CHAPTER V

THE CHROMOSOMES AS BEARERS OF HEREDITARY MATERIAL

The evidence in favor of the view that the chromosomes are the bearers of hereditary factors comes from several sources and has continually grown stronger, while a number of alleged facts, that seemed opposed to this evidence, have either been disproven, or else their value has been seriously questioned. We propose now to examine in some detail the observations and experiments that bear on the chromosome theory of heredity.

The Evidence from Embryology

Relating to the Influence of the Chromosomes on Development

It has been argued that since the sperm transmits equally with the egg, and since only the sperm head, consisting of the nucleus, enters the egg, inheritance is only through the nucleus. But it must be admitted that around the entering sperm nucleus there may be a thin enveloping protoplasm, which, however scanty, might suffice to transmit certain cytoplasmic factors. Moreover, while the tail of the sperm appears in some cases to be left outside the

egg, in other cases it appears to enter and to be absorbed.

Behind the head of the spermatozoon, and at the base of the tail, there is a middle piece which contains a derivative of the old centriole or division center. Since the centrosome carried by the sperm has been found in some forms to give rise to the new centrosomes that occupy the poles of the first cleavage spindle of the egg, it may appear that a paternal contribution can come about in this way. It is true that the continuity of the centrosome of the sperm with that of the dividing egg has been disputed in some forms; but it is difficult to prove that the sperm centrosome is lost, even though it may disappear owing to loss of staining power.

The nucleus contains a sap which is probably of cytoplasmic origin. The presence of this sap may again be appealed to by those who do not accept the chromosomes as the bearers of heredity, as a weak link in the evidence. It is true that the nuclear sap appears to be squeezed out of the nucleus of the sperm head, leaving a compact and apparently solid mass of chromatin, yet its complete elimination can not be proved. Hence, while those who favor chromosomal transmission find in the facts of normal fertilization strong *indications* favorable to that view, yet it is also true that those who are inclined to dispute this view find several loopholes in the argument of their opponents.

The importance of the nucleus in heredity has further been shown by experiments of Bierens de

Haans, Herbst, and Boveri on giant eggs of sea urchins fertilized by sperm of another species. The hybrid larvæ produced when normal eggs of one species are fertilized by sperm of the other species are intermediate in character between the two parental types of larvæ; while those from giant eggs of the same species fertilized by sperm of the other, also intermediate, incline more to the maternal side. The nucleus of the giant egg is double the size of that of the normal egg and according to Bierens de Haans the chromosomes are also double in number. Consequently, the amount of maternal chromatin should be double that introduced by the sperm, and might produce a corresponding influence on the hybrid character. But since in these giant eggs the cytoplasm is also doubled, it is not evident that the results are due to the chromosomes rather than to the cytoplasm. By means of the following ingenious comparison Boveri has shown that the results must be ascribed to the chromosomes rather than to cytoplasm. Normal eggs were broken into fragments, the nucleated pieces were fertilized with the sperm of the other species, and those fragments of half the volume of the normal egg were isolated. As is known, such fragments develop into whole larvæ, whose nuclei will have the usual chromatin content. The egg cytoplasm is, however, reduced to half. Nevertheless the larvæ did not incline to the paternal side, although these larvæ, like all larvæ from fragments, were often simpler than the normal. Hence since a relative decrease in the amount of

cytoplasm does not here affect the character of the larvæ, it is rational to suppose that an increase such as is present in the giant eggs likewise produces no such effects as observed in the larvæ. At the same time, normal eggs were cross fertilized and in the two-cell stage the blastomeres were separated. The contributions by the two parents were relatively the same as in the normal egg. These larvæ were like those from egg fragments, and serve as a control of those larvæ in so far as they bear on the question of how far size alone may affect the result. Moreover, in them, the relation of the chromosomes to the cytoplasm is the same as in the normal egg (whether the sperm does or does not bring in cytoplasm). Hence, since the amount of cytoplasm is shown to have no influence on the character of these larvæ, there is no reason for supposing that it had any influence in the case of the giant eggs.

