VERTEBRATE EMBRYOLOGY

PREFACE TO THE FIRST EDITION

THIS book is designed as an introductory text in Vertebrate Embryology, a work which seems to be justified on the following grounds: The older texts upon this subject, though in many cases excellent, do not cover exactly the field which is now covered in many colleges; these texts, moreover, are becoming somewhat out of date in various letails. Among the newer books the best ones tend to do one of two things. Either, in the interest of thoroughness, they confine their attention entirely to one form, e.g., the Chick, or else, for the sake of a broader viewpoint, they deal with a considerable number of animals, but in doing so touch only upon the earlier developmental stages of each. Now it is obvious that there is great value for the student, both in the accuracy gained by the careful intensive study of a single type, and also in the possession of less detailed knowledge of the history of other forms which are nearly related to it. Hence, what has seemed o be needed was a book which would, so far as is possible, make ıvailable both these advantages. To meet this need, the major part of the present text comprises a moderately complete account f the development of two typical forms, i.e., the Frog and the Chick, each of which, in the writer's opinion, has special features which justify such treatment. These relatively detailed discussions re then supplemented by chapters which present brief comparisons, ot only with the Mammal, but also with certain other significant nembers of the Vertebrate group. Furthermore, the essentially embryological portion of the book is preceded by an optional introductory chapter dealing with the elements of cytology. Upon this basis he effort throughout the work has been to produce something especially adapted to the requirements of the general student of Zoölogy, as well as to the individual particularly interested in premedical preparation.

As regards certain details concerning the method of handling the topics involved, the following remains to be said. Because of the haracter of the book the chapter upon cytology places special emphasis upon the structure, development, and function of the germ cells, th particular reference to nuclear phenomena and their genetic significance. The strictly embryological subject matter is then introduced by a short general discussion of the more fundamental and universal processes of Vertebrate development from the comparative standpoint. This includes a description of the various types of segmentation, gastrulation, and the formation of the rudiments of the nervous system and the main mesodermal structures. Following these introductory chapters, Amphioxus is the first particular type to be considered because of the relatively primitive character of most of its early history. The later development of this animal, i.e., that following the formation of the mesodermal somites, is, however, quite highly specialized in respects which distinguish it from the vast majority of Chordates. As these later stages are without great significance for the general student, they are omitted.

The Frog, as suggested above, is one of the two forms which have been treated at some length. The reasons for such extended consideration in this instance and in that of the Chick are presumably obvious to every Zoölogist. For the sake of the student, however, the value of these animals as subjects of embryological study are indicated in the paragraphs of the text which introduce them. In the case of the Frog, its early history has been presented under the head of certain fairly well recognized stages which lend themselves well to correlation with work in the laboratory. In further pursuance of this method the internal changes have been noted in alternation with those occurring externally. This was done in order that the reader might obtain, so far as possible, a correct idea of the really simultaneous character of these processes. It did not seem feasible, however, in a work of this scope to continue this plan throughout the entire course of development in this animal. The later external changes, therefore, are included under one heading, while the more advanced details of organogeny are described in terms of particular systems.

Following the treatment of the Frog, there has been introduced a very brief account of segmentation and gastrulation in the Teleosts and the Gymnophiona. This has been done despite the realization that in the case of the latter group laboratory consideration will in most cases be impossible. The reason for this is the author's opinion that segmentation and gastrulation in these two classes of animals are extremely valuable in assisting the student to relate these processes in the Frog to those which he is about to study in the Bird. Experience, moreover, has seemed to indicate that the relation of avian and mammalian gastrulation, to that in more primitive forms is always particularly difficult for the beginner to grasp, and it is believed, therefore, that any legitimate aid to this end is worth while.

In treating the early stages of the Chick a good deal of stress has been placed upon the method of segmentation and gastrulation. The latter especially has been emphasized because of its peculiar character, and the desirability of making clear its relationship to that in the forms already studied. The later history of this animal is then presented in daily periods, according to the well known plan of Foster and Balfour. This has been done because it seems to the writer that at least in a beginning course this method has certain marked advantages over that of studying the complete embryology of one system at a time. In the first place the Bird lends itself particularly well to treatment by periods, and secondly the simultaneous development of all the systems is what is actually seen to occur in any animal. This latter fact it would seem well to impress upon the student when possible by the method of presentation. Finally it has appeared not only possible but easier to conduct the class work in correlation with the laboratory when development is studied by periods rather than by systems. It should be noted, nevertheless, that in this book the material has been so arranged that the student can readily follow through the complete growth of any one system if the instructor so desires.

