CYTOLOGY

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CYTOLOGY

WITH SPECIAL REFERENCE TO THE METAZOAN NUCLEUS

BY

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PREFACE

For many years now it has been apparent to biologists that the nucleus —and especially its most conspicuous constituent, the chromatin—has special claims to be considered the master substance of the organism, not only determining its form and activities within the life-time of each individual, but by means of the gamete nuclei bringing about that organic continuity between parent and offspring which we call heredity. The absorbing interest of this conception has attracted many workers to the field of nuclear cytology, and consequently the advance in knowledge and the output of hypotheses have been very great in recent years. As must happen in any growing branch of science, the results of all this research are scattered through many publications, and any one whose duty it has been to lecture to students on this science must often have felt difficulty in recommending to inquirers a concise course of reading which will give a summary of the main results in this field of research, and at the same time indicate where more detailed information can be obtained. This book is an attempt to provide for this want.

The discovery of mitosis, and the recognition of all that is implied by that process, has led biologists to lay more stress on the nucleus than upon the cell, considered as units, and lately the " unit " has tended to shift to certain constituents of the nucleus, namely, the chromosomes ; still more recently the advance of knowledge has made it profitable to focus attention on the even more elementary " units " of which the chromosomes themselves are composed. In this book, consequently, problems concerning the organization and physiology of the cell as a whole are scarcely touched upon, practically the whole space being devoted to the nucleus and its constituent parts. Moreover, the field has been still further restricted by confining attention chiefly to the Metazoan nucleus. The nucleus of the Metaphyta is very similar, in structure and behaviour, to the Metazoan nucleus, and only rarely needs

special mention. With the Protista the case is different, but even these forms have been treated in a very brief and eclectic manner. This is partly because, like most cytologists, the author has unfortunately very little first-hand knowledge of the cytology of this group, and partly because Protistan cytology has not up to the present time helped us very much to gain an insight into those aspects of the nucleus and its activities with which we are here concerned.

As the science of Physiology is almost entirely founded on the physiology of the higher Vertebrates, so is Cytology mainly the cytology of the Metazoa and Metaphyta. In both cases the greater simplicity of the technique of dealing with the larger forms has no doubt been partly responsible for this state of affairs, but there is another and more important reason. The functions of organs and the meanings of processes are far easier to interpret when the organs are complicated and the processes specialized than when they are simple or generalized. The specialization of the nerve cells of the Metazoa for conduction of impulses, of their muscle cells for contraction, and of their gland cells for secretion has enabled physiologists to gain a far deeper insight into the physiology of these processes than they could have obtained from the study of the Protistan cell in which no one function dominates over the others. Similarly, the high specialization of the mitotic processes in Metazoa and Metaphyta has afforded cytologists an insight into their meaning far greater than could ever have been obtained from the study of the less specialized nuclei of most of the Protista. In order properly to understand any process, however, it is necessary to know as many variants of it as possible, as well as the steps by which it has been evolved. For this reason a very brief account of some of the more striking differences between the Metazoan and Protistan nuclei has been given.

Each branch of Science presents its own special technical difficulties to its students. Cytology has at least its full share of these. The objects with which the cytologist has to deal are extremely minute, and indeed his analyses are almost invariably limited merely by the imperfection of his optical instruments and technique. However powerful the former and perfect the latter, he is sure that below the limits of visibility there exists a morphological complexity at least as great as that which he has already revealed. At present he has no methods comparable with those of the chemist and physicist for dealing indirectly with ultramicroscopic

complexity, though a promising start in this direction has been made by Mendelian analysis and its correlation with hypothetical "factors" or "genes" in the idioplasm. Even when dealing with objects slightly above the limits of visibility, the difficulties of observation are often very great, so that two cytologists examining an identical object will often give a different account of it. Many examples will occur to any cytologist —for instance, the different accounts given of the changes undergone by the chromosomes when passing into the resting nucleus at telophase. The appearance of the disintegrating chromosomes of the same organism has been variously interpreted as (1) vacuolation, (2) a splitting into two threads, (3) the formation of a single spiral thread, (4) the formation of two intertwined threads.

Besides many other difficulties inherent in the nature of cytological research, the science suffers especially severely from one of the difficulties in the way of progress of all sciences. The researcher can only select for study a minute fraction of the mass of objects presented to him, and inevitably those objects appear to him significant, and therefore worthy to be studied, which fit into his preconceived ideas. If a cytologist sets out to study the gametogenesis of some animal, he will probably pass under review through his microscope many hundreds of thousands of cells. Out of these he can necessarily only select a minute proportion for detailed study. The cells which he thus selects are, of course, those which seem to him to represent stages in the process which he is endeavouring to reconstruct. If he has already formed a theory regarding this process, having a more definite mental image of the process as conceived by him than of the possible alternatives he more readily picks out for study those objects which appear to favour his theory than the others, which he rejects (as he is bound to reject the great majority) as equivocal or of no significance. This certainly appears to be the explanation of the partisan nature of so much cytological work.

The student must not get the impression from the above that it is hopeless to discover the truth in cytology. Gradually one or other of conflicting views becomes recognized as being nearer the truth than its rivals, or else some generally accepted principle is raised from the ashes of them all—either by the gradual accumulation of evidence or by some important discovery which is generally recognized as providing the key to the problem. In this way a science of cytology has grown up, firmly established as regards its main outlines.

A glance through the pages of this book will show that it makes no pretence to trace the historical development of the science, and consequently the works selected for special reference are not always those which contain the first, or even the most important, contribution to the matter under discussion. In most cases they have been selected either as giving a particularly clear account of it, or because they contain good general discussions and literature lists which would be useful to the student referring to them.

Since the book is intended to give a summary of the more important results of cytological research, it follows that the great majority of the figures are taken from the original works of other authors scattered through various scientific journals. They have in nearly every case been redrawn for the purpose by Miss Helen L. Ness, often with the omission of details not required for the purpose for which they are used.

CONTENTS

CHAPTER I

CHAPTER II

CHAPTER III

CHAPTER IV

CHAPTER V

CHAPTER VI

CHAPTER VII

CHAPTER I

NUCLEUS AND CYTOPLASM

In general a cell is composed of two principal morphological constituents, the *nucleus* and the surrounding *protoplasm*, or better, *cytoplasm*, since some authors use the former word to include both cytoplasm and nucleus. This division of the cell constituents is quite sharp in all but the lowest organisms, the nucleus being delimited from the cytoplasm by a membrane except during a certain period of its division processes. In certain unicellular organisms (Protista), however, the word "nucleus" is inappropriate, since the material which composes this structure in the higher organisms is scattered through the cytoplasm as minute granules, or *chromidia* (see Chapter VI.). In other Protista both nucleus proper and chromidia are present (*e.g. Difflugia*), while certain Bacteria are said to consist entirely of "nucleus." Even in multicellular organisms, both in Metazoa and Metaphyta, there are frequently minute bodies in the cytoplasm which are supposed by some cytologists to be derived from the nucleus, and to consist of true nuclear material, and therefore to be comparable to chromidia.

A. THE CYTOPLASM

The cytoplasm surrounding the nucleus includes a number of different structures of which the following are the most important :

(1) The cytoplasm proper.
(2) The cell membrane.
(3) The centrosome.
(4) Chondriosomes.
(5) Metaplastic bodies.

(1) *The Cytoplasm Proper*

The living cytoplasm itself consists of a viscid, nearly transparent substance, which often is clearly not homogeneous. Great difference of opinion exists as to its structure, chiefly owing to the fact that very little

organization can be made out in it in the living state (its constituents having nearly the same refractive indices), while when "fixed" by one of the various killing and hardening agents, and stained, the structure which is thus made visible varies considerably with the reagents used for fixation and the subsequent staining. It is not proposed therefore to do more than mention the most important views, especially as at present we have no means of correlating structure with function in the case of the cytoplasm. In our present state of knowledge, to decide between these various views is comparatively unimportant as regards general biological problems, apart from biophysics, with which we are not here concerned; but as we shall see later, the matter stands otherwise with the nucleus, where exact determination of structure and function is often of critical importance for theories of heredity and other problems.

Views as to the structure of the cytoplasm can be arranged as follows: (*a*) the reticular; (*b*) the fibrillar; (*c*) the granular; (*d*) the alveolar.

(*a*) The reticular theory. According to this, the cytoplasm consists of a more solid constituent forming a reticulum or network, like a sponge, containing in its meshes a more fluid substance known as the cell sap or *enchylema*. In addition, a greater or smaller number of minute granules or *microsomes* are embedded in the reticulum.

(*b*) The fibrillar theory. According to this view the reticulum is not continuous, but is composed of disconnected threads embedded in a matrix (Flemming,[1] 1882).

(*c*) The granular theory. As developed by Altmann (1893) this depends more upon theory and less upon observation than do the other views. The cytoplasm is supposed to consist essentially of granules, only the largest of which are visible through the microscope. The microsomes mentioned above are examples of these. Each granule is itself a living organism or *bioblast* and bears much the same relation to the cell as the cell itself to the whole organism. As regards the intergranular substance, Altmann supposed that this is mainly composed of granules below the limit of visibility. Any substance which may be left over between these ultimate granules is non-living matrix. This theory is mainly of historical interest.

(*d*) The alveolar theory. The alveolar theory of Bütschli (1892) supposes the cytoplasm to possess a frothy structure similar to that of the emulsion formed when two immiscible fluids are shaken together. The cytoplasm therefore consists of minute drops of one fluid suspended in a second, denser fluid, which, being generally small in bulk compared with the included droplets, forms thin films surrounding them like the films of soapy water surrounding the air in a foam of soap bubbles.

[1] References to the exact source of all authorities quoted will be found at the end of the book.

In optical section these films or lamellae surrounding the droplets are easily interpreted wrongly as a reticulum. This theory is supported by the behaviour of the artificial emulsions made by Bütschli, which exhibit many striking resemblances to cytoplasm.

Granules or fibrillae (chondriosomes, chromidia, etc., see Chapter VI.) may be suspended at the nodes of the apparent meshwork formed by the denser fluid. Thus this theory is not incompatible with the fibrillar theory, the alveolar structure applying to the matrix in which the fibrillae are embedded.

The alveolar theory is the one that fits in best with the known properties of the cytoplasm, and especially with its undoubtedly fluid nature. For living cytoplasm is, physically, a fluid—often very viscid indeed, but nevertheless fluid. During life streaming movements are often observable in it. Moreover, bodies such as the nucleus, which are very large relatively to the meshes or alveoli, can move through the living cytoplasm. Examples of rapid change of position of the nucleus in the cell are afforded by living Protozoa such as *Amoeba*, and by the movements of the pronuclei in fertilization in the Metazoa (Chapter III.). Many other proofs of the fluid nature of the cytoplasm could be cited, such as the spherical shape assumed by vacuoles and by fragments of cytoplasm extruded from a cell into water. These facts are impossible to reconcile with the presence of a permanent supporting reticulum forming part of the essential structure of the cytoplasm. In many kinds of cells supporting reticula and fibrillae are indeed undoubtedly present, but these are of a different order of structure and belong to the architecture of the cell as a whole, and not to the structure of the cytoplasm, which lies within the meshes of the supporting framework.

(2) *The Cell Membrane*

With few exceptions (certain Protozoa, leucocytes) animal cells are plainly delimited by an outer metamorphosed layer of cytoplasm, or by a membrane secreted by the cytoplasm, the distinction between the two being often very difficult to make and indeed unreal.

Sometimes, however, nuclear division is not followed by cell division with development of a membrane between the two cells, and in this case there arises a structure known as a *syncytium*, in which a number of nuclei are embedded in a continuous mass of cytoplasm. Well-known examples of syncytia are the plasmodia of Mycetozoa and the ectoderm of Nematodes.

In certain cells the membrane attains a much greater importance, and may lose its connection with the underlying cytoplasm which secreted it, so that the latter comes to lie more or less freely within it as if in a box with which it had no close organic connection. In such a

case of course the cytoplasm inside must form a new membrane round its periphery. Examples of cell membranes which have become relatively free from the cytoplasm by which they were secreted are the cellulose cell walls of most plant cells and the vitelline membranes of many eggs.

(3) *The Centrosome* (see also p. 21)

As we shall see, during division of the nucleus the centrosomes are the dynamical centres of the cell. Even when the nucleus is not actually in process of dividing, the centrosome may exert a powerful influence on the topographical arrangements both of the nuclear constituents and of the cytoplasm. This is specially well illustrated in what is known as the "bouquet" stage in gametogenesis (p. 33), where the chromosomes are in the form of U-shaped loops, so orientated that their free ends are directed towards the centrosome and the apices of the loops towards the opposite pole of the nucleus. In these cells the cytoplasm is heaped up round the centrosome, which is embedded in a mass of chondriosomes, "chromidia," etc., which it appears to have attracted round itself (p. 191).

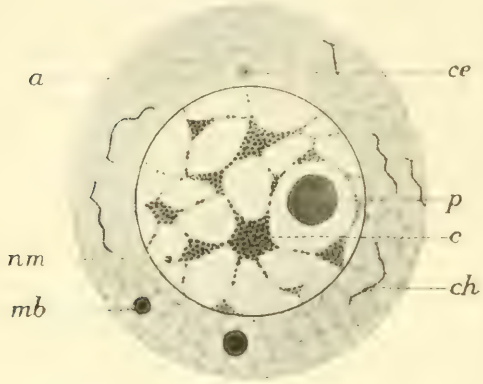


FIG. 1.

Diagram of a Cell. *a*, alveoli of cytoplasm; *c*, chromatin in the form of fine granules (chromioles) embedded in the linin meshwork; *ce*, centrosome, containing centriole; *ch*, chondriosome; *mb*, metaplastic body; *nm*, nuclear membrane; *p*, plasmosome.

(4) *Chondriosomes*

These are minute granules or filaments embedded in the cytoplasm. They are discussed in Chapter VI.

(5) *Metaplastic Bodies*

are non-living material included in the cytoplasm, such as yolk granules, fat globules, excretory and secretory granules, etc.

B. THE NUCLEUS

The study of the *nucleus* is in many respects easier than that of the cytoplasm. There are several reasons for this, amongst which may be mentioned the comparative coarseness of its structure, and the strong, selective affinity for stains possessed by its constituents.

The structure of the nucleus varies profoundly according as to whether it is going through the processes connected with nuclear division, or is in the phase between two division periods. In the latter condition, which we will consider first, the nucleus is said to be at "rest." It must, however, be understood that this word implies merely that the nucleus is at rest from division. The "resting nucleus" is doubtless in the phase of its greatest physiological activity.

A typical Metazoan resting nucleus consists of the following parts: (1) *chromatin*; (2) *linin*; (3) *nuclear sap* or *karyolymph*; (4) *karyosome*; (5) *plasmosome*—the two last mentioned both being known as *nucleoli*; they may or may not be present, and if present they may be multiple; (6) *nuclear membrane.*

The arrangement of these constituents varies greatly, the commonest disposition being such that the linin is a faintly staining substance forming a spongework stretching throughout the nucleus and containing embedded in it the chromatin—a substance which colours intensely with most stains. The meshes of the linin-chromatin reticulum—or rather spongework—so formed are filled with the fluid karyolymph. Karyosomes are larger aggregations of chromatin, but the term is incapable of exact definition. It is in practice restricted to comparatively large chromatin masses occurring in nuclei in which the rest of the chromatin (if any) is finely distributed. Nuclei of a coarser structure may contain equally large aggregations of chromatin at the nodes of the reticulum, though these are not generally called karyosomes.

Plasmosomes are composed of a substance called *plastin*, which is different in nature from any of the above-mentioned substances. Many nucleoli, however, are incapable of classification as karyosomes or plasmosomes, since they partake of the nature of both, consisting of plastin impregnated with chromatin. Examples of this kind of nucleolus (sometimes called an *amphinucleolus*) are found in the "karyosomes" of many Protista, and probably in the nucleoli of certain Metazoan oocytes. True plasmosomes disappear before nuclear division and are reformed in the young daughter nucleus. They are doubtless of a metaplastic nature.

Since it is impossible to appreciate the nature of the various constituents of the nucleus without knowledge of their behaviour during nuclear division, further discussion of them will be postponed till after we have studied this phase in the life of the nucleus.

A nucleus divides to form two nuclei in one of two ways, the one being known as *indirect division*, *mitosis* or *karyokinesis*, and the other as *direct division* or *amitosis*. The overwhelming majority of nuclear divisions among the Metazoa and Metaphyta are of the first or mitotic type, which we will now proceed to consider.

C. MITOSIS

The essential feature of mitosis is the rearrangement of the chromatin and linin to form a number of separate, thread-shaped bodies, the *chromo-*

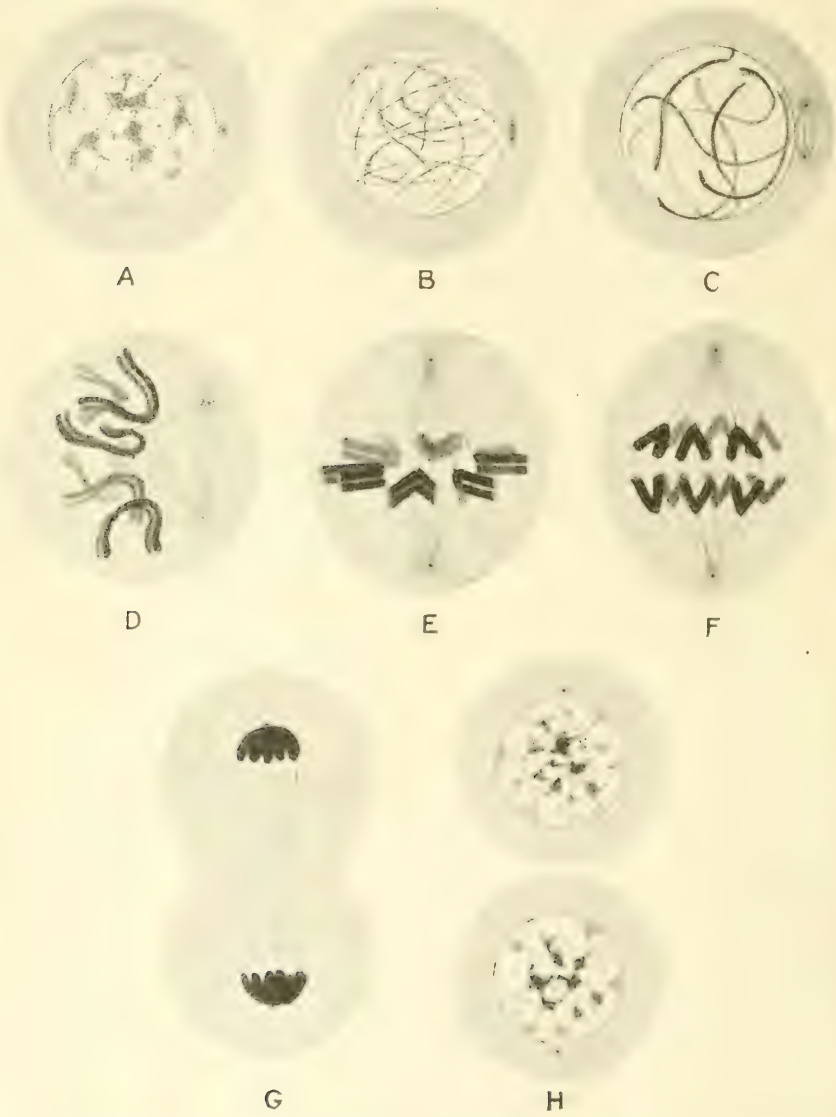


FIG. 2.

Diagram of Mitosis. The nucleus contains six chromosomes. A, resting nucleus; B, early prophase, individual chromosomes not yet distinguishable, centrosome dividing; C, middle prophase, appearance of spindle figure; D, late prophase. The nuclear membrane has disappeared and the chromosomes are becoming attached to the spindle fibres. E, metaphase; F, anaphase; G, telophase; H, nuclear and cell division complete, and daughter nuclei reconstituted.

somes, each of which subsequently divides into two *daughter chromosomes*. The original series of chromosomes is thereby duplicated into two exactly

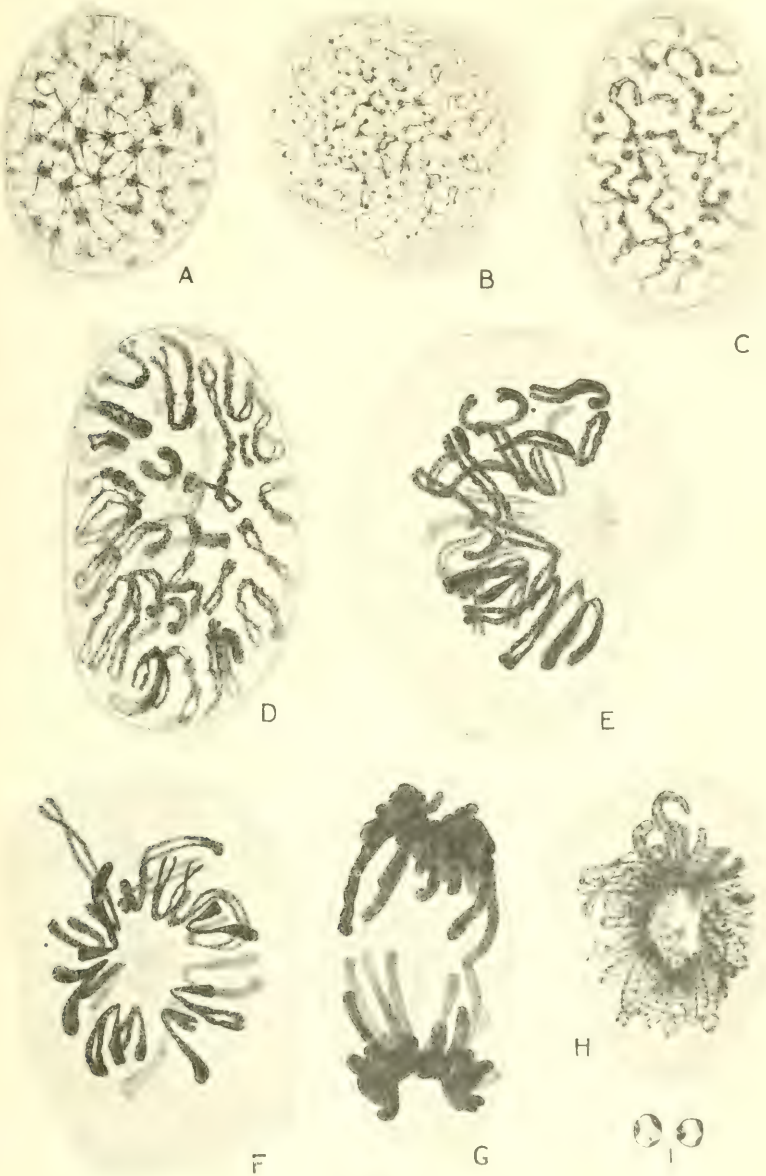


FIG. 3.

Mitosis in *Lepidosiren* (mesenchyme cell). A, resting nucleus; B, very early prophase; C, D, middle prophase; E, late prophase. The nuclear membrane has disappeared and the chromosomes are becoming attached to the spindle fibres. F, metaphase (seen from above). Only about half of the chromosomes are shown. G, anaphase; H, telophase, reconstruction of one of the daughter nuclei; I, two of the chromosomes from H, in transverse section and under a higher magnification.

similar groups, from each of which groups a new nucleus is constituted.

Certain cell structures — the centrosomes and spindle, forming together the *achromatic figure*—though generally outside the nucleus, are inseparably connected with mitosis and must be considered with it.

The process of mitosis is illustrated by the diagrammatic Fig. 2, while Figs. 3 and 4 show how the principal stages actually appear under the microscope. Fig. 3 shows a mitosis of a nucleus with abundant

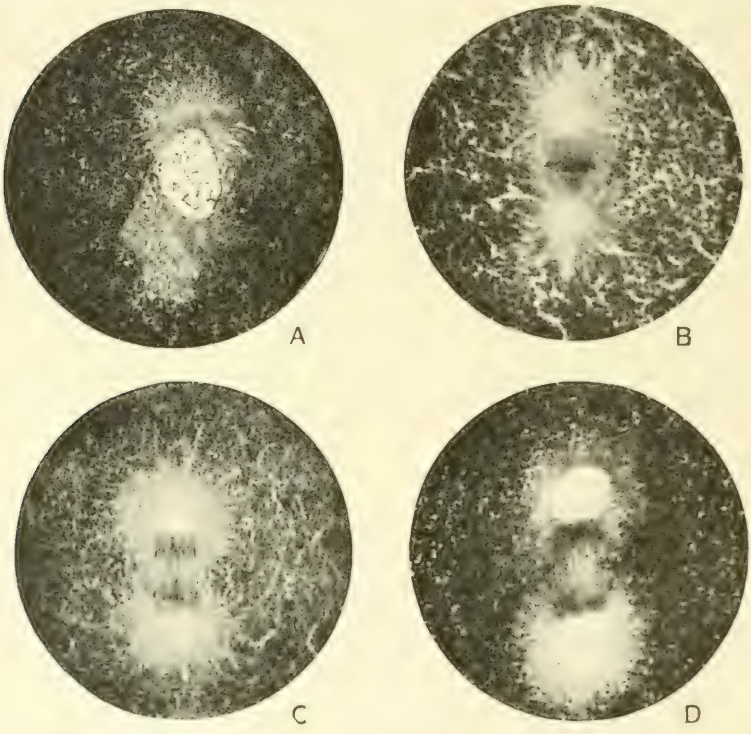


FIG. 4.

The first cleavage mitosis in the egg of *Echinus esculentus* (micro photographs by Professor T. H. Bryce). A, late prophase, nuclear membrane breaking down; B, metaphase, C, early, and D, late, anaphase.

chromatin, but not very voluminous achromatic figure, while Fig. 4 represents a mitosis of a nucleus poor in chromatin, but provided with a very well developed achromatic figure.

The sequence of events in mitosis is commonly divided into four main phases, namely, *prophase, metaphase, anaphase* (Strasburger, 1884) and *telophase* (Heidenhain, 1894). It must not be forgotten, however, that these are arbitrary divisions of a continuous process.

The *prophase* consists essentially in the reconstruction of the chromatin and linin of the resting nucleus into filaments, which by a process of

condensation ultimately form the relatively short and thick chromosomes of the later stages. Karyosomes, if present, since they are composed of chromatin, disappear, being used up with the rest of the chromatin in the formation of the chromosomes. If plasmosomes are present, they disappear either before or after the disappearance of the nuclear membrane (see below), apparently without participating in the formation of the chromosomes or playing any further part in the life-history of the nucleus.

For some time after the thread formation, which starts in the early prophase, has proceeded or even been completed (by conversion of the entire chromatin content of the nucleus into filaments), the length of the threads is far greater than the circumference of the nucleus (Figs. 3, 6, 7, 8), and hence the nucleus is filled with a complicated tangle—the *spireme* of Flemming—in which it is impossible to discern how many separate filaments are present. There may indeed be no apparent breaks in the thread, and when such do appear it is often difficult to determine whether they are real interruptions of continuity, or merely the optical effects of a sharp angle in the thread, etc. This gave rise to the old view that in the early prophase there is but a single greatly convoluted thread present, and so this stage was known as the *continuous* or *unsegmented spireme*, in contra-distinction to the *segmented spireme* of the later prophase, in which a number of separate threads is plainly present. However, careful examination, and especially comparison with forms in which the nuclei are poorer in chromatin and the prophase filaments consequently less voluminous, has led very generally to the conclusion that the conception of the continuous spireme as a constant stage in prophase is incorrect, but that at all stages of the prophase the spireme generally consists of as many separate segments as there will ultimately be chromosomes. In fact, the spireme is a tangle of very long thin chromosomes. It is not, however, uncommon for even fully formed chromosomes to cohere by their ends (Fig. 5), and there is no doubt that this occasionally happens in the case of the early prophase filaments also; in this way a continuous spireme might be formed, but its continuity would be, so to speak, accidental and not an essential feature of it.

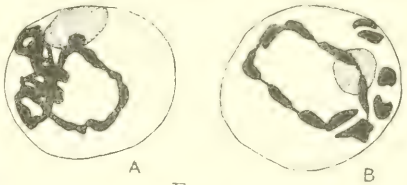


FIG. 5.

Meiotic prophase (?) in *Oenothera rubricalyx*, showing cohering chromosomes. (Gates, *Botanical Gazette*, 1908.) A, uncut nucleus showing single thick spireme; B, later stage showing the spireme segmenting into chromosomes.

A striking and important characteristic of the prophase threads or chromosomes in many species is their duplicity. This can often be observed from the very beginning of the prophase (Figs. 3, 8), and is caused by the longitudinal division of each chromosome into two

daughter chromosomes, by which the division of the nucleus as a whole is effected.

The remaining processes of mitosis are concerned merely with the separation of the daughter chromosomes and the reconstruction of new nuclei out of them.

In many species the division of the chromosomes is not apparent till a later stage (metaphase), and indeed the moment at which division occurs seems to vary greatly in the different cells of a single organism. It must, however, be remembered that we are dealing with very minute bodies, in which a narrow cleft may easily be obscured by the certain amount of distortion (swelling, shrinkage, etc.) inevitable during fixing and staining. The question of the division of the chromosomes will be returned to again (p. 13).

Another striking and important characteristic of the prophase chromosomes in many animals and plants is their alternate expansion and constriction, giving them the appearance of a string of beads. This is also a very variable phenomenon, generally most conspicuous in early or middle prophase. The beads are known as *chromomeres*, and are discussed in Chapters V. and VI.

In the later prophase shown in Figs. 2, C, 3, D, the spireme consists of obviously separate chromosomes. The nucleus, which has been steadily increasing in size since the inception of the prophase, has now attained its maximum volume, and the chromosomes are usually evenly spaced out through it. This stage of the prophase is conspicuous enough to have earned the special name of *diakinesis* (Häcker, 1897, *b*).

Except in the rare cases where the whole mitosis takes place within the nuclear membrane, diakinesis ends with the disappearance of this membrane, so that the chromosomes lie naked in the cytoplasm.

During prophase the centrosome (in animal cells) divides, if it has not already done so, and the two resulting daughter centrosomes move apart to take up positions at opposite poles of the nucleus. As they separate, connection is maintained between them by fine lines or fibres, the *spindle fibres*, and at the same time similar fibres radiate out from the centrosomes into the cytoplasm, the *asters*. The whole system of fibres (together with the centrosomes in animal and certain plant cells) is often known as the *achromatic figure*. After the dissolution of the nuclear membrane, some of the spindle fibres grow in and attach themselves to the chromosomes (Fig. 2, D).

Soon after the disappearance of the nuclear membrane the chromosomes typically become arranged in one plane, at right angles to the spindle fibres, in the manner shown in Figs. 2, 3, 4. The plate of chromosomes so formed is known as the *equatorial plate*.

As we have already seen, each chromosome is already, or now becomes,

longitudinally split into two daughter chromosomes. The *metaphase* in Strasburger's sense is the rather ill-defined moment when the two daughter chromosomes begin to move apart. Very often, however, the word is used to cover the whole period in which the complete mitotic figure persists, with the chromosomes arranged midway between the two poles of the spindle and the daughter chromosomes not yet completely separated.

At this stage it can be seen that each daughter chromosome is attached by one or more spindle fibres to one (and only one) centrosome.

The *anaphase* is concerned with the final separation of the two groups of daughter chromosomes, each of the latter travelling up the line of the spindle fibres towards one of the poles of the spindle. It is generally agreed that the fibres of the achromatic figure are the visible expression of the forces by which the movements of the chromosomes are effected, but there is considerable difficulty in determining the nature of their action (p. 23).

Often the separation of the daughter chromosomes takes place very regularly, so that by the splitting of the individual chromosomes which compose it the metaphase equatorial plate is divided into two *daughter plates*, which gradually diverge from one another. In other cases the movements of the chromosomes are not so regular, so that the separating daughter chromosomes travel up to the poles more independently.

The *telophase* comprises the metamorphosis of each of the two clumps of daughter chromosomes into a new resting *daughter nucleus*; the details of this process are discussed below.

During telophase, or late anaphase, the cell body becomes constricted between the two new nuclei, the constriction becoming deeper and deeper till finally two separate cells are produced, each containing one of the new daughter nuclei.

Each daughter nucleus thus contains one of the products of division of each of the chromosomes in the mother nucleus. As regards chromosome constitution, the daughter nuclei are therefore of like constitution with each other and with the mother nucleus.

A fact of fundamental importance for cytological theory, and one that has been established by innumerable observations, is that, with certain mostly well-understood exceptions which will be discussed in the later chapters of this book, the number of chromosomes in the nuclei of any given species is constant. Thus to take the species whose nuclei are figured in this chapter, the number of chromosomes in the nuclei of *Lepidosiren* is 38. It is indifferent in what tissue the nucleus is situated; whether it is a skin, nerve, muscle, connective tissue or other nucleus, the number of chromosomes which it exhibits at mitosis is 38. In

Allium cepa (the onion) the number is 16. The species *Ascaris megalocephala* contains two varieties, one of which, *bivalens*, has four chromosomes, the other, *univalens*, has only two. This last example has the smallest number of chromosomes yet recorded, or indeed conceivable, for an animal reproducing itself sexually, as will appear directly. The largest number of chromosomes so far accurately determined for any species is probably the 208 found in the crustacean *Cambarus immunis* (Fasten, 1914). Often nearly related forms differ widely from each other as regards the number of their chromosomes.

Another extremely important fact is that in many species, both of animals and plants, the chromosomes in any one nucleus are not all of the same length, and, moreover, that the relative sizes of the chromosomes are constant, in spite of the fact that the whole series of chromosomes may be longer in some tissues than in others. The relative size differences are also independent of the changes in length undergone by the chromosomes during mitosis, for these affect the whole series of chromosomes alike and approximately simultaneously. Indeed, throughout the whole mitosis, from the early prophase to the end of anaphase, the chromosomes are almost continuously shortening and thickening.

The constancy of the number of the chromosomes and of their relative sizes in individual organisms and species, together with many other considerations discussed in Chapter V., has led to an almost general agreement that the chromosomes, though not recognizable as such in the resting nucleus, nevertheless maintain their continuity from one mitosis to another, so that the substance—at least the essential living substance—of each chromosome, though diffused in the resting nucleus and indistinguishably intermingled with the substance of the other chromosomes, is nevertheless condensed together again in the next prophase. The same series of chromosomes that entered into the resting nucleus at telophase reappears therefore at the next prophase, each single chromosome of the one stage being continuous with one of the other.

An examination of those species in which the size differences among the chromosomes are strongly marked discloses at once the fact that there are two chromosomes of each size. If the chromosomes of such a form are designated, in order of magnitude, by the letters of the alphabet A, B, C . . ., we find in each nucleus two chromosomes of each kind, namely, A + A + B + B + C + C . . . We may here anticipate the later chapters by explaining that this double supply of chromosomes is due to the fact that in sexual reproduction the new individual is formed by the union of two germ cells, one from each parent, and that each germ cell has one complete set of chromosomes (A + B + C + . . .), so that the fertilized egg has the double set (A + A + B + B + C + C + . . .). Owing

to the continuity of the chromosomes, this double series is perpetuated throughout the nuclei of the growing embryo. Corresponding chromosomes, for example, the two A's, are known as *homologous chromosomes*.

The above remarks on the continuity of the chromosomes and the nature of the chromosome equipment of organisms are anticipatory of the later chapters of the book, where these points will receive more detailed consideration ; we will now return to certain problems of mitosis, the main steps of which we have just outlined.

(1) *The Division of the Chromosomes, and their Relation to the Resting Nucleus*

As we have seen, the prophase chromosomes are often from their first appearance double, *i.e.* split into two daughter chromosomes, possibly signifying that the chromosomes, or rather the elements of which they are composed, were already divided in the resting nucleus. It is even possible that the actual moment of division may be during the anaphase of the previous mitosis.

The problem of the mode and moment of division of the chromosomes is therefore intimately bound up with the question of the exact processes by which the compact chromosomes of the anaphase are changed into the resting nucleus, and those by which they are condensed out of it again in the following prophase. Moreover, a knowledge of these processes is essential to a proper understanding of the relation between the structure of the resting nucleus and that of the chromosomes.

Unfortunately the telophase and early prophase are two of the most difficult stages of the whole nuclear life-history to interpret, and very different accounts of them have been given by different workers, some of whom have claimed a large measure of generality for their conclusions, explaining the conflicting results of other researches by faulty fixation, wrong interpretation, etc. The contradictory conclusions reached by different cytologists must indeed be attributed to these factors to a certain extent, since they often refer to the same object. This is very well illustrated by the work on three forms which have been much studied on account of the size and clearness of their cytological elements, namely, the tissue cells of the larval salamander, the developing eggs of *Ascaris megalocephala*, and the root tips of the onion (*Allium cepa*).

The egg of *Ascaris* is specially favourable for this study owing to its small number of chromosomes, and to the fact that in the nuclear reconstruction at telophase the ends of the long chromosomes usually form projections from the main mass of the nucleus, thus greatly facilitating the study of the changes taking place in a single chromosome (these remarks refer to nuclei in the " germ track " only, see Chapter III.).

Figs. 6, 7, 8 show the process by which the chromosomes pass into the

resting nucleus at telophase, and reappear in the following prophase in *Ascaris*, *Salamandra* and *Allium* respectively, as observed by different workers.

The following are the principal views held regarding these three objects :

(1) The telophase chromosomes undergo a process of vacuolation, by which each becomes converted into a spongy cylinder ; this becomes further decomposed into a loose spongework. The spongeworks formed

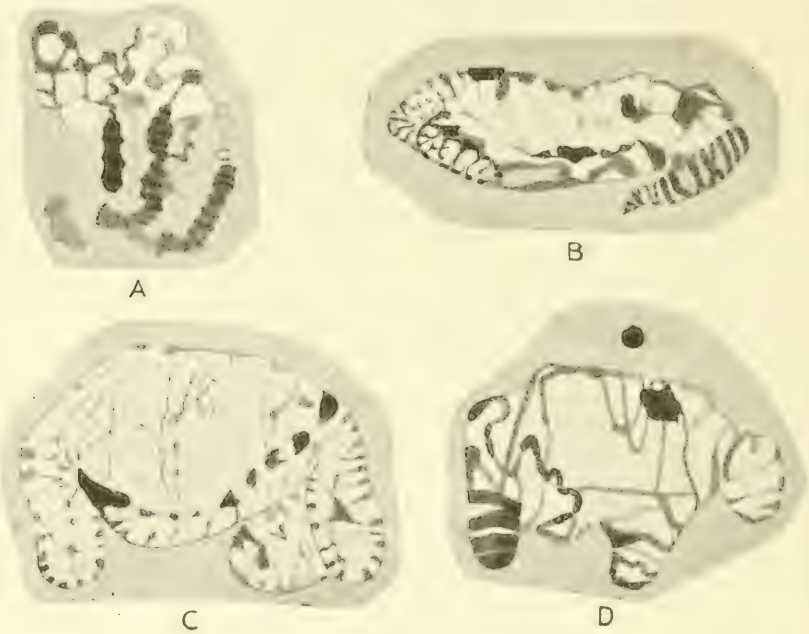


FIG. 6.

Blastomere nuclei of *Ascaris megalcephala*, showing the evolution of a single spiral thread from each telophase chromosome, and its reappearance as the prophase chromosome of the following mitosis. (After Bonnevie, *A.Z.*,[1] 1908.) A, B, telophase ; C, resting nucleus ; D, prophase.

by all the chromosomes become indistinguishably merged into one another, forming a " network of networks," which is the constitution of the resting nucleus. In the prophase a reverse process takes place, each chromosomal spongework becoming concentrated first into a spongy band, and then into a homogeneous thread. Division of the chromosomes into daughter chromosomes takes place in prophase. [Van Beneden and Neyt (1887), *Ascaris* ; Boveri (1909), *Ascaris* ; Kowalski (1904), *Salamandra* (Fig. 7) ; Grégoire (1906), *Allium* (Fig. 8).

(2) The telophase metamorphosis consists essentially in the formation of long threads from the chromosomes ; the reticulum of the resting

[1] For the abbreviations used in references to certain journals, see p. 217.

nucleus is formed by the intertwining of these threads, which at the same time become irregular and broken up (as regards the chromatin; the linin basis of the threads remains continuous) and connected with each other by anastomoses. Prophase consists of the reverse process. There are two variations on this view:

(*a*) Two threads are formed from each chromosome, and hence the prophase chromosomes are from the first double, and even the resting nucleus is duplex as regards its chromatin constituents. According to this view, therefore, the real division of the chromosomes into daughter chromosomes takes place not in prophase but in the previous telophase

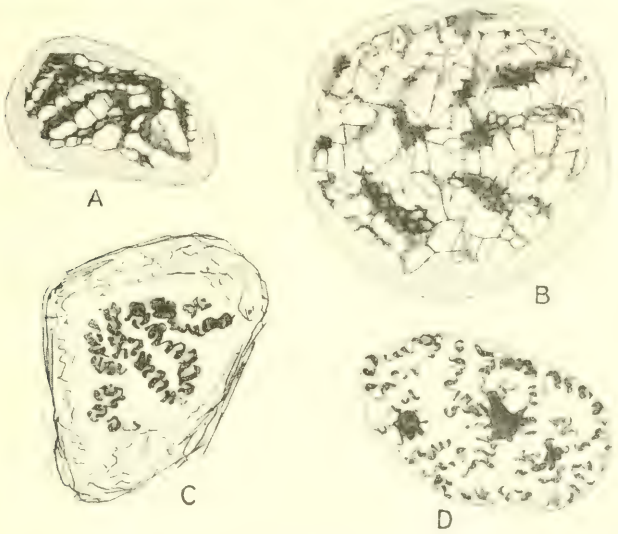


FIG. 7.

Larva of *Salamandra maculosa*. (A, B, after Kowalski, *L.C.*, 1904; C, D, after Schneider, *Fest. für R. Hertwig*, 1910.) A, C, telophase; B, D, prophase.

or anaphase. This view is held by many workers, *e.g.* Schneider (1910), *Salamandra* (Fig. 7); Dehorne (1911), *Salamandra* and (1911) *Allium* (Fig. 8), with, however, a different interpretation as to the part played by the anaphase division in the following mitosis; Lundegardh (1913), *Allium*; Schustow (1913), *Allium*.

(*b*) Only one thread is normally produced from each chromosome in telophase, the division of the chromosomes taking place in prophase. [Bonnevie (1908), *Ascaris* (Fig. 6) and *Allium* (Fig. 8); Boveri (1909), *Ascaris*, exceptionally; Vejdovsky (1911), *Ascaris*.]

It is clear that where different workers base such contradictory conclusions on identical material, the reason for their differences must be sought largely in the difficulty of interpreting these confused stages,

so that the same microscopical picture is interpreted by one cytologist as vacuolation, by another as the unravelling of a single twisted thread, and by a third as the intertwinings of two threads. Thus, even in the most obvious cases of thread formation, this is always accompanied by irregularities in the thickness and in the distribution of the conspicuous

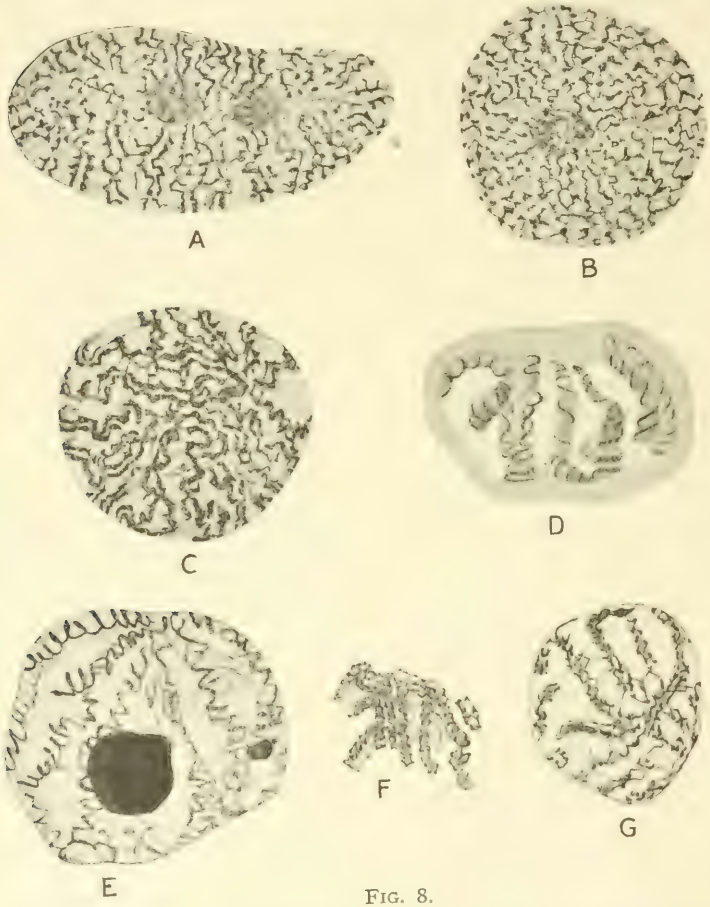


FIG. 8.

Root tips of *Allium cepa*. (A, B, C, after Dehorne, *A.Z.*, 1911; D, E, after Bonnevie, *A.Z.*, 1908; F, G, after Grégoire, *L.C.*, 1906.) A, D, F, telophase; C, E, G, prophase; B, resting nucleus.

chromatin along the inconspicuous linin basis of the thread, and also by outgrowths and anastomoses, which are generally sufficient to conceal entirely in the resting nucleus its essential construction out of comparatively few long threads. The same factors, acting in telophase at a still earlier stage of thread formation, may easily conceal the true nature of this process, and convert what is essentially an irregular and twisted thread into the appearance of a reticulum. On the other hand, the early

stages of the conversion of a long cylindrical object (like the anaphase chromosomes) into a spongework or reticulum is naturally the formation of a row of vacuoles down its axis, and this is easily mistaken in optical section for a splitting of the cylinder into two threads, or as the development of a coiled thread. Both these explanations have been invoked by cytologists to explain the different interpretations arrived at by their fellow-workers. Thus, it would be impossible to decide whether the structure of the telophase chromosomes of Fig. 3, H, as revealed by their transverse sections in Fig. 3, I, has been derived from that of the compact anaphase chromosome by its simple vacuolation, or by the formation within it of a spirally wound beaded thread, or even of two such threads intertwined.

The theory of thread formation in telophase is an attractive one, for it is highly probable, firstly, that the physiological meaning of the telophase metamorphosis of the chromosomes is the resulting increase of surface, compared with volume, of the chromosomes, and secondly, that the chromosomes, at any rate in prophase, consist of chromomeres or other constituents arranged in a linear series. The simplest way of conserving this linear arrangement, and at the same time increasing the surface, is by the outgrowth of the compact anaphase chromosome into a long thread ; this, moreover, explains the usually immense length of the early prophase chromosome compared with the same chromosome in metaphase or anaphase. Indeed, where the telophase metamorphosis of the chromosome consists of vacuolation and reticulation we must suppose that the linear arrangement is only obscured (as, for example, in the case of a long string of beads which has become tangled into a knot) and not lost, for it reappears in the following prophase (which we may compare with the unravelling of the knot).

Summing up, we must take as provisionally established the following propositions :

(1) The telophase transformation of the chromosomes consists essentially of an increase of their surface relatively to their volume. This may be effected either by the conversion of the compact anaphase chromosome into a long thread, or by its vacuolation, reticulation or other method [1] of irregularizing its outline, with consequent temporary loss of visible linearity of structure, though essentially this is retained.

(2) The division of the chromosomes into daughter chromosomes in preparation for the metaphase may take place in the anaphase or telophase of the preceding mitosis, or may not be demonstrable till the prophase or even till the beginning of the metaphase itself.

[1] For example, the curious forms assumed by the chromosomes in the germinal vesicles of many animals, Fig. 23.

(2) *Chromatin and Linin*

Chromatin is distinguished from linin by its much greater affinity for most stains. It is from this feature that it gets its name, while the linin is often called, in contradistinction, *achromatin*. By many cytologists chromatin is believed to be composed of very minute granules, or *chromioles* (see Heidenhain, 1911). The blocks of chromatin seen in most resting nuclei, or the chromomeres of prophase chromosomes, are aggregations of numbers of chromioles.

In many nuclei the meshes of the chromatin and linin spongework are filled with a granular mass, as if the karyolymph had been precipitated by the fixative. According to Heidenhain (see 1911), however, the granules are granules or chromioles of a substance allied to the true chromatin, and known as *oxychromatin*, the chromatin proper then being designated *basichromatin*. This terminology is based on the fact that the chromatin in the usual sense of the term, *i.e.* the basichromatin, has a special affinity for the basic aniline dyes, while the oxychromatin stains more readily with acid dyes. (These terms do not refer to the acid or alkaline reaction of the solutions of the stains, but to their chemical derivation.) Unless the contrary be stated, the word chromatin as used in this book refers to the basichromatin.

The interpretation of the granular mass in the meshes of the true chromatin spongework as composed of pre-existing granules and not due to precipitation of the karyolymph by the fixative is made more probable by the observations of Gross (1916), who was able to observe these granules in the living nucleus.

There is some reason to believe that the two kinds of chromatin (if indeed the oxychromatin be entitled to this designation) are different phases of the same substance, for they appear to be convertible into each other—as, for instance, in the growth stage of the oocyte (cf. Jorgensen, 1913). Moreover, in the prophase of all mitoses the oxychromatin disappears completely, either by solution, or by conversion into or absorption by the basichromatin. Hence a nucleus rich in oxychromatin presents a characteristically different appearance in the resting and prophase stages; in the latter the spireme filaments stand out sharply in a perfectly clear karyolymph, while in the former the basichromatin spongework is partially obscured by the mass of faintly stained oxychromatin. In the young daughter nuclei the oxychromatin is formed anew, probably at the expense of the basichromatin. Doubtless in correlation with its disappearance in mitosis, oxychromatin is generally very scanty, or altogether absent, in nuclei which are undergoing rapid multiplication.

It is generally supposed that the chromatin (in the form of chromioles)

is embedded in the linin, but a few cytologists (*e.g.* Grégoire and Wygaerts, 1904; Lundegardh, 1912) hold the view that there is no distinction between the two substances, and that the common aspect of the nuclear substance as consisting of a deeply staining material superimposed on a much finer and more weakly staining framework is not due to any chemical difference between the two parts, but simply to the fact that the stain is retained by the coarser masses (chromatin) but not by the very fine strands usually interpreted as linin. This view, however, seems to have little in its favour, and there are very great difficulties in the way of accepting it. For instance, in the prophase the young chromosomes are often connected by numerous fine transverse unstained threads (linin) (Figs. 3, D, 16, G). The chromosomes are all approximately of the same thickness, and the transverse threads, which are much finer than the chromosomes, are also approximately equal in thickness to one another. If these threads were merely thinner strands of the same material as the very much thicker chromosomes, it is hard to understand why we do not find all gradations in thickness between the chromosomes and transverse threads.

Heidenhain (1911) considers the linin to be the contractile substance by which the movements undergone by the chromatin in prophase and telophase are brought about. It is probably closely similar to cytoplasm in nature, though plainly of a firmer consistency.

(3) *Nuclear Membrane and Karyolymph*

The mode of formation and nature of the nuclear membrane is uncertain. It is possible that it is formed out of the linin framework of the nucleus, or, on the other hand, it may be a condensation or precipitation of the cytoplasm where it comes into contact with the karyolymph or nuclear sap which accumulates between and within the chromosomes at telophase. The telophase nucleus may in this case be conceived of as lying in a vacuole full of karyolymph, the cytoplasm round the circumference, and therefore in contact with the nuclear sap, becoming hardened to form the nuclear membrane. Whatever its mode of origin, the membrane becomes an integral part of the nucleus.

As a rule, the nuclear membrane disappears in the late prophase, but it may persist throughout the whole mitosis (*e.g.* many insects) or may disappear in early prophase (*e.g.*, *Mesostoma*, von Voss, 1914).

(4) *Karyosomes and Prochromosomes*

The term *karyosome* may be applied to any mass of chromatin large enough to stand out conspicuously from the general nuclear groundwork. Thus in a finely reticulated nucleus a comparatively small aggregation of chromatin may be alluded to under that name, while a nucleus with a

coarser structure may contain larger chromatin masses which would not receive that title.

No general rule can be given as to the relation of the karyosomes of the resting nucleus to the telophase or prophase chromosomes. In some cases they are portions of the chromosomes which have failed to undergo the telophase dissolution and remain as compact chromatin blocks. This is well seen in Fig. 9 (*Lepidosiren*). Here the anaphase chromosomes form a dense ring (daughter plate), the apices of the V-shaped chromosomes being on the inner circumference of the ring, while the limbs radiate outwards. At the end of telophase, when the daughter nucleus has been reconstructed, a ring of karyosomes occupies the place previously occupied by the apices of the V's. These bodies gradually get dispersed through the nucleus and disappear, so that in the middle of the resting period they are absent.

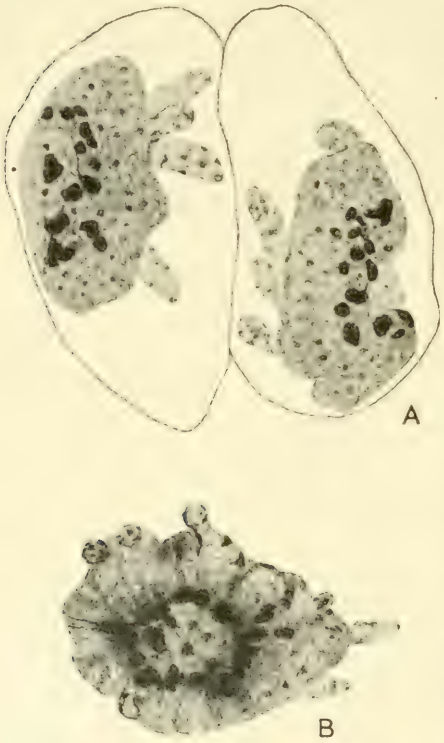


FIG. 9.

Telophases in mesoderm cells of *Lepidosiren*. A, side view; B, polar view.

On the other hand, in many forms in which karyosomes occur they are not traceable back to the telophase chromosomes, but are secondary formations, the newly reconstructed nucleus being without them (*Allium*, Lundegardh, 1913).

As a rule, the number of karyosomes in the nuclei of any organism is highly variable, but in other cases the number is found to be constant, and to be the same as the number of chromosomes in the species in question, as shown by Rosenberg (1904). Moreover, these karyosomes may act as centres of formation for the chromosomes in prophase, for which reason they have received the name of *prochromosomes* (Overton, 1906). They have been specially studied in plant cells (Fig. 10).

The presence of " prochromosomes " in the resting nucleus has been taken, with some justice, as additional evidence of the continuity of the chromosomes from one mitosis to another. It must be remembered,

however, that they only form a special case of karyosomes present in varying numbers, and often not traceable into the prophase chromosomes.

(5) *The Achromatic Figure*

This is the name given to the centrosome and system of radiating lines proceeding from it through the cytoplasm, which are plainly concerned with the separation of the daughter chromosomes. The term refers to the fact that (with the exception of the centrosome and centriole) the substance of which the system is composed (sometimes known as the *archoplasm*) has much the same weak staining reaction as the bulk of the cytoplasm and the linin. The main features of its development during mitosis and its general disposition have already been described (Fig. 2).

A complete achromatic figure at the metaphase of mitosis consists of the following parts: (1) A minute deeply staining *centrosome* occupies the centre of the radiations at each pole of the figure, and may contain (2) a still smaller central granule, the *centriole*. The centrosome is the point of insertion [1] of the so-called fibres of the achromatic figure, namely, (3) the radiating fibres composing the *aster*, and the *spindle fibres*. The latter are of two kinds, (4) the *mantle fibres*, which are attached to the chromosomes, and (5) the fibres of the *central spindle*, which run right through from one centrosome to the other. In many forms, however, a central spindle seems to be absent.

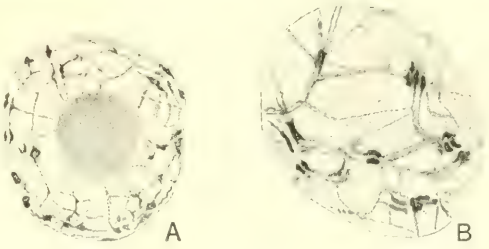


FIG. 10.

Prochromosomes in Pollen Mother cells of *Calycanthus floridus*. (Overton, *J.m.B.*, 1906.) A, resting nucleus; B, prophase.

The terminology of these various parts is unfortunately in some confusion, especially so far as concerns the centrosome and immediately associated structures. This is largely because the centres of the system are occupied by a substance arranged in concentric layers, and opinions differ as to how much of this should be called centrosome. The centriole is very often, indeed generally, indistinguishable from the rest of the centrosome, and in this book the latter term is used to cover the centrosome together with the contained centriole (when such is present).

Fig. 2 illustrates a case where the whole, or nearly the whole, achromatic figure arises from the cytoplasm outside the nucleus. Very often, however, the spindle at least is of intranuclear origin, probably derived

[1] The fibres cannot always be traced actually into the centrosome, but sometimes end in a clear spherical mass of cytoplasm, called the *centrosphere*, surrounding the centrosome.

from the linin ; and finally the whole achromatic figure, including the centrosomes, may be intranuclear (*e.g.*, *Ascaris megalocephala univalens*, Brauer, 1893).

In nearly all resting cells the achromatic figure disappears except for the centrosome, and occasionally a mass of differentiated cytoplasm surrounding it, called the *attraction-sphere* ; this corresponds to the central mass of the aster which surrounded the centrosome during mitosis. Even the centrosome can only be demonstrated on favourable material. In most resting cells both cytoplasm and chromatin seem to be disposed without reference to the centrosome, but in others this body obviously exerts a powerful influence on the disposition of the various cell constituents. A good example of such a cell is afforded by the gametocytes in the " bouquet " stage (Chapter II.).

Though so minute, the centrosome is often a conspicuous body owing to its intense affinity for certain common stains. In order to form the spindle figure it divides by simple fission into two daughter centrosomes, which separate from one another, spinning out the central spindle (when such is present) between them, and each becoming the centre of an astral radiation. These facts have led many cytologists to look upon the centrosome as a permanent cell organ, comparable in autonomy to the nucleus, and only arising by division of a previous centrosome. This view, however, is beset with grave difficulties. There is, for instance, strong evidence that a centrosome may arise *de novo* in the cytoplasm, and thereafter behave in precisely the same way as a centrosome derived by fission from a previous centrosome (p. 95). Moreover, in the higher plants, which have an achromatic figure otherwise essentially like that of animals, there are no centrosomes.

The division of the centrosome may, like that of the chromosomes, take place in anaphase, telophase or prophase. In the two former cases it is of course double in the resting cell.

It must be remembered that the *division* of the chromosomes is an autonomous process independent of the achromatic figure, for it often takes place before the spindle figure is formed or while it is still outside the nucleus. For the *separation* of the daughter chromosomes, however, a properly developed achromatic figure appears to be essential. Thus Wilson (1901) found that in the eggs of the sea-urchin developing by artificial parthenogenesis (p. 95), various abnormalities of the achromatic figure often appeared. One such irregularity was the failure to form a proper bipolar spindle, instead of which a single aster only was formed (*monaster*, as opposed to the *amphiaster* of a normal bipolar figure). In such eggs the ordinary nuclear cycle may be gone through many times. At each mitosis the chromosomes divide, but the daughter halves do not separate ; instead of forming two daughter nuclei, they enter into a

single resting nucleus again, and there is no cell division. Thus nuclei are produced with three or four times the normal number of chromosomes.

The mechanism by which the achromatic figure brings about the separation of the daughter chromosomes and subsequently cell division is still imperfectly understood. Two main theories are held: one that the "fibres" of the astral rays and spindle figure are actually what they appear to be, namely, fibres or threads, and the other that they are merely lines of force or stress. The first and simplest form of the fibrillar theory supposed that the mantle fibres are contractile, comparable to muscle fibres, inserted at one end into the centrosome and at the other into the chromosomes. The centrosome being held in place by the astral rays, contraction of the mantle fibres pulls the chromosomes

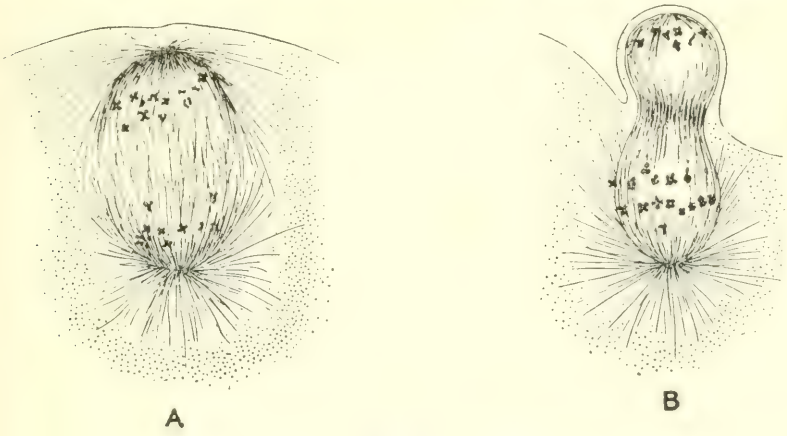


FIG. 11.

Early and late anaphase in the formation of the first polar body in *Echinus esculentus*. (Bryce, *Q.J.M.S.*, 1903.)

towards the centrosome. This simple theory, however, is met by insuperable difficulties. One of these is that in telophase of most mitoses the chromosomes come very close indeed up to the centrosome, thus demanding an apparently impossible amount of contraction on the part of the fibres, while such a contraction would of necessity be accompanied by a relatively enormous thickening of the fibres, which, however, is not observed. Again, in the formation of the polar bodies during the maturation of the egg, the spindle fibres appear to exert a *pushing* rather than —or at any rate in addition to—a *pulling* action in bringing about their extrusion from the surface of the egg (Fig. 11). These and many other considerations have led to the further hypothesis that the separation of the daughter chromosomes is aided by an elongation of the fibres which connect the separating daughter chromosomes, together with those of the central spindle, which pushes the centrosomes apart. How-

ever, no mechanical theory of contraction and expansion appears to be capable of giving a satisfactory explanation of all the facts, and consequently many cytologists look upon the rays of the aster and the spindle "fibres" merely as the expression of lines of force emanating from the centrosomes as centres. An analogy for this view is found in the position taken up by iron filings in a bipolar magnetic field. A very serious objection to this theory, however, is that the lines radiating out from the two centrosomes frequently cross each other, and this is incompatible with a system of lines of force. This is illustrated incidentally in Fig. 33, D.

The mechanism by which the achromatic figure brings about the movements of mitosis must therefore be for the present admitted to be quite unknown. An account of the whole problem of the achromatic figure, much fuller than attempted here, will be found in Wilson's text-book *The Cell*, and a discussion of the physical and mechanical problems involved in mitosis is given by Meek (1913).

D. AMITOSIS

In amitosis or direct division, the nucleus, without departing from the resting structure, divides by simple transverse fission. In the simplest case it first changes from a sphere into a dumb-bell or hour-glass shape (Fig. 12), and then becomes nipped across at the constriction to form two separate nuclei. In other cases the nucleus produces lobes which may become constricted off from the parent nucleus to form independent nuclei. In this mode of nuclear multiplication there is no chromosome formation, and apparently no mechanism to ensure that the daughter nuclei are each supplied with one of each of the chromatin elements of the mother nucleus, as is provided for by the longitudinal division of the chromosomes in mitosis. Therefore if each product of amitotic division, at least amongst the Metazoa and Metaphyta, were able to form a complete set of perfect chromosomes, it is plain that we should have to modify greatly the theory of chromosome continuity and differentiation outlined above, or else postulate some at present unknown mechanism for the precise partition of the chromatin elements in amitosis. This would be especially the case if it were shown that normal gametes could be produced from the descendants of cells which have divided amitotically, though it is conceivable that tissue cells could survive and multiply even though lacking some of the chromosomes or chromosome constituents.

The mere occurrence of amitosis therefore is of little significance unless it can be shown that nuclei produced in this way can afterwards proceed to complete chromosome formation and normal mitosis. In

many cases where amitosis has been described, no proof even that cell division follows has been given, and in these cases there is no evidence, even if mitosis does subsequently take place in the cell, that it is not preceded or accompanied by refusion of the nuclear fragments. In the spermatogonia of many animals the nuclei become deeply lobed or constricted during a prolonged resting period, and occasionally one or more of these lobes becomes nipped off, producing a cell with two or more nuclei, generally of unequal sizes. There is no evidence, however, that cell division follows the nuclear fragmentation, nor do we find any reason to believe that each of the nuclear fragments may proceed to a separate mitosis within the same cell. On the contrary, there is every reason for the belief that the lobing, or fragmentation, is merely a temporary phenomenon, probably correlated with the necessity for increase

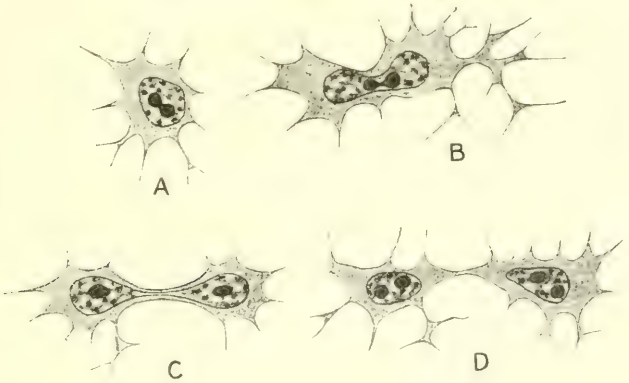


FIG. 12.

Four stages in "amitotic" division in a tendon of a new-born mouse. (Nowikoff, *A.Z.*, 1910.)

of surface relative to volume, and that the lobes are withdrawn or the fragments fuse together again before mitosis. In *Lepidosiren* the spermatogonial nuclei during periods of nuclear inactivity become very irregular and deeply lobed. Preparations for mitosis are only found, however, in approximately spherical nuclei, implying that the lobes have been withdrawn. Meves (1891, 1895) found that the spermatogonia of the salamander are lobed in the winter. In the spring the lobes are withdrawn and the now regularly rounded nuclei proceed to mitosis.

In the cleaving eggs of *Triton* (Rubaschkin, 1905) the nuclei are often completely divided into two, three or even more separate nuclei, though it is probable that in this case they have not been produced by the fragmentation of a single mother nucleus, but by the separation of the telophase chromosomes into groups, from each of which a separate little nucleus is formed. In prophase, chromosomes are formed in each one of the separate nuclei, which remain isolated till the nuclear membranes

break down at the end of the prophase. A single metaphase figure is then formed from the combined chromosomes of the various parts.

We therefore see that the fact that a nucleus is lobed, or divided into separate parts, is no evidence that it is dividing, or has divided, into daughter nuclei, each of which is capable of proceeding to a separate mitosis. On the contrary, it is probably a temporary phenomenon only, the lobes being withdrawn, or the fragments being fused to form a single nucleus again, at mitosis.

Irregular or fragmented nuclei are known as *polymorphic nuclei*, and are of fairly frequent occurrence. Besides the examples cited, they are particularly characteristic of certain leucocytes.

Even when amitosis is followed by cell division, as appears to be sometimes the case, it is very difficult to show that the nuclei can subsequently divide mitotically. Meves believes that normal mitosis may follow a peculiar type of amitosis, accompanied by cell division, in the spermatogonia of the salamander. The amitosis in these cases is not effected by a simple nipping off of a lobe of the polymorphic nuclei described above, but by a more complicated process in which the centrosome plays an important part, and which has not yet been found to be of general occurrence. The identification of nuclei in which true mitosis is taking place as the descendants of nuclei which have divided amitotically is, however, necessarily uncertain.

The amitotic multiplication of nuclei in the cleaving egg and germ track of tapeworms was first described by Child (1904), but has been contradicted by others (*e.g.* Richards, 1909; Harman, 1913). A considerable controversy has grown up over this matter, for a guide to the literature of which the reader is referred to Nakahara (1918).

An experiment by Nathansohn (1900), following one by Pfeffer, has had a good deal of importance attached to it. So-called amitosis was produced in the green alga *Spirogyra* by placing the living filaments in 1 per cent ether solution. The process of nuclear division was observed under the microscope, and takes twenty-five to thirty minutes. The nucleus, which contains (usually) one large nucleolus, becomes opaque, then presently clears again; two nucleoli are now found to be present. Then the nucleus becomes constricted and divides, apparently by amitosis, one nucleolus going to each daughter nucleus; cell division follows. Nathansohn kept these cells under observation and found that mitosis could take place in them, and even that conjugation occurred between descendants of nuclei produced in this way. The above processes were also examined in more detail in fixed and stained preparations.

Experiments by Häcker (1900), confirmed by Schiller (1909), on the effect of ether on mitosis in the cleaving eggs of the crustacean *Cyclops* have important bearings on the *Spirogyra* experiment. Living eggs of

Cyclops were placed in weak solutions of ether in water, with the result that, though amitosis was not produced, the normal course of mitosis was superficially much altered. The anaphase and telophase especially acquired a superficial resemblance to the later stages of amitosis, owing to a tendency of the chromosomes to start their telophase metamorphosis before they had completely separated in metaphase. The anaphase thus consisted of two confused masses of chromosomes, which began to draw apart while still connected with one another, forming an hour-glass figure not unlike that formed by a nucleus dividing amitotically into two. The modification of the normal course of mitosis thus induced does not result in death of the nucleus, for eggs removed from the ether solution into pure water resumed normal development.