Boveri's studies upon dispermic fertilization of the egg of the sea urchin bear directly upon the question at issue. He found that when two sperm simultaneously enter the same egg, each brings in a centrosome, so that a tetra- or tri-polar spindle is formed for the first division, as shown in Fig. 37. Instead of a double set of chromosomes, as in normal fertilization, there are three sets. At the first division, the chromosomes are irregularly distributed upon the multipolar spindles. In consequence, some cells may get one of each kind of chromosome, while other cells may get less than a full complement (Fig. 38). These dispermic eggs almost always give

rise to abnormal embryos, as several observers have recorded. The result can best be attributed to the irregular distribution of qualitatively different chromosomes; only those embryos in which each cell has a full complement developing normally.

Boveri's evidence went still further, for he separated the first cleavage cells of these dispermic eggs

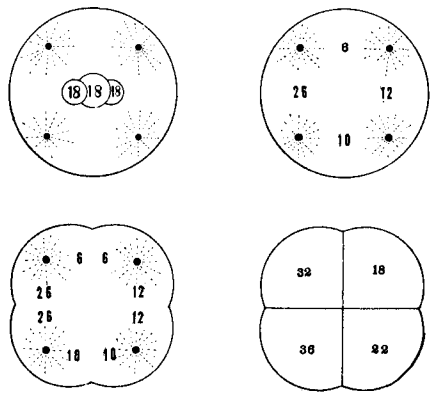

FIG. 37.—Dispermic fertilization of egg of sea urchin. The four centrosomes cause an unequal distribution of the fifty-four chromosomes, leading at the first division to four cells which contain different numbers of chromosomes.

and followed their history. Some of them gave rise to perfect dwarf larvæ. The number of normal embryos was small, but was that expected on the chance distribution of the chromosomes, for we should expect to find in a few cases an isolated cell that contained a full complement of chromosomes and from such a cell a normal embryo would be formed. The abnormality in development of the rest of the isolated cells was not due to any harmful

effect caused by isolation, for it had been shown by Driesch and others that when the first two cells of a sea-urchin egg that has been normally fertilized are separated, each forms a perfect embryo. Such cells, although containing only half the cytoplasm, contain

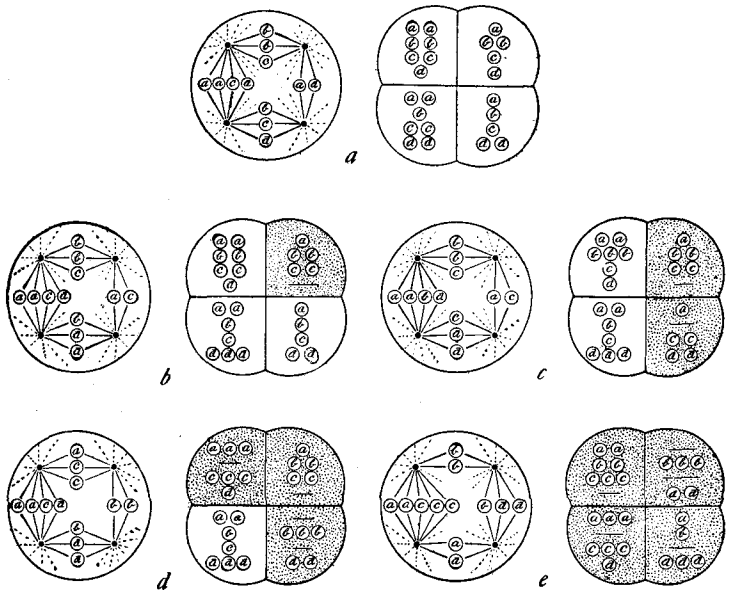

FIG. 38.—Diagram to show five combinations of chromosomes resulting from the first division of dispermic eggs, in which either each cell gets one complete set of chromosomes, *a*; or three cells get a full set, *b*; or two cells, *c*; or one cell, *d*; or none of the four cells, *e*, get a full set. (After Boveri.)

a full set of chromosomes. The difference, therefore, between these cells and isolated cells from dispermic eggs would seem to be due mainly to their different chromosomal contents.