As regards the Mammals, it is felt that the detailed differences between the organogeny of this group and that of the Birds, are not, on the whole, of great general biological significance. Of very considerable significance, however, are those unique characteristics of both mother and embryo connected with mammalian gestation. For this reason the discussion in this portion of the text is confined chiefly to the earlier developmental stages, which are treated largely from the comparative standpoint. The subject is introduced by a description of the structure and functions of the adult reproductive organs in the same manner as in the case of preceding forms. This involves the process of ovulation, and in that connection it has seemed worth while to describe briefly the peculiar cyclic phenomena which accompany this process in the mammalian female. Following this the comparative idea is pursued with particular reference to the development of the extra-embryonic appendages. This is believed to be especially important from an evolutionary viewpoint because it shows how these appendages,

PREFACE TO THE SECOND EDITION

In preparing this edition it has not been the intention of the author materially to alter the character or the content of the book. For the most part, therefore, the changes made involve merely the correction of errors, and the bringing up to date of those topics where advances in our knowledge have occurred. In conformity with this idea there have been no alterations in either text or figures simply for the sake of newness, substitutions being made only where it appeared that a real benefit would be derived with respect to accuracy or clearness.

With regard to one field, however, it has been felt wise to make a small addition. This is in connection with the Frog, where two short sections have been added dealing with a few of the more outstanding results of the experimental attack on the problems of the early stages. This has been done in such a manner that these additions can be readily omitted if this is desired. In view, however, of the current emphasis on the experimental, as compared with the purely descriptive aspect of embryology, it is believed that a slight introduction to this phase of the subject is not out of place, even in an undergraduate text. Examples of work on the Frog and other Amphibians were chosen, both because of the historical importance of these animals as experimental subjects, and because of the fundamental significance of some of the results.

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VERTEBRATE EMBRYOLOGY

PART I

CHAPTER I

THE CELL AND ITS FUNCTION IN REPRODUCTION

All organisms are made up of more or less specialized cells and their products, which had as their ultimate origin either a single germ cell, or more usually two fused germ cells, i.e., a fertilized egg or *zygote.* The adult animal or plant has then arisen chiefly through the processes of cell division, differentiation, and rearrangement. Furthermore, these processes are always ordered in such a way as to result finally in the production of an individual similar in its general characteristics to the parents from which the fertilized ovum was derived. Such processes applied to any given case, therefore, constitute individual development or *ontogeny*, and their study is termed embryology. Since, as has just been indicated, however, the processes in question depend chiefly or wholly upon the activities of cells, it is desirable to begin embryology by giving some attention to the character and behavior of these organic units.

It will be found convenient to describe first a typical or generalized cell, and to proceed from that to a discussion of the more specialized male and female reproductive cells. It seems best to divide the description of the typical cell into two main phases, i.e., that of rest and that of division. In this connection, however, it should be clearly understood that by a "resting" cell is meant merely one that is not dividing. Such a cell, nevertheless, is not really resting, but is undergoing all the metabolic changes which are a constant accompaniment of life. The appearance of rest results simply from the fact that these chemical changes are less obvious than the very evident act of division, and the terminology is therefore only one of descriptive convenience.

THE TYPICAL CELL

The Resting Phase

According to the definition of Leydig and Schultze, given over forty years ago, a cell is "a mass of protoplasm containing a nucleus"

(Fig. 1). This definition is applicable to the majority of cells, but in the light of present knowledge a more inclusive and accurate description may be formulated thus: a cell consists of nuclear material and

