THE ESSENTIALS OF CYTOLOGY

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AN INTRODUCTION TO THE STUDY OF LIVING MATTER

WITH A CHAPTER ON CYTOLOGICAL METHODS

BY

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PREFACE BY

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PREFACE

Cytology is a distinctly specialised study both in the scope of its observations and in the means of making them. For this reason a book specially describing its achievements and technique will be welcome to many. To the student of biology the interest of cytological facts is at the present time especially great, since their bearings upon problems of heredity and disease were never more evidently significant than now. A clear outline therefore of this relatively young branch of the tree of modern knowledge will, given as it is by one well acquainted with it at first hand, be a boon not only to the student of science but to general readers. The book will fulfil yet another admirable purpose if it attract to the field of cytological research, rich as that is in promise of harvest of discovery, additional workers in this country.

C. S. SHERRINGTON.

AUTHOR'S NOTE

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Liverpool, 1907.

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THE

ESSENTIALS OF CYTOLOGY.

CHAPTER I.

INTRODUCTORY.

Our knowledge of the properties of living matter generally as distinct from any other form of matter is very limited, but there is one fundamental character which, though it is perhaps the most important of any in this connection, does not always appear to be properly appreciated. This fundamental fact is that living matter, as far as our knowledge goes, exists only in one form. Whether it be in the shape of animals or vegetables, living matter is always in the form of definite and concrete masses of a complex material, each mass being composed of certain definite and distinct parts. The material of which these masses are composed is known as protoplasm. The masses themselves have, somewhat inaptly, been called cells. Living matter then can apparently only exist in the form of cells.

Until quite recently protoplasm was regarded as being the simplest form of living matter, "the physical basis of life" of Huxley. Recent research, however, has shown that no homogeneous mass of protoplasm is really alive. Unless

the constant constituents of a cell are present in a mass of protoplasm it will disintegrate with greater or less rapidity,[1] and the phenomena exhibited by living matter will never be exhibited by protoplasm unless the protoplasm be in the form of cells or a cell. All the supposed cases of undifferentiated protoplasm being alive, such as Huxley's Bathybius, have broken down under examination. Bathybius was first abandoned by Huxley himself. Haeckel's Monera have never been rediscovered.

Having realised that the cell is the unit of living matter, the simplest form in which it can exist, the conclusion naturally follows that most problems relating to living matter must ultimately resolve themselves into cell problems. Physiology is the study, the recording, and the measuring of the phenomena and processes taking place normally in groups of cells. Pathology is the study and recording of departures from the normal in similar groups of cells. That such problems are ultimately cell problems becomes evident when we realise that every part of the animal and vegetable body which is alive is formed of cells, and of cells only. Those parts which are not composed of cells, such as the hard parts of bones and the wood of trees, are not alive, but are merely the dead secretions or excretions of cells.

The similarity in structure, however, does not by any means end the likeness between the different forms of living matter. As we shall see later, some of the most complicated cellular phenomena, including many which are of the most fundamental importance, are similar, even to minute details, in both animal and vegetable organisms.

[1] See p. 17

Living organisms, whether animal or vegetable, may exist in the form of single independent cells—unicellular organisms, or may be composed of many cells associated together and forming an individual—multicellular organisms. In either case, however, these cells—units of living matter—have always been derived from pre-existing cells. We have no knowledge of any origin of cells at the present time excepting from other cells, and no alleged instance of any other origin of living matter has hitherto been able to bear investigation.

The whole of the cells forming the various parts of the multicellular organism are derived from a single cell, the ovum. We have seen that so far as our knowledge goes, no cells can arise from any other source than from pre-existing cells. New individuals of various kinds of multicellular organisms are produced from cells derived from similar multicellular organisms. Under normal conditions the new individual is produced from a single cell derived from an individual of the same species. This single cell divides into two cells, each of these two again divides into two more, and so on, until the whole soma or body of the individual is built up. At some stage in the life of the organism, cells are thrown off—separated from—the body of the parent to form new individuals in their turn. In the case of most multicellular organisms, however, two individuals are necessary to produce a new organism. This production of the new individual is brought about by two cells, one derived from each parent, fusing to form a single cell. This process is called fertilization, and from this single cell, the fertilized ovum, the body of the new individual is formed. The new individual thus formed will in its turn produce cells which

will be cast off from its body, and which will produce new individuals, and so on through each fresh generation.

If this series of events be represented diagrammatically (Fig. 1) it will be seen that there is a direct line of cell generations running through successive generations of multicellular individuals. Some of the cells, in fact, the repro-

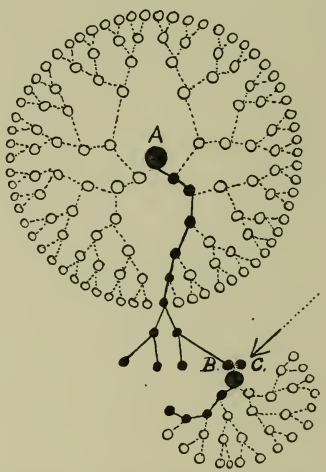


FIG. 1.—A, represents a fertilized ovum which has given rise to successive generations of cells, and thus has built up a multicellular organism. The cells forming the body or soma are represented by white circles, the line of cell generations destined to produce sexual elements by black. A sexual cell, B, is represented as having fused with a sexual cell C, derived from another individual. As the result of this fusion a new individual is being built up. (Modified from J. E. S. Moore.)

ductive cells and the intermediate generations of cells between them and the ovum, are potentially immortal. In most unicellular animals of whose life-cycles we have any accurate knowledge a very similar series of events takes place. A number of generations of individuals is produced by simple division of pre-existing individuals. Then a stage intervenes where the individuals conjugate,

exchanging portions of their nuclei with each other, or actually fusing into one cell (Fig. 2). This corresponds to the process of fertilization in multicellular organisms, and the result is also comparable—the fertilized individuals are able to go on dividing for a large number of generations, until conjugation again takes place.

In organisms which consist of one cell only all the

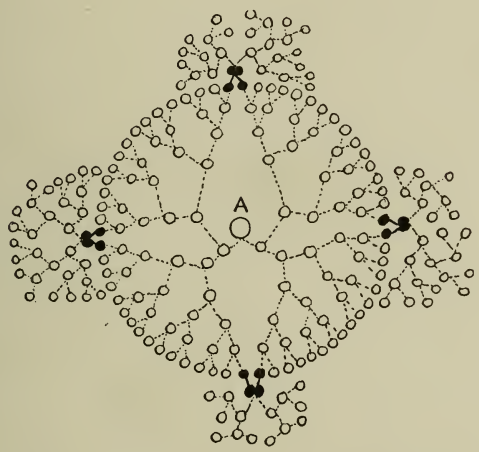


FIG. 2.—A series of divisions beginning in the unicellular organism A, has produced a large number of individuals. After many generations have been produced in this manner, the individuals conjugate. The black individuals are represented as having conjugated, and a fresh series of generations is being produced in each case by simple divisions. (After J. E. S. Moore.)

functions necessary to the well-being of the individual and to the reproduction of the species are performed by the one cell. Digestion, secretion, excretion, movement, and reproduction are all carried on by it. In multicellular organisms, on the other hand, the different functions necessary to the individual are carried out by different cells or groups of

cells. The members of these different groups differ in appearance and structure within certain limits, always retaining, however, the essential parts found in all cells. The differentiation in fact lies in the addition of various structures to these essential parts. Thus in the multicellular animal we find various groups of cells which normally carry out the different processes involved in the digestion of food, other groups which are so modified as to enable the animal to move, others which enable it to appreciate its surroundings, and so on. In every case, under normal conditions, the activities of the cells thus differentiated are confined to certain particular functions. Every multicellular organism, however, as we have already seen, begins its existence as a single cell. Thus it is evident that the ovum possesses all the potentialities subsequently exhibited by the cells derived from it. It may, in fact often does, possess all the properties of a unicellular organism. Now the differentiated cells of the multicellular organism appear to retain, to a greater or less extent, this general potentiality of reproducing the various other differentiated cells found in the particular organism. This is very obvious in the case of some plants. It has been proved experimentally that it is retained by the cells of some vertebrate animals to a certain extent. If, for instance, the lens be removed from the eye of a salamander a fresh lens will grow. The original lens was developed from the outer layer of cells, which were differentiated from the rest of the cells of the embryo at a very early period. The new lens, however, is developed from another group of cells which were differentiated also at an early period along quite different lines, and which normally would never have produced anything at all like the

lens. Again, the phenomena observed in cancer, as we shall see later on, show that even in mammalia differentiation does not entirely destroy the general potentiality of the cells forming the various tissues of the body.

In spite of this greater or less potentiality to form different kinds of tissues retained by the differentiated cells of the multicellular organism, the fact remains that, under normal conditions, cells belonging to differentiated groups retain their special characteristics and properties, and, when they divide, produce cells similar to themselves. It is therefore evident that in the multicellular organism there is some kind of interaction or mutual regulation which limits the characters and properties of each individual cell and each group of cells in some special manner in relation to the rest of the cells forming the organism. For want of a better term we will call this influence "Somatic Co-ordination." We know but little more with regard to it than that it is an interaction or common influence dominating the individual cells of a group or groups which causes them to be, to a considerable extent, dependent upon each other—in fact, to form an individual multicellular body or soma.

For instance, if a fertilized ovum has divided into two daughter-cells and each of these into two more, on the way to forming the body of a multicellular animal, each of the four cells, if the embryo is allowed to develop in the normal manner, will produce different tissues possessing different functions. Thus the derivatives of A (Fig. 3) may produce nervous tissue, and will eventually convey nervous impulses; B may, among other things, produce the liver and eventually secrete bile; C may produce the kidneys and secrete urine,

and so on.[1] If, however, the four cells be artificially separated without injury and are kept under suitable conditions, it has been proved experimentally that each of the four cells may produce a complete embryo possessing a full complement of tissues and functions.[2] A, therefore, in

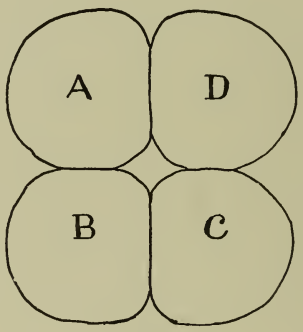


FIG. 3.

association with B, C, and D, will produce only one group of tissues. Out of association with them it will produce not only its own proper tissues, but also all those which would have been produced by B, C, and D. In the one case the tissues produced by A will be confined to one set of functions; in the other they will perform all the functions of the body. It is obvious then that there is some influence which dominates A, B, C, and D which interacts between them, and which limits the characters and functions of the tissues produced by each when they are associated, but which ceases to influence them or allow them to mutually influence each other when they are dissociated. This reciprocal influence is called Somatic Co-ordination.

[1] These derivatives of the four blastomeres are given merely as a hypothetical example, to facilitate the explanation.

[2] Roux, Wilson, Driesch, Morgan, Zoja, and others.

CHAPTER II.

THE STRUCTURE AND PARTS OF THE CELL.

We have seen that the whole soma or body of the multicellular organism is built up by generations of cells produced from the ovum. The process of cell division was regarded by the older observers as a very simple matter, no more complicated than the division of a drop of viscous fluid into two drops. Occasionally cells do divide in a manner that is not apparently very much more complicated than this, but it is now known that the phenomenon of division in the great majority of cases involves a very complicated process. The apparently simple form of cell division just referred to is known as "amitotic" division or "amitosis." It will be dealt with in more detail later on. The usual and more complicated mode of division is known as "mitotic" division, "mitosis," or "karyokinesis." Before considering the details of this process it is necessary to have some knowledge of the structure and constituent parts of a cell.

Cells are, unless they become specially differentiated or until they are pressed upon by contiguous cells or other things, more or less spherical in shape. Within the cell is a mass of protoplasm which is different in structure from the rest of the protoplasm which forms the cell. This contained mass is called the nucleus, and, like the cell itself, is usually spheroidal or spherical in shape. When the cell is examined

under a microscope the nucleus appears denser in structure than the rest of the cell, and if the cell be stained, this difference becomes more marked. The protoplasm which surrounds the nucleus, the rest of the cell in fact, is called the "cytoplasm."

Both nucleus and cytoplasm are composed of protoplasm. The protoplasm itself is a viscous translucent substance, sometimes showing a definite structure, sometimes appearing practically homogeneous. Some kind of structure is the more usual form, but the nature of this structure varies considerably. Perhaps it most frequently presents a finely granular appearance, or that of a fine reticulum or meshwork. A foam structure, like a number of minute bubbles crowded together, is not uncommon. The structural basis of the protoplasm forming both nucleus and cytoplasm is almost certainly the same, for, as we shall see later, at a certain period in the process of cell division, those portions of the protoplasmic ground substance which are separated into nucleus and cytoplasm become inextricably mingled together. When, however, the cell is not in actual process of division but is in a vegetative condition[1] the nucleus is surrounded by a membrane which separates it from the cytoplasm. The appearance of the contents of this membrane, the nucleus proper, may vary considerably. The most usual appearance however, is a reticulum or meshwork. In the substance of the threads of this reticulum, or attached to them, are masses of a darkly staining substance. This

[1] A cell which is not in any of the several phases which immediately precede, accompany, and follow the process of division, is sometimes described as being in a state of rest. This is an obvious inaccuracy, and is likely to be misleading, as a living cell can hardly, if ever, be in a resting condition: it will always be doing something.

threadwork is called "linin," and the darkly staining substance is called "chromatin."

It may here be pointed out that the different parts of the cell react differently to different stains. The cytoplasm and most of the structures contained in it and derived from it take an acid in preference to a basic stain. The linin of the nucleus also takes an acid stain, but the chromatin

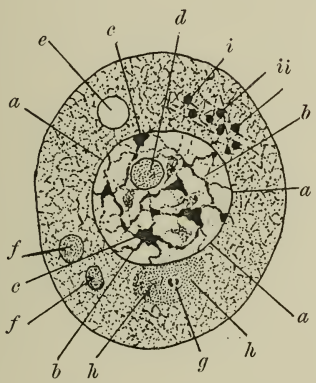


FIG. 4.—A semi-diagrammatic representation of a cell. *a*, nuclear membrane; *b*, linin reticulum; *c*, chromatin masses contained in envelopes of linin (chromatin nucleoli); *d*, true nucleolus; *e*, vacuole; *f*, plastids; *g*, centrosomes; *h*, archoplasm; *i*, food particles.

stains with a basic rather than with an acid dye. All the structures contained in a cell will stain with either an acid or basic dye to a greater or less extent, but if a basic and an acid stain be both used the different structures contained in the cell react to them as is here indicated.[1]

For a long time it was believed, indeed it is still very commonly held, that the one constant part of the nucleus, the substance through which all the hereditary characters were transmitted from one generation of cells to another,

[1] See p. 122.

was the chromatin. Very strong evidence has however come to light which seems to show that the chromatin is probably no more than a secretion of the linin, and that it is the linin, if it be any definite substance contained in the cell, which performs the functions generally attributed to the chromatin.[1] The linin, as has been already said, generally forms a reticulum in the ground substance of the nucleus. Often the linin is apparently in the form of threads or ribbons, but it may also, particularly during certain stages in the process of division, be in the form of tubes.

The chromatin is, as a rule, distributed in granules of varying sizes along the threads or tubes of the linin. In most nuclei chromatin is also found in a varying number of more or less irregular masses of a larger size, these masses sometimes occupying an appreciable portion of the space contained in the nucleus. These masses were called "nucleoli" by the earlier observers, but it was found that they were only some among several other kinds of structures to which this term was applied. They were then called "chromatin nucleoli," but as they are simply masses of chromatin of a large size it seems unnecessary to add a fresh word to an already large and rapidly increasing vocabulary to distinguish chromatin masses which are indistinguishable from their fellows except by their size. It would indeed be impracticable to specify what size gave a right to the special name.

The chromatin masses are almost certainly generally contained in an envelope of linin which is continuous with the threadwork or tubes.

[1] See p. 37, "Meiotic Phase."

There are other bodies in the nucleus which are commonly called "true nucleoli." They generally stain with an acid stain, in contrast to the chromatin masses which take a basic stain. Though there are many speculative theories as to the nature and function of these bodies, nothing certain is known with regard to them. It is even doubtful whether they are all similar. They are almost always spherical in shape, and generally appear practically homogeneous in texture. As they are very often absent altogether, and as during the process of cell division they generally seem to be absorbed or disintegrated completely, they are evidently not necessary constituent parts of all cells.

The spaces between the linin meshwork are occupied by a clear viscous fluid or jelly in which there is no apparent structure. This is known as the "nuclear sap."

The size of the nucleus in proportional relation to the size of the cell varies considerably in different classes of cells. It is usually large in those cells in which any active metabolic process is taking place, such as gland cells, and also in those cells which are destined to give rise to the reproductive elements. Its shape, though on the whole very constant, may also vary. Usually spherical, it may be elongated in highly differentiated cells such as those forming muscle fibres. It may also be definitely lobular, as is the case in some cells found in cancer, in bone-marrow, and in some leucocytes. The nucleus may also change in shape by movements which have been described as amœboid.

In some unicellular organisms (*e.g.*, Infusoria) two nuclei are present. These two nuclei are of different sizes and apparently perform different functions. The larger "macronucleus" seems to control various functions of the cell,

such as digestion and excretion. The smaller "micronucleus" seems to be concerned in the reproduction of the species.

The nuclear membrane does not appear to be absolutely constant. Apart from the fact that it disappears, as we shall see later, during the process of mitosis in cells where it is usually present, some forms have been described in which there appears to be no nuclear membrane. In these organisms the nucleus is described as being distributed in the form of chromatin granules, often collected in masses. If this description is accurate the chromatin masses are probably enveloped and possibly connected by linin. Although this class of nucleus requires further investigation, there is nothing with regard to it which is at all improbable. Indeed, it may be that this is the most primitive form of nucleus.

The cytoplasm seems to vary considerably in structure in different classes of cells. In some cells it is apparently almost homogeneous, in others it is granular, reticular, or of a formation which appears like a delicate foam. Though some cells, such as leucocytes, have been held to be naked and to possess no membrane enclosing the cytoplasm, it is almost certain that in all cells there exists a layer of differentiated protoplasm upon the surface. There certainly is such a layer in leucocytes. Between this differentiated layer of cytoplasm and a very definite cell wall, all stages exist. In animal organisms a differentiated layer of protoplasm is the more usual, while in plants a cell wall is the commoner. Where a cell wall exists it is regarded by most observers as having been produced by a secretion of the cell. The differentiated cell organs, such as cilia and flagellæ, arise from the cytoplasm, and the form of the

cytoplasm itself frequently undergoes the most striking modifications, as, for instance, in the case of the striped muscle fibres. Various cell secretions also appear to arise in the cytoplasm either in the form of granules or as liquids filling hollow spaces known as vacuoles. The digestion of food particles takes place in the cytoplasm, and masses of food material of varying sizes are frequently seen lying in its substance.

Other bodies, known as "plastids," are sometimes found in the cytoplasm. Several different kinds of plastids exist. Among those about which we know most are the "chromatophores." These are usual in plant cells, though they occur in but few animals. They arise, in some cases at any rate, if not in all, as colourless plastids in the embryonic cells, and, dividing with the other parts of the cell, go on from one cell generation to the next, eventually forming the pigment bodies in the adult cell.

Lying in the cytoplasm, as a rule near the nucleus, is a pair of minute bodies which are of great importance. These are the "centrosomes." They are very small as a rule, often being on the extreme limit of microscopic vision. So far as can be seen they are usually homogeneous in structure, though occasionally they appear to be granular. In the latter case they are generally comparatively large. They are usually kidney or bean shaped. Sometimes only one is present, but in other cells there may be several, as in certain leucocytes and the red blood corpuscles of Axolotl.

In a few cases the centrosomes are stated to be inside the nuclear membrane, but this statement requires further confirmation.

Centrosomes have not been demonstrated in the cells of

the higher plants, though this is not quite conclusive evidence that they are not present. They may be multiple and below the range of microscopic vision. They are present in the lower plants and in animal cells, and it is possible that they may have been lost in the higher plants in the process of differentiation.

When they are present, centrosomes go on from generation to generation dividing with the cell, one going to each daughter-cell produced by the division of the parent. They play a very important part in the phenomenon of mitosis, as will be seen later.

Often the centrosomes are surrounded by an area of protoplasm which is denser than the rest of the cytoplasm. This condensed mass is called the "archoplasm." The archoplasm is often particularly well marked in the case of cells which are about to produce sexual elements, in some leucocytes, and in some of the cells found in cancer. When no archoplasm is to be seen, the centrosomes are sometimes surrounded by radiations. This formation is known as the "aster." Frequently in cells that are in a vegetative condition the centrosomes lie in the cytoplasm with practically no differentiated area around them, and they may then be almost impossible to demonstrate.

Of all these constituent parts the two which are of vital necessity to the cell are the nucleus and cytoplasm. As we have seen, all the other cell structures may be absent. We may even go further, and say that only the linin with its chromatin and the general protoplasmic ground work are necessary, for the nuclear membrane disappears during the process of division, and seems to be absent altogether in some unicellular forms.

Besides this morphological evidence as to the necessity for both nucleus and cytoplasm there is a considerable amount of experimental evidence which appears to prove this necessity. At any rate the evidence that a portion of cytoplasm that does not contain a portion of nucleus upon separation from a living cell will certainly die, is conclusive. The majority of these experiments have been made with unicellular animals, and in them it has been found that while a portion of cytoplasm containing even a minute portion of nucleus will, when separated from the animal, develop into a complete animal, portions of cytoplasm devoid of any portion of nucleus, though they are able to move about and engulf food particles, are unable to digest, unable to regenerate the cell membrane or organs, and die in a comparatively short time.[1] An example of a similar piece of evidence with regard to multicellular forms is the well-known fact that, if a nerve fibre in one of the higher animals be cut, the portion which is separated from the nucleus of the nerve cell proceeds to degenerate, while a new fibre grows out from the part of the cell which is connected with the nucleus to take the place of the degenerated portion.

These facts indicate that the active metabolism of the cell, that is such processes as digestion and secretion, is performed or controlled by the nucleus. It also appears that growth and the regeneration of organs, or indeed of any part of the cell which may be destroyed, is dependent upon the presence of the nucleus. Chemical evidence, with

[1] Brandt, 1877 ; Nussbaum, 1884; Gruber, 1885; Verworn, 1888; Lillie, 1896.

which it is not proposed to deal, also seems to corroborate this view.

We shall see also that there is very strong evidence that certain hereditary characters, or rather the potentiality of producing them, is transmitted by the linin and possibly to a lesser extent by the chromatin of the nucleus.

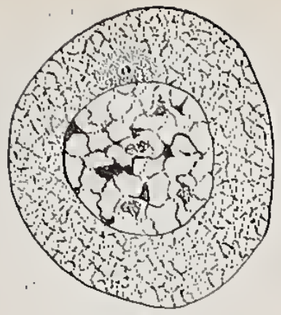


FIG. 5.