Further evidence in favor of the chromosomal hypothesis is found in certain cases of hybrids between

species of sea urchins. The best analyzed cases are those that Baltzer has worked out. Crosses were made between four species of sea urchins; one such cross will serve as an example (Fig. 39). The eggs of Sphærechinus were fertilized by the sperm of Strongylocentrotus. The division of the chromosomes proceeded in normal manner. The pluteus

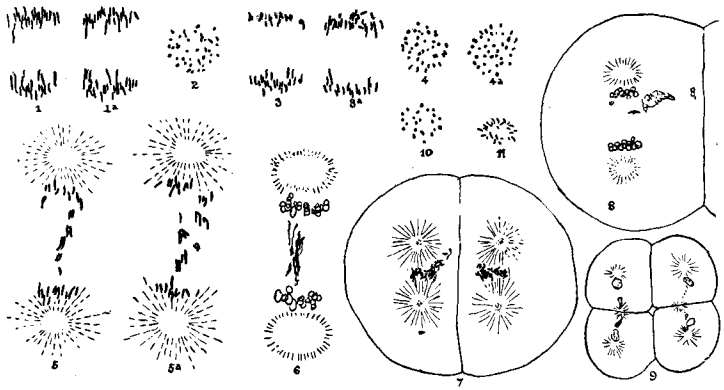

FIG. 39.—1 and 1*a*, chromosomes in the first normal cleavage spindle of Sphærechinus; 2, equatorial plate of two-cell stage of same; 3 and 3*a*, spindles of two-cell stage of egg of hybrid of Sphærechinus by Strongylocentrotus; 4 and 4*a*, same, equatorial plates; 5 and 5*a*, hybrid of Strongylocentrotus by Sphærechinus cleavage spindle in telophase; 6, next stage of last; 7, same, two-cell stage; 8, same, later; 9, same, four-cell stage; 10, same, equatorial plate in two-cell stage (22 chromosomes); 11, same, from later stage, 24 chromosomes. (After Baltzer.)

that developed was intermediate in character; or at least showed peculiarities both of the maternal and of the paternal types. The reciprocal cross was made by fertilizing the eggs of Strongylocentrotus with the sperm of Sphærechinus. At the first cleavage of the egg some of the chromosomes divide normally, while other chromosomes remain inactive and finally be-

come scattered in the region between the others that have retreated toward the poles. When the division is completed the belated chromosomes are found to be excluded from the daughter nuclei. They appear irregular in shape and show signs of degeneration. At the next division of the egg they may still be found, but they are lost later, and seem to take no part in the development. The difference between this and the other cross seems directly caused by the differences observed in the behavior of the chromosomes.

A count of the chromosomes in the hybrid embryos shows about twenty-one chromosomes. The maternal nucleus contained eighteen. It appears that only three of the paternal chromosomes have taken a regular part in the development—fifteen of them must have degenerated in the way described above. The hybrid embryos that developed were often abnormal; the few that developed as far as plutei were apparently entirely maternal in character. Since the reciprocal cross proves that the maternal characters are not dominant, the most reasonable interpretation is that, although the foreign sperm had started the development, it had produced little or no effect on the character of the larvæ, and this absence of effect would seem most probably to be due to the elimination of most of the paternal chromosomes. It might possibly be maintained that the same kind of effect produced by the egg of Strongylocentrotus on the chromosomes of Sphærechinus is likewise produced on the protoplasm introduced by the sperm. But there is here, in contrast to the case for the chromosomes,

no evidence of any abnormal cytoplasmic behavior which could account for the observed abnormal effect.

