embryonic and maternal tissues to serve as a mechanism for the transfer of food and oxygen from the mother to the embryo and of the waste products of metabolism from the embryo to the mother. As Man is a mammal this placental mechanism is present during human development, and is described in Chapter V. Many other examples of developmenatl adaptation to special environmental conditions are known (see Bonner, 1952, 1958; Willier *et al.*, 1955; Waddington, 1956; Beatty, 1957) and some are referred to in Chapter XVI.

HEREDITY AND ENVIRONMENT IN DEVELOPMENT

What an organism becomes in the course of development is the resultant of two factors, its heredity and its environment. Heredity acts by way of *internal* factors present in the fertilized egg itself. The modern science of genetics has demonstrated that many, if not all, of the internal factors are present in the nuclei of the gametes. They are the so-called *genes* which are probably complicated protein molecules situated in the chromosomes. As the genes of a zygote are derived from both maternal and paternal gametes the characters of the developing organism are derived from both mother and father.* It is now well established that the special characteristics of an organism (e.g., hair type, eye and skin colour, etc.) are due to its nuclear genic equipment. The more general characteristics (e.g., those enabling us to classify it as a man or a chimpanzee, as a primate or a carnivore, as a mammal or a reptile) are controlled by factors which are not yet understood. It is suspected, however, that in addition to the nuclear genes the general cytoplasm of the egg may have some influence in the establishment of the specific, generic and class characteristics of an organism (Needham, 1942; and Harvey, 1942).

Environment acts on the development of the egg as a whole by way of *external* factors not present in the egg itself. Such factors are gravity, temperature, light, chemical agents and nutritive substances. There is, however, an interaction within the developing organism of the various genes, chemical substances, and products from the different tissues and organs upon one another. This is often spoken of as the internal environment. All of this internal environment is, however, in the last analysis a part of heredity, in contrast to external environment which is what is ordinarily meant when the problems of heredity and environment are discussed.

* Owing to the nature of the hereditary mechanism, however, blending of particular characters of mother and father does not usually occur. On this point, and on genetics generally, the student should consult a textbook of genetics, e.g., Begg, 1959; Dobzhansky, 1955; Gruneberg, 1947; Ford, 1942; Castle, 1940; and Waddington, 1939.

Heredity and environment are often brought into a false antithesis, since it is assumed that they act in opposition to each other. The characters of an adult organism, however, are produced by the interaction between the genetic factors and the environmental ones. An alteration in either of these components may lead to variation, or if excessive, to abnormal development or even death. Genetic variation results from changes in the genetic constitution due to mutation or, more frequently, to changes in chromosomal or genic pattern (recombination). Environmental variation results from changes in the environment in which the genes operate. The actual organism produced by the normal interaction between heredity and environment is called a *phenotype*, an organism judged by its genetic constitution alone is a *genotype*. Phenotypically similar organisms may be genotypically different. Thus the genetically hybrid children of a pure dark brown-eyed parent and a pure blue-eyed one although possessing both blue-eye and dark brown-eye genes, all actually have eyes as dark as their dark-eyed parent. In other words, their phenotype is "dark-eyed," but their genotype is a hybrid between dark brown-eyed and blue-eyed. In genetical terms one parent is *homozygous* for dark brown eyes the other homozygous for blue eyes and the children are *heterozygous*, possessing genes for both dark brown and blue eyes. On the other hand alterations in the environment may result in the simulation of characters associated with one genotype in an organism possessing quite a different genotype. Thus, genotypically dissimilar organisms can be made to be phenotypically similar ("phenocopies").

Owing to intra-uterine gestation the environment of the developing mammalian embryo is tolerably constant and more or less optimal. Environmental variation in this vertebrate class, therefore, is difficult to study in the prenatal period. It is well established, however, that environmental differences of quite subtle kinds (e.g., number of embryos in uterus, age of mother, number of previous pregnancies and certain virus diseases such as *rubella* in the mother) do influence the course of development (Chapter VIII). Maternal hormones, too, may have some effect (Chapter V).