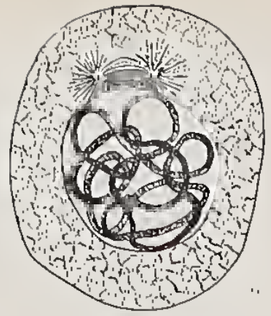
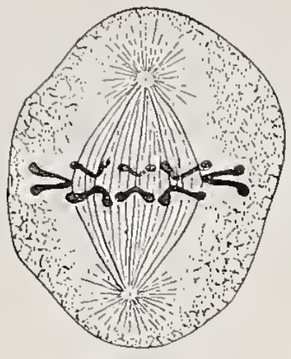


FIG. 6.

FIG. 7.

FIG. 8.



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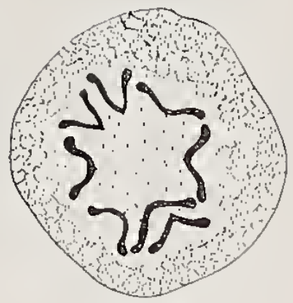


FIG. 10.

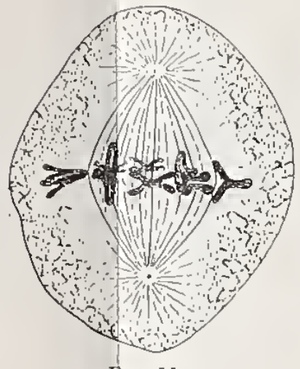


FIG. 11.

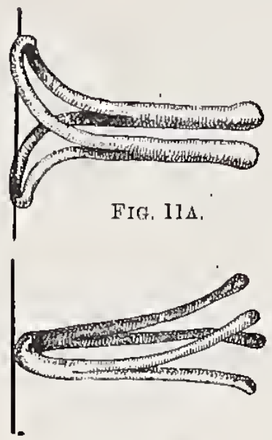
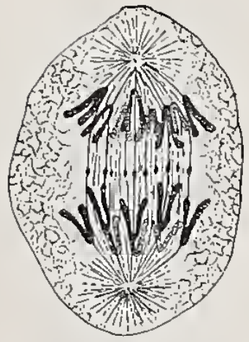


FIG. 11A.

FIG. 11B.



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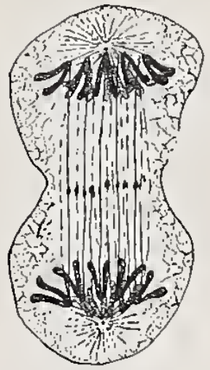

FIG. 13.

FIG. 14.

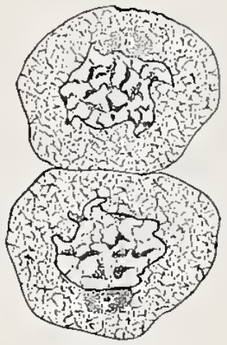

FIG. 15.

SEMI-DIAGRAMMATIC REPRESENTATION OF THE PROCESS OF MITOSIS IN A CELL EXHIBITING 8 CHROMOSOMES.

FIG. 5.—A cell with the nucleus in the vegetative condition.

FIG. 6.—Early prophase of division. The linin and chromatin have adopted the form of a coiled-up thread (spireme). The asters and spindle are forming in connection with the centrosomes.

FIG. 7.—The spireme has broken up into the chromosomes, and the spindle is in a more advanced stage of formation.

FIG. 8.—The nuclear membrane has disappeared. The spindle is fully formed.

FIG. 9.—Each of the chromosomes has become attached to a spindle fibre. Metaphase. [Lateral view.]

FIG. 10.—Metaphase. [Polar view.]

FIG. 11.—The chromosomes are splitting lengthwise.

FIGS. 11A and 11B.—The different ways in which the chromosomes split.

FIG. 12.—The halves of the chromosomes are travelling towards the opposite poles of the spindle. [Early anaphase.]

FIG. 13.—Later stage of anaphase.

FIG. 14.—The daughter-cells are separated from each other. [Telophase.]

FIG. 15.—The reconstruction of the daughter nuclei.

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CHAPTER III.

CELL DIVISION.

It is of the greatest importance to the right understanding of the phenomenon of mitosis that all mental concepts of the various phases of the process should be definitely three dimensional. It is extremely difficult to convey the idea of three dimensions in a diagram or even in the portrait of a cell. Under the high powers of the microscope, which are necessary for the observation of cell phenomena, but a very thin optical section of a cell can be seen at a time, so that actual observation of the mitotic figures themselves is frequently of but little help unless a mental picture be built up from the various optical sections as they successively come into focus. The cell is generally roughly spherical, and always extends in three dimensions. Therefore all the figures which are produced in the various cell processes occupy three dimensions, and this fact must be kept in mind in considering them.[1]

When a cell is in a vegetative condition a reticulum of linin containing granules and masses of chromatin is usually found in the nucleus. When, however, the cell is about to divide into two daughter-cells, the linin and chromatin

[1] The stereoscopic illustrations on cards in the cover of the book, will, when looked at through a stereoscope, give the reader a proper conception of the phenomenon of mitosis. A more complete set of sterescopic illustrations are published separately. (See advertisement at end of book.)

undergo some very striking changes in form and arrangement. Following the nomenclature introduced by Strasburger, Flemming, and others, the phenomenon of mitosis may be divided into (1) "Prophase," (2) "Metaphase," (3) "Anaphase," and (4) "Telophase."

The most usual sequence of events is as follows—

The chromatin and linin gradually lose their irregular arrangement, and adopt the form of a coiled-up thread. This thread is much thicker and more definite than any of the threads of chromatin and linin that are seen in the vegetative condition (Fig. 6). It is usually formed of a ribbon or tube of linin, in which are distributed chromatin granules. These granules are much more regularly arranged along this coiled thread than is the case with the threads of the resting nucleus. Sometimes the arrangement of the chromatin is so close that the thread appears almost solid. Again, in other cases the granules coalesce in small masses, so that the coil has the appearance of a thread of beads. In many cases the coils of the thread can apparently be followed indefinitely, and many observers hold that it may be continuous—endless, in fact. This coiled thread is called the "spireme." In other cases, however, the spireme is divided into definite segments from the time of its first appearance. Frequently the spireme is at first thin and very closely coiled, but later it spreads out and grows thicker and shorter. From the time of the appearance of the spireme the chromatin seems to increase in quantity, to become denser in texture, and to stain more darkly. Eventually the spireme thread breaks transversely in several places, thus forming a number of rod-like bodies which often retain the curve of the spireme, being in the shape of U's (Fig. 7). Frequently,

however, they are V-shaped, and sometimes they are nearly straight. These rod-like bodies are called "chromosomes," and in them the chromatin appears to reach its maximum of density and staining reaction.

The formation of a spireme is by no means a constant prelude to cell division. Sometimes the chromosomes appear directly, without any coiled thread being observable. Here the usual sequence of events is that certain areas in the nucleus appear to grow denser through the accumulation of linin and chromatin. At first more or less nebulous, these masses gradually acquire a definite shape, and become dense in structure. Though generally in the form of rods bent into the form of U's or V's these chromosomes sometimes appear as oval or round masses.

One of the most striking facts connected with the chromosomes is that every species of animal or plant appears to possess a characteristic number. Thus in man we find 32, in a mouse 24, in a donkey 36, in a cockroach 32, in *Ascaris megalocephala*, var. univalens 2, var. bivalens 4, and so on in different organisms. This characteristic number appears in all the cells forming the soma or body of the organism. Though dividing cells may exhibit a number of chromosomes which differs from the characteristic number of the organism to which it belongs, the number is extraordinarily constant, much too constant to be a matter of coincidence. The number of the chromosomes is known to be modified in the most marked manner by pathological conditions, so that a few cases of departure from the usual number among the cells of an apparently healthy multicellular organism is not in the least a surprising matter.

While the chromosomes have been in process of formation

very striking changes have been taking place with regard to the centrosomes. It has been stated that the centrosomes are sometimes surrounded by radiations, forming what is known as the aster. In the prophase of mitosis an aster generally appears, round the centrosomes if they are present, round a clear area of cytoplasm close to the nucleus in the case of the higher plants where no centrosomes are discernible. As the changes proceed in the nucleus the centrosomes begin to separate and travel away from each other rapidly. Besides the radiations extending into the cytoplasm around the centrosomes, fibres may be seen extending from one centrosome to the other, lengthening as the centrosomes get further and further apart (Figs. 6 and 7). From the form assumed by these fibres they have been called collectively the "spindle," and are known as "spindle fibres." In plants which exhibit no centrosomes the spindle is formed in an exactly similar manner, the areas of clear cytoplasm at the centres of each set of radiations acting apparently after the manner of centrosomes.

By the time that the chromosomes have been fully differentiated the centrosomes have become so far separated that they are often as distant from each other as the length of the diameter of the nucleus. Often the spindle partially envelops the nucleus, pressing in the membrane where it touches it.

At this stage the chromosomes are generally found at the periphery of the nucleus, lying just under the nuclear membrane, and then the nuclear membrane disintegrates, setting free the chromosomes (Fig. 8). The nuclear sap and the substance of the cytoplasm are thus obviously mingled

together by the disappearance of the nuclear membrane. This is the end of the prophase of mitosis.

As soon as the chromosomes are liberated by the disappearance of the nuclear membrane each of them becomes attached to one of the spindle fibres. The centrosomes are usually at opposite poles of the cell, and the chromosomes are always attached to the fibres at the equatorial plane of the spindle. This stage, when the chromosomes are attached to the spindle fibres at the equatorial plane, is the metaphase (Fig. 9).

It can easily be demonstrated by comparing the appearance of a cell in this stage of mitosis as regarded from the point of view of one of the centrosomes (polar view) with the appearance it presents when looked at from a point of view level with the equatorial plane (lateral view), that the U or V shaped chromosomes all tend to lie flat upon the equatorial plane (Figs. 9 and 10).

Very soon after the chromosomes have got into position upon the equatorial plane of the spindle, they begin to split lengthwise (Fig 11). A very common mode of splitting is that illustrated in Fig. 11 A, but often they split as shown in Fig. 11 B. This splitting is the commencement of the anaphase, during which the chromosomes are individually split into halves which are destined to be distributed, a half of each chromosome to each of the daughter-cells produced by the division.

Each of the halves produced by this longitudinal splitting travels along the fibre to which it is attached towards the nearest centrosome at the pole of the spindle until a half of each chromosome is quite close to each of the centrosomes (Figs. 12 and 13). There is thus a group of the longitudinal

halves of each chromosome collected round each of the centrosomes. The cell at the same time becomes elongated and eventually contracts in the middle in the situation of the equatorial plane, assuming, more or less, an hour-glass shape. This contraction continues, pressing in the spindle fibres which have become parallel, until the two parts of the cell are completely separated from each other (Fig. 14). Thus the two daughter-cells arise, each containing an exact half of each of the chromosomes contained in the parent cell. The stage of actual separation is the telophase.

The spindle fibres are still apparent, even after the two daughter-cells are completely cut off from each other. As the two halves of each chromosome separate and travel towards the respective centrosome they are still connected by a spindle fibre, and these fibres are at first parallel with each other, being subsequently pressed together at what was the equatorial plane of the spindle. They often show very marked thickenings at the point where the plane crossed them, and so, when they are all pressed together at one point when the two daughter-cells are separated from each other, a body is formed by these thickenings (Figs. 13 and 14). This is the "mid-body" or cell plate.[1] Though common and very well marked in the case of many plant cells this phenomenon is often not observable in any marked degree in the case of animal cells, and is frequently absent.

The asters round the centrosomes, both before and after the disappearance of the nuclear membrane, are generally

[1] In animals the bodies thus formed are known as mid-bodies, in plants as cell plates. Though far more definite, the cell plate in the case of the plant cell is probably analogous to the mid-body occurring in the animal cell.

extremely well marked in all divisions occurring among embryonic cells. As the organism approaches the adult condition the asters are often less and less marked and in the division figures seen in the cells forming adult tissues they are frequently absent altogether.

The chromosomes in the two daughter-cells are congregated together round the centrosomes. Here, as soon or even before the cytoplasm has contracted so far as to be definitely separated into two masses, they begin to form new nuclei. There are two usual modes by which this is accomplished, though there are many variations from them. The congregated chromosomes may form a figure which closely resembles the spireme of the prophase of division, except that the coil never seems to be continuous, and is comparatively very thick. The new nuclear membrane is then formed. In Ascaris this is apparently brought about by processes of linin growing out from the chromosomes to form the new nuclear membrane. Other processes grow between the different segments of the coil, the chromatin is distributed in them, and the nucleus gradually resumes the normal vegetative appearance (Fig. 15). The chromosomes may never assume such a position as to simulate a spireme, but processes may simply grow out from them in the way just described. In many cells on the other hand, each chromosome seems to become more or less vesicular, appearing to some extent like a small nucleus. These small structures then fuse to form the nucleus of the new cell.

It is most important to realise thoroughly that each daughter-cell receives an exact half of each of the chromosomes that is contained in the parent-cell. It is upon this

ground alone that any probable morphological theory as to the mode in which certain hereditary characters are transmitted can at present be based, as we shall see later. The single cell from which the multicellular organism is built up divides, and half of each chromosome contained in it goes to each of the daughter-cells produced. This process is repeated in each succeeding cell division, the substance of which the chromosomes are formed apparently growing in bulk at each succeeding cell generation. The obvious result is that every cell forming every tissue of the multicellular organism possesses individual chromosomes, each of which is probably derived by a direct succession of divisions with intervening periods of growth from the actual substance of the corresponding individual chromosome present in the single cell from which the organism was built up. It must be remembered too, that the division of the chromosomes being longitudinal there is apparently a derivative of every transverse section of every chromosome present in the parent-cell in all the cells produced in subsequent generations.

The general constancy in the number of the chromosomes exhibited by the cells of any given organism is so great as to completely outweigh the occasional departures from this rule. The fact that pathological conditions will produce irregular mitoses makes it evident that many exceptions to the normal number of chromosomes ought to be expected when many cells are examined. The fact that a number of exceptions *are* found cannot therefore be used as a valid argument that the number of the chromosomes varies normally to any extent in the cells of similar organisms, at least not on the evidence that is available at the present

time. On the other hand, as we shall see later, the variations that do occur, under exceptional circumstances, from the normal number of chromosomes in the cells of any given organism may have a special significance of their own.

The process of mitosis is subject to several modifications, some of which are normal, others being caused by abnormal conditions, some of which are pathological.

Sometimes the chromosomes divide and the daughter nuclei are formed without the cytoplasm of the cell dividing.

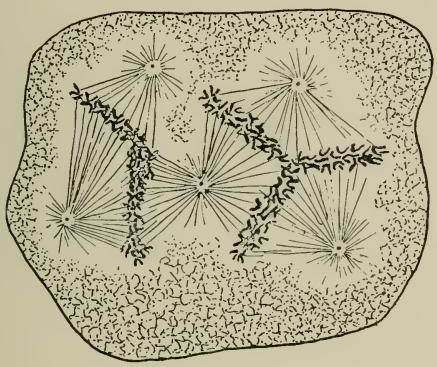


Fig. 16.

A condition is thus produced in which two or more nuclei are included in a common cytoplasm. When these daughter nuclei in their turn divide, the several spindles become intermingled, though there are three, four, or more poles. Such forms are known as "pluripolar mitoses" (Fig. 16). In organisms where multinucleate cells are common, pluripolar mitoses are also common. Though they have long been recognised as normal in the cells of certain plants, they were regarded as abnormal in the cells of the higher animals until quite recently. They have been shown,

however, to occur normally in certain cells even among mammals (*e.g.*, Myeloplaxes—certain large cells found in the bone marrow). Certain cells in many organisms divide amitotically, that is, without the appearance of chromosomes or the formation of a spindle. In such cells the nuclei often divide without the cytoplasm dividing, and this is another cause of pluripolar mitoses. The result of a pluripolar mitosis is usually that the cell divides into the same number of daughter-cells as there are poles in the figure. Sometimes however it may be that a similar number of nuclei are produced without a division of the cytoplasm occurring.

Among the pathological causes of irregular mitoses are certain poisons, which if allowed to act upon the cells without killing them, cause an unusual number of mitoses, many of which will be abnormal. Nicotine, cocaine, iodide of potassium, antipyrine, quinine, and other substances have been used experimentally for this purpose. Sometimes the irregularity of the mitoses is due to one centrosome dividing and not the other, which causes tripolar, quadripolar and other forms of spindles. In other cases the mitosis is asymmetrical, more chromosomes going to one pole than to the other, and so an unequal number of chromosomes in the daughter-cells results. Again, some of the chromosomes may fail to become attached to spindle fibres, and degenerate without being involved in the process of mitosis.

It would seem probable that the irregular mitoses observed in many diseases are due to the presence of a poison produced by parasitic organisms or by some other cause. At any rate it is obvious from the results of

experiments that conditions that are only slightly abnormal may produce abnormal mitoses.

As has already been pointed out, though cells generally divide by the process of mitosis, some cells divide by the

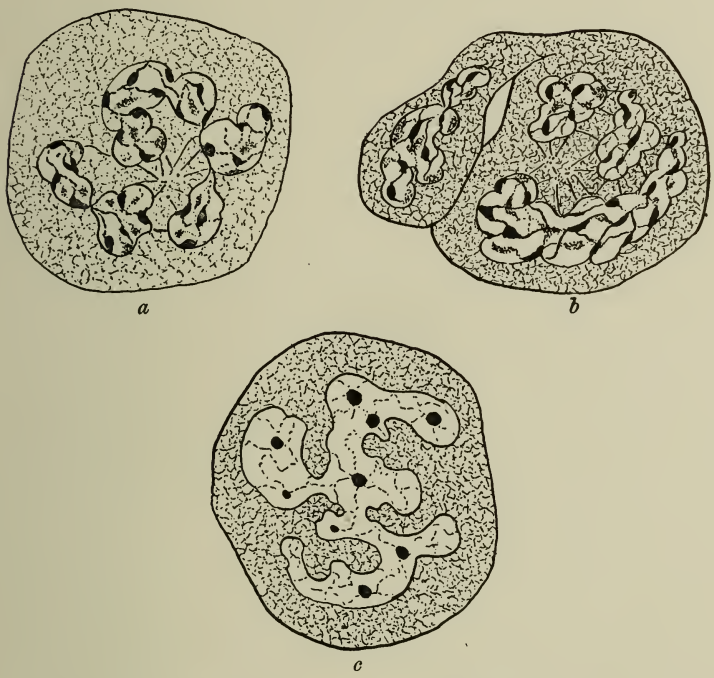


FIG. 17.—Amitotic division. *a*, a large multinucleate cell from the bone-marrow of a guinea-pig (myeloplax). The nuclei are dividing amitotically; *b*, a similar cell where a portion of a cytoplasm is dividing off amitotically; *c*, the nucleus of a cell in the testis of *Triton* dividing amitotically.

apparently simpler process of amitosis. Here both nucleus and cytoplasm divide in a similar manner to a drop of viscous fluid. The contents of the nucleus remain in the vegetative condition, and the nuclear membrane does not disappear. Both nucleus and cytoplasm are constricted at

one part until they separate. Generally the process occurs first in the nucleus independently of the cytoplasm, and thus we find multinucleate cells produced (Fig. 17). In most cases there is no evidence as to whether the centrosomes play any part in amitosis, though it would appear that they frequently divide at or about the same time as the nucleus.

It was generally believed, until quite recently, and many observers still hold, that amitosis never occurs except as a prelude to degeneration. There is, however, no doubt whatever that, in very many cases, amitosis cannot in any way be regarded as a sign of approaching degeneration. In certain animals (Amphibia), among the cells that are destined to produce the male sexual elements, amitotic generations are followed by several generations of mitotic divisions, and the mature sexual elements thence derived fuse with others to produce new individuals. Again, among the cells in the bone marrow, amitotic are followed by mitotic divisions.

As far as we can see at present, amitosis involves the division of the nucleus in bulk, just as though a portion were cut off hap-hazard. If this is really the case, amitosis is one of the most inexplicable phenomena exhibited by cells, when it is remembered how accurately individual parts of the cells involved are sorted out and preserved at each succeeding division both before and after this apparently undiscriminating mode of division occurs. It may be found eventually, however, that amitosis is anything but the simple process it appears to us at present.

CHAPTER IV.

THE MEIOTIC PHASE.

It has already been explained how, during the phenomenon of mitosis, all cells exhibit a definite number of chromosomes, and that though in different species of both animals and plants this number varies, in the cells of the same species the number of the chromosomes remains the same. This constancy in the number of the chromosomes holds good for all the cells of the co-ordinate soma or body of the multicellular organism. A period is reached, however, in the life of the organism when certain cells depart from this uniformity of behaviour and in these the phenomenon of division differs widely from what is seen to occur in the cells forming the somatic or body tissues. The period at which this divergence on the part of certain cells takes place is when the organism reaches sexual maturity, and the result is the production of cells which are in a suitable condition to fuse with cells that have passed through the same series of changes in a similar individual of the opposite sex, and so to create a fresh individual.

The main feature of the form of mitosis by which these sexual cells are produced is, that the number of chromosomes is reduced to one-half of that found in dividing cells forming the somatic tissues of the same organism. This makes the utility of the phenomenon obvious. If the number of the chromosomes exhibited by the sexual elements remained

the same as that of the cells forming the somatic tissues, the fusion of two cells would double their number, and all the cells forming the soma of the new individual would exhibit this double number. Not only would this happen but the number of the chromosomes would be doubled at each succeeding generation. We know that in the same species the chromosomes are not increased by the fusion of the sexual elements, and this fact is explained by the reduction to one-half in the number of the chromosomes that occurs in the production of the mature sexual cells.

The terminology with respect to this phenomenon of reduction is in a state of considerable confusion. In 1887 Flemming first described a form of mitosis which differed materially from the usual somatic form. This he called the "heterotype" mitosis. It has since been shown that it is by this peculiar form of mitosis that the chromosomes in the daughter-cells produced by it are reduced to one-half of the somatic number, and that this form of division always precedes the production of sexual elements.

In the following pages it is proposed to use the term "Meiotic Phase" for the whole period during which reduction is taking place, and "Meiotic Division" for that particular division which is the culminating point in the Meiotic Phase.

Though the series of phenomena observed during the Meiotic Phase is extremely complex, a great similarity exists between that occurring in animals and in plants, a similarity so striking that it may justly be said to differ only in minor details and not in essentials or in general outline.

It is necessary, in order to obtain a clear conception, not

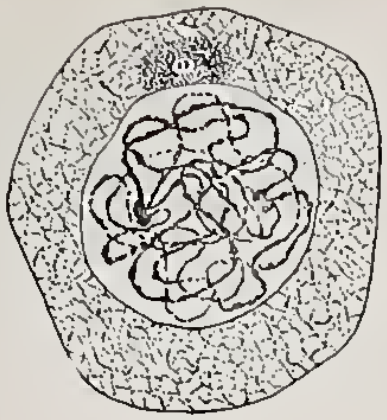


FIG. 18.