Tennent also has found that when the sea urchin Toxopneustes (♀) is crossed to Hipponoë (♂) no loss of chromatin occurs, and the larvæ are predominantly paternal, but in the reciprocal cross (Hipponoë ♀ by Toxopneustes ♂) some of the chromatin is eliminated and the larvæ are more like the maternal type.

Some experiments by Herbst also have an important bearing on the question. The eggs of Sphærechinus were put into sea water to which a little valerianic acid had been added. This is one of the recognized methods of starting parthenogenetic development. After five minutes the eggs were taken out and put into pure sea water to which sperm of Strongylocentrotus was added. The sperm fertilized a few of the eggs. The eggs had already begun to undergo some of the changes that lead to development. The belated sperm failed to keep pace with the division so that the paternal chromosomes did not reach the poles of the egg before the egg chromosomes reformed their nuclei (Fig. 40). In consequence, the paternal chromosomes formed a nucleus of their own that came to lie in one of the cells formed by the division of the egg. As a result one cell had a maternal nucleus and the other had a double, paternal and maternal, nucleus. In later development the paternal nucleus became incorporated with the maternal nucleus of its cell. Embryos were found later, in the cultures, that were on one side maternal

and on the other side hybrid in character and probably came from such half-fertilized eggs. It will be recalled that Baltzer has shown that when the cross is made in this direction both paternal and

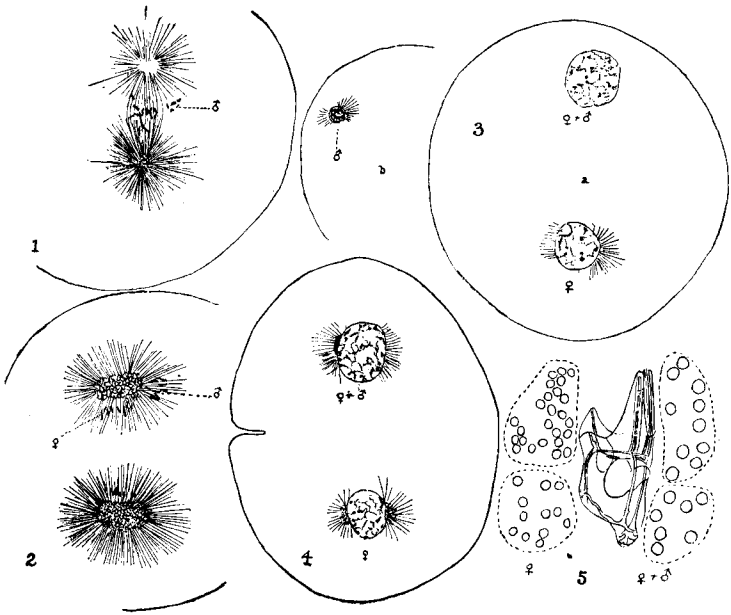

FIG. 40.—1, The chromosomes of the egg lie in the equator of the spindle, the chromosomes of the sperm at one side; 2, a later stage showing all of the paternal chromosomes lying at one side passing to one pole; 3 (to the right), later stage; the conditions are the same; there is also a supernumerary sperm in the egg (shown to the left, in another section); 4, same condition as last; 5, pluteus larva that is purely maternal on one side, and hybrid on the other. (After Herbst.)

maternal chromosomes behave normally at each division. The conclusion follows with much plausibility that the absence of paternal characters on one side is due to the absence of paternal chromosomes on that side.

The Individuality of the Chromosomes

The view that the chromosomes are persistent as individual structures in the cell has steadily gained ground during the last twenty years. The process of karyokinetic or mitotic division by means of which at each cell division the halves derived from a lengthwise split of each chromosome are carried to opposite poles, so that a genetic continuity is maintained between corresponding chromosomes (and parts of chromosomes) in mother and daughter cells, has been found to be almost universal in both plants and animals. It is true that several instances have been described in which the nucleus simply pinches into two parts, and there can be little doubt that such cases occur; but no one has been able to show in a convincing way that cells which have once divided in this manner ever return to the regular process of karyokinetic division. Case after case of amitosis that has been described for the germ cells has been either disproven, or found to rest on faulty observation, or else to relate to cells like those of the egg coats that take no part in the germinal stream.