The experiments of Walton and Hammond (1938) have demonstrated very clearly the effect of environment on mammalian development. These workers, by artificial insemination, produced reciprocal crosses of the Shetland pony and the Shire horse. At birth the cross-bred foal from the large (Shire) mother and the small (Shetland) stallion is three times as large as that from the small (Shetland) mother and the large (Shire) stallion. As the cells of both foals have similar chromosomal contents, and presumably similar genes, the size differences must be due to the environment provided by the mother. How the size is controlled to suit the size of the maternal organism has not been determined. It may be limited by the amount of nutrition provided by the maternal circulation, by the maximum size of the uterus or by some unknown (maternal, placental or foetal) hormonal influence.

The occurrence in Man of like (*identical*) and unlike (*fraternal*) twins provides excellent material for assessing the importance of changes in environment on the subsequent history of individuals of identical or different genetic structure. The results of such assessment are considered in Chapter VIII.

The question whether environmental influences affect only the individual organism concerned or whether such influences have a *transindividual* action (i.e., the effects are carried over to the next generation) has been much debated. Modern biologists are almost unanimous in their opposition to the so-called "transmission of acquired characters" in the Lamarckian or neo-Lamarckian senses of the term (Huxley, 1942). All animal characters are acquired in the course of development by the interaction of the genetic equipment with the environment and, as has been stated earlier, variations in either of these sets of factors may result in alterations in the course of development. There is, however, no acceptable evidence that a character of the body of an organism arising in response to an environmental stimulus is able so to impress itself upon the genes that in subsequent generations the character will appear in the absence of the stimulus. The internal factors (genes) can be permanently changed as the result of the direct action upon them of irradiation, which is, of course, an environmental stimulus.

However, environmental stimuli, such as those of light, temperature and food, which act directly on the body tissues, but have no direct action on the genes within the germ cells, cannot produce hereditary modifications.

EPIGENESIS AND PREFORMATION

The major problem in embryology is the appearance, during development, of complexity of form and function where previously no such complexity existed. Historically two contrasting points of view have been held on this problem. One of these, the so-called theory of epigenesis, considered that during development there is actually the creation of new structures; whereas the other, the theory of preformation, maintained that a pre-existing diversity is already present in the fertilized egg (or in the sperm) and that future development consists merely in the unfolding and rendering visible of this innate diversity. The embryological investigations of the past hundred years have demonstrated that the actual processes of development are of an epigenetic nature, the fertilized egg, possessing a simple form and exhibiting an apparently undifferentiated structure, undergoes a series of developmental changes which result in the spatial differentiation of the mature organism with its specialized types of cells, tissues and organs. Modern genetics, however, has shown that the genes located in the chromosomes of the nucleus of the zygote carry the necessary information enabling normal development to occur. The hereditary constitution is, therefore, determined by the panoply of genes in the chromosomes and, in this sense, ontogeny is essentially a gradual revelation of the plan stored in the *genome* (the collective term for all the genes). Hence current opinion is rigorously preformationist on the hereditary constitution of organisms. Exactly how and when the different genes play their part in the developmental processes is not yet established.

FUNDAMENTAL PROCESSES IN DEVELOPMENT

Development includes the formation of germ cells, the process of fertilization, cleavage, and the further growth and differentiation of the organism to maturity, but with particular emphasis on the period before birth. Three fundamental processes are involved in development; these are *growth*, *differentiation* and *metabolism*. Growth is increase in spatial dimensions and in weight; it may be *multiplicative* (increase by mitotic activity in number of nuclei and (or) cells),* *auxetic* or *intussusceptive* (increase in the size of cells) or *accretionary* (increase in the amount of non-living structural matter). Differentiation is increase in complexity and organization. This increase may be in the number of varieties of cells and may not at first be apparent ("invisible" differentiation, e.g., determination of fates, segregation of potencies, loss of competence, etc., see Chapter VIII), but, when apparent ("visible" or "manifest" differentiation), constitutes *histogenesis*. Differentiation may be manifested as an increase in morphological heterogeneity resulting in the assumption of form and pattern and in the appearance of recognizable organs or organ primordia (*organogenesis*). Metabolism includes the chemical changes in the developing organism.