Very similar results were obtained by Němec (1904) by the action of chloral hydrate on the root tips of several of the higher plants.

It is probable, therefore, that the apparent amitosis observed by Nathansohn was really a modified mitosis, *i.e.* a division preceded by chromosome formation.

Proliferation by amitosis has often been described in pathological growths, but here again there is no proof that normal mitosis may follow.

Summing up as regards the Metazoa and Metaphyta, it is extremely improbable that normal mitosis ever takes place in nuclei produced by true amitosis—that is to say, by direct mass division of the nucleus without any sort of formation of chromosomes and their division into daughter chromosomes for partition to the daughter nuclei.

A summary of the literature on the subject of amitosis in the Metazoa and Metaphyta is given by Nakahara (1918).

The question of amitosis in the Protista must be reserved to Chapter VII.

CHAPTER II

MEIOSIS

In the life-cycle of the great majority of organisms there occurs a moment when a new individual, the offspring, is formed by the fusion of two reproductive cells budded off from the parents. The reproductive cells are the gametes, and the cell formed by their union is the zygote. The male gamete is the *microgamete* or *spermatozoon*, and the female gamete the *macrogamete* or *ovum*.

This periodical fusion of cells at fertilization or *syngamy* involves the fusion of their nuclei, and hence a mechanism must exist to prevent a corresponding periodical doubling of the mass of nuclear constituents, or, to put it from the point of view of the hypothesis of the continuity of the chromosomes, we must look for a mechanism to prevent the doubling of the number of the chromosomes at each act of syngamy. This mechanism is found in the fact that each gamete is provided with only one-half of the number of chromosomes characteristic of the "species" (*i.e.* the zygote or ordinary individual).[1] Thus the gamete is said to be *haploid* and the zygote *diploid* in regard to their chromosome equipment. The process of reducing the number of chromosomes to one-half is known as *meiosis*.

The special cytological problems of the production of the gametes, or *gametogenesis*, centre in the manner by which meiosis is brought about. This always (in Metazoa) takes place in one of the last two mitoses involved in the production of the gamete. Hence these two mitoses are known collectively as the *meiotic phase*, though only one of them actually effects the halving of the chromosome number, and is therefore, strictly speaking, the meiotic division. In nearly all cases this division is the first of the two mitoses of the meiotic phase—*i.e.* the penultimate mitosis of that long series of divisions by which the gamete is produced from the primordial germ cell. The second mitosis of the meiotic phase differs in no essential from the ordinary mitoses of the body (somatic mitoses), except that it takes place in a nucleus containing only half the number of

[1] This applies to all Metazoa. In a few Protista, and in many Metaphyta, the haploid individual is the characteristic representative of the species, the diploid phase being very transitory. See Chapter VII.

chromosomes. Hence the first mitosis of the meiotic phase—*i.e.* the meiotic division proper—is often known as the *heterotype*, and the second one as the *homotype*, division. Before proceeding to a detailed consideration of this important part of the life-cycle it will be necessary to give a brief sketch of the course of gametogenesis.

The natural starting-point for this sketch is the *primordial germ cell*. This is of course one of the products of those divisions of the unicellular stage of the zygote by which it becomes transformed into the multicellular adult, and it may be defined as the first of the mass of cells thus produced to be dedicated to the formation of reproductive cells alone. It is only in a few cases that this cell has actually been identified in the developing embryo (see Chapter III.), but whether visibly recognizable or not it is plain that such a cell (or cells) must occur in the development of every organism.

In some cases, for example in *Ascaris megalocephala* (p. 80), the nuclei and the character of the mitoses of the primordial germ cell and its derivatives are distinguishable from those of the other tissues of the organism by various features, but in most cases they are essentially similar until the meiotic phase is ushered in by the prophase of the penultimate division before the formation of the gamete.

The general course of gametogenesis is very similar in the two sexes, differences in detail being associated with the relatively enormous size of the macrogamete. Correlated with this, only one of the four cells resulting from the two divisions of the meiotic phase becomes, in the female, a functional gamete, the other three forming the very minute "polar bodies."

A diagrammatic scheme of the course of gametogenesis of both sexes is given in the accompanying diagram (Fig. 13). A few words of general description will suffice, as cytological details will be given in actual cases.

The *period of multiplication* really involves a much greater number of divisions than shown in the diagram. The cells in this period are known as *spermatogonia* and *oogonia* (or *ovogonia*). In some animals, the earlier generations of these cells differ in certain visible characteristics from the later ones, with the result that the former are often termed primary spermatogonia (or oogonia) and the latter secondary.

The cells in which the first meiotic division occurs in the male are the *primary spermatocytes*. This division gives rise to the *secondary* spermatocytes. For the sake of brevity, these cells may be referred to as Spermatocyte I. (primary) and Spermatocyte II. (secondary), etc. Similarly, the two divisions of the meiotic phase may be referred to as Meiosis I., Meiosis II., and the various stages of the two mitoses as Prophase I., Metaphase I., Anaphase II., etc., according as they belong to the one or the other of the two divisions.

The spermatocytes II. give rise, by the second division of the meiotic phase, to the *spermatids*, which are nothing else than young spermatozoa.

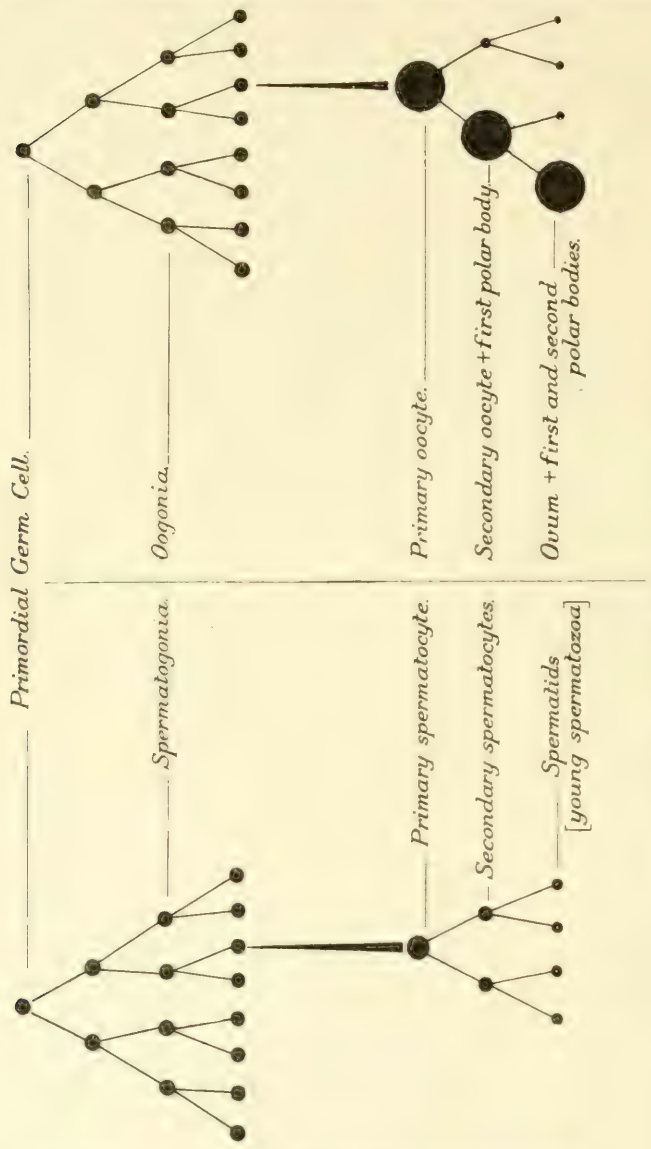


FIG. 13.
Diagram of the course of gametogenesis in the two sexes. (Boveri, 1891.)

That is to say, each spermatid metamorphoses into a spermatozoon. Thus four spermatozoa are produced from each spermatocyte I.

The fully formed spermatocytes I.—that is to say, just before the meiotic mitosis takes place—are considerably larger than the later generations of spermatogonia. Hence the interval between the last spermatogonial mitosis and the meiotic division is called the *growth period*. It is during this period that the changes of the long drawn-out meiotic prophase are found.

In the female the growth period is much more conspicuous than in the male, since it is during this period that the yolk, which is so abundant in most macrogametes, is deposited, and hence the *primary oocyte* (or *ovocyte*) is much larger than the primary spermatocyte. Meiosis I., instead of resulting in two similar oocytes II., is followed by a very unequal cell division, resulting in one oocyte II., very nearly as large as the parent cell, and a minute cell, the " first polar body." Meiosis II. is followed by a similarly unequal cell division, resulting in the mature ovum and " second polar body."

A. MEIOSIS IN THE MALE

The remarkable interest of the meiotic processes has resulted in a great deal of attention being paid to this phase, and it has been investigated in many species both of animals and plants. Unfortunately, the difficulties of observation and interpretation are great, and have resulted in a corresponding diversity of views as to the precise mode by which the halving of the chromosome number is effected.

In the first place we will describe the process as it occurs in the male of the Polychaete worm *Tomopteris* (as described by A. and K. E. Schreiner, 1906 *a*), after which the more important variations on this scheme, or different interpretations thereof, will be discussed.

(1) *Meiosis in Tomopteris onisciformis* (Fig. 14)

The spermatogonial or *pre-meiotic* divisions present no special differences from the somatic mitoses. The number of chromosomes is eighteen. (It will of course be understood that the figures, being depictions of actual sections, show only such chromosomes, or portions thereof, as occur in the sections.) We may, therefore, begin our detailed description with the last pre-meiotic telophase (Fig. 14, A, B). The daughter nuclei reconstructed from this telophase are the primary spermatocytes. In them the chromatin is arranged in a fine network, in which, however, chromosome areas are said to be discernible in the form of parallel bands along which the chromatin is more densely aggregated. A. and K. E. Schreiner believe that by means of these bands the chromosomes of the last pre-meiotic telophase can be traced continuously into the chromosomes of prophase I. A conclusion of this nature, involving as it does the negative demonstration that at no time between the two phases do the

chromosome areas lose their identity, is obviously a very difficult one to

FIG. 14.

Meiotic Phase in the male *Tomopteris onisciformis*. (After A. and K. E. Schreiner, *A.B.*, 1906.) A, B, telophases of last pre-meiotic (spermatogonial) mitoses; C, late telophase of same, passing into primary spermatocyte; D, leptotene stage; E, F, G, zygotene stage; H, pachytene stage; I, diplotene stage; J, diakinesis; K, nuclear membrane disappeared; immediate prophase of meiotic I.; L, metaphase I.; M, anaphase I.; N, prophase II.; O, metaphase II.; P, telophase II.

establish. Wilson, however (1912), who examined the Schreiners' own material, is inclined to agree with them on this point.

An early stage in the preparation for the first meiotic division is shown in Fig 14, D, where it can be seen that the chromatin is condensing into fine threads, and also that (1) this condensation is most marked at one pole of the nucleus (shown throughout the figure as the upper pole), and (2) at this pole the chromatin threads converge in pairs. Anticipating, we may say, that each chromatin thread (of which there are eighteen) is a chromosome, and that the pairing is not haphazard, but that each pair consists of two homologous chromosomes in the sense described on pp. 13 and 125.

In favourable preparations it can be determined that the centrosome is embedded in the cytoplasm just outside that pole of the nucleus to which the chromosomes converge.

Another important point to notice is that the condensed chromosomes at the pole of the nucleus are not smooth, but resemble strings of beads. These beads are the chromomeres, and will be further discussed in Chapter V. This stage (Fig. 14, D), where the chromosomes are still very fine, is known as the *leptotene stage.*

In the later stage, shown in Fig. 14, E, the condensation has spread away from the pole along a further length of the chromosomes, and now the homologous chromosomes which were paired in Fig. 14, D, are beginning to approximate themselves still more closely, till they come into actual contact. Like the preliminary condensation, this process begins at the polar end of the chromosomes and spreads away from this point. This coming together of pairs of chromosomes, which is of fundamental importance, is often known as the *conjugation of the chromosomes* from its resemblance to the conjugation of certain Protozoa, especially Infusoria such as *Paramecium.* It is also known as *syndesis*, while the nucleus is said to be in the *zygotene stage.*

Fig. 14, F, shows a more advanced stage of syndesis, and illustrates also the fact that the process is not necessarily synchronous in all the chromosomes. In the nucleus shown in the figure, three pairs of chromosomes are still in the leptotene stage.

In Fig. 14, G, syndesis is complete, and now instead of the eighteen thin chromosomes of the leptotene stage we have nine thick chromosomes formed by their fusion in pairs. Hence this stage is called the *pachytene stage.* The chromosomes are now seen to be horse-shoe shaped, with their ends directed towards the nuclear pole. A characteristic appearance is thus produced in the pachytene nuclei of *Tomopteris* and of many other forms which exhibit a similar polarization. This has earned for this stage the further term of *bouquet stage.* In many species, however, such a polar orientation is absent.

The chromosomes have by this time condensed and contracted sufficiently to make it possible to count them, and it is found that there

are nine of the thick bands, thus justifying the statement made above that there were eighteen of the thin threads in the leptotene nucleus.

The nine thick chromosomes now present, having been formed each by the conjugation of two homologous chromosomes, are said to be *bivalent*, in contra-distinction to the separate, unconjugated or *univalent* chromosomes.

Fig. 14, H, is a later pachytene nucleus. In this the shortening and thickening of the chromosomes, which proceeds throughout the meiotic as throughout the somatic prophase, has progressed further.

At the stage shown in Fig. 14, I, a process the reverse of what occurred in the zygotene stage is taking place, the bivalent chromosomes splitting into their two constituents again. Hence this phase is called the *diplotene stage*, or, since the members of each pair are often conspicuously twisted round one another, the *strepsitene stage*. The polar orientation of the chromosomes is now less pronounced, and by the stage shown in Fig. 14, J, it has quite disappeared. By this time the separation of the two constituents of each pachytene bivalent has proceeded considerably further, and indeed is complete in the case of some pairs. Others remain attached at one end, giving rise to U-shaped figures, or at both ends producing figures shaped O, or if twisted to figures of 8. Others, again, may remain united at their middles and separate at their ends, forming X or $\overset{V}{\underset{\wedge}{}}$-shaped figures.

The nucleus has now commonly attained its greatest volume, and the chromosomes are characteristically distributed round its periphery, immediately beneath the nuclear membrane. This is the phase called by Häcker *diakinesis*, and is a conspicuous stage in most gametogeneses. Soon after this the nuclear membrane disappears, and the chromosome pairs lie in the cytoplasm (Fig. 14, K).

Thus the long series of changes which the nucleus passes through from the first appearance of the leptotene threads to the point at which we have now arrived, all take place in a long drawn-out mitotic prophase, namely, the prophase of the first meiotic division, and are succeeded by the metaphase and anaphase of this division (Fig. 14, L, M). In these, the chromosomes which paired in syndesis and disengaged themselves again in the diplotene stage are finally separated, one member of each pair going into one daughter nucleus, and the other member into the other. This division is therefore the true meiotic or *reduction division*, since through its agency two secondary spermatocyte nuclei, each containing nine chromosomes, are formed from one primary spermatocyte nucleus containing nine pairs, or eighteen, chromosomes.

It will be noticed that each of the separating chromosomes in the anaphase is itself split along its length. This is an exaggeration, commonly found in meiosis, of the tendency (discussed in Chapter I.) of chromosomes to exhibit already in the anaphase that division into two

daughter chromosomes, which will become operative in the succeeding mitosis. In the present case, that mitosis is the second division of the meiotic phase which follows immediately after the first without the intervention of a resting stage. It is depicted in Fig. 14, N, O, P, and does not differ essentially from a somatic mitosis except in having only half the normal number of chromosomes. The last figure illustrates a not uncommon minor feature of spermatogenesis, namely, that the meiotic mitoses are not immediately followed by complete cell division, so that for a time the four young spermatids are united in a four-lobed cell.

At this point we may leave the description of spermatogenesis in *Tomopteris*, all the important nuclear phenomena being by now concluded. The development of a spermatid into a spermatozoon is described in the next chapter.

The fundamental fact in meiosis is the segregation, into separate nuclei, of the members of each pair of homologous chromosomes. This is brought about in the meiotic mitosis by the previous pairing of the homologues into double or bivalent chromosomes, which take up a position on the spindle such that the constituent chromosomes of each bivalent occupy the position taken in an ordinary mitosis by the daughter halves of each single chromosome. Thus the meiotic anaphase separates whole (homologous) chromosomes, instead of the daughter halves of single chromosomes, and therefore the daughter nuclei have only half the number of chromosomes present in the previous cell generations. In other words, the pre-meiotic nuclei have 2n *chromosomes, the primary spermatocyte has* n *double or bivalent chromosomes, and the post-meiotic nuclei have* n *chromosomes.*

The course of meiosis just described is schematized in Fig. 15, which is a diagrammatic representation of its course in a species with four chromosomes. Fig. 15, A, shows a pre-meiotic (spermatogonial) prophase. The remaining figures illustrate the fundamentally important fact that it is homologous chromosomes which pair together in syndesis, to break apart again in the diplotene stage, and finally separate in the first meiotic division. The proof of the statement that syndesis takes place between homologous chromosomes is found in the large number of species in which the chromosomes are of different lengths and even different shapes, so that homologous chromosomes can be identified by their relative sizes, just as they can be recognized in the diagram by their shading. Species with chromosomes of varying lengths are very numerous and will be met with frequently in the accounts in this book (see especially p. 125). One of them, *Lepidosiren*, will be described immediately.

It is clear that, in the case illustrated, meiosis will result in gametes having two instead of four chromosomes, and, moreover, that these two will consist of one member of each of the two pairs present in the

pre-meiotic nuclei. As the chromosomes behave similarly in meiosis in the female, the fusion of the male and female gametes results in the reconstitution of a diploid nucleus with four chromosomes in two pairs. Thus, if we designate each of the differently shaded chromosomes of the diagram by a different letter, the gametes contain chromosomes

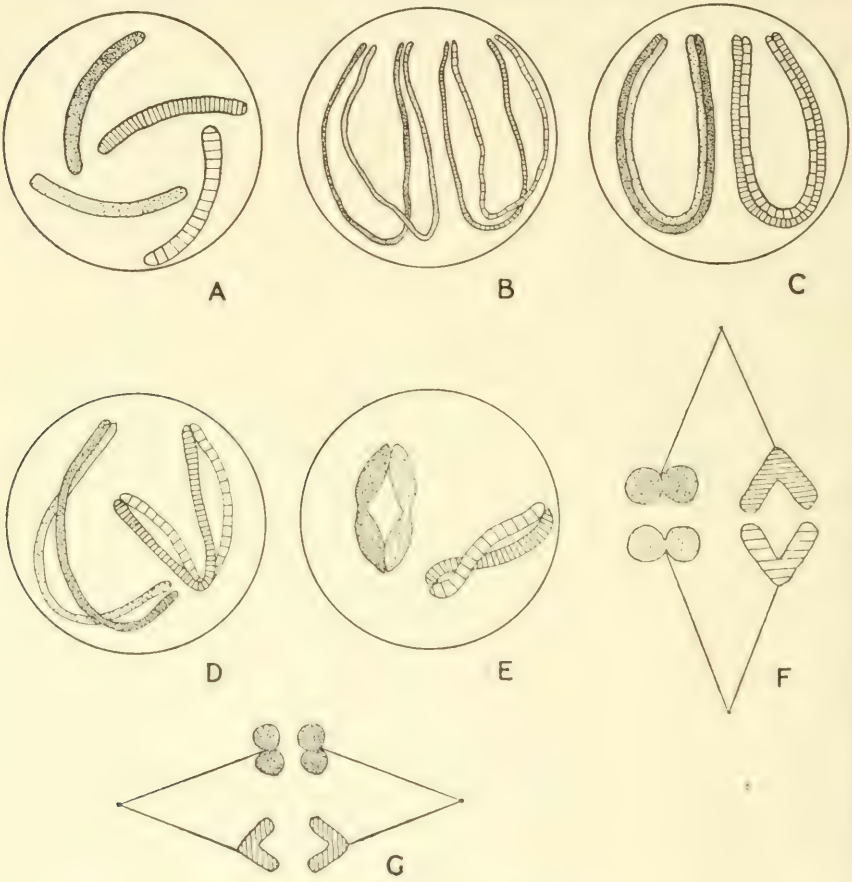


FIG. 15.

Diagram of the principal stages of meiosis by parasyndesis. Two pairs of homologous chromosomes are shown, the members of one pair being stippled, and those of the other cross-striped. A, pre-meiotic prophase, showing the four separate chromosomes; B, leptotene; C, pachytene; D, diplotene stages; E, diakinesis, showing the evolution of the definitive bivalents; F, meiotic metaphase; G, metaphase of second division of the meiotic phase in the secondary spermatocyte formed from the upper daughter nucleus derived from F.

A + B, and the zygote A + A + B + B. In syndesis pairing takes place in such a way that the pachytene nuclei contain two bivalents, forming the series AA + BB. We also see that one member of each bivalent was originally introduced by the male gamete and the other by the female.

While the most important features of meiosis are all to be found

in the above account of *Tomopteris*, various additional features and minor modifications are commonly met with in other cases, as will be illustrated by short descriptions of the meiotic phases in the lung-fish, *Lepidosiren paradoxa*, and in certain insects.

(2) *Meiosis in Lepidosiren paradoxa* (Fig. 16)

Lepidosiren is probably unsurpassed as an object for cytological research, owing to the great size of its nuclei and the clear sharp outlines of its chromatic elements as prepared for examination by the ordinary cytological methods. Another great advantage which it possesses is the fact that the chromosomes differ greatly from each other in size. The number of chromosomes in the body tissues is thirty-eight, and in the gamete nineteen.

The most important stages of the meiosis of this species are depicted in Figs. 16 and 16*a*, in which a few pre-meiotic figures are also shown. Fig. 16, A, is a spermatogonial nucleus, taken from that part of the testis in which active spermatogonial mitosis is proceeding. Its coarse structure as compared with that of the spermatocyte I. nucleus is to be noted. This is a common and conspicuous distinction between nuclei of these two grades.

The spermatogonial prophases are of an extremely simple nature, contrasting, therefore, strongly with the complicated series of events which takes place in the meiotic prophase. The coarse blocks of chromatin of the resting spermatogonial nucleus form themselves into long threads by lengthening and fusion, and then these threads shorten and thicken into the definitive metaphase chromosomes.

The further contraction of the chromosomes in anaphase (Fig. 16, D) brings to light a feature which is visible, though less conspicuous, among the longer prophase or metaphase chromosomes, namely, that they are of very different lengths (see also Fig. 65). Especially noticeable is the pair of very long chromosomes which are bent (at this stage) into the form of a V. As these chromosomes are about twice the length of the next largest pair, they are easily identified whenever the chromosomes are individually distinguishable.

As in *Tomopteris*, syndesis begins at the polar ends of the chromosomes and spreads along them from this point. It will be noticed that polar views of the nuclei are shown in this figure, while the corresponding figures of *Tomopteris* represent the nuclei seen from the side.

The onset of the diplotene stage (Fig. 16*a*, H) is considerably obscured in *Lepidosiren* by the simultaneous contraction of the greater part of the chromatin elements into a compact mass, leaving the larger part of the nuclear cavity free from chromatin. This is a frequent and extremely characteristic feature of the meiotic phase, though it is not universal.

It is not found, for example, in *Tomopteris*. Owing to its conspicuous nature and to the fact that it is confined to the meiotic prophase, it early attracted the attention of cytologists, by many of whom it used to be considered diagnostic of the fact that syndesis was in progress. In

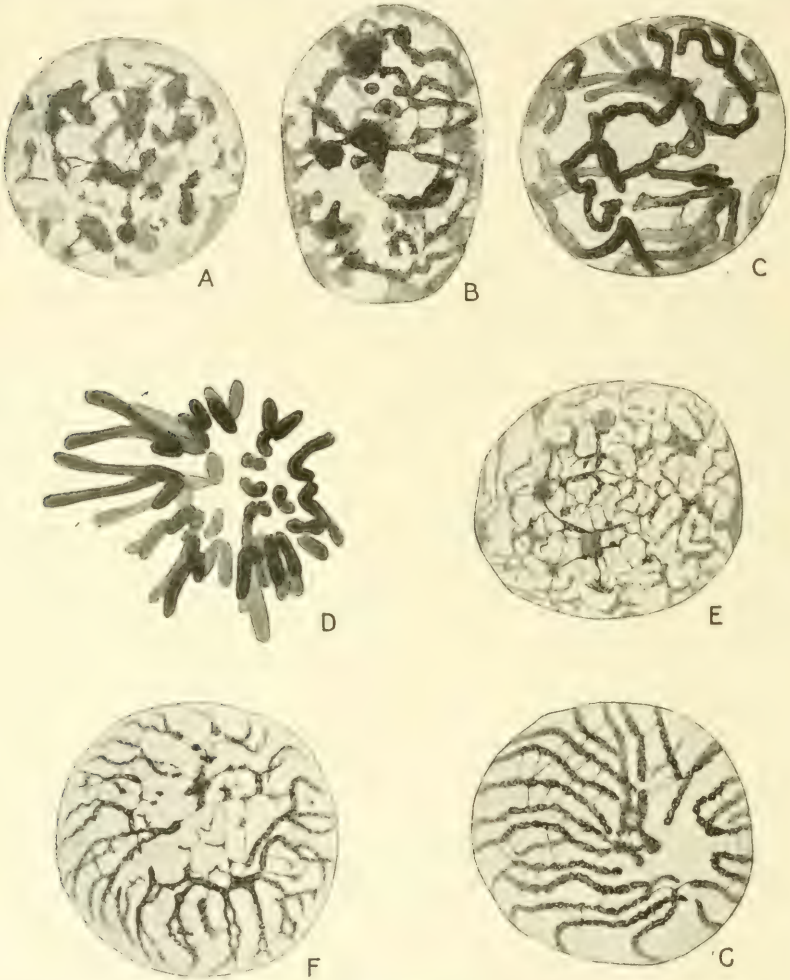


FIG. 16.

Meiosis in *Lepidosiren* (male). (Agar, *Q.J.M.S.*, 1912.) A, resting spermatogonial nucleus; B, C, spermatogonial prophase; D, daughter plate from a spermatogonial anaphase; E, resting spermatocyte I.; F, zygotene; G, pachytene nucleus.

consequence of this, the word *synapsis* proposed by Moore to cover the whole of that period of meiosis in which syndesis occurs, has been applied by many cytologists to its most conspicuous feature alone—namely, the contraction just described. It has been thoroughly established, however, that the contraction of the chromatin has no invariable relation to the

conjugation of the chromosomes. Hence the word *synizesis* was proposed for the contraction, and *syndesis* for the chromosome conjugation.

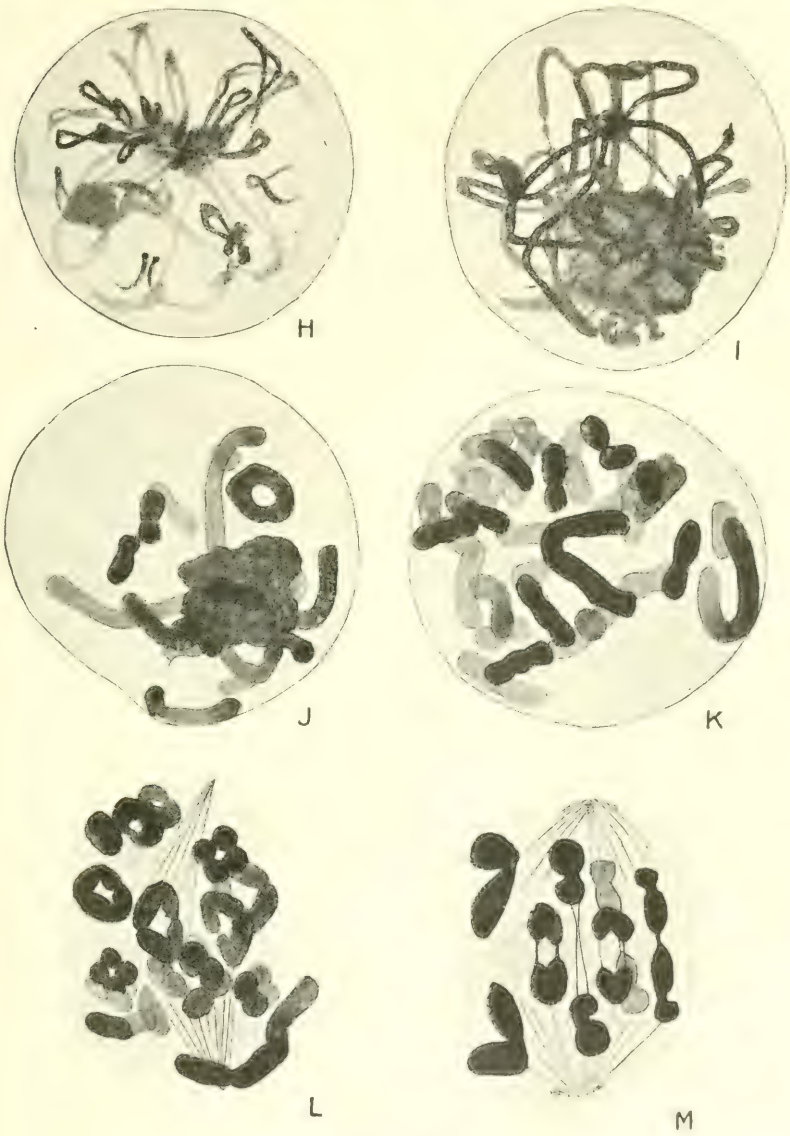


FIG. 16*a*.

Meiosis in *Lepidosiren* (male). (Agar, *Q.J.M.S.*, 1911.) H, diplotene stage and beginning of synizesis; I, synizesis further advanced; J, synizesis breaking up; K, diakinesis, showing that the bivalents which were formed in the zygotene stage are completely resolved into their univalent constituents; L, immediate prophase of the meiotic division, showing the univalents pairing again; M, early anaphase I.

Unfortunately, however, cytologists are not agreed in their use of the alternative words. Some continue to use the term synapsis for the

contraction, reserving syndesis for the chromosome conjugation. Others use synapsis for the conjugation and synizesis for the contraction. In this book the word synapsis is not used at all, on account of the confusion as to which of the phenomena included in the original description it should be reserved for. The term synizesis is employed throughout for the contraction and syndesis for the chromosome conjugation.

That separation of the constituents of the pachytene bivalents which occurs in the diplotene stage is carried to a much greater extent in *Lepidosiren* than in *Tomopteris*, so that by the time diakinesis is reached we frequently have all, or all except two or three, of the bivalents resolved completely into their univalent constituents (Fig. 16*a*, K). Examination of the stages leading up to this shows that in the beginning of the diplotene stage the bivalents separate first in the middle, remaining attached for a time at their ends and thus form long oval rings. Subsequently these rings break apart at the points of contact of the ends of their component chromosomes. In diakinesis we thus find thirty-eight univalents, or at least a majority of univalents with a few bivalents of which the components have not completely separated.

Since in metaphase I. we again find nineteen bivalents, the chromosomes must reunite before this stage is reached. This second pairing is apparently effected by the recently separated homologous chromosomes coming into contact again, first by one end and later by the other, to form closed rings similar to those present in the diplotene nucleus, with the difference that the rings are now much smaller and thicker. In the nucleus shown in Fig. 16*a*, L, there are fifteen complete rings, three pairs of univalents are in contact by one end only (one of these being the large pair) and two are still unpaired, accounting for the thirty-eight chromosomes in all.

A conspicuous feature which now presents itself is the transverse constriction or joint which is to be seen across each univalent chromosome. Sometimes (in the longer chromosomes) this takes the form of a sharp angle in the chromosome, in others (the shorter ones) it is merely a deep constriction making the chromosome dumb-bell shaped. The result of each univalent being constricted across in this way is to make each bivalent appear tetrapartite.

The development of these transverse constrictions can be traced by gradual stages which space prevents from figuring here. It is not apparent in the long chromosomes which are first formed by the disjunction of the bivalents in the diplotene stage, but as they contract it gradually makes its appearance. An exactly similar process is to be observed in the contraction of the long chromosomes of the pre-meiotic metaphase, as they recede towards the poles in anaphase (Fig. 16, D).

Here again the joint takes the form of a sharp angle in the longer chromosomes and of a deep constriction in the smaller ones. Evidently the form it shall take depends upon the relative length and breadth which a given chromosome has attained at a given moment.

As commonly happens in meiosis, there is usually no resting stage between the two meiotic divisions in *Lepidosiren*. As soon as the chromosomes approach the poles in anaphase I. new spindles are formed and the chromosomes, now longitudinally split, become arranged in the metaphase equatorial plates. Since each chromosome is still transversely constricted as in the first division, figures very similar in appearance to those of metaphase I. are obtained, although of course each tetrapartite chromosome is now constituted out of two transversely constricted daughter chromosomes, instead of out of two juxtaposed transversely constricted whole chromosomes.

The pair of long chromosomes already alluded to should be noted in Fig. 16*a*, K, L, M. From the study of favourable nuclei, it can be established that these are formed by the breaking apart of one of the rings of the diplotene stage. That is to say, they conjugated to form one bivalent in syndesis, an example of the evidence mentioned above that conjugation is not haphazard, but takes place between homologous chromosomes.

(3) *Meiosis in Certain Insects* (Fig. 17)

The Insects are a group which have attracted much attention from cytologists, the most important recent work on their meiosis being that of Wilson (1912) on certain Hemiptera (Fig. 17).

The spermatogonial telophase (Fig. 17, A) passes into a confused network (B) in which no chromosome limits are visible. The beginning of the meiotic phase is marked by the appearance of a number of massive chromatin bodies, of the diploid number (C). (The two denser bodies visible at this stage, and more conspicuous in D-J. are the sex chromosomes, to be described in Chapter IV.)

The leptotene nucleus is derived from this stage by the resolution of each one of the chromatin bodies into a spirally coiled filament, which spreads out and interlaces with the other similarly formed filaments to produce the leptotene nucleus. This is followed by synizesis, and this by the pachytene stage. The mode of syndesis cannot be traced, but since the leptotene threads are in diploid number and the pachytene threads haploid, and since each pachytene thread splits longitudinally into two in the diplotene stage (I), it is to be assumed that syndesis of the type described for *Tomopteris* and *Lepidosiren* took place during synizesis.

The fact is notable that neither the leptotene nor pachytene threads are orientated to form a bouquet as they are in *Tomopteris* and *Lepidosiren*.

This absence of a bouquet is not, however, characteristic of insects in general, for it is a conspicuous feature in many species.

Diplotene nuclei (I) show us that each pachytene thread proceeds to split into a pair in the same manner as we saw in the two forms already

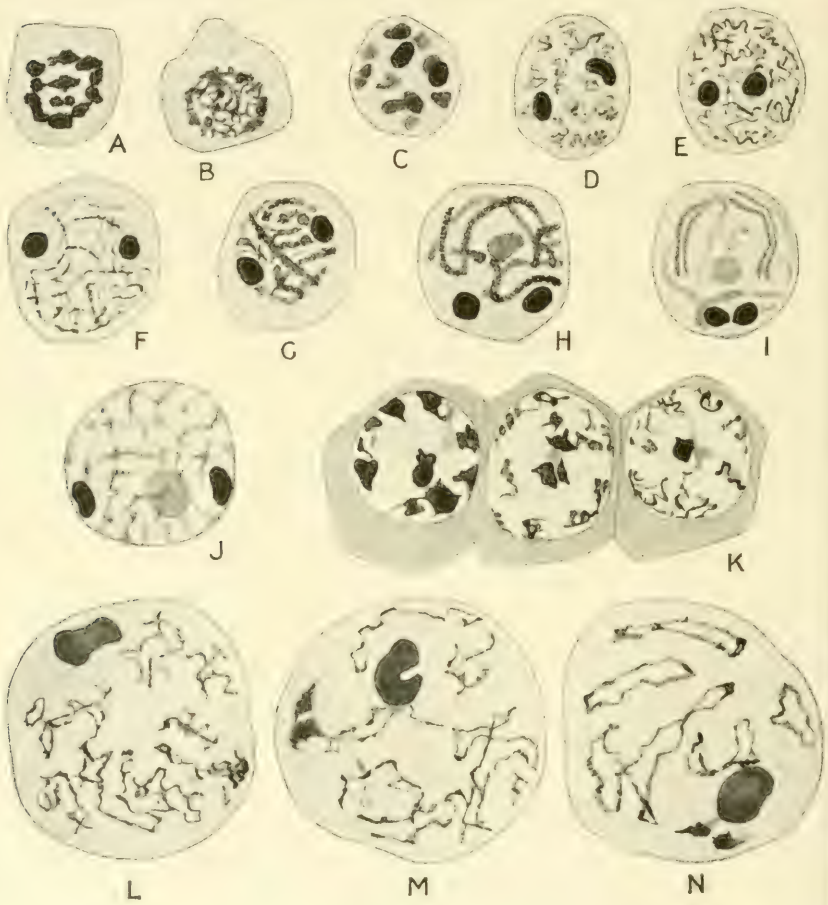


FIG. 17.

The formation of the meiotic bivalents in certain insects. (After Wilson, *J.E.Z.*, 1912.) A-J, *Oncopeltus*; K, *Anax*; L-N, *Protenor*. A, B, spermatogonial telophase; C, emergence of massive chromatin bodies in spermatocyte I.; D, E, each chromatin body (with the exception of two, the sex chromosomes) is giving rise to a single coiled thread; F, the coiled threads of E have given rise to the leptotene nucleus; G, synizesis; H, the pachytene stage; the large, faintly stained body in this figure, and in figures I, J, is the plasmosome; I, diplotene stage; J, "confused stage"; K, three contiguous cells showing the evolution of the coiled threads from the massive bodies; L, M, N, evolution of the bivalent rings from the confused stage.

described. Instead, however, of these pairs condensing progressively into the definitive bivalents of metaphase I., the early diplotene stage is immediately followed by one which Wilson calls the "confused stage" (J), in which the double chromosomes lose their visible identity and become merged into a vague, lightly staining reticulum. From this

reticulum the bivalents presently condense out (L, M, N) in the form of long loops, frequently twisted.

These further condense into "tetrad rods" or double dumb-bells, very similar to many of the bivalents in *Lepidosiren*. Wilson, however, believes that the condensation takes place in such a way that the transverse joints of the bivalents correspond to the original cleft which separates the two components of each diplotene bivalent.[1] If this be so, these transverse joints represent the points of junction of the constituent univalents of each bivalent, and hence they are of quite different origin and significance from the transverse constrictions of the *Lepidosiren* bivalents. In accordance with this mode of formation, the transverse joint is the plane of division in metaphase I., which therefore separates entire univalent chromosomes as it does in the two cases already described.

B. DIVERGENT VIEWS OF THE PROCESS OF MEIOSIS

While it is generally accepted that meiosis consists in the segregation of homologous chromosomes, it must not be supposed that all cytologists agree that the accounts of the processes leading up to that segregation which have been given above for *Tomopteris*, *Lepidosiren* and Hemiptera are either completely correct interpretations of what occurs in these forms or, even if this were granted, that the process occurs in essentially the same manner throughout the animal (and vegetable) kingdom. However, it is the general scheme of meiosis which is accepted in essentials by more cytologists—at any rate, students of animal cytology—than adhere to any other one scheme, and it is rapidly gaining new adherents. We will now briefly discuss certain other schemes of meiosis that have been proposed, together with a few general problems of this important phase, leaving out of account a few special hypotheses which have been put forward to explain the phenomena observed in certain individual cases, and which can lay no claim to generality. We may also leave out of discussion the pure—and, as it appears to the author, unjustifiable—scepticism of certain cytologists (for example, Meves) who deny the possibility of coming at present to any useful conclusion as to how the number of chromosomes is reduced in meiosis.

(1) *Parasyndesis and Telosyndesis* [2]

These two schemes of meiosis have much in common, and a great measure of generality is claimed for each by their respective supporters.

[1] Wilson, however, believes that fusion in syndesis is complete, the bivalent chromosome undergoing internal reconstruction so that it is not possible to homologize the two chromosomes which separate in the diplotene stage with those which united in syndesis. See p. 48.

[2] Called parasynapsis and telosynapsis by cytologists, who employ the term synapsis in the sense in which syndesis is here used (p. 39).

In many cases meiosis in one and the same species has been interpreted by one worker as parasyndesis and by another as telosyndesis. The point of difference between the two schemes lies in the observation and interpretation of the zygotene, pachytene and diplotene stages.

Syndesis as described above in *Tomopteris*, *Lepidosiren* and certain insects, and illustrated diagrammatically by Fig. 15, is *parasyndesis*, so called because in the zygotene nucleus the homologous chromosomes lie side by side and conjugate along their lengths. This interpretation of the zygotene stage, with its consequent reading of the pachytene and diplotene stages, was first given in its present form by von Winiwarter (1901), and has since gained wide acceptance. To the Louvain school of cytology is specially due the credit of having established the hypothesis on a firm basis, both by new observation and the review of old work in the new light (Grégoire, 1910).

The theory now known as *telosyndesis* was first proposed by Montgomery in 1903 for various Amphibia, and independently by Farmer and Moore in 1905 for several other forms. It must, however, be mentioned that Montgomery subsequently (1911) gave up his original view in favour of the theory of parasyndesis.

According to the theory of telosyndesis the thin threads which are seen joining together in pairs in the zygotene stage are not whole chromosomes conjugating to form a bivalent, but are the temporarily separated daughter halves of chromosomes split for forthcoming mitosis. On this view, therefore, the duplicity of the chromosomes in Figs. 14, D, E, F, and 16, F, is of precisely the same nature as the duplicity of somatic prophase chromosomes (cf. Figs. 3, 7, 8, etc.). The undisputed fact that the number of thick bands in the pachytene nucleus is haploid is supposed to be due to the fact that each consists of two homologous chromosomes joined end to end, as shown in the diagrammatic figure.

So far we have been dealing with what is mainly a matter of interpretation and not of observation, but the next stage in the process involves a difference of opinion on a matter more nearly approximating direct observation. The telosyndetic view requires that the double chromosomes of the diplotene stage are not formed, as described above, by the reopening of the space between two approximating chromosome threads of the zygotene nucleus, but by the approximation of the limbs of the horse-shoe shaped pachytene bands. They may then join at the free ends to form rings instead of U's, or break across at the point of junction to form two separate but adjacent chromosomes, together constituting a bivalent.

The constitution of the bivalents is now the same in both schemes (Figs. 15, E, and 18, C'), and the further course of meiosis is described alike in both cases. In Fig. 18 are shown three stages in the formation of the

definitive bivalents out of the pachytene loops as depicted by Farmer and Moore. It will be noticed that the longitudinal slit, faintly visible here and there in the pachytene stage (A, and A′) appears from these figures to be traceable into the slits occasionally indicated in each constituent of the bivalents, and therefore represents the division plane of the second division of the meiotic phase.

It would take too much space to discuss fully the relative merits of the two theories, but the most important *pros* and *cons* can be briefly summarized, remembering that it is impossible to reconcile the divergent

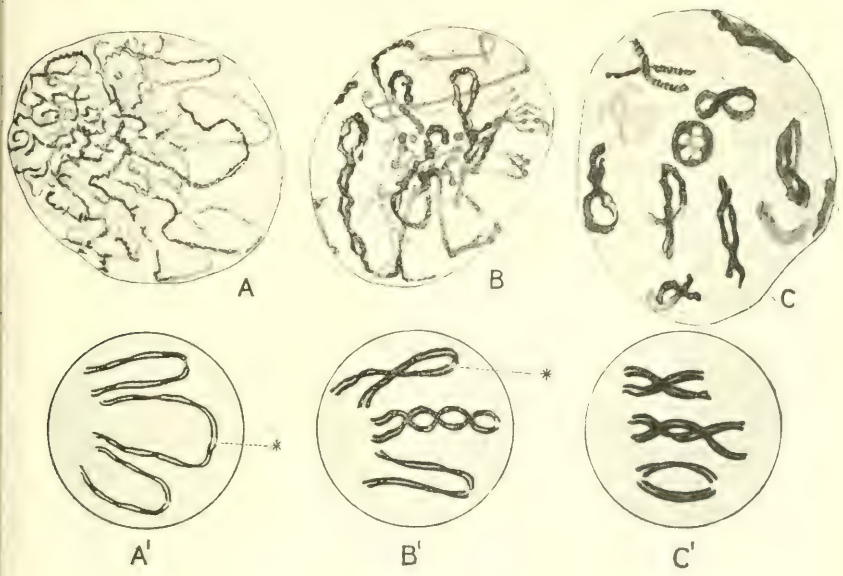


FIG. 18.

Illustrating telosyndetic view of meiosis. (A-C, after Farmer and Moore, *Q.J.M.S.*, 1905.) A-C, three stages in the formation of the bivalents in *Osmunda regalis*. A′-C′, their interpretation according to the telosyndetic view. * Supposed points of junction of the longitudinally split constituents of each bivalent.

views by the simple assumption that parasyndesis holds for some species and telosyndesis for others. For the disputed stages—zygotene and diplotene—are too similar in many of the species which have been interpreted in opposite senses to have been brought about by such different processes, and, moreover, in many instances opposite accounts have been given of the same species.

1. The overwhelming balance of evidence of actual observation appears to the author and many others (including the older cytologists who were not interested in either theory) to favour the view that the diplotene and diakinetic figures are produced by the reopening of the

slit within each bivalent pair in the zygotene nucleus. Such cases as *Tomopteris*, where there is no synizesis to obscure any stage in the process, seem to be decisive. This fact alone appears to dispose definitely of the theory of telosyndesis.

2. A considerable degree of similarity between the duplicity of the prophase chromosomes of many somatic mitoses and that of the zygotene pairs must be admitted. The threads which come together in the zygotene stage are, however, more distinct than those of somatic prophases. In the leptotene nucleus, or earlier, they often show little or no sign of paired arrangement, in this respect differing greatly from the duplex chromosomes of somatic prophases, where the two longitudinal portions of each chromosome are probably always closely approximated to one another. Moreover, we do not find in somatic prophase that regular fusion of the members of the pairs spreading away from their polar ends which is such a characteristic feature of the zygotene nuclei in those organisms which exhibit the bouquet orientation in this stage (*e.g.*, *Tomopteris*, *Lepidosiren*). The fact, therefore, that the theory of parasyndesis has to interpret the frequent duplicity of somatic prophase chromosomes as caused by fission, and the superficially somewhat similar duplicity of the zygotene nucleus as fusion, is no serious drawback to that hypothesis—especially when it is remembered that, as will be evident from the next paragraph, such a differentiation must in any case be made between the double prophase chromosomes of *Culex* and those of most other organisms.

3. There is commonly observed, even in somatic mitoses, a tendency for homologous chromosomes to lie side by side, and this tendency can be traced through all intermediate degrees up to its climax in *Culex*, where even in somatic, spermatogonial and oogonial mitoses, homologous chromosomes may be indistinguishably fused together, especially in prophase and anaphase. It is instructive to compare Figs. 14, I, 17, I, 19, B, E, with Fig. 56, C, D, E, H, remembering that in the latter there is no questioning the fact that the longitudinal components of the double chromosomes are each an entire chromosome, the pairs being formed by approximation of these, and not by fission of a single original one. Parasyndesis is therefore but the climax of a widespread tendency of homologous chromosomes to apply themselves side by side.

4. As stated in Chapter I., a tendency for chromosomes to adhere by their ends is sometimes observed in somatic mitoses. This may be taken as favourable to the view that syndesis is effected in the same way, *i.e.* by telosyndesis.

5. There is no direct evidence that the pachytene loops consist of two chromosomes united end to end. The fact that a break is often observed in the loop, dividing it into two portions connected by an

achromatic band, has indeed been cited in evidence (Montgomery, 1903; Schellenberg, 1911). As, however, this break is frequently not in the middle of the loop (Schellenberg), it cannot be taken as the point of junction of the conjugating chromosomes, since the limbs of the diakinetic and metaphase rings, loops, etc., are equal. There can indeed be little doubt that these breaks are the transverse constrictions which develop across the contracting chromosomes in *Lepidosiren* and elsewhere. It is

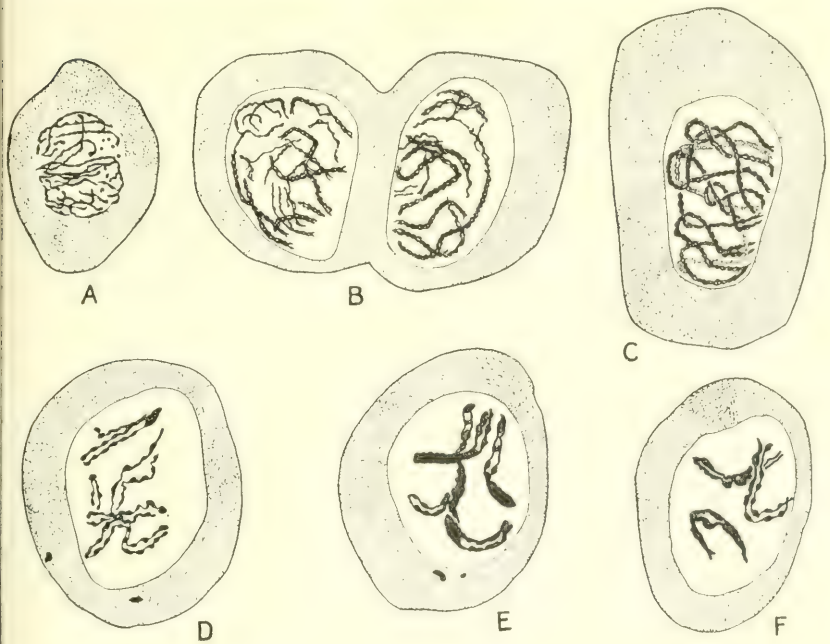


FIG. 19.

A-C, parasyndesis in *Planaria gonocephala*. (After Schleip, *Zool. Jahrb. Anat.*, 1907.) D-F, parasyndesis in *Dytiscus marginalis* (after Henderson, *Z.w.Z.*, 1907); A, D, leptotene; B, E, zygotene; C, F, diplotene stages.

characteristic that these transverse constrictions are often by no means in the middle of the chromosomes (Fig. 65).

This very short and incomplete summary of the arguments for and against the only two schemes of meiosis which can lay any claim to generality must suffice for the present. It is clear that parasyndesis is the hypothesis accepted in this book, and we shall find, especially in Chapters V. and VI., that we continually meet with observations as well as experimental results which are readily intelligible on the assumption that conjugation of the chromosomes takes place by parasyndesis, but are quite inexplicable on the theory of telosyndesis.

(2) *The Mutual Relations of the Homologous Chromosomes during Syndesis*

Three views are possible as to the mutual relations of the chromosomes which unite in syndesis, and these views have, as a matter of fact, all been upheld. They are :

1. Syndesis is a temporary fusion of the conjugating chromosomes, comparable to that of conjugating Infusoria, and the chromosomes separate again in the diplotene stage. (Many cytologists—*e.g.* Schreiner.)

2. The chromosomes fuse completely at syndesis, so that the two chromosomes which separate in the diplotene nucleus are not the chromosomes which came together in the zygotene stage, but new products formed by the longitudinal fission of the compound chromosome formed in syndesis (Bonnevie, 1906 ; Vejdovsky, 1907, 1911 ; von Winiwarter and Sainmont, 1909 ; Wilson, 1912).

3. Syndesis is only a temporary approximation of homologous chromosomes, which never become organically continuous (Grégoire, 1910).

The distinction between the last view and the other two is a real one, but the difference between the first two is largely a matter of words. If two chromosomes fuse, a mutual influence, if not actual exchange of substance, is implied ; whether or not the chromosomes which separate afterwards are to be considered as the same as those that entered into combination is a question of dialectics. Are two separating exconjugant *Paramecia*, after exchanging micronuclei, the same individuals as those which entered into conjugation ?

While there is therefore no need to attempt to decide between the first two views, the question of whether organic continuity is or is not established between the approximated chromosomes is a very important one. As we shall see later, the possibility of an exchange of substance between homologous chromosomes is an important—probably indeed essential—supplementary hypothesis to the chromosome theory of heredity.

As in so many cases, however, it is very difficult to arrive at a decision by direct observation. The view that the conjugating chromosomes do not enter into organic continuity is founded upon certain cases in which a dividing line between them appears to be present throughout the whole period of syndesis. It therefore really rests on negative evidence, namely, that at no time during syndesis is the dividing line absent—an observation which, as any cytologist will recognize, would be an exceedingly difficult one to establish. There is of course no need to suppose that fusion takes place simultaneously over the whole chromosomes. Indeed it is certain that in many forms it begins at one end and spreads thence to the other. The presence of slits here and there in a zygotene chromosome is therefore no evidence that the constituents are not fused at other points, or at other stages.

On the other hand, the view that organic continuity is established between the two participating chromosomes is much more securely founded on the very numerous cases where complete contact is observed between the constituents of each pachytene thread, either over the whole length of the thread at once, or now at one point and now at another.

(3) *Meiosis with Tetrad Formation*

Much error and confusion has been introduced into the study of the meiotic phase by the failure to recognize the true nature of the transverse constrictions of the meiotic chromosomes, and that they are often of no direct significance in the process of reduction. It was for long supposed to be a general rule that the quadripartite chromosomes, so commonly formed in meiosis by the junction of two bipartite chromosomes, are composed of four masses, one of which eventually reaches each of the four spermatid nuclei. These "tetrads," as they were called, were supposed to divide across one joint in the first meiotic division, giving two "dyads"—one to each spermatocyte II. In the second division the dyads were supposed to divide across at the remaining joint, giving one "monad" to each spermatid. Thus one division of the tetrad was said to be longitudinal and one transverse.

In the case of *Ascaris megalocephala*, the organism to which our knowledge of cytology is due probably more than to any other one species, this is indeed the fate of the tetrads. These are, however, produced in this species in a different way from that described for *Lepidosiren*, as will be shown below. A similar partition of the four parts of the tetrads among the four spermatids has been frequently described for insects also. Here again it appears that the apparently transverse constriction has an origin different from that in the lung-fish (p. 43).

In other cases, however, notably the Copepoda, accounts of the distribution of the four segments of each tetrad, one to each spermatid, were based on faulty observation. In this group it has more recently been shown (Lerat, 1905; Matschek, 1910) that the chromosomes which separate in anaphase II. are not monads formed by division of the dyads into their two parts, but are still bipartite, being formed by longitudinal division of the dyads of anaphase I. Thus the transverse joints of the Copepod tetrad are of the same nature as those in *Lepidosiren*, and do not represent a division plane.

The false view of the composition and fate of what may be called the Copepod type of tetrad was intimately connected with the older theories of meiosis. At an early period of cytological theory (Roux, 1883; Weismann) it was recognized that a chromosome consists of a number of smaller dissimilar elements arranged in linear series (see Chapter V.). Consequently, a longitudinal division of the chromosomes results in the

division of each one of these smaller elements, and hence the two daughter chromosomes derived by the longitudinal fission of the mother chromosome are identical. If, however, a chromosome were to divide transversely, the two resulting chromosomes would be dissimilar, as each would contain only a portion (half) of the smaller elements.

Now in a meiosis with tetrad formation of the Copepod type it follows that if both the joints represent division planes, one division must be longitudinal (or *equational*, since the resulting daughter chromosomes receive similar sets of chromatin elements), and the other division must be transverse (or *reductional*, since each resulting daughter chromosome receives only one-half of the set of chromatin elements). As we have seen, however, it was by an error of observation that the transverse joint in a Copepod tetrad was taken to be a division plane, and it is now almost universally held that "reduction" consists in the separation of entire homologous chromosomes, and not in their transverse division. Indeed, while there are many cases, notably amongst insects, still in need of elucidation, it is more than doubtful if transverse division of chromosomes in the above sense ever occurs as the result of mitosis.

As mentioned above, the "tetrads" of *Ascaris megalocephala* consist of four masses, one of which eventually reaches each of the four spermatids. In other words, both joints of the tetrad are division planes. An examination of the mode of formation of these tetrads reveals, however, that they are constituted differently from that which we have called the Copepod type, as exemplified in *Lepidosiren*, etc.

Ascaris has been the subject of a very great number of cytological investigations. The classical description of the meiotic phase (in the male) is that given by Brauer (1893) for the variety *A. m. bivalens*, in which, it will be remembered, the diploid chromosome number is four.

His description practically starts with synizesis, in which the chromatin is contracted to one side of the nucleus in a fairly compact mass, from which, however, chromatin threads project (Fig. 20, A). These threads are at once seen to be double, and when cut in transverse section, or seen in end view, they reveal themselves as quadruple, being divided longitudinally by two division planes at right angles to one another (Fig. 20, B). These quadruple threads are to be interpreted as formed by syndesis of two homologous chromosomes, each split longitudinally (in preparation for the second division of the meiotic phase). Brauer, whose investigations were carried out before the modern ideas as to syndesis had been formulated, did not interpret the quadruple threads thus, but as formed by the double splitting of a single thread. Later work on meiosis in this animal, however (de Saedeleer, 1912), has revealed all the principal stages as found in *Tomopteris*.

By the later stage shown in Fig. 20, C, synizesis has completely disappeared, and all the chromatin is in the form of a long, doubly split thread (only one split is visible in the plane of the figure). As there are really two bivalent chromosomes present, these must be joined tempor-

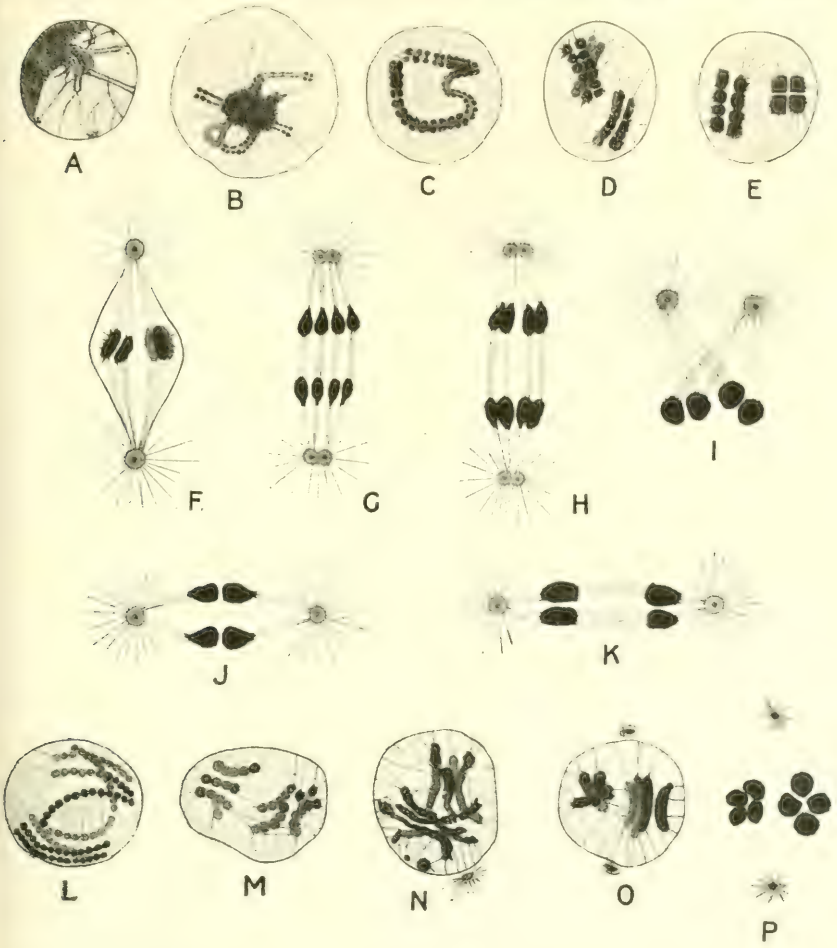


FIG. 20.

Meiosis in *Ascaris megalocephala bivalens*. (A-M, after Brauer, *A.m.A.*, 1893; N-P, after O. Hertwig, *A.m.A.*, 1890.) A, B, synizesis and syndesis; C, D, E, formation of the definitive bivalents. In E the left-hand bivalent is seen in side view and therefore only two of the constituents are visible; the right-hand bivalent is seen in end view, and therefore its quadruple constitution is revealed. F, metaphase I.; G, H, anaphase I.; I, preparation for second division; J, metaphase II.; K, anaphase II.; L-M and N-P, condensation of the definitive bivalents in nuclei in which the four constituents of each are well separated.

arily end to end to form an " unsegmented spireme " as described on p. 9. Later (D) this dissociates into its two components. Each of these is a bivalent chromosome, longitudinally divided into four owing to the fact that each constituent univalent is, as described above, itself longitudinally divided. The quadruple nature of each bivalent chromosome is well

illustrated by Fig. 20, L-P, which show the condensation of the tetrads in nuclei in which the parts of each bivalent are well separated.

Each bivalent tetrad therefore now consists of four parallel rods produced by the longitudinal apposition of two homologous univalent chromosomes each longitudinally divided into two daughter chromosomes. Before the metaphase is reached each of the four rods has so contracted as to be nearly spherical, and the tetrad now consists of four nearly spherical masses, one of which, as is sufficiently explained by the figures, is distributed to each of the four spermatids. Thus, as in *Tomopteris* and *Lepidosiren*, one of the two meiotic divisions is reductional in the sense of separating homologous chromosomes, and the other, like an ordinary mitosis, separates the daughter chromosomes produced by fission of the mother chromosome. While there is here no means of deciding which division does which, it may be assumed from analogy that the first division is the reductional one.

The resemblance between the "tetrads" of *Ascaris* and those formed by some of the chromosomes in *Lepidosiren* or in the Copepoda is very close, but obviously only superficial. In each case the tetrad is bivalent, but in the one case (*Ascaris*) the joint in each univalent is really longitudinal (as shown by its origin) and marks the plane of division of the second meiotic division. In *Lepidosiren* and the Copepoda, on the other hand, the joint in each univalent is a mere transverse constriction, and is not operative as a division plane.

The accompanying diagram illustrates the structure of a meiotic chromosome in *Lepidosiren*, *Ascaris*, and an insect such as *Oncopeltus*. The linear series of dissimilar elements of which the chromosome is composed is represented by the letters of the alphabet, one chromosome being represented by A B C D and its homologue by a b c d. The diagram illustrates the fact that the first and last stages (syndesis and gametes) are alike in all three cases, and also that in all three it is the first meiotic division which separates the homologous chromosomes.

The early appearance of the longitudinal fissure which becomes operative in the second division, and which is responsible for the "tetrad" form of the *Ascaris* bivalent, is a common feature of meiosis. When both this fissure and a transverse joint are present in each component of the bivalent, the chromosome assumes an "octad" shape. This is well exemplified in the bivalents of certain Copepods.

It would take us too far to consider other schemes of meiosis which have been proposed, especially as none of them can establish any claim to general application. A warning, however, is necessary against the too ready acceptance of accounts in which no syndesis proper is said to

occur, the homologous chromosomes being supposed to come together for the first time in diakinesis.

Probably such accounts have been due to failure to realize the complete temporary disjunction of the ex-conjugant chromosomes which may take place in the diplotene stage (cf. *Lepidosiren*). Hence the

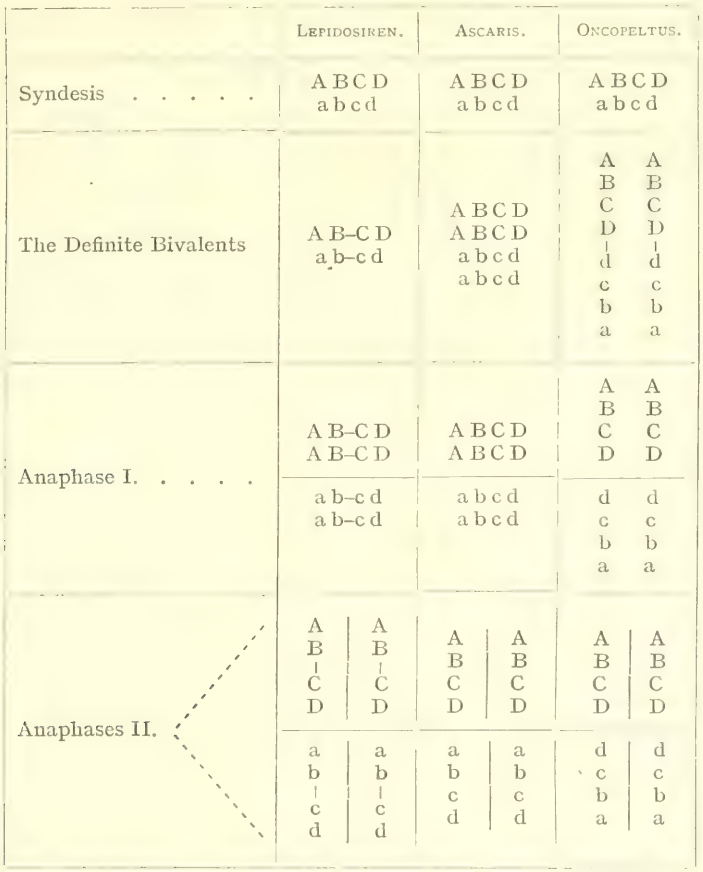


Diagram of the constitution of a pair of homologous chromosomes in *Lepidosiren*, *Ascaris* and *Oncopeltus*. The hyphens represent transverse joints in the chromosomes.

erroneous assumption is made that if the diploid number of chromosomes is found in diakinesis, no previous syndesis has taken place. The classical description of diakinetic pairing without previous syndesis is that of Korschelt (1895) for *Ophryotrocha*, but the further researches of A. and K. E. Schreiner (1906 *b*), and Grégoire and Deton (1906), have disclosed that stages of the usual type indicating parasyndesis precede diakinesis in this animal also.

(4) *Which of the two Divisions of the Meiotic Phase effects the Separation of the Homologous Chromosomes ?*

Korschelt and Heider, in their general account of meiosis (1903) give two subdivisions of the different modes of meiosis which they recognize. These are called *Pre-reduction* and *Post-reduction* according as to whether it is the first or second division of the meiotic phase which effects the reduction (by separating homologous chromosomes).

In *Tomopteris* and *Lepidosiren* it is plain that it is the first division which does this, and the great majority of modern accounts of meiosis agree in this. They therefore fall into the category of pre-reduction. Most of the earlier accounts, however, favoured post-reduction, but this was mainly due to those errors regarding the composition and fate of tetrads of the Copepod type which have been explained above, and may therefore be ignored.

As will be readily appreciated, it is often very difficult to decide which of the two divisions effects the reduction, especially in the case of tetrads formed, as in *Ascaris*, by two longitudinal planes. Here it is a question of tracing the two planes without break from their inception into metaphase I. in order to see which plane it is that is operative in this division.

Although pre-reduction appears to be by far the commoner, post-reduction has been quite definitely described by competent workers, especially in certain insects. Indeed in this group it appears that even in the same animal some bivalents may divide reductionally in the first and others in the second division (M'Clung, 1914). In the case of certain peculiar chromosomes, the "sex chromosomes" (to be described in Chapter IV.), no fact in cytology is better established than that these may undergo pre-reduction in some species and post-reduction in others (p. 102).

(5) *Synizesis*

The significance of this very characteristic stage in meiosis is quite obscure. The one thing certain is that it can have no direct necessary connection with syndesis, since in many forms (*e.g.*, *Tomopteris*) it is absent. The fact that it is not found in certain species has even led some cytologists, who happen to have worked chiefly with such species, to doubt its natural occurrence at all, and to ascribe the presence of it in forms used by other workers to their faulty methods of fixation. In many organisms, however, synizesis occurs whatever method of fixation is used, and the matter appears finally settled by the observation of synizetic contraction in living and fresh tissues. A few examples out of many such observations are those of Wilson (1909 *b*) in *Anasa*, Arnold (1909) in *Planaria*, Schleip (1909) in Ostracoda.

A comparison of the figures of *Lepidosiren* and *Oncopeltus* shows that the intensity of the contraction varies. *Oncopeltus* forms an intermediate stage between the extremely dense contraction in *Lepidosiren* and its complete absence in *Tomopteris*.

The exact moment at which synizesis begins and ends also varies considerably. In *Lepidosiren* it begins with the onset of the diplotene stage. In *Oncopeltus* it apparently corresponds with the zygotene stage —at any rate it occurs between the leptotene and pachytene stages. More frequently, perhaps, it sets in earlier still, when the leptotene threads are emerging from the resting nucleus.

Finally, in many forms the telophase contraction of the last spermatogonial mitosis has been described as passing directly into synizesis without an intermediate diffuse stage. As this conclusion is based upon the negative evidence of failing to find diffused stages between the two, these accounts should perhaps be accepted with some reserve. On the other hand, there seems to be no theoretical reason to doubt them, and so many different observers have given such accounts that it is difficult to doubt their combined testimony. As examples may be quoted *Peripatus* (Montgomery, 1901 *a*), and *Scolopendra* (Blackman, 1905).

A few cytologists have described two synizetic contractions, separated by a diffused stage. This observation has been upheld especially by Farmer and Moore (1905) and by the adherents to their telosyndetic scheme, in which the second contraction is supposed to bring about the doubling over of the pachytene bands to form the rings, etc., of the bivalents.