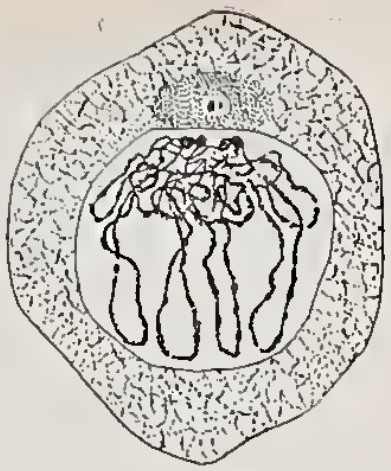


FIG. 19.

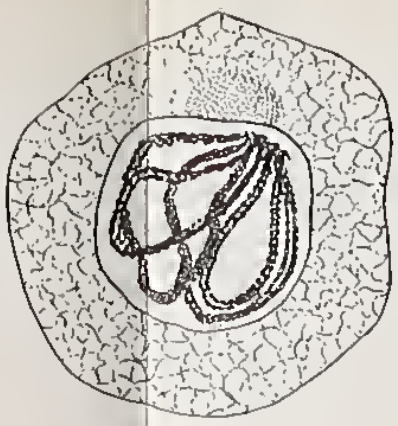


FIG. 20.

FIG. 21.

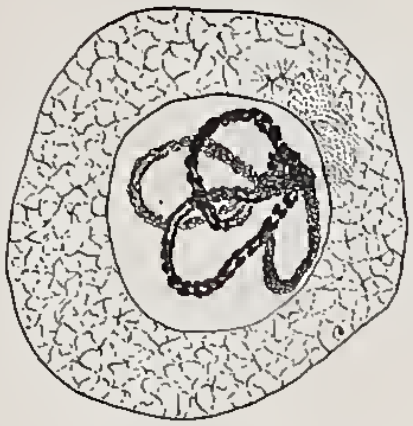


FIG. 22.

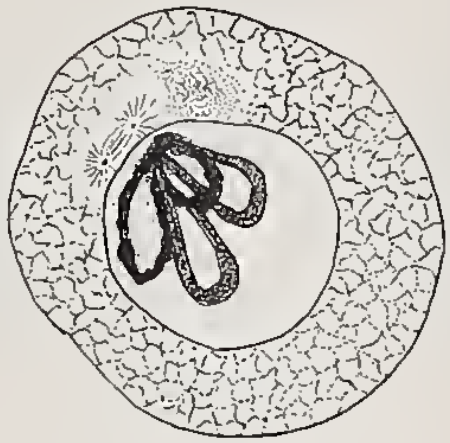
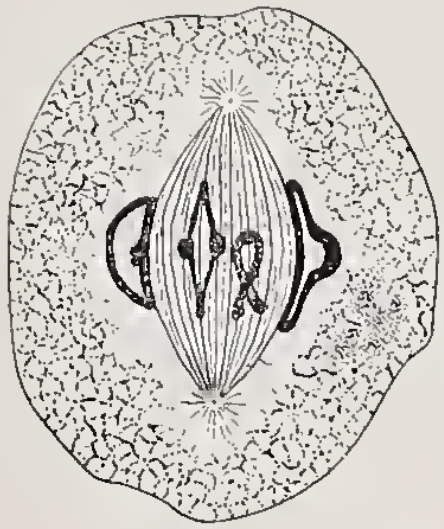


FIG. 23.

FIG. 24.

FIG. 25.



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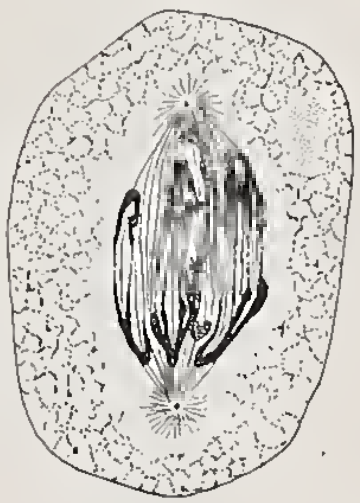

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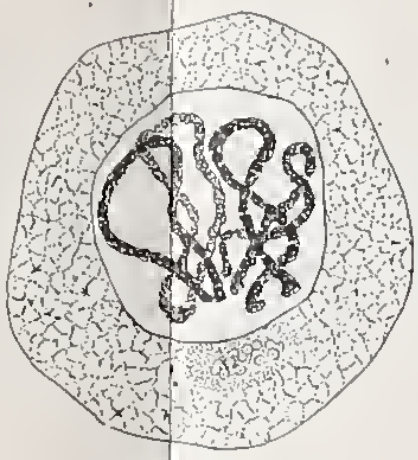


FIG. 28.

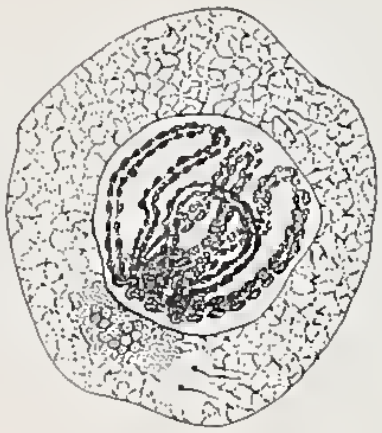


FIG. 29.

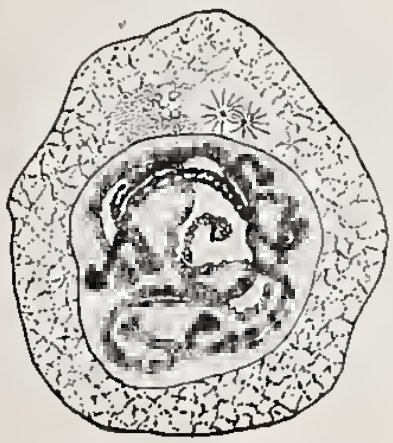


FIG. 30.

THE MEIOTIC PHASE (Semi-diagrammatic).

FIG. 18.—The early fine spireme. FIG. 19.—The first synaptic contraction figure. FIG. 20.—Thickening and double beading of the loops. The centrosomes are outside the archoplasm. FIG. 21.—The coarse spireme. FIG. 22.—Beginning of the second synaptic contraction. FIG. 23.—The second synaptic contraction figure. FIG. 24.—The meiotic gemini formed. FIG. 25.—The nuclear membrane has disappeared. FIG. 26.—The gemini attached to the spindle fibres. FIG. 27.—Separation of the meiotic gemini.

In vertebrates and in many other animals the changes are somewhat different to what is represented in Figs. 20 *to* 23. *They are as follows:* FIG. 28.—Loops are thickened. The centrosomes leave the archoplasm. The archoplasmic vesicles appear. FIG. 29.—The loops show double beading. Minute flagellæ grow from the centrosomes. FIG. 30.—The loops spread out.

[*To face* p. 33.

only of the Meiotic Phase itself, but of the multicellular organism as a whole, to divide the several series of cell-generations into Pre-Meiotic, Meiotic, and Post-Meiotic.

The Pre-Meiotic generations are all those extending from the first segmentation of the fertilized ovum to the prophase of the Meiotic division. Thus all the cells forming the various tissues of the organism that are not passing or have not passed through the Meiotic Phase must be classed as Pre-Meiotic.

The Meiotic Phase includes the series of changes following the last Pre-Meiotic division and the Meiotic division itself.

The generations following the Meiotic Phase are Post-Meiotic, and the cells of these generations retain the reduced number of chromosomes. Post-Meiotic cells may form actual sexual elements, or may form tissues possessing the characters and functions of tissues formed of Pre-Meiotic cells, that is, tissues similar to those of the co-ordinate soma.

The essential characters of the Meiotic Phase, common to both animals and plants, though very complex, are also very definite. It was seen that in the pre-meiotic cells the prophase of division is comparatively simple and brief. The linin and chromatin generally, though not always, appear in the form of a more or less definite thread or spireme, which immediately breaks up into a definite number of segments (chromosomes). The prophase of the meiotic division, on the other hand, exhibits a number of extremely characteristic and very striking stages.

The cells immediately destined to go through the meiotic phase grow considerably, thus being larger than most of the

somatic cells. The nuclear reticulum becomes closer, more chromatic and often polarised. The polarisation increases, and eventually it is seen that the linin and its contained chromatin is arranged in the form of a definite number of loops or rods. Where these loops can be counted, it is seen that there are half as many loops as there are chromosomes in the pre-meiotic cell when it divides. In those cases where special observations have been made, it is found that these loops have a particular origin and arrangement.[1] In such cells, in the species which have been specially observed, it is found that a more or less constant number of chromatin masses are found at this stage and just before. The number of these masses, which in some organisms are in pairs, is generally one half of that of the somatic number of chromosomes.[2] Where the number varies it is due either to the coalescence or to the unusually early division of some of these masses. From each mass a thread of linin, containing a varying amount of chromatin, passes into the substance of the nucleus, and if the course of these threads is traced it is seen that their other ends return into some part of the same chromatin mass from which they started (Fig. 18). This gives the appearance of two threads leading from each chromatin mass. In reality these threads are the ends of the loops whose number is half that of the somatic chromosomes.

Shortly after this stage has been reached the loops begin to contract away from the nuclear membrane. As the contraction takes place in these threads, the chromatin masses

[1] Moore and Walker.

[2] Where the masses are in pairs, the appearance of one quarter of the somatic number of chromosomes is given.

migrate to one part of the nuclear membrane, and that curious and striking arrangement of the nuclear elements arises to which the term "Synaptic" was originally applied.[1] This is the first "synaptic contraction" (Fig. 19).

Some of the chromatin masses frequently coalesce at this period, and the loops become thicker and better defined owing to the presence of an increased amount of chromatin in the linin forming them. After a short time the chromatin granules in the threads divide and give an appearance of splitting or double-beading to each individual loop[2] (Fig. 20).

Shortly after this has happened the chromatin masses divide, and it is eventually seen that each consists of the peripheral end of a loop. This implies that each original mass has divided into its two constituent parts, and that subsequently two ends of each loop are separated, each end carrying with it that part of the chromatin mass to which it was attached. There results, therefore, the same number of chromatic ends to the loops as there are somatic chromosomes in the pre-meiotic cell. Further, it is often easy to make out that each loop is more or less divided in the middle, so that there are the same number of bodies composed of linin containing chromatin in the cell at this stage of the meiotic phase as there are chromosomes in the pre-meiotic cell, though in the meiotic cell they adhere to each other in pairs (Fig. 21). As we shall see later, if it has not already become evident, each of these two components of the synaptic loops probably represents a pre-meiotic chromosome.

[1] Moore, 1895.
[2] Farmer and Moore

The moving apart of the ends of the loops is accompanied by a considerable increase in their thickness, and an accentuation of the splitting or double-beading, so that the threads are apparently longitudinally divided from end to end.[1] The ends of the loops are apparently attached to the nuclear membrane, and at these ends the splitting of the chromatin is often as distinct as it is at any other part of the thread. The divergence of the ends of the loops also causes the loss of that appearance of polarity that was so striking during the first synaptic contraction. The arrangement of the nuclear contents appears to be quite irregular, the threads seeming, upon anything but a most detailed examination, to be continuous, which has led to this stage being called the "coarse spireme."

The details with regard to the loops and segments of the coarse spireme being separate from the commencement of the meiotic prophase have not as yet been followed in many forms. Except for this, however, the process appears to be uniform.

This so-called coarse spireme stage is in many cases succeeded by another contraction in the loops in which the double beading is lost, and the two constituent parts of each are joined more or less completely end to end (Figs. 22 and 23). In this, the "second synaptic contraction," the loops become much thickened and their ends often travel to one area of the nuclear membrane, so that an apparent polarity in the nuclear contents is again observed. The second contraction figure lasts but a very short time in every case. While it is usual in plants, it is very often absent in animals. It appears to be absent in all vertebrates, but is very well

[1] Farmer and Moore.

marked in the cockroach. Gradually each loop assumes a definite shape, and in assuming these shapes the changed loops, which are pairs of pre-meiotic chromosomes, hereafter called "gemini," are dispersed irregularly to the periphery of the nucleus and lie just beneath the nuclear membrane (Fig. 24). The number of these gemini is of course half that of the pre-meiotic chromosomes.

When the second contraction is absent the separate lengths in the coarse spireme figure directly assume the form of the meiotic gemini (Figs. 28—30).

During the coarse spireme stage, particularly in the latter part of it, the cell frequently grows to a considerable extent, and in some cases it may be seen that the amount of chromatin diminishes. This decrease in the amount of chromatin contained in the tube or ribbon of linin sometimes goes on until there is but little if any chromatin left, and the linin loops stain entirely with the acid dye, showing no trace of any chromatin granules.

It is probable that the correct interpretation of this phenomenon is that the chromatin is used up in nourishing the cell during a period of unusually rapid growth. In any case it would seem to indicate that the chromatin is only a secretion of the linin, and is not in itself a permanent constituent of the nucleus handed on from generation to generation of cells, and growing during the intervening vegetative periods, as was supposed by the earlier observers.

While this has been happening in the nucleus the spindle has been forming in the cytoplasm of the cell just as occurred in the case of the pre-meiotic division. At this stage the nuclear membrane disappears, and meiotic gemini are let free in the mingled nuclear and cytoplasmic substance

(Fig. 25). The gemini then become attached to the spindle, a single spindle thread to each of the gemini, just as happened to the single chromosomes in the pre-meiotic division.

It has already been explained that in the pre-meiotic division the chromosomes, before they have split longitudinally, generally lie flat upon the equatorial plane of the spindle. The meiotic gemini on the contrary, lie upon the spindle at right angles to the equatorial plane, with their axes parallel to the axis of the spindle (Fig. 26). Though a longitudinal split may be, and often is, apparent in the gemini, they do not divide in this manner, but the pair of chromosomes that goes to form each of the gemini separates into its two constituent parts, giving the appearance of transverse division in what were originally supposed to be single chromosomes. As the process of division goes on therefore, the pre-meiotic chromosomes that are joined together to form the meiotic gemini are separated, one from each pair going towards each pole. When the cell divides into two daughter-cells, it is obvious that each daughter-cell will receive only half the pre-meiotic number of chromosomes in its nucleus.

A longitudinal splitting is often observable in the meiotic gemini while they are upon the equatorial plane of the spindle, as was stated in the last paragraph. This splitting becomes more obvious when each of the gemini is divided into its two constituent parts, so that in the later phases of division, when they are approaching the poles, each chromosome may, under favourable conditions, be seen to be longitudinally split. This split, which in the anaphase is quite distinctive of the meiotic division and not present in

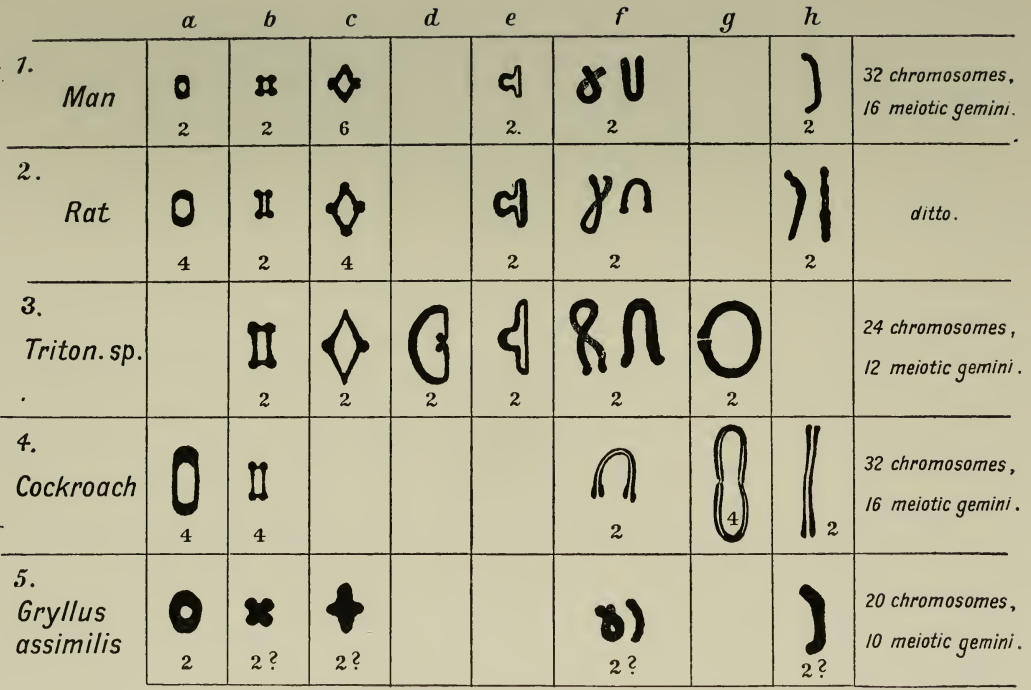


FIG. 31.—The forms of the Meiotic gemini in several different organisms. 1 to 4 after Moore and Arnold (Proc. Roy. Soc., 1906); 5, Baumgartner (Bio. Bul., 1904). Baumgartner states definitely that there are two rings (*a*). The other shapes *b*, *c*, *f* and *h*, with the numbers of each, have been gathered from a careful consideration of his figures in view of the subsequent work of Moore and Arnold, and of his own suggestions.

the similar stage of the pre-meiotic, is not consummated. It is by many, and with some reason, regarded as being the actual longitudinal fission of the chromosomes that takes

place in the succeeding post-meiotic division. Whether this is or is not the case, the longitudinal splitting of the chromosomes disappears during the reconstruction of the daughter nuclei produced by the meiotic division, except, as we shall see later, in the few instances where the daughter nuclei are not reconstructed after this division.

In the pre-meiotic divisions, by which as we have seen, all the cells of the soma were produced from the fertilized ovum, the chromosomes were all of the same shape. They take the form of rods, often bent into the shape of a U or a V. In the meiotic division, however, the gemini assume various definite shapes which are constant in the species of animal or plant in which they occur. They may be many different shapes in a meiotic cell of the same animal or plant, but there never appear to be less than two of the same shape in any such cell. If there be more than two of one shape, the number seems always to be a multiple of two. The shapes assumed by the meiotic gemini in several different organisms are shown in Fig. 31. It will be seen that though some or all of the shapes may be common to two or more species, the number of any particular shape present may vary in the two species. It will also be seen that in some cases forms are present which are absent in others, and that this happens usually in widely divergent forms. Thus of the form *c* there are six in man, four in the rat, and only two in Triton, while the form *d* has hitherto been observed only in Triton. As each of the gemini is formed of two pre-meiotic chromosomes, so when they divide, no matter the shape that has been assumed, they separate from each other when the meiotic division takes place. Being joined end to end, this separation of

the two component parts of the gemini gives an appearance of transverse division in what were originally supposed to be individual chromosomes.

At the end of the meiotic division the two groups of chromosomes are reconstructed into the two daughter nuclei in the same way as reconstruction took place after the pre-meiotic divisions. Each daughter nucleus, however, only receives half the somatic or pre-meiotic number of chromosomes.

In mammals, in many, if not in all vertebrates, and in some invertebrate animals, some remarkable events occur in the cytoplasm of the cell while the meiotic phenomena just described are taking place in the nucleus. They have as yet only been demonstrated in that meiotic division which precedes the production of the male sexual elements. These must be regarded as part of the phenomenon of reduction, as they are not found to occur in pre-meiotic cells, though they do occur in the post-meiotic during the process of spermatogenesis, and in some of the post-meiotic cells found in cancer. It is possible that they also occur during the maturation of the ovum.

The archoplasm becomes much more prominent in those cells which are destined immediately to go through the meiotic phase. As is the case in the pre-meiotic cells, the archoplasm at first contains the centrosomes, but when the meiotic gemini are beginning to separate out, it is seen that the centrosomes migrate out of the archoplasm and lie in the cytoplasm altogether detached from it. A delicate filament grows from each centrosome as it lies in the cytoplasm, but this disappears before the division takes place. When the centrosomes have migrated into the cytoplasm away

from the archoplasm a number of minute vesicles appear in the latter. These archoplasmic vesicles also disappear with the archoplasm when the cell divides (Figs. 28, 29, and 30).

Some observers state that the longitudinal splitting of the loops described here as occurring during the prophase of the meiotic division is really the approximation of two threads. This interpretation, however, would appear to be based upon observations which are too limited as regards the number of species of animals and plants investigated. While in some cases it would be permissible to uphold the statement that the apparent split is due to the approximation of two threads, in many cases it is quite obvious that nothing of the kind happens, but that a single thread is split lengthwise. It is therefore probable, considering the general uniformity of the phenomenon in other respects, that the approximation of the two threads does not occur, particularly as splitting would appear to be just as probable an interpretation as approximation, even in those cases that are considered most doubtful.

During the prophase of the meiotic division which precedes the production of sexual elements in animals a chromatin body appears in the cytoplasm. This is apparently lost during the process of mitosis, but a similar body appears in the post-meiotic prophase, disappears during mitosis, and another, larger than either of the preceding, appears in the daughter-cells that are destined to be converted into the mature sexual elements. This phenomenon is particularly well marked in the production of the male sexual elements. Hermann described these chromatin bodies several years ago, deriving them from the nucleus,

but there seems to be considerable evidence that they arise in the cytoplasm and do not originate from the nucleus. There is no evidence with regard to their function. It has been suggested that this may be the extrusion of the male or female element according to the sex of the organism in which they are produced, but there is no evidence as to this. The suggestion is purely speculative.

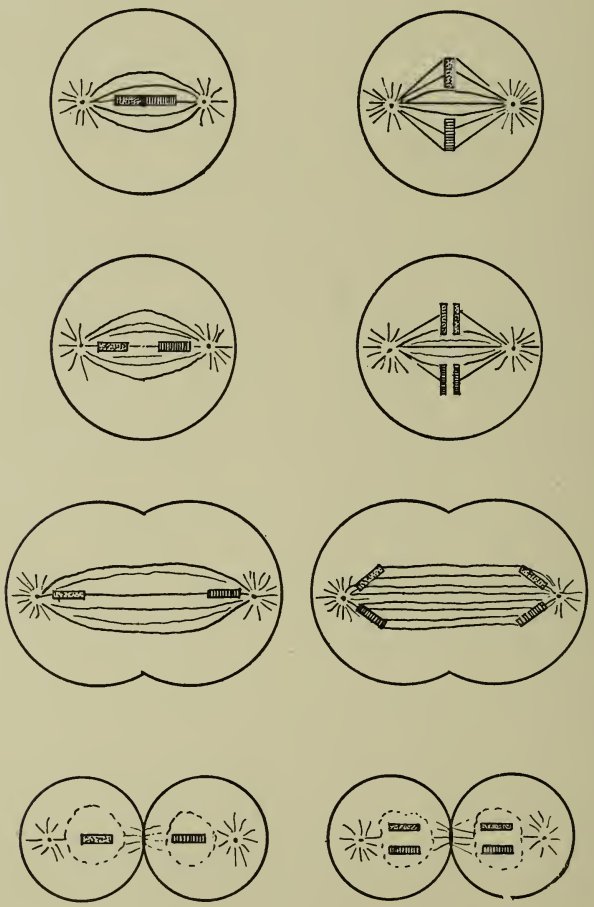
Several observers have described supernumerary chromosomes in the mitoses preceding the production of the male sexual elements in certain animals. Wilson regards some supernumerary chromosomes described by him as sex determinants. These chromosomes have, however, only been described in a few species, and there is no evidence that they occur in any widely divergent forms. The matter is still so much *sub judice* that it would be out of place to discuss it here.