In the normal development of an embryo these fundamental ontogenetic processes are all closely interlinked, constituting an integrated system. "They fit in with each other in such a way that the final product comes into being by means of a precise co-operation of reactions and events" (Needham, 1942).

Cell division is an essential part of most of the developmental processes. Growth is almost always reducible to enlargement and division of individual cells; differentiation is likewise largely a matter of changes in the nature of the individual cells resulting from growth and division; cleavage is primarily cell division; germ cell formation is a coupling of a highly specialized type of division with a special differentiation; and fertilization is in a sense the opposite of

---

* Increase in number of nuclei and number of cells is not necessarily growth in the sense of size expansion. Cell division, for example, occurs without size expansion in the earlier stages of cleavage (Chapter IV). Nevertheless nuclear and cell division are so intimately bound up with growth that Needham classifies them with true growth processes.

cell division—cell fusion—but its successful outcome depends upon the highly specialized type of division that produces the egg and sperm. Thus a clear understanding of the main features of cell division is necessary if one is to understand embryology.

CELL DIVISION

Mitosis. Mitosis is division of cells in which each chromosome has previously been duplicated, so that each daughter cell receives a replica of each chromosome of the parent cell. Except for the specialized form of the process which occurs during the "maturation" of germ cells, practically all cell divisions of any significance in a developing organism are of this type. Even nuclear divisions without cytoplasmic division, as in syncytia, are generally believed to be mitotic in nature. Of course atypical and abnormal mitoses do occur, sometimes as a feature of normal development (e.g., in the production of large polyploid cells in mammalian livers) and at other times as a pathological phenomenon such as is commonly observed in malignant tumours. Types of cell division formerly described as amitotic are now generally considered to be obscured mitoses, nuclear divisions without loss of the nuclear membrane, or simply a degenerative nuclear fragmentation.

Some of the more important features of mitosis are shown in Figures 1 and 2. Note that, typically, a cell grows to twice its resting volume before dividing; thus the range of cell volume for each cell type within a tissue is maintained within relatively narrow limits—a volume limit ratio of about 1 : 2 which is equivalent to a cell diameter ratio of about 1 : 1·26. Giant cells, however, either polyploid or multinucleate, sometimes occur as a normal feature in certain tissues.

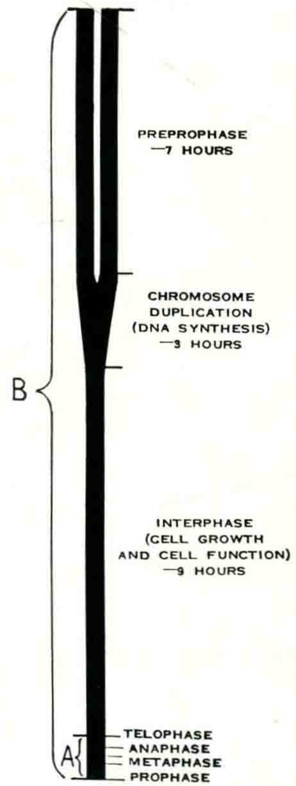


FIG. 1.—A scheme of the events in one hour (A) and in one mitotic cycle (B) of a fibroblast grown in tissue culture. (Reproduced by the courtesy of Hans Ris—based partly on Firket, 1958.)

It should be noted also that chromosome duplication takes place during the so-called, but obviously misnamed, *resting phase* of the mitotic cycle. Actually it is the amount of deoxyribonucleic acid (DNA) of the chromosomes that is known to be exactly doubled. This and much other evidence indicates that DNA is the chief component of genes, and that it is by this process of DNA synthesis within the nucleus that a replica of the genic components of each chromosome is produced. The *original* and the *replica* remain side by side as a *double* chromosome until anaphase, and are indistinguishable from one another. Thus, as a prerequisite to cell division, a complete set of double chromosomes is provided, one element of each couplet being destined for each daughter nucleus. While it is commonly stated that at mitosis each chromosome is split in half, it is more accurate to say that the two units of previously doubled chromosomes are separated, one replica of each chromosome of the set passing to each daughter nucleus.