There are several observations that lead to the view, at present generally accepted, that the chromosomes retain their individuality from one cell division to the next. These may now be given.

During the resting stage the chromosomes spin out in such a way that they appear to form a continuous network in the nucleus. They can not be identified individually during this period. When the chromo-

somes again become visible, preparatory to the next division, it has been found by Boveri in Ascaris, which is particularly well suited for the study of this point, that in sister cells the configuration of the groups of chromosomes is the same (Fig. 41). The similarity of the sister cells would be expected had the chromosomes retained during the resting stage the same shape and size and relative location that they had at the end of the last division. On no other

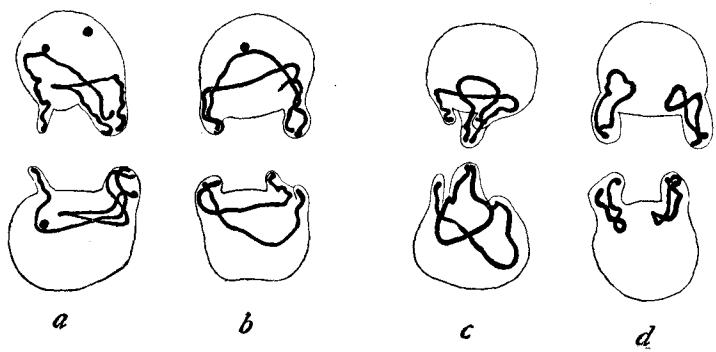

FIG. 41.—Four pairs of sister cells of Ascaris, in which the chromosomes are reappearing. Note the similarity of arrangement in the cells of each pair. (After Boveri.)

view can we so readily understand the similarities between the sister cells; for, in other cells of these same embryos that are not sister cells, a great variety of arrangements is found, and no two arrangements are so nearly alike as are those that are found in cells that have separated from each other at the last division. In a few instances certain observers believe that they have even been able to distinguish the separate chromosomes throughout the whole

resting period of the cells, but this must be received with some caution. In many animals and in some plants the chromosomes are of very different sizes and shapes, and many, or even all of them, can be identified at each division. It is found that these size relations hold throughout all divisions of the cells. While this evidence appears at first sight to show that the chromosomes are structures that perpetuate themselves, preserving their identity, yet it might be maintained, in fact it has been maintained, that each species has its own peculiar protoplasm from which chromosomes of a particular kind and number are, as it were, crystallized out anew before each cell division. This point of view can not, however, be reconciled with the evidence that follows. In Metapodius, Wilson has found that individuals may differ in the particular chromosome that he calls the m chromosome. While the normal individuals have a pair of m chromosomes, one individual had three m's; but all of the cells of any given individual have the same number. These chromosomes furnish strong support of the continuity of the chromosomes; for, in whatever number they enter the individual during fertilization, they retain that number throughout all the subsequent generations of cells. The same is true, of course, for the sex chromosomes.

Corroborative proof is found in certain hybrids, where the evidence is even more significant, because in such cases the chromosomes introduced by the male are, as it were, in a foreign medium. For example, Moenkhaus first pointed out that when

the fish Fundulus is crossed to another fish, Menidia, the two kinds of chromosomes present in the fertilized egg can readily be distinguished in later divisions. Similar observations have been made for many other crosses (Fig. 42) by Morris, G. and P. Hertwig, Federley, Doncaster, Rosenberg, etc. Despite the fact that the paternal chromosomes are in a foreign