It is of the greatest importance that the essential difference between the somatic or pre-meiotic and the meiotic divisions should be realised as regards the mode of distribution of the chromosomes to the daughter-cells. The accompanying diagram will make this point clear.

The right-hand column represents the series of changes as regards the chromosomes which takes place in the somatic division. A cell is represented containing two chromosomes, one of these being shaded by lines, the other by dots. It will be seen that each of these (both dots and lines) is represented in each of the daughter-cells produced by the division.

In the left-hand column the meiotic division is represented, also in a form possessing two chromosomes. Here it will be seen that in the prophase the two chromosomes

have become joined together, end to end, and when division takes place this pair of chromosomes simply separates into



its two constituent parts. The result of this is that one daughter-cell receives the chromosome shaded with lines, the other that shaded with dots. Thus if different

characters are contained in different chromosomes or parts of chromosomes, it is evident that the two daughter-cells, which are destined to produce sexual elements will convey different characters. Which character is transmitted to the offspring will therefore depend upon which of the daughter elements fuses with a similar cell derived from another individual.[1]

[1] See Chapter XI.

CHAPTER V.

POST-MEIOTIC DIVISIONS.

As a rule, in animals, only one cell generation intervenes between the meiotic division and the production of mature sexual elements.[1] The daughter nuclei produced by the meiotic division divide once, and the resulting cells are converted directly into spermatozoa in the case of the male, or the maturation of the ovum is complete in the case of the female, without any further division taking place.

In plants, however, an apparently unlimited number of generations retaining the reduced number of chromosomes may intervene between reduction and the production of mature sexual elements.

The first division following the meiotic is very commonly given a special name—the "homotype" division. This first division is supposed by some observers to differ from any succeeding generation of cells with the reduced number of chromosomes in that in some cases the chromosomes in the two daughter-cells resulting from the meiotic division do not apparently reconstruct fresh nuclei, but go on immediately to another division without any period of rest intervening. As the form of division does not, however, differ in any other particular even in these plants, or in any

[1] The only exception with which I am acquainted is in *Hydrophilus piceus*, where, apparently, two generations of cells intervene between the division and the production of spermatozoa (Moore and Walker).

particular at all in the great majority of cases, from the succeeding generations, a special name does not seem to be called for, as it would tend to create the apparently false impression that the first division following the meiotic differed essentially from following generations, which it does not.

In plants it frequently happens that comparatively few of the progeny of those cells that have passed through the meiotic phase are destined to form sexual elements. The rest form tissues which support and nourish the few among them that are destined subsequently to conjugate and produce new individuals. The cells that exhibit half the normal somatic number of chromosomes are in fact obviously capable of differentiation which does not lead to the formation of sexual elements, but which results in their performing functions and exhibiting the characteristics of somatic cells. This capacity of differentiation that does not end in mature sexual cells does not appear, however, to be limited to certain groups of cells in plants. As we shall see later, unfertilized mature ova may proceed to segment and to produce complete embryos, notwithstanding the fact that the cells which build up the various tissues exhibit but half the normal number of chromosomes. There is also evidence which seems to show that some leucocytes, in mammals at any rate, after passing through the meiotic phase, produce generations of cells that possess very different characteristics and functions from the sexual cells in the same organism.[1] The same is also stated to occur among the cells in malignant growths, where many generations containing the

[1] C. E. Walker, 1906.

post-meiotic number of chromosomes are said to be produced.[1]

In any case, whatever be the ultimate destiny of the progeny of the post-meiotic cells, the mode of division is similar to the ordinary pre-meiotic or somatic mitosis, excepting, of course, that the post-meiotic cells exhibit only

FIG. 32.—Prophase of post-meiotic division from the testis of a guinea-pig.

half the pre-meiotic number of chromosomes. The prophase of division is simple and short, just as was the case with the pre-meiotic cells. In post-meiotic cells, however, the formation of a spireme does not seem to be a usual occurrence even in the case of organisms where a spireme is found in the prophase of the pre-meiotic mitoses (Fig. 32). The fact that in some plants there is no apparent period of rest between the separation of the two daughter-cells produced by the meiotic division and the commencement of the first post-meiotic division might account for the absence of the spireme in the case of these plants, but there is nothing to account for its absence in the majority of cases,

[1] Farmer, Moore, and Walker, 1903, etc.

where there is a definite interval between the formation of the daughter nuclei of the meiotic and the commencement of the first post-meiotic division.

The chromosomes are usually in the form of short rods, often curved into the shape of a U, sometimes in the shape of a V. They are generally, if not always, shorter and thicker than the pre-meiotic chromosomes. They split longitudinally, just as was the case with the pre-meiotic chromosomes, and when the cell divides a longitudinal half of each chromosome is distributed to each daughter-cell (Figs. 33—35).

It was seen that in the anaphase of the meiotic division the chromosomes exhibited a longitudinal splitting as they approached the centrosomes. In some cases where the daughter nuclei of the meiotic division are not completely re-formed, and the individual chromosomes do not disappear, this longitudinal split is apparently never lost, and in some other cases, where the chromosomes in the daughter-cells are lost sight of for a time, they are longitudinally split when they reappear, the split taking effect and the two halves of the chromosome being finally separated in the first division following the meiotic.

Very often, more often than is the case in pre-meiotic cells, the chromosomes appear in an oval or in a more or less irregular form. The post-meiotic divisions in those cases where more than one such generation occurs, are all of a similar character.

The behaviour of the centrosomes and the formation of the spindle is similar to what happens in the meiotic division. In many cases in mammals, in Amphibia, in some fishes, and in some other animals, the centrosomes migrate

from the archoplasm and are found in the cytoplasm at some distance from it. They migrate further from the archoplasm than they do in the meiotic division. In some cases (in the spermatogenesis of mammals) the small vesicles appear in the archoplasm after the migration of the centrosomes just as they do in the meiotic phase (Fig. 32),

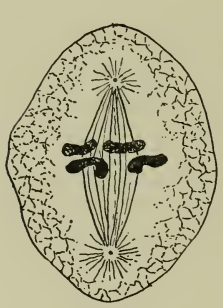


FIG. 33.

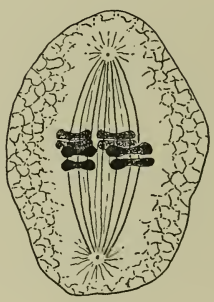


FIG. 34.

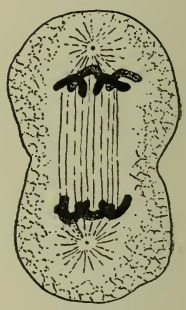


FIG. 35.

FIG. 33.—Post-meiotic division (Metaphase).
FIG. 34.—Post-meiotic division (Early Anaphase).
FIG. 35.—Post-meiotic division (Later Anaphase).

though in other cases where these vesicles are found in the archoplasm of the cells immediately before the meiotic, they are absent in the prophase of the division that follows (*e.g.*, Elasmobranch fishes). When they do appear they are generally more marked than in the meiotic cell, but they disappear in a similar manner when the post-meiotic division takes place.

Post-meiotic cells are always smaller than the meiotic, at any rate for some time after the meiotic division has occurred, and are usually smaller than many of the cells forming the soma from which they were derived—that is, smaller than many of the pre-meiotic cells.

CHAPTER VI.

THE MALE SEXUAL ELEMENTS.

The process by which certain cells derived from the soma of the parent organism become differentiated into the mature male sexual element—the spermatozoon—presents so many points of similarity in all the multicellular animals, excepting perhaps some of the lowest forms, that it is convenient to describe this process in general, remarking upon material exceptions as they occur.

It will already have been realised that those cells that ultimately give rise to spermatozoa have been derived from the fertilized ovum through many cell generations, all of which have been produced in the ordinary pre-meiotic manner. Long before the prophase of the meiotic division has commenced, however, these can as a rule be distinguished from the surrounding cells. They are larger, and show no signs of differentiation. That is, they remain as nearly spherical as surrounding conditions will allow, and do not develop any particular organs or appendages. The archoplasm becomes unusually well marked, often at a very early age. For instance, in the embryo of a mammal the cells that will eventually give rise to the spermatozoa can be distinguished by their comparatively large size and well-marked archoplasm, by the closeness of the linin reticulum, and by other features, long before the testis has begun to show any signs of its later constituent parts, and while it is

merely a mass of apparently undifferentiated tissue lying to one side of the yet unossified back-bone.

Later in the life of the organism the testis develops tubules in the case of mammals, which all lead to a duct that conveys the spermatozoa, and into pockets or more or less angular cavities in the case of most other vertebrates, of Arthropods, and many other animals.

It is in the testis that is divided into pockets and not into tubules that it is most easy to follow the exact sequence of events with regard to spermatogenesis. In many such animals the testes atrophy each year, only a few so-called "male ova," that is, cells destined in a few generations to produce spermatozoa, being left. The process sometimes commences by these few cells dividing amitotically, that is, without the appearance of any mitotic figure. Amitotic division may go on, as in the case of *Triton*, until several pockets of cells are well defined. Then those groups or pockets that were first produced, pass into a stage of mitotic division in which the full pre-meiotic or somatic number of chromosomes is present. This is followed rapidly by the prophases of the meiotic division, the meiotic division itself, and post-meiotic generation. There is, as has already been pointed out, usually but one post-meiotic generation in animals.

When the nuclei of the daughter-cells of the post-meiotic division have resumed the vegetative condition they generally increase in size to some extent. They are now known as "spermatids," and are destined to undergo modifications which will convert them directly into spermatozoa without any further division taking place.

In most cases it is to be noticed that the archoplasm in

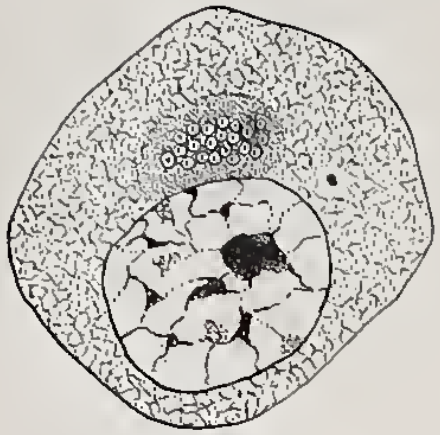


FIG. 36.

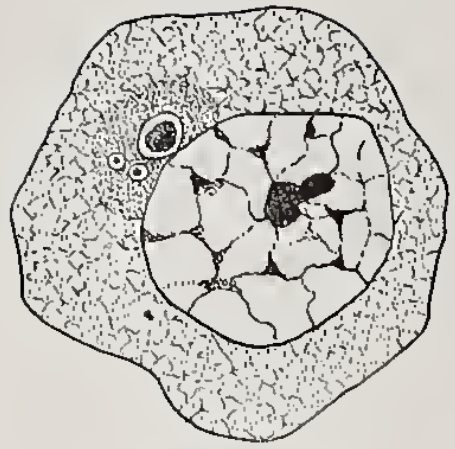


FIG. 37.

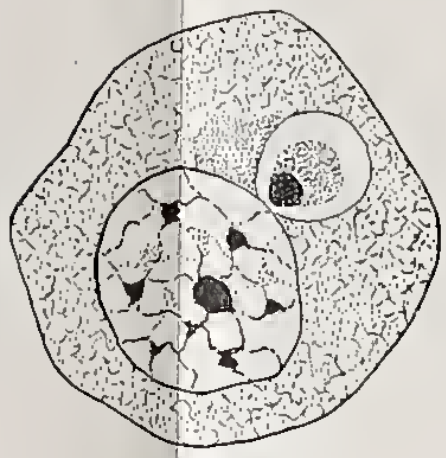


FIG. 38.

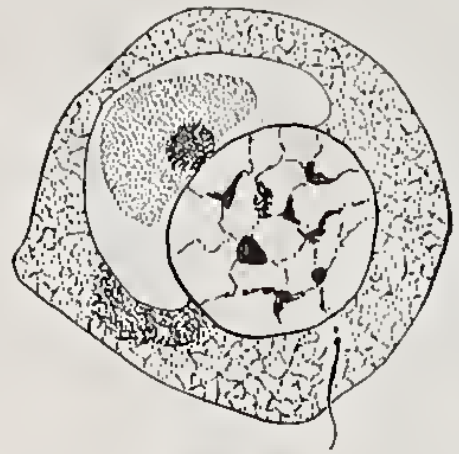


FIG. 39.

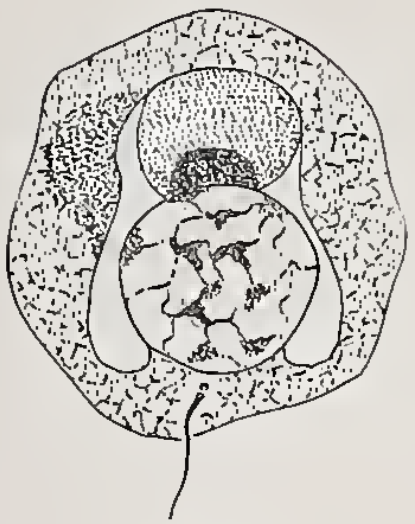


FIG. 40.

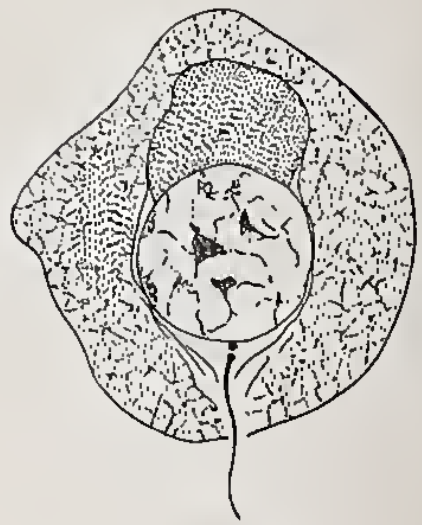


FIG. 41.

FIG. 42.

FIG. 43.

DIFFERENTIATION OF THE SPERMATOZOON IN VERTEBRATES AND MANY OTHER ANIMALS (SEMI-DIAGRAMMATIC).

FIG. 36.—Spermatid. The centrosomes are outside the archoplasm and the archoplasmic vesicles are well developed.

FIG. 37.—One of the archoplasmic vesicles has grown large, and the intermediate substance can be seen. Most of the rest of the vesicles have disappeared.

FIGS. 38, 39 and 40.—Growth and development of the single remaining archoplasmic vesicle.

FIG. 41.—Collapse of all of vesicle excepting cephalic cap.

FIG. 42.—Shrinking of the cytoplasm. Increasing density of nucleus.

FIG. 43.—A mature spermatozoon. (1), cephalic cap; (2), nucleus; (3), middle piece; and (4), flagellum or tail.

[*To face* p. 53.

the spermatids is large and very distinct. Soon after the nucleus has been completely reformed, and the cell has grown to some extent, the centrosomes are seen to have migrated from the archoplasm into the cytoplasm. It seems probable that in some cases they are never in the archoplasm of the spermatid.

The two centrosomes generally lie one outside the other in relation to the nucleus, and in those cases at any rate where the mature spermatozoon possesses a flagellum a filament grows out from the inner of the two centrosomes towards the periphery of the cell. In vertebrates, and in some other animals, the other centrosome seems sometimes to be modified into the form of a ring, through which the filament growing from the inner centrosome passes.

The nucleus, from the time it reaches a vegetative condition, begins to assume a finer structure than is usual among nuclei generally, and as differentiation proceeds the nucleus becomes more condensed and homogeneous.

In mammals, in many if not in all vertebrates, in some Arthropods, Mollusca, and Annelids some remarkable changes take place in the archoplasm which play an important part in the formation of the spermatozoon, and it is quite probable that this series of phenomena may be even more general among animals than is at present known, for but a limited amount of work has been done upon the subject.

We have seen that in mammals and in some other animals, a number of small vesicular structures appear in the archoplasm during the prophase of the meiotic and post-meiotic division, disappearing when division occurs. These vesicles reappear in the archoplasm of the spermatid (Fig. 36), but

instead of disintegrating, proceed to develop. One of two things appears to happen. Either one of the vesicles grows at the expense of the rest, which degenerate, or they fuse together to form one large vesicle (Figs. 37 and 38). This vesicle is bounded by a definite membrane, and its contents are clear and homogeneous, excepting that in the part adjacent to the nucleus, or perhaps in its centre, is a denser and more deeply staining area, containing a smaller and still denser spot. It was seen that in the centre of each of the vesicles that appeared in the archoplasms of the two preceding generations of cells there was a small darkly staining mass. If the history of the small vesicles appearing in the spermatid archoplasm be followed it will be seen that in the single vesicle that survives and grows it is from the small staining central mass that the intermediate and less dark area arises. As the vesicle grows the intermediate substance lies adjacent to the nucleus and it is seen that the nucleus and the vesicle reciprocally press upon each other so that the spherical shape of each is deformed (Fig. 39). The intermediate substance goes on growing until it almost fills up the vesicle, and in some cases, among mammals at any rate, it would appear that there is a small breach in the nuclear membrane where it is adjacent to the archoplasmic vesicle. In many cases the vesicle attains a size equal to or larger than that of the nucleus, and the remains of the archoplasm are seen lying to one side of it.

The vesicle gradually grows round the nucleus until it almost surrounds it (Fig. 40), and then it all collapses excepting at the place where it originally arose in the archoplasm, where it forms a more or less rounded cone with its concave base fitted upon the nucleus. The collapsed

membrane of the rest of the vesicle surrounds the rest of the nucleus excepting a small area at the opposite pole, and in the meantime the two centrosomes have found their way to this small area (Fig. 41). The centrosome which has in some cases assumed the shape of a ring, forms a plate contiguous to the nucleus, while the filament derived from the other has grown considerably, and forms the axial filament of the tail of the spermatozoon. The part of the cytoplasm immediately around the centrosome becomes modified into what is known as the middle-piece of the spermatozoon. It is stated that in mammals, and in those animals in which the archoplasmic vesicle is found, the remnants of the collapsed vesicular membrane play an important part in the formation of the middle-piece. In other cases it is stated that the middle-piece arises directly from one of the centrosomes. Other observers derive the middle-piece from various structures that would appear to be identical with the archoplasm or with some part of it.

The homogeneous conical mass which results from the archoplasmic vesicle forms the so-called "cephalic cap" of the spermatozoon.

There is a great deal of confusion about the sequence of events with regard to the differentiation of the spermatozoon, a confusion created to a certain extent by the nomenclature used by various observers. It is often obvious, in following the accounts of different authors, that different names are used for the same structure or parts of the same structure, and that different names are used for the same thing at different periods of its existence. The exact derivation of the middle-piece, however, is not a matter of fundamental importance, and possibly it may be

derived from different structures in different organisms. There is no doubt, however, with regard to the direct derivation of the nucleus of the spermatozoon from the nuclei of the preceding generations of cells. The development of the central filament of the tail of the spermatozoon in connection with one of the centrosomes seems to be equally certain. The account of the development of the cephalic cap from vesicles arising in the archoplasm is also probably correct in the case of the animals mentioned, and this is likely to prove a very general process.

When the various parts of the spermatozoon have become more or less differentiated the general mass of the cytoplasm shrinks, some portions of it being sometimes thrown off bodily according to some observers (Fig. 42). At the same time the nucleus becomes more and more dense, staining very deeply with basic dyes. Some part of the cytoplasmic substance grows out round the filament attached to one of the centrosomes in many cases, and the tail is thus formed, though often the tail would seem to consist of but little more than the filament itself.

The form of the mature spermatozoon varies to a very considerable extent in different animals, so much so that it is impossible to deal with the varieties here. As has already been said, the commonest form is the flagellate, in which the spermatozoon is more or less in the shape of a tadpole or worm, which swims about actively by means of its flagellum or tail. In this form the spermatozoon consists of (1) the cephalic cap at its anterior end, which is usually more or less conical in shape; (2) the nucleus, often elongated in shape and apparently always very dense and almost homogeneous in structure; (3) the middle-piece, containing

the centrosome, and (4) the tail or flagellum along the axis of which runs the filament attached to and derived from one of the centrosomes (Fig. 43).

In some cases, however, the spermatozoon does not possess any flagellum. This appears to happen among some of the crustacea, nematodes, and a few other animals. Though occasionally they exhibit amæboid movement these forms are usually incapable of movement. A nucleus is always present, but in most of these spermatozoa without flagellæ, the centrosomes have not yet been demonstrated.

The production of the spermatozoids in plants seems to be essentially similar to the process of spermatogenesis in animals. Here, however, a considerable amount of confusion has been caused by the fact that the centrosomes have been called "blephoroplasts" by many observers. There can be but little if any doubt that the so-called blephoroplasts in the spermatozoids of plants are centrosomes, though their history in the preceding cell generations is somewhat different according to some observers. It is generally accepted that the centrosomes disappear and arise *de novo* in the generation preceding the formation of the spermatozoids in certain plants.

It appears that in the development of the spermatozoids in some plants the centrosomes grow into the form of a thread, from which cilia are developed.

In the case of both animals and plants where there are motile organs attached to the male sexual elements, these organs appear to grow from and to be attached to centrosomes.

It is here necessary to refer to certain observations extending over several years (since 1903) which claim to show

that the meiotic phase occurs among the cells of malignant growths (cancer), and that a number of post-meiotic generations of cells are produced.[1] Cancer is known to occur among a number of vertebrate forms, and may be even more widely diffused than is at present known. It is also probable that the occurrence of the meiotic phase among the cells of these growths may possess a more general significance than its direct connection with cancer.

The following is a brief *résumé* of the observations upon this subject, and the deductions that have been made therefrom.

The cells forming a malignant growth at first divide in the ordinary pre-meiotic manner, exhibiting the full somatic number of chromosomes. Multinucleate cells are not uncommon at a slightly later stage, and these frequently exhibit pluripolar mitotic figures. At a later stage some of the cells are seen to pass through the meiotic phase. The nucleus exhibits the characteristic contraction figures; when the chromosomes appear they are in the forms characteristic of the meiotic division; and they are present in half the number found in the somatic or pre-meiotic cells. Apparently a number of post-meiotic generations are produced. A fairly exhaustive series of countings of the number of chromosomes exhibited by dividing cells in several different cases of cancer showed that there was a large proportion of cells in which only half the somatic number of chromosomes was present. The number of cells exhibiting half the somatic number of chromosomes was, in fact, equal to that exhibiting the full complement. Besides these nuclear phenomena there are also certain cytoplasmic

[1] Farmer, Moore, and Walker, Proc. Roy. Soc., 1903, 1904, 1906, etc.

structures common to cells found in reproductive tissue and cancer which are not found in any other class of cells. These are the archoplasmic vesicles, which, in spermatogenesis, become converted into the cephalic cap of the spermatozoon.[1] In the cancer cells in which they occur these vesicles are never developed very much beyond the stage in which they retain their spherical shape. They may, however, attain a size equal to that of the nucleus, and mutual pressure may flatten both nucleus and archoplasmic vesicle so much where they are contiguous that they may appear almost as two more or less equal hemispheres. Though the archoplasmic vesicles in the cancer cells are never differentiated to the same extent as they are in the cells that are converted into spermatozoa (spermatids) the structure of these vesicles is so characteristic and the process of development in both cases being identical, there can be very little doubt that they are homologous. There are thus two entirely independent sources of evidence, one from the nucleus and one from a cytoplasmic structure. On these grounds the tissue forming malignant growths has been called "gametoid tissue" to mark both the similarity and difference between it and "gametogenic tissue" which normally produces the mature sexual elements. The fact that the cells that are destined immediately to produce the sexual elements pass out of co-ordination with the parent soma and live upon it in a parasitic manner is considered, in view of the preceding observations, as being an explanation of the parasitic character of malignant growths. The parasitic nature of more or less independent post-meiotic generations is particularly striking in the case of certain

[1] See p. 53.

plants, where a number of post-meiotic generations of cells are produced, only a few of them being ultimately converted into sexual elements.