C. MEIOSIS IN THE FEMALE

So far we have confined our description of meiosis to that process as it occurs in the male. The behaviour of the chromatin in oogenesis is closely parallel to that in spermatogenesis, with, however, modifications connected with the long-growth period of the oocyte and the comparatively gigantic size of the mature egg. The deposition of the yolk and the growth of the oocyte I. take place between syndesis and metaphase I. During this period, which may endure for months or years (mammals), the chromosomes may not retain their chromosomal forms, but often nearly, or quite, vanish into a peculiar form of resting nucleus known as the *germinal vesicle*, to reappear immediately before the first meiotic division. This special feature of oogenesis does not, however, destroy the fundamental similarity between male and female meioses, and the above discussions regarding syndesis, pre- and post-reduction, synizesis, etc., apply as well to oogenesis as to spermatogenesis. Even the germinal vesicle stage, as we shall see, is paralleled in the spermatogenesis of certain animals.

Another difference between gametogenesis in the male and female has already been alluded to, namely, the fact that in the female each primary oocyte gives rise to only one functional gamete and three (or two, if the first polar body does not divide) minute and functionless cells (polar bodies) instead of to four functional gametes as in the male. This is also obviously correlated with the necessity for the female gamete to

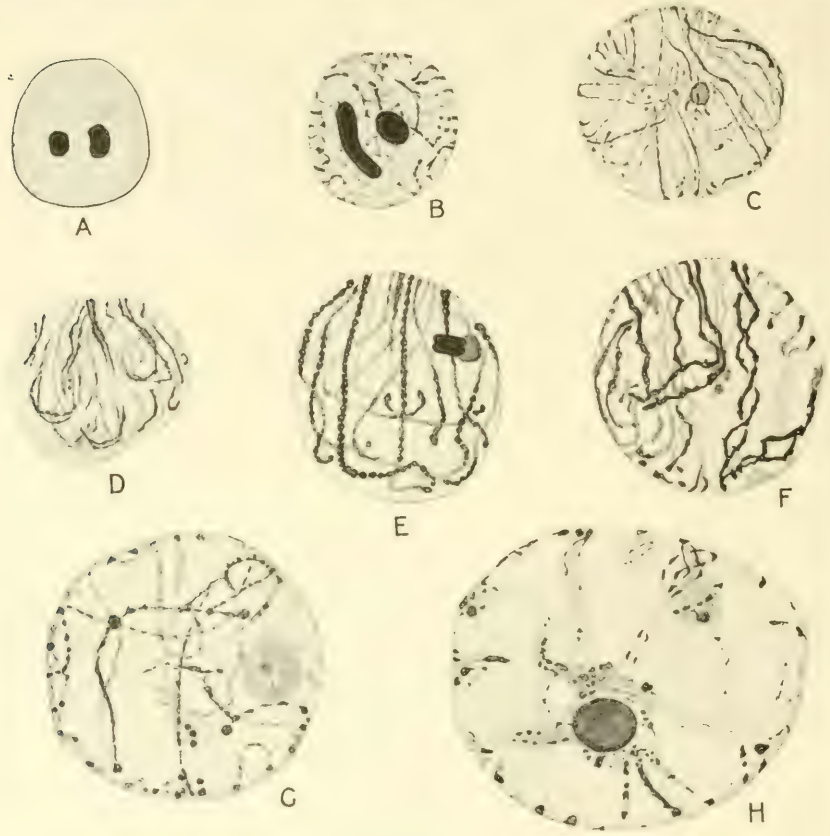


FIG. 21.

Early stages of oogenesis in the cat. (After von Winiwater and Sainmont, *A.B.*, 1909.) A, young oocyte I., chromatin mostly very finely divided; B, C, development of leptotene stage; D, zygotene; E, pachytene; F, diplotene stages; G, H, development of "germinal vesicle" stage.

be very large and richly provided with reserve food material; the achievement of this is materially assisted by the concentration of practically the whole of the reserve material into one macrogamete, instead of its partition among four.

The correspondence between the mature egg with its polar bodies and the four spermatids derived from one spermatocyte is of course only complete in those cases where the first polar body divides into two, giving

thus a set of four cells derived from the one oocyte I. Cases where the first polar body does thus divide are very common, but the division does not always occur, simply because the degeneration of the polar body as a cell has often gone too far. Stages in its loss of power to divide can be observed. In the sponge *Sycon* (Jorgensen, 1910 *a*), for example, the first polar body makes a beginning of a mitotic division which, however, generally remains uncompleted.

As a rule, the polar bodies disintegrate and disappear soon after the egg has been fertilized, but sometimes they can be traced, adhering to the outside of the developing embryo, for a long time (*Ascaris*).

An interesting confirmation of the essential homology between the polar bodies and the ripe egg is provided by an observation of Lefevre (1907) on *Thalassema*. The egg of this annelid, like that of so many others, can be induced to develop without fertilization if placed in a suitable excitant chemical medium (see Chapter III.). In some cases not only was the egg induced by this means to start cleavage, but the polar bodies also divided repeatedly, producing a morula-like cluster of minute cells—in some instances as many as sixteen.

The later stages of oogenesis—*i.e.* the two actual mitoses of the meiotic phase—are illustrated in Figs. 11, 32, 33, 82. As in the case of the male meiosis, the important stages are to be sought in the long-drawn-out prophase of the meiotic mitosis itself. The early stages of this process in the cat are illustrated in Fig. 21.

It will be seen that up to the diplotene stage (F) the process is closely parallel to the corresponding stages in spermatogenesis. Instead, however, of straightway condensing into the definitive chromosomes of the first meiotic division, the bivalents now lose their sharp contours, diminish in staining capacity and become distributed in an irregular fashion through the nucleus, while at the same time the nucleolus, which was already present in the young oocyte, increases in size. Thus the nucleus passes into the germinal vesicle condition.

The manner in which the bivalents pass into the germinal vesicle and re-emerge as the definitive chromosomes of the first meiotic division in the dog-fish is shown in more detail in Fig. 22 (Maréchal, 1907). There is a typical pachytene bouquet (A), the orientation of which is lost in the diplotene stage (B). The chromosomes now lose their sharp outlines by reason of the development of very numerous thread-like outgrowths (Figs. 22, D; 23). These being arranged more or less at right angles to the central axis of the chromosome give the whole structure a characteristic appearance which has often been compared to that of a cylindrical chimney brush. Synchronously with the development of these outgrowths the chromosomes as a whole lose their characteristic chromatin staining

reaction, appearing indeed to consist now entirely of achromatin or oxychromatin. At the same time the number of nucleoli increases.

At the end of the growth period the chromosomes undergo a reverse process of concentration, the filamentar outgrowths being apparently

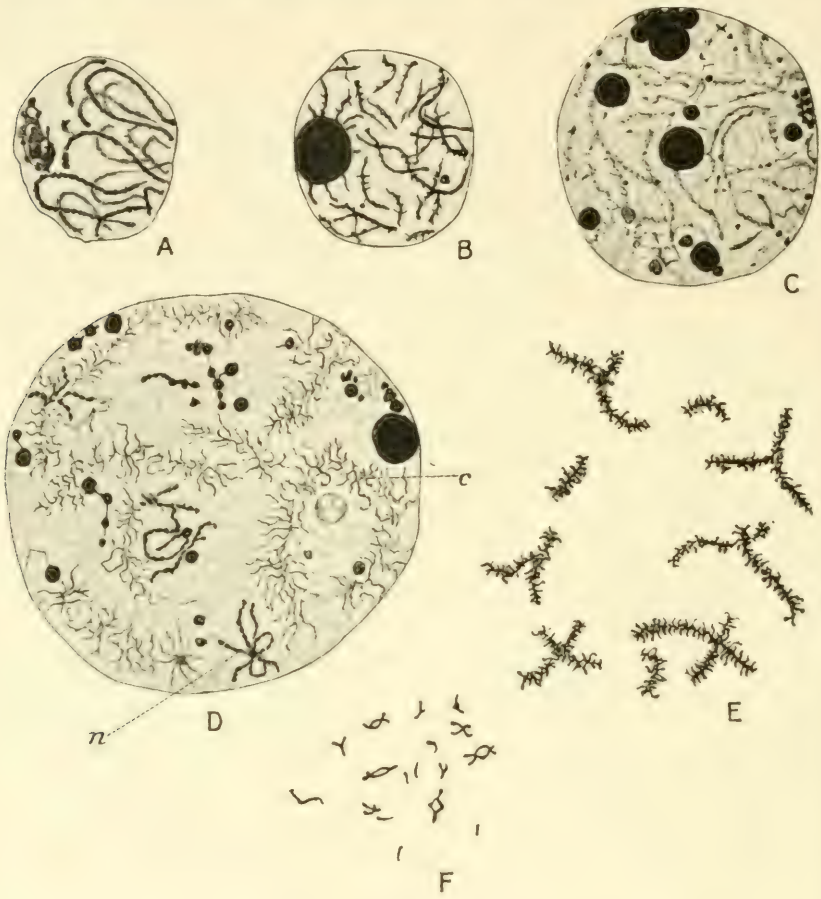


FIG. 22.

Stages in the oogenesis of the dog-fish, *Pristiurus*. (After Maréchal, *L.C.*, 1907.) A, pachytene stage, nucleolar mass already conspicuous; B, C, passage of the diplotene stage into the germinal vesicle. In C the number of nucleoli has increased. D, early stage in the reconstruction of the chromosomes; E, F, later stages in the condensation of the chromosomes into the definitive bivalents. These two figures are drawn at the same magnification.

c, chromosome; *n*, nucleolar filament.

retracted on to the central axis. At the same time the staining capacity of the chromosomes increases again, and they diminish enormously in size. The nucleoli break up, and their substance shows evidence of degenerative changes, forming small granules or droplets. Sometimes these get arranged one behind the other into filaments superficially not unlike chromosomes (Fig. 22, D) but having in reality no relation to these.

The particular problems raised by the germinal vesicle stage in oogenesis are :

(1) The continuity of the chromosomes throughout this period.

(2) The relation between the chromosomes and the nucleoli.

(3) The connection between the peculiar germinal vesicle stage and the synchronous enormous growth of the cytoplasm of the egg, together with the formation of yolk.

(4) Does any comparable stage occur in spermatogenesis ?

(1) *The Continuity of the Chromosomes*

The conditions in the germinal vesicle have been urged against the theory of the genetic continuity of the chromosomes, since in some species the fully developed germinal vesicle—which it must be remembered is interposed between syndesis and metaphase I.—shows no trace of

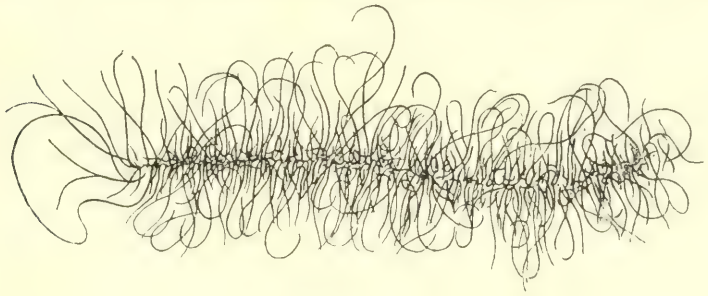


FIG. 23.

A chromosome from the germinal vesicle of *Pristiurus*. (After Rückert, *A.A.*, 1892.)

chromosomes. This condition occurs, for instance, in many Echinodermata, whose fully developed germinal vesicle consists of an enormous nucleolus suspended in a fine, very faintly staining, reticulum in which no trace of individual chromosomes can be detected.

As in the case of the resting stage between two ordinary somatic mitoses, however, we must ascribe the invisibility of the chromosomes in such germinal vesicles to their extreme diffusion and loss of staining power, and not to any loss of identity. This can be clearly determined by a comparative study of this period of oogenesis. In the Copepoda (a group which has been extensively studied in this connection) we find a great range of variation in the degree of certainty with which the chromosomes can be recognized throughout the growth period (*e.g.* Matschek, 1910). In *Cyclops gracilis* the chromosomes remain sharply individualized throughout, as is also the case in *Heterocope saliens* (Fig. 24). In *Diaptomus castor*, however (Fig. 25), the chromosomes become very diffuse at the height of the germinal vesicle stage, and their

exact limits cannot be made out. Other species of Copepoda exhibit intermediate conditions.

Häcker (1893) made the interesting observation that the degree of diffusion of the chromosomes during the germinal vesicle stage may vary in a single species. *Cyclops* and the allied genera of Copepoda mostly

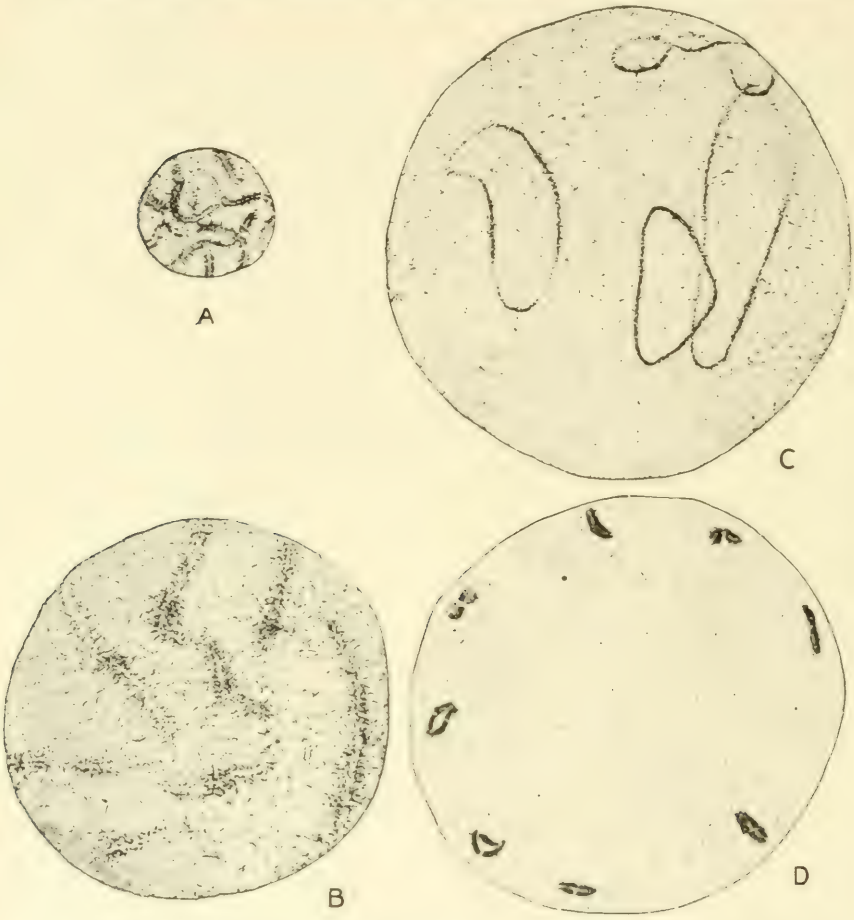


FIG. 24.

The chromosomes during the oogenesis of *Heterocope saliens* from the pachytene stage (A), through the germinal vesicle stage (B, C) to the condensation of the definitive bivalents (D). (Matschek, *A.Z.*, 1910.)

lay their eggs in batches, of about ten to about a hundred at a time. The eggs when laid do not leave the animal completely, but are cemented together into masses, the so-called egg-sacs, which are carried about by the animal until they hatch. A new batch of eggs is not laid till the previous batch has hatched.

In females of *Cyclops strenuus* which have not yet laid any eggs, no

germinal vesicles with diffuse chromosomes are to be found, and it is evident that the bivalents resulting from syndesis condense continuously

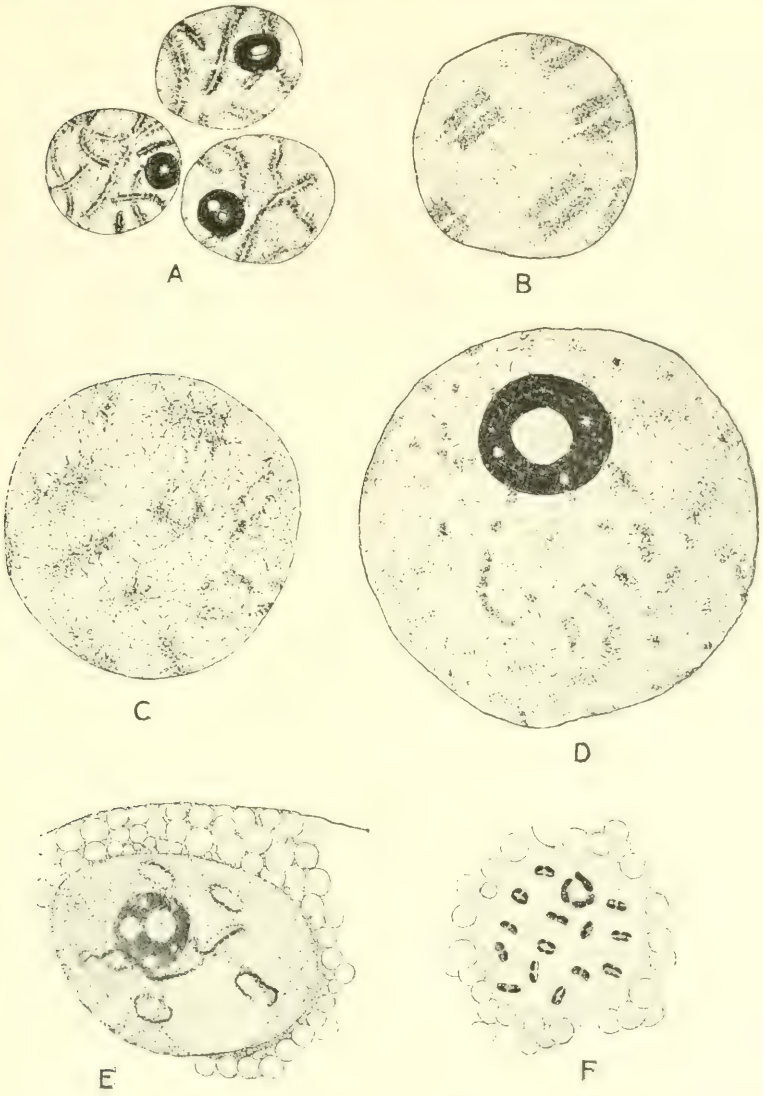


FIG. 25.

The chromosomes during the oogenesis of *Diaptomus castor* from the pachytene stage (A), through the germinal vesicle stage (B-E) to the condensation of the definitive bivalents (F). (Matschek, *A.Z.*, 1910.)

into the definitive bivalents of metaphase I. Animals, however, which are carrying egg-sacs contain in their oviducts oocytes with well-developed germinal vesicles with diffuse chromosomes. Häcker suggests that in

the latter case the oocytes are retained in the oviducts (waiting for the previous batch of eggs to hatch) longer than in the former, and that this delay accounts for the greater diffusion of the chromosomes.

This idea that the amount of diffusion of the chromosomes is in a certain degree a function of the length of time which elapses between syndesis and metaphase I. is supported by the observation of Matschek (1910) that those Copepods in which the chromosomes undergo only a moderate amount of diffusion in the germinal vesicle stage belong chiefly to those species which lay comparatively few eggs at frequent intervals (*Cyclops gracilis, Heterocope saliens*), while those in which diffusion is carried to great lengths mostly lay numerous eggs at long intervals (*Diaptomus castor*).

(2) *The Relation between the Chromosomes and the Nucleoli*

Many cytologists believe that the nucleoli of the germinal vesicle act as temporary storehouses of chromatin, receiving this substance from the chromosomes at the beginning of the growth period, and giving it back to them at the end of it. This conclusion is based on the facts (1) that in the beginning of the germinal vesicle stage the staining capacity of the chromosomes diminishes, while the size and number of the densely staining nucleoli increase; and (2) that at the end of this stage the chromosomes regain their chromatic character, while the nucleoli break up or give other evidences of degeneration—such as the development of vacuoles.

Many other cytologists, however, deny any such direct relationship between the nucleoli and the chromatin, and this is the view which appears to be best supported by recent researches (for example, Jorgensen's comparative study of the nucleoli of the germinal vesicles of a large number of animals, 1913).

In any case, only very little of the nucleolar mass in the germinal vesicle could contribute to the formation of the chromosomes, since these in their final form are, in combined bulk, very much smaller than the nucleolar mass (Fig. 26). The residue of this substance is thrown out into the cytoplasm when the nuclear membrane is dissolved in prophase I., and there degenerates.

In the descriptions of somatic mitoses and of spermatogenesis we have generally avoided the use of the term "nucleolus," substituting either "plasmosome" or "karyosome" as the case might be. The germinal vesicle nucleoli do not appear to come under either of these headings. Their chromatin staining reaction shows that they are not merely plasmosomes, while on the other hand their relations—or rather, lack of relations—to the chromatin structures of the nucleus prevent one from calling them karyosomes. In many cases they are of a double

nature (amphinucleoli), consisting of a plastin groundwork (plasmosome), covered or impregnated with chromatin or a chromatin-like substance; or the two constituents may be separate, so that the nucleolus consists of two parts, a chromatin and a plastin portion. These remarks refer especially to the main nucleolus, which persists right through the growth period. In many animals the secondary nucleoli which develop later

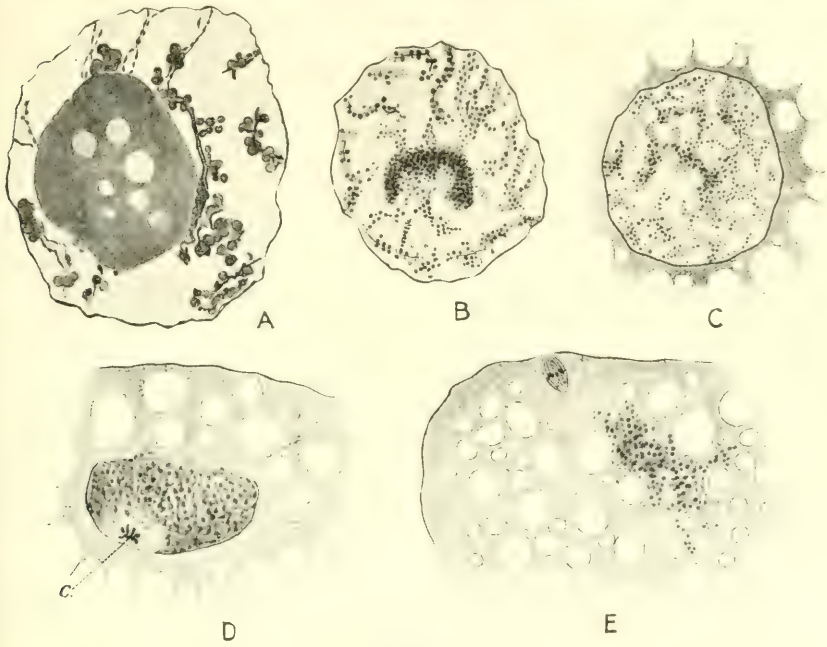


FIG. 26.

Showing the fate of the nucleolus in the oogenesis of *Daphnia pulex*. (After Kuhn, *A.Z.*, 1908.) A, the large central nucleolus is beginning to break up into much smaller bodies which are spreading over the thin threads representing the chromosomes; B, C, the continuation of this process. In C the nucleolus has completely disintegrated into granules or droplets which conceal the chromosomes. D, the condensed chromosomes (*c*) embedded in the disintegrated nucleolar mass; E, the meiotic division. Note the mass of nucleolar granules left in the cytoplasm by the rupture of the nuclear membrane. A is drawn under a higher magnification than the remaining figures, which are all to the same scale.

appear to be purely of the nature of plasmosomes—*e.g.*, *Cyclops brevicornis* (Häcker, 1893).

(3) *The Connection between the Germinal Vesicle and Yolk Formation*

The co-existence in the primary oocyte of two unique cytological occurrences, namely, the germinal vesicle and the enormous growth of the cell with its formation of reserve food material, naturally suggests a causal connection between them. We have already drawn attention to the fact that the diffusion of the chromatin in the ordinary resting nucleus has the result of increasing its area in proportion to its mass, and thus of favouring active metabolism. The excessive diffusion of

the chromosomes in the germinal vesicles of many animals has similarly been held to be an expression of the intense activity required of them in connection with the elaboration of the yolk. It has also been considered that the enormous mass of nucleolar substance present at this stage represents merely the accumulation of waste products of great metabolic activity. Finally, many cytologists have described the actual extrusion of chromatin from the germinal vesicle into the cytoplasm, either directly from the chromosomes or indirectly by way of the nucleolus. The extruded chromatin (chromidia) is supposed to take part in yolk formation, either by direct transformation into this substance, or by exerting a formative influence on the cytoplasm. This matter of the extrusion of chromatin is dealt with more fully in Chapter VI.

We are thus introduced to a body known as the *yolk nucleus* (not to be confused with the embryologist's yolk nuclei of Selachian, etc., embryos, which are derived from supernumerary spermatozoa; see p. 77). During the early growth period, intensely staining granules appear in the cytoplasm of the oocyte. These are variously interpreted as extruded chromatin, or as chondriosomes (see Chapter VI.). Sometimes, as in *Echinus* (Schaxel, 1911 *a*) and *Hydractinia* (Beckwith, 1914), they are scattered uniformly through the cytoplasm. In other cases they are concentrated into a more or less compact mass, often round the centrosome as a centre, forming a conspicuous body in the cytoplasm. Examples of such cases are found in *Antedon* (Chubb, 1906; Fig. 27), certain Copepoda (Fig. 27), Amphibia, etc. The supposed connection of these cytoplasmic bodies with yolk formation (whence their name of "yolk nuclei") rests chiefly upon the facts that their appearance precedes yolk formation, and that as this proceeds they disintegrate and finally disappear in the ripe oocyte, where no more yolk is being deposited.

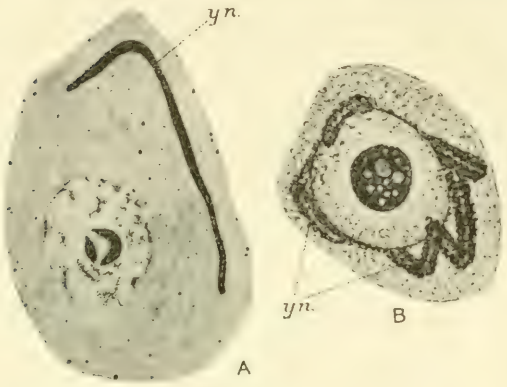


FIG. 27.

Yolk nucleus (*y.n.*) in the oocytes of (A) *Antedon bifida* (Chubb, *Phil. Trans.*, 1906), and (B) *Paracalanus parvus* (Morgan, *A.Z.*, 1909).

The term yolk nucleus has also been applied to what is probably an entirely different structure, namely, the centrosome and surrounding substance of the centrosphere, which sometimes forms a large conspicuous body (*e.g.*, *Enchytraeus*, Vejdovsky, 1907).

(4) *Does any Stage comparable to the Germinal Vesicle occur in Spermatogenesis?*

The undoubted correlation between the peculiar conditions of the germinal vesicle and the long duration of the growth period in the female meiotic phase, and probably also with the deposition of yolk, makes it

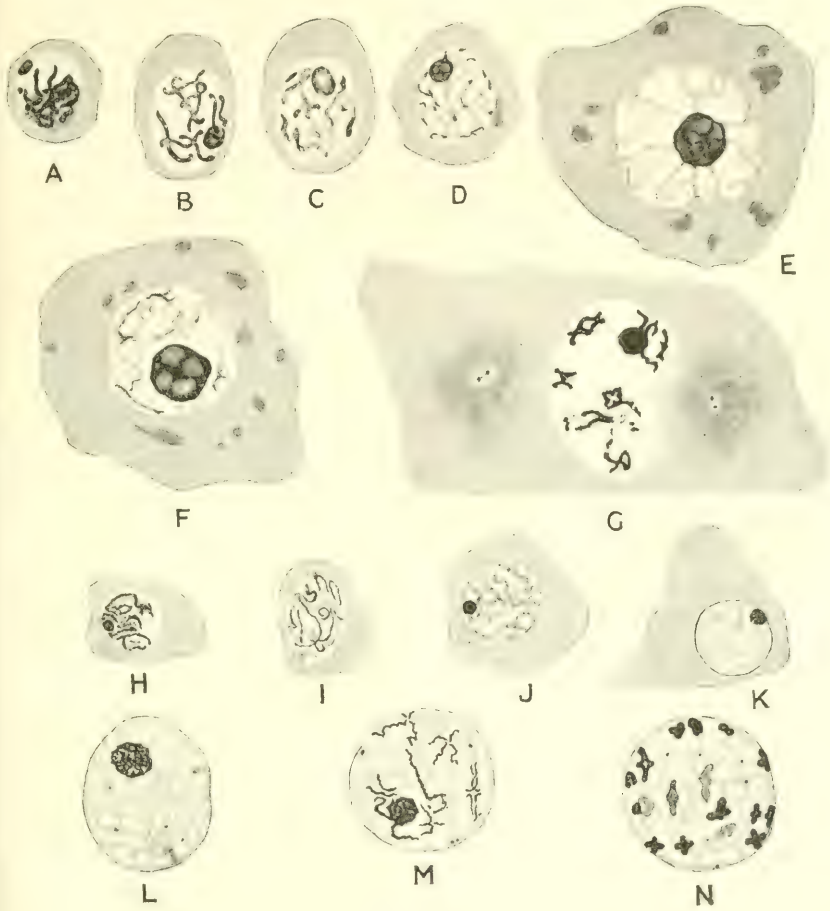


FIG. 28.

Germinal vesicle-like stages in spermatogenesis. A-G, *Notodromas monacha* (after Schmalz, *A.Z.*, 1912); H-N, *Scolopendra heros* (after Blackman, *B.M.C.Z.H.*, 1905). In both cases the principal stages between the pachytene nuclei (A and H) and the formation of the definitive bivalents (G and N) are shown.

a priori improbable that a fully developed germinal vesicle stage should be a normal occurrence in spermatogenesis, where the duration of the growth period is comparatively short, and there is little or no deposition of reserve food material.

Nevertheless, stages obviously corresponding with the germinal vesicle occur in the male meiotic phases of some animals. There is often

a temporary lengthening out of the chromosomes in the diplotene stage (*e.g.*, *Tomopteris*, Fig. 14, H, I), which may perhaps be considered an indication of a tendency to pass into a germinal vesicle condition. The " confused stage " of certain insects (Fig. 17) represents a slightly more marked, but still rudimentary, condition of the same stage. In some Ostracoda (Fig. 28) there is a stage in spermatogenesis closely corresponding to the germinal vesicle of the oocyte, the resemblance even extending to the formation of " yolk nuclei " in the cytoplasm (Schmalz, 1912). In the Myriopod *Scolopendra* (Blackman, 1905) where the primary spermatocyte undergoes an unusually pronounced growth, the resemblance to the oocyte germinal vesicle is even greater (Fig. 28).

CHAPTER III

SYNGAMY, EARLY DEVELOPMENT, PARTHENOGENESIS

The ripe microgamete or spermatozoon is a very minute motile cell, highly specialized for the purpose of conveying the chromatin and centrosomes from the male parent into the macrogamete. While varying greatly in form in different groups of the animal kingdom, by far the commonest form for it is a relatively large *head* containing the nucleus, to which is attached a flagellum or *tail*. The latter is the organ by means of which the movements of the microgamete in search of the macrogamete are carried out. It is not attached directly to the head, but through the intervention of the *middle piece*, which contains the centrosome (in many spermatozoa). As will appear directly, the spermatozoon also presents other structural features of some importance.

A. THE DEVELOPMENT OF THE SPERMATOZOON

It is necessary first to consider briefly the development of the spermatozoon from the spermatid, since this matter bears upon the interpretation of the rôle of the nucleus in heredity. Before we can understand either the structure or development of the spermatozoon we must have some knowledge of the cytoplasmic bodies known as *chondriosomes*.[1] The nature and significance of these bodies are obscure, and are discussed in Chapter VI., but they undoubtedly play an important part in the structure of the spermatozoon.

If material for cytological study be treated by appropriate methods of fixing and staining, the chondriosomes are revealed as very definite, though minute, bodies in the cytoplasm. Their behaviour in a particular case, the insect *Blatta germanica* (Duesberg, 1911 *a*), may be taken as sufficiently typical of the general course of events (Fig. 29).

Starting with the resting spermatogonium, the chondriosomes are here in the form of longer or shorter filaments distributed irregularly throughout the cytoplasm. In many other species they are granular instead of

[1] See note on terminology on p. 195.

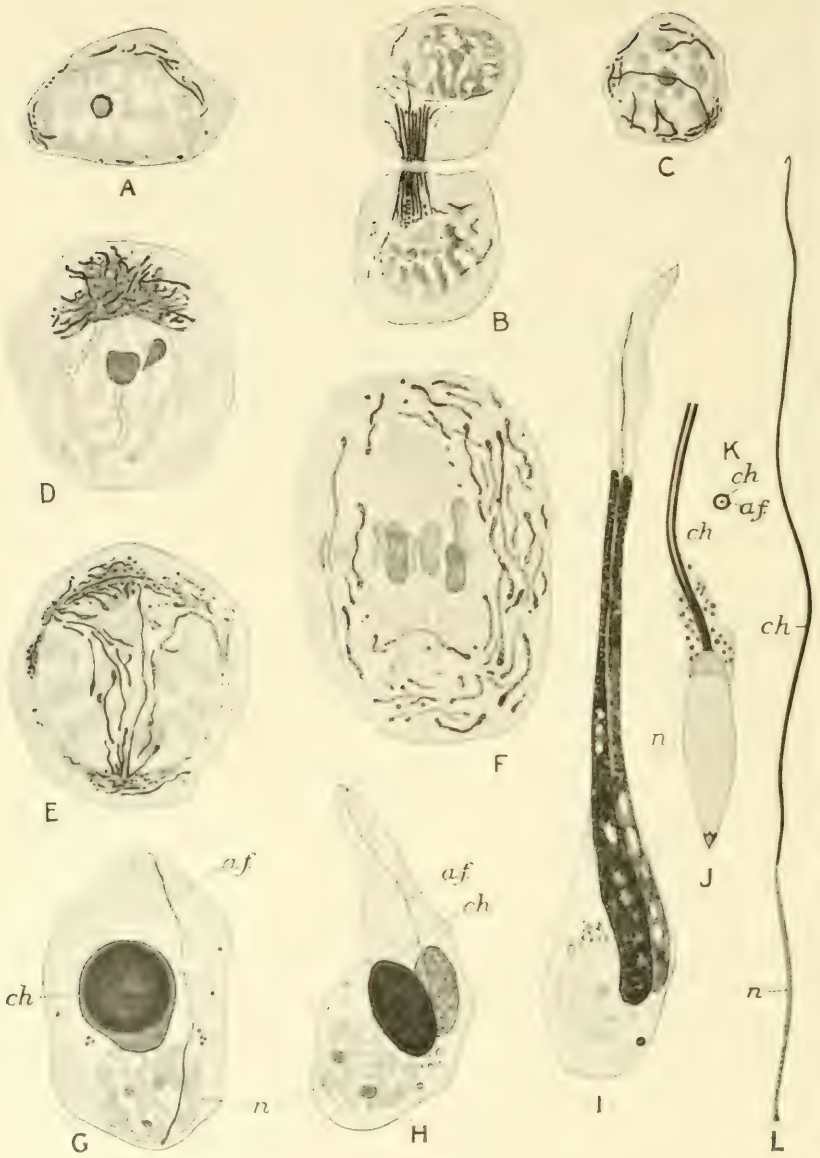


FIG. 29.

Chondriosomes in the spermatogenesis of *Blatta germanica*. (After Duesberg, *A.Z.*, 1911.) The material is prepared by Benda's method, and the chondriosomes are shown darker than the chromatin. A, resting spermatogonium, filamentar chondriosomes distributed through the cytoplasm; B, tel phase of a spermatogonial division; chondriosomes mostly congregated in a bundle which is just cut across; C, young spermatocyte I.; D, pachytene stage, chondriosomes temporarily concentrated outside pole of nucleus; E, later prophase I.; F, metaphase I.; G, young spermatid; chondriosomes massed into the very conspicuous "Nebenkern"; H, chondriosome mass divided into two; I, the two chondriosome masses growing out into the tail of the spermatozoon, and clasping between them the axial filament; J, the chondriosome mass now forms a sheath round the axial filament, as shown in K, transverse section of the tail; L, ripe spermatozoon under a lower magnification.

a.f, axial filament of tail; *ch*, chondriosome mass; *n*, nucleus.

filamentar. During cell division they congregate between the separating daughter nuclei. Finally, when cell division is almost complete, they occupy the bridge of cytoplasm that connects the two nearly separated daughter cells. When this is broken through, the bundle of chondriosomes is also broken across, and thus a portion of the chondriosome mass is left in each daughter cell.

In the young primary spermatocyte they are again scattered through the cytoplasm, but at the beginning of the growth period they concentrate round the centrosome, forming thus a cap at the pole of the nucleus.

They remain in this position throughout the growth period, but in the later prophase again become scattered through the cell and form during mitosis a mantle round the spindle figure. Their distribution between the two secondary spermatocytes is thus insured.

In the second division they behave in the same way, and thus each spermatid receives a share of the chondriosomes present in the primary spermatocyte.

In the spermatid they fuse together into a compact mass which may be as large as, or larger than, the nucleus. This is the supplementary nucleus, or "Nebenkern," of older cytologists (though this term has also been applied to structures of different natures).

Next, the "Nebenkern" divides into two, and elongates with the lengthening tail of the spermatid, the two portions clasping between them the axial filament of the tail. As the spermatid continues to elongate, its parts become more and more attenuated, the "Nebenkern" keeping pace with this elongation, and undergoing various minor changes. In the adult spermatozoon it forms a sheath for nearly the whole length of the tail, only a very short stretch of the latter projecting from the end of the sheath.

In the spermatids of many animals the chondriosomes are scattered instead of being concentrated into a "Nebenkern." In the spermatozoon they are found in a variety of positions, but apparently are never absent from the ripe spermatozoon.[1] This fact has led certain cytologists to assign a very important function to the chondriosomes (Chapter VI.).

We are now in a position to study, very briefly indeed, the general development of the spermatozoon from the spermatid. The development of the mammalian spermatozoon as worked out for the guinea-pig by Meves (1899) and Duesberg (1911 *a*) will serve as a type (Fig. 30). Very briefly, the course of development is as follows: After the second meiotic division the spermatid consists of a cell containing (1) the nucleus; (2) chondriosomes (here in the form of scattered granules); (3) two centrosomes; (4) the idiosome. The last-mentioned body corresponds more or less closely to the attraction sphere of other cells. In the spermatid

[1] An exception has been described in the case of *Peripatus* (p. 197).

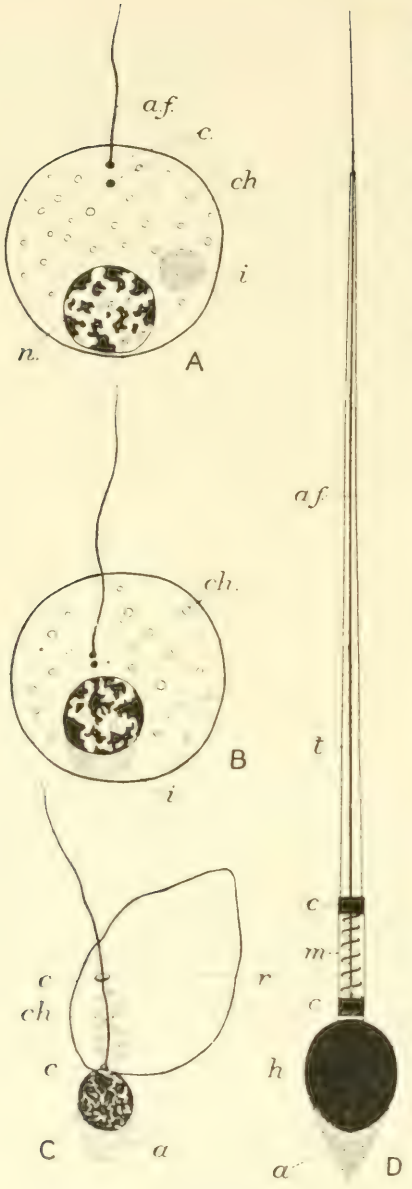


FIG. 30.

Metamorphosis of the spermatid into the spermatozoon. Diagrammatized and simplified from the accounts of the process in the guinea-pig by Meves (*A.m.A.*, 1899), and Duesberg (*A.Z.*, 1911). *a*, acrosome; *a.f*, axial filament of tail; *c*, centrosome; *ch*, chondriosomes; *h*, head (nucleus) of spermatozoon; *i*, idiosome; *m*, middle piece of spermatozoon with two centrosomes and spiral chondriosome band; *n*, nucleus; *r*, cytoplasm of spermatid which is thrown off; *t*, tail of spermatozoon.

this body is separated from the centrosomes, and comparatively large.

The changes undergone by the nucleus during the development of the spermatozoon are, so far as can be seen, little more than a progressive concentration.

The *idiosome* applies itself to the end of the nucleus farthest from the centrosomes, and there becomes

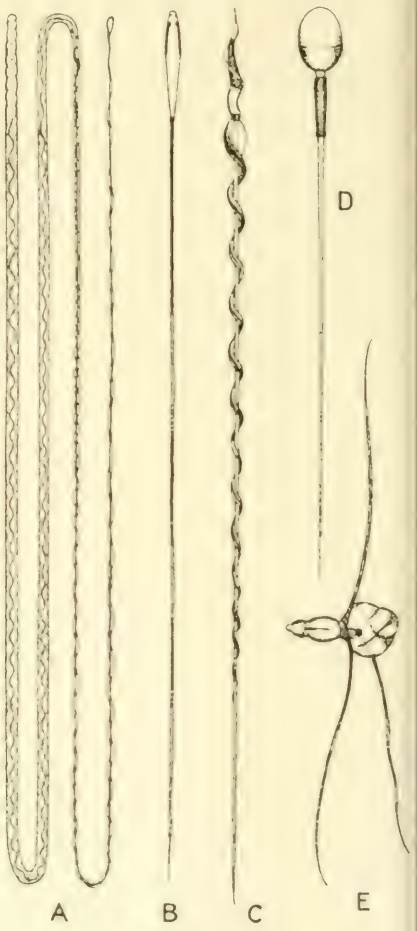


FIG. 31.

Various spermatozoa. (After Retzius, 1909.) A, *Notodromas*; B, *Locusta*; C, *Turdus*; D, *Homo*; E, *Galathea*.

metamorphosed into the *acrosome* which forms the anterior tip of the ripe spermatozoon ; it appears to act as a spear-head by which the spermatozoon perforates and penetrates into the egg.

In the spermatid shown in Fig. 30, A, a fine thread, is already growing out from the distal centrosome (*i.e.* the centrosome farthest from the nucleus). This is the beginning of the axial filament of the tail. Later, the two centrosomes move down towards the nucleus, coming into contact with one another at the same time, so that the base of the tail filament is now continued past the distal into the proximal centrosome. This comes into close contact with the surface of the nucleus and undergoes certain metamorphoses not shown in these figures. The distal centrosome parts company with the proximal one, grows into a ring surrounding the tail filament, and travels down it for a certain distance. The portion of the mature spermatozoon in which the centrosomes are lodged is called the *middle piece*.

The chondriosomes, which in the guinea-pig, as in other mammals, are scattered through the spermatid instead of being collected into a " Nebenkern," become concentrated round the tail filament between the two centrosomes, and there form a granular sheath for the proximal part of the filament, the granules being often specially dense along a spiral band round the middle piece (Fig. 30, D).

By far the greater part of the spermatid cytoplasm is not used up in the process of forming the spermatozoon, and is cast off about the stage shown in Fig. 30, C.

A few representative types of other spermatozoa are shown in Fig. 31.

B. SYNGAMY

With certain exceptions which will be described later on in this chapter, neither the male nor the female gamete is of itself in a position to start development. Before this can occur, *syngamy*, or fusion of a male and female gamete, must take place. The zygote thus formed is capable of development into a new individual of the species, and, in the great majority of cases, this development starts immediately after the zygote has been constituted. Owing to the fact that the zygote differs superficially but little from the ovum, except in its power of development, the process of syngamy in the Metazoa is generally known as the *fertilization of the ovum*.

The details of the process of syngamy vary but little, the essential features being the penetration of the motile spermatozoon or microgamete into the immotile egg or macrogamete, and the fusion of the two gametic nuclei to form the zygote, or cleavage, nucleus ; this proceeds to mitosis under the influence of the centrosome introduced by the male

gamete. The general course of events is illustrated by the diagrammatic Fig. 32 and is sufficiently explained by the legend of that figure. Fig. 33 on the other hand shows the process as it actually occurs in the Annelid,

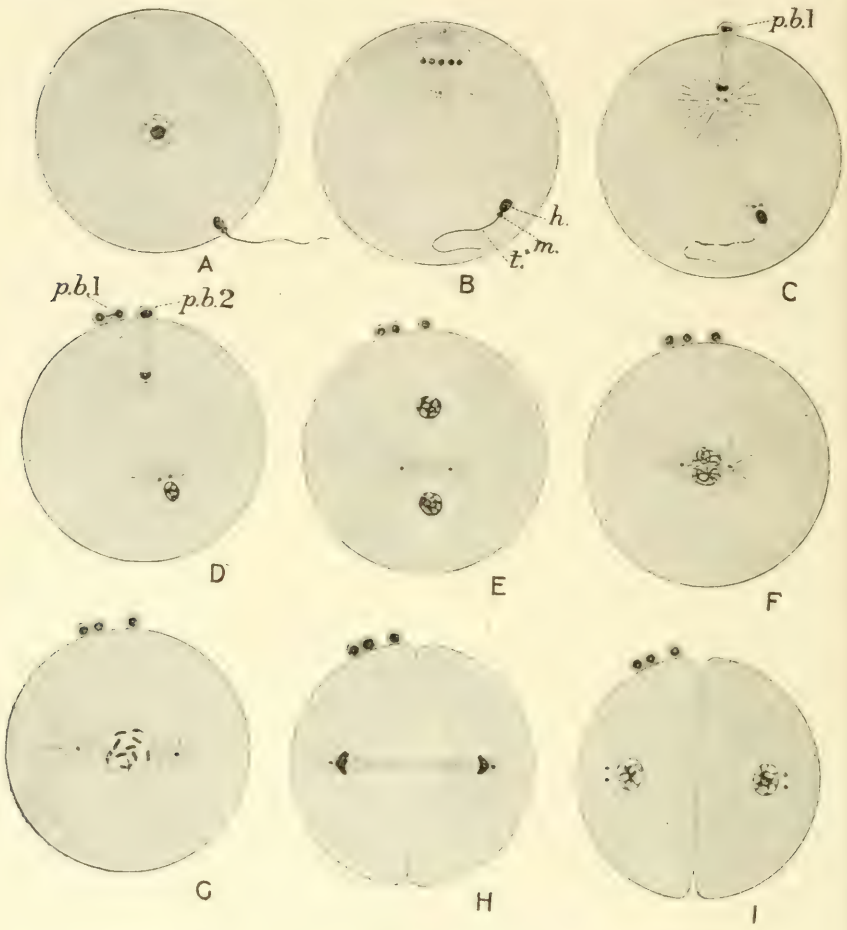


FIG. 32.

Diagram of fertilization. A, entry of spermatozoon into primary oocyte; B, the ♀ nucleus has moved to the surface and is in metaphase I.; C, ♀ nucleus in telophase I. The tail of the spermatozoon has broken off and is degenerating. The head of the spermatozoon has rotated so that the two centrosomes which have disengaged from the middle piece now precede the ♂ nucleus as it travels towards the centre of the egg. D, ♀ nucleus in telophase II.; first polar body dividing; E, approach of the ♂ and ♀ nuclei; F, the two nuclei, now of approximately equal size, and in early prophase, are in contact; G, the two nuclei have fused into a zygote nucleus: formation of spindle figure out of centrosomes and asters introduced by the spermatozoon; H, telophase of first (cleavage) division of the zygote nucleus; I, 2-celled stage.

h, head of spermatozoon (♂ nucleus); *m*, middle piece; *p.b.*1 and *p.b.*2, first and second polar bodies; *t*, tail of spermatozoon.

Chaetopterus. The chief variations from the examples depicted concern the moment of entry of the spermatozoon, and the condition of the gametic nuclei (often called the male and female *pronuclei*) at the moment of *karyogamy* or nuclear fusion.

The case illustrated in the diagram is one where the oocyte I. proceeds with the usual course of meiosis up to the end of the growth period, and then pauses with its nucleus either in the " germinal vesicle " stage, or in diakinesis, for the first meiotic division. The subsequent completion of meiosis is dependent upon the entry of a spermatozoon into the oocyte. Should this not occur, the oocyte perishes without further development. The entry of a spermatozoon, however, immediately sets the meiotic processes in operation again, and the nucleus moves towards the surface of the egg, undergoes its two meiotic divisions to produce the first and second polar bodies, and then returns to the centre of the egg to meet the male nucleus. Examples of eggs in which fertilization is of this type are *Asterias* (many workers) and *Fasciola* (Schellenberg, 1911). Rarely the spermatozoon enters the oocyte I. at a much earlier stage, *e.g.* in *Saccocirrus* (Buchner, 1914), where it enters at the beginning of the growth period and lies among the yolk during the whole of the long time which elapses before the meiotic divisions of the egg take place and consequently karyogamy becomes possible. Much more often, however, the spermatozoon enters at a later stage. For instance, the pause in the meiotic phase for the entry of the spermatozoon takes place in metaphase I. or anaphase I. in *Ophryotrocha* (Korschelt, 1895), *Chaetopterus* (Mead, 1898; Fig. 33), *Thalassema* (Griffin, 1899), *Physa* (Kostanecki and Wierzejski, 1896). It occurs in metaphase II. or anaphase II. in *Amphioxus* (Sobotta, 1897), the axolotl (Fick, 1893), and in the mouse and guinea-pig (Lams and Doorme, 1908). In other cases the egg completes the process of meiosis, forming both polar bodies, and the mature egg nucleus passes into the resting condition to await the entry of the spermatozoon. Examples of this type are the sea-urchins (many workers), and the sponge *Sycon* (Jorgensen, 1910 *a*).

As regards the fusion of the two gametic nuclei, the diagram shows each nucleus in the resting condition at the moment that karyogamy takes place. In certain cases, however, the two nuclei do not fuse to form a resting zygote nucleus, but they may fuse when in prophase for the first cleavage division. In other cases the nuclei do not fuse before the first cleavage mitosis at all, but each gamete nucleus forms its chromosomes while still independent, and the chromosomes are placed on the spindle in two separate groups. In these cases the chromosomes derived from the male and female parents are not united into a zygote nucleus until the telophase of the first cleavage mitosis. Examples of karyogamy of this type are *Ascaris megalocephala* (cf. Fig. 70) and *Ophryotrocha* (Fig. 34). Indeed, it not infrequently happens that the complete fusion of the gamete nuclei is postponed to a much later stage (p. 77).

The male nucleus, which is derived from the head of the spermatozoon, is at first very much smaller than the female. This is obviously due

merely to the fact that the chromatin is more concentrated in the former, for as it approaches the female nucleus it grows larger, and at the same time becomes looser in texture, till ultimately the two gamete nuclei are generally of the same volume and structure.

Since each gamete nucleus is haploid, it is obvious that the zygote nucleus is diploid again.

As we have seen, a typical spermatozoon consists of three main portions—head, middle piece, and tail. The fate of the head or nucleus

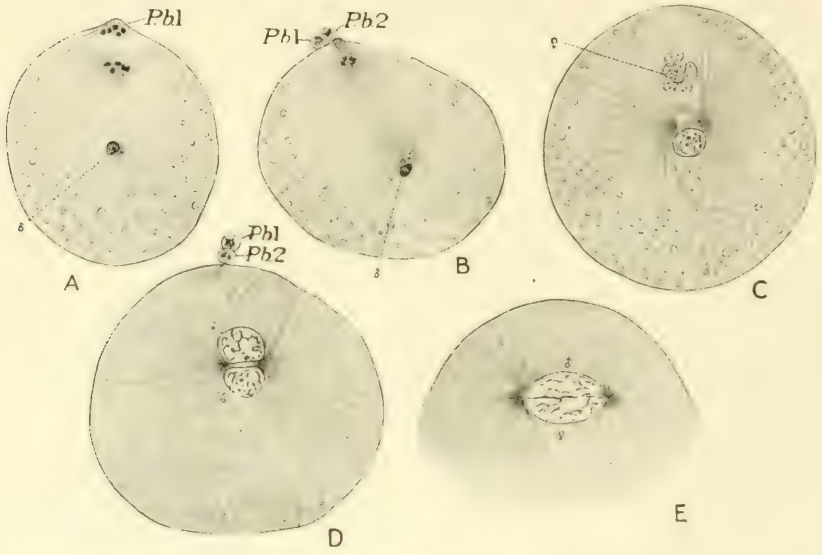


FIG. 33.

Syngamy in *Chaetopterus pergamentaceus*. (After Mead, *J.M.*, 1898.) A, soon after entry of spermatozoon; ♀ nucleus in anaphase I.; ♂ aster developing; B, ♀ nucleus in anaphase II.; C, ♀ nucleus in form of karyomeres (see p. 131); ♀ achromatic figure has disappeared; D, approach; E, fusion, of gamete nuclei. *P.b.1* and *P.b.2*, first and second polar bodies.

we have already followed. The tail apparently takes no part in fertilization. (For the fate of the chondriosomes, which are usually situated in the tail, see p. 198.) Sometimes it does not even enter the egg, being broken off from the middle piece as soon as this has penetrated into the egg (sea-urchins). When the tail does enter the egg it breaks off from the rest of spermatozoon soon after entry, and lies in the egg cytoplasm for a time, and then degenerates.

There remains to be considered the middle piece, with which the centrosome, as the development of the spermatozoon showed us, is generally related. Owing to the mode of entry of the spermatozoon—head first—the middle piece is at first behind the head. After entry, however, the head and middle piece rotate so that the latter is in front of the former during its journey towards the centre of the egg. The

centrosome soon becomes visible in the middle piece, and an astral radiation appears round it. It next divides into two (if it has not already done so in the spermatid), a spindle figure being spun out between the two daughter centrosomes, and thus a complete achromatic figure is

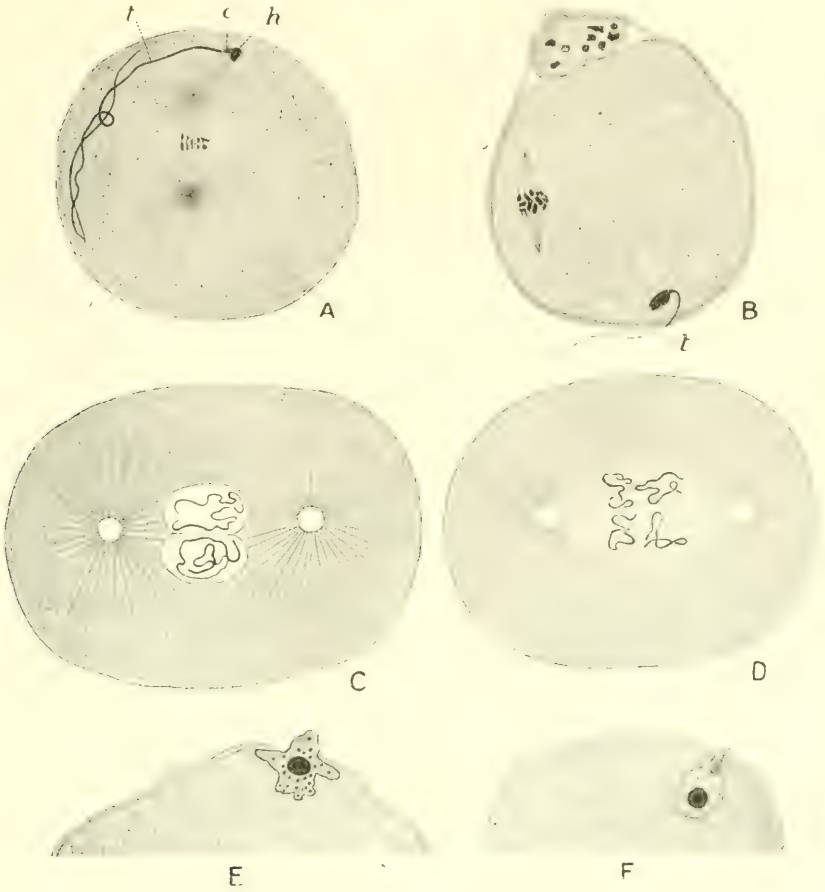


FIG. 34.

Syngamy in various animals. A, *Physa fontanalis* (after Kostanecki and Wierzejski, *A.m.A.*, 1896) just after entry of spermatozoon; B, the mouse (after Lams and Doorme, *A.B.*, 1908) spermatozoon in the act of entering the egg; C, D, successive stages in the fusion of the gamete nuclei in *Ophryotrocha puerilis* (after Korschelt, *Z.w.Z.*, 1895); E, F, the penetration of the amoeboid spermatozoon in *Ascaris canis* (after Walton, *J.M.*, 1918).

c, centrosome in middle piece; *h*, head; *t*, tail of spermatozoon.

formed under the influence of the sperm centrosome. This is the achromatic figure of the zygote nucleus, for after the formation of the second polar body the entire achromatic figure of the egg, including the centrosomes, disappears. Thus the centrosome, and hence the whole achromatic figure of the zygote, is entirely derived from the spermatozoon. This conclusion is based on numerous observations, and also is well

grounded on such experiments as those of Boveri (1907), who found that sea-urchins' eggs into which, from pathological causes, two spermatozoa had entered possessed four centrosomes, with a four-poled first cleavage mitosis (tetraster) ; if three spermatozoa had entered the egg, six centrosomes were found to be present.

The important part played by the centrosome of the spermatozoon introduces us to the thoroughly well established view that fertilization has two functions—(1) the stimulation of the egg to develop, and (2) the fusion of the gamete nuclei. These two processes are not necessarily interdependent. Thus an egg may be stimulated to development by the entry of a spermatozoon which belongs to such a distantly related species that nuclear fusion between them is impossible (p. 160), or the stimulus may even be provided by physical means without the intervention of a spermatozoon at all (artificial parthenogenesis, p. 94).

Since an egg under certain circumstances may develop without fusion of its nucleus with a microgamete nucleus, it is certain that karyogamy is not a necessary condition of development. The function of the fusion of the gamete nuclei must be looked for in its ulterior effects on variation and heredity. The incapacity of either gamete to develop, under ordinary circumstances, by itself, has indeed been looked upon as an adaptation to ensure that karyogamy shall take place, the loss of power to develop independently being attained in the case of the spermatozoon by its general specialization and the reduction of its cytoplasm to a minimal quantity, and in the case of the egg by the degeneration of the centrosomes and rest of the achromatic figure after the completion of the meiotic divisions. It is indeed difficult to believe that the developmental stimulus given by the spermatozoon is not connected with the introduction of the active male centrosome. It must however be remembered that in the case of artificial parthenogenesis no new centrosome is introduced from without. Moreover, in many eggs the pause in the meiotic processes in which the egg awaits fertilization takes place in metaphase I. or II., that is to say, at a time when its centrosomes and achromatic figure are fully developed and active. Thus here, as in so many other biological problems, a simple, almost mechanical, explanation is found to be inadequate to cover all the facts.

As a rule, only one spermatozoon enters the egg. Even when, as in the great majority of cases, the egg is surrounded by an enormous number of spermatozoa seeking to enter it, the penetration of a single spermatozoon usually immediately confers on the egg the power of resisting the entrance of any more. The means by which this resistance is effected is not fully understood, though in many eggs the exclusion of supernumerary spermatozoa is certainly aided by the secretion of a membrane

round the egg almost instantaneously after the entrance of the first spermatozoon.

In some eggs, however, more than one spermatozoon always enter at fertilization (*polyspermy*), though in no case does more than one normally fuse with the egg nucleus. In some cases the supernumerary spermatozoa merely degenerate and are absorbed (Axolotl, Fick, 1893) ; in other cases they maintain themselves for a considerable time in the egg cytoplasm, even forming nuclei which may increase in number by amitotic fragmentation (*Triton*, Braus, 1895). In yet other types the supernumerary spermatozoa metamorphose themselves into nuclei which multiply by mitosis (exhibiting of course the haploid number of chromosomes) and may persist for a long time in development. In the case of the Elasmobranchs, where this type of polyspermy has been most thoroughly studied (Rückert, 1899), the ultimate fate of these nuclei is not known, but it is extremely improbable that they take any part in the formation of the embryo. In the pigeon, the nuclei derived from the extra spermatozoa, which also multiply by mitosis, disappear much earlier, namely, about the 32-cell stage (Blount, 1909).

Cases such as the above, where several spermatozoa normally enter the egg (though only one fuses with the egg nucleus), are said to exhibit *physiological polyspermy*. This is specially characteristic of large, heavily yolked eggs (Insects, Elasmobranchs, Amphibia, Reptiles, Birds). In other cases, however, the entry of more than one spermatozoon into the egg is pathological (*pathological polyspermy*) and leads to abnormal development, owing to the multiplication of centrosomes and to the fact that more than one of the microgamete nuclei enters into relation with the female nucleus. If two spermatozoa enter the egg of *Ascaris megalocephala* or of *Echinus*, both male nuclei form chromosomes and both give rise to a pair of centrosomes. Thus there are three sets of chromosomes (two ♂ and one ♀) and four centrosomes. A four-poled spindle figure is thus produced, and at the first cleavage division the egg divides simultaneously into four blastomeres instead of two, the $3n$ chromosomes being irregularly distributed among the four nuclei. These special cases are more fully described later on (p. 162).

C. GONOMERY

We have seen that the gamete nuclei may fuse in the resting condition, or may carry through the prophases of mitosis while still separate, in which case the chromosomes of the two gametes come together for the first time on the first cleavage spindle. Sometimes, however, the two nuclei retain their individuality much longer (Fig. 35). In various species of *Cyclops*, for instance (Rückert, 1895 ; Häcker, 1895), the resting nuclei

of the early cleavage stages are double, each portion being the direct descendant of one of the gamete nuclei. Each constituent of such a double nucleus is called a *gonomere*. In prophase each gonomere forms its chromosomes separately from the other. The two groups of chromosomes thus formed are usually indistinguishable from one another after the break-down of the nuclear membrane, but in telophase they become recognizable again owing to the fact that the group of chromosomes derived from each gonomere again forms a nucleus distinct from, though closely applied to, that formed by the other group. Occasionally, however, the two groups are distinct during metaphase and anaphase as well (Fig. 35, A). In later cleavage stages the chromatin derived from the two gamete nuclei gradually mingles more and more, and double nuclei become consequently rarer. In *Cyclops brevicornis* double and bilobed

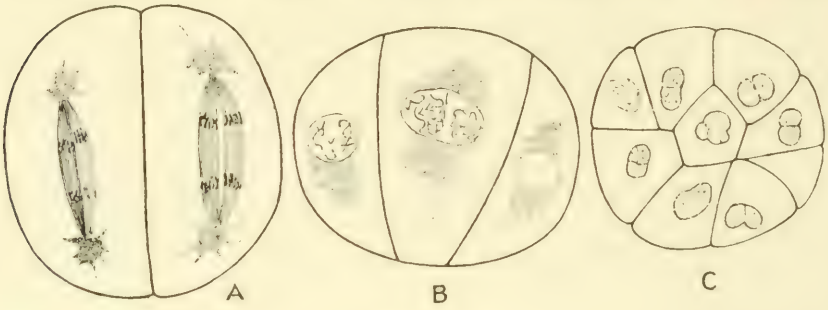


FIG. 35.

Gonomery in *Cyclops strenuus*. (After Rückert, *A.m.A.*, 1895.) A, 2-4-cell stage, the groups of chromosomes derived from ♂ and ♀ gametes quite separate; B, 4-cell stage. In the nucleus in prophase the two groups of chromosomes are seen. C, 32-cell stage. Gonomeres indicated in most of the nuclei.

nuclei are still common in the 64-cell stage, and in later stages bilobed nuclei with a nucleolus in each lobe are still to be found, as well as spherical nuclei with two symmetrically placed nucleoli, which Häcker interprets as the last remaining indication of gonomery. In the germ-track (see p. 79) evidences of gonomery can be found at a much later stage of development than in the somatic cells (Häcker, 1903).

A remarkable instance of gonomery is to be found in the Protozoan, *Amoeba diploidea* (Nägler, 1909). This animal possesses two nuclei, in close apposition to one another (Fig. 36), exactly like the double nuclei of early *Cyclops* embryos. The life history shows that these nuclei are the direct descendants of the two gamete nuclei—*i.e.* they are gonomeres. During the asexual reproduction of the animal the two nuclei divide separately, but simultaneously, so that each daughter cell again receives a double nucleus. Sexual reproduction begins by the coming together of two individuals which enclose themselves in a common cyst. Now in each individual the gonomeres for the first time fuse into a zygote

nucleus, so that each of the conjugating amoebae has now a single nucleus. These nuclei undergo a process of meiosis, comparable to the formation of the polar bodies of a Metazoan egg, converting each amoeba into a single gamete. The two gamete cells fuse together to form a zygote, their nuclei, however, remaining unfused. Thus the binucleate condition is restored, to be retained through an indefinite number of cell divisions

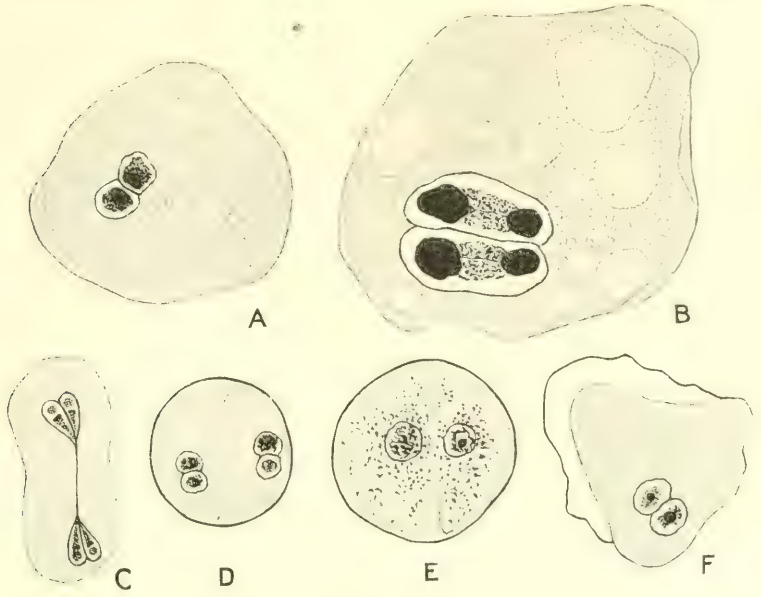


FIG. 36.

Amoeba diploidea. (After Nägler, *A.P.K.*, 1909.) A, the animal in its active phase, showing the double nucleus (gonomeres); B, C, division stages showing simultaneous division of the gonomeres; D, two individuals encysted preparatory to conjugation; E, in each individual the gonomeres have fused into a single nucleus; F, conjugation has taken place, and the zygote with the two gamete nuclei (gonomeres) is emerging from the cyst, thus bringing the life cycle back to A again.

during asexual reproduction, the two gonomeres fusing together for the first time immediately before gamete formation.

D. THE GERM-TRACK

One more feature of early development remains to be mentioned. In a large number of animals the primitive germ-cells—those cells, that is to say, that will eventually give rise to gametes—are visibly marked out from the remaining or somatic cells at a very early stage of development. The distinguishing marks may be features either of the nucleus or cytoplasm. The best-known case is that of *Ascaris megalocephala* (Boveri, 1899, 1904, 1910).

Nothing remarkable is to be observed in the first cleavage division,

but when the nuclei of the 2-cell stage are preparing for the next

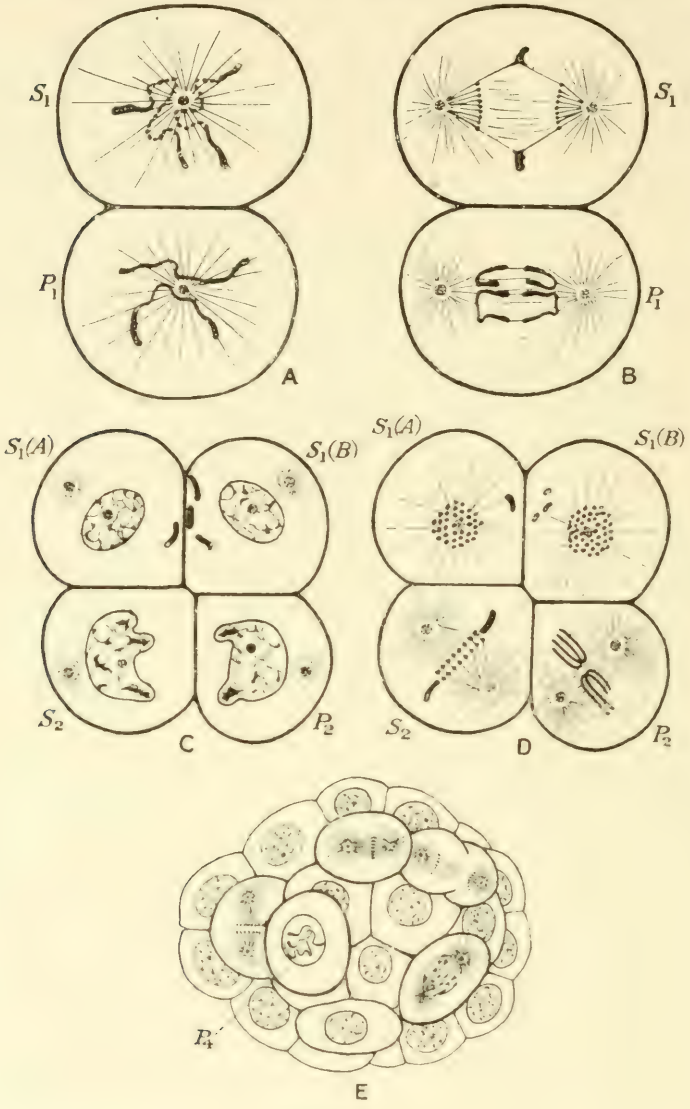


FIG. 37.