Mitotic cell division is, therefore, of paramount significance in the higher forms of living things, for it assures that each daughter cell can be genetically like, and therefore, in general, compatible with every other cell in the individual's body. The physico-chemical mechanisms by which gene replication and cell division are carried out is today one of the most intriguing and active fields of biological investigation (De Robertis *et al.*, 1960).

Cell Division in Germ Cells: Meiosis. The definitive germ cells of most bisexually reproducing plants and animals are formed from derivatives (oogonia and spermatogonia) of the primitive germ cells (*gonocytes*). The final ripening process (*maturation*) includes

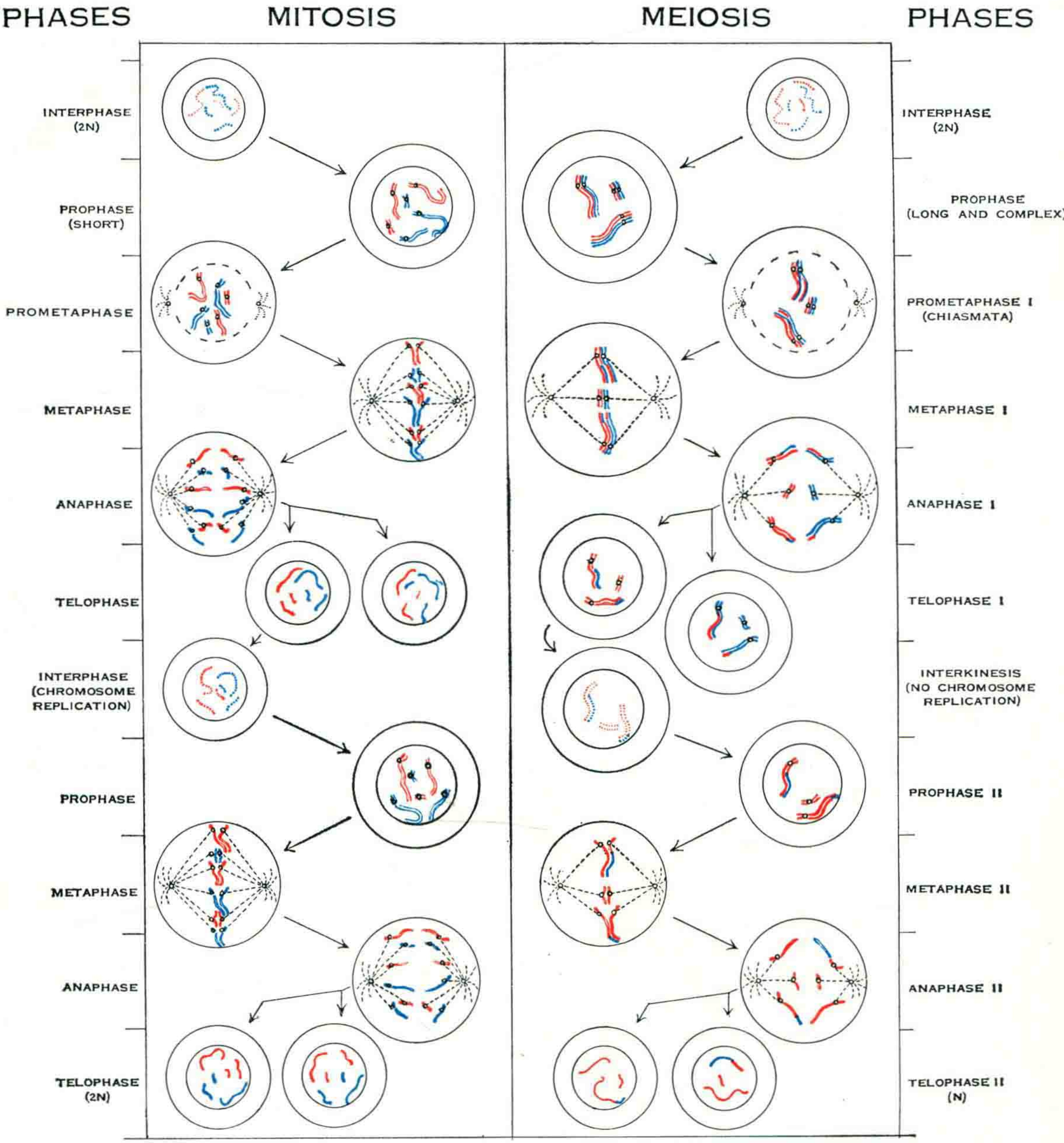


FIG. 2.—A diagram of gametogenesis and fertilization with particular reference to chromosome distribution and an approximate comparison of cell size relationships. For simplicity the diploid chromosome number (2n) is represented as 6, of which the blue can be assumed to be from the male parents of the individuals producing the gonocytes (primitive germ cells) and the red from the female parents. Note that in most mammals the secondary oocyte gives off the secondary polar body only if a sperm has penetrated it—hence ovum maturation and fertilization overlap.

two successive final and highly specialized mitotic cycles known together as *meiosis*. During these two meiotic divisions the following essential processes occur :—

(1) Duplication of the paired chromosomes (bivalents) in the interphase before the first meiotic division only.

(2) Pairing (conjugation or synapsis) of homologous chromosomes after duplication, and before the first meiotic division. This allows for the interchange of individual genes or groups of genes (segments) of homologous chromosomes, thus providing for most of the great variety of recombinations of hereditary characters seen in the offspring of biparental reproduction.

(3) Retention of the closely apposed bivalent condition of homologous doubled chromosomes at the equatorial plate of the first meiotic division, so that the two double homologous parts of these bivalent chromosomes are separated and pass to opposite poles of the spindle, each daughter cell thus receiving the haploid number of double (not bivalent) chromosomes.

(4) Random orientation of bivalent chromosomes on the first meiotic equatorial plate, so that the sets of chromosomes derived from each parent are usually shuffled and passed as mixed sets to each daughter cell. This, in addition to crossing over, provides for recombination of parental characters in the offspring.