The conclusion then is, that through the action of one or several different causes at present unknown, certain cells of the soma, passing out of co-ordination, go through the meiotic phase and produce a number of generations of cells that live upon the parent organism in a parasitic manner.

It has been claimed that some of the cells (leucocytes) in the bone-marrow of mammals pass through the meiotic phase, and subsequently produce several generations of post-meiotic cells, possessing but half the pre-meiotic number of chromosomes.[1]

[1] C. E. Walker, Proc. Roy. Soc., 1906.

CHAPTER VII.

THE MATURATION OF THE OVUM.

The process by which the ovum—the female sexual element—is prepared for fertilization is essentially similar to the process of spermatogenesis. The processes, however, differ considerably in detail. In the ovum the changes which precede fertilization and during which the number of the chromosomes is reduced to one-half of that found in the pre-meiotic or somatic cells, is known as "maturation."

In animals the ovum is of a very large size compared to the rest of the cells found in the body. With a very few exceptions among multicellular animals the ova are collected together and, with the cells that support and surround them, form a definite sexual gland, just as the cells destined to give rise to spermatozoa in the male are collected together in the testis. The sexual gland is the ovary.[1] In the very early stages of the development of the embryo it is impossible to distinguish whether the progenitors of the sexual cells are going to give rise to spermatozoa or to ova, but as development proceeds these cells are differentiated and the sex of the organism becomes apparent.

The cells that become differentiated into ova grow to an enormous size. The nucleus is very large, and though it is

[1] In some hermaphrodite forms (*e.g.*, certain Mollusca) the sexual gland produces both ova and spermatozoa. In these cases the cells representing the two sexes are often inextricably mixed together.

at first in the middle of the cell in most cases it usually travels towards the periphery as the ovum grows. The nucleus was known to the early observers as the "germinal vesicle." In the nucleus one or more spherical chromatin masses are generally present. In many forms only one large chromatin mass is found. This is the "germinal spot" of the earlier observers. A true nucleolus is also sometimes present. The probable or possible significance of these chromatin masses and nucleoli has been the subject of many theories. As, however, it is doubtful whether either possesses any special function, and as the theories are generally of a highly speculative nature, it is unnecessary to discuss the matter here.

The nucleus is usually finely reticular in structure but sometimes the chromosomes may be more or less clearly distinguished as ill-defined masses of a denser structure than the surrounding parts of the nuclear material, even when the cell is in the vegetative condition (*e.g.*, *Triton*).

There is much conflicting evidence with regard to the presence or absence of centrosomes in the ova of many animal forms. Some observers state that no centrosomes are present in the ova of certain species during the vegetative period, but that they appear *de novo* in the cytoplasm or nucleus when the nucleus enters upon the prophase of mitosis. Other observers state that centrosomes are present even in some of those species where they have been stated to be absent, and it would appear very probable that they may often be present but hidden in the cytoplasmic reticulum.

A number of granules or spheres, liquid or solid, are present in the cytoplasm of the ovum. These are the yolk

or deutoplasm granules. They vary in number, size, and shape in the ova of different animals. Some of them are fat globules. All of them serve a nutritive purpose. In some cases the yolk granules take the form of plates or rhomboids. In some forms, particularly where a large amount of yolk is present, the granules or spheres occupy only one part of the cytoplasm, often occupying a considerable portion of one hemisphere.

The ovum is often surrounded by a membrane—the "vitelline membrane"—and in this membrane there are usually one or more small openings—"micropyles"—through which the spermatozoon enters. Commonly there is but one micropyle, which closes after the entry of the spermatozoon, thus preventing more than one from getting into the ovum. In cases where several micropyles exist and several spermatozoa enter, all excepting one of these spermatozoa degenerate under normal conditions. In the ova which do not at first possess a vitelline membrane, one is frequently formed immediately after the entry of the spermatozoon, and the entry of others is thus prevented.

In plants the female sexual element is more commonly known as the oösphere than as the ovum. As is the case in animals, the oösphere is larger than the somatic cells, but not generally to so great an extent in proportion. It is often naked, that is, it possesses no membrane, nor does it generally contain yolk granules. Yolk is not, however, so generally necessary, for the ovum is often attached to the surrounding maternal cells and derives its nourishment from them. On the other hand it contains a number of leucoplasts which are stated to divide individually at the same time as the rest of the cell, and ultimately to give

rise to the chromatophores and the amyloplasts which produce starch.

In some animals, perhaps in most, the meiotic phase begins in the ova at a very early period, long before the body reaches maturity. The prophases of the meiotic division may be very much prolonged, the nucleus remaining at one stage for a considerable period of time. Again, among mammals at any rate, the nuclei of some of the ova may pass through the meiotic phase shortly after, if not actually before, the birth of the individual in which they are produced. In any case, however, the process is essentially similar, whether it occurs early or late in the life of the organism producing the ova, and whether some stages be prolonged or not.

The nucleus of the ovum passes through the meiotic phase, dividing into two daughter nuclei, each possessing half the pre-meiotic or somatic number of chromosomes. The ovum as a whole does not divide, but one of the daughter nuclei is thrown out, carrying with it a small amount of cytoplasm. This is the "first polar body." The first polar body, either while it is still in the cytoplasm of the ovum or shortly after it is thrown off, divides again into two daughter nuclei, both of which degenerate without performing any further function. The remaining daughter nucleus also divides again, one of the resulting nuclei remaining in the cytoplasm of the ovum, the other being thrown off as the "second polar body." The second polar body degenerates without any further division. The ovum is thus left with one nucleus containing half the somatic number of chromosomes. The division of the first polar body and the division which produces the second

polar body are post-meiotic, with half the pre-meiotic number of chromosomes. They correspond to the post-meiotic division in spermatogenesis. It will be seen, however, that whereas in spermatogenesis the four cells resulting from the meiotic and one post-meiotic division are all converted into mature sexual elements, in the maturation of the

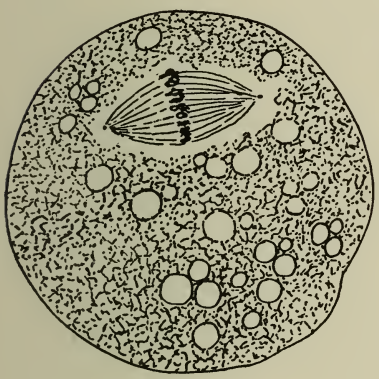


FIG. 44.

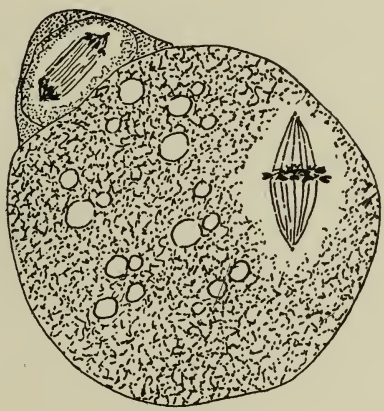


FIG. 45.

SEMI-DIAGRAMMATIC REPRESENTATION OF THE FORMATION OF THE POLAR BODIES IN A MAMMALIAN OVUM.

FIG. 44.—Nucleus of ovum dividing to form the first polar body.

FIG. 45.—Spindle in formation of the second polar body. The first polar body is dividing outside the ovum.

ovum three of the nuclei produced by these two divisions are apparently waste products, only one of the four entering into the process of fertilization, that is, into the formation of a new individual.

The result of the meiotic and post-meiotic division in the ovum is that the ovum is left with a single nucleus with the post-meiotic number of chromosomes, and is ready for fertilization.

CHAPTER VIII.

FERTILIZATION IN MULTICELLULAR FORMS.

In unicellular plants and animals, as we shall see later, fertilization may take the form of conjugation of two individuals, during which an exchange of nuclear material takes place, or two individuals may actually fuse together permanently. In the higher plants and animals, however, fertilization takes the form of a permanent fusion of two post-meiotic cells, one male and the other female.[1]

Among animals the process of fertilization is extraordinarily uniform. The spermatozoon enters the ovum, which consequently for a time, contains two nuclei, the male and female "pro-nuclei."

In some cases (mammals, etc.), the male and female pro-nuclei fuse to form one nucleus. Here the polar bodies are usually both thrown off long before the spermatozoon enters the ovum. In other animals (*Ascaris megalocephala*, etc.), the male and female pro-nuclei never form a common nucleus, but remain separate until the first division of the ovum takes place. In the latter case the polar bodies are not thrown off until the spermatozoon has entered the ovum, or they may be extruded during the entry of the spermatozoon. The latter appears to be the more common sequence of events during the process of

[1] Parthenogenesis, which is dealt with later, is, in a sense, only a modification of this process.

fertilization, but in various animals it may take any form intermediate between these two extremes.

The nucleus of the spermatozoon is very much smaller than that of the ovum, but where the two do not fuse the male pro-nucleus grows rapidly, and soon attains the same dimensions as the female (Fig. 46). Where the two pro-nuclei fuse, the male becomes attached to the female, generally assuming a concave shape on the surface which

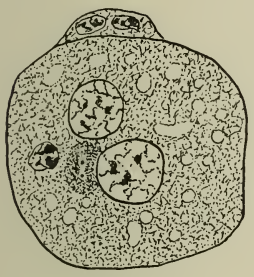


FIG. 46.

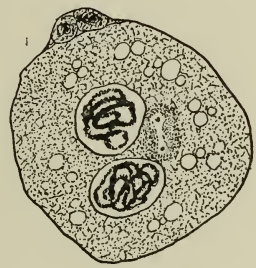


FIG. 47.

FIG. 46.—Fertilized ovum of Ascaris. The male and female pro-nuclei are in a vegetative condition. The centrosomes are separating. To the left of the pro-nuclei is the second polar body which is being thrown out of the cytoplasm. Above is the first polar body which has divided after being thrown off.

FIG. 47.—The same at a later period. Spiremes have formed in both pro-nuclei.

touches the female pro-nucleus. In either case the linin and chromatin contained within the nuclear membrane soon assume the appearance usual in the ordinary pre-meiotic or somatic prophase of division. Generally, therefore, a spireme is formed (Fig. 47), which later on breaks up into chromosomes, and the nuclear membrane or membranes (according to whether the two pro-nuclei have or have not fused) disappear, leaving the chromosomes free in the cytoplasm.

While these nuclear changes have been taking place the spindle has been forming. It would appear that the spindle figure is always derived from the centrosomes or other elements introduced by the spermatozoon. The spermatozoon may or may not leave its flagellum outside the ovum. If it is brought in it soon disintegrates. An aster, however, is developed in connection with the middle-piece, and this can often be shown to arise in connection with the centro-

FIG. 48.

FIG. 48.—The membranes of the pro-nuclei have disappeared, and the two chromosomes derived from each, four in all, have become attached to the spindle fibres.

some belonging to the spermatozoon, the rest of the middle-piece disappearing gradually.

By the time that the chromosomes are set free in the cytoplasm the spindle is fully developed, and the chromosomes attach themselves to the spindle fibres (Fig. 48), divide lengthwise, and the process of mitosis proceeds in the usual manner, the two daughter nuclei being reconstructed in the daughter-cells, each of which receives a longitudinal half of each chromosome.

It will have been realised that in the fertilized ovum the full pre-meiotic number of chromosomes is present, and that

they are all involved in the first segmentation or division. As the nuclei of both ovum and spermatozoon have passed through the meiotic phase, the number of chromosomes contributed by each has been reduced to one-half of the pre-meiotic number. Therefore in the nuclei of the daughter-cells of the first division of the fertilized ovum half the chromosomes are derived from the male, half from the female sexual cell. Thus, for instance, in the formation of the human embryo where the normal pre-meiotic number of chromosomes is thirty-two, at the stage where the fertilized ovum has divided into two cells, sixteen chromosomes in each will have been derived from the father and sixteen from the mother. But when these daughter-cells divide, and the cells thus produced divide again and again in the building up of the embryo, it is evident, if the chromosomes are permanent individual entities,[1] that, as each of them divides lengthwise at each mitosis, there is a chromosome in every cell which has been directly derived from and actually represents each of the thirty-two chromosomes present in the fertilized ovum, sixteen of which were derived from the male and sixteen from the female parent. This brings us to the interesting conclusion that every cell forming the multicellular body exhibits, when in process of division, a definite number of chromosomes which is normally a multiple of two, and that half of these chromosomes have, in all probability, been produced by consecutive processes of division and growth from each parent, each having contributed half of the number of chromosomes appearing in the first division of the fertilized ovum.

[1] See Chap. X.

The process of fertilization in plants is generally essentially similar to what occurs in animals. There are, however, some remarkable modifications in certain cases. Both the male and female sexual elements are sometimes multinucleated, that is to say, there are a number of nuclei present in what is apparently a common cytoplasm. Here the male and female nuclei appear to fuse in pairs. Again, in some cases two male sexual cells are produced, one of which fuses with the female nucleus, and thence the new individual is produced, while the other fuses with a polar nucleus, whence cells are produced that serve to nourish and to support the embryo.

In all cases of fertilization among multicellular organisms the general result is the same, and on the whole the process itself is extraordinarily uniform. Irregularities are, however, not very infrequent, though the causes for these departures from the normal course are, as a rule, very evident.

It has already been said that in many different organisms it is usual for only one spermatozoon to enter the ovum, while in other forms several spermatozoa generally find their way into it. Where it is normal for several spermatozoa to enter the ovum all excepting one of them degenerate. It sometimes happens, however, that more than one spermatozoon finds its way into an ovum where normally only one enters. This may happen because the vitelline membrane is not formed soon enough, or because the micropyle does not close up after the first spermatozoon has entered. When this happens the mitotic figure of the first division in the ovum is deranged, and pluripolar mitoses often occur. This causes irregularities in the division of the ovum and of

the daughter-cells produced [polyspermy]. In some such cases development ceases at a comparatively early period, and the cells degenerate without producing an embryo. There are other irregularities which will be dealt with later on.[1]

In several species of plants and animals new individuals are produced without the fusion of male and female elements. The new individuals are produced by the females without any fertilization by the males taking place. This is known as parthenogenesis. In many such cases the female produces ova which develop into mature females, and this goes on for many generations without any males appearing. On the approach of winter, however, the existing generation of females produces both males and females, and then fertilization by the males occurs. In some species, however, there is apparently no fertilization at any period.

Without going into details, which would be out of place in a short work of this nature, it may be said that there are two common modes by which new individuals are produced parthenogenetically. In one the ovum passes through the meiotic phase, and the number of chromosomes in what under ordinary circumstances would be the female pronucleus is thus reduced. Instead of the second polar body degenerating, however, it either remains in the ovum or returns into it, and plays the part usually filled by the spermatozoon, thus restoring the full pre-meiotic number of chromosomes to the first cleavage figure in the ovum, and consequently to all the cells subsequently derived from it. In other cases no reduction takes place, and so the full

[1] See p. 91.

number of chromosomes is retained. In the latter case we appear to have an example of continuous multiplication and growth without any fertilization, a phenomenon which, as we shall see later, though unusual, has been demonstrated as existing in several other instances both among animals and plants.[1]

The question as to the nature of the impulse which causes the ovum to begin dividing and building up the embryo has been much discussed, and has been the subject of many experiments. In spite of the fact that in the vast majority of cases the fusing of elements derived from two individuals of the same species is necessary to produce a new individual, it is evident that this fusion is not necessarily the impetus that starts segmentation in the ovum. Apart from the many normal instances of parthenogenesis, it has been shown that if the nuclei be removed from the eggs of certain animals, and spermatozoa be then allowed to enter them these eggs will segment and the embryo will develop up to a certain point. Other experiments show that the impetus is not necessarily due to the spermatozoon. If, for instance, sea-water, in which carbonic acid gas has been dissolved under pressure, be poured over the unfertilized eggs of an Echinoderm these eggs will segment and will produce complete embryos. Both in the case of fertilization of enucleated eggs and in that of the mechanical stimulus applied to the eggs of the Echinoderm, the cells in the embryo exhibit only half the pre-meiotic number of chromosomes, which shows conclusively that fertilization is not necessary to start segmentation in the ovum. The only deduction that can be

[1] See p. 81.

made legitimately from these facts is that there are probably several stimuli, very likely quite different in nature from each other, which are sufficient to start segmentation in the ovum, and that while the fusion of two separate elements or the presence of the sperm may be the exciting cause in the majority of cases, they are certainly not the only causes that will bring about this result.

CHAPTER IX.

FERTILIZATION IN UNICELLULAR FORMS.

We have seen that among multicellular animals and plants new individuals are produced by the conjugation of two cells derived from separate individuals, generally of opposite sexes, and that departures from this rule are exceptional. Our knowledge of a similar process in unicellular forms is comparatively limited, but in the case of those species in which the details of the life-cycle are known we find an essential similarity to what happens in the multicellular organisms.

The most complete observations have been made in the case of certain unicellular animals (Infusoria), and the classical observations of Maupas upon several species probably represent in outline what happens in a great many cases. Maupas found that in swarms or races of these animals conjugation took place after a number of generations had been produced by the simple division of individuals. Under the conditions of his experiments, which he made as normal as possible, he found that the period of conjugation was followed by another of simple division, and so on. If, however, the individuals were prevented from conjugating, the whole race grew senile. The organisms were markedly decreased in size and the cytoplasmic organs (cilia, etc.) and the nuclei degenerated. These organisms possess two nuclei, and it was the micro-nucleus that

degenerated first.[1] Though it has since been found that, under certain conditions of food and temperature, the period of simple divisions without conjugation can be prolonged, apparently indefinitely,[2] yet it is almost certain that the cyclical character of the life-history of these species as shown by the experiments of Maupas and others,[3] is what happens under normal conditions, and that under ordinary circumstances the race will degenerate and die out rapidly if conjugation does not take place at intervals. Though it is probable that fertilization occurs in the majority of unicellular forms, it is also probable that there are some, perhaps many, in which it does not occur.[4]

The observation of nuclear phenomena among unicellular forms generally presents such great difficulties that it is not surprising that our information with regard to the occurrence of the meiotic phase among these organisms is very meagre. Apart from the vast amount of trouble involved in securing organisms with their nuclei in any particular stage, the chromosomes are so small and frequently so massed together that it can be only under the most favourable conditions that anything like accurate observations can be made.

So far as it goes, however, the available evidence shows that a reduction in the number of chromosomes to about one-half of the normal number does occur in some forms immediately before conjugation takes place. Hertwig first observed in *Paramecium caudatum* that the number of the

[1] See p. 13.
[2] G. N. Calkins.
[3] R. Hertwig, Bütschli, Minot, etc.
[4] See p. 81.

chromosomes in the nuclear divisions which took place some time before conjugation was eight or nine, while in the divisions immediately preceding fertilization the number was from four to six. Like all subsequent observers, however, he was unable to make quite certain of the actual numbers. In view of the uniformity of the process in multicellular forms, this evidence makes it almost certain that the process is very similar in the case of *Paramecium*, and in other unicellular forms where similar observations have been made.

This conclusion does not, however depend entirely upon the counting of the chromosomes, which is always difficult and often impossible. It has been observed in a large number of unicellular animals and plants that portions of the nucleus are thrown off and degenerate before fertilization takes place. This throwing off of portions of the nucleus is accomplished by a series of mitotic divisions, in many respects analogous to the throwing off of the polar bodies from the ovum in the case of multicellular forms. This suggestion is strengthened by the fact that in some cases that have been accurately observed the portions of nucleus are thrown off in a manner almost identical with the throwing off of the polar bodies, excepting that it is generally impossible to count the number of chromosomes or even to identify individual masses of chromatin.

In *Paramecium* the series of events accompanying conjugation are known with considerable accuracy, and are very similar to what is known to happen in many other forms.

Two individuals approach each other and lie side by side. The macro-nuclei degenerate, and play no part in the process of fertilization. The micro-nuclei divide twice,

each producing four daughter nuclei. These daughter nuclei are in the form of spindles. The spindle is of the form already described in the mitotic figures in multicellular organisms, excepting that there is no aster at either end. Although spoken of as nuclei, no definite membranes are apparently formed. The chromosomes are distributed

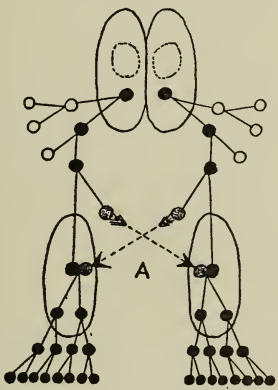


FIG. 49.—Divisions of the Micro-Nucleus in *Paramecium* during conjugation (modified from Maupas). The white circles represent the products of division which are thrown off and degenerate. The shaded circle represents the male pro-nucleus. The partners in the conjugation are represented as separating at A. The divisions following the fusion of the male and female pro-nuclei produce eight nuclei. Of these four are converted into macro-nuclei, and the individual divides into four, each new individual receiving a macro- and a micro-nucleus.

on the spindle in the usual manner but the spindle formation is retained. Of the four nuclei produced in each individual by these two divisions, three degenerate, leaving one. This process closely resembles what happens in the throwing off of the polar bodies in the maturation of the ovum. The remaining nucleus in each *Paramecium* divides again, with the result that there are two in each. One of these acts as the male fertilizing element, and penetrates into the other individual. The other nucleus remains, and fuses

with the male element from the other partner in the process of conjugation. The spindle formation is retained during the fusion, the two spindles lying side by side and the chromosomes being equally distributed to the daughter nuclei subsequently produced. Each individual now contains a nucleus composed of a fusion of an element derived from its own pre-existing micro-nucleus with a similar element derived from another individual. The process of fertilization is thus complete and the two individuals separate. The fertilized nucleus divides, the daughter nuclei thus produced in their turn also divide, and the four nuclei thus produced divide again. There are therefore eight nuclei present in the individual. Four of these become differentiated into macro-nuclei, and fission of the whole cell takes place, four individuals being produced, each of them receiving a macro- and a micro-nucleus. This is apparently the simplest process occurring among *Paramecia*. In some species there are some complications that occur after fertilization has taken place, but it is not necessary to enter into the details of them here. A process almost identical with this, differing only in some details, is known to occur in a large number of forms besides *Paramecium*. In some unicellular animals, micro- and macro-gametes are produced, the former acting as the male, the latter as the female sexual cell. Here the two gametes fuse bodily and new individuals are produced by more or less complicated processes. Except with regard to details, however, the processes in all these forms are essentially similar. In many unicellular animals it has been observed that a very rapid multiplication of cells takes place immediately after fertilization, and this is known to occur after the fertilization

of the ovum in the case of multicellular animals. This is the opposite of what appears to happen very frequently in the case of unicellular plants after fertilization. The two nuclei, after fusing in the case of plants, often remain in a vegetative condition for a considerable period. In unicellular plants complete fusion of the conjugating cells appears to be common, the fused cells often becoming surrounded by a thick membrane. Sometimes, however, they remain separate, and male and female pro-nuclei are formed.