Differentiation of the germ-track in *Ascaris megalocephala univalens*. (After Boveri, *Fest. f. Kupffer*, 1899, and *Ergebnisse*, 1904.) A, 2-cell stage. Diminution taking place in the cell S_1, chromosomes intact in P_1. B, passage of the 2-cell into the 4-cell stage. In S_1, numerous small chromosomes formed by the fragmentation of the two large ones. The broken-off ends of the latter are seen outside the equator of the spindle. C, 4-cell stage. In $S_1(A)$ and $S_1(B)$ the cast-out ends of the chromosomes are seen in the cytoplasm. D, mitosis which will give rise to the 8-cell stage. Diminution taking place in S_2, chromosomes intact in P_2. E, later embryo, process of diminution now complete. The two original chromosomes are still intact in P_4 only.

mitosis, one of them is marked out from the other by the fact that its

chromosomes have each broken up into a number of small pieces (Fig. 37, A). The ends of the chromosomes break off as comparatively large club-shaped pieces, and the central portions become divided into a great number of much smaller, more or less spherical, fragments. The larger pieces derived from the ends of the chromosomes are cast out into the cytoplasm, where they degenerate and disappear. For this reason the phenomenon is known as the *diminution* of the chromatin.

The cell in which chromatin diminution has taken place is now left with a large number of small chromatin granules, and each of these acts in future mitoses as a single chromosome. The embryo at this stage (prophase of second cleavage division) consists therefore of two cells, the one with two large chromosomes (in *A. m. univalens*, four in *A. m. bivalens*), the other with a large number (about sixty in *A. m. univalens*) of very small ones. The total chromatin content of the latter nucleus is much less than that of the former, owing to the loss of the large pieces from the ends of the original chromosomes.

All the descendants of the cell which has undergone diminution have the same nuclear composition as their parent cell, the mitosis of the numerous small chromosomes apparently taking place regularly, and there being no further loss of chromatin. It is not so, however, in the case of the cell with the two original chromosomes intact. For three more successive mitoses this cell divides into one which undergoes diminution and one which does not, so that in the 16-cell stage we have one cell with the original chromosomes intact, one in which diminution is in progress and fourteen with the diminished amount of chromatin and numerous small chromosomes. After this, there is no further diminution, so that after the next cleavage there are two cells with the original chromosomes intact, and the remainder with the numerous small chromosomes, and in all future mitoses the daughter nuclei remain of the same composition as their mother nucleus. *The two cells with intact chromosomes are the primitive gonad;* the remaining cells will develop into the soma. Thus all the somatic cells of *Ascaris megalocephala* have the numerous small chromosomes and lack the large portion of the chromatin which was thrown out from the ends of the original chromosomes, while all the germ-cells contain the original chromosomes, with all the chromatin, intact (Fig. 38). The line of cells with intact chromosomes leading from the undivided zygote to the gametes is called the *germ-track*.

Phenomena, similar in principle but differing in detail, have been observed in other species of *Ascaris*. In *A. lumbricoides*, however (Bonnevie, 1902), only the ends of the chromosomes break off (and are got rid of); there is no fragmentation of the middle portions of the chromosomes. In *A. canis* (Walton, 1918) the chromosome ends are

thrown out and the middle pieces break up, as in *A. megalocephala*, except that only two instead of about thirty small chromosomes are produced from each original one.

In many animals the germ-track is marked out from the undivided egg onwards by characteristics of the cytoplasm instead of the nucleus. The fresh-water crustacean *Cyclops* furnishes an example. The germ-track in this animal was first worked out by Häcker in *C. viridis* (1897 *a*), his results being confirmed in all essentials by Amma in 1911, who also

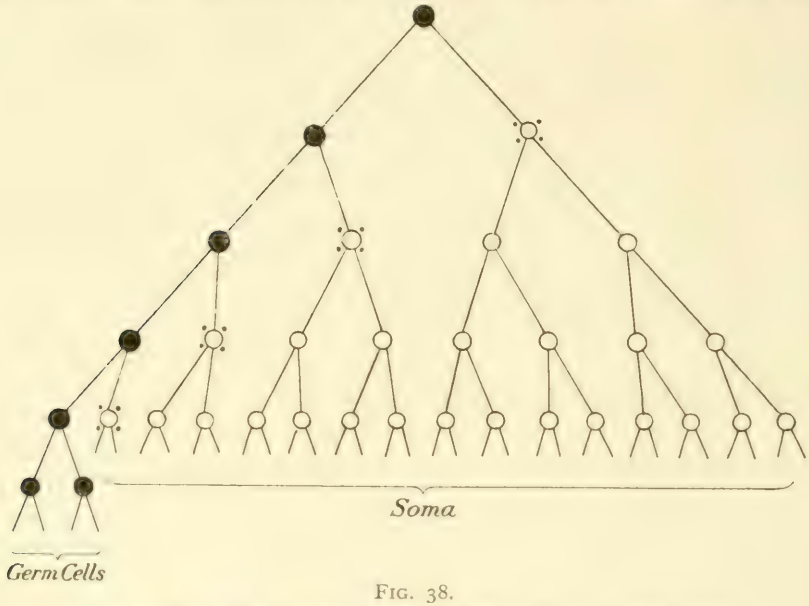


FIG. 38.

Scheme of the cleavage divisions in *Ascaris megalocephala*. (Boveri, *Ergebnisse*, 1904.)
The uppermost cell is the fertilized ovum.
● Cell in which chromatin diminution has not taken place.
◌ Cell in which chromatin diminution takes place.
○ Cell with diminished chromatin.

discovered the same process in several different species of *Cyclops* and in the allied genera *Diaptomus* and *Canthocamptus*. The account given by the latter author for *Cyclops fuscus* will serve for an example (Fig. 39).

In the prophase of the first cleavage division one attraction sphere is distinguished from the other by a group of granules which surrounds it, these being completely absent from the other sphere. Consequently, at cell division all the granules pass into one of the first two cells or blastomeres and none into the other; nor do they ever appear in the descendants of the latter cell. In the case of the blastomere containing the granules the process is repeated in the following mitosis. After the first cell division is completed the granules become clumped together

and gradually disappear, and a new set of granules makes its appearance in the next prophase. As the granules of the preceding mitosis have not quite disappeared by the time the new set develops, the cell is never altogether without them, and this fact makes its continuous identification possible. The new granules appearing at prophase are again concen-

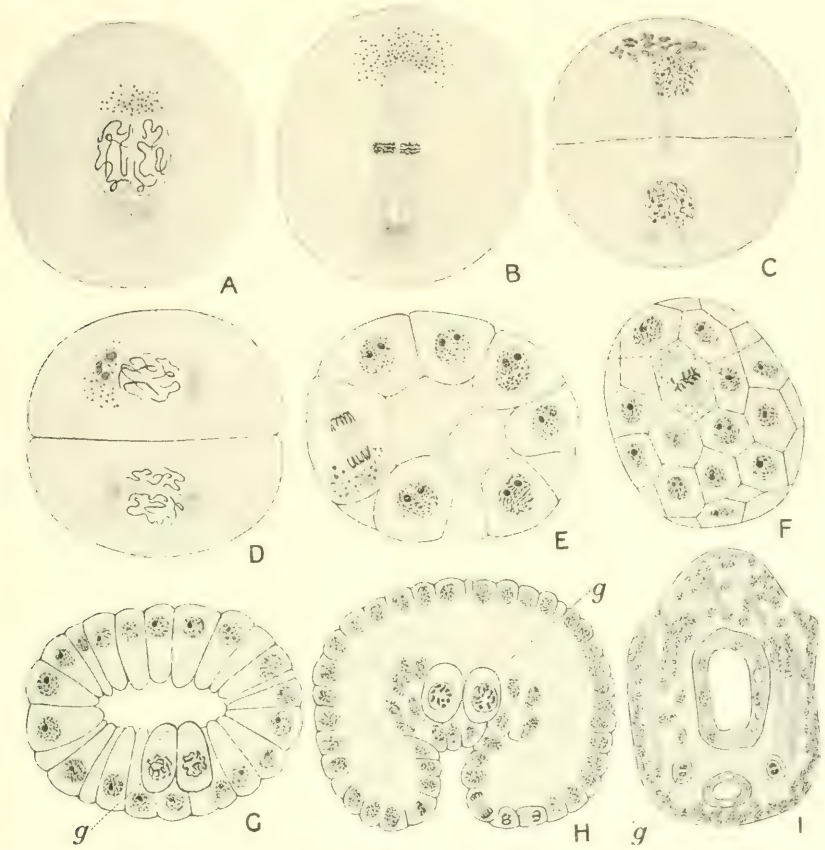


FIG. 39.

Differentiation of the germ-track in *Cyclops fuscus* (A-H), and *Diaptomus coeruleus* (I). (After Amma, *A.Z.*, 1911.) A, prophase of first cleavage mitosis, granules congregated round one attraction sphere; B, same mitosis, metaphase; C, 2-cell stage, resting nuclei (note gonomery); D, prophase of second cleavage division; E, 16-cell stage. All the nuclei have completed their division, and entered into the resting stage, except that of the granule cell which is still in anaphase. F, division of the granule cell into the two primitive germ-cells; G, H, I, later stages.
g, primitive germ-cells.

trated round one attraction sphere only, and thus again pass into only one of the daughter cells. A differential cell division of this sort takes place four times, so that the cleaving egg up to the end of the 16-cell stage contains one, and only one, cell with granules, or *granule cell*. The nucleus of this cell does not as yet differ markedly from those of the other cells, except that it constantly lags a little behind the others in mitosis.

By the time that the 16-cell stage is reached this lagging has gone so far that, when the remainder of the cells divide to form what should be the 32-cell stage, the granule cell fails to divide at all, so that the blastula at this stage contains only thirty-one cells.

After the fourth division of the granule cell—*i.e.* in the 16- and 31-cell stages—the granules are no longer confined to one attraction sphere, but are scattered throughout the whole cell. Consequently, when the granule cell divides next time, which it does at the close of the 31-cell stage, it produces two similar granule cells. These cells, which remain without further division for a considerable time, are the primitive germ-cells, from which the right and left gonads develop respectively.

Thus the germ-track in *Cyclops* and the allied genera is quite as clearly marked out as in *Ascaris*. At first sight it might, however, appear that the two processes were very distinct, the one being a case of nuclear, and the other of cytoplasmic, differentiation. Nevertheless, while we do not know the significance of the diminution of the chromatin and fragmentation of the chromosomes in the somatic cells of *Ascaris*, there is little doubt that in both cases it is the nature of the cytoplasm of the cell which determines whether it shall be a somatic or a gonadic cell. This is fairly plain in the case of *Cyclops*, where it is clear enough that the granules are of cytoplasmic origin. Amma gives reasons for the belief that they are temporary metabolic products of a special portion of the cytoplasm. We may conceive of this special cytoplasm as concentrated in the undivided egg near one of the centrosomes, and consequently passing into only one of the daughter cells, until, after four such divisions, the cells have become so much reduced in size that now this substance occupies the whole, or nearly the whole, of the cell instead of one pole only of it. Henceforth division of this cell, or of its descendants, must result in the passage of this substance into both daughter cells.

By the study of the diminution process in dispermic *Ascaris* eggs, Boveri (1910) has shown that in this species also it is the nature of the cytoplasm which determines whether diminution shall or shall not take place in a particular cell. The eggs in question are the very rare abnormal cases where two spermatozoa have entered the egg. Both centrosomes introduced by the spermatozoa divide, and then form a quadripolar spindle figure (cf. Fig. 74). On this spindle the $3n$, or 6, chromosomes derived from the female and the two male gamete nuclei take up their position (the account deals with *A. m. bivalens*). As a rule the first cleavage mitosis of such an egg divides it simultaneously into four blastomeres, and the twelve daughter chromosomes of the original six are distributed among the four nuclei in a most irregular manner; for example, one nucleus may get only one chromosome, another three, and the other two four each, etc.

Now in the normal monospermic egg all the daughter chromosomes of the zygote nucleus which pass into the one blastomere (designated by Boveri, *S*) undergo diminution, and all that go into the other (*P*) undergo their next mitosis intact. Thus, if the inducement to diminution were furnished by the chromosomes themselves, we would have to suppose that every chromosome of the normal zygote nucleus divides at metaphase into two daughter chromosomes, one of which is predestined to undergo diminution and the other is not. If this were the case in the dispermic egg, it would follow that six of the daughter chromosomes

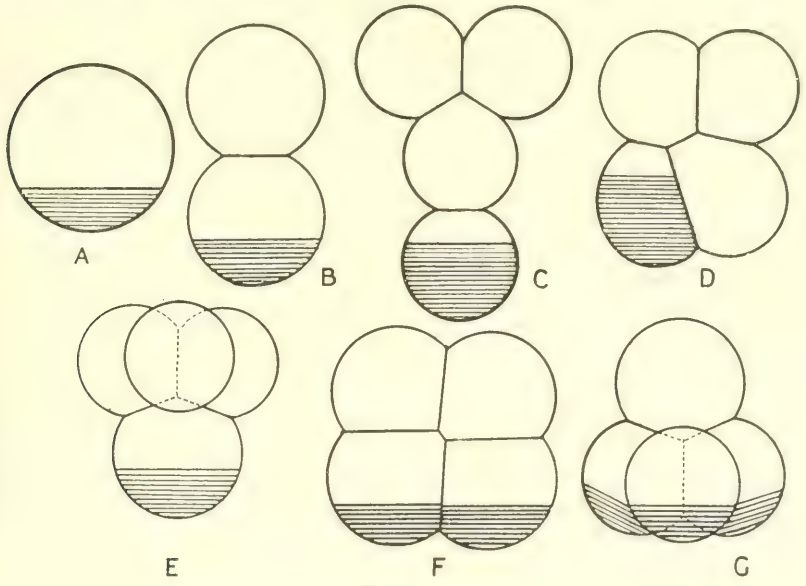


FIG. 40.

Diagram illustrating the part played by the cytoplasm in determining the diminution of the chromatin in *Ascaris megalocephala*. (After Boveri, *Festschr. f. Hertwig*, 1910.) The shaded portion represents the "vegetative" cytoplasm. In every case the chromosomes remain intact in the cells containing this substance, and undergo diminution in the cells which lack it. A, undivided egg; B, C, D, stages in the production of the 4-cell stage in the normal, monospermic egg; E, F, G, results of the first cleavage of dispermic eggs. According to the orientation of the spindles with regard to the egg axis, one of the three types shown is obtained.

produced by the metaphase of the first cleavage mitosis were predestined to undergo diminution, while their six sister chromosomes were not. Moreover, it would often result from the unequal distribution of the daughter chromosomes among the four primary blastomeres, that the same nucleus would contain a mixture of chromosomes predestined for diminution and of those predestined to remain intact. As a matter of fact, neither of these expectations is realized. Diminution may take place in one, two or three of the four cells formed by the first cleavage division. All the chromosomes in any one nucleus behave alike, and the number of chromosomes which escapes diminution, instead of being always six, varies from two to twelve in different eggs.

The reasonable explanation which Boveri offered is that the uncleaved *Ascaris* egg, like the eggs of so many other animals, possesses an internal cytoplasmic differentiation into an upper, or "animal," and a lower, or "vegetative," pole. In the normal monospermic egg the first cleavage, being horizontal, divides it into "animal" and "vegetative" blastomeres, the former being less rich in yolk than the latter. The chromosomes in the "animal" blastomere (*S*) undergo diminution, those in the "vegetative" blastomere (*P*) do not. The second cleavage divides both blastomeres into two, and again the chromosomes in the cell nearest to the "vegetative" pole alone remain intact.

Dispermic eggs, as stated above, divide simultaneously into four cells, and this may take place in one of three ways, according as to how the spindles are arranged in relation to the polarity of the cell—namely, one *P* and three *S* cells, two *P* and two *S* cells, or three *P* and one *S* cell, as illustrated in Fig. 40. Chromatin diminution takes place in all the *S* cells but not in the *P*'s, that is to say, it takes place in all cells which fail to contain a portion of the cytoplasm from the vegetative pole of the egg.

A fair number of animals are now known in which the germ-track is thus visibly marked out, the distinguishing marks being of the most varied, and sometimes surprising, nature, though probably all ultimately of cytoplasmic rather than nuclear origin. Thus in the crustacean *Polyphemus pediculus* (Kuhn, 1911), when the egg is laid it has attached to it the remains of one to three "nurse cells" (oocytes which have failed to develop into eggs but have been absorbed by other oocytes). When the vitelline membrane is secreted it is formed in such a way as to include this little mass of nurse cell remains within the egg, and it soon becomes embedded in the egg cytoplasm. As long as the included mass lies passive, it follows that it can only be present in one of the blastomeres, and consequently at the 16-cell stage it is found in one blastomere and is absent from the other fifteen. At this stage the little mass breaks up and becomes scattered through the blastomere in which it lies. Consequently, at the next division of this blastomere, both its daughter cells contain portions of it. These two cells are the primordial germ-cells.

A useful summary of the various forms of germ-track differentiators at present known is given by Hegner (1914 and 1915). There appears to be nothing corresponding to the animal germ-track in the higher plants (see, however, footnote to p. 144).

E. PARTHENOGENESIS

It is plain that the development of the egg without fertilization must involve some modification of the general rule that the mature egg has

only half the number of chromosomes present in the other tissues of the body. Otherwise this number would be halved in each generation, and very soon brought to vanishing point. This eventuality is avoided by one of two methods.

(1) The halving of the chromosome number in oogenesis is omitted, and the ripe egg is diploid. This is usually accompanied by the formation of only one polar body instead of two, and is the commonest form of parthenogenesis. It is found in the Cladocera, Ostracoda, Aphids, etc., amongst Arthropods and in Rotifers, etc. Occasionally, however, two polar bodies are formed, without reduction of chromosomes (the Hymenoptera *Nematus* and *Rhodites*).

(2) True meiosis takes place, the resulting ripe egg being haploid, but reduction is omitted when the haploid individual developing from this egg forms its own gametes. This form of parthenogenesis has been found in several Hymenoptera. In all cases so far known, the haploid individual thus produced is a male. This, it will be observed, is in accordance with the fact that in the great majority of animals in which a difference in the chromosomes of the sexes has actually been observed the male lacks the second sex chromosome, or has it replaced by an inert chromosome (Chapter IV.). For it is obvious that a haploid animal can have only one of each kind of chromosome, including the sex chromosome.

The eggs of type (1) being diploid are incapable of fertilization, and therefore committed to develop parthenogenetically; they are said to exhibit *obligatory parthenogenesis*. The haploid eggs of type (2) differ in no way from eggs destined for fertilization. They seem indeed to be equally capable of fertilization or parthenogenetic development. If fertilized they give rise of course to an ordinary diploid individual, which in all cases known is a female. If they are not fertilized they develop parthenogenetically into a haploid male. Parthenogenesis in these cases is therefore said to be *facultative*.

Further, the eggs of many animals which normally develop only after fertilization can be induced to develop parthenogenetically by the application of appropriate stimuli. Such eggs produce either haploid or diploid individuals according as to whether meiosis had or had not taken place before the parthenogenetic development was induced. This phenomenon is known as *artificial parthenogenesis*.

The cytology of these three types of parthenogenesis will be considered in order.

(1) *Obligatory Parthenogenesis*

By far the greater number of these cases are accompanied by the formation of only one instead of two polar bodies, and the chief interest

centres in the details of the meiosis, as we may call it, though it does not result in a reduction of the chromosome number.

A puzzling feature about the changes undergone by the nucleus in preparation for the single maturation division is their extraordinary similarity to the typical phases of a true meiosis resulting in chromosome reduction. The definitive chromosomes of the single maturation division are also strikingly similar to those found in true meiosis, though the former are univalent and the latter bivalent (Figs. 41, 42).

Thus, in both sexual and parthenogenetic Ostracods (Schleip, 1909) there occurs a synizesis from which in the former the haploid, and in the latter the diploid, number of chromosomes emerges. These chromosomes are remarkably alike in appearance in the two types of eggs, being conspicuously double in both (Fig. 41). In the one case, however, the duplicity is due to bivalency, in the other to the prophase division of univalents. Kuhn (1908) found in parthenogenetic Cladocera a stage with conspicuous duplicity of chromatin threads, strongly suggesting syndesis (Fig. 41). This stage, however, is both preceded and followed by one in which the chromosomes are obscured by their relation to the nucleolus, thus making correct interpretation difficult.

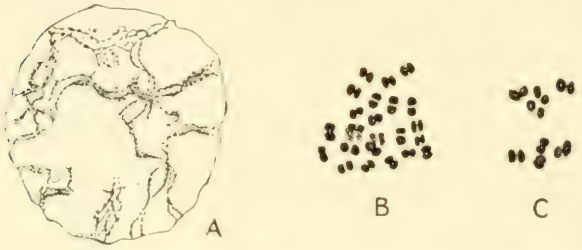


FIG. 41.

A, parthenogenetic oocyte of *Daphnia pulex* during the growth stage (after Kuhn, *A.Z.*, 1908); B, C, chromosomes of the maturation divisions of two Ostracods. B, *Notodromas monacha* (sexual); C, *Cypris fuscata* (parthenogenetic) (after Schleip, *A.Z.*, 1909).

The similarity between the meiotic processes of sexual and parthenogenetic species has indeed been cited by some cytologists as reason for denying altogether the connection of the ordinary meiotic phenomena (zygotene stage, etc.) with the reduction of the chromosomes. This is undoubtedly going to an unjustifiable length, for (1) none of the animals which exhibit parthenogenesis are really favourable objects for cytological study. They cannot be compared in this respect with, say, *Tomopteris*, *Lepidosiren*, or the Amphibia, and consequently the details cannot be said to be satisfactorily known; and (2) the fact that the diploid chromosome number appears in late prophase and metaphase in parthenogenetic eggs is no proof that syndesis did not take place in earlier prophase. We have only to remember the complete dissociation of the ex-syndetic homologous chromosomes in the spermatogenesis of *Lepidosiren* and in many oogeneses to agree with this proposition. It is not impossible that, in the examples of parthenogenesis now under

discussion, syndesis takes place in the usual manner, and that the homologous chromosomes become completely, instead of partially, dissociated in the diplotene stage. Instead of pairing again to form the bivalents of the meiotic metaphase, each chromosome behaves from now onwards as it would in a somatic mitosis.

Strasburger (1907, 1909) was the first to elaborate this view in the case of plants, and has in fact described the dissociation of the haploid bivalents into diploid univalents in the parthenogenetic (" apogamous ") development of the macrospore of the cryptogam *Marsilia drummondii*.

For animals, however, it still remains nothing more than a conjecture.

Although in the parthenogenetic species considered so far the meiotic prophases are scarcely distinguishable from those of sexual forms (except for the diploid chromosome number and the occurrence of only one instead of two maturation divisions), there exist other forms where the meiotic prophase stages are markedly different in allied parthenogenetic and sexual species. This again was first described in plants (Urticaceae) by Strasburger (1910).

In animals, Fries (1910) found in the crustacean *Branchipus* (Fig. 42), which reproduces sexually, all the ordinary phases of meiosis, namely, a leptotene stage followed by synizesis, during which the leptotene threads arrange themselves in parallel pairs ; these apparently fuse to form pachytene bands which on the dissolution of synizesis are found in the haploid number. In the nearly allied but parthenogenetic *Artemia salina*, however, there is no synizesis, nor parallelization of leptotene threads, nor fusion of these to form pachytene bands. On the contrary, each leptotene thread condenses into a single chromosome, and consequently these are present in diploid number. Again, however, we find the definitive chromosomes—in the one case bivalents, and in the other split univalents—surprisingly alike in appearance in the two genera.

Morgan (1915 *a*) also found in the Aphids *Phyllaphis* and *Phylloxera* that in the ovaries of sexual females the meiotic prophases include a synizesis, into which the full number of chromosomes (6) enter, and from which three bivalents emerge. In the ovaries of the parthenogenetic members of the same species, the six chromosomes of the early meiotic prophase contract continuously into six univalents without ever being condensed in a synizetic contraction.

A very few cases of obligatory parthenogenesis are known in which two meiotic divisions occur as in sexual reproduction, but without reduction of the chromosome number. The mature egg is therefore in the same condition as in the ordinary cases of obligatory parthenogenesis with only one meiotic division. This very puzzling phenomenon was first (with the exception of the special case of *Artemia*) described by

Doncaster (1907) for the parthenogenetic eggs of the saw-fly, *Nematus ribesii*, and later by Schleip (1910) for *Rhodites rosae*, a gall-fly.

A very peculiar phenomenon observed by Brauer (1894) in *Artemia* also falls into this category. As we have already seen (p. 89), the normal procedure is for only one maturation division to take place, without

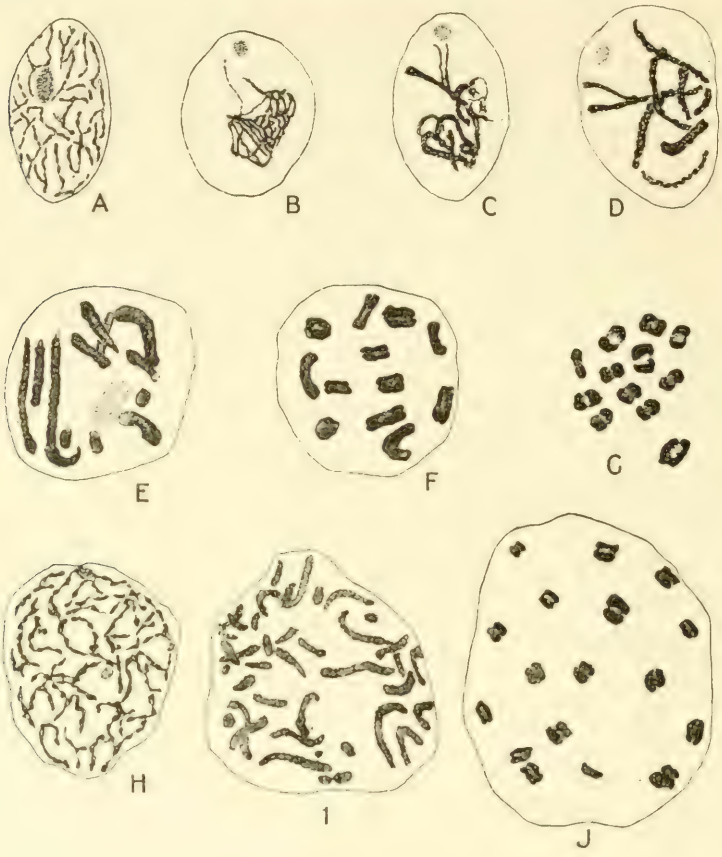


FIG. 42.

Meiotic prophase in the sexual egg of *Branchipus* (A-G), and in the parthenogenetic egg of *Artemia* (H-J). (After Fries, *A.Z.*, 1910.) A, young oocyte I.; B, synizesis and leptotene stage; C, zygotene, D, pachytene stages; E, F, G, contraction of the chromosomes into the definitive bivalents; H, young oocyte I.; I, late prophase, chromosomes longitudinally split; J, definitive chromosomes of the maturation division.

reduction of chromosome number. As a rare exception, however, Brauer found that a second division took place, again without reduction. The two nuclei resulting from this second mitosis must be regarded as equivalent to the nuclei of the mature egg and second polar body. The latter, however, is not extruded from the egg, but remains close to the egg nucleus and moves with it to the centre of the egg. Thereafter these two nuclei act like the male and female gamete nuclei in fertilization,

and fuse together. The egg behaves, indeed, as if it had been fertilized by the second polar body, except that, since no halving of the chromosome number has taken place in either nucleus, the resulting zygote nucleus has double the normal diploid number, that is to say, 168 instead of 84.

It should be noted that Brauer found this mode of meiosis much rarer than the ordinary mode with only one maturation division, and that Fries did not find any instance of it among his material.

(2) *Facultative Parthenogenesis*

The second type of parthenogenesis, where the egg matures in the ordinary way and develops with the haploid number of chromosomes, has been described in a few Hymenoptera. The classical example is the honey-bee (*Apis mellifica*). It has long been known that the fate of the bee's egg depends upon whether it is fertilized or not. The queen bee is impregnated by the drone during the nuptial flight, and the spermatozoa are stored up in her receptaculum seminis. As the eggs pass down the oviduct they pass the mouth of this receptacle, and according as to whether a spermatozoon issues from it or not, the egg is or is not fertilized. If fertilized, the egg develops into a female (either a queen or a worker according to the food supplied to the larva) ; if not fertilized it develops into a male.

The cytology of the bee has been thoroughly worked out by Meves (1907) and Nachtsheim (1913). The diploid chromosome number is thirty-two, though, as in many other animals (*e.g. Ascaris*, p. 81), this number is greatly exceeded in the somatic tissues outside the germ-track, and may reach as high a number as sixty-four. On the other hand, in the oogonia the chromosomes tend to come together in pairs, giving sixteen double chromosomes, much as in some Diptera [1] (p. 126). Thus the determination of the chromosome number is fraught with some difficulty, but it is revealed by the number in the gamete, which is sixteen, and in the female embryo, where it is thirty-two.

No difference is to be expected, nor as a matter of fact was observed, between the meiotic processes of those eggs that are to be fertilized and those that are not. After the second meiotic division the egg nucleus leaves the surface of the egg (in which position, as usual, the maturation divisions take place) and travels towards the centre. If the egg has been fertilized it there meets with the male nucleus and an ordinary zygote nucleus is formed. If it has not been fertilized, the egg nucleus continues to travel right across the egg to the opposite side, as if in search of the

[1] A similar but less strong tendency to pair is observed in the spermatogonia (Nachtsheim) which is particularly noteworthy, because these nuclei are haploid. Moreover, the sixteen double chromosomes in the oogonia unite into eight (tetravalent) chromosomes in the meiotic prophase. See p. 92.

male nucleus, and there forms the first cleavage spindle of the developing embryo.

Great interest attaches to the cytology of the spermatogenesis of the individual developing from these eggs, for since it is already haploid a further reduction of chromosomes must be avoided. How this is accomplished has been worked out by Meves.

The main features of the meiosis in the drone are shown in Fig. 43. None of the usual meiotic prophase stages, such as leptotene or zygotene nuclei, are found; no essential stages seem to intervene between that shown in Fig. 43, A, and that of Fig. 43, B, by which time the definitive chromosomes have appeared. There are sixteen of these as in the spermatogonia, each being conspicuously split longitudinally. An intra-nuclear mitotic figure is formed and the chromosomes congregate at the equator as if for an ordinary metaphase. This, however, is not consummated; the daughter chromosomes do not separate, but the mitotic figure degenerates and the chromosomes become clumped together again, generally at one pole of the elongated nucleus. Although the nucleus does not divide, cell division proceeds, with the result that one of the daughter cells lacks a nucleus. The non-nucleated cell is very much smaller than the other, and of course takes no further part in gametogenesis. Meanwhile the nucleus of the other cell prepares for the second meiotic division, sixteen chromosomes again appearing as in the abortive first division. The second division is carried through in the normal way in so far as the nucleus is concerned, each chromosome dividing into two daughter chromosomes which separate in anaphase. Curiously enough, however, the cell again divides very unequally, though this time both daughter cells receive a nucleus. These of course each contain sixteen chromosomes (though here again they tend to come together in eight pairs—see footnote to p. 91).

In spite of their unequal size, both spermatids begin the series of changes which should convert them into spermatozoa, but it is probable that only the larger one completes the process.

The essential feature of this spermatogenesis is of course the omission of the true meiotic division, by which a further halving of the already haploid group of chromosomes is avoided. In conformity with this, no such stages as leptotene or zygotene nuclei have been described in the male honey-bee. That these stages are really absent is made still more probable by Armbruster's (1913) observation on the spermatogenesis of the solitary bee *Osmia cornuta*. He states that though he specially looked for these stages he failed to find them; the telophase of the last spermatogonial division appears to pass without important intermediate stages into the diakinesis of prophase I.

The unequal cell division of the secondary spermatocyte in the honey-

bee is puzzling, and does not appear to have any significant connection

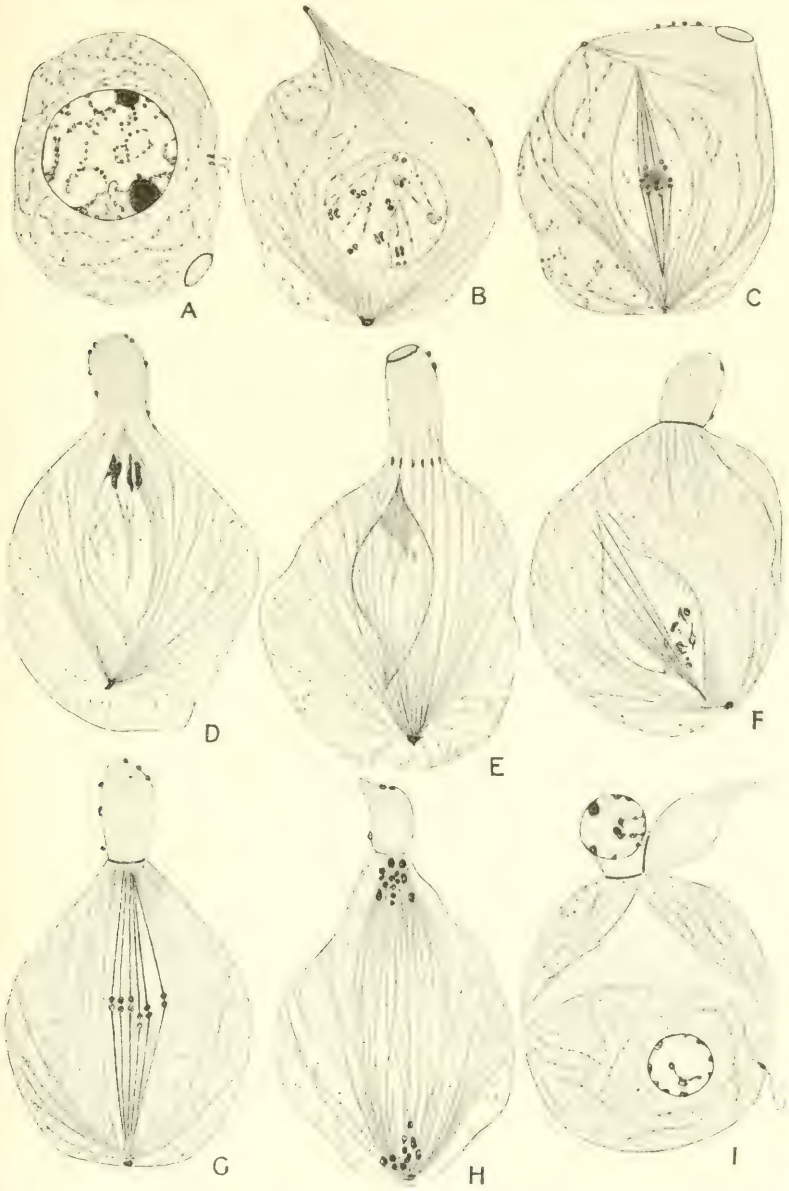


FIG. 43.

The meiotic phase in the drone of the honey-bee (*Apis mellifica*). (After Meves, *A.m.A.*, 1907.) A, primary spermatocyte; B, prophase I.; C, abortive metaphase I.; D, E, degeneration of the mitotic figure, beginning of cell division; F, prophase II. The cell has completed its division into a larger nucleated and a smaller non-nucleated portion. G, metaphase II.; H, anaphase II.; I, cell divided into two unequal spermatids. The non-nucleated cell derived from the first cell division is still attached.

with the peculiarities of the first division. In the hornet (Meves and

Duesberg, 1908) the first division is abortive as in the bee, but the second is normal, resulting in two equal and similar spermatids.

(3) *The Homology of the Meiotic Divisions in Obligatory and Facultative Parthenogenesis*

The above account of the spermatogenesis of the bee shows that it is the first division of the meiotic phase which is omitted, and this is in accordance with the consensus of recent opinion that it is this division which usually brings about the segregation of the homologous chromosomes. It would be of great interest to determine which of the two divisions it is which is omitted in the maturation of eggs preparing for obligatory parthenogenesis, if indeed the single maturation division of these eggs can be homologized with either of the divisions of an ordinary sexual meiosis. It used generally to be assumed that it is the second division which is omitted, but there is little evidence either way. What there is, is perhaps in favour of this view. Weismann and Ishikawa (1887) found that the single polar body produced by the parthenogenetic eggs of Cladocera frequently divides into two or more cells—a feature perhaps more characteristic of the first than of the second polar body of an ordinary oogenesis. Again, Brauer's observation that in *Artemia*, after the first polar body has been formed in the usual way, a more or less abortive attempt is in rare cases made to form another, probably indicates that the single maturation division of the majority of eggs corresponds to the first one of a sexual meiosis. As, however, the second division is still unaccompanied by reduction of chromosome number (like the two divisions without reduction in *Nematus* and *Rhodites*[1]) it is possibly useless to attempt to homologize the two divisions with the first and second divisions respectively of an ordinary meiosis.

Even though it were shown that the single division of obligatory parthenogenesis has all the characteristics (except that of halving the chromosome number) of the normal first meiotic division, this need cause little surprise. For few things are better established in cytology than the fact that in the case of one type of chromosome—the sex chromosomes (Chapter IV.)—the first is the reduction division in some animals and the second in others, even nearly allied species differing in this respect.

(4) *Artificial Parthenogenesis*

It has long been known that eggs which normally only develop after fertilization may be induced by certain agents to develop without fusion

[1] It is perhaps significant that there is found a variation in the chromosome number both in *Nematus* and *Rhodites* somewhat similar to that in the bee (p. 91).

with a spermatozoon. The accurate study of this phenomenon dates from the experiments of O. and R. Hertwig on Echinoderm eggs (1887). The methods have been specially elaborated by Loeb and others, and consist, so far as the eggs of marine animals are concerned, in placing them in sea water of which the chemical composition has been altered in various ways; for details the reader is referred to any of the works cited below.

From the point of view of cytology, two points claim special attention —the achromatic figure and the nucleus. As we have already seen, in the mature egg the centrosome and other parts of the achromatic figure apparently disappear; a new centrosome, which in turn gives rise to the rest of the figure, being provided for the zygote by the spermatozoon. Eggs which have started development under the stimulus of chemical reagents are, however, provided with typical centrosomes and spindle figure, etc. Two alternatives as to the origin of these are possible: (1) that the old egg centrosome does not disappear entirely, but is merely rendered latent and is reawakened to activity by the stimulus which induces the parthenogenesis; or (2) that the centrosomes arise *de novo* in the stimulated egg. Although the first alternative may be true in some cases, it has been demonstrated beyond question that the latter may occur also. Wilson (1901) showed this for *Toxopneustes*. If the eggs of this sea-urchin are subjected to sea water to which $MgCl_2$ has been added, a large number of division centres (*cytasters*) may appear simultaneously in the cytoplasm. Each centre is provided with a centrosome and rays, exactly as in a typical aster. These asters divide again like normal asters, before nuclear division, their division being preceded by that of the centrosome. Both Wilson and M'Clendon (1909) even obtained cytasters in eggs from which the nucleus had been removed. Such enucleated eggs of *Asterias* segmented for several hours, dividing into a large number of irregular blastomeres by the action of these cytasters. We are therefore compelled to conclude that the centrosome and its derivatives, though normally a permanent cell structure derived by division of a previous one, can arise *de novo* in the cytoplasm.

As regards the nuclear cytology of artificial parthenogenesis, we must distinguish between the types of eggs mentioned on p. 73, according as to whether maturation normally takes place before or after entry of the spermatozoon or, in the case of artificial parthenogenesis, the stimulus which takes the place of this.

The simplest case is afforded by those eggs which mature before the entry of the spermatozoon; for example, those of the Echinoidea. These eggs mature in the ovary, and therefore when, after being laid, they are subjected to the stimulus which is to cause them to develop, they are

already haploid. In such eggs, if caused to develop parthenogenetically, the haploid egg nucleus acts in cleavage exactly like the zygote nucleus in a fertilized egg. The resulting embryo is in consequence haploid, as has been demonstrated by many observers, though originally Delage erroneously supposed that the diploid number was restored by an act of auto-regulation (Wilson, *Toxopneustes*, 1901; Hindle, *Strongylocentrotus*, 1911, etc.).

Eggs which do not normally mature until the spermatozoon has entered vary in their reaction to the artificial developmental stimulus. Some, such as the annelid *Thalassema* (Lefevre, 1907), on being subjected to the appropriate chemical stimulus undergo maturation, throwing out

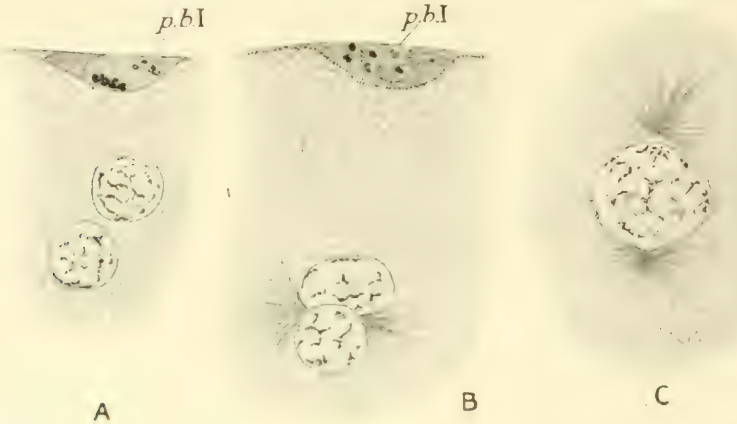


FIG. 44.

Artificial parthenogenesis in *Asterias*. (Buchner, *A.Z.*, 1911.) A, telophase of second maturation division. The second polar body nucleus, instead of being thrust out of the egg, remains close to the egg nucleus. B, the egg and second polar body nuclei have come into contact. Achromatic figure developing. C, the two nuclei have fused.
p.b.I., first polar body.

two polar bodies, as if they had been properly fertilized. The haploid egg nucleus then proceeds to divide as in Echinoids, and a haploid embryo results. In *Asterias*, on the other hand (Buchner, 1911), while the first maturation division is carried through normally and the first polar body is cut off, the second division only proceeds normally as far as telophase. Instead of the outer telophase group being extruded from the surface of the egg in the second polar body, it is retained within the egg and there forms a nucleus lying close to the inner group or egg nucleus (Fig. 44). The egg and second polar nuclei now approach each other again and fuse precisely as if the latter were the male gamete nucleus. Thus the diploid number of chromosomes is restored. The resemblance between this process and the rarer method of maturation of the parthenogenetic egg of *Artemia* described by Brauer (p. 90) is striking.

The immediate cause of the retention of the second polar nucleus within the egg seems to be that the tendency of the chromosomes to form karyomeres (see p. 131) in Echinoderm eggs is accentuated, or rather accelerated, so that the chromosomes begin their telophase metamorphosis before the daughter plates of the second meiotic division have fully separated. Consequently the two daughter nuclei come to lie close together.

The fertilization of Amphibian eggs by spermatozoa which have been injured by radium emanation or by the action of certain poisons, such as strychnine and nicotine, is in a sense intermediate between artificial parthenogenesis and natural fertilization. G. Hertwig (1913) established the, at first sight paradoxical, result that whereas if the eggs of the common toad are fertilized by the spermatozoa of the frog, very few zygotes result and these never develop so far as gastrulation, yet if the frog spermatozoa be injured by subjection to radium emanation before being added to the toad eggs, a large proportion of embryos pass the hatching stage. The interpretation put forward is that in the first case development is prejudiced by the incompatibility of the chromatin of the two animals, while in the second case the frog spermatozoa are so weakened that, though they enter the eggs, they have not the power to fuse with the female pronucleus. Their entry, however, stimulates the eggs to develop. This explanation is borne out by the further investigations of O. Hertwig (1913), who counted the haploid chromosome number in newt larvae which had developed from eggs fertilized by spermatozoa previously subjected to treatment with radium.

These experiments are closely comparable to the cross-fertilizations between Echinoderms, Molluscs, etc., described on p. 161.

Nearly related to artificial parthenogenesis is the phenomenon of *merogony*, the term applied to the fertilization of an egg fragment containing no nucleus. Echinoderm eggs will survive being broken into fragments, one only of which of course can contain the nucleus. The non-nucleated fragments can be fertilized by spermatozoa and then develop into dwarf and haploid, but otherwise normal, larvae. This phenomenon is discussed in greater detail in Chapter VI.

CHAPTER IV

THE SEX CHROMOSOMES

(1) *The Sex Chromosomes in Insects*

In 1891 Henking, working on the spermatogenesis of the Hemipteran insect *Pyrrochoris*, discovered that, contrary to the general rule in other groups of the animal and vegetable kingdoms, the diploid number of chromosomes as determined in the spermatogonia is an *uneven* one, namely, twenty-three.

Consequently, instead of all the spermatozoa having eleven chromosomes as if the diploid number were twenty-two, or all having twelve as happens when it is twenty-four, half the spermatozoa have eleven chromosomes and half have twelve. Taking a large stride in the historical development of the case, we now know that the female *Pyrrochoris* has twenty-four as its diploid number, and hence all its eggs have twelve (Wilson, 1909 *a*).

Now it is obvious that if an egg is fertilized by one of the spermatozoa containing eleven chromosomes, the resulting zygote will have twenty-three, which as we have seen is the diploid number of the male. If on the other hand an egg is fertilized by a spermatozoon with twelve chromosomes, the resulting zygote will have twenty-four, the number of the female. Thus it appears that in these insects the sex of the individual is determined by the nature of the spermatozoon which fertilizes the egg—the eggs being all alike, or indifferent, and the spermatozoa of two kinds in equal numbers, namely, male-producers with eleven chromosomes, and female-producers with twelve chromosomes.

As the odd chromosome of the male was first supposed to be an *additional* one, it was for a time known as the " accessory chromosome " (M'Clung). It is now known, however, that the female has one more chromosome (in these forms) than the male, so that the unevenness of the number in the male is not due to the addition, but to the subtraction of a chromosome. Hence the term accessory is obviously unsuitable. Other terms have been proposed, such as *heterochromosomes* (Montgomery) and *heterotropic* chromosomes (Wilson). The now generally accepted

term of *Sex Chromosomes* is, however, a sufficient and obvious description of them, and by this name they will be called here. Owing to the comparative ease and certainty of the observations, and to the interest due to their relation to sex, a great mass of knowledge of these sex chromosomes has been accumulated in recent years, American cytologists having been particularly active along these lines.

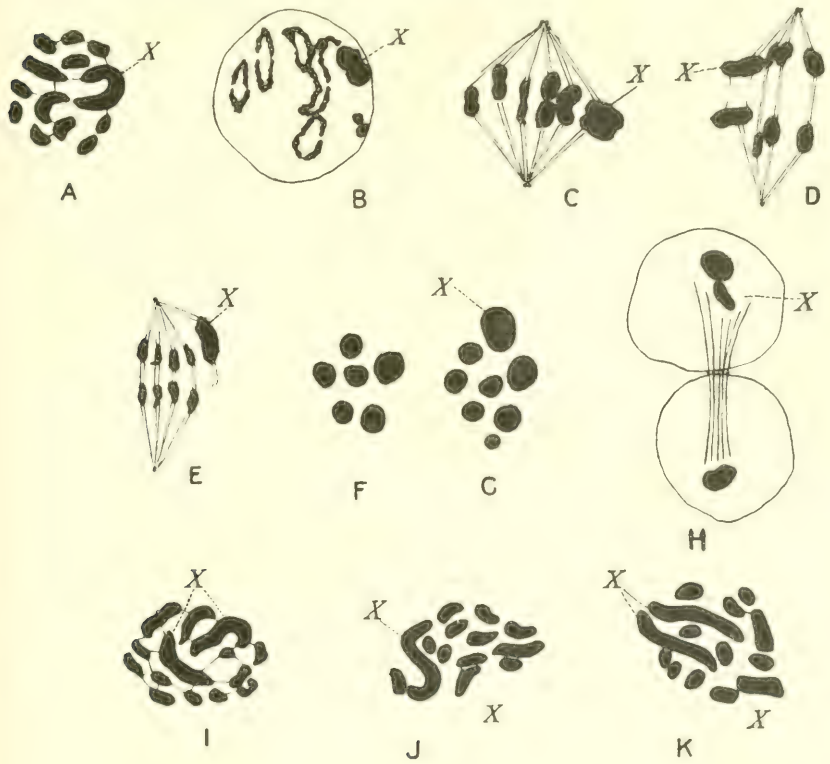


FIG. 45.

The chromosomes of *Protenor belfragei*. (A, I-K, from Morrill, *B.B.*, 1910; B-E, H, after Montgomery, *Trans. Amer. Phil. Soc.*, 1901; F, G, from Wilson, *J.E.Z.*, 1906.) A, spermatogonial metaphase group. Twelve ordinary and one sex chromosome. B, prophase I., ♂; C, metaphase I., ♂; D, anaphase I., ♂; E. early anaphase II. in the ♂. Sex chromosome passing undivided to one pole. F, G, polar views of the chromosome groups at each pole of the spindle in anaphase II. One group with the sex chromosome, the other without. H, late anaphase II., showing one spermatid with the sex chromosome, the other without I, oogonial metaphase group, twelve ordinary and two sex chromosomes; J, chromosome group from a male embryo; K, chromosome group from a female embryo.
X, the sex chromosome.

As a typical example of an animal with sexual dimorphism of chromosomes we may take the Hemipteran insect *Protenor belfragei* (Fig. 45). Its chromosome cycle has been worked out by Montgomery (1901 *b*), Wilson (1906 *b*, etc.) and Morrill (1910).

The spermatogonial chromosome groups (A) show thirteen chromosomes, one being more than twice as large as any of the others. This

is the odd sex chromosome, and on account of its large size it is easily identified at all stages.

B is a late prophase I., showing six bivalents and the sex chromosome, which, being without a homologue, must remain univalent. Corresponding with the difference in valency, the behaviour of the sex chromosome in the two meiotic divisions is remarkably unlike that of the other chromosomes. In metaphase I. the bivalents separate into their constituents in the usual way. The unpaired sex chromosome, on the other hand, divides longitudinally as if the mitosis were somatic, thus anticipating the normal division of the chromosomes in metaphase II. Each spermatocyte II. therefore contains a similar chromosome group of six ordinary chromosomes and one sex chromosome. In metaphase II. the ordinary chromosomes divide longitudinally in the usual way, but the sex chromosome, which has already undergone this division in metaphase I., does not divide again, but passes intact to one or other pole of the mitotic figure (E). Hence the two spermatids formed by each secondary spermatocyte differ in their chromosome equipment, one (G, and upper cell in H) containing the sex chromosome, and the other (F, and lower cell in H) lacking it.

The female *Protenor* has fourteen chromosomes instead of thirteen, there being two of the large sex chromosomes instead of only one. Hence all the mature eggs will have 6, or 5 + X (X standing for the sex chromosome). If now an egg is fertilized by a spermatozoon of the composition shown in F—*i.e.* without the X chromosome—the resulting zygote will have 13 chromosomes thus :

$$\underset{\text{♀}}{(6+\text{X})} + \underset{\text{♂}}{6} = 12 + \text{X} \quad (\text{♂, Fig. J}).$$

If fertilized by the spermatozoon shown in Fig. G the result will be :

$$\underset{\text{♀}}{(6+\text{X})} + \underset{\text{♂}}{(6+\text{X})} = 12 + \text{XX} \quad (\text{♀, Fig. K}).$$

Protenor is an example of the simplest case known, in which the male differs from the female in possessing one chromosome fewer. Several more complicated conditions than this are known, however.

For instance, in *Lygaeus*, another Hemipteran, the male has the same number of chromosomes as the female, each having a pair of sex chromosomes, but while in the female these are equal, like any other pair of homologous chromosomes, in the male they are unequal, one being much smaller than its mate ; this latter is of the same size as those of the female. The relation between the male chromosome groups in *Lygaeus* and *Protenor* may therefore be expressed by the statement that in *Lygaeus* one of the pair of sex chromosomes is reduced, while in *Protenor* one is absent. Or, using the convenient notation now in

general use, X standing for the large sex chromosome and Y for the small one, the chromosome formulae of the two species is as follows (*Lygaeus* having like *Protenor* six pairs of ordinary chromosomes or "Autosomes"):

	Diploid.	Haploid.
Protenor ♀	12 + XX	eggs all 6 + X.
♂	12 + X	spermatozoa 6 + X or 6.
Lygaeus ♀	12 + XX	eggs all 6 + X.
♂	12 + XY	spermatozoa 6 + X or 6 + Y.

The cases are seen to be exactly parallel. There are two classes

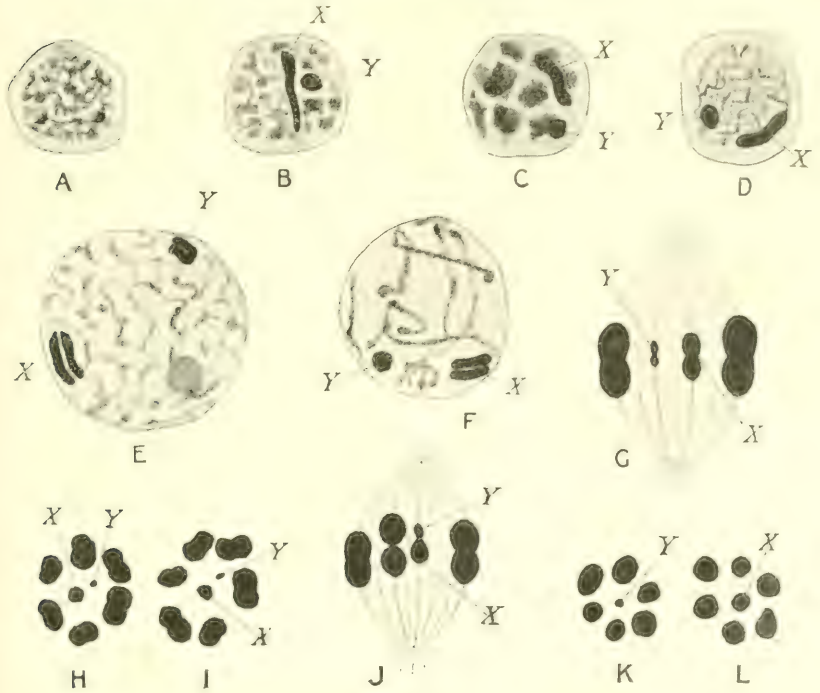


Fig. 46

The chromosomes of *Lygaeus turcicus*. (After Wilson, *J.E.Z.*, 1905 and 1912.) A, spermatogonial telophase; B, later telophase; C, emergence of the massive chromatic bodies in the primary spermatocyte; D, leptotene stage; E, "confused stage"; F, evolution of the bivalents; G, metaphase I.; H, I, daughter chromosome groups of anaphase I.; J, metaphase II., X and Y, now paired to form a bivalent; K, L, daughter chromosome groups from anaphase II.

of spermatozoa in *Lygaeus*—one, the 6 + X form, will on fertilizing an egg produce a female, the other (6 + Y form) will produce a male. The process of meiosis in the male *Lygaeus* is illustrated in Fig. 46. This figure demonstrates the important fact that the sex chromosomes of the male often (though not in all cases; see below) remain compact throughout the whole meiotic prophase, including the leptotene and zygotene stages.

It will be noted in Fig. 46, G, that X and Y are not paired to form

a bivalent like the other chromosomes, but are quite separate from one another, and that each is constricted preparatory to division, as if it were a somatic mitosis. Thus we now find six bivalent ordinary chromosomes and two univalent sex chromosomes (only two of the bivalents are shown in this figure). Hence each anaphase group contains both X and Y. In prophase II. X and Y conjugate to form the unequal bivalent shown in Fig. 46, J (metaphase II.), which results in the two kinds of spermatid chromosome groups shown in K and L.

In still another Hemipteran (*Oncopeltus*, Wilson, 1912) the X and Y chromosomes are so nearly equal that in many individuals no inequality could be demonstrated, though in others a distinct size difference was detected. Even where they are equal the two sex chromosomes are nevertheless easily identified by their compact form throughout the meiotic prophase. This compact phase is, however, by no means a universal feature of the sex chromosomes, and hence the possibility is at once suggested that forms exist in which there are X and Y chromosomes differentiated physiologically, but not visibly distinguishable from each other or from the other chromosomes. Hence the sexual differentiation of chromosomes, which has been demonstrated for a comparatively small number of animals, so far from being peculiar to them, may be a universal characteristic revealed by the lucky accident that such differentiation is in some animals visible by ordinary methods of microscopic technique.

We will now consider some other features of the sex chromosomes.

(2) *Which of the two Meiotic Divisions acts as the Reduction Division for the Sex Chromosomes?*

It is a surprising fact that, in the cases just described, the sex chromosomes divide longitudinally in the first meiotic division, while the second is the actual reduction division, separating the X from the Y (*Lygaeus*) or sending the single sex chromosome into the one spermatid and leaving the other spermatid with no sex chromosome (*Protenor*). In these cases, therefore, the reduction division for the ordinary chromosomes is the first, and for the sex chromosomes the second, meiotic division. What is perhaps even more surprising is that the behaviour of the sex chromosomes varies in this respect in different forms, and sometimes in nearly related species. An example of an insect in which the first division is the differential one is given in Fig. 47.

The following table, compiled from the exhaustive summary of the numbers of chromosomes in the Metazoa given by Harvey (1917), shows how the orders of insects vary in this respect. It will be noticed that most of the species within any order are alike, but in most orders there are one or two exceptions to the general rule. In compiling this table

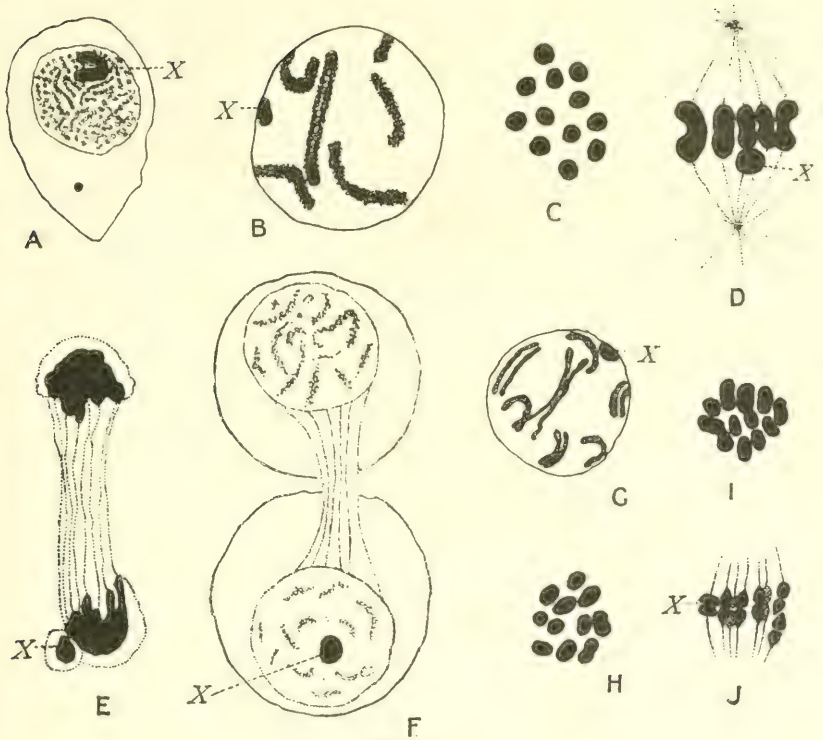


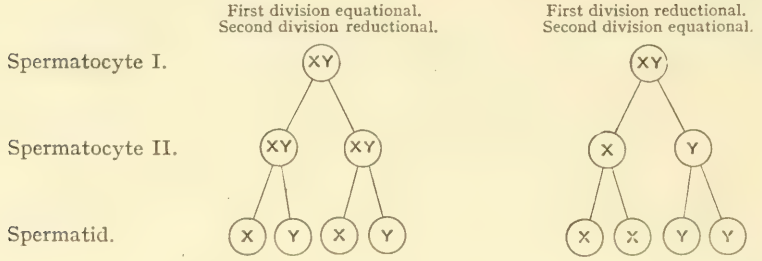
FIG. 47.

The chromosomes of the beetle *Blattella germanica*. (Stevens, *Carnegie Inst. Pub.*, 1905.) A, primary spermatocyte; B, prophase I.; C, metaphase I., polar view, showing twelve chromosomes, X not distinguishable from the others in this view; D, portion of metaphase I., side view, showing X passing undivided to one pole; E, late anaphase I.; F, telophase I., X in one daughter nucleus only; G, prophase II. of the secondary spermatocyte which contains the X chromosome; H, polar view of metaphase II. of secondary spermatocyte lacking the X chromosome; I, similar figure of secondary spermatocyte containing the X chromosome; J, side view of metaphase II., showing the X chromosome dividing.

cases which are doubtful or which present any special complication have been omitted.

GROUP.	Number of Species in which the Reductional Division for the Sex Chromosomes is the	
	First Division.	Second Division.
Neuroptera . . .	1	1
Orthoptera . . .	88	0
Coleoptera . . .	38	2
Diptera	11	0
Hemiptera heteroptera .	2	68
Hemiptera homoptera .	43	1
Total .	183	72

The two different ways of obtaining the same end result—the production of the two types of spermatozoa in equal numbers—may be represented in a diagram as follows :



(3) *Various forms of the X and Y Chromosomes*

A frequent feature of the X chromosome is that it is compound,

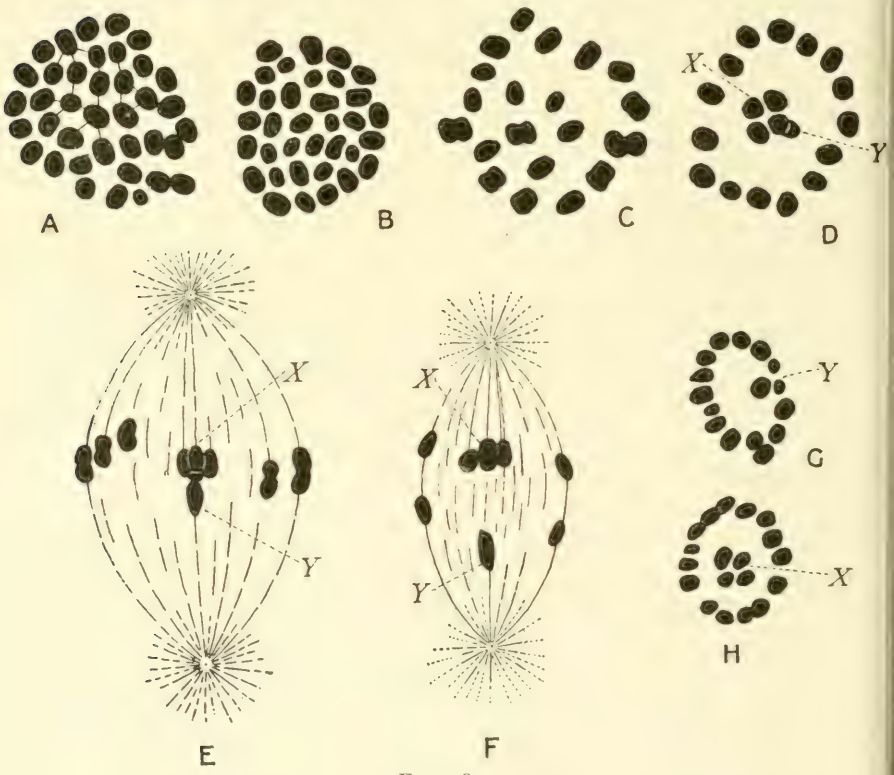


FIG. 48.

The chromosomes of *Gelastocoris*. (Payne, *B.B.*, 1909.) A, ♀ diploid group, $30+2X_4=38$ chromosomes; B, ♂ diploid group, $30+X_4+Y=35$ chromosomes; C, metaphase I., ♂, $15+X_4+Y=20$ chromosomes; D, metaphase II., ♂; the sex chromosomes are in the middle of the ring, the four components of the X chromosome in a group opposed to the single Y chromosome which is partly underneath them; E, metaphase II., ♂; F, anaphase II., ♂; G, H, polar views of anaphase II., ♂—G with the Y chromosome, H with the X group.

consisting of two or more components. These are separate in the somatic or premeiotic nuclei of both sexes, and in all nuclei of the female (with the probable exception of *Phylloxera caryaecaulis*, see p. 117); in the meiotic phase of the male, however, they commonly become associated in a degree varying from merely a more or less close grouping (*Acholla*) to an actual junction (*Syromastes*) (Figs. 48, 49, 50). The Y chromosome is always simple, even in those species where the X is compound. The

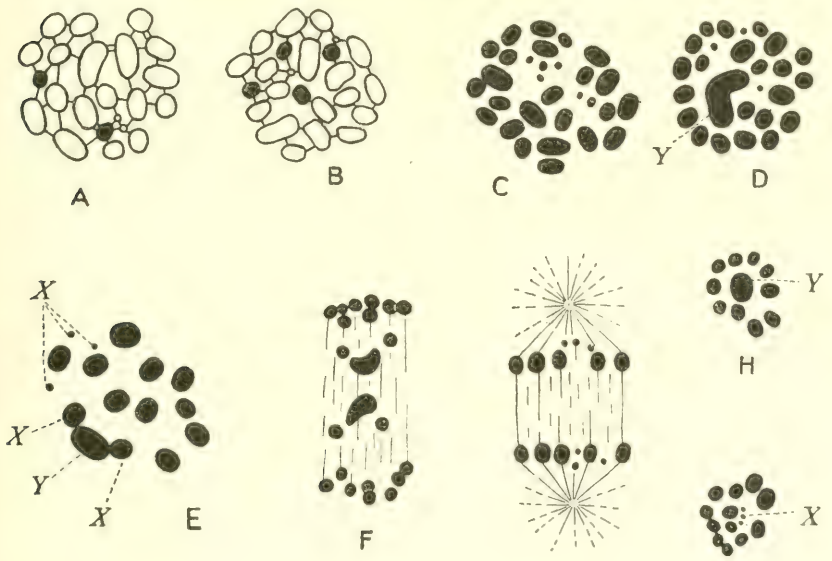


FIG. 49.

The chromosomes of *Syromastes* (A, B) and *Acholla multispinosa* (C-I). (Wilson, *B.B.*, 1909 *a*, and Payne, *B.B.*, 1910.) In A and B the X chromosomes are blackened. A, ♂ diploid group, $20+X_2=22$ chromosomes; B, ♀ diploid group, $20+2X_2=24$ chromosomes; C, ♀ diploid group, $20+2X_5=30$ chromosomes. The three pairs of small X elements are easily seen, the other two pairs are not at this stage distinguishable. D, ♂ diploid group, $20+X_5+Y=26$ chromosomes; E, metaphase I., ♂, polar view. The Y element is characteristically in association with the two largest of the X elements. F, G, anaphase I., side view cut in two sections. The section in F contains the Y and the two large X elements, and that in G the three small X elements. H, I, anaphase II., showing the results of the segregating division.

following examples illustrate the range of variation in the constitution of the sex chromosome (Fig. 51):

(A) No Y chromosome present.

1. X Chromosome single (*Protenor*).
2. X ,, double (*Syromastes*).
3. X ,, pentad (*Ascaris lumbricoides*).

(B) Simple Y chromosome present.

1. X Chromosome single (*Lygaeus, Oncopeltus*).
2. X ,, double (*Fitchia*).
3. X ,, triple (*Prionidus*).
4. X ,, quadruple (*Gelastocoris*).
5. X ,, pentad (*Acholla multispinosa*).

The last case is perhaps the most remarkable, the X group consisting of two large and three very small constituents (Figs. 49, 50).

The chromosome equipment of the various examples is as follows (omitting *Protenor* and *Lygaeus*, which have already been dealt with): n' stands for the haploid number of ordinary chromosomes, and the

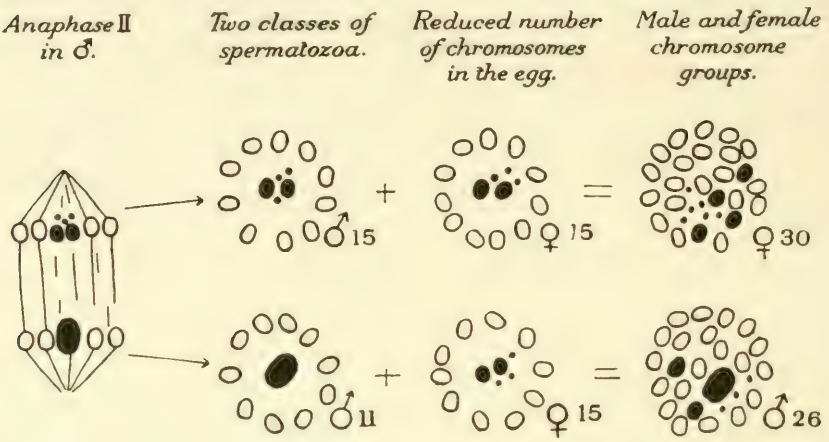


FIG. 50.