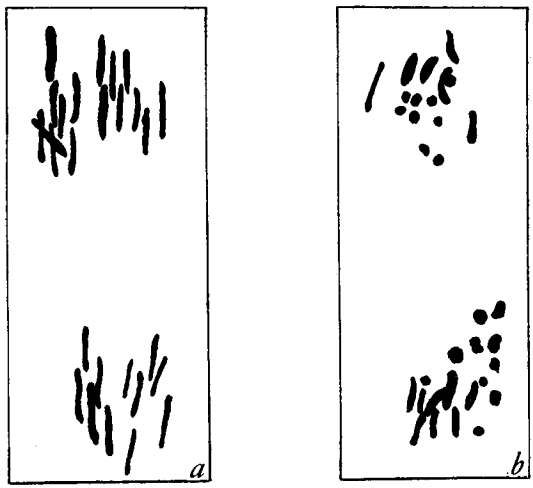

FIG. 42.—*a*, Telophase, division of an embryonic cell of Fundulus; *b*, telophase, division of an embryonic cell of egg of Fundulus fertilized by sperm of Ctenolabrus. (After Morris.)

medium they retain their characteristic size, form, and number. The embryos from these eggs are abnormal, and often die, not because chromosomes are eliminated but because the combination does not work out successfully. On the other hand, in hybrid embryos (studied by Herbst, Baltzer, and Tennent), in which paternal chromosomes are eliminated, they

seem never to re-appear subsequently, while those not eliminated always re-appear at the next cell division. Other cases of the same sort are known.

In general it may be said that even an abnormal set of chromosomes, once established in a cell, tends to persist through all succeeding cell generations. This evidence indicates that the chromosomes are not mere products of the rest of the cell but are self-perpetuating structures.

The Chromosomes during the Maturation of the Germ Cells

On the most essential point concerning the maturation of the egg and sperm there is no dispute: the observed number of chromosomes is reduced to half. It is generally agreed that this lowering of the number is due to the union of similar chromosomes in pairs, each chromosome derived from the father conjugating with the homologous chromosome derived from the mother. In cases where different chromosomes can be distinguished by their shape or size relations, the relations of these pairs correspond exactly to what they should be if like chromosomes conjugated.

When we come to consider how this union of chromosomes is brought about, there is much divergence of opinion, for the evidence is fragmentary or contradictory on almost every point. The reason for this uncertainty is clear: the stages at which the reduction in the number of the chromosomes takes place are extraordinarily difficult to interpret, be-

cause at this time the chromosomes are in the form of what seems to be a dense tangle of long threads. When this stage has been passed through, and the chromosomes are distinguishable again, the pairing has been completed. For any information that is worth while we have to rely on the best material available. It may be disputed which material is the best, but it will be generally conceded that a few types have shown themselves superior to others. The account of maturation that is here followed confines itself to two types—one for the male and the other for the female. These are selected cases, it is true, but they are those that give, in the opinion of the writers, two of the most complete accounts of these stages. The selection is admittedly not without bias, for these types can be most advantageously utilized to illustrate how crossing over can take place between the members of homologous pairs of chromosomes.

The salamander, Batracoseps attenuatus, has furnished some of the best material for the study of the ripening of the germ cells of the male. The account that follows is taken from Janssens' elaborate and detailed study of the spermatogenesis of Batracoseps.

At the end of the multiplication period (spermatogonial divisions) the nucleus appears as shown in Fig. 43, *a*. It then passes into a condition resembling a resting stage, *b*. Later the chromosomes begin to emerge in the form of long thin threads as shown in *c, d, e*. In the last figure (the leptotene stage) the ends of the thin threads are directed toward one pole

where some of the ends can be seen to be arranged in pairs. As they unite in pairs these thin threads often have the appearance of twisting tightly around