It is necessary to point out here that a considerable amount of confusion has arisen with regard to the sexual stages in the life-cycles of the parasitic unicellular animals (Trypanosomes, etc.). Not only has the centrosome been generally termed the blephoroplast, as has been done in certain cases among plants, but this term has also been applied to several other minute structures in the cell, about the nature of which the observers have been uncertain. A more serious cause of confusion is that the term "reduction" has generally been used in a sense entirely different from that in which it is used with regard to the whole of the animal and vegetable kingdoms. The commonly accepted sense of the term reduction is the process in the production of the sexual elements by which the number of the chromosomes is reduced to one-half of the somatic or pre-meiotic number. Unfortunately, many of those who have published observations upon the life-history of parasitic unicellular animals have apparently not been acquainted with, or have at least ignored, the almost universal phenomenon of reduction in connection with the sexual phenomena in multicellular organisms and those unicellular organisms of which we have any

accurate information. Schaudinn described a process in these unicellular animals where the nucleus is said to throw off a body which is extruded from the cytoplasm and proceeds to degenerate or to form a sexual element. This is stated to occur by a process of "heteropolar mitosis." Schaudinn's interpretation of his observations is practically a revival of the old theory advanced by Balfour and Minot. They explained the throwing off of the polar bodies in the ovum and of the so-called "residual corpuscles" detached from the male elements by supposing that this was the way in which the nucleus got rid of the element of the opposite sex before fertilization. Schaudinn revived this theory in spite of the fact that we know now that it was not correct, at least not in the sense intended by Balfour and Minot. They had no means of knowing what really takes place. It is conceivable, but very improbable, that the first polar bodies might on some occasions take away all the chromosomes derived from the male parent, but there is no evidence that this ever takes place, and there are the strongest suggestions that it does not. It certainly cannot happen in the case of the male sexual element. Even did this happen it would not be similar to the idea of the casting out in bulk of the element of the opposite sex from the cell.

We must therefore regard Schaudinn's interpretation of his observations as being more than questionable, for it would form an isolated exception among both animals and plants. Besides this, there seems to be nothing in the observations themselves which suggests anything at all inconsistent with the process of reduction as it occurs in other forms.

In any case it is absolutely necessary, in forming a

general idea of the phenomena connected with the reproduction of living organisms, to realise that the term "reduction" as used in Schaudinn's sense (unfortunately also the sense of almost all who have studied parasitic unicellular animals) is entirely different and at variance with the sense in which it is used, and has for some time been used, with regard to every other form of living organism.

It has already been said that there is no evidence of fertilization taking place among a number of unicellular forms. Although on account of the great difficulties of observation this does not indicate necessarily that conjugation and fertilization do not take place in these species, still it is quite probable that there may be many cases in which they do not. Fertilization does not appear to be always necessary to a continuous and apparently unlimited production of generations of cells. We have many instances of cell multiplication involving the production of new individuals, where there appears to have been no fertilization for centuries. For instance, we may graft a plant; when the graft has grown we may graft again, and so on, in some cases apparently indefinitely. It seems probable that several trees, which are all of one sex, are examples of some of these growths of centuries' standing. Again, there are certain tumours among animals that may be transferred from individual to individual of the same species by a process of grafting. Thus in a sufficient number of generations of grafts a quantity of this tumour, hundreds of times the weight and bulk of the whole animal in which it originally occurred, has frequently been produced without any signs of loss of vitality on the part of the tumour. Many partheno-

genetic organisms should probably also be placed among these examples of continuous growth.

Whether those cases should be considered as examples of continuous growth in which no male has been discovered, though diligently sought for during many years, is doubtful, for the ova of some are known to be fertilized by a polar body. How this differs from continuous growth in any essential manner it is difficult to see, but there are also cases where no reduction takes place and where there is no fertilization of any kind at all. These facts suggest that there probably are unicellular forms in which no fertilization takes place.

The typical life-cycle of a unicellular form has suggested a very interesting comparison between a race or collection of such unicellular individuals and the multicellular organism.

We have seen that in the multicellular organism the cells forming the soma or body become differentiated in groups to perform various functions, the function of each cell being limited, under normal conditions, to the functions carried out by the particular organ or tissue of which it forms a part. In the unicellular organism all the functions are performed by the same cell, so it is evident that there is here an essential difference. In a limited sense, however, the multicellular organism may be regarded as similar to a swarm or colony of unicellular forms. The tissue cells of a multicellular organism have all arisen by successive generations from a single cell, and in structure are similar to unicellular forms. Thus far the comparison is clear. Bütschli and Minot carried the comparison further. They held that cell divisions tend to run in cycles, in which simple division

and conjugation alternate with each other. In the unicellular forms the cells remain separate from each other and lead an independent existence. In the multicellular organisms the cells do not separate but cohere to form the body. In the former case the individuals conjugate after a certain number of generations produced by simple division; in the latter some of the cells of the same lineage as the rest, all having been derived from the fertilized ovum, are thrown off and conjugate with a similar cell derived from another organism of the same kind. The generations of unicellular forms between the periods of conjugation are compared to the group of cells forming the multicellular body including those which are destined to produce sexual elements. The comparison is heightened when we consider that, under normal conditions of food, etc., among some of the unicellular animals, the race appears to degenerate and die out if conjugation be prevented; and that while the cells forming the body or soma of the multicellular organism degenerate after a number of generations, the sexual cells which conjugate are potentially immortal.

It has been stated that after passing through the meiotic phase and several post-meiotic generations having been produced, the leucocytes in vertebrate animals normally conjugate.[1] This conjugation is said to be accomplished in a somewhat complicated manner. The nucleus of one leucocyte sends out a process which penetrates the cytoplasm belonging to itself and to that of the partner in the conjugation. This process is in the form of a tube, and through it the linin and chromatin of the one nucleus are

[1] C. E. Walker.

drawn into the other. The absorption of one cell by another is a well-known phenomenon, but is a comparatively simple affair. The absorbed cell is taken into the cytoplasm of the absorbing cell, and is there digested. No nuclear changes take place, and the absorption is apparently carried out entirely in the cytoplasm without the nucleus being directly involved. It is claimed that the appearance of a special and complex apparatus with no apparent result but the transference of the one nucleus to the other without exposing the contents so transferred to the action of the cytoplasm, shows that some process other than mere absorption of one cell by another is taking place, and that fertilization is the probable explanation. It is also suggested that this may be a form of fertilization not hitherto observed in unicellular forms, and that its occurrence among leucocytes is a case of phylogenetic reversion.

CHAPTER X.

THE PROBABLE INDIVIDUALITY OF THE CHROMOSOMES.

In the vast majority of the various species of animals and plants there must, under present conditions, be a considerable element of doubt as to the number of chromosomes appearing in any given cell. The number of chromosomes characteristic of different species has therefore generally been arrived at by counting the chromosomes in a large number of cells as accurately as possible, and by depending upon the average of the results of these counts. In most cases this method has been considerably facilitated by the fact that in the cells that are passing through the meiotic phase the chromosomes are reduced to one-half of the characteristic number, and are considerably increased in size. On the whole we may take it that our knowledge with regard to the characteristic number of chromosomes in those species of animals and plants which have been the subjects of special investigation is fairly accurate.

Hitherto it has been found that the characteristic number of chromosomes in the species is extraordinarily constant, not only in the cells forming each individual, but also from generation to generation of new individuals. In only a few species, however, is it possible to count the number of chromosomes in any given cell with such accuracy as to practically eliminate errors, and frequently even in these cases only in a limited number of generations of cells

derived from the fertilized ovum. The classical example among such forms is *Ascaris megalocephala*. Here, where any variations from the characteristic number of chromosomes occurs, the causes of the variation would appear to have been sufficiently demonstrated. Therefore we may, without any undue assumption, take it that in the two varieties of *A. megalocephala*, one of which possesses two, the other four chromosomes, the number of these chromosomes is constant, under normal conditions, in all the cells of the organism, and through the successive generations of individuals produced from it.

Unfortunately *Ascaris* is almost a solitary instance in which the number of the chromosomes in all the cells can be arrived at with accuracy. In almost every other form which has been the subject of investigation the number of the chromosomes would appear, according to the countings, to vary within certain limits, though with but two or three isolated exceptions the limits of the variation seem to be small.

In counting the chromosomes of almost every known form the probabilities of error are very great. Chromosomes may be superimposed in the position in which the cell is presented to the eye when under the microscope, and in this case the count would be short. In other cases one or more chromosomes may have divided a little earlier than the rest, and then the apparent number would be increased. The probability of such errors cannot be avoided in the great majority of instances, and the probable margin of error seems usually to be within the limits of variation in number as shown by countings. That the evidence for the normal occurrence of considerable variations in the number

of chromosomes under the conditions usually encountered in the observations on the cells of any given species is, with a few exceptions, insufficient, is all that it is necessary to assume to emphasize the probability of the characteristic number being as constant in them as it is in *Ascaris*. Though it has been proved by experiment that certain stimuli will produce mitoses that are asymmetrical, and consequently variations from the normal number of chromosomes, it appears that the progeny of such pathological mitoses generally die out in a comparatively short time. This fact nevertheless suggests an explanation for a certain number of departures from the characteristic number.

Two explanations of this remarkable retention of the characteristic number of chromosomes by the cells of the various species of animals and plants suggest themselves, and both theories have adherents.

One theory is that the material forming the chromosomes—the linin and chromatin—is inextricably mingled when the daughter nuclei produced by mitosis are formed, and pass into the vegetative condition. When the next mitosis occurs, the chromosomes that appear are not individually the same chromosomes, but are similar aggregates derived from the common mass of material which was formed by the preceding generation of chromosomes, and which may have increased its bulk by a process of growth in the meantime.

The appearance of the characteristic number of chromosomes when the cells divide, is supposed to be due to properties inherent to the particular kind of protoplasm, that is, to the linin and chromatin of which the chromosomes are composed. Variations in the number of chromosomes

are brought about by changes in the environment or in the condition or constitution of the substance of which the chromosomes are composed. While the environment and constitution of this substance remain the same it will always produce the same number of chromosomes. When the environment and constitution change, the number of chromosomes is liable to change. Thus the stability of the characteristic number of chromosomes and all variations from this number are accounted for.

The other theory is that the chromosomes never lose their individuality. Though it may not be possible to render them perceptible to observation, by any means at present available, during the period when the cell is in the vegetative condition, it is supposed that they still continue to exist as individual concrete entities. When the chromosomes are apparently lost as the daughter nuclei reach the vegetative condition they still remain separate, and at the onset of the next mitosis they again become visible. In fact the chromosomes are, according to this theory, permanent entities, each of them individually multiplying by division, the halves produced growing at each successive generation.

Without considering any of the special evidence bearing upon these two theories, the phenomenon of mitosis itself would seem to suggest that the latter is the more probable. The obvious result of mitosis, uniform whenever it occurs under normal conditions, is the selective and equal division of each of the chromosomes and of no other parts of the cell. That the only probable, perhaps possible, result, accomplished with such accuracy by so complicated a process, and repeated with such regularity at each

succeeding cell division, should be completely obliterated as regularly and as frequently as it is effected, seems very improbable. Our knowledge of other biological phenomena leads us to look with some suspicion upon an interpretation which involves an apparently objectless waste of energy or material. Yet the mitotic phenomena would appear to be quite objectless if the substance of the chromosomes is mingled so as to form a common mass when the nucleus is in a vegetative condition.

The main points in favour of the first theory are that the chromosomes as individual entities disappear entirely, as far as can be seen, when the cell is in the vegetative condition in the great majority of species; the obviously considerable variations in the number of the chromosomes that occur in a few isolated cases; and the interposition of amitosis between two series of mitotic generations, which certainly happens in some species. The disappearance of the chromosomes has already been referred to, and will be dealt with again later on. Variations in the number of chromosomes such as occur in certain species of plants are certainly remarkable, and would seem to be too considerable to be accounted for by errors in counting. Such instances are, however, exceptional, and it would seem more reasonable to seek the cause for an exception than to ignore what happens in the great majority of cases because exceptions exist. It must also be remembered that comparatively slight abnormal stimuli will produce irregular mitoses.

Amitosis seems at present to be quite inexplicable. This again must be regarded as an exception, though in some cases a very regular one. As far as morphological evidence goes there is nothing to show that just such a mingling of

the substance of which the chromosomes are formed does not take place during amitosis as is assumed to take place according to this theory during the time that the nucleus is in the vegetative condition. As far as can be seen, amitosis involves an undiscriminating division of the nucleus in bulk. The probability that amitotic division is selective as regards the chromosomes is suggested by the fact that it is preceded and followed by mitotic divisions, but there is no direct evidence that anything of the kind takes place.

The second theory assumes the permanence and individuality of the chromosomes through succeeding generations of cells. The general arguments in favour of this theory based upon the phenomenon of mitosis have already been stated. It is also held that as the characteristic number of the chromosomes is apparently constant in those species where they can be counted with accuracy, their number is also constant under normal conditions in the cells of the great majority of animals and plants where the number is constant within small limits but the difficulties of counting are great. Most of the variations in number are considered as being sufficiently accounted for by the probable errors in counting and by the fact that pathological mitoses are asymmetrical in many cases. The fact that in some species the individual chromosomes can be distinguished throughout the period during which the nucleus is in the vegetative condition, is regarded as suggesting that they continue as discrete entities in other cases, where, however, they escape observation. Thus in the cells of *Triton* and *Periplaneta*, the chromosomes often remain quite apparent during the vegetative stage. Again, between the meiotic division and that division which follows it, there is sometimes no

vegetative period, and the chromosomes remain distinct throughout.

The rest of the evidence in favour of this theory had been derived largely from observations upon the early divisions of *Ascaris megalocephala*. It has been found that in certain cases of abnormal fertilization and early development in this animal nuclei are formed in which a number of chromosomes different from that characteristic of the organism is included. When such nuclei again divide, after passing through a vegetative stage during which the chromosomes disappear, exactly the same number of chromosomes as went to build them up, reappear.

It sometimes happens, through some irregularity in the sequence of the events connected with the process of fertilization in the egg of *Ascaris*, that one or both of the chromosomes usually included in the second polar body remain in the ovum. In such cases the additional chromosomes form an additional nucleus which is indistinguishable from either of the true pro-nuclei. When the first mitosis in the ovum takes place each of the three nuclei gives rise to chromosomes—the male and female pro-nuclei to two each, the nucleus derived from the second polar body to one or two, according to the number that went to form it. Thus two daughter nuclei are formed, each of which receives five or six chromosomes instead of the usual four, and when these daughter nuclei divide in their turn the abnormal number is reproduced.[1]

The ova of *A. megalocephala*, var. bivalens, have been fertilized by the spermatozoa of var. univalens. The result

[1] Boveri, van Beneden, and others.

has been that the female pro-nucleus has given rise to two chromosomes, the male to one. The three chromosomes included in the first segmentation figure have continued to appear in the succeeding generations of cells produced. Other variations from the normal number of chromosomes produced by irregularities in the process of fertilization have been found to continue in the progeny of the cell in which they first appeared.[1]

All these observations indicate very forcibly, that the number of chromosomes appearing in a nucleus during mitosis, is the same as the number of chromosomes from which it was originally formed.

In *Echinoderm* eggs that are artificially stimulated to segment without being fertilized and in enucleated eggs fertilized by spermatozoa only half the characteristic number of chromosomes appears when mitosis occurs, and this reduced number is retained in all the generations of cells that are produced in the building up of the embryo.[2]

When the ovum of *Ascaris* segments and the half chromosomes distributed to the daughter-cells proceed to form nuclei they are always in the same position in relation to the centrosome to which they are adjacent. The loose ends point away from the centrosome and the bends, which are near the middle of the chromosomes, point towards it. When the chromosomes reappear at the onset of the next mitosis they do so in this position.[3]

The paternal and maternal chromosomes remain distinct from each other, at any rate in certain species of *Cyclops*

[1] Herla, Zur Strassen.
[2] Boveri, Morgan, Loeb.
[3] Rabl, Boveri, van Beneden.

and in *Ascaris*, to a comparatively late stage in the former case, to the twelve-cell stage, and possibly later, in the latter.[1]

The chromosomes appear in two distinct groups in the nucleus of the ovum of *Ascaris* before reduction takes place, and it has been suggested that these are the paternal and the maternal chromosomes that have remained distinct throughout the generations of cells that have been produced to build up the organism.[2]

Finally there is the fact that in those species where the shapes of the meiotic gemini have been specially investigated, these shapes are apparently permanent in the species. In every meiotic mitosis there are at least two of each shape and if there be more than two of the same shape the number will be a multiple of two. The number of any given shape is stable in the species, as also is the number of different shapes, though those present in one species may not be found in another.[3]

The direct evidence for this second theory is obviously very strong, almost to demonstration in certain species of cells. It must however be remembered that much of it applies to only a few particular species, and is largely dependent upon one. On the other hand, although direct evidence is lacking with regard to several of these points in an overwhelming proportion of cases, there is very little against their holding good, and a considerable amount of indirect evidence in their favour in all. There are, however, several general points of view that seem to carry the hypothesis further.

[1] Häcker, Rückert, Herla, Zoja.
[2] Rückert.
[3] Moore and Arnold, Baumgartner. See p. 39.

If the chromosomes are permanent entities that individually divide at each mitosis and grow during the intervals, any variation in the number of chromosomes going to form a nucleus would, excluding accidents and given suitable conditions, produce a race of cells containing this variant number. The variation would in fact, given the permanency of the chromosomes, be permanent for as long as the race of cells continued to exist, or until a similar variation occurred in one of the cells belonging to one of the subsequent generations thus produced. There is very strong evidence that the characteristic number of chromosomes in the majority of species does not vary under normal conditions, but is retained from generation to generation. If the chromosomes are permanent, the stability of the characteristic number in any given species is accounted for. The perpetuation of any variation from this number is also accounted for. It is known that there are several ways in which the number of chromosomes entering into the formation of a nucleus may be varied, both in the ovum before the first segmentation and on other occasions. There are also observations and experimental evidence to show that, whatever number of chromosomes enters into the formation of a nucleus, that number reappears when the nucleus divides, whether the number be characteristic of the species from which the cell was derived or not. It has also been shown that, as far as the progeny of such cells have been followed, the variation in the number of the chromosomes has been retained. We thus have two opposing influences which, acting synchronously, tend to produce permanent variations in the number of the chromosomes. The process of mitosis under normal conditions ensures that equivalent halves of every

chromosome entering into the formation of a nucleus will be distributed to each daughter nucleus produced when division takes place. But on the other hand there are many possibilities of an unusual number of chromosomes entering into the formation of a nucleus particularly during the process of fertilization, which, though irregular, cannot be regarded as pathological. While the progeny of asymmetrical mitoses,[1] which are probably always pathological, seem to die out, this other class of irregularities is rendered permanent by the process of mitosis, the influence which is always present. It is also possible that there may be other causes, of which we at present know nothing, that produce variations in the number of chromosomes included in the first segmentation figure of the ovum, which would, under favourable circumstances, be perpetuated.

Thus, by assuming the permanence of the individual chromosomes, for which there is a considerable amount of evidence, we can account both for the stability of the number of chromosomes in any given species, and for the difference in the characteristic number of chromosomes in different species. If, however, we refuse to accept the permanence of the chromosomes, we are driven to adopt a number of assumptions for which there is no evidence, to account for facts which are well established. A theory which assumes that the substance of which the chromosomes are formed is mingled into a common mass after each mitosis, accounts for the adherence in the cells of a species to a particular number of chromosomes while the environment and constitution of those cells remains

[1] This is not intended to include mitoses where the "supernumerary chromosome" is stated to occur.

the same. A change in these conditions will produce a change in the number of chromosomes. There is, however, great difficulty in this case in accounting for the stability in the number of chromosomes in the face of certain facts already mentioned. We know that the number of chromosomes may be altered by environment, but such changes are pathological, there is no evidence to show that they are permanent, and some that indicates that they are not. There is no evidence to show that changes in environment which are insufficient to produce pathological mitoses can modify the number of chromosomes, while there is a considerable amount to show that they do not. We know however that there may be a change in the number of chromosomes without any change in environment, and that this change when once established continues through successive generations of cells. If nuclei are formed from a number of chromosomes which differs from the characteristic number, the same number reappears that went to their formation when these nuclei divide. If the forming of a particular number of chromosomes were a property inherent to particular forms of material it is difficultto account for this phenomenon. If this were a fact, a mass of a particular kind of this substance should always produce a similar number of chromosomes, no matter its quantity, just as the crystal of a particular salt has the same number of facets irrespective of its size. In the case of the chromosome substance, however, even when the sizes of the nuclei are the same, the number of the chromosomes appearing during mitosis is that number which was concerned in its formation, even when that number is not the characteristic number of the species.

It seems probable then that the hypothesis of the permanence and individuality of the chromosomes is either the truth or something very near the truth. In spite of the fact that except in a very few cases, there is no direct evidence that the chromosomes remain as discrete entities in the nucleus when in a vegetative condition, and as far as can be seen disappear at regular intervals in most species of cells, it is difficult to avoid the conclusion that, in the majority of organisms, they probably retain their individuality from generation to generation under ordinary circumstances.

CHAPTER XI.

THE MORPHOLOGICAL ASPECT OF THE TRANSMISSION OF HEREDITARY CHARACTERS.

Many theories with regard to the transmission of hereditary characters from parent to offspring have been propounded. We are here concerned only with the morphological evidence showing how this transmission may be effected. From the morphological point of view there seems to be one means particularly adapted to the transmission of characters from one individual to another. This is through the chromosomes. A careful consideration of the phenomena of cell multiplication, the only process by which new individuals can be produced, will show that no other part of the cell divides in a discriminative manner. There is no other part of the cell which constantly appears in all species and which divides individually as the chromosomes do. Certain other parts may divide individually, but they are not necessarily constituent parts of a cell, being absent in many cases. The rest of the cell divides in bulk, and there is apparently no selection shown in the manner of division.

Given the permanent individuality of the chromosomes, the probability of which has already been discussed, we appear to have a very satisfactory explanation of how certain hereditary characters are transmitted. Of the chromosomes appearing in the first segmentation of the ovum half are derived from the male and half from the female parent. In

the cells subsequently produced which build up the body of the organism the chromosomes are derived from those in the fertilized ovum. Furthermore, each of these chromosomes has been derived from its parent chromosome in such a way that it is an exact replica of it, and possesses a portion of every part of the chromosome from which it was originally derived.