Diagram of the results of fertilization in *Acholla multispinosa*. (Payne, *B.B.*, 1910.) The sex chromosomes shown in black.

number of components of the X chromosome is represented by the suffixed numeral.

	Male. Diploid Group.	Gametes.	Female. Diploid Group.	Gametes.
Syromastes (Wilson, 1909 *a*), $n'=10$	$2n'+X_2$	$\begin{cases} n'+X_2 \\ n' \end{cases}$	$2n'+X_2X_2$	$n'+X_2$
Ascaris lumbricoides (Edwards, 1910), $n'=19$	$2n'+X_5$	$\begin{cases} n'+X_5 \\ n' \end{cases}$	$2n'+X_5X_5$	$n'+X_5$
Fitchia (Payne, 1909), $n'=12$	$2n'+X_2Y$	$\begin{cases} n'+X_2 \\ n'+Y \end{cases}$	$2n'+X_2X_2$	$n'+X_2$
Prionidus (Payne, 1909), $n'=11$	$2n'+X_3Y$	$\begin{cases} n'+X_3 \\ n'+Y \end{cases}$	$2n'+X_3X_3$	$n'+X_3$
Gelastocoris (Payne, 1909), $n'=15$	$2n'+X_4Y$	$\begin{cases} n'+X_4 \\ n'+Y \end{cases}$	$2n'+X_4X_4$	$n'+X_4$
Acholla (Payne, 1909), $n'=10$	$2n'+X_5Y$	$\begin{cases} n'+X_5 \\ n'+Y \end{cases}$	$2n'+X_5X_5$	$n'+X_5$

Thus in *Acholla* the female group has four more chromosomes than the male, the actual numbers being 30 and 26.

(4) *Behaviour of the Sex Chromosomes during Syndesis and the Meiotic Prophase, and outside the Meiotic Phase*

As was illustrated in the case of *Lygaeus*, the sex chromosomes in the male retain a compact form, while the other chromosomes are in

the linear or diffuse stages characteristic of syndesis and other phases of the meiotic prophase.

Since where Y is absent it is obvious that the X chromosome, having no mate, cannot go through the process of syndesis like the other chromosomes, the natural conclusion is that the visible difference between the behaviour of the ordinary and sex chromosomes is the expression of the fact that the latter is not taking part in syndesis. Even when a Y chromosome is present, both it and the X chromosome often remain

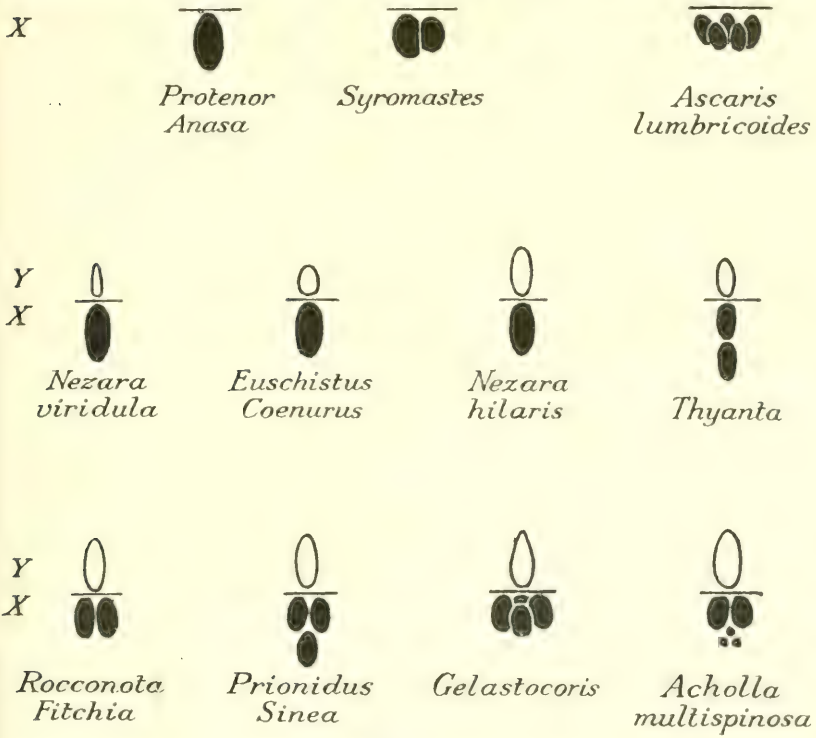


FIG. 51.

Diagram of the relations of the sex chromosomes in various animals. (After Wilson, *A.m.A.*, 1911.)

compact throughout the meiotic prophase, and no evidence of syndesis between them can be found. The absence of conjugation is not surprising in view of the physiological differentiation between the X and Y chromosomes which must underlie the frequent difference between them in regard to size and composition. Moreover, as we shall see later, the facts of sex-linked inheritance (p. 179) lead to the conclusion that the Y chromosome is inert.

If the absence of syndesis be the explanation of the compactness of the X and Y chromosomes during the zygotene stage in the male, it

follows that in the female, which has two equivalent X's, these should participate in syndesis, and that therefore they should not have the compact form noticeable in the male at this stage. While the female meiotic phase has not been so carefully studied as the male, the facts, so far as they are known, are in general accordance with this hypothesis. In the female syndetic and meiotic prophase nuclei in, *e.g.*, *Anasa*, *Harmostes*, *Alydus*, *Euschistus*, *Coenus* and *Podisus* among Hemiptera (Wilson, 1906 *b*) and *Ancyracanthus* in Nematodes (Mulsow, 1913), the sex chromosomes are as extended and diffuse as the others, though they are compact in the corresponding stage in the male meiosis of these species.

Another fact bearing out the same view is, that the X and Y chromosomes are not usually compact in the oogonial, spermatogonial and somatic prophases, even in those species where they are so in the male meiotic prophase, *e.g.*, *Anasa*, *Harmostes*, *Alydus*, *Euschistus*, *Coenus* and *Podisus* (Wilson, 1906 *b*), *Archimerus*, *Anasa*, *Protenor*, *Chelinidea* (Morrill, 1910), *Euschistus* (Montgomery, 1911).

A more striking example is afforded by the hermaphrodite generation of *Ascaris nigrovenosus* (p. 113). This is of the female build of body, but produces both eggs and spermatozoa in its reproductive organ. The diploid group is $2n' + XX$, where $n' = 5$. The primitive germ cells, at first all alike, differentiate later into oogonia and spermatogonia. In the oogenesis the XX pair acts exactly like the other bivalents. In the male meiotic prophase, however, one of the X's may be said to take on the characteristics of a Y chromosome. This XY pair condenses out sooner than the other chromosomes, though in the female meiosis, where both sex chromosomes retain their X character, they do not do so.

In some species (*Aphis*, Fig. 53) the X chromosome is filiform like the others during syndesis, although, there being no Y chromosome, it cannot be participating in this process. Even here, however, the X chromosome is clearly distinguishable from the others in the diplotene and later stages, being single or inconspicuously split for the second meiotic division, unlike the conspicuously double bivalents formed by syndesis of the other chromosomes (Fig. 53, E, F, G).

Taking everything into consideration, therefore, it is hard to escape from the conclusion that in these species (by far the majority) where the sex chromosomes remain compact during syndesis and the rest of the meiotic prophase in the male, this is an expression of the fact that they are not themselves engaged in the act of conjugation. It must be admitted, however, that compactness at a time when the other chromosomes are linear or diffuse is not always due solely to the fact that the one set is engaged in syndesis and the other not, for in some species the same difference between the consistency of the sex and ordinary chromosomes is found outside the meiotic phase. The case of *Aphis* also shows

that the failure to conjugate does not necessarily result in the sex chromosomes remaining compact at this period.

Wilson (1912) has made some interesting observations which possibly indicate a slight tendency to syndesis on the part of the sex chromosomes of the male, which, however, does not culminate in actual conjugation. In the case of *Oncopeltus* he examined a hundred nuclei in syndesis. In seventy-five of these X and Y were entirely separate ; in twenty-five they were side by side, just in contact. In not one case, however, out of hundreds examined, were they fused or even flattened together. In *Lygaeus* the tendency for X and Y to come together is stronger, for out of a hundred synizetic nuclei forty-five showed them separate and fifty-five showed them in contact (thirty-six times end to end, and seventeen times side by side), often pressed together, but never fused into a single body. Again, in *Ascaris nigrovenosus* (p. 113) Schleip found that in the metaphase I. of the spermatogenesis in the hermaphrodite the XY pair were usually separate, but in one individual were always, and in others rarely, united into a bivalent like the other chromosomes.

Another very common, but not universal, feature of the sex chromosomes is their tendency to travel to the poles of the spindle either in advance of, or more often behind, the other chromosomes in the anaphase of the reduction division. Here again this distinction is not found in the corresponding phase in the female, except in the special case of the male-producing parthenogenetic egg of *Phylloxera* (p. 119). Examples of forms in which the X chromosomes lag in the male meiosis but not in the female are *Anasa*, *Archimerus* (Wilson, 1905 ; Morrill, 1910) ; *Aphis* and *Phylloxera* (Morgan, 1909 ; von Baehr, 1912).

(5) *Sex Chromosomes in Animals other than Insects*

So far, except for occasional references, we have confined ourselves to the consideration of the sex chromosomes of insects, since this group by itself serves to illustrate all the main variations. They have, however, been found in many other forms, in some of which they can be observed as clearly as in the insects already described, while in others the evidence for their presence is not so satisfactory.

Nematodes provide some very clear cases, the simplest being *Ancyracanthus cistidicola*, a parasite in the swim-bladder of various fresh-water fish (Mulsow, 1913). The male diploid group is eleven (10 + X) and the female twelve (10 + XX). During the diffuse stages of the male meiotic prophase the X chromosome retains its compact character. Metaphase I. effects the differential division, the secondary spermatocytes having six and five chromosomes respectively. In the second meiotic

division X divides equationally in those nuclei where it is present. The four young spermatids formed from each primary spermatocyte remain attached together, and it is easy to verify the fact that two have five chromosomes and two have six. The chromosomes remain individually distinguishable even in the ripe spermatozoon, so that fertilization of the eggs by the two different kinds of spermatozoa can be traced. The whole case can be followed as clearly as in a text-book diagram.

Similar simple conditions (presence of a single X chromosome, no Y chromosome) have been described by Gulick in five species of *Heterakis* and *Strongylus* (1911).

Conditions are more complicated in the genus *Ascaris*. In *A. lumbricoides* Edwards (1910) found that the X element consists of a group of five chromosomes which pass undivided to one pole in anaphase I. There is no Y chromosome, and two classes of spermatozoa are thus formed, one with nineteen and the other with twenty-four chromosomes.

In *A. Canis* (Walton, 1918) the X group consists of six chromosomes, the two types of spermatozoa having respectively twelve and eighteen chromosomes; all the mature ova have eighteen.

The problem of the sex chromosome in *A. megalocephala* has been attacked by several workers. In this species the X chromosome appears to be single, and there is no Y. As a rule the X element is attached to one of the larger chromosomes, and hence difficult or impossible to recognize. In rare individuals, however, it is a separate element. In the ♀ the two X's can also be recognized, again generally, but not always attached to the larger chromosomes. For further information and literature in regard to this species the reader is referred to Frolowa (1913).

It is noteworthy that in Nematodes the division which is differential for the sex chromosomes varies as it does in insects. In most species so far described this is the first division, but in *A. nigrovenosus* (described below) it is the second.

Among Vertebrates, sex chromosomes have been studied principally in Birds and Mammals.

Sex chromosomes have been described in many species of the latter group, but in most cases the evidence cannot be considered quite conclusive. The clearest example is perhaps the Opossum (Jordan, 1912).[1] A summary of the work done on mammalian sex chromosomes will be found in Jordan (1914).

[1] The most circumstantial account of sex chromosomes in a mammal is probably that of Wodsedalek (1913) for the pig. Until, however, some means is found of reconciling the extraordinary discrepancy between this author's account of the chromosomes of the spermatogonia of this animal and that given by Hance (1918 *a*), it is difficult to appraise the value of the evidence.

(6) *Cases where the Differential Sex Chromosome is present in the Female*

In all the cases described so far it is the spermatozoa which carry the determining factor for sex; the male produces two kinds of gametes, male-producing and female-producing, and is said therefore to be *heterozygous* for sex. The eggs, on the other hand, are all similar, or indifferent, and so the female is said to be *homozygous*. So far there is comparatively little cytological evidence of the condition of the sexes being reversed in this respect. In 1909 Baltzer described such a case in Echinoderms, but in 1913 he withdrew the statement, and meantime Tennant (1911) found that the sea-urchin *Hipponoe* conforms to the usual rule, that the male produces the two kinds of sex-determining gametes, while the female gametes are all alike.

The Lepidoptera and Birds present specially interesting features in this connection, since experiments on sex-linked inheritance (see p. 180) require that the female should be heterozygous and the male homozygous.

Unfortunately neither of these groups are favourable objects for cytological study. In the Lepidoptera the number of chromosomes is usually very high, and in Birds the conditions for observation seem especially difficult, chiefly owing to a pronounced tendency of the chromosomes to become agglutinated together.

In the latter group Guyer (1909) described an X chromosome in the male fowl and guinea-fowl, resulting in the formation of the usual two classes of spermatozoa, and thus apparently indicating *male* heterozygosity in birds as in insects. These observations have been questioned by Boring and Pearl (1914), but Guyer's subsequent observations (1916) confirm his original description and at the same time show that it may not be incompatible with homozygosity of the male and heterozygosity of the female. It appears that in the somatic tissues of the male fowl there are eighteen chromosomes, of which sixteen are rod-shaped and two are U's. The latter are the sex chromosomes, the chromosome formula being therefore $2n' = 16 + XX$. In the female only one of the U's is present, the formula being $2n' = 16 + X$. In the male meiotic prophase all the chromosomes pair, giving nine bivalents, one of which is larger than the others and curved; this is the X bivalent. At metaphase I. this fails to dissociate, passing undivided to one pole, so that the secondary spermatocytes contain either eight univalents or eight univalents and the X bivalent. The latter dissociates in metaphase II., so that two kinds of spermatids are produced with the chromosome formulae 8 and $8 + X$ respectively. Guyer gives reasons for believing that the spermatids without the X chromosome degenerate without metamorphosing into spermatozoa, though this could not be actually demonstrated. The meiosis of the female was not worked out, but the

fact that she possesses an unpaired sex chromosome in her somatic cells leads to the assumption that she produces two classes of eggs, of formulae 8 and 8 + X. All the surviving spermatozoa being probably of the 8 + X class, it follows that the female is heterozygous and the male homozygous for sex, as the phenomena of sex-linked inheritance in birds demand. The cytological evidence, however, is in need of confirmation from other species.

In the Lepidoptera the cytological evidence of female heterozygosity is stronger. The simplest case so far known is that of *Talaeoporia tubulosa* (Seiler, 1917). In metaphase I. of the female meiosis there are thirty chromosomes. In anaphase, one of these lags behind the rest, but ultimately gets included in one or other of the telophase groups, *i.e.* in the nucleus either of oocyte II. or of the first polar body. This chromosome has evidently not divided, since the group which receives it has 30 chromosomes, while the other has 29. In metaphase II. all the chromosomes appear to divide. Thus two classes of eggs are produced, one with 29 and the other with 30, or 29 + X, chromosomes. In the male, 30 (bivalent) chromosomes appear in metaphase I., and all behave alike, so that all the spermatozoa have 30 chromosomes. Two types of embryos were also found, one with 59 chromosomes, presumably females (58 + X), and the other with 60, or 58 + XX chromosomes, which are presumably males.

It is interesting that in the anaphase of the first polar division the unpaired X chromosome seems to go rather more frequently into the polar body than into the oocyte nucleus, and that this corresponds with the fact that females are more numerous than males in this species.

In *Phragmatobia fuliginosa* (Seiler, 1913) the heterozygosity of the female is expressed in an unusual manner. The metaphase I. figures of the two sexes are alike, containing twenty-eight bivalents, one of which is very much larger than the others; this large one divides normally in the male, but in the female the two chromosome groups formed in anaphase I. differ from one another. One of them contains the expected twenty-eight chromosomes, as in the secondary spermatocytes, but the sister group contains twenty-nine chromosomes; moreover, in this group the large chromosome, though still much larger than any of its fellows, is not so large as its mate in the group of the twenty-eight chromosomes at the other end of the spindle, and Seiler concludes that the twenty-ninth chromosome has been produced by the breaking up of the very large chromosome into a large and a normal-sized one. Thus there is a physiological difference between the members constituting the large bivalent in the female, for in anaphase I. one of them breaks up into two and its homologue does not. This can clearly be compared

with the distinction between the X and Y pair in the males of many other animals.

In *Abraxas grossulariata* Doncaster (1914 *b*) found that whereas 56 is the typical somatic number both for males and females, yet females of certain strains have only 55 chromosomes and produce two classes of eggs with 27 and 28 chromosomes respectively. It is not an unreasonable hypothesis, therefore, that the females with 56 chromosomes have an X chromosome paired by an inert Y, the latter having been lost in the strain with 55 chromosomes.

(7) *Some Special Life Histories*

The apparently simple and obvious relation between the presence or absence of the X chromosome in one of the gametes and the sex of the resulting zygote in the examples already dealt with raises at once some interesting questions regarding certain cases of reproduction of a different type. What is the condition of the sex chromosomes, for instance, in a case of alternation of bisexual and hermaphrodite generations, such as is found in *Ascaris nigrovenosus*; or where a female produces parthenogenetically both males and females, the sex therefore being determined by something other than the spermatozoon (Cladocera, Aphids, etc.); or again where all fertilized eggs develop into females (Aphids, Apidae, etc.), males only developing from unfertilized eggs?

The chromosome cycle in a number of these life histories has been worked out.

(*a*) *Ascaris nigrovenosus* (Fig. 52).—The life history of this species exhibits an alternation of hermaphrodite and bisexual generations, the former being parasitic in the lung of the frog, while the latter is free-living. The chromosome cycle in this species was worked out independently by Boveri (1911) and Schleip (1912), the two accounts agreeing in all important points.

In the free-living bisexual generation the male has eleven chromosomes and the female twelve; the male produces two kinds of spermatozoa, one with five and one with six (5 + X) chromosomes, while all the eggs have six (5 + X). Now all the animals developing from the zygotes formed by the union of these gametes are hermaphrodites, which are of the female form of body and have twelve chromosomes (10 + XX). It is therefore to be supposed (though this matter was not actually determined by observation) that the spermatozoa without the X chromosomes do not take part in fertilizing the eggs (cf. *Aphis* and *Phylloxera*, below).

The hermaphrodites produce eggs and spermatozoa in the same reproductive organ, and up to the onset of the meiotic phase the cells

(primary oocytes) which will give rise to eggs are indistinguishable from

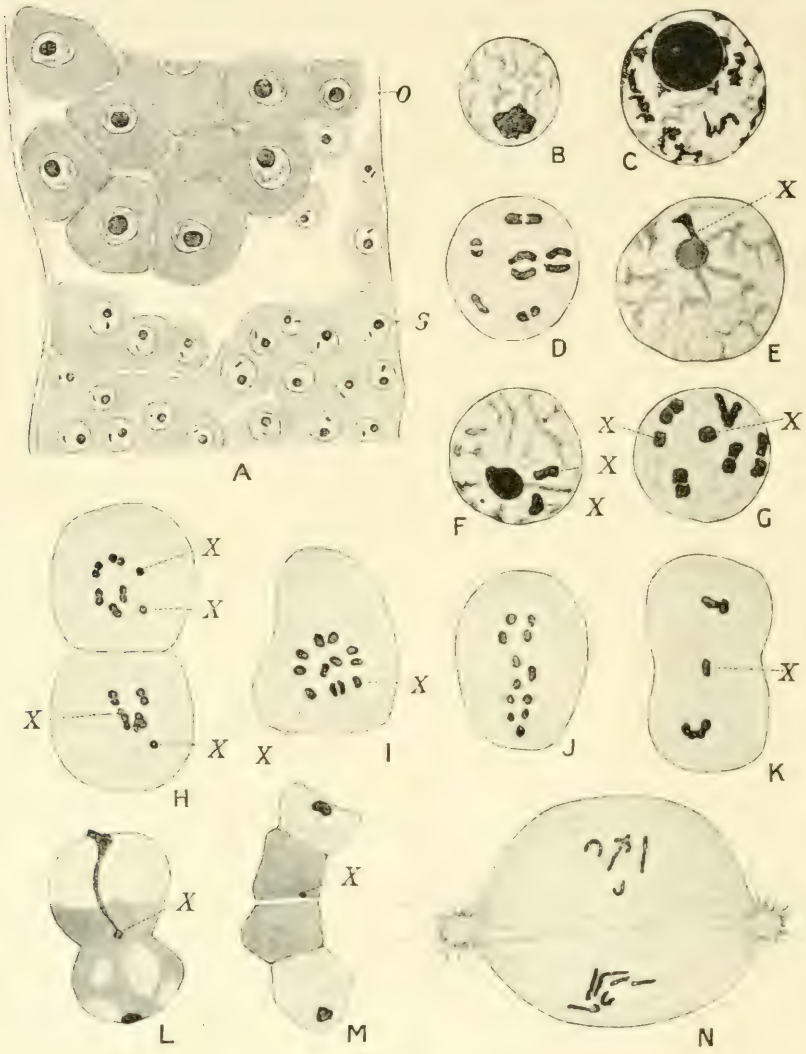


FIG. 52.

Spermatogenesis in *Ascaris nigrovenosus*. (After Schleip, *A.Z.*, 1912.) A, portion of gonad after the primitive germ-cells have differentiated into oocytes and spermatocytes. In both, note the plasmosome, and in the spermatocytes the single sex chromosome (cf. E). B, oocyte just before synizesis; C, oocyte during growth period. In both B and C note absence of compact sex chromosome. D, metaphase I., ♀, six bivalents; E, spermatocyte I.; one sex chromosome has condensed out; F, later stage, both sex chromosomes condensed; G, late prophase I.; H, two secondary spermatocytes; I, metaphase II.; J, anaphase II., 5+X chromosomes passing to each pole; K, late anaphase II., one X chromosome lagging behind; L, M, two pairs of spermatids. In one of each the sex chromosome has been left out of the nucleus. N, first cleavage division of an egg fertilized by a spermatozoon, without the X chromosome, and which will therefore develop into a male. The groups of chromosomes from the ♂ and ♀ gametes still separate, showing the five chromosomes of the one and the six of the other.

o, primary oocytes; *s*, primary spermatocytes; X, the sex chromosomes; the distinction between X and Y made in the text is not shown here.

those (primary spermatocytes) which will give rise to spermatozoa. All

of course contain 10 + XX chromosomes. From now onwards, however, the sex chromosomes of the two kinds of cells behave differently.

In the oogenesis there is nothing noteworthy, all the chromosomes behaving alike, and all the mature eggs possessing six (5 + X) chromosomes.

In those cells which are going to give rise to spermatozoa, however, and which may therefore now be called primary spermatocytes, one of the X chromosomes undergoes a change which may perhaps legitimately be expressed by saying that it turns into a Y chromosome. This chromosome condenses out of the diffuse stage sooner than any of the others (Fig. 52, E), its mate, the remaining X chromosome, following soon after (Fig. 52, F). From now onwards the meiotic phase proceeds in the typical manner for an animal with an XY pair, the second division being the differential one so far as they are concerned.

Two classes of spermatids are formed, one with 5 + X, the other with 5 + Y chromosomes. Of each pair of spermatids one (of the formula 5 + X) develops in the usual way into a spermatozoon containing six chromosomes. In the other, however, the Y chromosome fails to enter into the nucleus, but remains outside in the cytoplasm, to be ultimately cast off with the excess cytoplasm (cytophore) when the ripe spermatozoon is freed. Thus two classes of spermatozoa are formed, one with 5 and one with 5 + X chromosomes.

It should be noted that Boveri found the process less regular than this, but with the same end result—namely, the same two classes of spermatozoa, in which five and six chromosomes can be counted respectively.

The conjugation of these spermatozoa with the ova brings us back to our starting-point—the bisexual generation, the males of which have eleven and the females twelve chromosomes.

(*b*) *Aphids and their Allies.*—The eggs laid in autumn are fertilized and in the spring hatch into females, which reproduce parthenogenetically (with one maturation division and no reduction of chromosomes). After a lapse of one or more parthenogenetic generations, sexual forms (*i.e.* males and sexual females) are produced ; copulation takes place, fertilized eggs result, and the life-cycle is complete.

Here the double problem arises :

(1) How is it that all fertilized eggs produce females only ?

(2) What determines the sex of the individual developed from an unfertilized egg, and which is sometimes male and sometimes female ?

The answer to the first problem is very clearly given by von Baehr (1912) for *Aphis saliceti*. In this species the diploid formula for the female is 4 + XX and for the male 4 + X. Examination of spermatogenesis (Fig. 53) shows that the X chromosome does not divide, but passes

intact to one pole, so that in anaphase I. there are three chromosomes

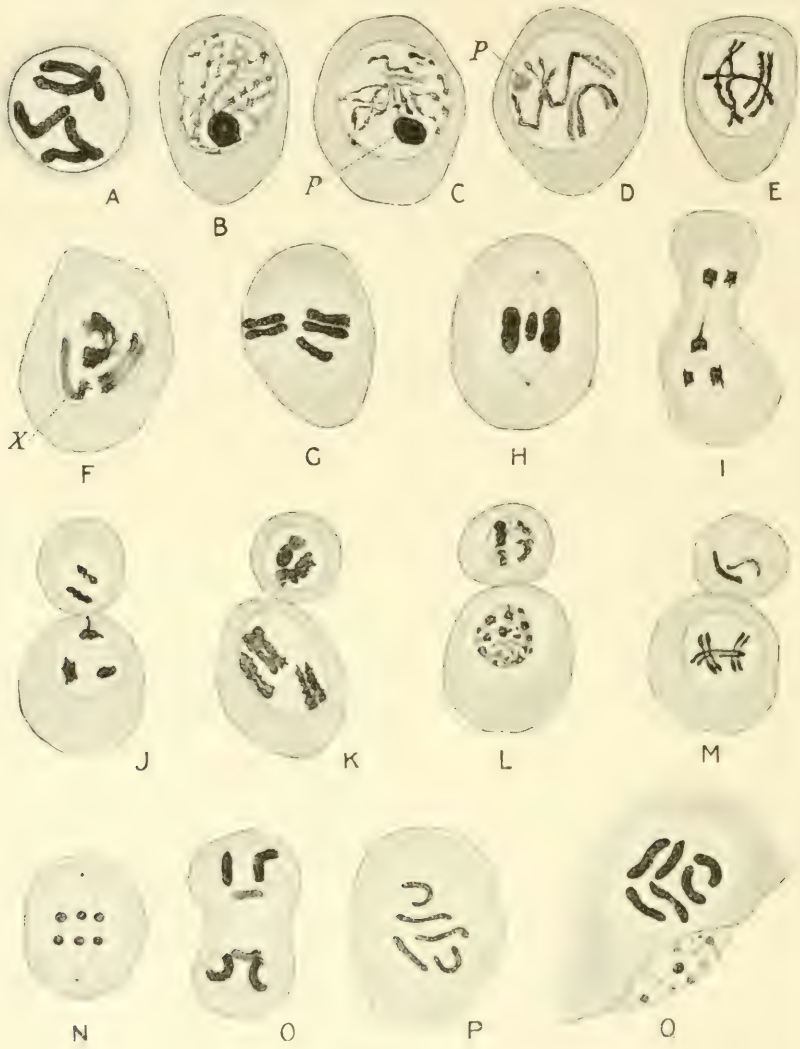


FIG. 53.

The chromosomes in the life-cycle of *Aphis saliceti*. (After von Baehr, *L.C.*, 1912.) A, spermatogonial prophase; B, primary spermatocyte, beginning of the meiotic phase; C, D, E, F, G, evolution of the definitive chromosomes. Note the two bivalents and the single X chromosome. H, metaphase I.; I, anaphase I.; J, K, telophase I. All the chromosomes now alike, all being univalent and split in preparation for the second division. L, resting stage between the two divisions; M, prophase II.; N, metaphase II.; O, telophase II. (N and O illustrate the case of the spermatocyte II., which contains the X chromosome.) P, cell from a segmenting egg with five chromosomes (*i.e.* a ♂); Q, cell from an embryo with six chromosomes (*i.e.* a ♀).

p, plasmosome; X, the sex chromosome.

(2 + X) at one pole and only two chromosomes at the other, in the familiar manner. When cell division takes place, however, this is unequal,

the cell (secondary spermatocyte) containing the X chromosome being larger than the other one and, moreover, receiving the whole of the chondriosomes. The larger cell proceeds to the second meiotic division in the usual way, the X chromosome dividing this time so that both the resulting spermatids have an X chromosome. On the other hand, the smaller spermatocyte II. without the X chromosome proceeds as far as prophase II. (Fig. 53, M), but degenerates without completing the second division. In other words, only the one class of spermatozoon, namely, the X-bearing or female-producing ones, are formed. Hence it is clear why all fertilized eggs develop into females.

(2) The second problem is not completely cleared up by von Baehr's account for *Aphis*. The same individual gives rise parthenogenetically to both males and females, so that presumably the mature egg is sometimes left with five chromosomes (male-producers) and sometimes with six (female-producers); indeed, segmenting eggs and embryos are found to have sometimes six and sometimes five chromosomes in their nuclei. The mechanism by which one chromosome is eliminated in the formation of the male-producing egg could not, however, be determined in this animal. The elimination of the second X chromosome during the maturation of the male-producing eggs was, however, observed in the following case.

Phylloxera (Morgan, 1909, 1915 *a*; Fig. 54). The life history of this genus is the same in principle as that of *Aphis*. All fertilized eggs give rise to females (stem mothers) which hatch in the spring. These produce, parthenogenetically, other females which in turn produce parthenogenetically males and sexual females. The eggs which will develop into males are smaller than those which will develop into females. In *Phylloxera caryaecaulis* all the daughters produced (parthenogenetically) from one stem mother produce the same kind of egg—*i.e.* all are either male-producing or female-producing. In the case of *P. fallax* both kinds of daughters appear to be produced from the same stem mother, and perhaps both kinds of sexual eggs from the same daughter.

Taking the case of *P. fallax*, which presents fewest complications (conditions in *P. caryaecaulis* being the same in principle), the diploid number is twelve in the female and ten in the male, the X chromosome consisting of two components. The male may therefore be represented by the formula $8 + X_2$ and the female by $8 + X_2X_2$. The two components of the X chromosome act as a single compound chromosome as in *Syromastes*, etc.

Spermatogenesis proceeds in the same way as in *A. salicti*; in anaphase I. the two X components pass intact into one secondary spermatocyte, which is larger than its sister cell, and alone proceeds to the second division; thus all spermatozoa are female-producers.

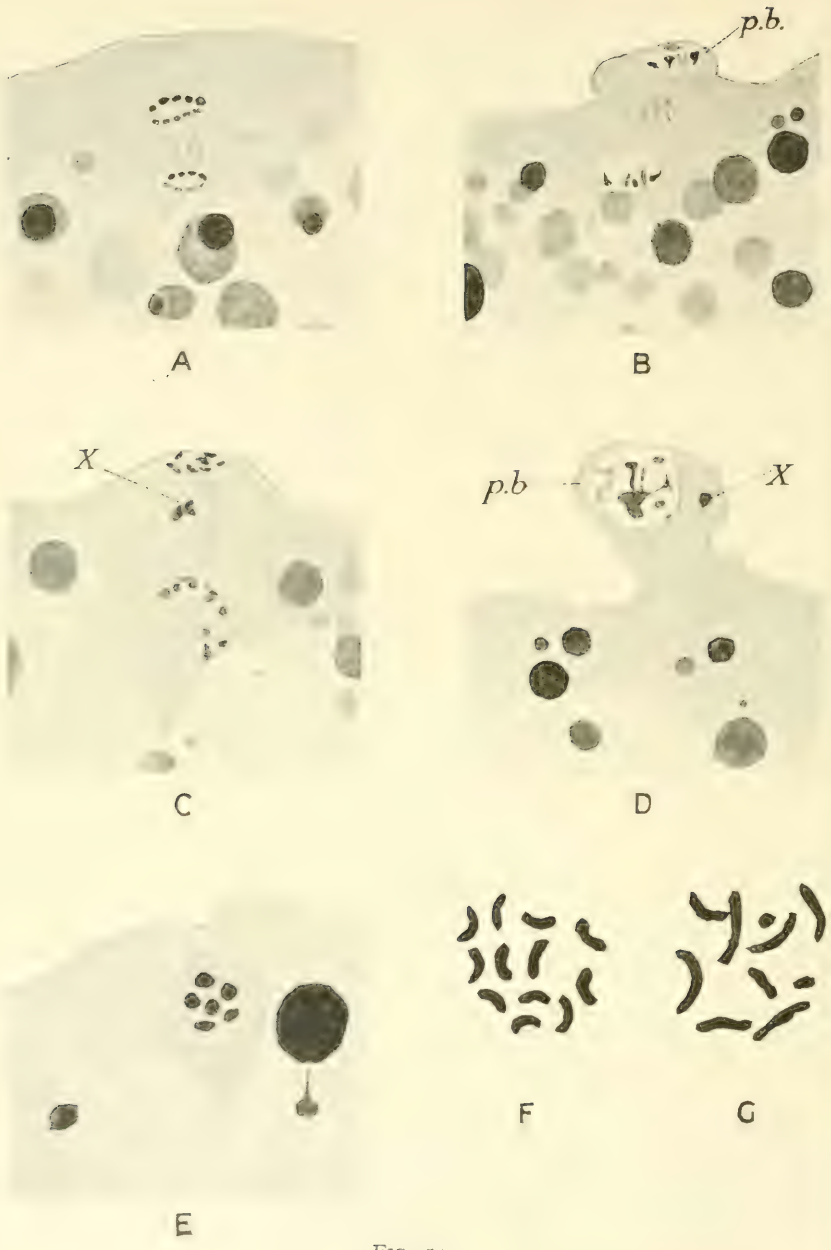


FIG. 54.

The chromosomes in the life-cycle of *Phylloxera fallax*. (After Morgan, *J.E.Z.*, 1909, and 1915 *a*.) A, B, polar body formation in a ♀-producing parthenogenetic egg: all the chromosomes retained; C, D, polar body formation in a ♂-producing parthenogenetic egg, showing elimination of the X chromosome; E, metaphase I., sexual egg; F, chromosome group from a ♀ embryo, twelve chromosomes; G, chromosome group from a ♂ embryo, ten chromosomes.

p.b. polar body; X, the (double) sex chromosome.

An analogous process is found to take place in the male-producing eggs. It will be remembered that there are three kinds of eggs :

(1) The sexual eggs, which need fertilization and which all develop into females. In them meiosis takes place in the usual way, and the mature egg is left with $4 + X_2$ chromosomes. When these are fertilized by spermatozoa, which, as we have just seen, all have the chromosome formula of $4 + X_2$, all the resulting zygotes are $8 + X_2X_2$, *i.e.* females.

(2) Parthenogenetically developing eggs which are going to develop into females. These produce only one polar body without reduction of chromosomes, and at the single maturation division all the chromosomes divide as at a somatic mitosis ; the ripe egg (and also the polar body) is therefore left with $8 + X_2X_2$ chromosomes.

(3) Parthenogenetically developing eggs which are going to develop into males. Again only one polar body is produced, but at the single maturation division one X_2 chromosome is left behind in the anaphase and does not enter into the mature egg nucleus. This is consequently left with ten chromosomes of composition $8 + X_2$. This is shown in Fig. 54, C, D, where an X_2 chromosome (plainly double in Fig. 54, C) is seen left behind when the groups of chromosomes separate at anaphase. The dark body in the cytoplasm which is being nipped off with the polar body in Fig. 54, D, is presumably this double X chromosome.

A very important point for the general theory of the sex chromosomes is the fact that the sex of the individual which is going to develop from the parthenogenetic egg is in these cases determined *before* the distribution of the sex chromosomes at polar body formation ; for the male-producing and female-producing eggs are already differentiated from one another by their relative sizes before this point is reached ; the eggs which are going to eliminate an X_2 chromosome, and therefore develop into males, are smaller than the female-producers. While therefore we may probably still speak of the presence or absence of the X_2 chromosome as determining the sex of the individual, we must realize that in this case its presence or absence is not a matter of chance, but that there is some earlier factor which determines whether it shall be eliminated at maturation or not, and which consequently is a sex-determining factor earlier in the chain of causation.

In *P. caryaecaulis* this prior factor in sex determination must be sought very far back, for not only are the male-producing and female-producing eggs thus early differentiated from each other, but they are produced by different females. Moreover, all the offspring of a single stem mother are alike in respect to the type of eggs which they produce. Thus the sex of the members of the sexual generation is determined by the con-

stitution of their grandmother or even earlier ancestor. Morgan (1909) has, however, suggested a way in which even this determination may be preceded and hence "caused" by a differential partition of the chromosomes.

(*c*) *Some other Cases.*—An entirely different method of sex regulation is found in the gall-fly *Neuroterus lenticularis* (Doncaster, 1910, 1911). This species produces two generations in a year. The generation which hatches in the spring consists of females only, which reproduce parthenogenetically. Their eggs hatch out into males or sexual females. All offspring produced by one individual parthenogenetic female are of the same sex; *i.e.* her eggs are all either male-producing or female-producing. It is also found that the parthenogenetic eggs fall into two types: (1) in which there are the usual two meiotic divisions resulting in the formation of two polar bodies; (2) in which there are no meiotic divisions or maturation processes. Embryos developing from type (1) have haploid chromosome groups, and from (2) have diploid. Moreover, all eggs laid by one individual are alike in this respect, being either all of type 1 or all of type 2. Finally, the female of the sexual generation has the usual diploid group in its pre-meiotic nuclei, but in the male this is haploid.

Combining these observations, therefore, it becomes clear that the parthenogenetic eggs with two polar bodies produce haploid embryos which are males, while those with no polar body produce diploid embryos which are females. The maleness of the haploid individuals is in accordance with the general rule for facultative parthenogenesis in the Hymenoptera (Chapter III.).

In the case of some groups of animals with alternation (though in most cases irregular alternation) of sexual and parthenogenetic generations, nothing is yet known of any accompanying changes in the chromosome complex. In the Cladocera the common individual is the parthenogenetic female, which produces females like itself for a variable number of generations. After a time, however, males and sexual females are produced. The sexual eggs fertilized by the spermatozoa of these males invariably, so far as is known, develop into females.

One great difficulty in the cytological investigation of this group is that males and females are produced by the same parthenogenetic female and it is not possible to determine into which sex an egg will develop. So far as has been observed, all the parthenogenetic eggs produce only one polar body, but whether this applies to the comparatively rare parthenogenetic eggs which will develop into males, as well as to the majority which will develop into females, is not known, though it is probable from the fact that the male is diploid. The other problem,

why all fertilized eggs develop into females, is also quite unsolved. Chambers (1913) described degeneration of large numbers of spermatozoa in *Simocephalus*, and suggested that these, though not visibly different from the others, were the male-producing spermatozoa. Taylor, however (1915 *b*), found no evidence of degeneration of a whole class of spermatozoa in the allied genus *Daphnia*.

(8) *The Relation between the Sex Chromosomes and the Determination of Sex*

This question, having been fully discussed in a recent publication (Doncaster, *The Determination of Sex*, 1914). will be treated very summarily here to avoid unnecessary repetition.

In the cases where a dimorphism of the spermatozoa exists, it appears plain that the sex of the zygote depends upon whether the egg was fertilized by a spermatozoon with the X chromosome or without it ; and similarly that where the spermatozoa are all alike and the eggs dimorphic, it depends upon the nature of the egg which was fertilized. It therefore seems legitimate to say that in these cases sex is determined, or caused, by the sex chromosomes, but we must remember that the sex chromosomes are only one of a number of causes. We have already seen that in *Phylloxera fallax* a female gives rise parthenogenetically through her descendants both to male-producing and to female-producing eggs. It appears, therefore, that we must here look for an earlier cause of sex than the presence or absence of the X chromosome—namely, something which determines whether the X chromosome shall or shall not be eliminated at maturation. Similar considerations apply to the hermaphrodite *Ascaris nigrovenosus*.

In certain other cases it appears that the sex of the individual can be determined by factors acting after fertilization, and at a time therefore when we must suppose the chromosome equipment of the embryo to be fixed. The experiments of King (1912) on toads, and of R. Hertwig (1912) on frogs, make it probable that the sex of these animals can be influenced by external factors acting on the egg either before or after fertilization. An example from nature of the determination of sex by environment is afforded by the life history of the marine worm *Bonellia viridis* (Baltzer, 1914). Here it appears to be the environment of the larva which determines whether the adult shall be male or female.

Finally, it has long been known that the secondary sexual characters of one sex may appear in individuals of the opposite sex as the result of castration or other causes.

Probably in every zygote, and indeed in every gamete, both sexes

must be considered as potential, and which sex shall develop, or dominate over the other, may depend upon a multitude of factors of which the sex chromosomes are only one. In most cases where sex chromosomes are differentiated, however, the presence or absence of the second X chromosome appears to be overwhelmingly the most important immediate factor in sex determination, so that in the vast majority of such cases when once the chromosomal constitution of the zygote has been fixed, its sex is irrevocably determined. In certain rare cases, however, other factors may be more powerful and thus be the immediate determiners of sex.

As to the nature of the relation between the presence and absence of the second X chromosome and of the sex of the zygote we have practically no conception. Any attempt to ascribe the influence merely to the difference in the mass of chromatin is probably doomed to failure. It is true that in the majority of cases the male has less chromatin than the female, owing to the absence of the second X, or to its representation by the Y chromosome, which is usually smaller than its mate. In *Acholla multispinosa*, however, the single Y chromosome is considerably larger than the sum of the five X chromosomes (Payne). The chromatin content of the moth *Talacoporia* (p. 112) is also greater in the male than in the female, since in this species it is the female which lacks the second X chromosome.

Probably the problem of the determination of sex by the sex chromosome (on the occasions when this acts as sex determiner) is the same as that of the dependence of any bodily characteristic upon hereditary factors residing in the chromosomes (see Chapters V. and VI.).

CHAPTER V

THE CHROMOSOMES

Observation of the minute structure of the nucleus, together with the evidence from experimental work on heredity, has led to the formulation of a hypothesis which can be stated as follows :

The nucleus—and in particular that part of the nucleus constituting the chromatin or, at least in most phases of the nucleus, indistinguishably bound up with the chromatin—is the seat of the agency which initiates and controls morphogenesis and function, and hence, since the chromosomes are carried on from one generation to another through the gametes, it is also responsible for the phenomena of heredity. The chromatin appears to act in this respect not as a homogeneous whole, but rather as an aggregate of smaller bodies, each of which plays a different part though the sum of them all is necessary to the general economy of the organism (analogous to the parts played by the lungs, heart, liver and other organs in the higher animals). These smaller bodies, which constitute the lowest order of living units which need be considered for the purpose of this hypothesis, are aggregated during mitosis in linear series into bodies of a higher order, the chromosomes. The sum of the chromosomes again forms the nucleus.

As the haploid number of chromosomes is sufficient to enable a normal individual to develop (cf. facultative parthenogenesis and merogony), each gamete must contain a complete set of all the units necessary for the production of a normal individual of the species. Hence the diploid zygote must contain a double set of these units, *i.e.* two of each kind.

While it is impossible completely to separate the discussion of the morphological and physiological aspects of this thesis (for their interdependence is the chief evidence of the correctness of both) it is the former aspect that will be the main consideration in this chapter, while the physiological and more theoretical sides will be specially dealt with in the following chapter.

A. THE CONTINUITY OF THE CHROMOSOMES

The first morphological hypothesis to be established is what has come to be known as the *individuality*, or better, the *genetic continuity* (Wilson) of the chromosomes. The meaning of this phrase is that the material of the chromosomes is not resolved at telophase into a common nuclear reticulum from which new chromosomes differentiate out in the next prophase (as crystals might dissolve in a solvent and recrystallize out again), but that the substance of each telophase chromosome is concentrated again into a corresponding chromosome in prophase. Thus each individual chromosome is the direct descendant of the corresponding chromosome in the previous cell generation as described on p. 128.

This conclusion is at once suggested by the fact that the number of chromosomes in any one species is constant (with certain, mostly well-understood, exceptions) although it may vary greatly in nearly allied species ; that the number is constant (with the same qualification) in different tissues though the total amount of chromatin may vary greatly from tissue to tissue ; and that the number of chromosomes is halved at gametogenesis and subsequently restored at syngamy.

The truth of this hypothesis is indeed very generally accepted, being supported by a great body of observations as well as by indirect evidence. Indeed it would be exceedingly difficult to write a general treatise on nuclear cytology without accepting the hypothesis as a basis, and the reader will doubtless have noticed that it has frequently been implied in this work. It will be necessary, however, to discuss briefly a few of the problems which are raised thereby.

While the earlier cytologists were content with demonstrating the constancy of the number of chromosomes in a given species it became evident, with the extension of the study to a wider range of forms, that in many species the chromosomes are not all alike, but differ from each other in size, and especially in length ; as, except in the case of the meiotic chromosomes, this is apparently the only dimension in which constant differences occur, the thickness of all the chromosomes in a given nucleus being approximately equal. Moreover, the length differences are constant, so that in every nucleus (in mitosis) not only the same number of chromosomes, but the same series, ranging from the largest to the smallest, can be recognized. Again, it was found that there were in each diploid nucleus two chromosomes of each size, so that if the chromosomes are designated in order of size A, B, C, etc., the chromosome complex could be designated thus :

$$A+A+B+B+C+C+\ \ldots$$

In the meiotic prophase the two chromosomes of each type, usually

called *homologous* chromosomes, pair together to form the bivalents. The nucleus of the primary oocyte or spermatocyte can therefore be written :

$$AA + BB + CC + \ldots$$

At the reduction division the homologous chromosomes are separated, as described in Chapter II., so that each gamete nucleus has the formula

$$A + B + C + \ldots$$

The diploid nucleus of the first formula is of course reconstituted at syngamy.

Thus it follows that one member of each pair of homologous chromo-

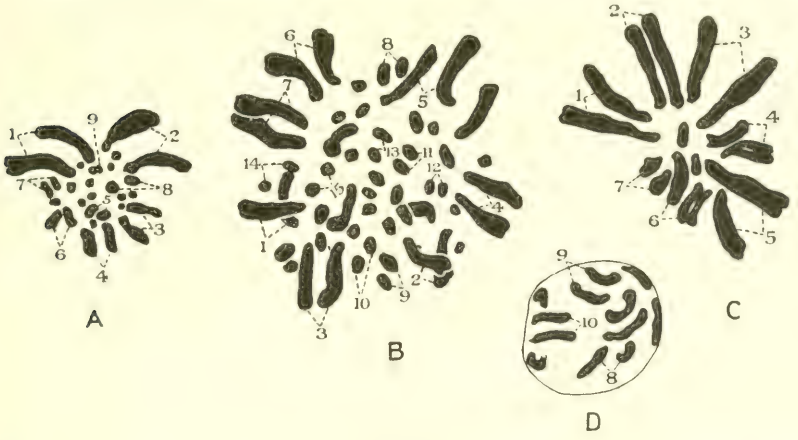


FIG. 55.

Illustrating the tendency of homologous chromosomes to lie near each other in somatic nuclei. (Müller, *A.Z.*, 1912.) A-C, polar views of equatorial plates; D, prophase. A, *Eucomis bicolor*; B, *Albuca fastigiata*; C, *Galtonia candicans*; D, *Dahlia coronata*. Some of the pairs are numbered.

somes in a diploid nucleus has been derived from the male and one from the female parent.

This morphological fact, together with its theoretical consequences for heredity (to be discussed in the next chapter) was first pointed out clearly by Sutton in the case of the insect *Brachystola magna*.

The degree to which the chromosomes of a single nucleus differ from one another in length varies greatly, and indeed in some species no certain differences are detectable. Such species are of course of negative value as evidence in this respect, the generalization being founded on those numerous other forms in which the chromosomes exhibit marked size differences. The seriation of the chromosomes according to size is often facilitated by a tendency on the part of homologous chromosomes to lie near or next to one another on the equatorial plate. This tendency also varies in different species, in some indeed apparently not existing, while in others (*e.g.*, *Yucca*; Muller, 1912) it is pronounced (Fig. 55).

The paired arrangement of homologous chromosomes is most strikingly shown in the Diptera (Metz, 1916), in some species of which homologous chromosomes are very closely approximated. This culminates in *Culex*, where they are often so closely applied to one another as to be distinguished only with difficulty. Fig. 56 shows the chromosome

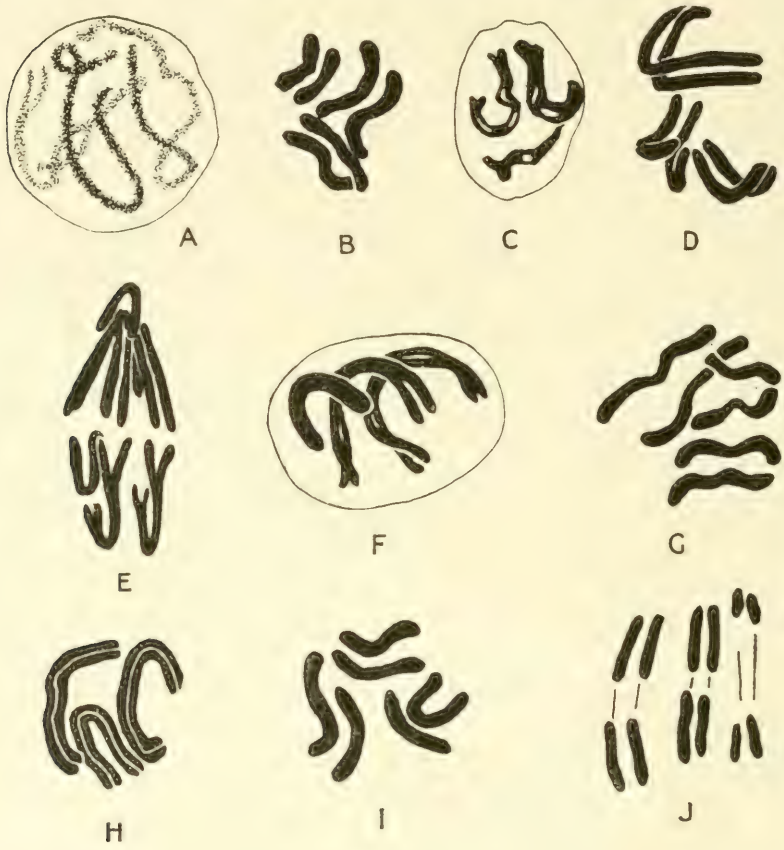


FIG. 56.

The chromosomes of *Culex*, as figured by different workers, showing the close approximation of homologous chromosomes. (A, B, from Stevens, *J.E.Z.*, 1910; C, from Taylor, *Q.J.M.S.*, 1915; D, E, from Whiting, *J.M.*, 1917; F, G., from Hance, *J.M.*, 1917; H, I, J, from Metz, *J.E.Z.*, 1916.) A, spermatogonial prophase; only one of the three chromatin threads is visibly double; B, spermatogonial metaphase, three pairs of separate chromosomes; C, somatic prophase; D, spermatogonial prophase; E, spermatogonial anaphase; F, ovarian (diploid) prophase; G, spermatogonial metaphase; H, I, J, prophase, metaphase, and anaphase of somatic mitoses.

complex of this mosquito (where $2n = 6$) as depicted by various workers. As will be seen, the homologous chromosomes in prophase (Fig. 56, A, C, D, F, H) are generally intimately applied to or twisted round one another, quite as closely as are the daughter halves of split prophase chromosomes in the somatic mitoses of many other forms (Figs. 3, 8).

As they condense for metaphase the homologues become more distinct from one another but still remain very closely paired (Fig. 56, B, G, I). In some strains they remain indistinguishably fused even at this stage (Taylor, 1915 *a*). In anaphase they again come into close application or fusion (Fig. 56, E, J).

It is interesting to note that in Taylor's material (1915 *a*, 1917), although, in general, fusion of the homologous chromosomes was so intimate that in somatic mitoses there appear to be only three chromosomes present, yet in the early cleavage divisions of the egg the six chromosomes are as well separated from one another as in other animals.

The case of the Diptera, and especially of *Culex*, leads to the conclusion that the fusion of the chromosomes in syndesis is only the climax of a general mutual attraction between homologous chromosomes.

Further indirect evidence of the continuity of the chromosomes is furnished by those animals in which the bivalents of the meiotic phase appear in various different shapes. In these animals it is found that the same shapes, and the same number of each shape, reappear in every meiotic nucleus (Figs. 57, 65).

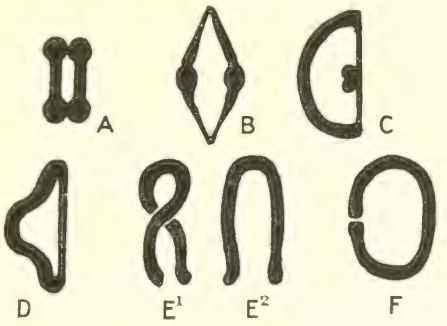


FIG. 57.

The different forms of bivalents found in the meiotic phase of the newt. (After Moore and Arnold, *P.R.S.*, B 1906.) Two of each type are found in all primary spermatocytes (E^1, E^2, being alternative forms of the same type).

The one difficulty in the way of the hypothesis of the continuity of the chromosomes is the fact that in the great majority of cases they lose all visible signs of their identity in the resting nucleus. This, however, is a piece of negative evidence which cannot be allowed to outweigh the overwhelming indirect evidence from their constancy in number, relative sizes, etc., which indicates that they do actually maintain this continuity. Moreover, in many cases direct evidence has been obtained that the chromosomes which enter into the resting nucleus at telophase do not become diffused throughout the whole nucleus and inextricably mingled up with one another, but retain a definite localization in the nucleus though their boundaries may not be visibly distinguishable. The classical piece of evidence on this head is Boveri's work on the cleavage nuclei of *Ascaris megalocephala*, which on account of the small number of its chromosomes is plainly a favourable object for such investigation.

Originally carried out on the eggs of *Ascaris megalocephala bivalens* (Boveri, 1888), the work was repeated by Boveri in 1909 on the *univalens*

form. He found that in anaphase the daughter chromosomes separate in such a way that their arrangement in the two telophase groups is the same. Thus in Fig. 58, A, one chromosome is bent in a U-shape, so that the two ends lie close together, while the other one is stretched out so that one end lies with the two ends of the first chromosome, while the opposite end is far removed. In D both chromosomes are U-shaped,

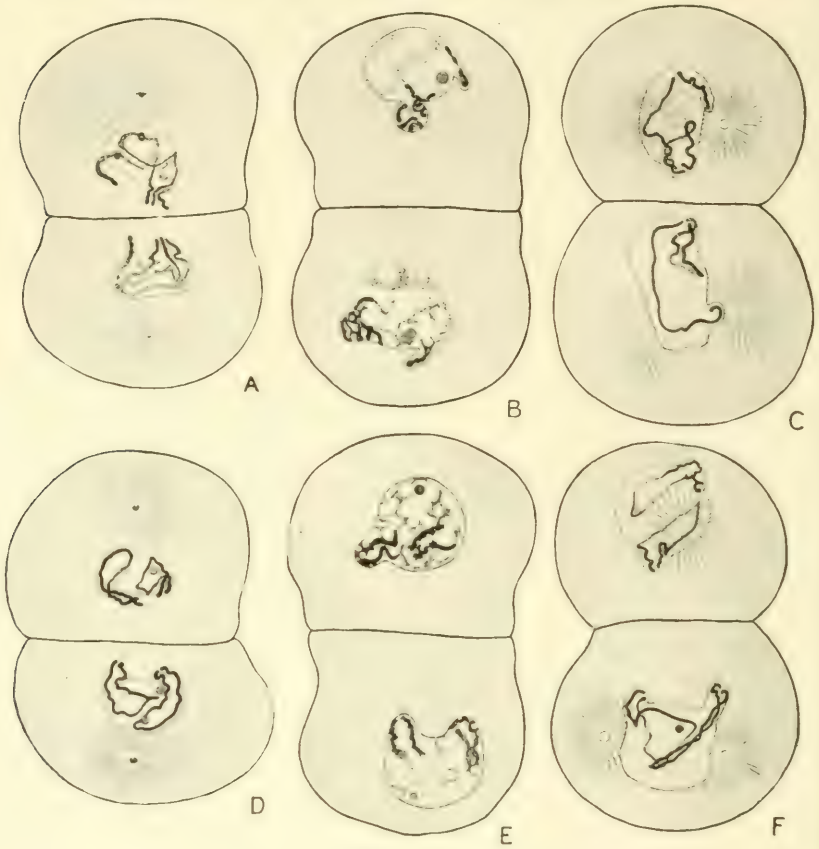


FIG. 58.

Telophase of the first cleavage mitosis to prophase of the following mitosis in *Ascaris megalocephala univalens*. (Boveri, *A.Z.*, 1909.) A, B, C, telophase, resting nucleus and following prophase of a nucleus with the chromosomes so grouped in telophase that one chromosome end projects by itself while the other three form a common projection; D, E, F, similar series in a nucleus in which each chromosome is bent upon itself, so that one projection contains the two ends of the one chromosome and another projection those of the other chromosome.

forming two separate groups of chromosome ends. When the telophase becomes resolved into the resting nucleus it is found that the chromosome ends form projections from the main mass of the nucleus, telophase groups of type A resulting in resting nuclei such as shown in B, where both sister nuclei have one thick and one thin projection (containing three and one chromosome ends respectively). Telophase groups of

type D produce the nuclei shown in E, which have two equal projections, each containing two chromosome ends. In the next prophases (C, F) the chromosomes reappear in the same arrangement as they exhibited in the previous telophases. It will be noticed that the orientation of the nuclei towards each other in the two daughter cells has changed slightly owing to the rotation of the nuclei within the cells. This however does not affect their internal architecture.

It must be understood that what we have here described as a *process* is, like all similar work in cytology, really pieced together from a series of fixed stages. Thus it cannot actually be observed that the prophase nuclei of types C and F are the outcome of telophase nuclei of types A and D respectively, but this can be inferred without reasonable doubt since (1) they are connected up by a close series of intermediate stages of which B and E are examples; and (2) in the prophase, as in the

FIG. 59.

Showing similar orientation of telophase (A) and prophase (B) in the epidermis of the salamander. (Rabl, *M.J.*, 1885.)

telophase, the arrangement of the chromosomes in the two sister nuclei derived from the previous mitosis is the same.

An orientation of the chromosomes in telophase similar to that in the succeeding prophase has been described by many cytologists from Rabl (1885) onwards (Fig. 59), though the conditions are seldom so favourable for observation as in *Ascaris megalocephala*.

Another fact directly supporting the hypothesis of the genetic continuity of the chromosomes is that each chromosome may undergo its telophase metamorphosis in a more or less separate vesicle within the nucleus. This is especially characteristic of Orthopteran spermatogenesis (Fig. 60). Sutton (1903) described the larger chromosomes of the spermatogonial telophase of *Brachystola magna* as forming each its own reticulum in a separate vesicle, which however is in communication with the other vesicles at their polar ends, forming there a common compartment from which the vesicles project like the fingers of a glove. This general account has been confirmed by several cytologists. In *Phrynotettix* (Wenrich, 1916) the telophase chromosomes first form closed

vesicles, then the walls of these vesicles break down to produce a common nuclear cavity, in which, however, the regions of the vesicles can still be recognized by the slightly denser core of chromatin occupying what was formerly their axes. In prophase the chromosomes condense again in the limits of these vesicles (Fig. 60, A-E).

In *Locusta viridissima* (Otte, 1907) the chromosomes of the spermato-

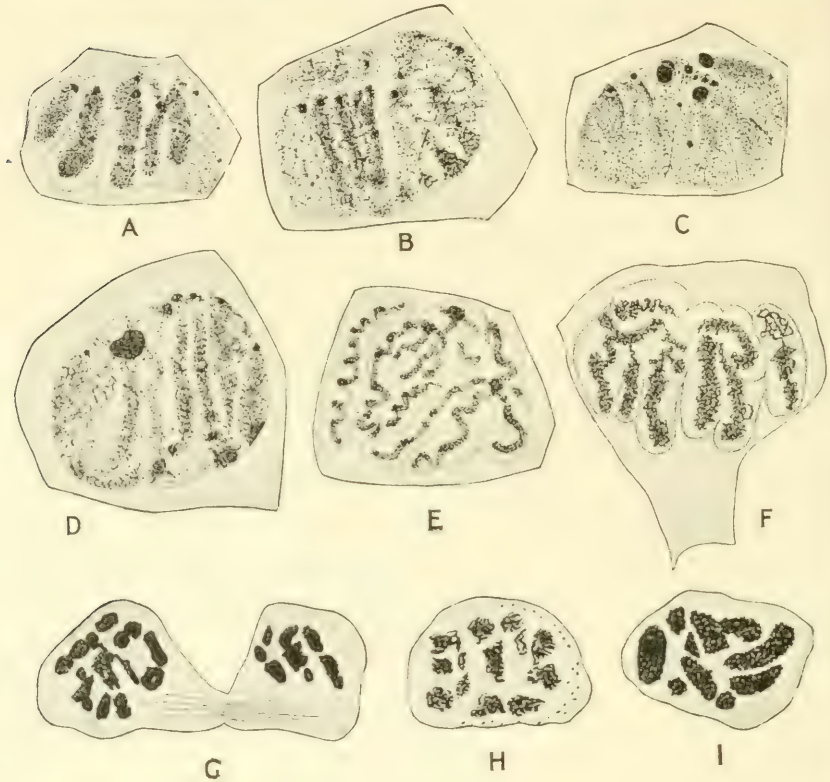


FIG. 60.

Formation of chromosome vesicles in the spermatogonia of Orthoptera. (A-E, *Phrynotettix magnus*, after Wenrich, *B.M.C.Z.H.*, 1916; F, *Brachystola magna*, after Sutton, *B.B.*, 1903; G, H, I, *Locusta viridissima*, after Otte, *Z.J.A.*, 1907). A, B, C, successive stages in the formation of the resting nucleus out of the telophase chromosomes; D, E, prophase; F, early prophase; G, telophase; H, resting "nucleus"; I, prophase.

gonial telophases do not come into contact at all, but each one forms a separate little nucleus, or *karyomere*, by itself (Fig. 60, G-I.) No common nuclear membrane is formed to enclose them, but they remain separate from one another, with cytoplasm extending in between them. This account refers to the earlier spermatogonial divisions. In the last one before the meiotic phase a compound nucleus is formed in the usual way.

Karyomere formation—*i.e.* the formation of a separate little nucleus

by each chromosome—is a common occurrence in the cleavage divisions of many animals. Instead of a single nucleus, we therefore find a mass of small ones corresponding in number to the number of the chromosomes (Fig. 61). As a rule this condition is temporary, the karyomeres generally fusing later into a single nucleus. Certain abnormal conditions, *e.g.* high temperature (Tobias, 1914), accentuate the tendency to karyomere formation.

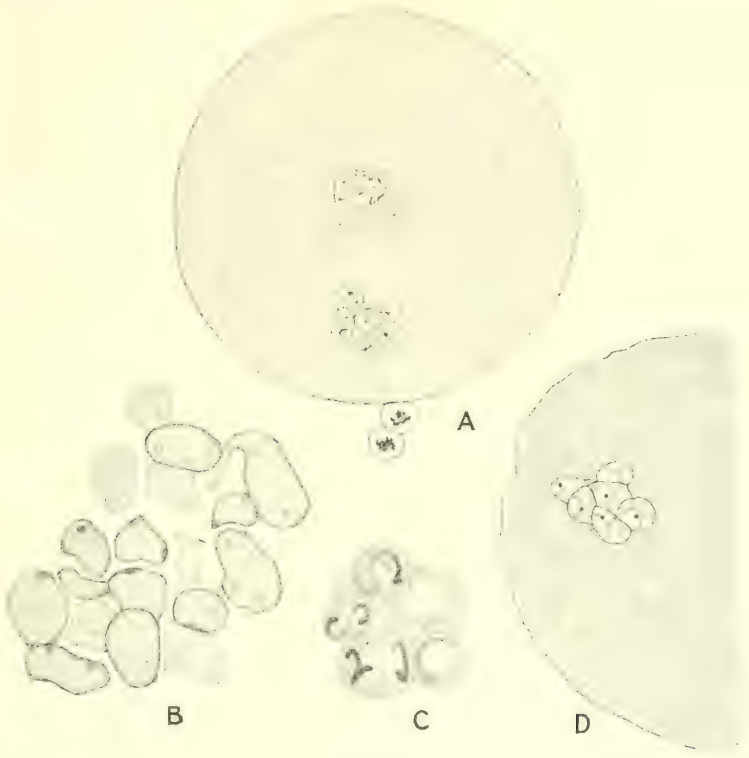


FIG. 61.

Formation of karyomeres in cleavage nuclei of various eggs. A. *Chaetopterus pergamentaceus*: ♀ nucleus has completed its maturation divisions, and, in the form of a group of chromosomic vesicles is moving inwards to meet the ♂ nucleus (after Mead, *J.M.*, 1895); B, C, resting and prophase nuclei from cleaving eggs of *Cyclops viridis*, subjected to a high temperature (after Tobias, *A.m.A.*, 1914); D, one nucleus of the 2-cell stage of *Polyphemus pediculus* (after Kuhn, *A.Z.*, 1908).

A difficulty which has been urged against the view of the continuity of the chromosomes is the supposed power of the nucleus to form chromosomes after amitotic division. As this matter has already been discussed (p. 24) it need not be dealt with again.

Very strong evidence in favour of the continuity of the chromosomes has been obtained from the study of certain hybrids. Moenkhaus (1904) crossed the Telostean fishes *Fundulus heteroclitus* and *Menidia notata*.

The former has long (2·18 μ), slender and generally straight chromosomes, n being 18. The latter has about the same number of chromosomes, which are short (1·00 μ) and generally curved. The eggs of each species are fertilizable by the sperm of the other, and the hybrids so formed develop for a certain period apparently normally, though in later embryonic stages abnormalities make their appearance, and the eggs seem incapable of hatching (see Loeb, 1912). The chromosome conditions during the development of the hybrid embryo are especially interesting (Fig. 62). After fertilization the male and female gamete nuclei fuse in the resting condition. When the chromosomes appear for the first

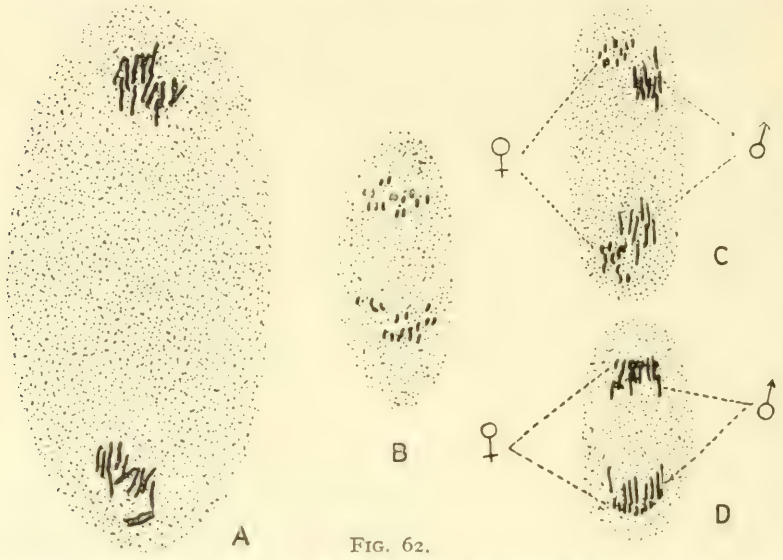


FIG. 62.

A, anaphase of first cleavage mitosis in the egg of *Fundulus heteroclitus*; B, similar figure from *Menidia notata*; C, D, anaphase of first (C) and later (D) cleavage mitosis of a hybrid (*Menidia notata* ♀ × *Fundulus heteroclitus* ♂) showing the two distinct types of chromosomes, separately grouped in C and mingled in D (Moenkhaus, *Amer. Journ. Anat.*, 1904).

cleavage mitosis, however, it is found that the chromatin of the two species has remained distinct, for the chromosomes appear in two groups, one consisting of long chromosomes easily identifiable as derived from the *Fundulus* parent, the other of short chromosomes derived from *Menidia*. In telophase the two groups of chromosomes again become indistinguishably merged into the resting nucleus, to reappear in the same grouping at the next mitosis (2nd cleavage division). At the 3rd cleavage the two types of chromosomes are still as sharply distinct from one another, though they are no longer completely segregated into two groups. By the 4th cleavage division the grouping is almost, and in later cleavages quite, lost, the two types of chromosomes—still, however, perfectly distinct—being intermingled with each other.