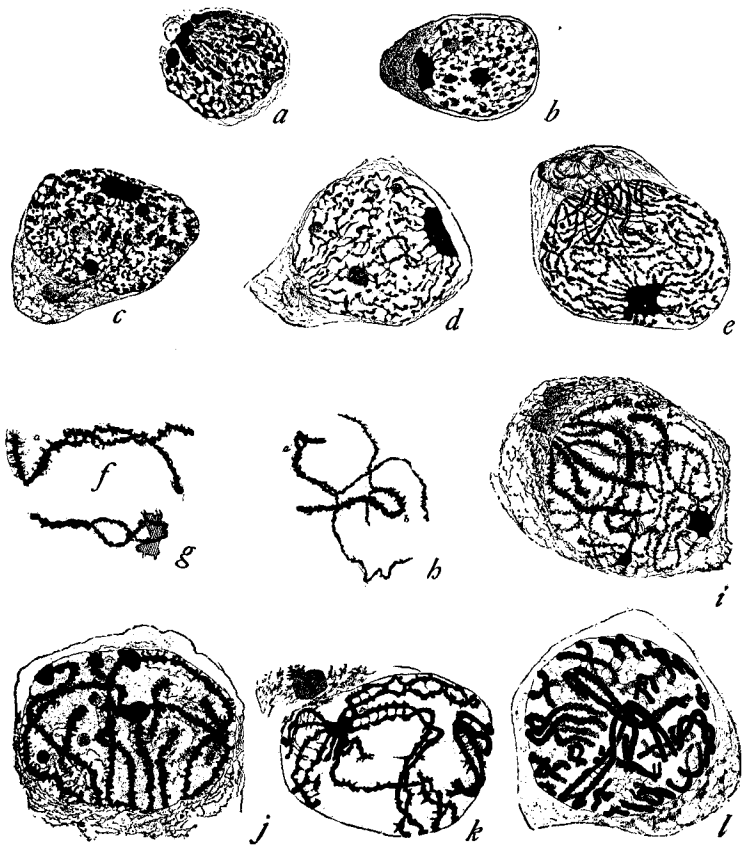

FIG. 43.—Spermatogenesis of Batracoseps attenuatus. *a*, late telophase of spermatogonial division; *b*, resting stage after the last spermatogonial division; *c*, appearance of the spireme; *d* and *e*, later stage of last (bouquet grele); *f*, *g*, *h*, twisting of leptotene threads around each other (amphitene stage); *i*, amphitene stage (entire cell); *j*, pachytene stage (bouquet pachytene); *k*, longitudinal splitting of threads (strepsinema stage); *l*, shortening and thickening of the chromosomes. (After Janssens.)

each other, beginning at the end where they first approached each other. The details of the union of the threads are further shown in *f*, *g*, *h*. As they unite they contract until they are in the form of a thicker thread, as seen in *i*, where the process of fusion has progressed as far as the middle of the nucleus. Later, *j*, the threads become fused throughout their length (pachytene stage). Still later the thick threads begin to show a longitudinal split (diplotene stage), and cross connections, uniting the halves of the threads, appear in different places. The threads thicken until finally a stage is reached like that shown in *k*, which, by further contraction, reaches the condition shown in *l*, a stage preparatory to the first maturation division. The threads of each pair, in all the stages of the latter part of the diplotene stage, are much twisted around each other; they are now so thick that they show the twisted condition very plainly.

The egg undergoes a series of changes during its maturation which parallels those of the sperm, and which leads also to the reduction in the number of the chromosomes to half of the full number. The eggs of a shark (Pristiurus melanostomus) have been described by Maréchal as passing through the following stages. At the end of the period of multiplication the eggs pass into a resting stage (Fig. 44, *a*) in which the chromatin appears as a delicate reticulum. A later stage is shown in *b*, *c*, when the separate thin threads begin to make their appearance, and take parallel courses, *d* (leptotene stage). These