It has been held that *every* hereditary character is represented by a chromosome or individual part of a chromosome, but this suggestion is hardly compatible with what seems to happen in the production of the sexual elements. If, as would appear to be the case, whole chromosomes are distributed to the daughter-cells produced by the meiotic division, almost insuperable objections to this theory arise at once. Whether whole chromosomes are distributed to the daughter-cells of the meiotic division or not it is evident that this form of division is of such a nature that it would be very difficult to uphold the theory that each individual character is contained in a certain part of a certain chromosome.

A consideration of the mode of distribution of the chromosomes to the daughter-cells at the meiotic division will show that if a character is contained in an individual chromosome or part of one, this character will be contained in one of the daughter-cells but not in the other, in half of the progeny of the meiotic division, but not in the other half.[1] For instance, if the growth of hair on the scalp were a character in man conveyed by a single chromosome or part of a chromosome, only half of the spermatozoa produced by an individual man would contain the chromosome that bore

[1] See p. 44.

this character. In a similar manner the first polar body may contain this particular chromosome in the case of the maturation of the ovum, or it may remain and be included in the female pro-nucleus. It is therefore obvious that a large number of cases of fertilization must occur where this chromosome would be absent from both male and female pro-nucleus. Individuals possessing no hair on the scalp at any time during their lives are, however, so rare that they may be neglected in this connection. It would therefore appear certain that *all* characters are not contained in and transmitted through individual chromosomes or parts of chromosomes.

At the same time it is quite conceivable that some particular characters might be contained in individual chromosomes or parts of chromosomes, while the potentiality of producing the general characters of the race or species may be a common property of all the chromosomes or even of the whole mass of protoplasm forming the cell.

The evidence at our disposal suggests that there are two classes of characters that are transmitted from parent to offspring in different ways. The transmission of the common characters of the race or species which have been produced in the normal course of evolution appears to be regulated by different laws from those governing the transmission of the characters that have been produced in a comparatively short period by artificial selection. Archdall Reid has pointed out the opposing influence which bi-parental reproduction must present to the perpetuation of variations, even when natural selection is working in their favour; it has in fact no part in producing characters but only in eliminating

them.[1] Bi-parental reproduction must tend to keep the characters of the race at a uniform level and to eliminate variations in individuals, and only such variations as occur very frequently and come very directly and continuously under the influence of natural selection will have a chance of being perpetuated. Common racial characters can therefore only be produced by the influence of natural selection and bi-parental reproduction acting upon successive generations of individuals and influencing variation during an immense period of time.

When we come to study the transmission of characters rapidly produced by artificial selection it is brought home to us in the most forcible manner that we are dealing with a class of characters which differ very materially from those produced under natural conditions. For instance, if artificial selection be abandoned in the case of a prize strain of pigeons which differs so much from the parent stock that, did we not know its history, it would be classed as a separate species, this breed will revert to the characters of the form from which it was produced in a comparatively short time—in a few generations, apparently, in some cases. Much the same thing appears to happen in the case of all the characters produced in various animals and plants by artificial selection, given a sufficient number of generations under natural conditions.

While artificial selection does not seem to have been acting long enough in any case to establish a character on the same footing as those produced under the combined influence of bi-parental reproduction and natural selection, there is a very

[1] "The Principles of Heredity."

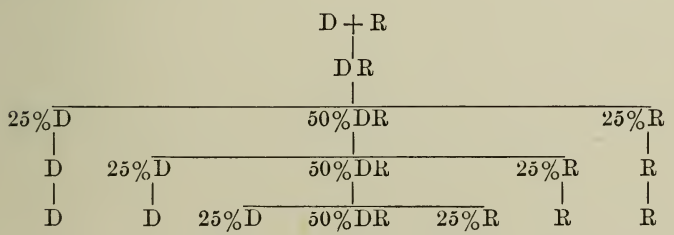
considerable amount of evidence to show that the transmission of these rapidly-produced characters occurs in a regular manner while they continue to appear. The way in which these characters are transmitted has been so well established, and has been shown to be so regular in many cases, that it is regarded by many as a law. This "law" was first formulated by Mendel as the result of experiments in breeding made by him, and is consequently known as "Mendel's law."

Mendel crossed a pea which produced a tall plant with another of which the plant was a dwarf. In the first generation produced from this cross all the plants were tall. In the second generation, however, 25 per cent. of the plants were dwarfs and 75 per cent. tall. The dwarf plants continued to produce dwarf plants through succeeding generations when crossed among themselves. Of the tall plants, 25 per cent. and all their descendants, continued to produce tall plants when self-fertilized in the same way as the 25 per cent. dwarf plants continued to produce dwarf plants. Of the remaining tall plants (50 per cent. of the whole), 25 per cent. produced dwarfs and the remaining 75 per cent. produced tall. Of the next generation, the dwarf plants produced succeeding generations of dwarf plants, while of the tall plants 25 per cent. produced tall plants which gave rise to nothing but tall plants, and the remaining 50 per cent. of the tall produced the same percentage of tall and dwarf as in the preceding generations. These relative proportions of tall and dwarf appeared in subsequent generations.

Mendel concluded from these experiments that the tall character was dominant over the dwarf in those plants

where both were present. He therefore called this character "dominant" and the other "recessive."

He concluded from these and other similar experiments that when a cross was produced between two individuals exhibiting a difference in a particular character of this kind, the offspring would in the first generation exhibit the dominant character. These individuals he called "impure dominants." In the second generation, however, 25 per cent. of the individuals are pure dominants, 50 per cent. impure dominants, and 25 per cent. pure recessives. The pure dominants bred together give rise to nothing but pure dominants, and the pure recessives to pure recessives, but the impure dominants again give rise to 25 per cent. pure dominants, 50 per cent. impure dominants, and 25 per cent. pure recessives. This goes on apparently indefinitely, pure dominants always producing pure dominants, pure recessives recessives, and the impure dominants the same proportions of dominants, impure dominants, and recessives. The following diagrammatic representation makes the sequence of events clear. D represents the dominant, R the recessive, and DR the impure dominant.



This "law" seems to govern the transmission of characters from parent to offspring in the most strikingly

regular manner in so far as some, at any rate, of the characters are concerned in the case of races and varieties that have been produced by artificial selection. The evidence for its operation in the case of characters which have appeared under the influence of natural selection and bi-sexual reproduction is, however, wanting. Indeed, it would appear that generally such natural characters definitely do not come under Mendel's law of hereditary transmission. For instance, if we take the case of a cross between a white and a black race of men, the characters of the progeny are more or less intermediate between the two, and are directly increased or diminished according to whether future crosses are with black or white individuals. Colour is a character which, in domesticated races that have for a long time been subject to artificial selection, is transmitted according to Mendel's law. In the case of man the colour of the progeny of a cross is intermediate between the two parents, and varies according to the amount of black or white blood introduced at each generation. Definite proportions of pure black and pure white individuals do not appear to be produced, but individuals exhibiting various degrees of brownness.

Secondary sexual characters appear to be far more stable than Mendelian characters. They have appeared without the action of artificial selection, and in spite of the continual intervention of bi-sexual reproduction. Though the female does not exhibit the secondary male characters, these characters are transmitted through her. Occasionally also we find a female individual exhibiting the male secondary characters, and the male exhibiting the female. Thus, though the mode of transmission of the

secondary sexual characters may perhaps differ from the mode of transmission of the common characters of the race, it certainly differs from the mode in which characters acquired by artificial selection are transmitted. The secondary sexual characters continue to appear in cases where artificial selection has ceased to operate. Characters created by artificial selection tend to disappear when artificial selection is discontinued.

In this connection it must be remembered that all the cells forming a multicellular organism retain, to a greater or less extent, the potentiality of producing the various tissues characteristic of the species.[1] The loss of this potentiality seems to be most marked in certain groups of cells included in the soma in the case of mammals, where differentiation is carried to a very high degree and where a large number of generations of differentiated cells is produced. From what we know of the results of castration and of the appearance of secondary sexual characters in individuals of opposite sexes, it is extremely probable that the potentiality of producing the secondary sexual characters is present in the cells of all individuals of both sexes, but that these appear only in the presence of a suitable stimulus. In view of the evidence regarding the phenomena of mitosis and meiosis, it seems impossible that the potentiality of producing the permanent specific and secondary sexual characters can be transmitted from generation to generation of cells through the chromosomes.

When we consider these matters from the morphological standpoint certain suggestions become more or less obvious.

[1] See p. 6.

Particular characters which appertain rather to the individual or to a limited number of individuals than to the whole race or species appear to be transmitted in certain proportional relations to the number of the offspring according to a definite law. These characters are, however, among those which will be eliminated in a certain number of generations under the unrestrained influence of bi-sexual reproduction. They can only be retained by selection. Now the way in which the chromosomes are distributed to the daughter-cells during the process of mitosis in the pre-meiotic, meiotic, and post-meiotic forms of division, appears to give the same mathematical probabilities of combinations of chromosomes during fertilization as would be expected from the proportions of dominants, impure dominants, and recessives produced in successive generations from the crossing of two individuals by Mendel, and since then by many other observers. If the dominant character is supposed to be carried by a chromosome represented by *a*, the recessive by the chromosome represented as *b*, we have a cell containing both *a* and *b* when the cross fertilization is effected. The chromosomes in the cell of the impure dominant may thus be represented as *ab*, in the dominant by *aa*, and in the recessive by *bb*. When a cell derived from an impure dominant passes through the meiotic phase it produces two daughter-cells, one of which will contain the chromosome *a*, the other the chromosome *b*—pure dominant and pure recessive in fact. If we start with a number of individuals possessing both *a* and *b* in each cell, as we should in the case of a cross, the sexual cells produced by these individuals would contain either *a* or *b*, and the number of cells containing *a* would be equal

to the number containing *b*. There would obviously be double the chance of a cell containing *a* combining with a cell containing *b* than there would be of either an *a* combining with an *a* or a *b* with a *b*. Thus we arrive at the exact relative numbers shown by the Mendelian experiments.

It appears, however, that the other class of characters which have been produced during the course of evolution without the intervention of artificial selection, and which are permanent as compared with the artificially produced characters, are not transmitted according to Mendel's law.

While it is strongly suggested that temporary variations are transmitted by means of the chromosomes, it is practically certain that specific or racial characters that are comparatively permanent are transmitted in some other manner. It may be that the latter class of characters is dependent upon the intrinsic potentialities of the particular kind of protoplasm which exhibits them, these potentialities having developed under the combined action of natural selection and bi-sexual reproduction. It is also conceivable that these permanent characters commenced as temporary variations and, passing through a period of comparative instability when they were transmitted by particular chromosomes or particular parts of particular chromosomes, the potentiality of producing them was gradually acquired by the common protoplasm of the chromosomes as a whole, or even of the cell, and thus were brought to the condition of stability in which we now find them. It would not be until the same character had been constantly represented by a chromosome or a part of

a chromosome for many generations in both male and female sexual elements, that the character would become a permanent and intrinsic potentiality of the chromosomes and subsequently of the cell as a whole, and not liable to elimination by bi-parental reproduction. The mode of distribution and division of the chromosomes in the pre-meiotic and meiotic forms of mitosis, and the fusion of two cells when fertilization occurs, suggest that the potentiality of producing any given character may be present in more and more chromosomes in succeeding generations, until at last it is represented in all of them.

CHAPTER XII.

CYTOLOGICAL METHODS.[1]

The recent rapid advance in our knowledge of cell phenomena is at least as much due to improvements in the methods of preserving and preparing microscopical specimens as to improvements in the microscope itself. While it is possible to observe the details of the process of mitosis with the microscope of fifty or sixty years ago with considerable accuracy in properly preserved material, nothing of such phenomena can be seen with the best of the most modern microscopes in material that has not been treated in a suitable manner. Cytological methods are absolutely necessary where the structures contained in cells are to be examined.

There are three principles that must be kept in mind in preserving cell structures :—

(1) That the material to be preserved shall be put into the fixative—that is, the fluid which is to kill the cells and begin the process of preservation—without any delay.

(2) That the fixative shall be of such a nature as to alter

[1] While it would be impossible in a small book like the present to give a complete account of the various methods used in the preparation of microscopical specimens, an attempt is made to accentuate the points where the methods used in making the more delicate cytological preparations differ from those employed in ordinary microscopical work. A few fixatives and stains that have been found by the author to give the most satisfactory results are described.

the structures in the cells as little, and that it shall be as quick in action, as possible.

(3) That after fixation is complete the material shall not be subjected to any treatment that will alter the form of the cells or of their contents during the process of rendering their preservation permanent, and preparaing them for microscopical examination.

Fixation.

The object of fixation is to precipitate or coagulate as rapidly as possible the contents of the cells forming the material to be examined, and at the same time to preserve the appearance of the form of the cells and their contents as they were during life. When cells die gradually, they die in the vegetative condition. The only way, therefore, of obtaining permanent preparations showing the structures exhibited by cells during an active process of change is to subject the cells to some treatment which will kill them instantly, and precipitate or coagulate their contents at the same time. The whole phenomenon of mitosis occupies but a short space of time—in the higher animals but a few minutes—so it is obvious that there must be no delay in placing the material to be preserved in the fixative. All the cells forming a portion of any multicellular organism will very likely begin to pass into the vegetative condition, on their way towards death and disintegration, as soon as they are removed from the body. The rapidity with which this occurs will vary greatly in different organisms, reaching its maximum probably in the case of portions of tissue removed from the body of a mammal or a bird. Here the piece of tissue to be preserved should, if possible, be placed

in the fixative in less than a minute after removal from the body of the living animal, or within that period of its death. While the period before fixation may be lengthened in other cases, particularly with certain vegetable tissues, no harm can be done by fixing the material as soon as possible, while there is always a risk in delay.

As fixation cannot occur until the fixative acts directly upon a cell, it is evident that if large pieces of tissue are used, the fixing fluid will not be able to act upon the cells that are in the middle of the piece of tissue until long after it has acted upon those that are upon the outside. All the cells will not be fixed until the fluid has completely penetrated the tissue. It is therefore necessary to use thin pieces of tissue in order that the fixative may penetrate rapidly. The actual size does not matter, but the tissue should always be cut into thin slices, not more than one-sixteenth or one-eighth of an inch thick. The thickness desirable depends upon the density of the tissue and the nature of the fixative used.

Different reagents produce very different effects upon cell structures. Some will cause distension, others will cause contraction. Thus the action of acetic acid results in the swelling up of cells, while alcohol will shrink them. As no single reagent will act indifferently in this way, fixative fluids are mixtures of two or more reagents in such proportion that their distortive action upon the protoplasm of the cells is counterbalanced. Acetic acid and alcohol may be used together in such proportions that the distension caused by the one is counterbalanced by the contraction caused by the other. As the texture of different tissues varies to an enormous extent, so the proportions of the

different reagents in the different mixtures should be modified to suit the particular tissues that are to be dealt with. The formulæ given at the end of the chapter should therefore be regarded as somewhat empirical. They give the proportions of the various ingredients that have been found most generally successful, but they must not be regarded as the best proportions for every kind of tissue. It may be pointed out, however, that with but two or three exceptions which are indicated, they are the formulæ that have been found least liable to require alterations in the proportions of the ingredients.

Besides the property of causing swelling or shrinking, there are other qualities about which it is desirable to have some knowledge. It has been pointed out that fixatives should kill the cells as quickly as possible. This property depends to a considerable extent upon the rapidity with which the reagent penetrates the tissues. Acetic acid and corrosive sublimate both possess this property in a high degree, but used by themselves the results they produce are otherwise unsatisfactory. Acetic acid causes a great amount of distension, and corrosive sublimate causes contraction. There is another disadvantage about acetic acid, which is that the precipitate or coagulum formed by it is very coarse. Now, it is obvious that the finer the precipitate, the more nearly the fixed tissue will represent the appearance of the structures as they were during life. It is also obvious that a very fine coagulum or precipitate will prevent the penetration of the fixative to a certain extent, because, as it acts upon the outer layers of cells first, it will prevent it from reaching those in the deeper layers. There is another disadvantage, that the cell structures that have been fixed

in this otherwise desirable manner are very difficult to stain. The reagent which gives the finest precipitate or coagulum is osmic acid, but excepting, perhaps, in the case of unicellular forms, it should never be used alone, for the reasons indicated above. Even when used in combination with other reagents it is generally found that the outer layers of cells in a piece of tissue are fixed in a different and more satisfactory manner than those in the inner layers. This is due to the fact that osmic acid penetrates very slowly. Experience will teach the observer to judge with considerable accuracy whether too much or too little of any particular ingredient has been used. There is no royal road to arriving at a correct judgment in these matters; experience used judiciously is the only guide.

There is, however, one very useful aid to estimating the value of any fixative. The best fixative is that which produces the appearance most like that which was presented by the cell during life. Unfortunately it is not possible to observe phenomena in the living cell in the majority of cases. In some, however, it is comparatively easy. Some vegetable and a few animal forms may be stained to a certain extent during life. A comparison between these and the cases where *intra vitam* staining is impossible is a very useful check. In some living cells we are able to observe, particularly with an oblique light, a considerable part, if not all, of the process of mitosis actually taking place in the living cells without any staining at all. We are thus able to make sure that the outlines of mitosis are correct as seen in the fixed specimen. We can go further, and say that as we get precisely the same appearances with different reagents that

are known to act upon protoplasm in different manners, these appearances are not likely to be due to the action of the reagents themselves, but must be due to something actually present in the cell. It must not be forgotten, however, that when we look at a fixed specimen the appearances are those of the precipitates or coagula produced by the reagents, and that if a structure is really present in the living cell it should be demonstrable when any suitable fixative is used, though very probably it will be rendered more clear by one than by another. Also, it must be realised that, though it might under ordinary circumstances be practically impossible to discover a particular structure in the living cell, it may easily be demonstrated in the living cell, when the structure has been rendered more clear, and has been studied in a fixed specimen.

Dehydration.

The tissue having been fixed, the next proceeding is to get rid of the water present in it. This is accomplished by passing the tissue through solutions of alcohol, gradually increasing the strength until absolute alcohol is reached. Before doing this, however, it is necessary in the case of most fixatives to wash the specimen in running water in order to remove the various reagents. In some cases also it is necessary to use some reagent to remove one of the constituents of the fixative. Thus, when a fixative containing corrosive sublimate has been used, the tissue must be treated with a weak solution of iodine at some stage before staining. This may be done while the tissue is in bulk,

just after washing, or when the sections are mounted on the slides. A convenient method is to put a little iodine in the weaker solutions of alcohol, thus saving time by carrying out two processes at once. The solution of iodine should be of the colour of pale sherry. The pieces of tissue are, in the case of some fixatives, put directly into absolute alcohol,[1] but in the great majority it is necessary to wash and dehydrate gradually. A sudden great change in the strength of alcohol produces very disastrous results upon the cells by setting up violent osmotic currents and by causing shrinking. It has been, and is still, believed by many microscopists that when once a piece of tissue has been fixed it will stand a great amount of rough treatment. The treatment to which it is necessary to submit it is indeed somewhat drastic, but there are very definite limits to what even fixed tissue can tolerate without being fatally damaged. One of the things that generally produces disastrous results is the sudden change from an aqueous solution to strong alcohol.

At the same time it is extremely dangerous to leave material for more than about two hours in water, or in the lower alcohols—that is, under about 70 per cent. It is frequently recommended that material should be washed in water for several hours after fixation, but this is likely to cause maceration in the case of many tissues, particularly in mammalian tissues, and should be avoided.

The most convenient receptacles in which to carry out the process of dehydration and clearing are flat-bottomed glass tubes about half an inch in diameter and from two to three

[1] The subsequent treatment of the tissue is stated with each formula given at the end of the chapter.

inches long. These should be kept corked during the process.

The best results are obtained by increasing the alcohol by 10 per cent. at a time, beginning with 10 per cent. of alcohol in water, and finishing with absolute. It is necessary to have the pieces of tissue in each solution long enough for it to be completely penetrated; and here again the advisability of using very small pieces, or at any rate very thin slices, again becomes evident. When the material is in very thin slices it is not necessary to leave it in each of the lower alcohols for more than ten minutes or a quarter of an hour, and a similar length of time may be devoted to washing in running water. All risk of maceration is thus avoided. The material should be left in absolute alcohol for at least an hour—longer if possible—and the alcohol should be changed at least once during this time.

Having thus been completely dehydrated, the material is transferred to some clearing agent in which the imbedding material is soluble. The best of these all round is cedar-wood oil, particularly in the case of animal tissues. The absolute alcohol is drawn off from the material, leaving enough to cover it completely. The tube is then held obliquely, and cedar-wood oil is poured in gently. The oil will sink to the bottom of the tube, and the pieces of tissue will float at its surface covered by the supernatant alcohol. As the oil penetrates it the material will sink to the bottom of the tube. Both alcohol and oil should then be drawn off and enough fresh oil poured in to cover the material. When the tissue is completely saturated with the oil, which it will be when all opacity has disappeared, it is ready for imbedding. Other clearing agents instead of cedar-

wood oil may be used. In the case of some plant tissues xylol works quite satisfactorily. The process is simpler, as the pieces of material may be directly transferred from the alcohol to the xylol. There is, however, a risk that harm may be done to the structures in the cells by the violent osmotic action produced by the change, and xylol should not be used for animal tissue at this stage of the proceedings.

Imbedding.

The most satisfactory medium in which to imbed material preparatory to cutting sections for microscopical examination is solid paraffin. "Celloidin" is used for some special tissues and for special purposes, but it is not proposed to deal with it here. It is only upon special occasions that paraffin is not the best medium for imbedding.

The most serious risks of injury to the cell structures involved in the process of imbedding are overheating and exposing to heat for too long a time. In most cases it is inadvisable to expose the tissues to a higher temperature than 45° C. Paraffin that melts at this temperature must therefore be used, and small dishes of the melted paraffin should be kept ready in the incubator. When the material is thoroughly saturated with the oil the pieces are transferred separately with a pair of forceps to the paraffin in the incubator. If the pieces are not larger than they should be, the paraffin should completely penetrate them in about an hour's time. The paraffin should be changed at least once during this time, and oftener if the smell of the cedar-wood oil has not disappeared. Some tissues, particularly plant tissues, will require a longer period in the paraffin than

others, and several hours may be necessary in some cases. The time should, however, be as short as is compatible with complete impregnation.

When the material is completely saturated, some melted paraffin is poured into the space formed by imbedding-L's, or some other receptacle which will produce a solid block of paraffin of a suitable size, and the tissue is transferred into this while the paraffin is still liquid. The forceps with which the pieces of tissue are moved must be warmed in the flame of a spirit lamp. The pieces of tissue are so arranged in the paraffin as to be in a suitable position for cutting, according to whether transverse or longitudinal sections are required. It is a good thing to rub glycerine over the imbedding-L's and plate, as this prevents the paraffin from adhering to them when it solidifies. When a skin has formed over the surface of the paraffin it should, with the imbedding-L's, be placed carefully in cold water and the paraffin allowed to solidify.

If the material is to be kept for some time without being cut this is the best condition in which to keep it. While it spoils comparatively soon if kept in alcohol or oil, it will keep indefinitely when it is imbedded in paraffin.

Very thin sections are generally to be avoided. The section should be at least two or three cells thick, as otherwise there will be a large number of parts of cells in the sections, and but few whole ones. Cells vary enormously in size, so the thickness desirable in a section will depend upon the tissue which is being dealt with.