The grouping of the chromosomes derived from the male and female parents which is to be seen in the first few cleavage divisions is of course an example of gonomery, such as occurs in *Cyclops*, etc. (Fig. 35), and, as usual, disappears in later cleavages. The important fact is that the two types of chromosomes introduced by the two parents, though mingled together, are recognizable in all mitoses. From this we conclude that their loss of identity in the resting nucleus is apparent only, and not real.

In the Lepidopteran cross *Lycia hirtaria* × *Ithysia zonaria* (Harrison

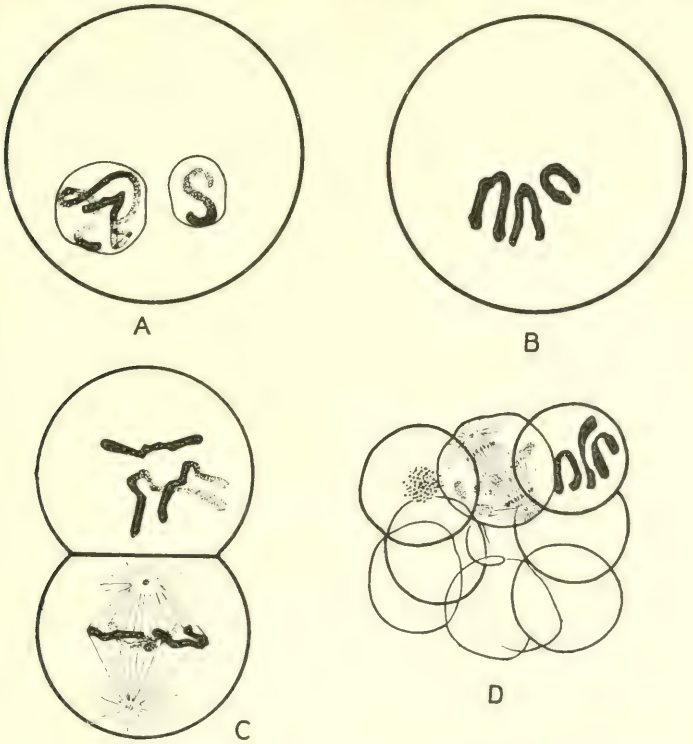


FIG. 63.

Fertilization of the egg of *Ascaris megalocephala bivalens* by spermatozoa of *A. m. univalens*. (After Herla, *A.B.*, 1895.) A, approach of the gamete nuclei; B, the chromosomes of the zygote nucleus; C, egg divided into two blastomeres; D, twelve blastomeres.

and Doncaster, 1914) the large chromosomes of *Lycia* and the small ones of *Ithysia* are distinguishable in the hybrid right up to the formation of its gametes (Fig. 185).

The persistence of an unusual number, though not of distinct types, of chromosomes in a hybrid was observed by Herla in 1895. He found five females of *Ascaris megalocephala bivalens* which had been fertilized by the *univalens* variety. Consequently the nuclei of their hybrid embryos had three chromosomes, two derived from the female and one from the

male parent (Fig. 63). This number could be counted (in the germ track) up to at least the 12-cell stage, which was the latest stage examined. This case must not be confused with the *A. megalocephala* with three chromosomes described by Boveri (p. 145), though the two cases furnish equally strong evidence for the continuity of the chromosomes.

B. THE COMPOSITION OF THE CHROMOSOMES OF SMALLER UNITS

While the definitive chromosomes of the metaphase generally appear homogeneous, they characteristically present a different appearance in prophase, where they are often markedly moniliform, *i.e.* consisting of a row of bead-like swellings of chromatin, called *chromomeres*, joined to each other by a thinner linin thread. This condition, which forms one of the most characteristic sights met with by the cytologist, can be illustrated by reference to almost any work dealing with mitosis, whether in the soma, germ track or during meiosis. Many figures in this book illustrate this point incidentally (*e.g.* Figs. 20, 77). It is equally characteristic of animals and plants (Fig. 64).

In the early prophase the chromomeres, if visible, are commonly very small and numerous (when not visible it probably means that they are so closely distributed along the chromosome that their boundaries are not distinct). As prophase proceeds they become larger and fewer, obviously by fusion in groups. At about this stage they are often extremely prominent, constituting comparatively large spheroidal swellings joined to their neighbours by short stretches of very fine threads. As the chromosomes contract, the now composite chromomeres become more and more pressed together, the boundaries between them gradually becoming obliterated till in the metaphase chromosome they are generally no longer distinguishable from one another, and in consequence the chromosomes appear homogeneous. Finally, in the greatly contracted chromosomes of meiosis, or somatic telophase, the chromomeres appear to lose their linear arrangement. We must however suppose that the loss of the linear arrangement is only apparent, and that essentially it is maintained so that the chromomeres appear in the same order in the prophase chromosomes of successive mitoses.

In the early prophase the chromomeres are often the points of departure for the linin threads which run out from the chromosomes into the vanishing nuclear reticulum (*e.g.* Figs. 3, 16). This suggests that the substance forming the chromomere has travelled down the linin fibre to the main trunk of the chromosome.

The correspondence, as regards number and sizes of the chromomeres, between the daughter threads of the split somatic chromosome (Fig.

64, A, D) and between the conjugating chromosomes in the meiotic prophase (Figs. 64, C, and 66) is very striking. The latter phenomenon is specially significant, suggesting that syndesis does not concern the chromosomes as wholes, but that it takes place between the separate elements of which they are composed.

The thesis formulated at the beginning of this chapter requires that the chromosomes should not merely be composed of smaller units, but that these should be differentiated among themselves. It further follows

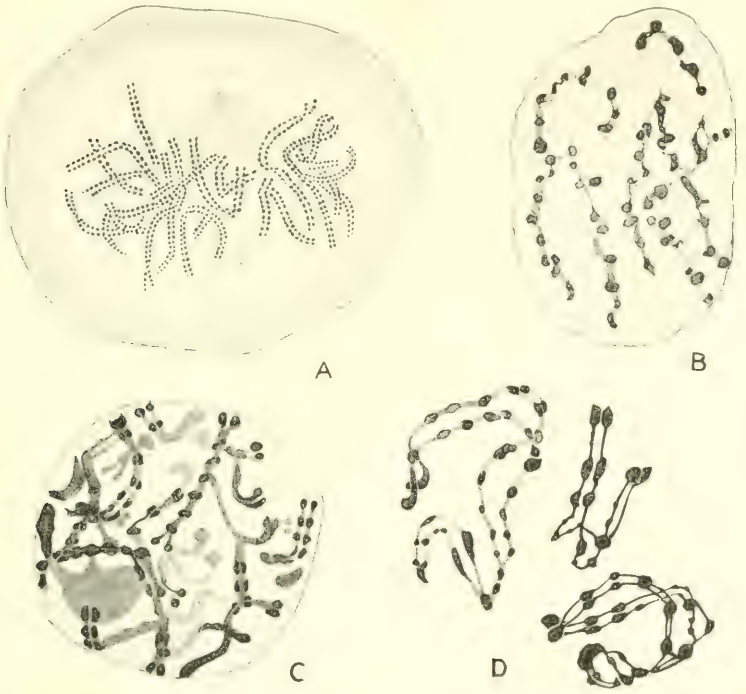


FIG. 64.

Chromomeres. A, epithelial cell of the salamander (Flemming, 1882); B, prophase of nucleus in root tip of *Najas marina* (after Müller, *A.Z.*, 8, 1912); C, zygo-pachytene nucleus of oocyte I. of *Enteroxenos* (after Bonnevie, *J.Z.*, 1906); D, prophase chromosomes from alimentary canal of *Culex* (after Holt, *J.M.*, 1917).

that if in syndesis corresponding elements of the homologous chromosomes pair together, these elements must always be arranged in the same order along the length of the chromosome.

Evidence that the longitudinal differentiation of the chromosomes is of a definite and relatively constant nature has been presented in the case of *Lepidosiren* (Agar, 1913). In this animal the 38 somatic chromosomes are usually V-shaped, but in the shorter ones the limbs of the V's tend to diverge, till at length the chromosome, by straightening out, becomes rod-shaped. The point of bending of the V can however be

traced through the gradually widening out V into the rod, where it persists as a constriction, or an actual break in the chromatin, dividing the chromosome into two portions connected by a linin bridge. This transverse constriction is the same as that so characteristic of the meiotic bivalents which causes them to appear in " tetrad " form as discussed on p. 40. The significant fact is that the transverse break always occurs in the same region in the same chromosome. It will be noticed from Fig. 65 that one pair of chromosomes is much larger than any of the others. The break in this chromosome—whether exhibited as the angle of the V, or as a transverse constriction—is always found at about

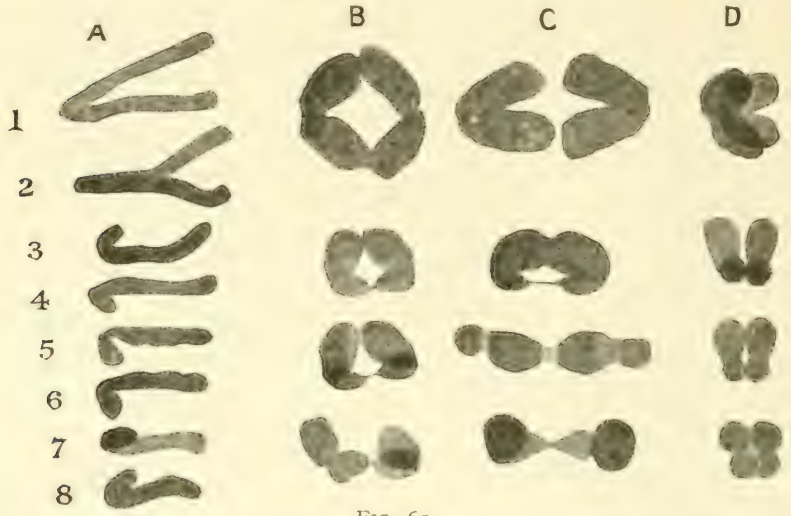


FIG. 65.

The eight largest chromosomes of *Lepidosiren*. (Agar, *Q.J.M.S.*, 1913.) A, from a spermatogonial anaphase; B, the four bivalents formed by the pairing of the eight chromosomes (late prophase I.); C, metaphase I.; D, anaphase I., each univalent split for the second division. Note that chromosomes 1 and 2 form a V with equal limbs in A, and that each constituent of the corresponding bivalent is similarly constricted into equal portions. The other three pairs of chromosomes have unequal limbs, both in A and in the bivalents.

the middle of the chromosome. The next two pairs of chromosomes are of much the same size, but easily distinguishable both from the large pair just described and from the next smaller pair. These two pairs constantly have the break excentrically placed. Now if the break were always in the middle of the chromosome, or varied in position in the same chromosome, it would be without significance for the present purpose, as it might then be due to purely accidental mechanical causes. The fact that—however caused—it is constant in position in a given chromosome, but differs in different ones, indicates that the chromosomes possess a constant differentiation in a lengthwise direction.

Wenrich (1916) has found that in the prophase chromosomes of the Orthopteran, *Phrynotettix magna*, the principal chromomeres are

constant in their arrangement in a given chromosome. Fig. 66 shows one example of the corresponding chromosome from thirteen different individuals. It is taken from the pachytene stage of spermatogenesis, and is therefore bivalent, as indicated by frequent signs of duplicity. The five principal chromomeres are numbered 1-5 and it will be seen how noticeably constant in arrangement they are. This regularity extends also to the smaller chromomeres. For instance, in the segment between Nos. 3 and 4 there are always two small granules of about the same size, while there are never any prominent ones between Nos. 2 and 3.

Of course, the constancy is not perfect. A certain amount of variation in the relative sizes of the principal chromomeres, and in the lengths of

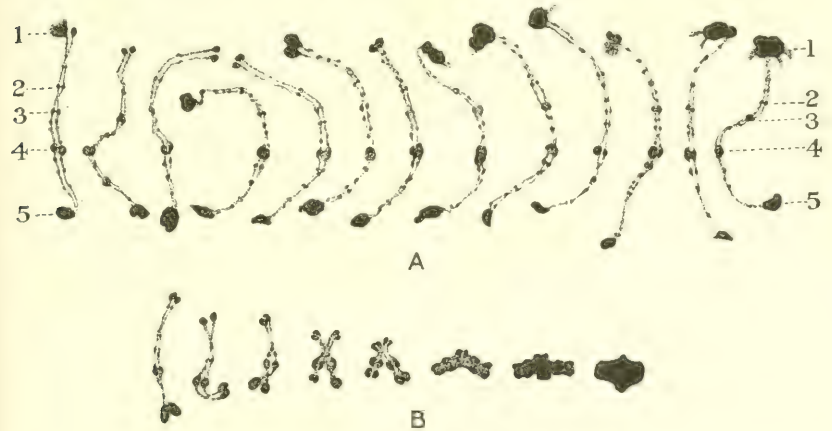


FIG. 66.

Various examples of the same chromosome in *Phrynotettix magnus*. (After Wenrich, *B.M.C.Z.H.*, 1916.) A, the chromosome (bivalent) from the pachytene stage of thirteen different individuals. The principal chromomeres are numbered 1-5. B, successive stages in the contraction of the pachytene chromosome to form the definitive chromosome of metaphase I.

the segments separating them, can be observed, as well as in the number and arrangement of the smaller granules in between. This variation may be due to several causes, partly to errors of technique—for instance, distortion by fixing agents, optical effects, etc.—partly to difference in the extent to which fusion of smaller granules to form larger ones has proceeded, but partly probably to real biological variation. The thesis outlined at the beginning of this chapter requires that all genetic differences in organisms should be referred to preceding variation in the idioplasmic elements, and hence it is no more surprising to find variation in homologous chromosomes than in the somatic characteristics of organisms.

Wenrich finds indeed, in the case of the particular chromosome under consideration, that the chromomere numbered 5 is often absent. In

some individuals this chromomere is present in both members of the pair, in some it is absent from both members, and in others it is present in one chromosome but absent from its homologue. The same combination is of course constant for all the nuclei of a given individual. The existence of the three possible combinations indicates promiscuous syngamy between gametes which possess and those which do not possess the chromomere in question.

Inequalities, or other visible differences, between the two members of a homologous pair have also been described in Orthoptera by Carothers (*Brachystola*, 1913; *Trimerotropis* and *Circotettix*, 1917) and by Robertson (*Tettigidea* and *Acridium*, 1915).

In *Trimerotropis* (one of the grasshoppers) the metaphase chromosomes are either rod-shaped, or bent into V's (with equal or unequal arms). These shapes are not transitory forms impressed on the chromosomes by temporary forces acting in mitosis, but they mark the different methods of attachment of the spindle fibres to the chromosomes, and are constant in all the nuclei of an individual. In the case of the rod-shaped chromosomes the spindle fibre is attached to one end, and in the case of the bent chromosome it is attached to the angle of the V. Often homologous chromosomes differ in this respect, as shown by the shape of the bivalents in metaphase I. (Fig. 67). A bivalent may be formed by two straight chromosomes (Fig. 67, A) or by two bent chromosomes (B) or by one straight and one bent chromosome (C), all these figures representing the same bivalent as found in three different individuals.

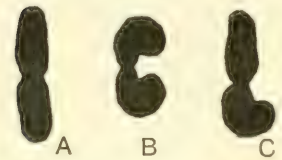


FIG. 67.

The corresponding bivalent from three different individuals of *Trimerotropis*. (After Carothers, *J.M.*, 1917.)

A number of other cases of inequalities between homologous chromosomes have been recorded, of which further mention need only be made of *Gryllotalpa borealis* (Payne, 1913 *a*) where the inequality is connected with the sex chromosomes and may be of the same nature as found in the unequal XY pair, which of course constitutes the most striking example of unequal homologous chromosomes.[1]

C. VARIATION IN THE NUMBER OF CHROMOSOMES

Variations from the number of chromosomes typical for the species may be due to the following causes:

(*a*) Transverse fracture of the chromosomes (fragmentation) leading to increase, or end-to-end fusion (linkage) causing decrease, in number.

[1] Boveri (1904) found a female *A. megalocephala bivalens* in which *both* the tetrads were often formed of two rods of unequal length.

(*b*) Irregularities of mitosis, by which the chromosomes are unequally distributed between the daughter nuclei, or certain of them are left out of either nucleus.

(*c*) Ordinary longitudinal fission of the chromosomes without a mitosis to separate the daughter chromosomes, thus leading to a doubling, quadrupling, etc., of the number. Or longitudinal fusion of the chromosomes as in parasyndesis leading to an apparent halving of the number.

(1) *Variation in Chromosome Number due to Fragmentation or Linkage*

Since, as we have seen, the chromatin units are arranged in linear series in the chromosomes, the total chromatin content of the nucleus may be considered as ideally arranged in such a linear series along a single long thread, which becomes divided into a number of segments varying in number in different species.

Thus in the genus *Cyclops* (Braun, 1909) it may be segmented into 3 (*C. gracilis*), 5 (*C. vernalis*), 6 (*C. viridis*), 7 (*C. fuscus*), 9 (*C. bicuspidatus*), or 11 (*C. strenuus*), these being the haploid numbers.

The older cytologists were indeed of opinion that this segmentation of a single linear series actually occurred in the prophase of every mitosis, the first stage in this process being the formation of the "continuous spireme," which in later prophase gave place by transverse segmentation to the "segmented spireme." Though a continuous spireme probably does not occur, at any rate as a regular stage, in prophase (see p. 9), the process probably represents substantially the method by which in evolution the varying chromosome numbers have been produced.

A special study of the phylogenetic derivation of chromosome numbers has recently been made by American cytologists. The number of chromosomes in the grasshoppers (*Orthoptera*) is relatively constant. Thus in ten species belonging to five genera of the family Tettigidae, Robertson (1916) found in every case $2n = 13$ (male) and 14 (female). In over forty genera of Acridiidae, $2n$ is 23 (male) and 24 (female) in all except three, of which one (*Chorthippus*) has 17 and the other two from 20 to 24 chromosomes.

Robertson (*loc. cit.*) has shown how the smaller number in *Chorthippus* (= *Stenobothrus*) has probably been derived from the type number for the family. The Acridiid spermatogonial chromosome is typically rod-shaped. In *Chorthippus*, however, only eleven of the chromosomes, including the X chromosome, are of this shape, the remaining three pairs being V-shaped. The angles of the V's—that is to say, the points of junction of the limbs—are marked by a constriction or non-staining bridge between the limbs. Robertson makes the very reasonable suggestion that the V's have been formed by association or linkage of couples

of the chromosomes of the type form, each limb corresponding to a whole rod-shaped chromosome, thus accounting for the usual 23 chromosomes by 11 simple + 6 double elements.

This association has of course not taken place between homologous chromosomes, but between non-homologous ones. Thus, using the notation on p. 124, linkage has occurred between each A and B, C and D, E and F, etc., to form composite chromosomes of the formula AB, CD, EF, etc. This interpretation is borne out by the facts that the six V's form three equal pairs, and also that the limbs of each V are not equal, showing that the linkage has been between non-homologous chromosomes.

M'Clung (1917) has come to similar conclusions regarding the variation in the number of chromosomes in *Hesperotettix viridis*, one of the Acridiidae. In this species the type number for the family (23 in the male) is found in some individuals, but others exhibit fewer. An examination of the bivalents of the primary spermatocytes shows that in the latter individuals one or more of the chromosomes have a transverse constriction which is not found in those individuals which possess the full 23 chromosomes, probably indicating that the chromosomes in question are compound.

Thus, in their primary spermatocytes—

5	individuals had	12	separate chromosomes	= 11 bipartite + the sex chromosomes.
7	,,	11	,,	= 10 bipartite + 1 tripartite (the sex chromosome attached to one ordinary bivalent).
5	,,	10	,,	= 8 bipartite + 1 quadripartite + 1 tripartite.
7	,,	9	,,	= 6 bipartite + 2 quadripartite + 1 tripartite.
7	,,	10	,,	= 7 bipartite + 2 quadripartite + the sex chromosome.
6	,,	11	,,	= 9 bipartite + 1 quadripartite + the sex chromosome.

It will be seen that the number of chromatin segments in all cases adds up to 23, the bipartite chromosomes being of course the ordinary bivalents formed by the pairing of two simple homologous chromosomes, the quadripartite ones being formed by a pair of composite homologous chromosomes (each of which has been formed by the linkage of two non-homologous chromosomes as in *Chorthippus*), and the tripartite forms by the junction of the single sex chromosome with an ordinary bivalent. The condition presented by the first example in the table is the typical one for the family when there is no linkage of chromosomes.

Again, all the nuclei of a given individual have the same type of chromosome complex. What happens in fertilization between different classes of individuals is not known. M'Clung suggests that the linkage is resolved, and re-formed, at syngamy.

Woolsey (1915) found similar relations in the locustid genus *Jamaicana*

(Fig. 68). In this genus the type number is 35 for the male; the chromosomes are rod-shaped. In some individuals, however, the number is reduced to 34 or 33 (spermatogonia). The 34 type has 33 rod-shaped and one V chromosome, while the 33 type has 31 rods and two V's. The former case is interesting as an example of homologous chromosomes behaving differently, for since there is only one V instead of a pair of them, linkage must have taken place between one member of each of two pairs of homologous chromosomes, the other member remaining free. That is to say, the chromosome formula is AB + A + B + C + C + . . . What happens in this case at syndesis? While we do not know the details of this process, the result is clearly shown by the structure of the chromosomes of the first meiotic division. They consist, besides the sex chromosome, of fifteen ordinary bivalents and one tetrapartite V of the type shown in the figure. As the spermatogonial divisions show, the original linkage was between two chromosomes of considerably different sizes, so that a V with unequal limbs is formed. The tetrapartite bivalent at meiosis is formed by the pairing of each limb of this V with its homologue, and may be represented thus: A B / A B (Fig. 68, C). The composite bivalent divides at the points of junction of the homologous

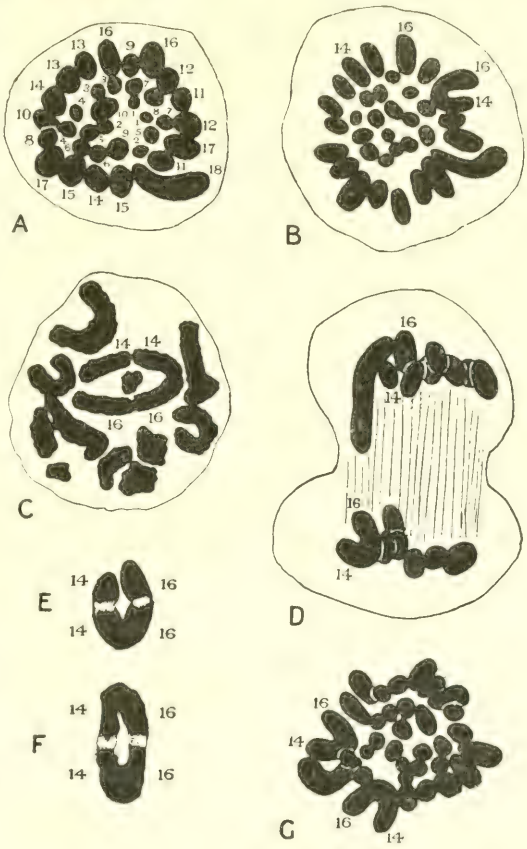


FIG. 68.

The chromosomes of *Jamaicana subguttata* (A-E) and *J. unicolor* (F-G). (Woolsey, *B.B.*, 1915.) A, spermatogonium of an individual with the type number (35) of chromosomes. The chromosomes are numbered in pairs from the smallest to the largest. No. 18 is the sex chromosome. B-E, chromosomes of an individual in which one member of two homologous pairs (14 and 16) have become associated; B, spermatogonium; C, late prophase I.; D, anaphase I.; E, another view of the bivalent 14-16; F, G, isolated bivalent and spermatogonial group from an individual in which both members of the two homologous pairs are associated.

chromosomes composing it, so that the V (AB) goes to one pole, and the two non-linked chromosomes (A and B) to the other (Fig. 68, D).

In the case of the individuals with 33 chromosomes—*i.e.* with two V's, or, in other words, in which linkage has occurred in both homologous couples—we find instead of the open tetrapartite V in metaphase I. a closed tetrapartite ring like the usual type of bivalent formed by syndesis of two V's (cf. *Lepidosiren*, Fig. 16).

Other similar cases could be cited, *e.g.*, *Notonecta* (Browne, 1913). Here $n = 13$ or 14, the former number being produced by the linkage of two chromosomes which are separate in the latter.

Fig. 69 illustrates five types of chromosome complexes found in various species of the genus *Drosophila*, with their possible relationships

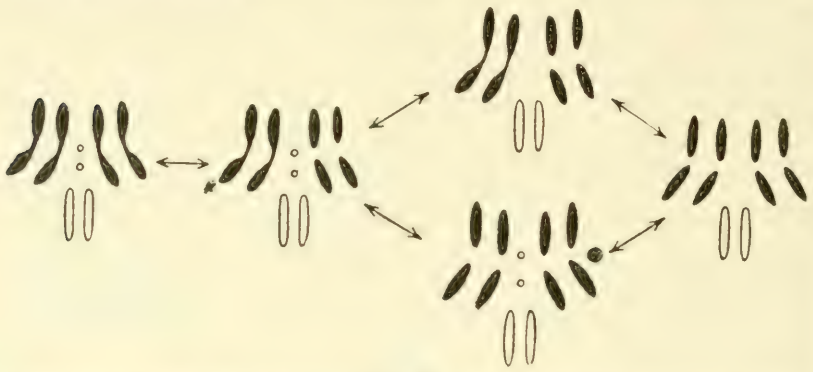


FIG. 69.

Five types of chromosome complex found in the genus *Drosophila*, showing their probable relationships. (After Metz, *J.E.Z.*, 1914.)

(Metz, 1914). The remaining four types are all derivable from type I. by (1) breaking across of one or both pairs of V's, and (2) disappearance, or attachment to another pair, of the pair of very small chromosomes. More recently (1916) Metz has added several other types, all, however, simply related to the above.

So far we have dealt with linkage or fragmentation of chromosomes permanent for the individual or species. A fragmentation of the type chromosomes, leading to a variation of chromosome number in different tissues or cells of a given individual, has also often been observed or inferred.

The classical case is that of *Ascaris megalocephala*, in which the somatic chromosomes undergo fragmentation, so that somatic mitoses exhibit far smaller and more numerous chromosomes (about 60 in *A. m. univalens*) than do those in the germ track (see Chapter III.). It is noteworthy that Payne (1913 *b*) found that subjecting eggs to radium emanation causes the chromosomes in the germ track also to fragment.

In *Ascaris canis* (Walton, 1918) the chromosomes in the somatic cells fragment into two, so that these mitoses have double the number of chromosomes present in the germ track. This seems to be a not uncommon phenomenon. Thus the bisexual form of *Ascaris nigrovenosus* (Schleip, 1912) has in the germ track eleven (♂) or twelve (♀) chromosomes, the somatic tissues having double that number of much smaller ones. Doncaster (1910) found one male of the gall-fly *Neuroterus lenticularis* in which the number of chromosomes in the somatic tissues was double that in the spermatogonia. In the bee (*Apis mellifica*—Meves, 1907; Nachtsheim, 1913) $2n$ is 32, judging from the meiotic divisions, but the somatic cells often show 64. Armbruster (1913) found also in the case of the solitary apid *Osmia cornuta* that the number of chromosomes is much higher in the mitoses of the soma than in those of the germ track.

Sometimes the process of fragmentation is less orderly, affecting only certain cells, and these to varying degree, resulting in apparently capricious variation of chromosome number. This has been studied by Hance in the pig (1918 *a*) and in *Oenothera* (1918 *b*).

In the pig the spermatogonial chromosomes always number 40. In the ninety-one somatic cells in which the chromosomes were counted, the number varied from 40 to 57. It will be noticed that none had less than the type number. Most of them had more, only four having exactly 40. In order to obtain evidence as to whether the increase is due to fragmentation or multiplication, Hance measured the sum of the lengths of all the metaphase chromosomes in the spermatogonia and in the somatic cells. In the former the sum of the lengths of all the chromosomes varied from 118·6 to 177·6 units of measurement. The combined lengths of all the chromosomes in the different somatic cells, in spite of the variation in their number, fell within the same limits, with one exception in which they totalled 117 units. This evidence, while not conclusive in view of the variation of chromosome lengths in different tissues, the possibility of regulations, and so on, is certainly greatly in favour of the numerical increase having been caused by fragmentation of the 40 " type " chromosomes.

Closely similar results were obtained from *Oenothera scintillans*. In this evening primrose $2n = 15$, it being, like *O. lata* (p. 146) one of the *Oenothera* mutants which possesses an extra chromosome. In the somatic tissues, however, while the number 15 is the commonest, it varies from 15 to 21. By measuring the lengths of all the chromosomes and adding them together, he found that the average total amount of chromatin was approximately the same in nuclei with 15 chromosomes as in those with 16, 17, 18, 19, 20 or 21. Hence he concludes that the higher numbers have been derived from the lower by fragmentation of one or more of

the " type " chromosomes. It is significant that he found no fragmentation in the germinal tissues.[1]

It is plain that variation in chromosome number by linkage or fragmentation is not by any means incompatible with the general thesis laid down on p. 123. The primary object of the arrangement of the units in linear series, which is to allow of their accurate distribution to the daughter nuclei after fission in mitosis, is not in the least prejudiced thereby. Syndesis of homologous chromosomes, or rather of the units composing them, would, however, certainly be complicated if irregular fragmentation were to occur in the germ track. It is precisely here, however, as the examples above quoted suffice to show, that the chromosome number is particularly constant. Only a few cases of fragmentation or linkage within the germ tracks of individuals have been described, and these are mostly of a simple and orderly nature. The evolution of a species with a chromosome number differing from that of the parent species must indeed have been accompanied by a rearrangement of chromatin units, or by a fragmentation or linkage of existing chromosomes. This case, however, goes far to furnish proof of the necessity for the constancy of the linear arrangement, since there is strong reason to believe that interspecific sterility arises through incompatibility of the chromosomes of the two incipient species in syndesis (Chapter VI.).

The remaining two causes of variation in chromosome number do not call into question the continuity of the chromosomes, since they concern the multiplication or disappearance of whole chromosomes.

(2) *Variation in Chromosome Number due to Irregularities of Mitosis*

This is an abnormality which, if leading to an extensive loss of chromosomes, must lead eventually to the death of the cell in which it has occurred. Since each diploid nucleus contains a double set of chromosomes, and since one set contains all the units required for a perfect organism, it follows that one member of each homologous pair might be lost without much disturbance, but if both members of a pair disappeared serious consequences must result.

Though mitotic irregularities usually lead to a loss of chromosomes owing to one or more of these failing to get included in the daughter nuclei, they sometimes have the effect of increasing the number. Probably the earliest of these cases to be described was in *Ascaris megalocephala bivalens* (Fig. 70). Boveri (for summary see 1904) found that sometimes the spindle of the first meiotic division in the egg is placed tangentially

[1] This perhaps is an indication of a differentiation between soma and gonad as displayed in the germ tracks of so many animals.

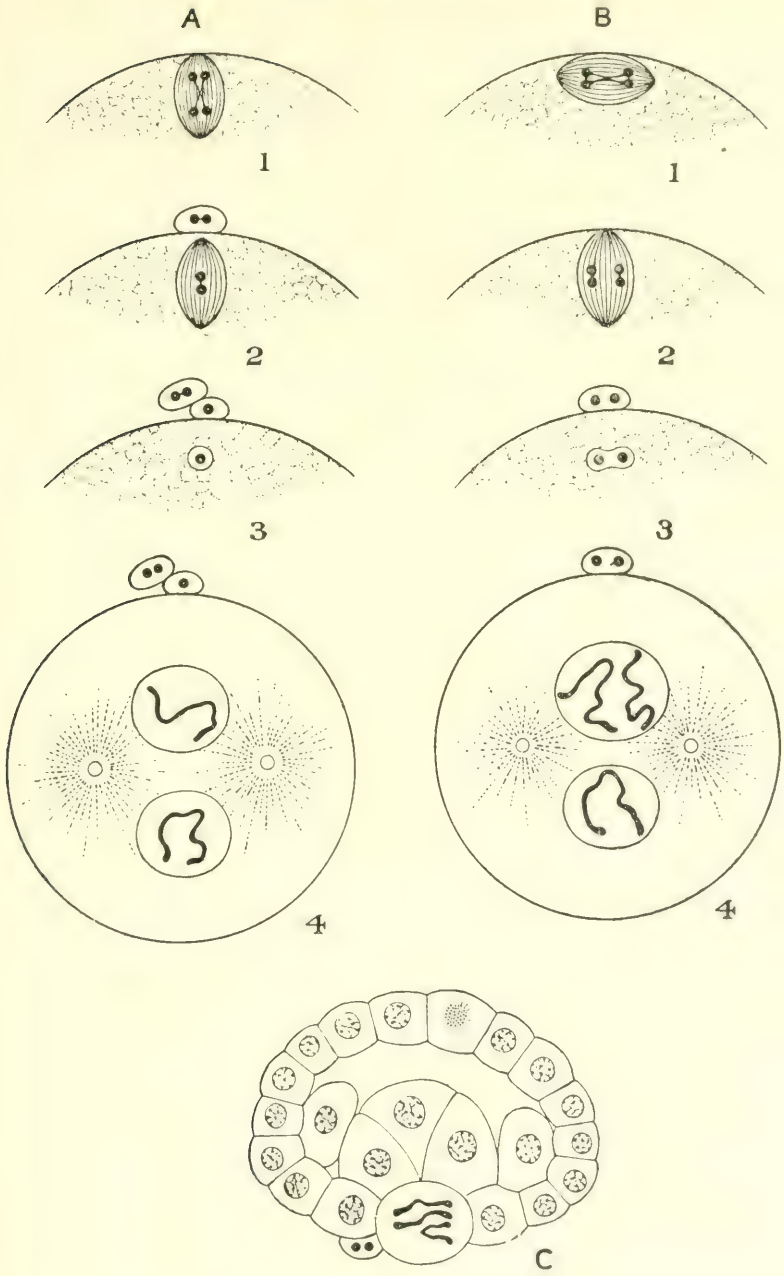


FIG. 70.

Normal and abnormal polar body formation in *Ascaris megalocephala univalens*. (Boveri, *Ergebnisse*, 1904.) A, normal series; B, abnormal series. In each, 1 is anaphase I.; 2, metaphase II.; 3, polar body formation completed; 4, fertilization, the ♂ nucleus below in each figure. C, embryo derived from series B, showing three chromosomes in the germ track, and only one polar body, with two chromosomes.

instead of radially. Hence the first polar body is not extruded, but all the chromosomes—*i.e.* two bivalents—remain in the egg. At the second meiotic division all these chromosomes enter the spindle, with the consequence that the egg and the single polar body are each left with two univalents instead of one. When the egg is fertilized, the zygote nucleus has therefore three chromosomes, two from the egg and one from the spermatozoon. These three chromosomes appear regularly in all the subsequent mitoses in the germ track. As the polar bodies in *Ascaris* are conspicuous objects for a considerable time in the early development of the embryo, the fact can be verified that these rare embryos with three chromosomes have been produced in this way, for they have only one polar body (containing two univalents) instead of two (one with one bivalent and the other with one univalent).

Some of the *Oenothera* mutants, *e.g.*, *O. lata*, probably owe their origin to an irregular distribution of chromosomes in meiosis, since they differ from the parent forms in possessing an additional chromosome ($2n = 15$ instead of 14). *O. lata* has been produced several times among the *Oenothera* mutants, occurring in the frequency of about ·4 per cent (Gates and Thomas, 1914). It presumably owes its origin to the union of a normal gamete with one bearing eight instead of seven chromosomes. In the meiosis of this form the chromosomes are normally separated in groups of seven and eight, though sometimes more irregularly, the anaphase groups consisting of six and nine chromosomes respectively, etc. (Gates and Thomas, 1914). Thus forms arise with still more varied chromosome numbers, when gametes bearing these abnormal numbers of chromosomes participate in syngamy.

In view of that dependence of somatic characters upon the chromatin elements which is required by our thesis, it thus becomes of great interest to know what are the characteristics of the offspring produced by *O. lata*. For causes at present obscure, this plant produces practically no fertile pollen grains. Consequently its behaviour in breeding can only be studied in crosses in which other forms are the male parents. Crossed with *O. lamarckiana*, in which $2n = 14$, it gives a mixture of *lamarckiana* and *lata* offspring (de Vries, 1910). This of course is in accordance with expectation, since all the microgametes have seven chromosomes, and some of the macrogametes seven and others eight. As we have just seen, however, the fifteen chromosomes of *O. lata* do not always separate into seven and eight at meiosis, but sometimes into more unequal groups. Lutz (1912) determined the number of chromosomes for fifty-two offspring resulting from a cross between *O. lata* and *O. gigas*. In the latter $2n = 28$. The numbers of chromosomes ($2n$) found, together with the numbers of plants exhibiting each number, were as follows :

Number of Chromosomes	15	21	22	23	29	30
Number of Plants . .	2	16	25	3	2	4

This variation is probably due to variation in the *lata* gametes, since *O. gigas* breeds true and therefore presumably always has the expected fourteen chromosomes in its gametes. It will be noticed that the most frequent numbers are 21 and 22, which corresponds with the above-mentioned fact that the gametes of *O. lata* most commonly have seven or eight chromosomes. These combining with the fourteen of the micro-gamete produce of course these two diploid numbers. The six plants with 29 and 30 chromosomes are probably examples of a phenomenon to be described in the next section; namely, they have presumably been produced by fertilization of *diploid* egg cells.

(3) *Variation in Chromosome Number due to Multiplication or Fusion*

Longitudinal fission of the chromosomes, as in ordinary prophase, but without subsequent mitosis leading to separation of the daughter halves, has frequently been described. By this means giant nuclei are formed containing twice the normal number of chromosomes if the process has taken place once, four times if it has happened twice, and so on.

One of the most striking cases described is that of *Culex pipiens* (Holt, 1917). Here $2n=6$. During metamorphosis, while the alimentary canal of the pupa is developing, the chromosomes in the cells of the old larval alimentary canal undergo repeated fission, forming nuclei with a large number of chromosomes, but always in multiples of three, and generally in multiples of six. Nuclei were found, for example, with 9, 12, 18, 24, 36 and 72 chromosomes. Nuclei with this enormous increase of chromosomes can proceed to apparently normal mitosis. In telophase the chromosomes fuse into three masses (it will be remembered that in the normal somatic mitoses of *C. pipiens* the pairing of the homologous chromosomes is very pronounced, so that three double chromosomes result, p. 126), from each of which in the next prophase one chromatin filament is formed, which divides up by multiple longitudinal fission so that three groups of chromosomes are produced. The chromosomes within each group are of closely similar lengths, though the length type is different in the different groups.

These cells, being in the larval alimentary canal which is being replaced by that of the pupa, are destined to perish. Nuclei with a multiple supply of chromosomes seem often to be produced in this way, however, and the process as such does not seem to have a necessarily deleterious effect.

Multiplication of chromosomes has been obtained by experimental methods by Němec (1904, 1910). If the root tips of plants (*Vicia*, *Pisum*, *Allium*) are subjected to the narcotizing action of chloral hydrate, any cell divisions that are in progress at the time are inhibited to this extent, that the telophase reconstruction of the nuclei takes place, but cell division is not completed. Thus binucleate cells are formed. If the root tips are allowed to recover from their narcotization, the two nuclei, which lie close together, may fuse into a single giant nucleus. This may proceed to mitosis, in which case it exhibits $4n$ chromosomes.

If the root tips are repeatedly narcotized, being allowed to recover after each narcotization, the above process may also be repeated, resulting in giant nuclei with $8n$ or probably even $16n$ chromosomes.

From the fact that such *polyploid* nuclei become fewer in proportion to the length of time that has elapsed since the plants recovered from the narcotization, Němec concludes that a reduction to the normal number of chromosomes ultimately takes place, and indeed found certain mitoses which he believed to be the reduction divisions; this interpretation is, however, necessarily uncertain.

There are many cases known where of two nearly allied species one has double the number of chromosomes of the other, and the possibility naturally suggests itself that the chromosome number may have been doubled in this way, namely, by a fission of the chromosomes unaccompanied by nuclear division. Forms thus derived would obviously have four sets of chromosomes; that is to say, they would be *tetraploid* instead of diploid in their somata, and diploid instead of haploid in their gametes. It must not, however, be assumed without special evidence that any species is tetraploid, since in the great majority of cases where one species has twice as many chromosomes as nearly related species, the larger number has almost certainly been derived from the smaller either by the aggregation of the chromomeres into double the number of chromosomes or by the transverse fragmentation of all the chromosomes into two—as, for instance, happens in the somatic cells of *Ascaris canis* and the other examples mentioned on p. 143. If transverse constrictions in chromosomes are signs of a weak point where fragmentation is liable to take place, there is no difficulty in understanding how the number of chromosomes comes to be exactly doubled, for it may happen that every chromosome exhibits this constriction (*e.g. Lepidosiren*, p. 40), and there are very few references in literature to a chromosome exhibiting more than one such transverse joint.

Out of the numerous cases of numerical doubling of chromosomes known, the following few striking examples may be quoted:

Species.	Number of Chromosomes (Diploid).	
Ascaris megalocephala univalens	2	Many authors.
,, ,, *bivalens*	4	
Cypris reptans, parthenogenetic	12	Schleip, 1909.
,, *fuscata*, ,,	24	
Ophryotrocha puerilis, some individuals	4	Korschelt 1895.
,, ,, other ,,	8	
Oenothera lamarckiana	14	Various authors.
,, *gigas*	28	Gates, etc., 1909 *a*.
Musa sapientum (the banana), one variety	16	Tischler, 1908.
,, ,, another variety	32	
,, ,, ,,	48	

In a species in which distinct size differences exist between the different chromosomes, the question of tetraploidy should be capable of easy solution, since in this case the chromosomes should be grouped according to sizes in fours instead of in pairs. Practically nothing seems to be known about this matter in supposed tetraploid forms. Montgomery (1909), however, has described the chromosomes of the *bivalens* variety of *A. megalocephala* as consisting of a longer and a shorter pair. The difference between the pairs is indeed slight, but if established it would make it probable that this form is not tetraploid. This case is especially instructive, as here if anywhere one might have expected the four chromosomes to have been derived by doubling of the two chromosomes of *univalens*, since the total volume of the four chromosomes in *bivalens* is even more than double that of the two in *univalens* (Brauer, 1893).

Two genera of the Oligochaete family Enchytraeidae present similar relations (Vejdovsky, 1907). In *Fredericia hegemon* $2n=32$, and in *Enchytraeus humicultor* $2n=64$. Nevertheless, the chromosomes in the latter genus are much longer and thicker than in the former.

In certain cases, however, especially those in which the forms with doubled chromosome number have arisen under experimental conditions, the comparison of the volumes of the chromosomes is of value in deciding how the doubling has occurred. Two such cases are found in the plant genera *Oenothera* and *Primula*.

Oenothera gigas is one of the well-known " mutants " of *O. lamarckiana*. It first appeared as a single individual in de Vries' cultures of 1898, and in the next twenty years the appearance of six more individuals was recorded from the cultures of de Vries and others, so that its origin from the parent form was observed altogether seven times in that period. Once arisen, it breeds pure to its peculiar characteristics (except for a tendency to give off exceptional mutants of the same order as those produced by the parent species). These characteristics are the possession of 28 chromosomes in place of 14, and the greater size of nearly all its parts, such as stalk, leaves, petals, etc.

Gates (1909 *a*) has compared the relative sizes of the cells in *O. gigas*

and *O. lamarckiana*. Those of *O. gigas* are 1·5 to 3·8 times as large as those of *O. lamarckiana*, averaging about 2·5 times as large (cells of anther epidermis). Since in general the size of the cell is proportional to the surface of the nucleus, which again is proportional to the volume of chromatin (Boveri, 1904, 1905), we may assume that *O. gigas* has twice as much chromatin as *O. lamarckiana*, and therefore that its 28 chromosomes comprise a double set of those present in the parent form. *O. gigas* is probably therefore a true tetraploid species.

The origin of such a form as this can be conceived as being due either to (1) syngamy of two diploid gametes, *i.e.* gametes in whose formation the meiotic division has been omitted; or (2) a doubling of the chromosomes in the zygote nucleus owing to fission of the chromosomes not followed by mitosis.

It is at present impossible to decide between these alternatives. As we have seen, a doubling of the chromosomes may take place under certain experimental conditions (p. 148), and there appears to be no reason to deny that this might happen in nature under abnormal conditions. If the doubling occurred in the undivided zygote cell, it might result in the whole individual being tetraploid. On the other hand, diploid egg cells are normally formed in obligatory parthenogenesis and may possibly occur, and be capable of fertilization, in sexual reproduction. Diploid microgametes are, however, not known to occur, though the giant spermatozoa occasionally found in many animals are sometimes supposed to be due to the omission of the meiotic division.

If diploid male and female gametes do occur, they are certainly very rare, and it must be still more rare that two such gametes should meet. In the enormous majority of cases a diploid gamete must meet a normal haploid gamete. This, if syngamy took place, would result in a triploid zygote. Triploid *Oenotheras*, with 21 chromosomes, have indeed frequently been described (Lutz, 1912; Stomps, 1912). They have been observed as "mutants" both in pure cultures of *O. lamarckiana* and from crosses between the various *Oenothera* mutants. Their characteristics vary to a certain extent according to their origin, but in their general growth they appear to stand mid-way between *O. gigas* and the ordinary diploid *Oenotheras* (Stomps, 1912).

Stomps estimates that three triploid ("Hero") plants are found among about 1000 plants in certain *Oenothera* cultures, and that *O. gigas* appears at most in the proportion of 1 : 10,000, though the total number of *O. gigas* mutants known is too small for the percentage to be reliably estimated. So far as the figures go, however, the greater rarity of tetraploid forms compared with the triploid varieties favours the view that they have arisen through syngamy of diploid gametes, rather than from a multiplication of the chromosomes in the zygote.

While *O. gigas* is probably a true tetraploid form, a case of chromosome doubling has been described by Farmer and Digby (1914), in which it appears probable that the doubling was produced by fragmentation. *Primula floribunda* crossed by *P. verticillata* gave a hybrid of a distinct type known as *P. kewensis*. In both parents $2n = 18$, and this was also the number in the hybrid. The hybrid plant produced only "thrum" flowers, and was therefore self-sterile. It was reproduced vegetatively by cuttings, and eventually a single "pin" flower appeared, which allowed of fertilization by one of the thrum flowers, and from this a fertile race of *P. kewensis* was obtained. This race was found, however, to have double the number of chromosomes present in the original *P. kewensis*, *2n* being 36. The chromosomes are also smaller in the fertile race, measurements giving the following results:

	Mean volume of single Chromosomes.	Total volume of all Chromosomes.
Race with 18 chromosomes	·8141	14·65
Race with 36 chromosomes	·4088	14·71

These measurements clearly suggest that in this case the doubling of the chromosome number has been brought about by transverse fragmentation, and not by chromosome fission unfollowed by nuclear division. A true tetraploid *Primula* has, however, been found in another case, as described in the next chapter (p. 170).

The last cause of variation in chromosome number which we have to consider is fusion of chromosomes. An example of this has already been given in the case of *Culex pipiens* (p. 126). It is obvious that here we are dealing with an *apparent* variation only, since all the chromosomes are present, though they may be indistinguishably fused in pairs.

A Sycon sponge (Jorgensen, 1910 *a*) probably presents a case similar to *Culex*. In this sponge the number of chromosomes in the gamete is eight, and in accordance with this sixteen chromosomes are found in the metaphase of the first cleavage division. In all other cells examined, however (mesoderm cells, oogonia, oocytes), the number is always eight, except in the early prophases, when it is more, though the number could not be exactly counted. Jorgensen brings forward evidence to show that a fusion of pairs of homologous chromosomes takes place in the telophase of the first cleavage division.

A particular class of cases of the halving of the chromosome number falls to be mentioned here. A union of the chromosomes—now in the haploid number—in pairs sometimes takes place in the anaphase of the first meiotic division or between the two divisions. This has been described in Birds (*Numidia* and *Gallus*—Guyer, 1909, 1916; *Columba*—Smith, 1913), Mammals (Man—Guyer, 1910; *Didelphys*—Jordan, 1912);

Lepidoptera (*Phragmatobia*—Seiler, 1913); Hymenoptera (*Osmia cornuta*—Armbruster, 1913).

The chromosome numbers in prophase are as follows in these cases :

	Spermatogonia.	Spermatocyte I.	Spermatocyte II.
Man	22[1]	12	5 or 7
Didelphys virginiana	17[2]	9	4 or 5
Numidia meleagus	17[3]	9	4 or 5
Gallus gallus	18[4]	9	4 or 5
Columba	16	8	4
Phragmatobia fuliginosa	[56][5]	28	14
Osmia cornuta	16[6]	16	8

The only cases in which we have any further information as to the behaviour of these secondarily united chromosomes are *Phragmatobia* and *Gallus*. In these the secondary spermatocyte pairs are resolved into their elements (though only exceptionally in *Gallus*) before the second meiotic division takes place. It is to be presumed that in the other cases mentioned they also separate before syngamy.

Another instance of pairing between chromosomes in a haploid nucleus is found in the spermatogonia of the bee (see p. 91, footnote). In the female bee, according to Nachtsheim (1913) the 32 chromosomes unite into 16 bivalents in the oogonia. In oocyte I. they pair a second time to form 8 tetravalents. The mature egg has therefore 8 bivalents, which, however, fall apart into univalents about the time of fertilization.

At present the above cases cannot be said to fit without forcing into any general scheme of chromosome behaviour. Probably, however, fusion of the chromosomes should be regarded, not as a *pairing* strictly speaking, but as a general tendency to fusion which usually stops short at fusion in couples. Cutler (1918) found that in the pheasant, where $2n$ is probably 20-22, the number of chromosomes in the first meiotic division is 10-11, while in the secondary spermatocytes 1-8 masses of chromatin appear, indicating a tendency to fusion carried to various degrees. Even in *Gallus*, though the fusion in couples is usually complete, giving 4 chromosome masses in secondary spermatocytes which lack the X-chromosome, and 5 chromosome masses in those which contain it, yet metaphases II. with six or seven such masses are not unusual.

[1] Two are sex chromosomes. Other workers, however, have given the number of chromosomes in man, in whom they are very difficult to count, in varying figures up to 40.

[2] One is a sex chromosome.

[3] One is a sex chromosome. The exact number of chromosomes was, however, impossible to determine beyond doubt. In view of Guyer's more recent conclusions as regards *Gallus* it seems probable that there are two sex chromosomes, with a total of 18 chromosomes.

[4] Two are sex chromosomes.

[5] Spermatogonial number inferred from the number of bivalents in meiosis.

[6] This is the haploid number, the case being parallel with that of the bee (p. 91).

CHAPTER VI

HEREDITY AND MORPHOGENESIS

In this chapter we have to consider the thesis that the nucleus and not the cytoplasm is the substratum by which hereditary qualities are transmitted from parent to offspring, and the correlative hypothesis that it is the nucleus which is the initiator and controller of the activities of the cell and especially of morphogenesis. It is obvious that these two theses are mutually interdependent, and if either were established the other would follow as a corollary without the necessity of further proof. In the meantime, any evidence obtainable for or against the one is in equal degree evidence for or against the other, and therefore the two may be considered together.

Both these theses are capable of expansion far beyond the limits of a volume of this scope, and have, moreover, so often been made the subject of special treatises that they can only be dealt with briefly here.

The student must also realize that in this chapter the amount of theory bulks larger in proportion to the amount of fact than in the preceding chapters, and therefore that it is all the more necessary for him to keep in mind the proper scientific spirit which is always ready to modify its ideas when the discovery of new facts makes this necessary.

The principal threads of evidence under this head may be classified as follows :

(*a*) The equality of inheritance from male and female parents.
(*b*) The process of mitosis and its implications.
(*c*) The process of meiosis.
(*d*) The parallel which exists between chromosome behaviour and the results of breeding experiments.
(*e*) The case of sterile and partially sterile hybrids.
(*f*) Morphogenesis and mode of action of the nucleus.
(*g*) Chromidia and chondriosomes.

We will consider these points in order.

A. THE EQUALITY OF INHERITANCE FROM MALE AND FEMALE PARENTS

The fact that, on the average, offspring inherit with approximately equal intensity from both parents, whereas the macrogamete as a whole is nearly always enormously larger (often a million or even more than a billion times larger) than the microgamete, immediately suggests that the hereditary substratum is not the substance of the gametes as a whole, but some special portion of them which is of more approximately equal mass in the two cells. This consideration led Nägeli in 1884 to postulate two substances in the gametes, one of which is present in equal amount in the micro- and macro-gamete. This is the bearer of hereditary qualities—the *idioplasm*. The other has mainly a nutritive function and is present in far greater amount in the egg and is indeed responsible for its larger size. Knowledge of the processes of fertilization naturally led to the idioplasm being identified with the nucleus (independently by O. Hertwig and Strasburger in 1884), since the nuclear substance appears to be the only one that is contributed in approximately equal amounts by the two gametes. Mere study of the anatomy of the gametes therefore at once leads us to suspect the all-importance of the nucleus and the essential passivity of the cytoplasm in the transmission of hereditary qualities.

It is of course not necessary to assume that equal quantities of living matter produce equally powerful effects, nor that inequal masses cannot produce equal effects. It is certain also that the amount of chromatin in the nucleus cannot always be taken as denoting the amount of idioplasm therein. Nevertheless, in corresponding stages of the life-cycle such as the ripe male and female gametes, one would expect to find approximate mass equality of the idioplasm, at any rate not the enormous disproportion that exists between the cytoplasm of the egg and that of the spermatozoon.

The statement just made, that the amount of chromatin in the nucleus cannot always be taken as an indication of the quantity of idioplasm is supported by many considerations. Thus the amount of chromatin in the nucleus varies greatly in more or less closely allied forms (Fig. 71). We cannot suppose that the amount of idioplasm in the nucleus of *Lepidosiren* is much greater than in that of the salamander, and enormously greater than in the nucleus of a rabbit. Moreover, the amount of chromatin varies in different tissues of the same organism, or even in different periods of the life-cycle of the same nucleus. Rückert pointed out long ago that the amount of chromatin in the oocyte nucleus in the growth period (Selachians) is very much greater than the combined bulk of the chromosomes on the spindle of the first maturation division,

though here a complete complement of hereditary substance is bound to be present. This is probably a universal rule in oogenesis (cf. Fig. 22, etc.). Thus Gardiner (1899) calculated that in *Polychaerus* not more than $\frac{1}{500}$ part of the chromatin of the germinal vesicle at its most chromatic stage is used up in the formation of the chromosomes of the meiotic divisions.

In the sea-urchin *Strongylocentrotus*, Erdmann (1909) finds that the volume of the chromosomes in the pluteus is only $\frac{1}{96}$ of their volume in the two-cell stage.

Such considerations have led to the hypothesis of two kinds of chromatin—*idiochromatin*, the essential hereditary substance, or idioplasma proper, and *trophochromatin* (see later under chromidia). Prob-

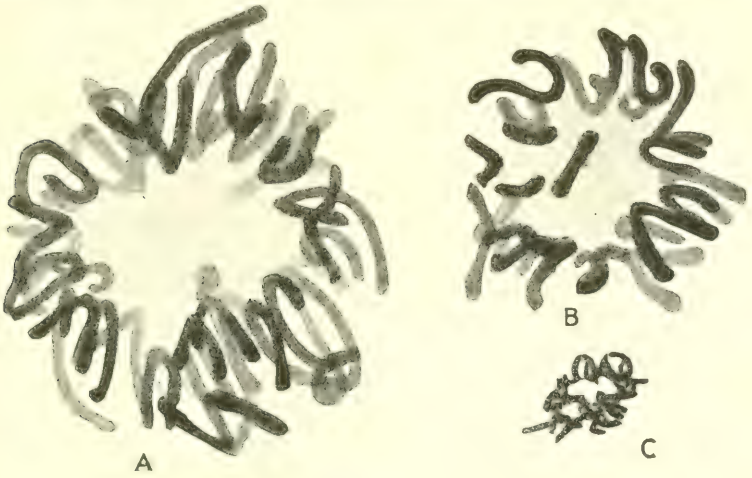


FIG. 71.

Equatorial plates of spermatogonia of three Vertebrates. A, *Lepidosiren*; B, salamander; C, rabbit. The three figures are drawn to the same scale to show the relative amounts of chromatin present in each.

ably, however, the idioplasm cannot, strictly speaking, be identified with any *substance*. The hereditary factors should probably be considered as *elementary organisms*, consisting mainly indeed of chromatin but possessing an organic structure on which their activities depend.

The argument therefore from equality or otherwise of mass, though weighty, is not conclusive in deciding the claims of the various constituents of the gametes to be considered as the idioplasm. There is no doubt that in animals, at any rate, a certain very small amount of cytoplasm is introduced by the male gamete along with the nucleus (see also under chondriosomes, below). This has, however, been denied in the case of some of the higher plants where it has been held that no cytoplasm enters the egg cell with the nucleus (Strasburger, 1908), but a negative is proverbially hard to prove.

The statement made above, that inheritance from male and female parents is on the average of approximately equal intensity, besides being a matter of common knowledge, is established beyond question experimentally. Contrary opinions have, however, been expressed by workers in one special field of biology—namely, the study of hybrids (especially of Echinoderms) in embryonic or early larval stages, where it has been held that the influence of the female parent is predominant. From this the conclusion has been drawn that the cytoplasm also contains idioplasm.

The Echinodermata have shown themselves to be very adaptable to these experiments, crosses being comparatively easily effected between species, genera or even classes, and an efficient technique has been evolved for dealing with this work, which has attracted a large number of investigators. Many of these have found that the hybrid embryos and larvae from certain crosses exhibit purely maternal characters. Thus Vernon (1898), making reciprocal crosses between seven different genera of sea-urchins, found that, as a rule, the hybrid larvae (plutei) were of the maternal type. The results varied considerably in this respect, however, in different seasons. Thus the cross *Sphaerechinus* ♀ × *Strongylocentrotus* ♂ gave in May-July mostly maternal larvae, while in December-January the larvae from the same cross were all paternal in type. Shearer, de Morgan and Fuchs have also shown (1913) how the prepotency of one or other species may vary at different times. Their crosses were made between *Echinus esculentus* or *acutus* and *miliaris*. The pluteus larvae of the former two species exhibit, in the older stages, posterior ciliated epaulettes but no masses of green pigment, while the larvae of *miliaris* at the same stage have masses of green pigment but no posterior epaulettes. In 1910–11 crosses between *esculentus* or *acutus* and *miliaris*, in whichever direction they were made, produced larvae resembling the species used as female parent (in regard to these two features). In 1912, however, similar crosses gave a different result, *miliaris* appearing practically incapable of transmitting its characters at all, so that when this species was used as the female parent the larvae resembled the male parent species. This result appeared to be due to something unfavourable to *miliaris* in the environment, since pure cultures of this species, which in 1910–11 were easy to rear, proved very difficult to bring up in 1912.

Godlewski (1906) succeeded in fertilizing *Echinus* eggs by the sperm of *Antedon*, which belongs to a very different class of the Echinodermata. Union between male and female gamete nuclei took place, and the two sets of chromosomes, though of such different origins, appeared to harmonize on the spindle and to co-operate at telophase to form the resting nucleus as in normal fertilization and cleavage. The hybrids

developed as far as gastrulation and in a few cases even reached the pluteus stage. They exhibited solely the characters of the female parent (*Echinus*). Godlewski even managed to fertilize eggs of *Echinus* from which the nuclei had been removed (by shaking) with *Antedon* sperm. These all developed to embryos of pure *Echinus* type. None of them, however, passed the gastrula stage. From these experiments Godlewski argues that the egg cytoplasm and not the nucleus is the determining factor in early development.

Boveri's classical experiments on the cross *Sphaerechinus* ♀ × *Echinus* ♂ led to exactly the opposite conclusion (1896). These larvae are normally intermediate between the two parents. By violently shaking the eggs before fertilization, Boveri succeeded in breaking many of them into two or more fragments, of which of course only one contains a nucleus. On adding *Echinus* sperm to a mass of these broken-up *Sphaerechinus* eggs a culture was obtained which consisted of a mixture of—(1) normal larvae, intermediate between the two parents; (2) dwarfs, also intermediate; and (3) dwarfs, resembling the male parent exclusively. Having previously, by direct observation, ascertained the power of non-nucleated egg fragments to be fertilized and to develop normally, Boveri interpreted these three classes as having originated as follows: (1) from unfragmented eggs, (2) from egg fragments containing a nucleus, (3) from egg fragments containing no nucleus—all of course fertilized by *Echinus* sperm. From this result Boveri draws the conclusion that the nucleus is the sole bearer of hereditary factors. Taking, however, a general view of the results of Echinoderm crosses, it undoubtedly is the case that the hybrids in the larval stages do tend to resemble the female parent more than the male, though the tendency is by no means a universal rule, and though the prepotency of one or other parent is strongly influenced in some way or other by factors of the external environment.

One cause of the frequent prepotency of the female parent in Echinoderm crosses has been discovered by Baltzer (1910) in a work which is of the greatest importance in interpreting the results of crosses between distantly related forms. To take the case of two Echinoderm genera, *Strongylocentrotus* and *Sphaerechinus*, the cross *Sphaerechinus* ♀ × *Strongylocentrotus* ♂ gives embryos which develop regularly and without pathological phenomena into plutei, which as regards the skeleton are intermediate between the two parent species. Cultures from the reciprocal cross, however, *Strongylocentrotus* ♀ × *Sphaerechinus* ♂ run a different course. When the hybrid embryos reach the blastula stage, instead of turning into a hollow sphere, the cavity (blastocoele) becomes filled with masses of degenerating cells and nuclei, and the larvae, which should be transparent, become in consequence opaque and the great

majority die. Those that reach the pluteus stage are found to exhibit exclusively the characters of the female parent (*Strongylocentrotus*).

Cytological examination of these hybrids (*Strongylocentrotus* ♀ × *Sphaerechinus* ♂) provides a satisfactory explanation both of the pathological course of development and of the purely maternal characteristics of the plutei.

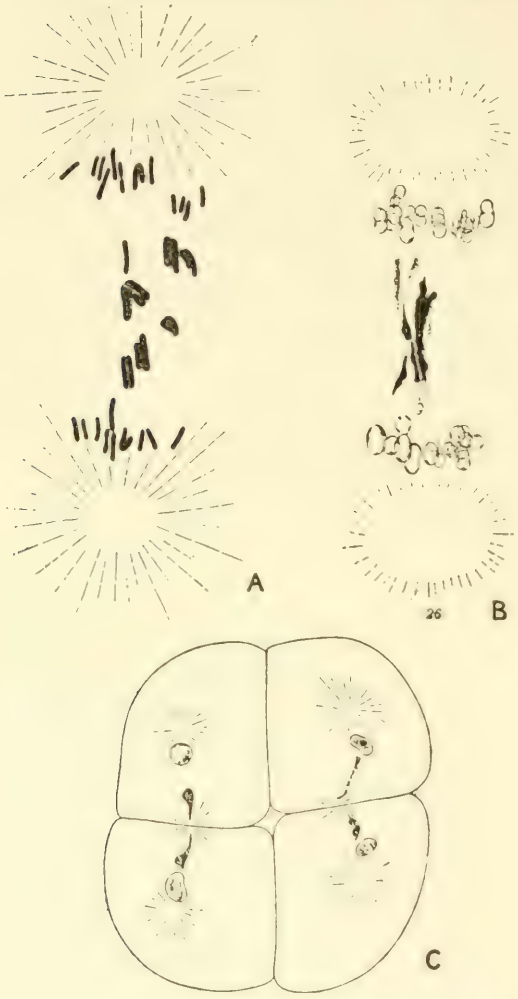


FIG. 72.

The chromosomes of the hybrid *Strongylocentrotus* ♀ × *Sphaerechinus* ♂. (Baltzer, *A.Z.*, 1910.) A, B, anaphase and telophase of the first cleavage division. A number of chromosomes fail to enter either daughter nucleus. C, 4-cell stage. The eliminated chromosomes form irregular masses of chromatin between the daughter nuclei.

In the anaphase of the first cleavage division a number of chromosomes fail to travel up to the poles with the other chromosomes, but remain lying in between the two daughter groups (Fig. 72). They fail to enter into the daughter nucleus, remaining as extra-nuclear masses of chromatin in the blastomeres. A further number of chromosomes get left out in the second cleavage mitosis. The nuclei from now onwards have only about twenty or twenty-one chromosomes (varying in different larvae from nineteen to twenty-four) instead of the expected thirty-six (*n* in both parent species being eighteen). Thus about fifteen chromosomes have been eliminated. These rejected chromosomes are, in all probability, those brought in by the male gamete. For instance, two pairs of the *Sphaerechinus* and one pair of the *Strongylocentrotus* chromosomes are distinguishable by their shapes and sizes. After the elimination we never find these two *Sphaerechinus* chromosomes, but the

Strongylocentrotus chromosome is still present. In regard to these three chromosomes, therefore, there is no doubt that it is the male chromosomes that are eliminated. There are other reasons, which cannot be gone into here, for supposing that this is so in regard to all the fifteen chromosomes which are got rid of. Thus the overwhelming influence of the female parent in the formation of the pluteus skeleton is easily intelligible on the hypothesis that the idioplasm is contained in the nucleus, since the larval nuclei contain eighteen chromosomes from the female parent but only about three from the male.

Furthermore, the pathological phenomena in the blastulae owe their origin to the same process. The eliminated paternal chromosomes do not lie altogether passive in the cytoplasm, but undergo repeated fission, forming great masses of chromatin. At the formation of the blastula these chromatin masses with surrounding cytoplasm are extruded into the blastocoele, where they form the masses mentioned above. In the cross *Strongylocentrotus* ♀ × *Arbacia* ♂, which also results in plutei with almost purely maternal characters, the paternal chromatin also appears to be eliminated, but by an entirely different method. The development of these larvae runs a somewhat similar course to that of the *Strongylocentrotus* ♀ × *Sphaerechinus* ♂ hybrids, only the pathological phenomena in the blastulae make their appearance slightly later.

An examination of the cleavage mitoses shows that there is here no elimination of the chromosomes, all the expected 38 being present at all stages up to the blastula (in *Strongylocentrotus*, $n = 18$; in *Arbacia* $n = 20$). In correspondence with this we find that the size of the nuclei (which is proportional to the number of chromosomes) is about the same in the hybrid larvae as in those of the pure parent species (*Strongylocentrotus*). The nuclei in the pluteus stage, however, have many fewer chromosomes (about 18, judging from direct counts), and correspondingly their nuclei are smaller than those of pure bred nuclei, in the proportion of about 21 : 36. Hence it appears that somewhere between the beginning of the blastula stage and the pluteus an elimination of about 20 chromosomes has taken place. Sections of the blastulae at the time of the critical period of their development show numbers of nuclei obviously pathological, others apparently in the process of extruding chromatin into the cytoplasm, while still others have lying beside them in the cytoplasm an extra-nuclear mass of chromatin. The conclusion to be drawn from these observations is that the *Arbacia* chromatin is eliminated from the hybrid nuclei at this stage. As in the case of the hybrids described above, the blastocoele is filled up with these masses of degenerating chromatin and their surrounding cytoplasm.

While this explanation of the female prepotency in this cross is not quite conclusive (for exceptions occur in the form of plutei, also purely

maternal in character, but with apparently the full complement of chromosomes), it may be accepted as reasonably satisfactory.

Elimination of chromosomes in cleavage of other Echinoderm crosses has also been described by Tennant (1912) and by Doncaster and Gray (1913). The results of Baltzer's crosses can be tabulated as follows:

(1) Development is normal. There is no elimination of chromosomes or chromatin. Plutei are intermediate between the two parents, (*Echinus* ♀ × *Strongylocentrotus* ♂, *Strongylocentrotus* ♀ × *Echinus* ♂, *Sphaerechinus* ♀ × *Echinus* ♂, *Sphaerechinus* ♀ × *Strongylocentrotus* ♂).

(2) Development is pathological. Most of the parental chromosomes are eliminated in the first two cleavages. Plutei are of maternal type (*Echinus* ♀ × *Sphaerechinus* ♂, *Strongylocentrotus* ♀ × *Sphaerechinus* ♂, *Arbacia* ♀ × *Echinus* ♂, *Arbacia* ♀ × *Strongylocentrotus* ♂).

(3) Development is pathological. The chromatin is eliminated in the blastula stage. The plutei are of maternal type (*Echinus* ♀ × *Arbacia* ♂, *Strongylocentrotus* ♀ × *Arbacia* ♂).

(4) Development is pathological. No chromatin is eliminated. The plutei are maternal, either predominantly (*Sphaerechinus* ♀ × *Arbacia* ♂) or completely (*Echinus* ♀ × *Antedon* ♂, *Strongylocentrotus* ♀ × *Antedon* ♂).

It will be noticed that the first three of the above results accord well with the view that the idioplasm is exclusively contained in the nucleus, classes 2 and 3, if finally established, even constituting strong evidence in favour of that hypothesis. As regards the 4th class, the following considerations suggest themselves.

(*a*) All these three crosses, it will be noticed, are between distantly related genera—in the case of the two last indeed, between different classes. Now, granting that the idioplasm is contained in the nucleus, it can only exert its morphogenetic function through, and on, the cytoplasm. When idioplasm finds itself in such a strange environment as cytoplasm belonging to a distantly related genus, or even class, it is not unlikely that it may be unable to exert any of its normal functions, though able to nourish and maintain itself, as is shown by the fact that the parental chromosomes multiply in mitosis with the maternal.