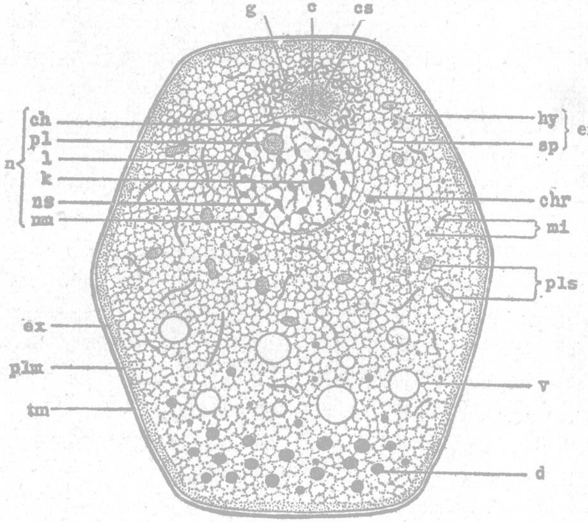

Fig. 1. — A generalized diagram of a cell. *c.* Centriole. *ch.* Chromatin. *chr.* Chromidia. *cs.* Centrosome surrounded by a latent aster. *d.* Deutoplasm. *en.* Endoplasm. *ex.* Exoplasm (cortical plasma). *g.* Golgi bodies or apparatus in the form of a concentrated vesicular network surrounding the centrosome and aster. *hy.* Hyaloplasm, *k.* Karyosome or chromatin nucleolus. *L.* Linin. *mi.* Mitochondria. *nm.* Nuclear membrane. *n.* Nucleus. *ns.* Nuclear sap. *pl.* Plasmosome or true nucleolus. *plm.* Plasma membrane. *pls.* Plastids, the lower one in process of division. *sp.* Spongioplasm. *tm.* "True membrane" or cell wall. *v.* Fluid vacuole.

the protoplasm within its sphere of influence.[1] Such a definition is preferable to the older one because it takes account of certain protoplasmic masses containing several nuclei, and termed *syncytia*, as well as of cells whose nuclear substance is diffuse. The protoplasm in its unstained condition is a grayish substance which may appear granular, recticular, or alveolar (see below), depending on the type of cell and the circumstances under which it is being observed. Furthermore, the content of the nucleus itself is really only another sort of protoplasm differing somewhat in its composition and organization from that outside. For this reason it is often customary to distinguish the protoplasm within the nucleus as *karyoplasm* and that

[1] Even the second definition does not include such specialized cells as the enucleated red blood corpuscles of mammals.

surrounding it as *cytoplasm*. Lastly the nucleus and the cell as a whole are usually each enveloped in a membrane. The membrane of the cell, however, is not infrequently lacking or invisible, while in other cases there may be more than one. Likewise the nucleus may lack a membrane, and in some instances the nuclear material may even be widely scattered throughout the cytoplasm. This last condition, however, is relatively exceptional, and is found more particularly among certain of the Protozoa and the Bacteria.

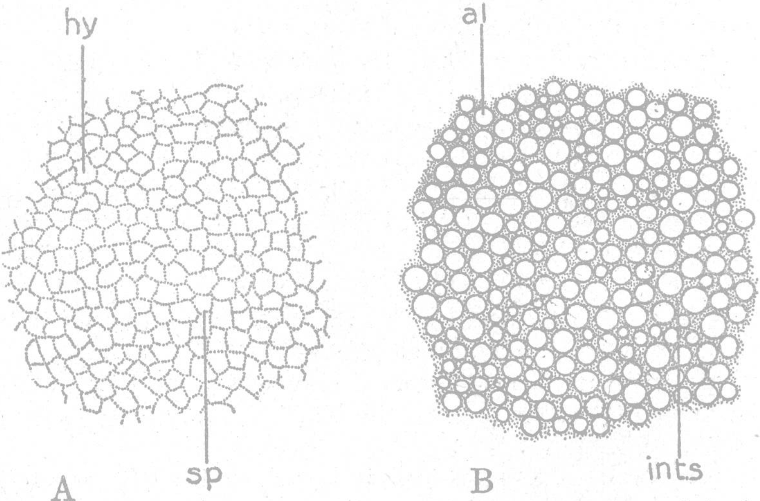

Fig. 2. — Diagram representing, *A*, reticular, and *B*, alveolar protoplasm. *al*. An alveolus. *hy*. Hyaloplasm. *int.s*. Interalveolar sustance. *sp*. Spongioplasm.