(5) Division of the monovalent but double chromosomes at the second meiotic division, so that each mature germ cell receives the haploid number of single chromosomes. At the combination of these haploid sets of an egg and sperm at fertilization the normal diploid number of chromosomes of the species is restored and ready for the long series of mitotic cycles which ensue as the fertilized egg develops into a new individual.

Meiosis is thus seen to occur only in germ cell production. It differs from typical mitosis chiefly in the pairing of homologous chromosomes derived from the egg and sperm which formed the individual in which the meiosis is taking place. This is the basis for shuffling of hereditary characters (genes) derived from each parent, and for dealing them out in random fashion to each definitive germ cell, but in only one-half the quantity normal for somatic cells of the species, thus typically maintaining, upon fertilization, a constant species number of chromosomes and presumably a constant quantity of genes. (See Chapter VIII for a discussion of the aberrations and abnormalities in chromosome behaviour which may occur and the results which these exceptional conditions produce.)

STAGES IN EMBRYOLOGY

From a descriptive point of view the principal stages in metazoan embryological development are :—

(1) **Maturation.** This is the process associated with the formation of mature female and male germ cells (gametes—ova and sperms) from the oogonia and spermatogonia of the female or male gonads. During maturation of both oocyte and sperm, as has been described above, a reduction of the chromosomes to one-half of the somatic number occurs. During maturation the female sex cell grows to a relatively large size due to storage of yolk. The male sex cell remains small but undergoes changes in shape and internal structure which make it a motile organism. Consequently the mature gametes are highly specialized cells which when fully differentiated do not usually live long unless they take part in fertilization.

(2) **Fertilization.** This is the fusion of a female and a male gamete. It results in the formation of the zygote or fertilized ovum. This process has two fundamental objectives; first the initiation of embryonic development of the egg; second the restoration of the chromosome number of the species and hence the achievement of biparental inheritance with all its important implications. The zygote, although resulting from the fusion of two highly specialized cells, is regarded as being the most unspecialized (undifferentiated) of all metazoan cells.

(3) **Cleavage.** Cleavage is the term applied to the series of rapid cell divisions of the fertilized egg before growth or obvious cell differentiation begins. Eggs can also be activated to cleave without fertilization, as in normal or artificial parthenogenesis. As a result of cleavage the unicellular zygote is converted into a multicellular organism. There are two obvious