Extreme examples of the behaviour of chromatin in foreign cytoplasm are furnished by the crosses made by Kupelweiser (1909). In these the male chromosomes were unable even to maintain themselves and multiply. He succeeded in fertilizing eggs of *Echinus* with sperm of Molluscs and Annelids (Fig. 73). The sperm nucleus did not fuse with the egg nucleus, but remained practically unchanged in the egg cytoplasm, obviously inert. The egg indeed developed, but the rôle of the sperm in this case was plainly merely to supply the stimulus to development. These cross fertilizations may indeed be considered as another of the methods of bringing about artificial parthenogenesis. Under these

circumstances it is not surprising that the embryos were of the purely maternal type.

(*b*) We have already just emphasized that the nucleus can only exert its morphogenetic function through the cytoplasm. Now the earlier stages of development proceed with little or no increase of cytoplasm. They consist indeed largely in the remodelling of the substance of the egg into that of the embryo. Hence in these very early stages the nucleus of the zygote has little opportunity to exert its morphogenetic function. This can only get full scope after the embryo has begun to form new cytoplasm by assimilation either of food substances supplied from without, or of reserve food material stored within it. Thus the

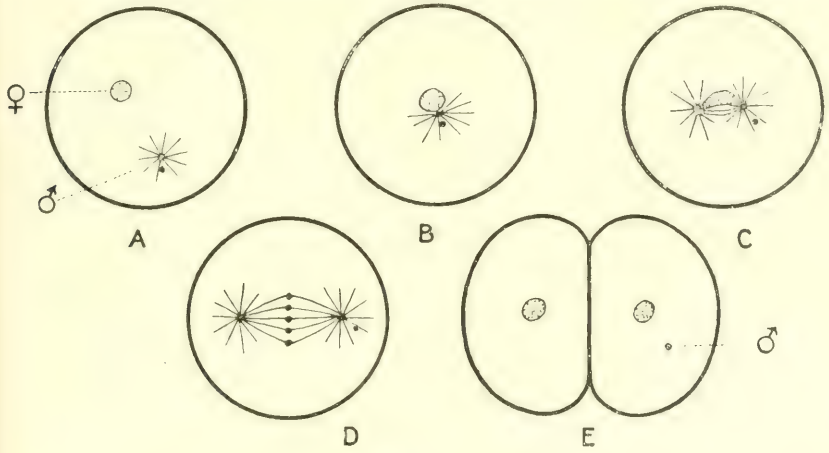


FIG. 73.

Diagram of the fertilization of the egg of *Echinus microtuberculatus* by the sperm of *Mytilus galloprovincialis*. (Kupelweiser, *A. E-M.*, 1909.) A, B, ♂ nucleus, preceded by aster, approaching ♀; C, D, first cleavage nucleus formed entirely from the ♀ nucleus, ♂ nucleus unchanged; E, 2-cell stage. The ♂ nucleus, still unchanged, lies inert in that one of the blastomeres to which cell division has chanced to relegate it.

very early form of the embryo must be determined to a large extent by the physical constitution of the egg cytoplasm.

The resemblance to the maternal species during cleavage, gastrulation, etc., which is brought about by the purely mechanical factors of size and number of yolk granules, viscosity of cytoplasm, etc., could only by a confusion of ideas be brought into the same category as the resemblance to parents due to the presence of the living, self-reproducing idioplasm. To apply the word "Heredity" to both these cases would be to confuse the meanings of the term as used biologically and socially, as is done when a child who has been infected *in utero* with *Spirochaeta pallida* is said to have *inherited* the disease of syphilis.

Finally, it must be mentioned that the only Echinoderm hybrids which have been examined in the adult condition, namely, the offspring of the crosses *Echinus miliaris* ♀ × *Echinus acutus* ♂ (Shearer, de Morgan

and Fuchs, 1914) are found, like all other hybrids, to exhibit certain characters of one parent and certain of the other. MacBride (1911) examined larvae from the cross *Echinocardium* ♀ × *Echinus* ♂ at a much later stage of development than has usually been employed by workers on Echinoderm hybrids, and also found them to exhibit characteristics of both parents.

Summing up, the predominance of maternal characters sometimes found in the young stages of hybrids, mostly between distantly related forms, is reconciled with the equality of inheritance from male and female parents which is the general rule in those vastly more numerous hybrids which have been studied in the adult condition, by (1) the observation of elimination of paternal chromatin from the hybrid nuclei ; (2) the hypothesis of the impotence of idioplasm in a foreign cytoplasm ; (3) (in the case of very early embryos) by the fact that the first stages of development consist in the remodelling of the substance of the egg into the embryo, without the formation of any new substance under the influence of the zygote nucleus.[1]

B. THE PROCESS OF MITOSIS AND ITS IMPLICATIONS. THE FUNCTIONAL DIFFERENTIATION OF THE CHROMOSOMES

The complicated processes of mitosis — the rearrangement of the apparently irregularly distributed chromatin of the resting nucleus into linear series forming long chromatin threads, the longitudinal fission of these involving the division of each element of the series, and the separation of the daughter threads in anaphase, one of each pair going to the one daughter cell and the other to the other—are obviously adapted to ensure the accurate division and distribution of a mass consisting of a number of differentiated elements, such as we must suppose the hereditary substance to be. On the other hand, nothing of the kind is recognizable in the division of the cytoplasm of a mother cell into two daughter cells.

This lack of a distributing mechanism for the cytoplasm at cell division argues an essential homogeneity of the cytoplasm. This does not imply that it may not contain a mixture of different substances nor possess a definite structure, but that it is not composed of localized elements of differentiated function essential for the life of the organism, and incapable of being regenerated if lost. We must qualify this thesis by an apparent exception, of a parallel nature to that already made to the thesis of equality of inheritance from the two parents, which we

[1] See also the discussion on "organ-forming substances," p. 188.

have just discussed, for experimental embryology has established the fact of the local differentiation of the cytoplasm of the egg (the so-called "organ-forming" substances). This matter is discussed on pp. 188, etc.

We have already found morphological evidence of the differentiation of the chromatin in the genetic continuity of the chromosomes, the constant size differences often visible between them, their composition out of chromomeres of different but constant sizes, etc., and it is a necessary corollary of the hypothesis of the dependence of Mendelian phenomena upon the chromosomes (see below under (D)). Direct experimental evidence of the functional differentiation of the chromosomes has been obtained by Boveri (1907) from his remarkable experiments on the development of polyspermic Echinoderm eggs.

Among normally fertilized Echinoid eggs it occasionally happens

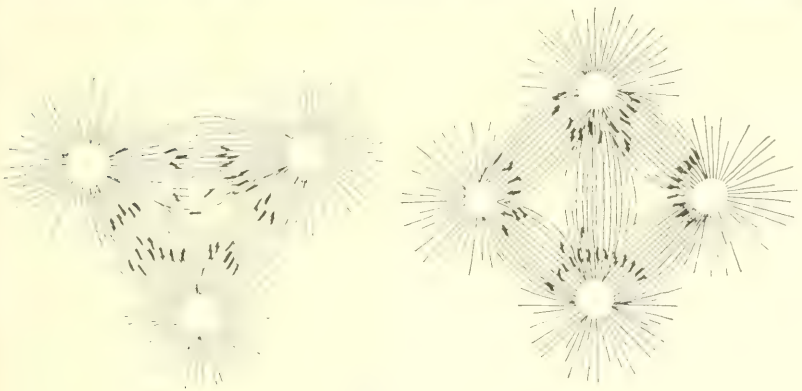


FIG. 74.

Triaster and tetraster mitotic figures from a sea-urchin, *Strongylocentrotus lividus*. (After Baltzer, *Verh. Phys. Med. Gesells*, Wurzburg, 1908.)

that two spermatozoa enter the egg instead of one. By increasing the concentration of sperm, the percentage of such *dispermic* (or polyspermic) eggs can be enormously increased. Thus in two parallel experiments, eggs placed in water with only a few spermatozoa resulted in a hundred normal monospermic and no di- or polyspermic fertilizations. On the other hand, eggs placed in very concentrated sperm gave only eleven monospermic and eighty-nine di- or polyspermic fertilizations.

When an Echinoid egg is fertilized by two spermatozoa both sperm nuclei (typically) fuse with the egg nucleus, and the centrosome introduced by each spermatozoon divides as if it were the only one—hence we get a zygote nucleus with $3n$ (=54) chromosomes and four centrosomes. A four-pole spindle figure is then produced (*tetraster*), and at the first division the egg divides simultaneously into four blastomeres instead of into two.

In the most usual type of tetraster (and the only type which we will

here consider) the two cleavage planes separating these first four blastomeres are identical in position with the two planes formed by the first two cleavages of the normal monospermic egg. Hence the four simultaneously formed blastomeres of the tetraster are identical as regards cytoplasmic contents with the first four blastomeres of the monospermic egg.

By shaking the eggs immediately after fertilization it may happen that the sperm centrosome fails to divide, and in the case of dispermic eggs it often chances to be brought about that one centrosome divides and the other does not. In this case the $3n$ zygote nucleus is provided with three centrosomes and a *triaster* spindle results, the egg dividing into three blastomeres at the first cleavage.

Now, it is obvious that in the tetraster the $3n$ chromosomes—or rather the products of their division—that is to say, $6n$ chromosomes have to be distributed amongst four nuclei; and as the arrangement of the chromosomes on the various parts of the four-pole spindle seems to be by chance, the number and combination of chromosomes received by each nucleus are various. As an illustration, one of the very many possible arrangements in the spindle, and the resulting number of chromosomes in the daughter nuclei, is given in Fig. 75.

Even when a blastomere receives more than n ($=18$) chromosomes there is still a strong chance that it may be lacking in one or more members of the series. Among the four nuclei, the daughters of three members of each series are to be distributed—that is to say, there are two ($3A+3B+3C+\ldots 3R$) to be distributed among the four nuclei. Hence in the vast majority of cases one or more of the blastomeres will lack a representative of one or more members of the complete series of chromosomes. A pictorial example of one out of the many possibilities, showing only four of the eighteen chromosomes, is given in Fig. 75, D, E.

Once the first four blastomere nuclei have been constituted, of course the number and series of chromosomes contained in them will be perpetuated in all the nuclei descended from them.

Now, it has been established by many workers, including Boveri himself, that if the first four blastomeres of the normal Echinoid egg are separated (which can most conveniently be effected by placing the egg in sea water from which the calcium salts are lacking—as discovered by Herbst) *each* will develop into a dwarf pluteus of one quarter the normal size. The plutei generally indeed have minor defects, and the four plutei derived from a single egg may exhibit differences from each other, but these are comparatively slight. Hence the great abnormalities among the embryos developing from isolated blastomeres of tetraster eggs, which will be described immediately, cannot be ascribed to their cytoplasm, since this does not differ from that in the first four blastomeres

of monospermic eggs, which, as we have just seen, develop practically normally. The same applies in principle to the triaster egg.

Boveri found, however, that if the blastomeres of tetraster eggs are separated, some or all of them develop abnormalities which lead to their early death, while very rarely, or never, do they all grow into normal plutei. Moreover, and this is very important, the abnormalities appearing in the four embryos which are derived from the four separated blastomeres of a single egg are often of quite different types. Thus, to take one

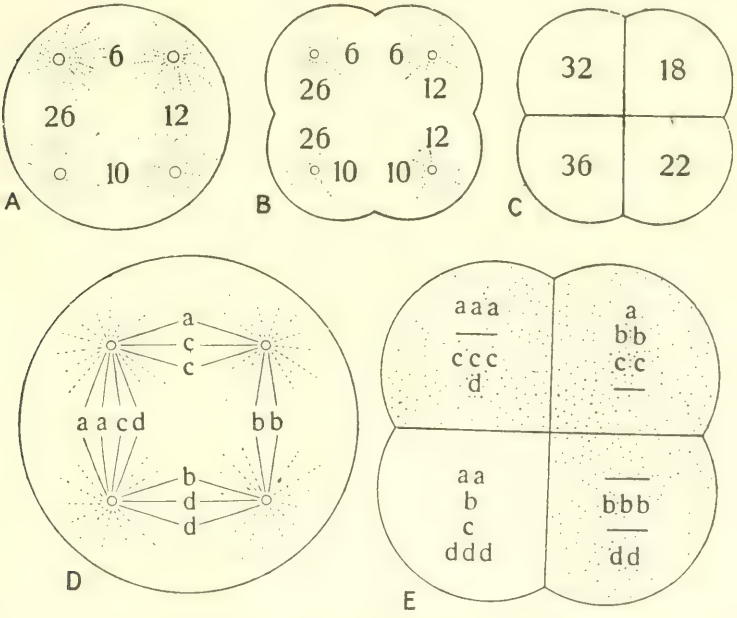


FIG. 75.

Diagram of distribution of chromosomes in the first cleavage division of dispermic sea-urchin eggs. (Boveri, *Zellen-Studien*, 1907.) A, B, C, showing one of the many possible arrangements of the 54 chromosomes on the tetraster spindle figure, and the consequent distribution of the 108 daughter chromosomes between the four blastomeres; D, a possible arrangement of the three sets of chromosomes belonging to the three gametic nuclei (only 4—designated *a*, *b*, *c*, *d*—out of the 18 chromosomes of each gamete are shown); E, the four blastomeres resulting from D. Only the cell in the bottom left-hand corner has a representative of each of the four types of chromosomes, and therefore it is the only cell that can develop normally.

example from the twenty-one given by Boveri, the four blastomeres of one dispermic egg gave :

One good gastrula.

One very thick-walled stereoblastula (*i.e.* blastula with blastocoele filled with cell masses).

One compact clump of cells.

One heap of isolated cells.

Similar differences were found amongst the larvae developed from isolated blastomeres of triaster eggs, only here a far greater proportion of them developed normally. This, it is to be noted, is in agreement with the

fact that the first three nuclei of a triaster have a far greater chance of each getting a representative of all the chromosomes of the series than have the four nuclei of the tetraster.

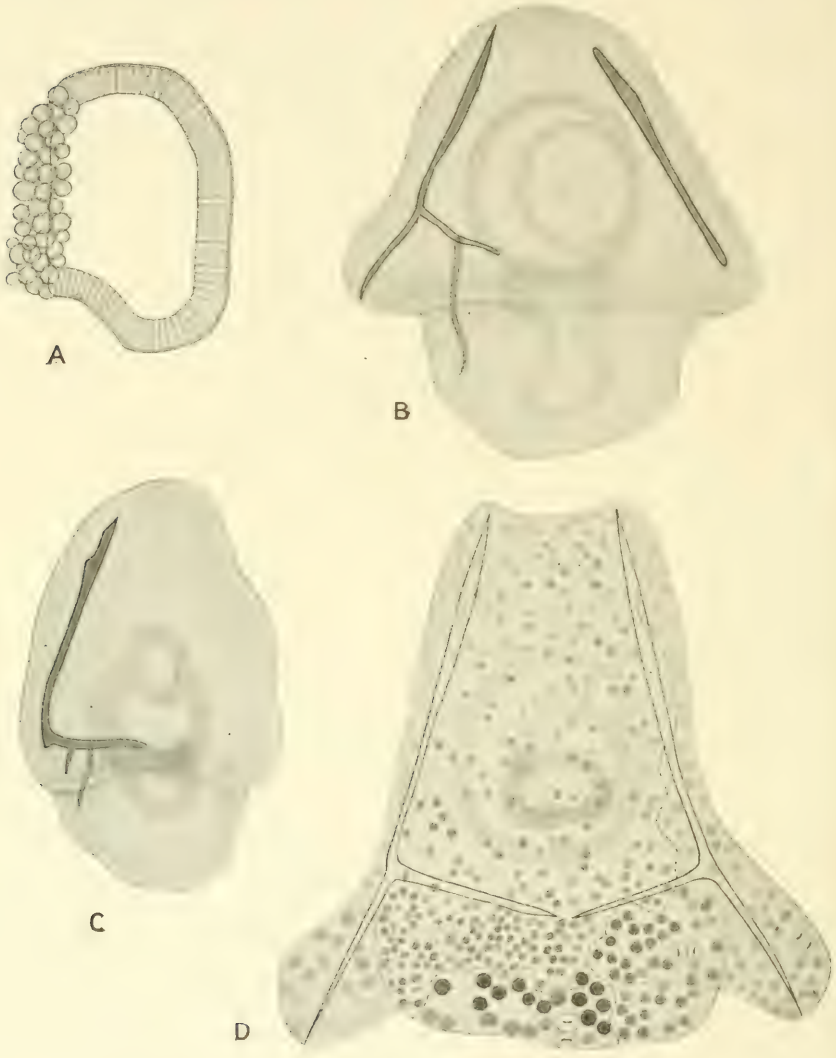


FIG. 76.

Development of dispermic Echinoderm eggs. (After Boveri, *Zellen-Studien*, 1907.) A, blastula of the tetraster type (*Echinus*). About one quarter of the wall of the blastula is falling into separate cells; B, C, D, plutei from triaster eggs (*Strongylocentrotus*). B, with a normal skeleton on the left side, rudimentary on the right; C, with skeleton present on left side only; D, a perfect pluteus. The dotted lines indicate the boundaries of the regions derived from the three primary blastomeres. Note the difference in the size of the nuclei in the different regions.

Boveri also reared tetraster and triaster dispermic eggs as whole embryos (*i.e.* without separating the first four or three blastomeres

respectively) and found that these larvae frequently or generally showed various and different abnormalities in one or more quarters (or thirds) of their bodies. That is to say, the regions of the larval bodies derived from the first four (or three) blastomeres were different from each other (Fig. 76).

Finally, Boveri found that the quarters or thirds derived from different blastomeres often had nuclei of different sizes. Now, as Boveri has shown, in Echinoid larvae the surface of the nucleus is proportional to the number of its chromosomes. Hence the different sizes of the nuclei in various regions of the body give additional evidence that the blastomeres from which they were derived differed as to the number of their chromosomes. To give specific examples: he calculated the number of chromosomes present in the nuclei of four larvae derived from whole triaster embryos and found that the numbers in the three thirds derived from the three primary blastomeres were as follows in the different larvae: (1) 18, 36, 54; (2) 18, 45, 45; (3) 29, 43, 36; (4) 28, 40, 40. Fig. 76, D, shows the first of these larvae.

Now, as we have seen, cytoplasmic differences between the first four or three blastomeres of tetraster and triaster eggs cannot account for the varying manner in which the primary blastomeres develop. It follows therefore that we must look to the nuclei for the cause of this variation. A moment's consideration shows us that it is not the *number* of the chromosomes that is of primary importance, since we have abundant proof that, at any rate, n, $2n$, $3n$ or $4n$ chromosomes are perfectly compatible with a normal organism (see Chapter V., etc.). It is only very rarely that even one, and still more rarely that two, of the primary blastomeres of a tetraster or of a triaster will have less than n chromosomes. Moreover, the four triaster larvae, the number of whose chromosomes are given in the preceding paragraph, were *normal* in all their parts, although the number of chromosomes was so varied. We are left with the conclusion that the cause of the abnormalities is the *lack of certain members of the chromosome series* in certain of the primary blastomere nuclei (cf. Fig. 75, E). The fact that the abnormalities which arise in development are not identical in all the blastomeres of a single egg, is due to the fact that *different* members of the series are lacking in the different blastomeres. This, then, is direct experimental evidence of the functional differentiation of the chromosomes.

C. THE PROCESS OF MEIOSIS

If we take it that the hereditary substance is composed of smaller differentiated elements, each with its own particular function, it is plain that some arrangement must be sought by which a doubling of the number of these elements in each successive act of syngamy is

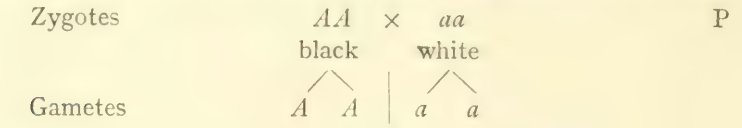
avoided. Moreover, since the elements are differentiated, a mere mass reduction—as, for instance, by diminished growth of the chromatin between mitoses—would not meet the case. Weismann indeed long ago postulated from theoretical considerations the occurrence of a reduction such as takes place in gametogenesis. The process of meiosis has now been universally recognized as peculiarly adapted to bring about the qualitative reduction required. This is especially the case since the discovery that, of the two chromosomes which unite to form each bivalent, the one is derived from the male and the other from the female parent. The consequences of this for the processes of heredity are discussed under the next section (D). The existence of meiosis, and the entire absence of any discoverable analogous process for the cytoplasm, is such an obvious argument in favour of the view that the whole of the idioplasm is contained in the nucleus, that it is unnecessary to enlarge further upon it.

D. THE PARALLEL WHICH EXISTS BETWEEN CHROMOSOME BEHAVIOUR AND THE RESULTS OF BREEDING EXPERIMENTS.

The results of Mendelian work find a ready interpretation in the discoveries of cytologists, and this forms one of the most fascinating chapters of Biology, which, however, can only be treated in brief fashion here.

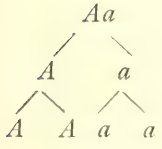
The fundamental step in establishing the parallel—to call it for the moment by no more committal name—between breeding experiments and cytological observations was taken by Sutton (1903), when he pointed out that the nucleus contains a double series of differentiated chromosomes, one series contributed from each parent, and that in syndesis the chromosomes from the one parent pair with the homologous chromosomes of the other.

If we consider the simple Mendelian case, involving one character only—say the feather colour in certain fowls (*A* = black, *a* = "splashed white"; see Bateson, Saunders and Punnett on the Andalusian fowl)—the results of crossing the two forms may be represented in the usual way:



The F_1 and F_2 represent what is actually found in the breeding-pen. To bring the notation into conformity with cytological observation we must suppose that the colour of the feather is dependent on a "factor" or *gene* residing in a chromosome, and we may call this chromosome *A* when the factor is present in the black-producing form, and *a* when it is present in the white-producing condition.

In the case of the original pure (homozygous) black parent, both of the homologous chromosomes are present in the *A* form, and in the white parent in the *a* form. Hence the gametes of the two birds contain one *A* or one *a* chromosome respectively, and the zygote contains both *A* and *a*, *i.e.* it is a hybrid or heterozygote. When this hybrid comes to form its gametes, *A* and *a* being homologous chromosomes, pair together in syndesis, *A* going to one spermatocyte II. (or oocyte II.) and *a* going to the other (or 1st polar body). Thus the *segregation* of the hereditary factors, and hence of the characteristics which they represent, is brought about. In the second division of the meiotic phase each of the chromosomes of course divides longitudinally, and therefore gametogenesis has proceeded (having regard to this pair of homologous chromosomes only) according to the following scheme :



— somatic cell or germ track.

— syndesis.

— spermatocyte II., oocyte II., and polar body I.

— spermatozoon, or mature egg and polar bodies I. and II.

In the male, therefore, we have obviously an equal number of spermatozoa carrying the *A* and the *a* chromosomes respectively, and as in the female it is a matter of chance which chromosome stays in the egg and which goes into the polar body, we have, on an average, approximate equality of the two kinds of female gametes also. If now two of these hybrids are mated together, we get the following possible combinations of gametes, all of which will occur, on the average, in equal numbers :

A ♀ may be fertilized by *A* ♂ = zygote *AA* —black.
A ♀ ,, ,, *a* ♂ = ,, *aA* } 2 greys.
a ♀ ,, ,, *A* ♂ = ,, *Aa* }
a ♀ ,, ,, *a* ♂ = ,, *aa* —white.

If two or more independently inheritable characters are under consideration we can again describe, by an interchangeable notation,

the results of breeding and the distribution of the factors in gametogenesis, on the assumption that these are located in different chromosomes. Thus supposing we are dealing with two characters—say colour (green or yellow) and surface (smooth or wrinkled) of the pea cotyledon—we may call the chromosome in which resides the colour factor by the first letter of the alphabet *A* or *a* in accordance with whether the factor is present in its yellow (*A*) or green (*a*) producing form, and similarly we may call the chromosome which contains the surface factor *B* (smooth) or *b* (wrinkled). Regarding these two chromosomes only, the pure parent plants, say the one green and smooth, the other yellow and wrinkled, possess chromosomes *aaBB* and *AAbb* respectively, their gametes being of course *aB* and *Ab*, and the hybrid offspring *AaBb*.

Now in the meiosis of this hybrid, chromosomes *A*-*a* pair together in syndesis and likewise *B*-*b*, and at the reduction division *A* and *a* go one to each spermatocyte II. and *B* and *b* behave similarly. As now it is a matter of chance whether *B* goes into the same secondary spermatocyte as *A* or as *a*, there are formed four different classes of spermatocytes II. and therefore of gametes, namely, *AB*, *Ab*, *aB* and *ab*. These gametes, uniting at random when the hybrids are bred together, combine to give the same classes of zygotes, and in the same proportion as the various classes of individuals found in F_2 in the actual breeding experiment.

Since this is a treatise on cytology and not on heredity, it would be out of place to give any account of the immense number of characteristics, both in animals and in plants, which have now been found to be inherited in accordance with Mendel's Law. One or two interesting side lines of evidence as to the correspondence between the distribution of characteristics in heredity and of chromosomes in gametogenesis, may, however, be mentioned.

(1) *The Genetics of a Tetraploid Plant*

Gregory (1914) obtained two tetraploid individuals of *Primula sinensis* (of independent origin), and as they proved to be heterozygous for certain characteristics, he was able to carry out breeding experiments with them. As we have just seen, if we fix our attention on a single characteristic, represented in the idioplasm by a factor which we may call *A*, or *a*, according to the form in which it is present, the formula for a pure homozygous individual is *AA* or *aa*, and for the heterozygote *Aa*. In a tetraploid individual, however, if its quadruple set of chromosomes means a quadruple set of factors, the formulae for the pure forms will be *AAAA* and *aaaa*, while there are three kinds of heterozygotes possible, namely, *AAAa*, *AAaa* and *Aaaa*.

The zygote *AAAa* produces gametes *AA* and *Aa*, and therefore if bred with its like will produce among its offspring no pure *aaaa*.

The zygote *AAaa* produces four kinds of gametes, *AA*, *Aa*, *Aa* and *aa*, and therefore will produce one pure *aaaa* among every sixteen offspring, or 1 : 15.

The zygote *Aaaa* produces gametes *Aa* and *aa*, and therefore we will find one *aaaa* among every four offspring, or 1 : 3.

Similarly, if crossed with a pure *aaaa* plant (producing gametes *aa*) the first kind of heterozygote (*AAAa*) will produce no *aaaa* among its offspring, the second kind (*AAaa*) will produce one to every three others, and the third (*Aaaa*) equal numbers of *aaaa* and *Aaaa*.

The last two of the three types of heterozygotes described above can be identified among the tetraploid Primulas. Thus, taking the characteristics green style (dominant) and red style (recessive), one heterozygous plant, self-fertilized, gave forty-four green and two red, *i.e.* approximately 15 : 1. It was therefore an *AAaa* plant. Ten others gave ninety-nine green and thirty-four red, *i.e.* 3 : 1. They were therefore *Aaaa* plants.

Again, taking the characteristics short style (dominant) and long style (recessive), one heterozygous short styled plant crossed with a long styled plant (*i.e.* pure recessive, *aaaa*) gave thirty-seven short and fifteen long, or approximately 3 : 1; *i.e.* this heterozygote was *AAaa*. Another plant gave, with the same cross, forty-nine short and forty-seven long—approximate equality, so that this plant was *Aaaa*.

Thus the quadruple set of chromosomes is shown to be associated with a quadruple set of hereditary factors.

(2) *Segregation and Parthenogenesis*

If the segregation of characteristics which is found in inheritance is due to the separation of homologous chromosomes in gametogenesis, it is clear that there should be no such segregation in obligatory parthenogenetic reproduction, since all the offspring contain the same chromosomes as their parent. Many species of the plant genus *Hieracium* produce some egg cells in which meiosis takes place (and therefore, having the reduced number of chromosomes, are capable of fertilization), and also others in which reduction does not take place and which develop parthenogenetically. In many species the latter egg cells are much more numerous and hence reproduction is mainly parthenogenetic; the occurrence, however, of a certain number of haploid egg cells permits the possibility of sexual reproduction and hence allows of crossing between different species. It is found that the hybrids so formed are constant under parthenogenetic reproduction—that is to say, the parental char-

acteristics do not segregate among their offspring, these all resembling their hybrid parent (Ostenfeld, 1904, and Rosenberg, 1907). Indirect evidence of the absence of segregation in parthenogenesis has also been obtained in the case of the Cladoceran *Simocephalus* (Agar, 1914). A " population " of females hatched from fertilized eggs was allowed to reproduce itself parthenogenetically. The females included representatives of numerous size-types, and having been collected from the same locality must have included many heterozygotes between the various types. Now, if segregation were taking place, the resemblance between parent and offspring, as measured by the correlation co-efficient, must get more and more perfect generation after generation in parthenogenesis, since heterozygotes could split into homozygotes, but, in the absence of syngamy, these could not recombine into heterozygotes. Thus the original mixed population must get more and more nearly homozygous, and the correlation between parent and offspring must consequently rise from generation to generation. This correlation co-efficient was measured for the first five parthenogenetic generations from the fertilized eggs and was found to remain practically constant—at any rate it showed no sign of increasing. Hence we conclude that no segregation was taking place.

(3) *Segregation and Bud-Variation*

A few cases have been described where segregation appears to take place in vegetative (somatic) growth in plants. The most famous case is the laburnum *Cytisus adami*, which is a hybrid between the purple *C. purpureus* and the yellow *C. laburnum*. *C. adami* has dingy red flowers which are sterile. It occasionally, however, gives rise to pure, or almost pure, branches or single flowers of *C. laburnum* and *C. purpureus*. The flowers on these branches are fertile, and give rise to *C. laburnum* and *C. purpureus* plants respectively (accounts differ as to whether these plants are quite pure or show traces of the other species).

Many cases have been described where a dominant plant hybrid has produced, by bud-variation, branches or flowers with the characteristics of the recessive parent. Before these can be put down as examples of vegetative segregation, however, two possibilities have to be taken into account. Firstly, do the recessive flowers breed true to the recessive character ? Otherwise the appearance of the recessive character in one part of the plant may be due to some somatic condition preventing the dominance of the normally dominant characteristic—and many cases are known where dominance of one or other characteristic in a hybrid is affected by somatic conditions (for examples, see Cramer, 1907). Secondly, even if the recessive bud-variations do breed true to the recessive character, showing that they no longer contain the dominant

factor, we must always reckon with the possibility that mutation, and not segregation, has taken place, as in the case of *Mirabilis* (p. 182). Suppose a plant with blue (dominant) flowers is crossed with a white variety (recessive) of the same species, the hybrid then contains, according to hypothesis, a pair of homologous chromosomes, one containing the colour factor in its blue condition, which we may call A, the other one the same factor in its white state (*a*). Now in many instances we know that *a* was originally derived by mutation from A, and there is no reason to suppose that this may not happen again leaving the heterozygote with two *a* chromosomes, *i.e.* pure white.

While many cases of supposed vegetative segregation may probably be explained in this way, there can be little doubt that true vegetative segregation does take place as a very rare occurrence.

A case which can hardly be explained otherwise has been described by Bateson and Pellew (1915). Many varieties of peas (*Pisum*) produce a small percentage of " rogues," or plants with a somewhat vetch-like habit. The genetic behaviour of heterozygotes between rogues and typical plants is remarkable. As young plants they usually differ very little from the type form, but as they grow older the rogue characters appear in their upper parts, and as adults they are always pure rogues. Moreover, though of heterozygous origin, they produce, when self-fertilized, exclusively rogue offspring. These exhibit the rogue characters even as young plants.

Thus, as the above-mentioned authors point out, the normal and rogue characteristics of the heterozygote seem to separate during the growth of the plant, the normal characteristics being left behind in the older or lower parts of the plant, leaving purely rogue characteristics in the upper parts, and therefore also in the gametes.

What the underlying cytological conditions of vegetative segregation may be we do not know, but it may be fairly confidently conjectured that something analogous to the separation of homologous chromosomes in meiosis is concerned in it.

Summing up, we see that, except as extremely rare exceptions of which nothing is known as to their cytology, segregation in heterozygotes does not take place unless meiosis occurs, and that when meiosis does occur segregation does take place, thus adding direct experimental evidence to the other considerations which lead us to suppose that the separation of homologous chromosomes in meiosis is the cause of segregation.

It is not to be expected that the distinction between the members of a pair of homologous chromosomes which differ in regard to one or even more of their factors should be visible in all cases under the microscope —though we have seen some examples of visible differences between

homologous chromosomes in the last chapter, and it is a plausible hypothesis that these differences are due to their different factorial composition.

(4) *The Interchange of Hereditary Factors between Homologous Chromosomes*

The simple proposition that the characteristics of organisms are represented by factors in the chromosomes and that their distribution, according to the classical Mendelian scheme, depends upon the movements of these chromosomes in meiosis, requires some elaboration, however. For if each independently inheritable character resided in a separate chromosome, it is clear that there could only be as many such characters as there are chromosomes in the gamete. By separately inheritable characters we mean those which can be separated from each other and made to enter into fresh combinations by crossing. To put it in another way, all the characteristics of an organism should be capable of correlation into the same number of groups as there are chromosomes in the gamete, each group of characters behaving in heredity as a unit.

Now organisms present far more characters which are separately inheritable than they have chromosomes. This has been shown definitely for some organisms, and there can be little doubt that it is the general rule. Thus in the fruit-fly *Drosophila ampelophila*, which has been the subject of such exhaustive study in America, more than a hundred separately inheritable characters are known, though the number of chromosomes (haploid) is only four.

The hypothesis that hereditary factors are located in the chromosomes has therefore to be supplemented by the supposition that there are many factors in each chromosome, each located in a definite part of the chromosome (*i.e.* represented by a definite unit in the structure of the chromosome) and that exchange of corresponding factors may take place between homologous chromosomes. It is natural to suggest the chromomeres as the seat of the separate factors, and syndesis as the moment at which exchange takes place. By chromomeres, in this case, must be understood the numerous small bead-like bodies often observable in syndesis, not the few, much larger swellings on the chromosomes, sometimes found in late prophase.[1] According to this view, a chromosome is to be considered as containing a linear series of factors ABCDE . . . and two chromosomes in syndesis can be represented thus :

ABCDE . . .
a b c d e . . .

[1] Although even in syndesis the chromomeres are probably often already compounded of several smaller units.

During their apposition exchange of corresponding factors takes place and the chromosomes after separation may be constituted in various ways, *e.g.*, *AbCdE* and *aBcDe* ; or *abCDe* and *ABcdE*, etc., etc.

By this means the Mendelian inheritance of any number of separate characters can be accounted for.

The necessity of assuming the interchangeability between homologous chromosomes of the chromosome components makes it highly desirable to determine whether parasyndesis is or is not of general occurrence. Parasyndesis obviously offers a favourable opportunity for the mutual exchange between conjugating chromosomes of their elements which, as we have seen, are arranged in linear series — and indeed at this stage the corresponding chromomeres of the two chromosomes are often most regularly and conspicuously in close apposition (Fig. 77). The evidence for parasyndesis was discussed in Chapter II., and its general occurrence provisionally accepted.

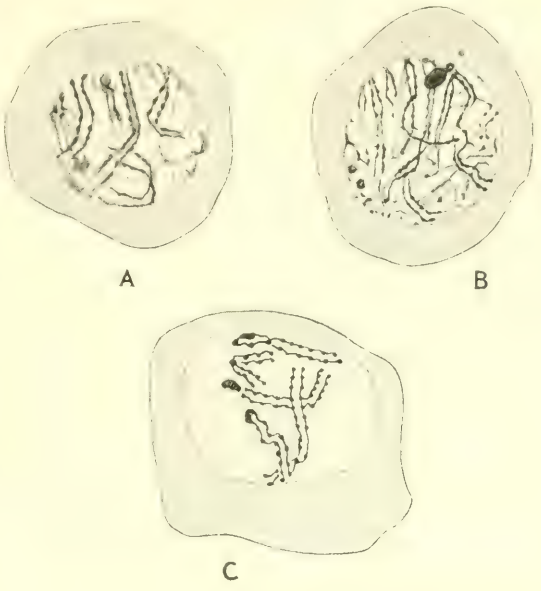


FIG. 77.

Examples of the correspondence between the chromomeres in homologous chromosomes during syndesis. A, *Spinax niger* ♂ ; B, *Myxine glutinosa* ♂. (A and B, after Schreiners, *I.B.*, 1906.) C, *Dytiscus marginalis* ♂ (after Henderson, *Z.w.Z.*, 1907).

We have, moreover, direct reason for believing that the function of syndesis is not merely that of bringing the members of homologous pairs into apposition so as to effect their sorting out into different daughter nuclei at meiosis. For syndesis often takes place months or years (mammalian oocytes) before the reduction division, and is frequently followed by complete separation of the ex-syndetic chromosomes (many oogeneses, spermatogenesis of *Lepidosiren*, etc.). Between syndesis and metaphase I. the chromosomes may even undergo metamorphoses as great as those undergone in the resting nucleus (most cases of oogenesis). The separated homologous chromosomes then pair again in prophase I. immediately before the metaphase (oogenesis, *Lepidosiren* spermatogenesis). This second pairing, which is not of the

late nature of the syndesis proper, has obviously the function of bringing the homologous chromosomes in pairs on to the meiotic spindle. This being effectively achieved by this late prophase pairing, we are compelled to look for another function for syndesis in these cases, and this function, as indicated above, we believe to be the exchange of hereditary factors.

In what way may we conceive that this hypothetical interchange of factors is effected? Two possibilities have been suggested—the first is that which naturally presents itself, namely, that while the chromosomes are longitudinally apposed to one another in parasyndesis, an exchange of chromatin units takes place analogous to the exchange of nuclei between two conjugating Infusoria. The other suggestion was first made by Janssens (1909) (chiasmatypie) on purely cytological grounds. Since the homologous chromosomes separating after syndesis are often spirally twisted round one another (strepsitene stage), he suggested that fusion may occur at the points of junction, and that when these break apart at final separation the chromosomes may be composed of alternate segments of the original chromosomes (Fig. 78).

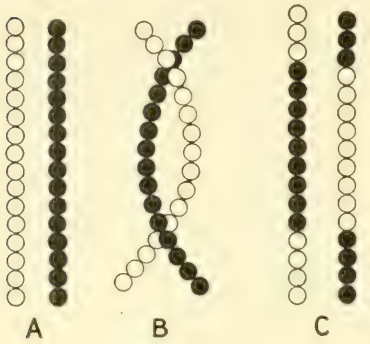


FIG. 78.

Diagram illustrating the hypothesis of "chiasmatypie" or "crossing over." A, a pair of chromosomes before syndesis; B, diplotene (= strepsitene) stage; C, the chromosomes fully separated after syndesis.

A view similar to Janssens' has been supported by Morgan, etc., as forming a cytological foundation for certain breeding results with *Drosophila* and other forms. It is known as the "crossing over" hypothesis, and is supported by the following experimental evidence.

A number of cases are now known in which characters which *can* be separated in crossing, nevertheless show a preference to remain in the combinations in which they were present in the parents, rather than to become rearranged in different combinations. Thus if two forms, differing as regards two characters, are crossed, say $AB \times ab$, the F_1 hybrid will of course form four classes of gametes AB, Ab, aB, and ab. In most cases these classes are formed in equal numbers, but in some cases the gametes representing the parental combinations are found in excess of the others. In the cross $AB \times ab$ we have an excess of the gametes AB and ab, and a deficiency of the Ab and aB classes. On the other hand, the cross $Ab \times aB$ gives an excess of Ab and aB and a deficiency of AB and ab. It must not be supposed that this is a mere exception to the

general Mendelian scheme and the theory of its cytological basis, and therefore invalidates their generality. On the contrary, the departure from the ordinary equality of different classes of gametes is quite orderly and, as we shall see, intelligible. Thus if $4x$ is the total number of gametes produced we find that they occur in the following proportions :

In the first case $(AB \times ab)$ the gametes are :

$$(x+y)\ AB + (x-y)\ Ab + (x-y)\ aB + (x+y)\ ab.$$

And in the second case $(Ab \times aB)$:

$$(x-y)\ AB + (x+y)\ Ab + (x+y)\ aB + (x-y)\ ab.$$

This will be apparent from the following experiment with the sweet pea (*Lathyrus*) described by Bateson and Punnett (1911) and by Punnett (1913) [1] : the characters concerned are the colour of the flower, blue (B) and red (b), and the shape of the pollen grain, long (L) or round (l). Blue is dominant over red and long pollen is dominant over round. Now the F_2 from the cross $BL \times bl$ is different from the F_2 from the cross $Bl \times bL$, as the following parallel experiments show :

Generations :

Blue, long × red, round				P	Blue, round × red, long			
Blue, long				F_1	Blue, long			
3915	312	294	1079	F_2	226	95	97	1
Blue, long	blue, round	red, long	red, round		Blue, long	blue, round	red, long	red, round

The F_1 is of course the same in both cases (blue, long), since blue is dominant over red, and long over round. The number to be expected in F_2, if it were a simple Mendelian case with no connection between colour and pollen shape, would be the same for both experiments, and would be in the proportion 9 blue, long : 3 blue, round : 3 red, long : 1 red, round. These proportions are what would be obtained supposing the four kinds of gametes BL, Bl, bL and bl were produced in equal numbers. The actual F_2's obtained [2] show that this cannot have been the case in this instance. Supposing, however, that the gametes had been produced in the first experiment (blue, long × red, round) in the proportion

$$7\ BL : 1\ Bl : 1\ bL : 7\ bl,$$

[1] The hypothesis of *reduplication* advanced by these authors in explanation is fundamentally different from Morgan's view of "crossing over," which is here adopted.

[2] Included in F_2 are a number of F_3 families descended from heterozygous F_2 plants.

this would give a proportion in F_2 of

177 blue, long ; 15 blue, round ; 15 red, long ; and 49 red, round ;

or, out of the total of 5600 in the above F_2, the numbers :

3871 blue, long ; 328 blue, round ; 328 red, long ; and 1073 red, round.

This we see is a very close approximation to the numbers obtained in the experiment, and it is to be noted that the gametes produced in excess are those which exhibited the same combinations of characters as the original parents (*BL* and *bl*). Similarly, if the gametes in the second experiment were formed in the proportions

$$1\ BL : 7\ Bl : 7\ bL : 1\ bl,$$

the numbers which would be given in F_2 are

210 blue, long ; 103 blue, round ; 103 red, long ; and 1·5 red, round ;

again a sufficiently close approximation to the numbers actually obtained.

Thus, we conclude that the hybrid produces about seven times as many gametes with the parental combinations of characters as with the reciprocal combinations.

The explanation of this, on the "crossing over" hypothesis, is that the factors for colour of flower and shape of pollen grain lie in the same chromosome.[1] To take the first case ($BL \times bl$) : during syndesis in the hybrid, the *BL* and the *bl* chromosomes come together, and separate again in the diplotene stage, during which they become twisted round one another and liable to exchange segments as described above. There is, however, a greater chance of the *BL* and the *bl* factors remaining together than of the combination having been broken up, and consequently of *B* and *l* being found in one of the separating chromosomes and *b* and *L* in the other ; for (1) the chromosomes may have separated without any crossing over of the particular segments containing the flower colour and pollen grain shape factors respectively ; or (2) if crossing over has taken place in this region, a length of chromosome sufficient to include both factors may have crossed over. It is plainly only when one of the factors has crossed with its mate, and not when both or neither have done so, that a recombination of the *B* and *b* with the *L* and *l* will have taken place. It is also plain that the nearer to each other the two factors are located in the chromosome, the more likely they are to be included in a segment behaving as a unit in crossing over—or, in other words, the less likely is a cross over and rupture of the chromosome to take place between them. Acting on these considerations Morgan and his colleagues (1915 *b*) have mapped out the arrangement of a large number of factors in the chromosomes of *Drosophila*, calculating their relative

[1] Such factors are said to be "linked."

distances from one another by the relative frequency of crossings over between them.

In *Drosophila*, for some unknown reason, crossing over takes place in the female only, never in the male. This would be explicable if only the sex-linked characters were concerned (see below), since the factors for these are presumably carried in the X chromosome, of which the male has only one (its mate, the Y, being apparently inert). The rule applies equally, however, to characters which are not sex-linked and of which the factors must therefore be carried in other chromosomes. Thus the characters for body colour (grey, dominant over black) and shape of wing (long, dominant over vestigial) behave in the male as an inseparable couple, all the gametes of a male hybrid between a grey, long and a black, vestigial, being either grey, long or black, vestigial. The corresponding female hybrid, however, gives indeed a majority of grey, long and black, vestigial gametes, but also a small percentage of grey, vestigial, and black, long. Conversely, a male hybrid between a grey, vestigial, and a black, long, gives only gametes grey, vestigial, and black, long, while the female hybrid gives a majority of these, with, however, a small percentage of grey, long and black, vestigial. It appears therefore that in *Drosophila* exchange of substance between homologous chromosomes occurs in syndesis of the female but not in that of the male; a fact which seems to indicate that the full explanation is not given by the hypothesis of crossing over in its simplest form.

Tanaka (1915) has described a case among silkworms where the reverse condition obtains, crossing over occurring in the gametogenesis of the male hybrid but not in that of the female.

The above brief summary of the points of contact between the observations of cytologists on one hand, and the results of the Mendelian method of studying heredity on the other, must suffice, for to follow it up by multiplication of detail and instances would lead us beyond the subject-matter of this book. Moreover, it is not necessary, as the subject has quite recently been treated in special publications *e.g.* Doncaster, *The Determination of Sex*, 1914 *a*; and Morgan, Sturtevant, Muller and Bridges, *The Mechanism of Mendelian Heredity*, 1915—to which the reader desirous of further information is referred. These works also deal with the problem of sex-linked (or sex-limited) inheritance (the connection between the sex chromosomes and the determination of sex has already been dealt with in Chapter IV.). This term covers those cases in which certain characters are transmitted only by gametes bearing the sex chromosome; *e.g.* in *Drosophila* certain characters such as red eye can be transmitted by any egg, but only by the female-producing spermatozoa. Similar cases are known in the cat and in man (*e.g.* colour

blindness and haemophilia). It is an obvious hypothesis that the reason for this is that the factors of the characters in question are located in the X chromosome. As the female has two X chromosomes, and as consequently all her egg cells have an X chromosome, therefore any of them are in a position to transmit this character. Since, however, half the spermatozoa lack the X chromosome altogether, or possess in its place the Y chromosome (which we must suppose to be inert) only half of them, that is to say the X-bearing (female-producing) spermatozoa, can transmit the character. Thus in *Drosophila* a red-eyed female can transmit this character to any of her offspring, but a red-eyed male only to his daughters, *i.e.* through the X-bearing spermatozoa.

In other cases (Lepidoptera, Birds) it is the male which transmits certain characters to all his offspring (*i.e.* through all gametes), and the female only to her sons (*i.e.* through male-producing gametes only), thus indicating that the chromosome formula for male and female is reversed, male being XX and female XY (or X –). This again corresponds with the somewhat scanty cytological observations on these two groups.

For further information on the subject of sex-linked inheritance, which furnishes one of the strongest pieces of evidence in favour of the chromosomes (chromomeres) being the seat of the Mendelian factors, the reader is referred to the above-mentioned works, where he will also find reference to certain exceptions and the supplementary hypotheses which have been put forward to account for them.

(5) *The Cytological Basis of Mutation*

If morphogenesis and heredity have their physical basis in the nucleus, heritable variation must be due to changes in one or more of the chromosomes, or, rather, of their constituents. This of course is, in the present state of our knowledge, impossible to prove directly, but certain relative evidence can be obtained from the study of the origin of variation, or mutation.

If a hereditary factor undergoes a mutational change, whether in gametogenesis or at some other time, the first individual to possess this altered factor in its diploid nuclei will presumably most often possess one chromosome containing the factor in its new state, and its homologue possessing it in its old state. A moment's consideration will make this clear. If the variation has taken place in gametogenesis, the mutated gamete will almost necessarily have to fertilize a non-mutated gamete from another organism, for its chances of meeting another gamete which has undergone a similar mutation will in most cases be negligible, and hence the first individual to possess the new factor will be heterozygous between it and the old form. If on the other hand the mutation takes

place in the fertilized (diploid) egg, the individual will again be in the heterozygous condition unless *both* members of the homologous pair concerned have undergone the same mutation.

A number of cases of mutants appearing in the first instance in the heterozygous form are on record.

Nilsson-Ehle (1911) described such a case in oats. He found in pedigree cultures of this cereal a small proportion (one in ten to twelve thousand) of plants exhibiting certain atavistic features in respect to the awns and the hairiness of the flower base. When these atavists were self-fertilized they gave offspring of three classes, viz. normals, atavists like themselves, and more pronounced atavists, in the proportion of 1 : 2 : 1. Obviously therefore the original semi-atavists, as we may call them, were heterozygotes between the normal and fully atavistic forms—that is to say, they were produced by syngamy of a mutated and a non-mutated gamete.

Gates (1914) has shown that in the case of *Oenothera rubricalyx*, which arose as a mutation of an *O. rubrinervis*, the first *rubricalyx* individual was a heterozygote between *rubricalyx* and *rubrinervis*, the new character *rubricalyx* being in this case dominant. Its heterozygous nature was therefore not obvious from its external characters, but was only disclosed by breeding.

There is one great obstacle in the way of discovering the mode of origin of mutations, and that is that a large number, probably the great majority of them, are partially or completely recessive to the type condition, and therefore the heterozygotes are indistinguishable from the type form. In these cases, since it is only the homozygous recessives which exhibit the new character, this will make its first external appearance in the homozygous condition. As moreover the new factor may have been in existence a considerable time, and may have become widely distributed, but invisible, owing to being always concealed in individuals possessing also the dominant type character, these recessive mutants are liable to appear suddenly in relatively large numbers, when sufficient heterozygotes have accumulated in the population to allow of the meeting between two mutated gametes to take place fairly frequently.

Also it must be remembered that mutation, striking enough to be recognized and investigated in the first animal or plant exhibiting it, is rare.

It must not be supposed, however, that mutation can only take place in the germ-cells, nor is there any theoretical reason for supposing that it should, like segregation, be connected with meiosis, except in that limited class of mutations due to irregular distribution of the chromosomes or their constituents in the reduction division.

An example of a mutation which almost certainly did not originate

during gametogenesis is afforded by the origin of the peculiarly malformed "cretin" sweet pea, described by Bateson and Punnet (1911) and Punnet (1919). The malformation concerned is recessive to the normal condition, and therefore only manifests itself in homozygous individuals. The "cretin" arose in a pedigree culture, and was the only one of its kind among a large number of direct and collateral ascendants and descendants (excluding, of course, its own offspring). Had the mutation occurred during gametogenesis two possibilities are open: (1) it might have occurred during the gametogenesis of the grandparent, so that the immediate parent of the cretin was heterozygous, though normal in external appearance. In this case, however, it should have produced one cretin among every four of its offspring, whereas it actually produced only the one cretin and 51 normals. (2) The mutation may have occurred during the gametogenesis of the immediate parent. If this were the case, more than one gamete must have been similarly affected, since the cretin itself, being homozygous, must have been produced by syngamy of two such affected gametes. As, however, the parent plant produced only one cretin among a large number of normals (the latter again producing only normal offspring) it is plain that the number of mutated gametes produced must in any case have been very small, and the chances against two of them having united to form the cretin very great. The evidence seems to indicate therefore that the mutation occurred in the zygote cell, and affected both members of the homologous pair of chromosomes concerned.

A well-known class of mutations occurring in somatic cells constitutes the phenomenon of bud-variation in plants. Innumerable examples of this could be quoted, but very few of them have been thoroughly investigated. As an example of one which has been more fully worked out, we may take a case described by Correns (1910) in *Mirabilis*. It was found that plants with variegated foliage occasionally gave rise to branches with pure green leaves. Seeds from flowers on these green-leaved branches yielded 25 per cent variegated plants and 75 per cent green. On further breeding, twenty-five of these 75 per cent of green plants gave only green plants, and the remaining fifty gave again 25 per cent variegated and 75 per cent green offspring. Thus it is plain that the green branches appearing as bud-variations on the variegated plants were heterozygous between variegated and green, green being dominant, and produced the usual proportion of offspring for this type of heterozygote, namely, 3 dominant : 1 recessive.

In terms of cytology, we must suppose that the factor for chlorophyll distribution exists in two forms—one to produce a uniform distribution of chlorophyll, giving green plants (*G*), and the other giving a patchwork distribution, resulting in variegated plants (*g*). In the variegated plants

both of the homologous chromosomes bearing this factor have it in the *g* form. Sometimes, however, one of these, by mutation, changes into the *G* condition, and since *G* is dominant over *g*, all future somatic descendants of this cell will be green; thus we find green branches occasionally appearing on the variegated plants. These branches are now in exactly the same condition, cytologically, as if they belonged to a hybrid between a green and a variegated plant, with the result that the ordinary Mendelian proportions which are to be expected among their offspring are realized.

E. STERILE AND PARTIALLY STERILE HYBRIDS

We can provisionally distinguish two types of these hybrids: (1) where the disturbance in meiosis seems to be mainly of a mechanical nature, depending upon a numerical, rather than a physiological discrepancy in the chromosomes of the two parents; (2) where the disturbance is mainly physiological. We must remember that this distinction, even if justifiable, is by no means sharp, the two types overlapping and grading into each other.

As an example of the first of these two types of crosses, we may take the cross between the two species of sun-dew *Drosera longifolia* and *D. rotundifolia* (Rosenberg, 1909). This cross results in a hybrid intermediate in character between the parent species, and known as *D. obovata*. In *D. rotundifolia* $2n$ is 20 and in *D. longifolia* it is 40, the latter species being probably a tetraploid form. In *D. obovata* the somatic number is 30.

In syndesis of the hybrid, Rosenberg found ten bivalents and ten univalents –that is to say, syndesis had taken place between the ten *rotundifolia* chromosomes and one of the two sets of their homologues from the tetraploid *longifolia*, leaving the other set of ten unpaired. In anaphase the constituents of the bivalents were regularly, but the univalents irregularly, distributed to the daughter nuclei, which therefore sometimes received very unequal numbers of chromosomes; for example, 18 and 12. Fertile pollen grains were seldom or never formed, but fertile ova fairly frequently.

Probably a closely analogous case is afforded by the crosses between *Oenothera gigas* and other *Oenotheras*, which may be considered here though they do not necessarily result in any notable degree of sterility.

We have already (p. 149) seen reason to believe that *O. gigas* is, like *D. longifolia*, tetraploid; its somatic chromosome number is 28. It therefore becomes of interest to see how this form behaves in crosses with the diploid forms ($2n = 14$). The cross most studied is *O. lata*[1] × *O.*

[1] The fact that this *Oenothera* has 15 instead of 14 somatic chromosomes (p. 146) need not concern us here.

gigas. In the male meiosis of this hybrid, which has 21 chromosomes (14 + 7), according to Geerts (1911), we get 7 bivalents and 7 univalents; *i.e.* of the triple set of chromosomes, two sets of homologues have paired, leaving the other set free, as is probably also the case in *Drosera*. Gates, however (1909 *b*), thinks it probable that there is no syndesis, and finds that there are 21 univalents in metaphase I. (in the *Oenotheras*, the associa-

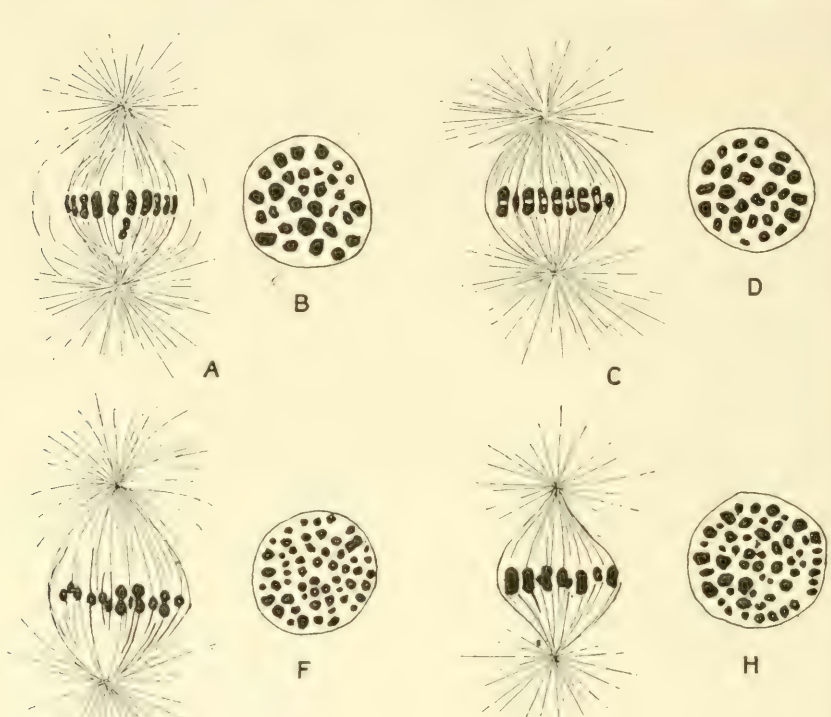


FIG. 79.

Side and polar views of the equatorial plate in the meiosis of certain Lepidoptera and their hybrids. (After Federley, *Z.A.V.*, 1913.) A, B, *Pygaera anachoreta*, 30 bivalents; C, D, *P. curtula*, 29 bivalents; E, F, *P. anachoreta* × *P. curtula*, hybrid; in E, 3 chromosomes are bivalent, the rest univalent; in F, 56 or 57 chromosomes can be seen, *i.e.* two or three are bivalent, the rest univalent. G, H, secondary hybrid obtained by crossing the first hybrid back with *P. anachoreta* ♀; in G a mixture of univalents and bivalents; in H, 56 chromosomes can be counted; namely, about 30 large bivalents (the *anachoreta* chromosomes), and about 26 small univalents (the *curtula* chromosomes).

tion between the constituents of the bivalents in the meiotic division is characteristically very loose). According to the latter authority these 21 chromosomes are separated at anaphase into groups of 10 and 11, rarely 9 and 12. According to Geerts the constituents of the bivalents separate normally, sending 7 to each pole, while the remaining 7 univalents suffer various fates—some being irregularly distributed to the

daughter nuclei and some being left behind and failing to enter either nucleus.

The nature of the progeny obtained by breeding from this hybrid has already been described (p. 146).

Somewhat intermediate between the two types which we have provisionally distinguished as mechanical and physiological, stand probably the Lepidopteran crosses examined by Federley (1913) and by Harrison and Doncaster (1914).

Federley's crosses (Fig. 79) were between *Pygaera curtula* ($2n = 58$) and *P. anachoreta* ($2n = 60$). The hybrid has about $29 + 30 = 59$ chromosomes. In meiosis of the male hybrid very little syndesis takes place, only two or three chromosomes being paired, so that there results a few bivalents while the remainder are univalent. The former separate into their constituents in the usual way, while the others divide as in a somatic mitosis. Thus each gamete has the diploid number of chromosomes, except for those few which paired at syndesis, in respect of which it is haploid.

This hybrid was crossed back with *anachoreta* ♀. The number of chromosomes to be expected in this secondary hybrid is $59 + 30 = 89$; *i.e.* a double set of *anachoreta* and a single set of *curtula* chromosomes—or nearly so, as the hybrid gametes have not quite the full 59 chromosomes. This of course is a very high number to count satisfactorily, but in several nuclei over 70 were counted. In meiosis of this secondary hybrid we find a mixture of bivalents and univalents, leading to the presumption that the *anachoreta* chromosomes introduced by the mother have paired with those of the same species introduced by the hybrid gamete, while the *curtula* chromosomes of the hybrid are left univalent.

Harrison and Doncaster's cross was between *Lycia hirtaria* and *Ithysia zonaria*, and gave results closely comparable to those of Federley (Fig. 80). This cross exhibits an advantage over the last one described, in that the chromosomes of the two parent species are distinguishable from one another in the hybrid by their relative sizes.

The chromosomes of *L. hirtaria* ($2n = 28$) consist of 11 pairs of large, 1 pair of small, and 2 pairs of very small ones. Those of *I. zonaria* ($2n = 112$) are all very small, the largest being no bigger than the smallest of *L. hirtaria*.

In the hybrid diploid cells (spermatogonia) the two types of chromosomes are easily recognizable, the total number being of course $14 + 56 = 70$. In the first meiotic division it is found that there can be counted rather less than 70, but always many more than 35 (varying from about 53 to 63). It is therefore to be presumed that about a dozen pairs of chromosomes have entered into syndesis, and that the rest remain unpaired.

These hybrids are completely sterile.

In the following three cases the meiotic disturbance must be ascribed to physiological causes, since the degenerative changes that take place in the nucleus are more profound, even though in some cases the number of chromosomes in the parent species is the same.

Matings between the magpie pigeon (♂) and dove (♀) (Smith, 1913) result in male offspring only. These are found to exhibit a meiosis differing from the normal in that there is an irregular metaphase I. and, doubtless in consequence of this, the second meiotic division appears to be omitted. In any case it was never found, though spermatogenesis proceeds without it, resulting in the formation of spermatozoa, 77 per cent of which are about twice the size of those of either parent. The

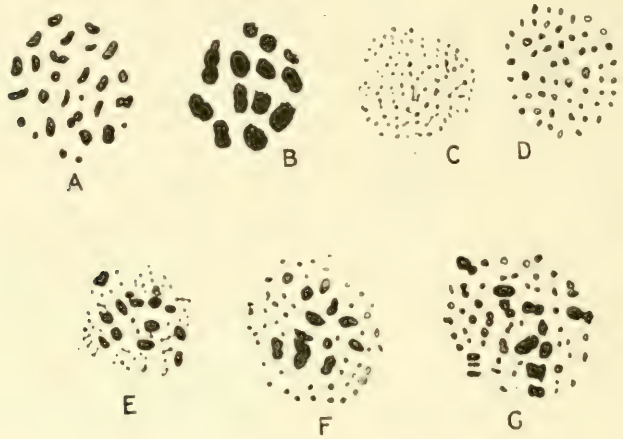


FIG. 80.

The chromosomes of certain Lepidoptera and their hybrids. (After Harrison and Doncaster, *J.G.*, 1914.) All are polar views of equatorial plates. A, B, *Lycia hirtaria*. A, oogonium showing 28 chromosomes; B, spermatocyte I., 13 bivalents (one compound). C, D, *Ithysia zonaria*. C, spermatogonium, about 112 chromosomes; D, spermatocyte I., 56 bivalents. E, F, the hybrid, *I. zonaria* × *L. hirtaria*. E, spermatogonium, 14 large (*hirtaria*), and about 56 small (*zonaria*) chromosomes; F, spermatocyte I., 63 chromosomes. G, spermatocyte I. of the reciprocal hybrid, about 60 chromosomes, showing the two parental types.

experiments indicate that the spermatozoa are not functional, for two of the hybrid males were paired with female pigeons, which laid and incubated eggs which, however, proved unfertile.

In the hybrids between different species of pheasant (Smith and Thomas, 1913), and pheasant and domestic fowl (Cutler, 1918), no normal stages can be observed after synizesis. These hybrids are of course sterile. When they are females the resulting anatomical abnormality of the ovaries is very marked, since oogenesis ceases at synizesis and therefore there is an entire lack of yolked oocytes. Thus the ovaries remain minute and sometimes invisible.

The best known sterile hybrid is of course the mule. The male meiotic phase of this animal has been worked out by Wodsedalek (1916),

who also examined the spermatogenesis of the horse. That of the ass has, however, not yet been described. The number of chromosomes (diploid) in the mule is 51, one being a large unpaired X chromosome. As $2n$ in the horse is $36 + X$, the ass may be presumed to possess $64 + X$ chromosomes. Evidences of physiological disturbance appear early in the meiotic prophase of the mule, the diplotene stage found in the horse being replaced by a reticular stage showing only occasional and irregular duplicity of threads. The majority of cells perish in this stage; in those that reach the late prophase the number of chromosomes varies between 34 and 49, the commonest numbers being 40 to 45, *i.e.* there are about 5 to 10 bivalents, the remainder being univalents. The few cells that reach metaphase I., or even anaphase I., are marked by numerous abnormalities, especially multipolar mitoses and the failure of many of the chromosomes to gain attachment to the spindle fibres. No secondary spermatocytes or any later stages were ever observed.

F. THE NUCLEUS IN MORPHOGENESIS

All arguments in favour of the nucleus being the bearer of the hereditary factors are of course equally arguments for its control of morphogenesis. As to its mode of action in this respect, however, we are almost wholly ignorant. One thing which does seem certain is that all the nuclei of the body—at any rate up to a late stage of development—are identical in their potentialities, *i.e.* contain a complete (double) set of hereditary factors. There are no differential nuclear divisions in the embryo by which the endoderm factors are sorted out into the nuclei of the cells which are to form endoderm, mesoderm factors into the mesoderm nuclei, etc., as originally supposed by Weismann and Roux.

This has been abundantly proved by the pressure experiments of Driesch (1893), etc., as well as by many other well-established facts of embryogeny, regeneration, etc. The particular experiments referred to consisted in making sea-urchin (*Echinus*) eggs undergo their early development under pressure between two glass plates. Under such conditions the eggs may continue to develop as far as the 4th cleavage (16 cells). Instead of forming a spherical blastula, however, the cells are all arranged in one plane in a flat plate. On removing the pressure, the embryo gradually recovers its proper shape and proceeds to normal development, in spite of the fact that, as can be easily verified, the cells have a quite different mutual arrangement and contain different parts of the cytoplasm from those which they have in the normal larva. Thus a cell which in the normal larva would have given rise to ectoderm, now gives rise to endoderm, etc.

What is it then that causes the cell differentiation which leads to the

formation of the various tissues and structures of the body? A discussion of this question would again lead us far beyond the scope of this book, and would indeed involve nearly the whole subject of experimental embryology. Here we can only allude to the question of the so-called "organ-forming substances," referred to on p. 163. Lack of space and the uselessness of repeating what has already been made the subject of recent text-books must restrict us to a very brief, and therefore necessarily dogmatic, summary. The reader is referred for further information to Korschelt and Heider's *Lehrbuch* (1902), or to the smaller text-book of Jenkinson (1909), where all the essential facts are given, and where references to the more important original works may be found.

It is found that a single blastomere isolated from an embryo of the 16-cell stage (termed a $\frac{1}{16}$ blastomere) of a sea-urchin will live and develop, at least as far as gastrulation, as if it were a whole miniature egg. It is only blastomeres from the lower or vegetative pole of the cleaving egg, however (*i.e.* that part of the egg from which gastrulation starts in a normal, whole embryo), which will gastrulate. Cells from other parts develop irregularly and do not gastrulate; $\frac{1}{2}$ blastomeres, however, whichever part of the egg they are taken from, will develop into embryos which gastrulate. If the egg is divided into its blastomeres at the two-cell stage, both develop into a perfect normal, though dwarf, pluteus.

This is expressed by saying that the blastomeres of an Echinoid egg are equipotential as far as the eight-cell stage, then gradually become inequipotential. The eggs of a large number of animals can be arranged in a series, according as to how long they retain their totipotentiality, down to the forms where even the undivided egg is inequipotential in its various parts.

Thus in the radially symmetrical Ctenophora, if a segment of the cytoplasm of the undivided egg is removed, the resulting larva lacks the organs on the radius represented by the removed egg cytoplasm.

The eggs of several Molluscs and Chaetopods exhibit a swelling of the cytoplasm (after fertilization but before cleavage has begun) termed the yolk lobe. If this is cut off, the egg will nevertheless develop to a certain stage, but the resulting larva, though it may become free-swimming, does not develop any mesoderm.

These and many other experiments have led to the hypothesis of the presence of "organ-forming substances" in the egg cytoplasm exhibiting a stratified arrangement, generally according to their specific gravities. For instance, ectoderm-forming substance is most concentrated towards the upper pole of the egg and least concentrated towards the lower pole, and endoderm-forming substance has the reverse arrangement. In the cleaving Echinoderm egg, up to and including the eight-cell stage, there is sufficient of all organ-forming substances in all the blastomeres

to allow of them all developing as complete organisms. By the time that the 16-cell stage is reached, however, successive cleavages have resulted in the cells in the upper pole having so little endoderm-forming in proportion to ectoderm-forming substance, that they are now no longer able to gastrulate, though isolated cells from the lower pole (which contain the endoderm-forming substance) can still do so—as can even a $\frac{1}{32}$ blastomere, if taken from this pole.

The action of the cytoplasm in determining which cells shall develop into soma and which into gonad in *Ascaris* (p. 85) is clearly that of an "organ-forming substance."

It must be granted that the egg cytoplasm contains substances that are necessary to the formation of various tissues and organs. There is, however, no reason to suppose that these substances play an active formative part, or that they are anything other than the conditioning environment or the releasing stimulus through which the nucleus exerts its activities. The external environment of the developing egg contains elements which act in quite as specific a manner as the so-called organ-forming substances in the egg cytoplasm. Thus if Echinoderm eggs are made to undergo their development in water identical with that of sea water, except that it lacks the SO_4 radicle, the gut of the larva is not properly formed; if the calcium normally present is absent, the blastomeres fail to cohere into a blastula, but fall apart, swim away by means of their cilia, and eventually die without undergoing any differentiation. Eggs of the Teleostean fish *Fundulus heteroclitus*, developing in sea water to which $MgCl_2$ has been added, produce embryos with a single median "Cyclopean" eye, instead of a pair of lateral ones (Stockard, 1909). It would clearly be possible to speak of the SO_4 radicle as a gut-forming substance, of calcium as a blastula-forming substance, and of $MgCl_2$ as a monocularity-producing body, with as much justification as we call the substances in the egg cytoplasm which we have just discussed, organ-*forming* (rather than organ-*conditioning*) substances.