The Cytoplasm. — Considering this part of the cell first, we find that it in turn has sometimes been divided into *active* cytoplasm and *metaplasm*. Under this classification the former was said to consist of the more homogeneous and finer grained substance, and was regarded as the essentially "living" cytoplasmic material. The metaplasm, on the other hand, has been thought of as comprising the larger granules, oil drops, vacuoles of various sorts, and certain other objects. It may thus represent food materials (*deutoplasm*), excretory substances, and a variety of other cell inclusions or "formed bodies" (Wilson), and hence has sometimes been regarded as relatively inert and "dead." It must, nevertheless, be strongly emphasized that it is impossible to draw any hard and fast line between those substances which are active cytoplasm and those which are mere metaplasm. As a matter of fact one shades insensibly into the other, and the distinction is one of descriptive convenience rather than reality. Thus with regard to what is really alive and what is not, we can only say at present that the cell as a whole is living. It is the smallest unit of matter we know of which is so organized as to exhibit this characteristic for any extended period of time, and frequently even when isolated from other similar units. Concerning the distribution of the above types of cytoplasm we find that there is considerable variation. In the general type of cell under discussion, however, the metaplasmic material may be imagined as scattered rather indiscriminately.

Turning now to a more detailed consideration of the general structure of cytoplasm, exclusive of the larger vacuoles and other more gross inclusions, we find that historically there have been three main conceptions as follows:

Some of the earliest students of cells including Schultze, and later especially Hanstein ('80), regarded the essentially living substance as made up of a viscid liquid called *hyaloplasm*, filled with fine granules termed *microsomes*, a name meaning literally merely small bodies. Subsequently Altmann ('90) considerably elaborated this idea, putting particular emphasis upon the granules. These he regarded as self propagative entities, and therefore the primarily "living" material. Hence this later development initiated by Altmann is sometimes referred to as the *granular* theory of protoplasm.

Other students of this subject developed the *fibrillar* conception. According to them the essential "living" material consists of delicate threads or *fibrillae* lying in a homogeneous ground substance similar to or identical with the liquid hyaloplasm of Hanstein. This general idea includes two subsidiary views regarding the relationships of the threads or fibrillae, and these views are known respectively as the *reticular* and the *fillar* theories. The former was promulgated particularly by Leydig ('67) and Van Beneden ('83) who thought that the fibrillae constituted a network or reticulum which Leydig called *spongioplasm* (Fig. 2, *A*). The fillar theory is more especially associated with the names of Flemming and Heidenhain who saw the fibrillae not as a reticulum, but as separate and unbranched threads. Both of these fibrillar conceptions seem to have been due mainly to the fact that these observers were studying material which had been coagulated and stained, a condition in which the appearance of threads, reticular or otherwise, is not uncommon.

The third main conception concerning the fundamental nature of protoplasm was the *alveolar* theory proposed by Bütschli in 1892. He again studied living cells, mostly Protozoa, and to him the active or "living" substance appeared like minute bubbles or globules of one liquid packed closely together and suspended in another, i.e., it had the character of an emulsion. The globules he called *alveoli*, and the material between them the *interalveolar substance*. The latter, he admitted, often contained microsomes (Fig. 2, *B*). Up to this point, it should be noted Bütschli's conception is not dissimilar from the view now generally accepted, as described below. He, however, went further, and distinguished rather sharply between the "living" or "primary" substance comprising the finer alveoli and interalveolar material just indicated, and the larger vacuoles and granules which he held were metaplasmic.

Finally as regards the present belief concerning the structure of cytoplasm the following may be said: Cells which have been killed and stained by different methods may exhibit any of the appearances indicated above according to the method employed. It is now commonly held, however, that when seen in the living state cytoplasm practically always seems to contain a certain amount of homogeneous material or ground substance presumably identical with the hyaloplasm of the granular and reticular theories and with the interalveolar substance of the alveolar theory. Besides this it may then contain granules, vacuoles, or alveoli of various kinds and sizes, and in some cases reticular fibers. With respect to the relation of these substances to one another, and to the older terms "active" and "metaplasmic" we may conclude as follows: At present it is quite generally believed that the entire cytoplasmic complex has the physical and chemical properties of a colloid. In contrast with Bütschli's view, however, the materials of the dispersed phase are now thought to comprise all the objects from the larger vacuoles, down through the smaller, but visible droplets, granules and threads, to ultra microscopic particles. In correlation with this, as previously suggested, it is no longer logically possible to separate sharply the "active" or "primary" substance from the "metaplasmic," and no such attempt is made.