For making preparations of small organisms which are almost or quite invisible to the naked eye, or of blood corpuscles, a modification of the processes described above

gives very satisfactory results. In the case of microscopic forms, as large a number of the organisms as possible are put into as small an amount of water as is practicable at the bottom of a test tube. The test tube is then filled with the fixing fluid and stood upright until all the organisms have settled at the bottom. If blood is to be fixed it is dropped as it is drawn into a test-tube containing the fixative, and then allowed to settle as a sediment at the bottom.

The further treatment is the same in both cases, the organisms or blood being allowed to settle as a sediment at each stage. When the sediment has settled at the bottom of the tube, the supernatant fixative is drawn off with a pipette, and the tube is filled with a 10 per cent. solution of alcohol in water. This process is repeated, increasing the alcohol by 10 per cent. at each change until absolute alcohol is reached. The sediment settles but slowly in the weaker alcohols, and in order to avoid maceration a certain number of blood corpuscles, or of organisms which have not settled, must be sacrificed. The 70 per cent. alcohol stage should be reached within an hour and a half or two hours after the 10 per cent. alcohol was introduced. After changing the absolute alcohol at least once, cedar-wood oil is poured into the tube in the manner previously described. The sediment will sink into the oil gradually and settle at the bottom of the tube. The alcohol and oil are then drawn off and fresh oil poured in. When the sediment has again settled, as much of the oil as possible is again drawn off, and the tube is stood in the incubator for about ten minutes. It is then filled with melted paraffin, and the sediment again allowed to settle. The paraffin is then drawn off with a *hot* pipette without removing the tube

from the incubator. This is repeated several times until the smell of cedar-wood oil is not perceptible. The sediment having settled at the bottom, the tube is then removed from the incubator and stood in cold water until the paraffin has solidified. When the paraffin is quite solid, the tube is broken, and the block of paraffin is cut in serial sections in the usual manner. These sections can be treated just as the sections of pieces of tissue, and the separate cells will adhere to the slide in a perfectly satisfactory manner during the process of staining and mounting.

Mounting Sections.

There are many methods of mounting the sections in series upon the slides. I have found the following very convenient: A little Mayer's albumen[1] is rubbed on the slide with the finger, taking care that very little is left. The ribbon of paraffin containing the sections is then laid gently upon the slide. If the ribbon be examined it will be seen that one side is shiny and the other dull. The shiny surface must be placed next to the glass. If the sections are perfectly flat already they may be pressed gently into contact with the glass with a piece of damp blotting paper. If, however, they are at all wrinkled, which is very frequently the case, a little water should be dropped on the slide with a pipette, sufficient to completely float the sections, but not enough to overflow the edge of the slide. A china plaque or small metal plate is then warmed, and

[1] White of egg, 1 part; glycerine, 1 part; salicylate of soda, 1 part in 100 of the mixture. Mix well together and filter. Filtering may take several days.

the slide placed upon it. If the plaque is at the right temperature the sections will gradually spread out and become perfectly flat. The paraffin should not on any account be allowed to melt, as injury to the sections is likely to result, either from undue heating or by the crystallising and contraction of the paraffin when it sets again. The plaque must therefore not be made too hot, and the slide must be carefully watched and removed before melting takes place. When all the wrinkles have disappeared from the sections, the water should be poured off, and damp blotting paper pressed very gently over the sections. The slides should then be set on edge and allowed to dry. When dry the slides are put into xylol or chloroform to remove the paraffin from the sections. Xylol is generally preferable. As soon as the paraffin has been dissolved, the slides are transferred to absolute alcohol. This coagulates the albumen and makes the sections adhere firmly to the slides. The sections are now ready for the process of staining, and further operations depend upon what method is to be used.

Staining.

In case aqueous stains are used, jars of 20 per cent., 40 per cent., 60 per cent., 80 per cent., and 90 per cent. alcohol should be available, as well as one of absolute. Whenever it is necessary to transfer a slide from an aqueous to an alcoholic solution it should be passed through these various percentages of alcohol ; a few seconds in each will be sufficient.

The largest and most useful group of stains is that of the aniline or coal tar dyes. Indeed, in the great majority of

cases it is possible to demonstrate the different constituent parts of cells without going beyond this class of stains, and they generally give better results from the cytologist's point of view than any other.

The coal tar stains have been divided into "basic," "acid," and "neutral." The basic stains are those in which the colouring matter in the compound plays the part of a base, in the acid stains it plays the part of an acid, and in the neutral stains it is neutral. It must be understood that these terms in practice are often only relative. Thus while gentian violet is a strong basic stain when used with acid fuchsin, it will be found to play the part of an acid stain if used with saffranin. The groups are, however, distinctly marked in relation to each other as groups, though there may be some confusion between two stains belonging to the basic group, one of which is more basic in character than the other. Broadly speaking we may say that the basic stains act upon the chromatin and that the acid act upon the rest of the cell. As a matter of fact, however, all of them will, to a greater or less extent, stain every part of the cell. All that is meant is, that the basic stains show a greater affinity for the chromatin, the acid to the other parts of the cell. These relative affinities form the principle upon which differential staining is based. The slide bearing the sections is immersed in a solution of a basic stain, and is left there until every part of the section is stained. It is then removed, and the slide is washed until all, or nearly all, of the stain is removed from the sections, excepting that which is retained in the chromatin. The slide is then placed in a solution of acid stain, and is left until the desired degree of colouring is obtained. In many cases

the slide may be transferred directly from the solution of the basic to the solution of the acid stain, the basic stain being automatically replaced by the acid in almost every part of the cell except in the chromatin. It is impossible to enter here into any details with regard to many stains, but a list of useful combinations, and the method of employing them, is given at the end of the chapter.

It is of the utmost importance to avoid even partial drying of the sections on the slide during the process of staining and mounting. From the time the paraffin is dissolved until the cover-glass is placed over the balsam or other medium in which the section is finally mounted, any approach to drying is likely to produce the most disastrous results upon the cells and their contents.

If the sections become accidentally even partially dry, the slide is probably not worth preserving from the cytological point of view. It should be thoroughly wet all the time, and its transit from one jar of fluid to another should be rapid.

It is also extremely important not to keep the sections in an aqueous solution for any length of time. More than half an hour in water involves the risk of maceration, particularly with animal tissues. There is an impression that some of the aqueous stains—Heidenhain's iron alum and hæmatoxylin in particular—take a considerable time. As a matter of fact the iron alum and hæmatoxylin stain, which is described at the end of the chapter, can generally be completed very satisfactorily in twenty minutes, or less. It must be remembered that penetration by any fluid must take place in a comparatively short time in the case of the thin sections upon the slide. With strongly alcoholic

solutions there is no need for haste, as there is no chance of maceration.

It often assists staining considerably if the sections are immersed for ten minutes in a weak solution of iodine, with or without a small amount of iodide of potassium, particularly if a fixative containing mercury or osmic acid has been used. The difficulty in staining is, however, often due to the paraffin not having been completely dissolved out of the sections.

In the case of aqueous stains, sections may be examined from time to time with a low power under the microscope. When alcoholic stains are used, however, this should not be attempted, as the alcohol will evaporate very quickly, and partial drying will take place. Experience will teach the correct tint to be obtained in the case of any particular stain in a comparatively short time.

After staining, the sections are brought into absolute alcohol and then into xylol. When they have become transparent, a small quantity of Canada balsam is dropped upon them and a cover-glass is placed over them. The slide is then placed upon a warm plaque and the cover-glass pressed gently down with blotting paper.

In many cases it is desirable to examine cells in the fresh condition. This is best accomplished by teasing out a piece of tissue in salt solution normal to the organism from which the tissue is taken, and after adding a drop of Polychrome Methylene Blue solution or Methyl Green, placing a cover-glass over the tissue and examining at once under the microscope. The normal salt solution prevents any undue osmotic action, and the Methylene Blue stains the cells rapidly without distorting their contents. In some cases,

however (*e.g.*, the fertilized eggs of *Ascaris*), much may be seen without any staining at all. These are, however, special methods, and cannot be dealt with in an elementary treatise.

Appendix.

Fixatives.—The quantity of fixative used should be at least twenty times the bulk of the tissues to be fixed.

(1) Glacial acetic acid . . . . 1 part
Absolute alcohol . . . . 3 parts

This fixative is particularly useful when an examination of the specimen is desired within a short time, as it penetrates quickly and the material is transferred immediately from it to absolute alcohol. The alcohol into which the tissue is put after fixation is changed two or three times, until no smell of acetic acid remains. The time required for fixation varies considerably with different tissues. Mammalian tissue and most animal tissue is generally fixed within ten minutes or a quarter of an hour. In vegetable tissues where thick cell walls exist two or three hours may be necessary. The fixation is generally somewhat coarse, particularly in the case of animal tissues.

(2) Glacial acetic acid . . . . 1 part
Absolute alcohol . . . . 6 parts
Chloroform . . . . . 3 „

The penetrative qualities of this mixture are even greater than those of the last. It is particularly useful in the case of some eggs (*e.g.*, *Ascaris*), which are contained in envelopes that are practically impermeable to most fixatives. It is to be used in the same way as the preceding mixture.

(3) Acetic sublimate (van Beneden).

25 per cent. of Acetic Acid in water. Corrosive Sublimate to saturation.

The pieces of tissue are left until they become opaque, which will be in a few minutes. They are then washed with weak solution of Iodine and dehydrated.

(4) Zenker's fluid (G. Arnold's modification).

Potassium bichromate . .	2·5 grammes
Copper sulphate . . .	1 gramme
Glacial acetic acid . . .	10 cc.
Saturated solution of Corrosive Sublimate in water . .	100 cc.

This is an excellent all-round fixative, and is particularly good for mammalian tissues. Leave tissue in fluid for from one to four or five hours. Wash in weak solution of Iodine, and dehydrate.

(5) Flemming's fluid (strong formula).

1 per cent. Chromic acid . .	15 parts
2 per cent. Osmic acid . . .	4 ,,
Glacial acetic acid	1 part

This is one of the best all-round fixatives, and is particularly successful in the case of animal tissues. Animal tissue should be left in it for from one to four or five hours, vegetable tissues generally for longer, both according to the density of the tissue. This is followed by washing in running water, and then by dehydration.

(6) Hermann's fluid.

The same as the last, excepting that a 1 per cent. solution of Platinic Chloride is substituted for the Chromic

Acid. It is often very useful where chromatin is to be examined specially. It is used in the same way as Flemming's fluid.

Stains.—Iron Hæmatoxylin (M. Heidenhain).

Solution 1.—Ferric alum, 3 per cent. in water. (Ammonio-ferric sulphate, clear violet crystals. The crystals become yellow and opaque when they degenerate; they are then useless.)

Solution 2.—Pure Hæmatoxylin, 0·5 per cent. in water.

After bringing the slide into water the edges and back are dried, and as much of Solution 1 is dropped on to the sections as is practicable without allowing it to overflow. This is left for about ten minutes. The solution is then poured off, and Solution 2 dropped on in the same manner. This is left until the sections are stained a very dense black, which will take about ten minutes, or less. It is often advisable to pour off the solution after two or three minutes, and to add some fresh. The slide is then rinsed in water and Solution 1 dropped on to the sections. Solution 1 dissolves out the stain, and the progress must be carefully watched. The object is to leave the black precipitate in the chromatin and certain other parts of the cells, but to dissolve it out of the rest. At first the amount of washing out can be verified by looking at the sections with a low power of the microscope from time to time, but experience will teach the observer the precise tint of grey desirable for each kind of tissue without a microscopic examination. When the correct degree of washing out has been reached the slide is washed in water for two or three

minutes and then taken up through the alcohols. The sections may with advantage be counter-stained with Bordeaux red, Acid Fuchsin, or Orange G.

Basic Fuchsin.—Saturated solution in 85 per cent. alcohol, with a few drops of aniline. The slide should be left in the stain until the sections are of a dense red colour. This often takes two or three hours, but there is no reason why they should not be left longer. The stain is then washed out of the sections. The amount left in will depend upon the result desired. If no counter-stain is to be used the chromatic structures should be bright red and the other parts of the cell different shades of pink. If a counter-stain is used the washing out should be carried further, so that but little colour is left excepting in the chromatic structures. Orange G is a very suitable counter-stain. Basic Fuchsin may also be used as a counter-stain with iron hæmatoxylin. When used as a basic stain it is often necessary to wash out with alcohol rendered slightly acid with hydrochloric acid.

Saffranin.—Saturated solution in 85 per cent. alcohol with a few drops of aniline. Treatment, the same as with Basic Fuchsin. Saffranin is a more strongly basic stain. If Orange G is used as a counter-stain the sections should be washed out with acid alcohol. This is a very excellent stain where chromatic structures are to be specially examined.

Very beautiful preparations may be made by using Thionin or Toludin blue as a counter-stain. Here, however, the slide is not even rinsed after being in the saffranin, but is transferred directly into the Thionin or Toludin solution. It is left in the latter only for a few

seconds; often it will be sufficient to dip the slide into the counter-stain two or three times. It is then slightly rinsed in 90 per cent. alcohol and quickly transferred to absolute and then to xylol.

Thionin.—Saturated solution in 85 per cent. alcohol. This stains the chromatic structures very sharply, but unfortunately the colour fades in the course of a few months. When used as a basic stain, Bordeaux red is probably the best cytoplasmic stain to use with it.

Toludin Blue.—Same as Thionin. The stain is a little more diffuse.

Bordeaux Red.—Saturated solution in 85 per cent. alcohol. This is an excellent cytoplasmic stain.

Acid Fuchsin.—0·5 per cent. solution in water. This stain is very active, and not more than two or three minutes are usually required. It stains centrosomes remarkably well as a rule.

Orange G.—Though a saturated solution in water may be used, as this stain sometimes does not act quickly, it is best used dissolved in clove oil. It takes some days to obtain a saturated solution in the oil. The slides are put into this solution from absolute alcohol. It is best to take the slide back to absolute alcohol and thence to xylol, when the desired intensity of staining has been obtained, as this ensures the superfluous orange being removed before mounting. If this is not done the basic stain may be rendered too faint by the action of the orange.

Method of preparing and staining wet films (A. Breinl).—A thin layer of Mayer's albumen is spread upon a slide. A drop of blood is spread on this layer, and while the blood is still wet the slide is dropped into Flemming's fluid,

where it is left for about five minutes. It is then washed in water and brought into absolute alcohol, the alcohol being increased by 10 per cent. at a time. It is thence put into a solution of equal parts of iodine and potassium iodide in 80 per cent. alcohol. This solution should be of a dark-brown colour. It is left in this solution for from five to ten minutes, and then taken down to 30 per cent. alcohol and stained for about half an hour in either of the following solutions:—(1) "Saffranin nach Babes," or (2) equal parts of concentrated solutions of saffranin "O," soluble in water, and alcoholic saffranin. A few drops of anilin oil should be added to this mixture, which should be allowed to ripen for at least three months, being well shaken from time to time. After staining with the saffranin the slide is rinsed and stained with the following solution: Polychrome Methylene Blue (*Purissima medicinale*), 7 grammes; distilled water, 100 cc.; sodium carbonate, $\frac{1}{2}$ gramme. This is placed in an incubator to ripen. The older the solution, the better its staining properties. After a dark-blue colour has been attained the slide is washed and then differentiated with Unna's Orange Tannin as long as clouds of blue stain are thrown off. The slide is then brought up through the alcohols and put into anilin oil, which removes the excess of blue stain. After being cleared in xylol the slide is ready for the Canada balsam and the cover glass. [The greatest caution must be exercised when using this method in order to prevent partial drying of the film before it is fixed. There should be no more risk of this occurring after fixation than in the case of any other kind of preparation.]

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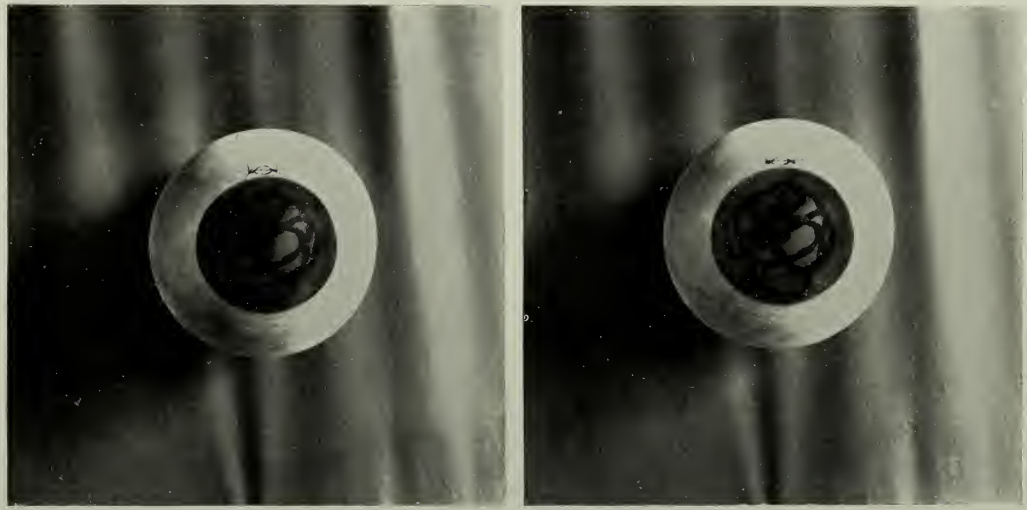
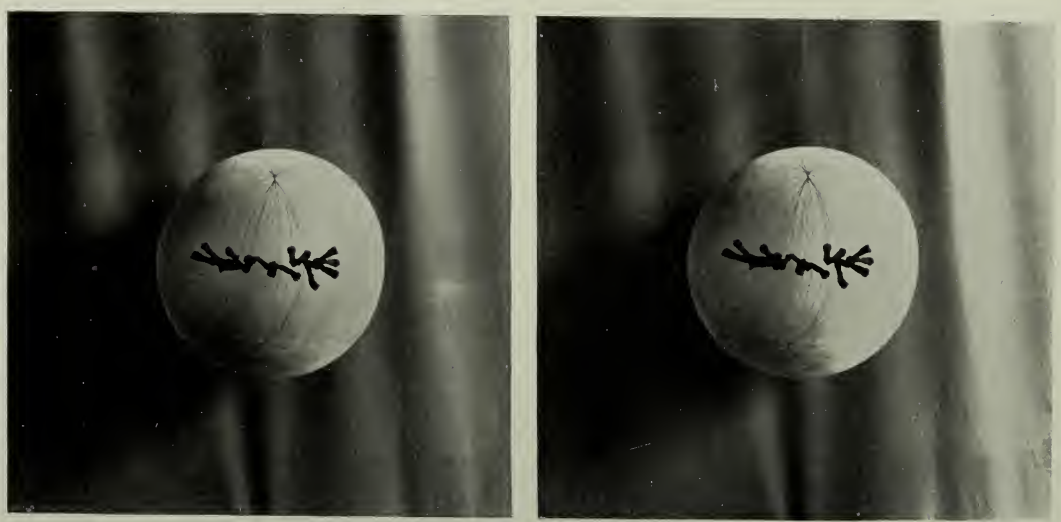
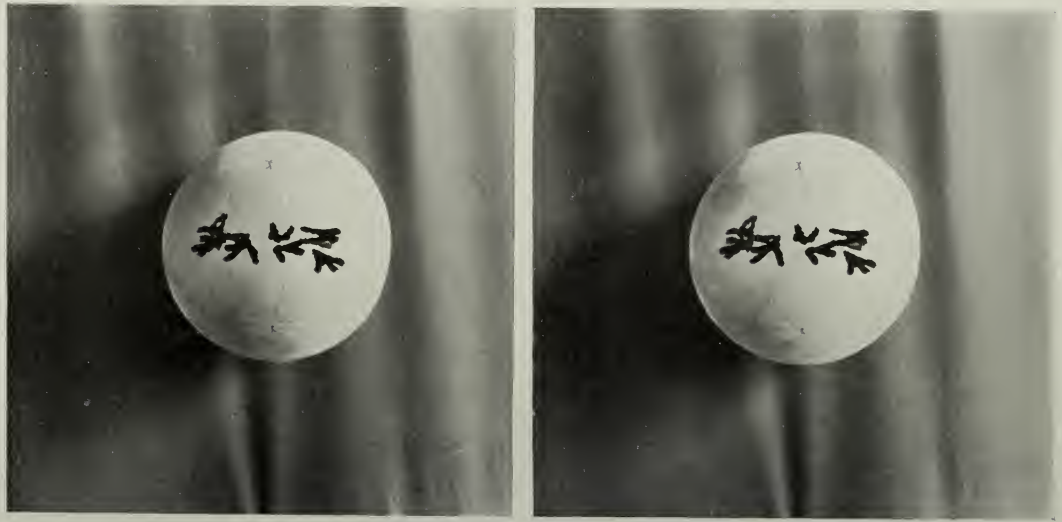


1 Early prophase showing the chromatin and linin in the form of a spireme. The centrosomes are beginning to separate, and radiations may be seen extending from one to the other and into the cytoplasm.

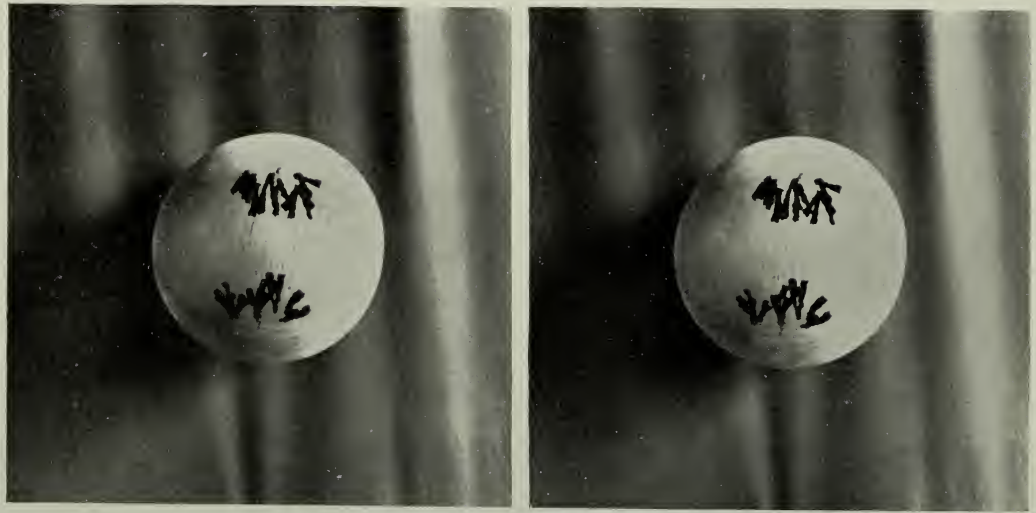
2. The spireme has broken up into segments. [Chromosomes.] The centrosomes have travelled further apart and the spindle is nearly fully developed.



3. The nuclear membrane has disappeared, and the chromosomes have become attached to the spindle, each chromosome to a fibre.



4. The chromosomes are splitting lengthwise.



5. The longitudinal halves of the chromosomes have travelled towards the opposite poles of the spindle, and are grouped together round the respective centrosomes.