The fact appears to be that all these substances, whether within the cytoplasm or without, including yolk and vitelline membrane (the degree of the permeability of which is of vital importance to the embryo), or the relation of the embryo to the mother in viviparous forms, are all alike components of the environment of the morphogenetic factors residing in the nucleus. The fact that certain of them, such as the vitelline membrane, the fine and coarse grains of the yolk, and the "organ-forming substances," are not uniformly distributed, but are more or less localized in various parts of the cytoplasm, does not seem to raise a problem different from that raised by any other adaptive arrangement.

Finally, we may point out that the local differentiation, or *anisotropy*, of the egg cytoplasm belongs to the class of exceptions which prove the rule. Not only is there no mechanism for an equal partition of these substances among the daughter cells at cell division, but their mode of action depends upon the fact that they are *not* so distributed. It is therefore very improbable that they can retain their continuity through the numerous cell divisions leading from the unfertilized egg through the cleavage divisions and all the divisions in the female germ track till the cycle is complete with the formation of the next generation of oocytes. It would appear therefore that they must be formed anew in these cells in each generation, and all the arguments in favour of the general morphogenetic activity of the nucleus in moulding the cytoplasm apply equally in favour of the view that these substances also are formed by the agency of the nucleus. Whatever view therefore is taken of the "organ-forming substances" of the egg cytoplasm, their presence does not affect the question of the monopoly by the nucleus of the hereditary substance, which stands or falls on other grounds.

G. CHROMIDIA AND CHONDRIOSOMES

We now come to a very difficult chapter of cytology, in which statements of fact and theory are so contradictory that it is at present scarcely possible to do more than give an abstract of the work done and the interpretations put upon it; the questions involved are, however, too important to pass over, in spite of the necessity of reserving judgement on the issues.

(1) *Chromidia*

As we have already pointed out, we are in almost complete ignorance as to the way in which the nucleus exerts its regulative and morphogenetic functions, but these appear to be usually exerted on the cytoplasm through the nuclear membrane. Occasionally, however, the chromatin comes to lie naked in the cytoplasm instead of forming part of a nucleus with a definite architecture enclosed by a membrane. This is very commonly the case in Protista, where granules of chromatin called *chromidia* often lie in the cytoplasm. These may be in addition to the formed nucleus, or may take the place of this at definite stages of the life history, or again may constitute the whole chromatic garniture of the animal, a formed nucleus being absent. Sometimes these chromidia are destined to take part in the reproduction of the organism (generative chromidia); in other cases they are finally absorbed by the cytoplasm without playing the part in reproduction usually allotted to

the nuclear material. They are then known as vegetative chromidia, and have been compared to the macronucleus of Infusoria, which is absorbed at the time of conjugation without playing any part in that process. Often, however, the extrusion of vegetative chromidia from the nucleus seems to be merely a means by which a nucleus which has through unfavourable conditions become hypertrophied, gets rid of its excess chromatin. The classical example of this is *Actinosphaerium* (R. Hertwig, 1904). Under certain unfavourable conditions (either starvation or over-feeding) the nuclei become greatly enlarged and hyperchromatic; the excess of chromatin may then be got rid of by the emission of large quantities of it into the cytoplasm, where it degenerates into brown pigment.

The formation of vegetative chromidia in the Metazoan oocyte I. has been described by many writers, and some have described it in other Metazoan cells also.

In oocyte I. it is said to take place at the beginning of the growth period, characteristically at the bouquet stage, when there is to be observed in many animals a deeply staining mass in the cytoplasm just outside the polar surface of the nucleus (Fig. 81), *e.g.*, *Proteus* (Jorgensen, 1910 *b*), *Paludina* (Popoff, 1907), *Gryllus* (Buchner, 1909).

This mass consists of granules or filaments which stain like chromatin, and are often so closely applied to the nuclear membrane that they appear to be continuous through this with the intranuclear chromatin. This fact has led many cytologists to conclude that the mass above referred to consists of chromatin extruded through the nuclear membrane into the cytoplasm, that is to say, of *chromidia*.

Often, however, the extrusion of chromidia occurs after the bouquet orientation has been lost; in this case, as in the forms where there is no orientated bouquet stage, the emission takes place diffusely through the nuclear membrane instead of only at the polar surface, *e.g.*, *Aricia* (Schaxel, 1912; Fig. 81).

Often when emission is diffuse, and sometimes even when it takes place from the polar surface only, in the bouquet stage (various Orthoptera—Wassilieff, 1907; Buchner, 1909), the nucleolus has been described as acting as an intermediary in the process, the chromatin which is to be extruded being first collected into it, and thence emitted into the cytoplasm.

A precisely similar process has also been described in spermatocyte I.; *e.g.*, *Blatta* (Wassilieff, 1907; see Fig. 81).

While descriptions of chromidia formation in the Metazoa have mainly been restricted to oocytes and spermatocytes, they have recently been extended to somatic cells. Here—doubtless in correlation with the absence of a well-marked bouquet stage—the emission is diffuse,

taking place apparently from any part of the nuclear surface (Fig. 81, G).

As to the way in which chromidia get out of the nucleus, opinions

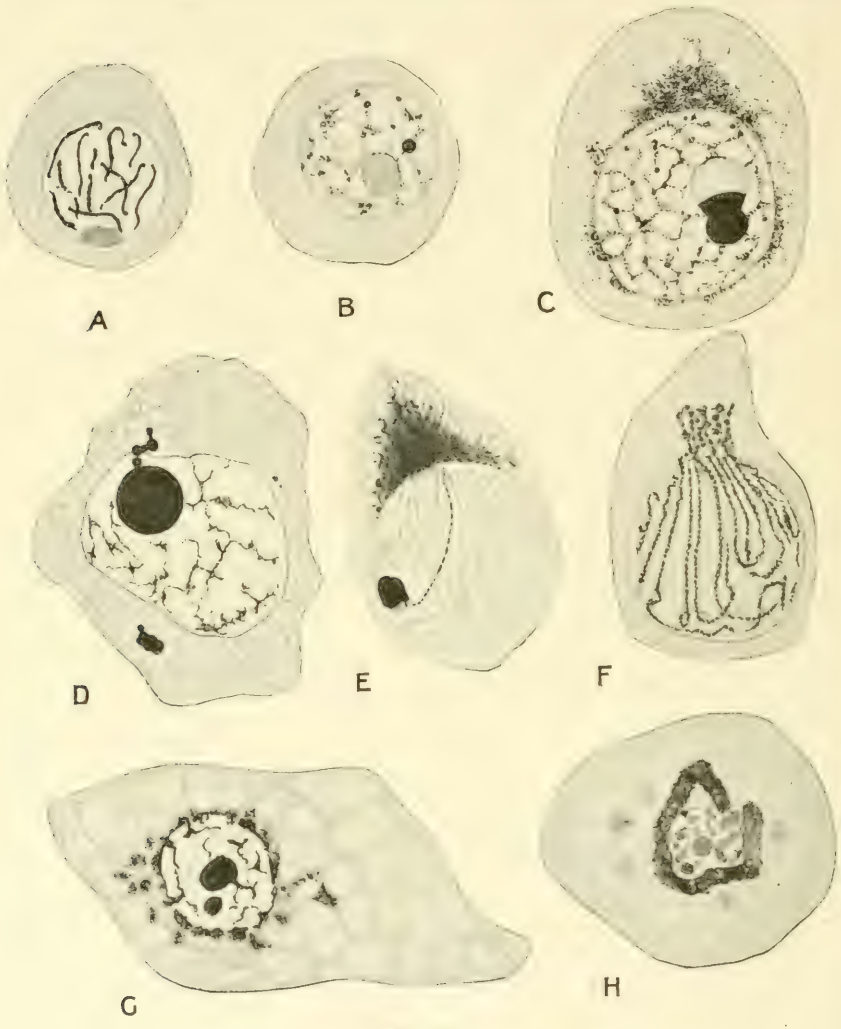


FIG. 81.

Illustrating the supposed emission of chromidia from various Metazoan cells. A, B, C, oocyte of *Aricia foetida* (after Schaxel, *Z.f A.*, 1912). A, young oocyte, chromosomes still filamentar, cytoplasm destitute of chromidia; B, older oocyte, still no chromidia in the cytoplasm; C, still older oocyte, showing emission of chromidia from the nucleus into the cytoplasm. D, oocyte of *Ancistrus bifida* (after Chubb, *Phil. Trans.*, 1906). Discharge of comparatively large masses of chromatin from the nucleolus. E, spermatocyte I. of *Blatta germanica* (after Wassilieff, *A.m.A.*, 1907). F, oocyte of *Proteus anguineus* (after Jorgensen, *F.H.*, 1910). G, H, somatic cells of *Musca* (after Popoff, *F.H.*, 1910). G, emission stage; H, the chromidia congregated into a band round the nucleus.

are divided as to whether they pass as formed bodies through deficiencies in the nuclear membrane (Buchner, 1910), or whether they are passed

through in a state of solution. Various views are held regarding the meaning of the chromidial formation. Following Hertwig, it has been supposed (Popoff, 1907) that it is a means by which the mass relations between nucleus and cytoplasm are restored, if for any reason the quantity of chromatin relative to the cytoplasm has become too great. Chromidia have also been supposed to give rise by degenerative transformation to reserve food material such as yolk (Popoff, 1907 ; *Paludina* oocyte : Moroff, 1909 ; Copepod oocyte) or fat (Popoff, 1910 ; *Musca* fat cells).

Others again have ascribed to them a much more important rôle, supposing that they have a formative function, being in fact the intermediaries through which the nucleus produces the necessary changes in the cytoplasm to bring about the differentiation of indifferent embryonic cells into the specialized cells—muscles, nerves, etc.—of the soma. According to Goldschmidt, the originator of this view, chromidia may give rise to cell structures by direct transformation into them. Thus in oocyte I. they are transformed into yolk granules, in embryonic cells into muscle fibrillae, zymogen granules of gland cells, etc.

This view was founded partly on observation of muscle and gland cells in *Ascaris* (Goldschmidt, 1905, 1910), which have not been supported by subsequent observers on the same material, and partly on analogy with certain Protista ; for example, Trypanosomes, where a darkly staining body (" kinetonucleus ") which is in close anatomical relation to the flagellum and therefore apparently concerned with the function of locomotion, is supposed by many to have been derived from the nucleus and to consist of chromatin. At present, however, we cannot be said to possess reliable evidence of the direct transformation of chromidia into functional cell structures, though it seems not unlikely that they may by fatty degeneration be transformed into yolk, fat, etc.

Goldschmidt, however, also allows of a different mode of action of the chromidia, considering that besides giving rise to functional cell structures by direct transformation into them, they are in other cases the formative bodies under whose agency the cytoplasm is moulded into its various forms ; in other words, the morphogenetic activity of the nucleus, to which we have so often alluded, is not exerted directly by the nucleus as a whole, but by portions emitted through the nuclear membrane, as chromidia, into the cytoplasm which it is to mould. Goldschmidt is thus led to distinguish between two kinds of nuclear material, propagative and somatic, or to select one of the various terms that have been proposed by different workers—*idiochromatin* and *trophochromatin*. Idiochromatin is the idioplasm, which we have sufficiently characterized already. Trophochromatin is derived from the idioplasm, and is the intermediary by which the latter, the master

substance of the body, reacts upon the cytoplasm on which, in the last instance, the forms and functions of the cells, and therefore of the organism, directly depend.

This view is again largely founded upon the analogy of the Infusoria, where the nucleus is divided into two portions, the micronucleus, which alone takes part in conjugation, and the macronucleus, which is supposed to be concerned with the physiological activities of the cell, and which disappears before conjugation, a new one being derived from the micronucleus after syngamy. The ordinary Metazoan nucleus contains both kinds of nuclear substances, the trophochromatin being separated from it from time to time as required in the form of chromidia, which therefore correspond to the Infusorian macronucleus. The only nuclei in the Metazoa which exactly correspond with the Infusorian micronucleus, consisting entirely of idiochromatin, are the gamete nuclei, from which all the trophochromatin is supposed to have been eliminated during the growth period of the oocyte or spermatocyte (Goldschmidt, 1905, 1910).

While probably no useful purpose is to be served by labouring the distinction between the two kinds of nuclear substances (since in any case the one is directly derived from the other), if it were established that the nucleus exerts its morphogenetic action through the agency of chromatin extruded naked into the cytoplasm, a step, though a small one, would undoubtedly have been taken towards the understanding of this dark problem. Practically our only direct evidence in favour of this view is to be found in a series of papers by Schaxel.

His results in a large number of forms are remarkably uniform, and may be illustrated by the development of the Polychaete *Aricia foetida* (1912) and *Strongylocentrotus* (1911 *a*).

There is no emission of chromidia during the process of cleavage (cell multiplication), but before cell differentiation begins, *e.g.* before the endoderm cells take on the specific character of the cells of the alimentary canal, or mesoderm cells develop into muscle cells, an emission of chromatin takes place.

To take a specific example: the conversion of an undifferentiated mesenchyme cell of *Strongylocentrotus* into a skeletal cell is preceded, according to Schaxel, by an emission of chromidia from the nucleus into the cytoplasm. These chromidia congregate into a mass, in the middle of which a globule of the skeletal secretion soon appears. This increases in size at the expense of the chromidia—that is to say, the chromidia are destroyed or used up by their own formative action, though it is not suggested that they are actually transformed into the secretion. As the Echinoderm skeleton is extracellular, this secretion has to be extruded from the cell to take part in the formation of the skeletal spicule. At present the work of Schaxel stands in need of con-

firmation. Indeed, even the occurrence of chromidial extrusion is not undisputed. Thus Duesberg (1911 *a*) denies the nuclear origin of the "chromidia" described by Wassilieff in *Blatta* (Fig. 81, E) and holds them to be chondriosomes (see below), and therefore of purely cytoplasmic origin. Meves also denies the derivation from the nucleus of certain bodies, which have been described by the upholders of the chromidia theory as having been so derived. Beckwith (1914) has subjected the egg of *Hydractinia* to a very careful examination, and comes to the conclusion that the staining particles there present, which must undoubtedly be of the same nature as the similar bodies described by Schaxel in so many oocytes (including those of several Hydrozoa), are of cytoplasmic, and not of nuclear, origin. She bases this conclusion on the fact that they make their first appearance scattered through the cytoplasm and not concentrated round the nucleus, and also on the fact that though they react similarly to chromatin to many stains, they show striking differences in their reaction to others. The doubts thus thrown on the nuclear origin of the "chromidia" in the Metazoa lead us on to a consideration of the chondriosomes, which, as we shall see, may or may not be identical with chromidia.

(2) *Chondriosomes* [1]

These are granular or filamentar bodies present in the cytoplasm, about the nature of which there has been much controversy during the last few years. Some cytologists have ascribed to them a rôle in morphogenesis and heredity equal to that of the chromosomes. This theory has been especially developed by Meves (1908, 1911, etc.) and Duesberg (1911 *b*, etc.), following on the work of Benda. An exhaustive review of the literature on the subject up to the year 1911 is given by Duesberg (1911 *b*).

An account of the chondriosomes in the spermatogenesis of *Blatta* has already been given (Chapter III.). They may take the form of granules, chains or filaments. They stain strongly with many stains, including the commoner chromatin stains, though towards others they react differently from chromatin, which fact is an important argument against their being the same as chromidia. By certain fixatives, especially

[1] This subject has suffered, like most other branches of science, from changes of nomenclature accompanying extension of knowledge or change of view. The following summary will be of use to the student who wishes to follow up this subject in the original literature:

General term	= Chondriosome (Meves) = Plastosome (Meves).
Chondriosomes in form of granules	= Mitochondria (Benda) = Plastochondria (Meves).
Chondriosomes in form of rods or filaments	= Chondrioconts (Meves) = Plastoconts (Meves).
Chondriosomes in form of chains of granules	= Chondriomites (Benda).

The change in the prefix in Meves' latest set of terms signifies his view of their histogenetic function (1911). Paraplastic bodies (Meves) are bodies developing from Plastosomes.

acetic acid, their non-chromatic nature is made especially evident, since in their usual condition chondriosomes are dissolved, or at any rate caused to disappear, by this reagent, which on the contrary forms one of the commonest and most useful of chromatin fixatives. Largely for this reason, doubtless, chondriosomes do not often appear in cytological figures, which are mostly taken from material which has been fixed with regard to the preservation of the nuclear structures. Chondriosomes are readily seen, and their movements followed in detail, in living cells (see, for example, Lewis and Robertson, 1916).

Meves (1910) identifies the chondriosomes with the filaments of Flemming (except that the latter included certain structures under this term, such as the fibres of the achromatic figure, which appear to be of a different nature) and with the granules of Altmann.

That the chondriosomes possess a peculiar significance in morphogenesis and heredity is based on the following claims :

(1) They are permanent cell structures, persisting from one cell generation to another, reproducing themselves by fission.

(2) There are indications that in some cases arrangements exist for at any rate an approximately equal partition of the chondriosomes between two daughter cells at cell division.

(3) They are carried into the egg by the spermatozoon at fertilization.

(4) The chondriosomes found in the fertilized egg can be traced in the tissues of the developing embryo, and have been described as actually giving rise to permanent cell structures of the adult—for example, neurofibrillae, muscle fibrillae of striated muscle fibres (Meves, 1908 ; Duesberg, 1910) and to various organs of plant cells (see Meves, 1918).

It must suffice to comment very briefly on these four points.

(1) Since the chondriosomes lie in the cytoplasm, and since the cytoplasm of the mother cell is divided among the two daughter cells, it follows that the chondriosomes also must be passed on from cell to cell ; the spermatogenesis of *Blatta* illustrates this (Fig. 29). There is no evidence, however, to show that they may not disappear and re-form in the cytoplasm from time to time ; their origin *de novo* in the cytoplasm has indeed been described in certain cases (Schaxel, 1912 ; Beckwith, 1914), while evidence of their regular multiplication by fission is practically non-existent.

(2) So far very little evidence exists for this, though at cell division the chondriosomes are often doubtless distributed together with the cytoplasm into two approximately equal masses. On the other hand, certain cases have been described where the division is unequal. This is manifestly the case in the polar body formation, where practically the entire mass of the chondriosomes of the oocyte I. are left in the ripe egg, exhibiting nothing corresponding to the reduction of chromosomes.

In the division of the spermatocytes the chondriosomes are presumably generally more or less equally divided, along with the cytoplasm, between the daughter cells, but definitely unequal distribution of chondriosomes among spermatids has been described in *Myxine* (A. and K. E. Schreiner, 1908) and *Euschistus* (Montgomery, 1911). An unequal distribution of the chondriosomes among the daughter cells also takes place in the developing embryo of Ascidians (Duesberg, 1915). Thus precise quantitative and qualitative distribution of the chondriosomes between the daughter cells at cell division is not at any rate of general occurrence, nor is there any reduction in their mass at oogenesis. These facts weigh heavily against the theory that the chondriosomes are the seat of morphogenetic factors.

(3, 4) The chondriosome apparatus always[1] plays a part, though a variable one, in the structure of the adult spermatozoon. In what may be called the "typical" spermatozoon it occupies the middle piece of the tail, forming a sheath for some distance round the axial fibre. In the tailless forms of spermatozoon, such as are found in Nematodes, Arthropods, and a few other animals, it assumes various forms and positions.

It is quite possible that chondriosome apparatus, or part of it, always enters the egg in fertilization. In several cases, it is true, the tail of the spermatozoon fails to enter the egg, but in many spermatozoa only the extreme base of it would be necessary, or in others (*Ciona*; Duesberg, 1915) none of it, the chondriosome apparatus being alongside the nucleus in the head. If the chondriosomes are to be accepted as hereditary substance, however, it is necessary to show that having entered, they mingle with the egg chondriosomes, and are distributed with them to the cells of the developing embryo.

The first form in which the behaviour of the chondriosomes at fertilization was worked out was *Ascaris megalocephala* (Meves, 1911), and this appeared to give brilliant support to the theory that the chondriosomes are hereditary material. The chondriosomes in the spermatozoon are in the form of a number of comparatively large granules, those in the egg being much smaller, and scattered throughout the egg cytoplasm. After the spermatozoon has entered the egg, the egg chondriosomes concentrate round it; the male chondriosomes leave the spermatozoon and break up into numerous small granules of the same size as those of the egg. The two sets of chondriosomes now become indistinguishably mingled.

It soon appeared that the case of *Ascaris*, in the spermatozoon of which the chondriosomes are unusually bulky, is not typical. Meves

[1] Montgomery (1912), however, describes it as being thrown off by the developing spermatozoon of *Peripatus*.

(1912) himself found that in sea-urchins the chondriosome apparatus introduced by the spermatozoon undergoes no metamorphosis, but is relegated unchanged to one of the first two blastomeres. An exactly similar process has been described for various mammals by van der Stricht (1910) in the bat, and by Lams (1913) in the guinea-pig (Fig. 82). In order to save the theory of chondriosome inheritance, it was suggested that the blastomere lacking the chondriosomes takes no part in the formation of the adult, but that in the mammals (van der Stricht) the trophoblast develops from it, and in Echinoderms (Meves) that part of the pluteus larva which is cast off at metamorphosis. This conjecture must be considered very improbable, for the pluteus is an organism quite as specific as the adult sea-urchin and quite as much in need of

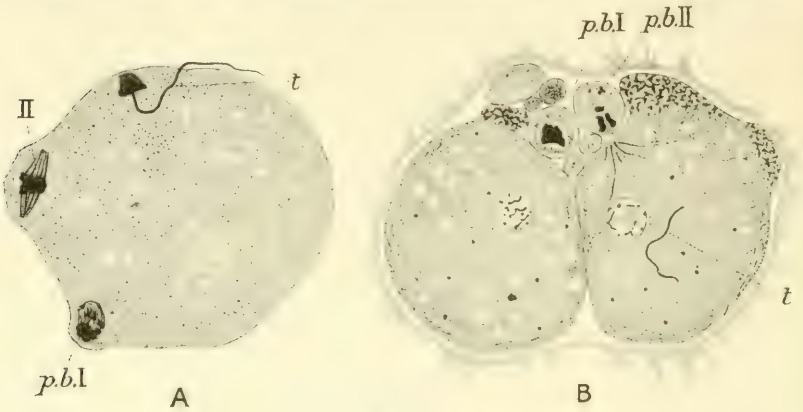


FIG. 82.

The chondriosomes in the fertilization and cleavage of the guinea-pig's ovum. (After Lams; *A.B.*, 1913.)
A, entry of the spermatozoon; B, 2-cell stage, the tail (including the chondriosome apparatus) of the spermatozoon lying unchanged in one blastomere.
p.b. I., first polar body; *p.b.* II., second polar body; *t*, tail of spermatozoon; II., metaphase II.

a complete hereditary outfit. It has, however, been rendered still more improbable—if not indeed definitely disproved—by Meves himself, who later (1914) traced the chondriosome mass of the male gamete in *Parechinus* up to the 32-cell embryo. At this stage it is still compact and unchanged, and is therefore of course only to be found in one cell. All the other 31 cells therefore contain no part of the male chondriosome apparatus, so that it is established that this is not essential to the development of the Echinoderm.

Whilst thus the behaviour of the male chondriosome mass in fertilization is alone enough to destroy all claim to the idioplasmic nature of chondriosomes, unless and until new knowledge of an unforeseen nature is forthcoming, there are several other almost equally convincing items of evidence. It is not necessary to labour the point of the colossal excess of the chondriosomes in the egg over those brought in

by the spermatozoon. More serious perhaps is the fact that these egg chondriosomes may be very unevenly distributed amongst the blastomeres in cleavage (Duesberg, 1915). An unequal distribution of the chondriosomes may be brought about experimentally in *Hydractinia* (Beckwith, 1914) by centrifuging the egg. This does not prejudice the normal development of the larvae.

The persistence of the chondriosomes which are undoubtedly present

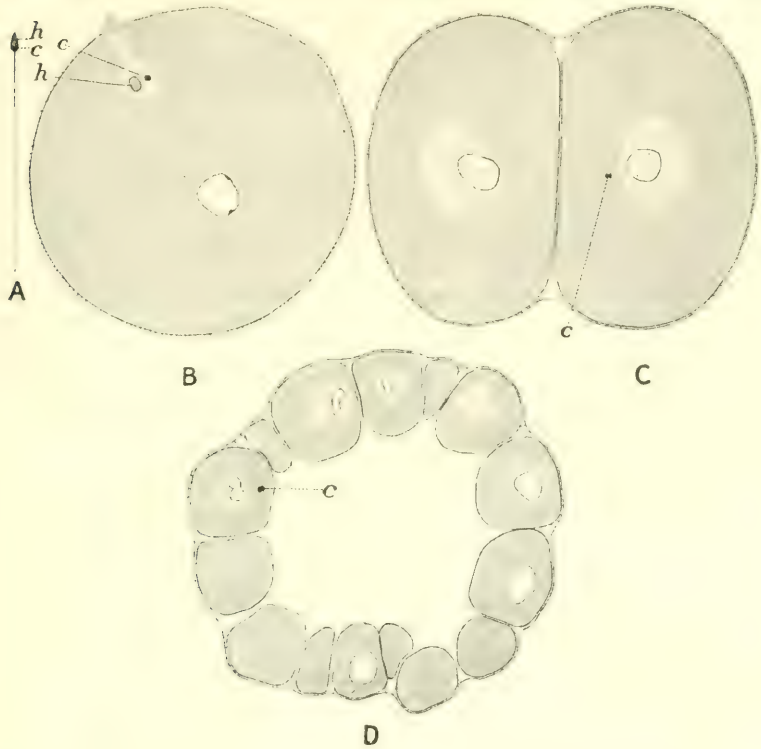


FIG. 83.

The chondriosomes in the fertilization and cleavage of the egg of *Parechinus miliaris*. (After Meves, *A.m.A.*, 1912 and 1914.) A, spermatozoon; B, shortly after entry of the spermatozoon into the egg; C, 2-cell stage; D, 32-cell stage.
c, chondriosome apparatus; *h*, head (nucleus) of spermatozoon.

in the fertilized egg and embryo, and their development into certain specific cell structures of the adult, especially into neurofibrillae and muscle fibrillae, have been described by Meves (1908) and Duesberg (1910) for the chick, and also for some mammals. According to Arnold (1912) they give rise to the zymogen granules in the pancreas.

(3) *The Relation between Chromidia and Chondriosomes*

This last supposed characteristic of the chondriosomes obviously raises at once the question whether these bodies are not the same as chromidia, and here we are once again face to face with a controversy which must at present be left undecided. There can be no doubt that in certain specific cases the same thing has been described by one worker as chondriosomes and by another worker as chromidia—we may compare Fig. 29, D, and Fig. 81, E, both representing the primary spermatocyte of *Blatta germanica*. We have indeed all possible views of the relation between the two structures supported by different workers.

(1) Chondriosomes have no relation with chromidia, though in some cases they have been erroneously described as such (Meves, Duesberg).

(2) Chondriosome is simply a name given to chromidia by those who have failed to recognize their true origin from the nucleus (Goldschmidt, Popoff, Buchner).

(3) Chondriosomes and chromidia are independent bodies, found side by side in the cytoplasm, the one of cytoplasmic, the other of nuclear origin (Schaxel, Jorgensen).

Finally, it should be mentioned that both chondriosomes and chromidia have been interpreted in some cases as metamorphosed parts of the achromatic figure. This applies to certain of the very diverse bodies known under the comprehensive terms of "Nebenkern" and yolk nucleus, but it appears certain that most of the structures described as chondriosomes and chromidia cannot be so explained.

CHAPTER VII

THE NUCLEUS OF THE PROTISTA AND PLANTS

A. PROTISTA

THE nucleus of the Protista is not constructed on such a uniform plan as that of the Metazoa or Metaphyta. Certain bacteria indeed are said to exhibit no differentiation into nucleus and cytoplasm, being alternatively interpreted as consisting wholly of the one or the other. Dobell (1911), however, who has made a special study of the larger forms of bacteria (on which alone reliable cytological observations of this kind are possible) finds a differentiation between nucleus—or, at least, chromatin—and cytoplasm in all the forms which he examined.

In many other Protista there is no *organized* nucleus, the chromatin, etc., being scattered through the cell in the form of chromidia. This may possibly be the permanent condition of the nucleus in some of the more lowly organized forms, but much more often it is a temporary phase, a compact nucleus being formed at other phases of the life cycle, as in certain bacteria (Dobell, *loc. cit.*) and many Protozoa, of which examples are given below.

Even when an organized nucleus is present in the Protista, its form is more varied than it is in the higher organisms.

Two chief types of organized nucleus are commonly distinguished in the Protista, namely, the vesicular and the granular. In the commonest type of the former, which appears to be the less specialised form of nucleus, the greater part or possibly sometimes all the chromatin is aggregated into a single central mass or karyosome, which lies in a vacuole containing fluid, probably of the same nature as the Metazoan karyolymph. The chromatin in the karyosome is probably in the form of granules bound together by linin or plastin (as in the amphinucleolus of Metazoa). In certain Protista the karyosome also contains a body which has been identified as the centrosome, which is therefore intranuclear in these forms.

In the granular type of nucleus the chromatin is distributed in the form of granules or small masses over a linin framework. This type

of nucleus approaches therefore the commoner type of Metazoan nucleus. The vesicular and granular types of nuclei grade into one another, however.

Many Protista have two nuclei differing greatly in structure, and presumably in function. Two principal types of this *binuclearity* are found.

(1) In the Infusoria, one of the two nuclei (macronucleus) is much larger than the other (micronucleus). The former divides amitotically during reproduction by fission, while the latter exhibits a form of mitosis. Moreover, the macronucleus disintegrates before conjugation, and takes no part in that process. In the exconjugant it is formed anew from the zygote micronucleus. It is thus clear that the macronucleus is a nucleus physiologically of a lower order than the micronucleus, and it is supposed to be concerned exclusively with the somatic functions of the cell.

(2) In the second type of binuclearity, found in the Trypanosomes and their allies, the two nuclei are called respectively the *trophonucleus* and the *kinetonucleus*. (It must be mentioned, however, that the nuclear nature or origin of the "kinetonucleus" has been questioned in many cases.) The former is the larger, and the one most nearly corresponding to the nucleus of uninucleate Protista. The kinetonucleus is in close anatomical relation to the flagellum, and is therefore supposed to be specially concerned with the movements of the animal.

A general review of binuclearity in the Protista is given by Dobell (1909).

Compared with the Metazoa and Metaphyta, the modes of nuclear multiplication in the Protista are of bewildering variety. This refers both to the behaviour of the chromatin and to the development of the achromatic figure. The latter we will not consider specially, except to point out that the centrosomes when present in the Protista are not uncommonly intranuclear, as indeed is the whole achromatic figure (in cases where this is identifiable). In such cases the whole mitosis may take place without rupture of the nuclear membrane. Centrosomes are, however, sometimes absent; *e.g.* in certain Amoebae.

As regards the chromatin, we may classify the modes of nuclear multiplication into three principal categories—amitosis, mitosis, and through the intermediation of chromidia formation.

Before discussing mitosis and amitosis in the Protista we must make quite clear the precise meaning which we attach to each of these terms. The word mitosis was coined to describe nuclear division in the Metazoa and Metaphyta, and correctly emphasizes the most significant feature of the process—namely, the linear arrangement of the chromatin elements into the threadlike chromosomes. The function of this is universally assumed to be to facilitate the exact partition among the daughter nuclei

of the products of division of differentiated chromatin particles. In the Protista, however, we must extend the word to cover all cases of nuclear division accompanied or preceded by rearrangements of the chromatin which can be interpreted as having this function of bringing about a qualitative rather than a purely quantitative division of the chromatin, even though they do not result in the formation of regular chromosomes.

The term amitosis is confined, as in the case of the Metazoa and Metaphyta, to a purely mass division of the nucleus without any attempt at a qualitative equality among the daughter nuclei.

Amitosis, in the sense just defined, has been described repeatedly in the Protista, both in the vesicular and granular types of nucleus. Undoubtedly, however, the trend of modern research in Protistology is to discover in more and more supposed cases of true amitosis a preliminary internal readjustment of the chromatin, which suggests that it undergoes a qualitative rather than a purely quantitative partition. Very often these readjustments do not go so far as the formation of the regular chromosomes which we find universally among the higher organisms, but they in all probability represent a primitive form of mitosis. It would seem as well therefore to suspend judgement for the present as to whether purely quantitative mass division of simple nuclei ever does take place. The term " simple " is a necessary qualification, for the Protozoan nucleus sometimes has a very different composition from that of the higher organisms, and in these cases the problem of " amitosis " bears a different complexion.

Pure mass division of the macronucleus of Infusoria and Acinetaria does indeed appear to be demonstrated. Especially in the latter group is it difficult to see how the division can be qualitative during the process of bud formation in such a form as *Ephelota*. Here a process of the macronucleus grows out into the developing bud and then becomes nipped off to form the macronucleus of the bud. The macronucleus in these groups, however, appears to be composed of trophochromatin only. It is at any rate destined only to last during the asexual portion of the organism's life cycle ; before conjugation the macronucleus breaks up and disappears, only the micronucleus taking part in syngamy. In both groups the latter nucleus, in contrast to the macronucleus, divides by mitosis throughout the whole life cycle, thereby retaining its qualitative composition.

The process of mitosis in the Protista ranges in complexity from the merest indication of a sorting out of the chromatin elements before division of the nucleus as a whole, to a mitosis as perfectly developed as any found in the Metazoa, with fully formed chromosomes, an equatorial plate, centrosomes and complete achromatic figures.

As examples of primitive mitosis we may take the nuclear division at

two different phases of the life cycle of *Coccidium schubergi* (Schaudinn, 1900). In the schizont (the asexual cycle), before nuclear division the chromatin granules become massed together in little clumps and irregular threads, in which, however, no definite longitudinal splitting can be made out, and they do not get collected into an equatorial plate. They sort themselves out in some way or other into two groups which appear to be pushed apart by the elongation of the karyosome, which contains, or takes the place of, the centrosome and achromatic figure.

The nuclear divisions in the oocyst of the same species, which cover

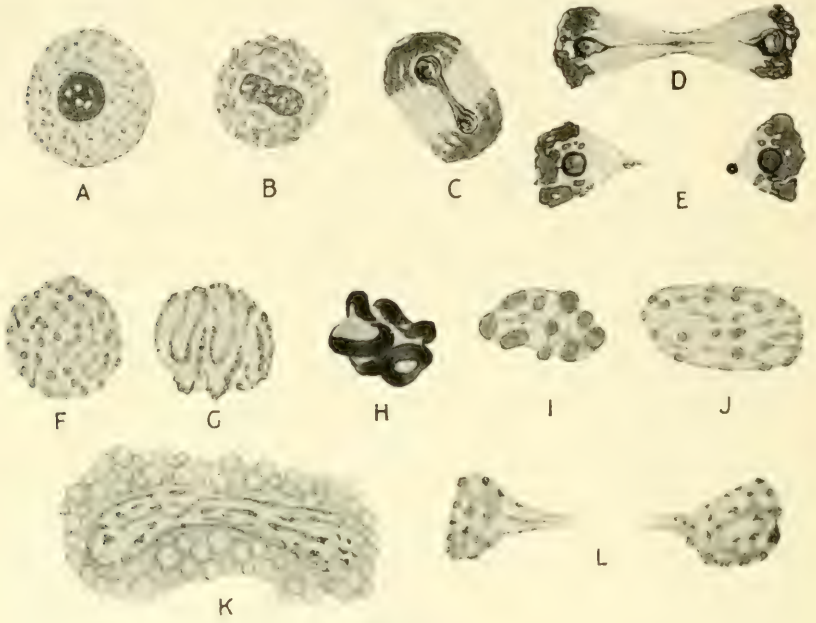


FIG. 84.

Nuclear division in the asexual cycle of *Coccidium schubergi*. (After Schaudinn, *Z.J.A.*, 1900.) A-E, the schizont; F-L, the oocyst.

the first few divisions of the zygote nucleus after syngamy, are instructive as showing how nuclei which appear to divide in the most purely amitotic fashion may have undergone a previous reorganization which is presumably connected with the accurate partition of differentiated chromatin elements between the daughter nuclei (Fig. 84, F-L).

There is a prophase closely resembling that of a Metazoan mitosis resulting finally in the formation of a relatively very thick and short spireme. This, however, breaks up into irregular fragments which become united to form a reticular nucleus again, and in this condition the nucleus divides. Although, therefore, the actual division appears to be amitotic it is difficult to avoid the conclusion that the previous arrangement of

the chromatin granules in linear series had the same function as postulated for the chromosomes of a Metazoan mitosis, namely, to effect their accurate division and partition among the daughter nuclei.

In some Protista very well-developed mitosis, closely resembling that in the Metazoa and Metaphyta, is found (Fig. 85).

In the Coccidian, *Aggregata eberthi* (Dobell and Jameson, 1915) the nuclei of the primary gametocytes (♂ and ♀) show in mitosis six chromosomes of very different sizes (labelled, from largest to smallest, *a-f* in Fig. 86). The macrogametocyte is transformed into the macrogamete without any reduction of chromosomes, the macrogamete having therefore the same series of six chromosomes. The primary microgametocyte nucleus undergoes repeated division to form the microgamete nuclei, the same series of six chromosomes appearing throughout, though becoming greatly reduced in size. Both gametes have therefore, like the gametocytes, six chromosomes. Syngamy results in a zygote with twelve chromosomes which can be sorted out into pairs as in a typical Metazoan diploid nucleus.

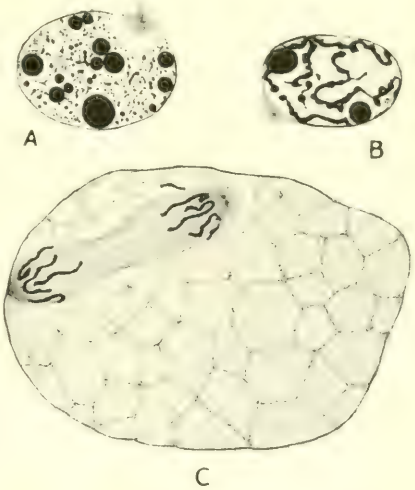


FIG. 85.

Mitosis of the gametocyte nucleus of *Monocystis*. (After Brasil, *Arch. Zool. Exp. Gén.*, 1905.) A, B, prophase; C, anaphase.

In the metaphase of the first division of the zygote nucleus the homologous chromosomes become united into bivalents, the constituents separating at anaphase. This division therefore is a reduction division and the daughter nuclei have only six chromosomes. This number is retained throughout all the subsequent nuclear divisions of the life cycle, which include spore formation, the asexual multiplication of the schizont, and the gametocyte divisions again. Thus in this animal the relation between the duration of the haploid and diploid phases is the reverse of what obtains in the Metazoa, the nuclei being haploid throughout all the life cycle except in one cell generation—the zygote cell—while meiosis takes place, not at gametogenesis, but at the first division of the zygote nucleus, which is comparable to the first cleavage mitosis of the Metazoan egg. Many plants also exhibit a much longer duration of the haploid generation relatively to that of the diploid part of the life

cycle than is found in the Metazoa, where with the exception of certain cases of parthenogenesis the haploid stage is represented by at most two cell generations (p. 214).

A closely similar account of the nuclear cycle of the Gregarine *Diplocystis schneideri* is given by the same authors (*loc. cit.*).

The third principal mode of nuclear multiplication in the Protista—by the intermediation of chromidia formation—is supposed to be of frequent occurrence. The three forms, *Mastigella*, *Coccidium* and *Arcella* will serve as examples.

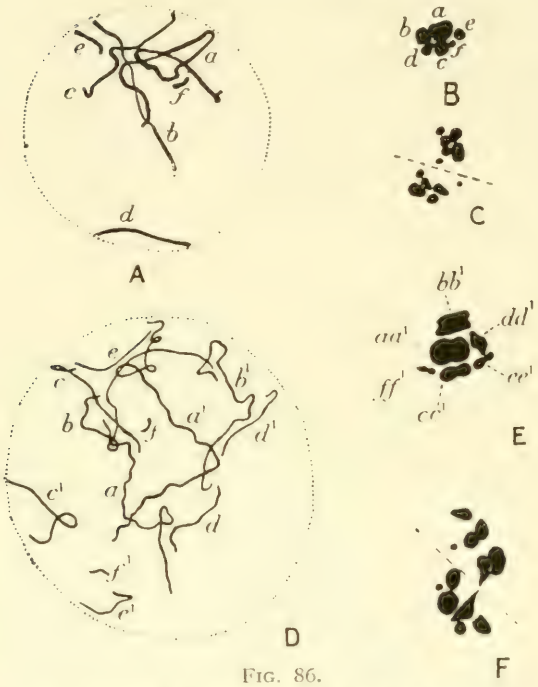


FIG. 86.

The chromosomes of *Aggregata eberthi*. They are designated *a f*, from the largest (*a*) to the smallest (*f*). (Dobell and Jameson, *P.R.S.*, 1915.) A, microgametocyte nucleus in prophase, with six chromosomes; B, equatorial plate, and C, anaphase of same; D, zygote nucleus in prophase for its first division: E, equatorial plate of same division—homologous chromosomes united into bivalents; F, anaphase of same (reduction division).

During the asexual multiplication of *Mastigella vitrea* (Goldschmidt, 1907) the nucleus divides by mitosis with well-developed chromosome formation. In gametogenesis, however, the gamete nuclei are produced from the nucleus of the gametocyte by a very different process (Fig. 87). There is a copious emission of chromidia from the nucleus into the cytoplasm. In the mass of chromidia numerous nuclei (up to two or three hundred) are formed by aggregation of numbers of chromidia into clumps. The nuclei thus formed then (in the case of the macrogamete) divide by mitosis (with chromosome formation) at least once. Goldschmidt interprets this as a reduction division, as it results in only one of the two daughter groups of chromosomes forming a functional nucleus, the other degenerating into a body strikingly reminiscent of the Metazoan polar body.

The micro- and macrogametes unite in syngamy, the zygote nucleus dividing by mitosis to introduce the asexual cycle with which we began.

Another example of multiplication of nuclei by chromidia formation alternating with multiplication by mitotic division, though attended by more complications, is afforded by *Arcella* (Fig. 88), the life history of

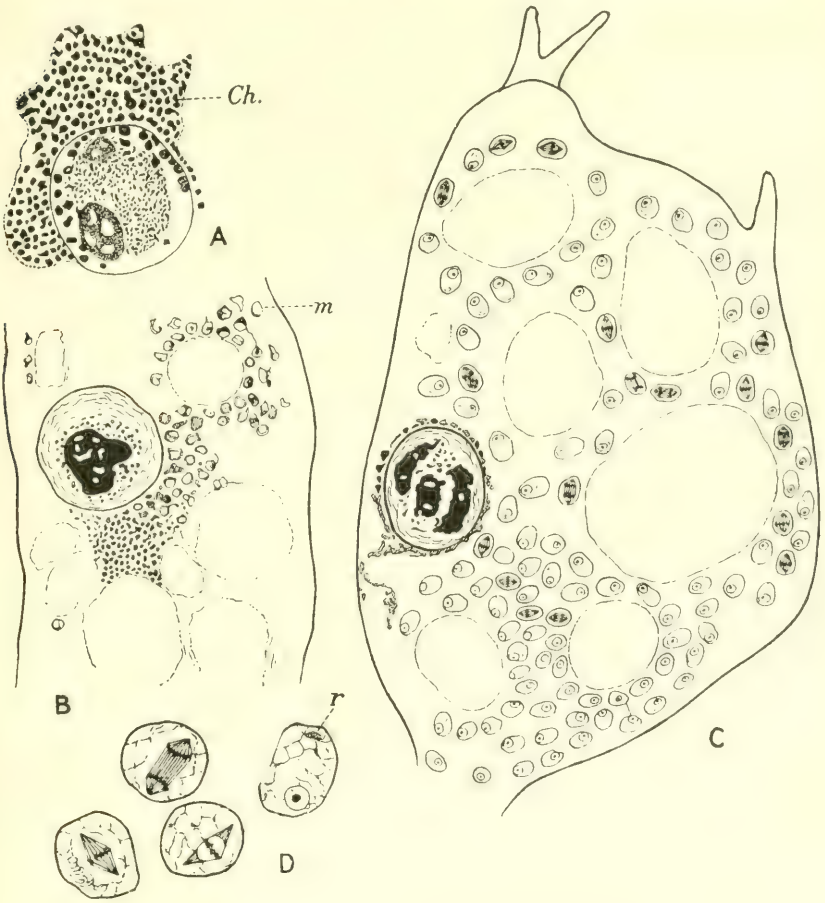


FIG. 87.

Stages in the formation of macrogametes in *Mastigella vitrea*. (After Goldschmidt, *A.P.K.*, 1907.) A, macrogametocyte nucleus surrounded by extruded chromidia. B, a later stage. As the chromidia get further away from the nucleus they are formed into the macrogamete nuclei. C, gametocyte full of macrogametes. Some are undergoing the (? reduction) division. D, four of the macrogametes from C on a larger scale. Three are in mitosis, and the fourth has completed the mitosis and contains the mature gamete nucleus and the polar body-like "reduction body."

ch, chromidia; *m*, macrogamete nuclei; *r*, "reduction body."

which has been studied by many workers. The essential features from our present point of view are as follows. At the beginning of the asexual cycle the young *Arcella* consists of a minute uninucleated cell. Soon the nucleus divides into two, by mitosis. At about the same time a great number of chromidia are emitted from the nuclei. These become

massed together into a "chromidial net" which forms a ring round the edge of the cell. The *Arcella* therefore now consists of a cell with two nuclei (primary nuclei) and a chromidial net.

At certain stages of the life history secondary nuclei are formed in large numbers out of the chromidial net, by the aggregation of chromidia into small masses. These secondary nuclei multiply by mitosis, and ultimately may give rise either to asexual buds or to gametes.

An unusual type of syngamy is sometimes observed in this animal, involving fusion or mingling of chromidia (chromidiogamy, Swarczewsky) rather than of formed nuclei. Two *Arcellas*, in which the primary nuclei have degenerated and all the chromatin is in the form of finely scattered chromidia, come together. Their cytoplasms—and hence the chromidia—mingle together, and then separate again into the two individuals, each

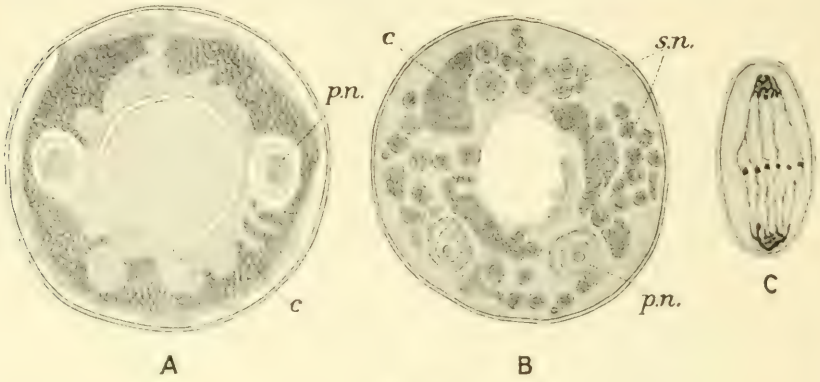


FIG. 88.

Arcella vulgaris. (A, B, after R. Hertwig, *Festschr. Kupffer*, 1899; C, after Swarczewsky, *A.P.K.*, 1908.) A, active phase, with two primary nuclei and a chromidial net; B, degeneration of primary nuclei and formation of secondary nuclei out of the chromidial net; C, mitosis of a secondary nucleus.
c, chromidia; *p.n.*, primary nucleus; *s.n.*, secondary nuclei.

containing presumably a mixture of chromidia from both conjugants. Out of these chromidia secondary nuclei are organized in a manner similar to that described above.

In *Coccidium schubergi* also, according to Schaudinn, the microgamete nuclei are produced from that of the microgametocyte by the passage of the chromatin of the latter out of the nucleus into the cytoplasm in the form of chromidia which rise to the surface of the cell, and there become aggregated into the microgamete nuclei.

In *Coccidium*, *Arcella*, and still more definitely in *Mastigella*, therefore, during certain periods of the life history the chromatin is aggregated into a formed nucleus, and all the usual mechanism of mitosis is present to ensure its accurate quantitative and qualitative partition among its descendants. At other periods this methodical mode of nuclear division is interrupted by a method of nuclear multiplication which affords no direct

evidence of qualitative chromatin distribution among the nuclei so formed. Since, however, during nuclear multiplication by means of chromidia the chromatin is resolved into small particles which are not improbably its structural units, it is possible that there takes place a qualitative sorting out of these units into the new nuclei which are formed by their aggregation into masses. Moreover, in assessing the significance of accounts of nuclear multiplication through the intermediation of chromidia much caution must be used. It must be remembered on the one hand that the study of the cytology of the Protista is often beset with much greater difficulties of technique than in the case of the Metazoa, owing to the minuteness of the elements concerned. Dobell and Jameson, as the result of their study of *Aggregata* and *Diplocystis*, are inclined to doubt accounts of chromidia formation in the Coccidia and Gregarines, such as, for instance, that of Schaudinn for *Coccidium schubergi* mentioned above. In the anaphase of the first division of the microgametocyte nucleus of *Aggregata* (the mitosis shown in Fig. 86, C) the chromosomes which were spheroidal in the metaphase become filamentar again. The asters at the two poles of the spindle divide repeatedly, and at each division the chromosomes divide longitudinally, becoming at last very minute. They are finally sorted out in groups of six, each group forming a nucleus at the periphery of the gametocyte cell, there to multiply by mitosis to form the microgamete nuclei. It can hardly be doubted that this process corresponds to the chromidial formation described by Schaudinn as above, a conclusion which suggests that there the term "chromidia" might be translated into "minute chromosomes"—a change in terminology implying that the process of their formation involves an exact division and partition of differentiated elements.

It must also be remembered that the Protistan nucleus may have a composition very different from that of the Metazoa or Metaphyta. In the latter groups the nucleus always, so far as we know, contains either a single or a double series of differentiated elements (with the special exception of the triploid, tetraploid, etc., nuclei considered on page 150). In the Protista, however, it appears that the nucleus may be *polyploid*, containing, not one or two, but a great number of series of elements. Examples of such *polyenergid* nuclei (Hartmann, 1909) are afforded by the great nuclei of the Radiolaria. The nuclear cycle of one of these, *Aulacantha* (Borgert, 1901, 1909), is as follows:

Reproduction may take place asexually by binary fission of the cell, or sexually through the intermediation of gametes. The division of the nucleus in the first type of reproduction takes place by a form of mitosis superficially very similar to a Metazoan mitosis (Fig. 89), but accompanied by the formation of an enormous number of chromosomes. The number of these is far more than a thousand, but varies greatly in

different individuals. These chromosomes, however, are said to undergo two longitudinal divisions in every mitosis. Each is said to divide once during prophase, each daughter chromosome dividing again in the metaphase. Another important point is that the spindle fibres do not converge to a single centrosome, but run parallel with one another.

Gamete formation (Fig. 89, D) begins with the emission of chromatin particles from the nucleus into the cytoplasm. Each of these particles becomes enclosed in a vesicle to form a minute secondary nucleus. Closer examination shows that the chromatin particles which are emitted from the original or primary nucleus are the individual "chromosomes" which appear in the mitosis of this nucleus. The minute secondary nuclei, each thus constituted out of a single "chromosome" of the primary nucleus, multiply by repeated mitosis to form the gametes. The number of chromosomes appearing in these mitoses is, however, not one, but ten to twelve.

Each of the enormous number of chromosomes of the primary nucleus is therefore equivalent to an entire gamete nucleus containing ten to twelve chromosomes. It may in fact be compared with the "unsegmented spireme" found in the prophase of certain Metazoan and Metaphytan mitoses (p. 9).

The emission of the "chromosomes" of the primary nucleus into the cytoplasm obviously suggests "chromidia formation," though in this case it is merely the breaking up of a compound polyploid nucleus into its constituent haploid (or diploid ?) nuclei, and therefore raises no special problem.

Amitosis, which, according to Borgert, occurs in *Aulacantha* in addition to mitosis, also raises no difficulties in the case of a polyploid nucleus, since each daughter nucleus may still have hundreds of representatives of each individual chromatin element.

Summing up, further knowledge is required before we can decide whether nuclear multiplication in the Protista by means of "amitosis" and "chromidia" formation is to be conceived as an exception to, or as a variant of, the orderly division of the chromatin elements which appears to be universal in the Metazoa and Metaphyta and to be at least common in the Protista.

B. ANIMALS AND PLANTS—HAPLOID AND DIPLOID CONDITIONS

As will have been gathered from the occasional references to plant cytology in the previous pages, the cytology of plants and animals is on the whole so similar that detailed comparison is unnecessary. Plants, however, exhibit a much more varied relation to the haploid

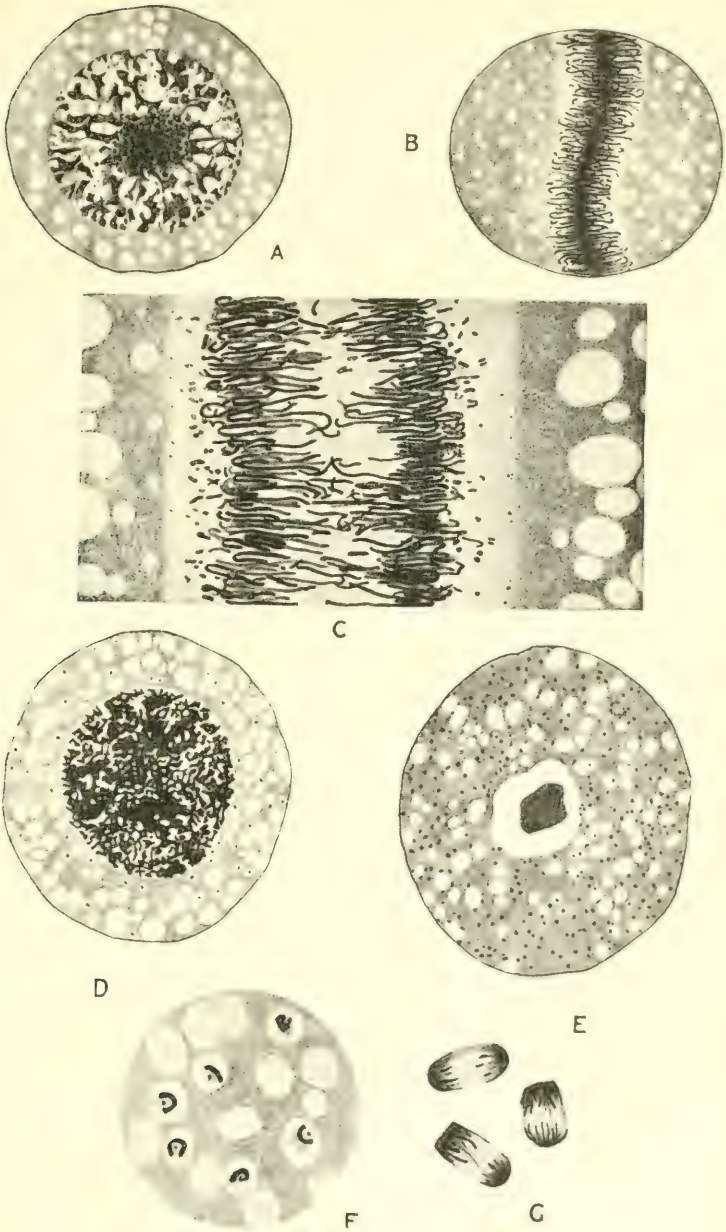


FIG. 89.

Nuclear multiplication in *Aulacantha*. (Borgert, *Z.J.A.*, 1901, and *A.P.K.*, 1909.) A-C, stages in the division of the primary nucleus during binary fission of the animal; D-G, preliminary stages in gamete formation. A, resting nucleus; B, mitosis, equatorial plate; C, portion of an anaphase shown on a larger scale; D, formation of secondary nuclei (the little dark specks in the cytoplasm) from the primary nucleus; E, the primary nucleus is almost used up in the production of the secondary nuclei; F, a few secondary nuclei under a higher magnification, showing the single chromatin thread in each; G, secondary nuclei in mitosis.

and diploid states than do animals. In the latter—at least in the Metazoa, with certain rare exceptions such as the males of the Hymenoptera—the individual is always diploid. In each life cycle occur one (post-reduction) or probably two (pre-reduction) haploid cell generations, namely, the gamete itself and generally the secondary oocyte or spermatocyte. In plants, however, the processes of meiosis and syngamy are often separated by a long section of the life history giving rise to an alternation of haploid and diploid generations. One of the best illustrations of such an alternation is in the ferns, where, as is well known, the ordinary fern plant or sporophyte is diploid, and the prothallus or gametophyte is haploid. The sporophyte produces, with reduction of chromosomes, haploid spores from which grows the prothallus. This produces gametes, without of course any further reduction of chromosomes, and from the zygote cell develops the next sporophyte. In the fern therefore the dominant phase in the life history is the diploid generation. This is still more so in the case of the flowering plants, where the haploid generation or gametophyte is reduced to a very few cell generations (5 in the female and 4 in the male), and does not lead an independent life, but is borne on and nourished by the sporophyte, which is the plant body as we know it.

The cell generations involved in the haploid phase of the flowering plants are shown diagrammatically in Fig. 90. The diagrams start with the *pollen mother-cell* in the male and the *embryo-sac mother-cell* in the female—in each case the last cell generation of the diploid phase. These cells divide twice in rapid succession, giving rise each to a group of four cells. Reduction takes place in the first of these two divisions. The process therefore is closely parallel to the meiotic phase in animals. The four haploid cells formed by these two divisions are *spores*, homologous with the spores of ferns, mosses, etc. In the male, the spores are *microspores* or *pollen-grains*. The nucleus of the pollen grain divides into two, one being a *vegetative nucleus* and the other a nucleus which again divides to give two gamete nuclei.

In the female, typically only one out of the four spores (*megaspores*) derived from a single embryo-sac mother-cell develops, the other three degenerating and thus again reminding us very strikingly of the ovum and polar bodies in animals. The nucleus of that megaspore which is destined to proceed with its development divides three times, thus producing eight nuclei. Since cell division does not follow these mitoses, these nuclei are all contained in one large vacuolated cell, the *embryo-sac*. Of the eight nuclei, only one is a functional gamete nucleus (ovum); three of the remainder become the *antipodal nuclei*, two the *synergidae*, and the remaining two come together in the middle of the cell and fuse to form the *central fusion nucleus*, which is therefore diploid.

At fertilization the two male gamete nuclei which are formed in each pollen grain are both introduced into the embryo-sac; one fuses with the ovum nucleus to produce the zygote, while the other fuses with the central fusion nucleus, forming thus a *triploid* nucleus. This afterwards gives rise to the endosperm, or reserve food material of the seed.

For a more detailed account of the gametophyte and fertilization in the flowering plants the reader is referred to any comprehensive work on botany, such as that of Bower (1919).

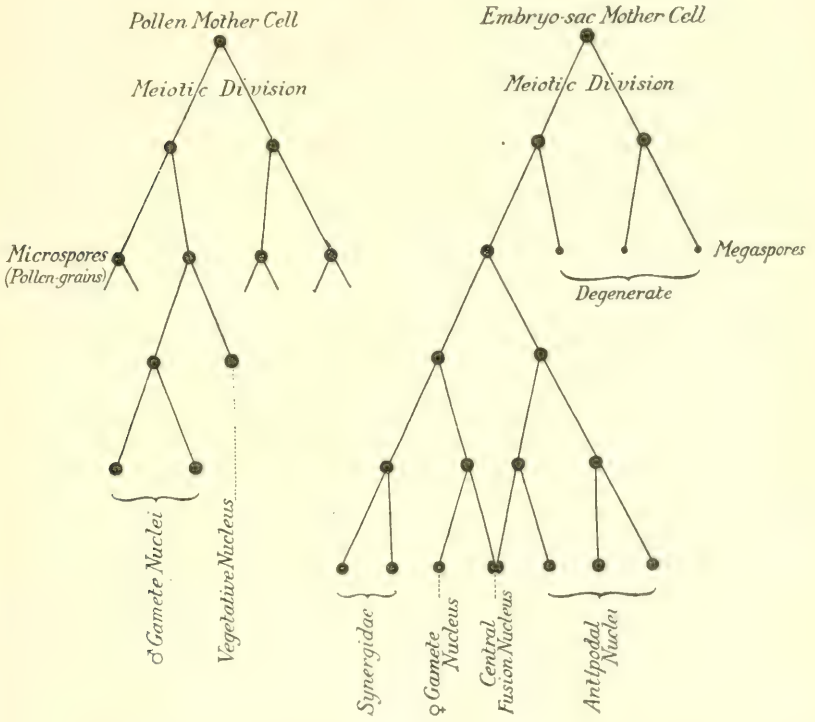


FIG. 90.

Diagrams of the cell generations involved in the haploid phase of the flowering plants.

Mosses and liverworts present the reverse case to the flowering plants, for the dominant phase (the ordinary moss plant, etc.) is the haploid gametophyte. The zygote grows into a comparatively simple sporophyte which is retained on and nourished by the gametophyte, and produces spores with reduction of chromosomes. These are set free to produce the new generation of gametophytes.

The relations of the haploid and diploid phases of the life cycle in animals and plants is summarized in Fig. 91; the figures A-F form a progressive series in the rise of the diploid and reduction of the haploid

generations. Although the examples mentioned as illustrating the

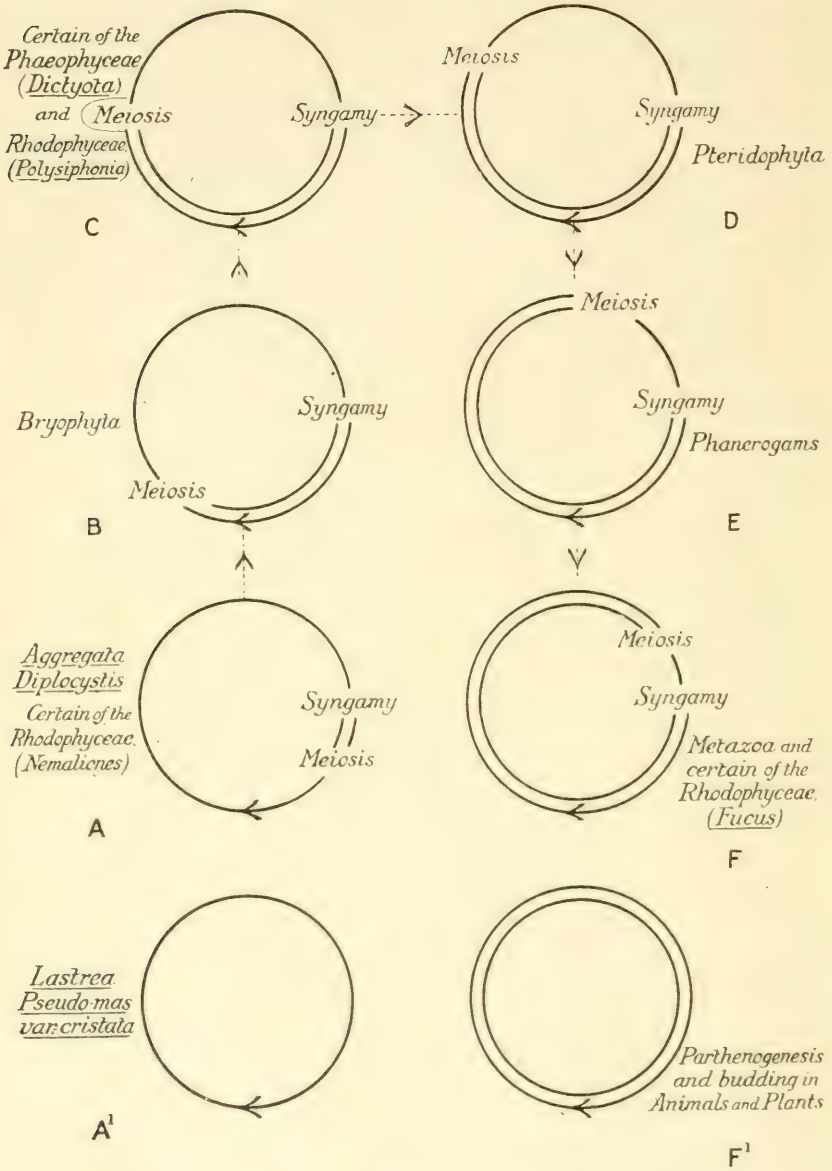


FIG. 91.

Diagrams illustrating the relations of the haploid and diploid phases in various organisms. In each case the single line represents the haploid, and the double line the diploid, condition.

different stages manifestly do not in most cases form a phylogenetic series, yet in the main it is probable that the condition shown in Fig.

91, A, is phylogenetically the oldest, from which the other conditions have been derived. While all such discussions are of course highly speculative, it is certainly probable that the earliest organisms, before sexual reproduction was evolved, were haploid. It is scarcely possible to avoid the conclusion that the condition in a diploid organism with its duplex set of all hereditary factors is a secondary one and the direct consequence of the introduction of syngamy, while the function of meiosis is to bring back the organism to its original haploid condition.

At present we cannot say for certain whether any organisms exist in which sexual reproduction has not yet been evolved, and which therefore have no diploid stage ; obviously it would be a difficult matter to identify such organisms, since in the absence of an alternation of diploid and haploid generations there would be no certain criterion for deciding whether the number of chromosomes (in the unlikely case that this could be determined in such a primitive organism) were the haploid or diploid number. Moreover, it would be necessary to prove a negative —namely, the non-occurrence of occasional syngamy and diploid stages.

Nevertheless it is possible to cite at least one case of a life cycle which has secondarily become completely haploid—namely, the fern *Lastraea pseudo-mas*, var. *cristata*. In this fern the sporophyte and the gametophyte have the same number of chromosomes, and this (as can be determined by comparison with its near allies) is the haploid number. One generation passes into the other without syngamy in the one case or meiosis in the other. This is a feature which has obviously been acquired but recently from an evolutionary point of view, and therefore it is shown as outside of the series in Fig. 91. The converse case, where organisms have entirely eliminated the haploid phase from their life histories, can be illustrated by many cases of parthenogenesis and asexual reproduction in animals and plants.

It is interesting to note that the haploid and diploid conditions in plants are not necessarily associated with a particular type of structure. For instance, in ferns, the above-mentioned *Lastraea pseudo-mas*, var. *cristata*, has a haploid sporophyte of the usual type of structure, though in ferns generally the sporophyte is diploid and the haploid condition is associated only with the prothalloid type of structure. The converse is the case with another fern, *Athyrium felix-foemina*, in which the prothallus is diploid as well as the sporophyte. Analogous cases in animals are the haploid individuals developing in certain cases of artificial parthenogenesis and the males of many Hymenoptera.

For a general discussion of the problems of alternation of generations in plants, the reader is referred to Bower (1919).

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The titles of certain Journals and other publications which are referred to several times are abbreviated as follows :

A.A. Anatomische Anzeiger. (Jena.)
A.B. Archives de Biologie. (Liége.)
A.E-M. Archiv für Entwicklungsmechanik der Organismen. (Leipzig.)
A.H.E. Anatomische Hefte : Ergebnisse der Anatomie und Entwickelungsgeschichte. (Wiesbaden.)
A.m.A. Archiv für mikroscopische Anatomie. (Bonn.)
A.P.K. Archiv für Protistenkunde. (Jena.)
A.Z. Archiv für Zellforschung. (Leipzig.)
B.B. Biological Bulletin. (Woods Holl, Mass.)
B.C. Biologisches Centralblatt. (Leipzig.)
B.G. Botanical Gazette. (Chicago.)
B.M.C.Z.H. Bulletin of the Museum of Comparative Zoology at Harvard. Cambridge, Mass.)
F.H. Festschrift zum sechzigsten Geburtstag Richard Hertwigs. (Jena, 1910.)
J.E.Z. The Journal of Experimental Zoology. (Philadelphia.)
J.G. Journal of Genetics. (Cambridge.)
J.M. Journal of Morphology. (Philadelphia.)
J.w.B. Jahrbücher für wissenschaftliche Botanik. (Leipzig.)
J.Z. Jenaische Zeitschrift für Naturwissenschaft. (Jena.)
L.C. La Cellule. (Louvain.)
M.J. Morphologisches Jahrbuch. (Leipzig.)
Phil. Trans. Philosophical Transactions of the Royal Society of London.
P.R.S. Proceedings of the Royal Society of London
Q.J.M.S. Quarterly Journal of Microscopical Science. (London.)
Z.A. Zoologischer Anzeiger. (Leipzig.)
Z.A.V. Zeitschrift für induktive Abstammungs- und Vererbungslehre. (Berlin.)
Z.J.A. Zoologischer Jahrbücher, Abteilung für Anatomie und Ontogenie der Tiere. (Jena.)
Z.w.Z. Zeitschrift für wissenschaftliche Zoologie. (Leipzig.)

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INDEX

Numbers in thick type refer to pages on which figures will be found

THE END

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