It remains to note that within recent years there have been distinguished among the microscopically visible bodies just alluded to three rather special kinds. The real character, functions, and possible relationships of these special materials to others in the cell are still uncertain despite intensive and widespread study by numerous investigators. Because of the emphasis they have received, however, brief reference must be made to them. One variety termed *chondriosomes*, which can be demonstrated by particular methods of killing and staining, or by certain ways of staining the tissue while alive (vital staining), may appear as either granules (*mitochondria*), or threads and rods (*chondrioconts*). The second variety designated as the *Golgi bodies* or *Golgi apparatus* after their discoverer Golgi ('98), also appear following certain special treatments. They may be seen as scattered rods, or more commonly as either a network, or a vesicular aggregation concentrated in one side of the cell. The third, *chromidia*, seem to be chromatin granules extruded from the nucleus.

There is next to be mentioned a rather important cell organ which usually lies in the cytoplasm near the nucleus, and which is often visible during the resting state. It is a minute granule (there is sometimes more than one), which stains darkly with nuclear stains, and is termed the *centriole*. Immediately surrounding it there is usually a somewhat clearer cytoplasmic area which is known as the *centrosome* (formerly the *attraction sphere* or *centrosphere*). The structure as a whole is intimately connected with the division mechanism of the typical cell, and hence its parts are frequently known collectively as the *division-center*, or the *central body*. Certain portions of this body, as will appear later, are probably involved in the organization of yolk in the egg, and in the formation of parts of the male germ cell.

Besides this central body, whose occurrence is very widespread, there also exist in the cytoplasm of certain cells two other types of cell organ. The first of these types consists of bodies of various shapes, sizes, and colors, often apparently located at random. They are termed *plastids*, and are most frequently found in plant cells, where they contain chlorophyl (*chloroplastids*) and are active in the formation of starch. In other kinds of cells other varieties of plastids are present, differing from the chloroplastids not only in composition and color, but also, as would be expected, in the substances which they produce. In all cases, however, these bodies are to be distinguished from typical metaplasmic material because of their activity and also because they are thought in many instances to be self-propagative. The second type of cytoplasmic organ is the *contractile vacuole.* Vacuoles of this sort differ from those of a more strictly metaplasmic nature in respect to their contractile character, their constancy of occurrence in those cases where they occur at all, and in many instances in their constancy of position. Furthermore, they are said in certain cases to reproduce, like the plastids, by fission. They are found most frequently among the Protozoa.

Lastly there are often used in connection with the cytoplasm the terms *exoplasm* and *endoplasm*. The former naturally indicates the cytoplasm close to the surface, and the latter refers to that nearer the center. Frequently, however, it is impossible to distinguish sharply between the kinds of protoplasm in the two localities, and when it is, the kinds in different types of cells are found to differ widely in their relative appearance. Thus these terms are indicative of the location in which a substance is found rather than of its character.

This brings us to the covering of the cell, which may be essentially of two sorts. Either it is a very thin layer of the exoplasm itself, the *pellicle* or *plasma membrane*, or it is a secretion from this exoplasm, a "*true membrane*." Frequently, as in the sea urchin egg, the former is transmuted into the latter so that the distinction is not fundamental.

The Nucleus. — Considering now the nuclear protoplasm or karyoplasm, we find that in two respects at least it differs quite definitely from the cytoplasm. It lacks any obvious metaplasmic inclusions, such as the larger vacuoles and fat droplets, but at the same time it contains another substance which seldom occurs regularly or permanently in the cytoplasm. This latter material is termed *chromatin*, a compound which derives its name from the fact that it stains readily with haematoxylin and certain basic tar-colors. This quality of the chromatin in turn is probably due to the fact that it is especially rich in a complex phosphorus-containing acid known as nucleic acid. In preparations properly stained with the above dyes therefore the chromatin material is characteristically colored. In unstained cells, on the other hand, it is quite colorless, thus causing the nucleus to appear lighter than the surrounding protoplasm. As will be observed later, this chromatin is probably the most important substance which the cell contains, and is the vital center of all its activities.

Besides the chromatin the nucleus also contains certain other materials whose character and arrangement with respect to the chromatin has been commonly described as follows: First, there is a more or less liquid medium, supposed originally to be unstainable, and serving as a sort of basis or ground substance, known as the *nuclear sap*. Secondly, suspended in this is a fine reticular network, stainable ordinarily by acid or cytoplasmic stains, and termed *linin.* Finally lying in or on this linin network is the chromatin in granules or clumps of varying size. According to this description, therefore, the nucleus may be thought of as having a structure quite similar to the cytoplasm as conceived of under the reticular theory, the nuclear sap corresponding to the hyaloplasm, and the linin to the spongioplasm, with of course the addition of chromatin.

It should at once be added, however, that this conception, though still held by many cytologists, is being attacked upon the same ground as was the similar theory with respect to the cytoplasm; i.e., that the reticular appearance is chiefly seen in killed material, and may be only a coagulation product. Hence it is believed by many investigators that the contents of the nucleus like that of the cytoplasm is really colloidal. From this point of view it is of course held to consist of numerous alveoli. The contents of these alveoli then represent the nuclear sap, while their surfaces are presumably the linin in which the chromatin has been supposed to be embedded. On the other hand it must be said that certain nuclei seem to show a genuine reticulum during life, and in so far as this is true it is clearly impossible to state that the older reticular conception is entirely erroneous.

Not only, however, is the structure of the nuclear material thus in doubt, but the unique staining capacities of its different elements are actually less uniformly characteristic than has been indicated above. It appears in fact that whatever the structure of the non-chromatic elements (linin and nuclear sap), it is certainly not always possible sharply to distinguish between these materials on the one hand and what has previously been defined as chromatin on the other. This follows from the fact that it has been clearly shown that the portion of the nuclear material which will take the basophilic or "chromatin stains" depends largely upon the condition of the cell, and the methods of its preparation. Consequently many authorities (Wilson) now regard all the contents of the nucleus as chromatin. They then apply the term *basichromatin* to those parts of it which at a given time stain with the chromatin stains, and the term *oxychromatin* to the remainder.[1]

[1] It should be added that rather recently there has been discovered a method of staining which is supposed to be specific for chromatin in so far as the latter is defined as any substance containing nucleic acid. It is known as Feulgen's method, and depends upon the hydrolysis of the nucleic acid by hydrochloric acid, thus giving rise to an aldehyde. When the material is then properly treated with reduced basic fuchsin, the resulting color is a sure test for the presence of the aldehyde, and hence

In addition to the configurations of basichromatin and oxychromatin just described the nucleus also frequently contains bodies termed *plasmosomes* or *true nucleoli*, and *karyosomes* or *chromatin nucleoli*. The former consist of one or more rounded masses of material which ordinarily, though not always, take the acid or cytoplasmic stains, and whose functions are little understood. The karyosomes on the other hand stain typically with basophilic stains and are in fact merely larger clumps of chromatin. As such they contribute their material at certain periods in the life of the cell to aid in the formation of aggregations of the chromatin called chromosomes, important bodies whose character and significance will be noted in subsequent sections. Indeed in some cases a karyosome is actually identical with a particular chromosome.

Finally, as already noted, the nucleus like the cell is almost always surrounded by a more or less definite membrane. Its character is not certainly known, but it is quite commonly regarded as consisting of oxychromatic material like that inside.

The Cell in Division

There are two types of cell division, one of which is termed *amitosis* and the other *mitosis*.

Amitosis. — This form is relatively rare and is believed by many to occur only in cells that are degenerating or in some other respect abnormal. In any event the process is very simple and will be described here only briefly. The nucleus does not undergo any apparent internal change but simply constricts itself into two approximately equal parts. This is followed in most cases by a similar division of the cytoplasm in such a manner that each portion contains a part of the original nucleus. In some instances, however, which can hardly be termed real cell division, only the nucleus thus divides, thereby starting the production of a syncytium.[2]

Mitosis or Karyokinesis. — This is the more usual method of cell division and is far more complicated than that just described. There

for the nucleic acid. It should be noted, however, that the specificity depends upon the assumption that the nucleic acid is the only source of the aldehyde. This is not the case in plant tissues nor in material fixed in any fixative containing formalin. The technique has not been sufficiently used as yet to enable us to say just how many of the structures staining with either the old basichromation or oxychromatin stains are actually chromatin according to the above definition.

[2] It is not to be understood that syncytia arise only as a result of amitosis.