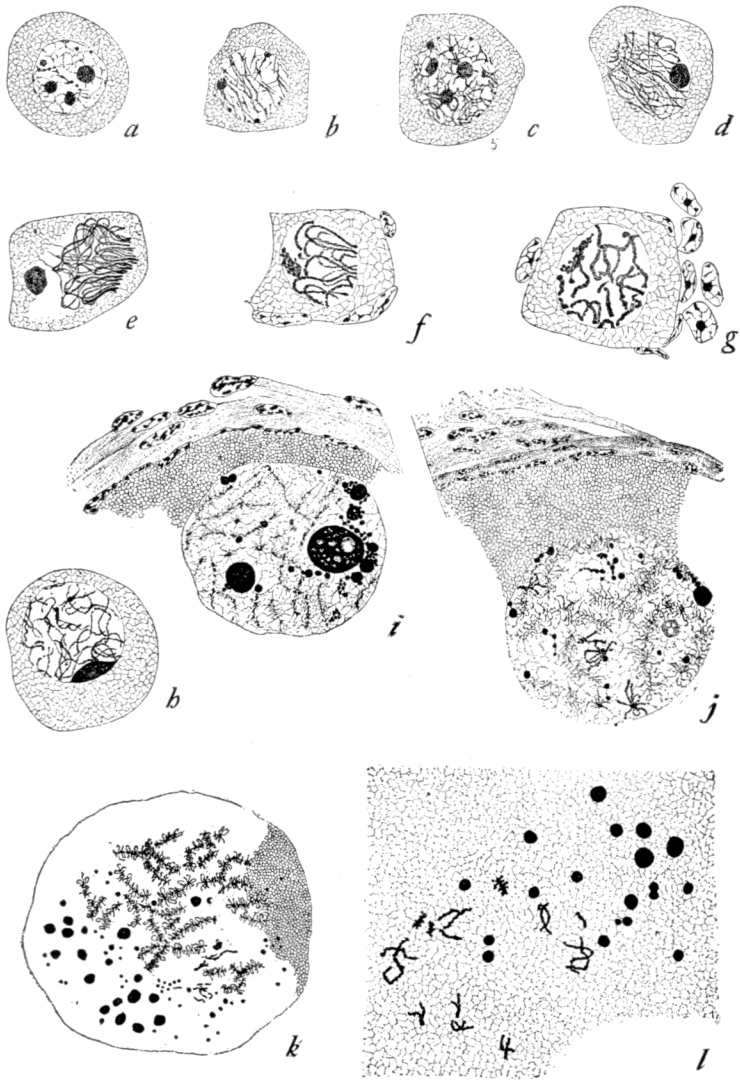

FIG. 44.—The growth, synapsis, and reduction stages in the egg of Pristiurus melanostomus. (After Maréchal.)

thin threads next assume the form of loops with their free ends pointing toward one pole, *e* (bouquet stage, also called the period of synapsis). At their free ends the threads soon appear to meet in pairs, *d* and *e*. Each pair, by the apparent fusion of its threads, leads to the formation of a thick thread in the form of a loop, *f*. Further condensation and separation of the threads leads to the condition shown in *g*. The thick double threads next show a lengthwise split, the halves being often twisted around each other (diplotene stage) *h*. The pairs of threads now begin again to become longer and to occupy more of the interior of the nucleus as seen in *i*. The eggs have grown larger meanwhile and the yolk appears. As the nucleus grows still larger, keeping pace with the growth of the cell, the chromosomes begin to lose their staining capacity. Despite the difficulty of tracing the chromosomes throughout the remaining period, Maréchal has succeeded in following them, step by step. His drawings of the chromosomes give the impression of the existence of a central core or filament remaining, as shown in Fig. 44 *i*, *j*, *k*. Delicate loops and threads are attached to this core and may be traced out into the region of each side of the chromosome. During these stages deeply staining balls of material, the nucleoli, appear in the nucleus. Finally the chromatin threads begin to condense again and once more take the stain; the chromosomes are found lying in pairs often twisted around each other as before, as seen in *l*. They pass in this condition on to the first

polar spindle, which develops in the egg as the nuclear membrane breaks down.

At the time when the double chromosomes of the sperm and the egg are about to pass onto the first

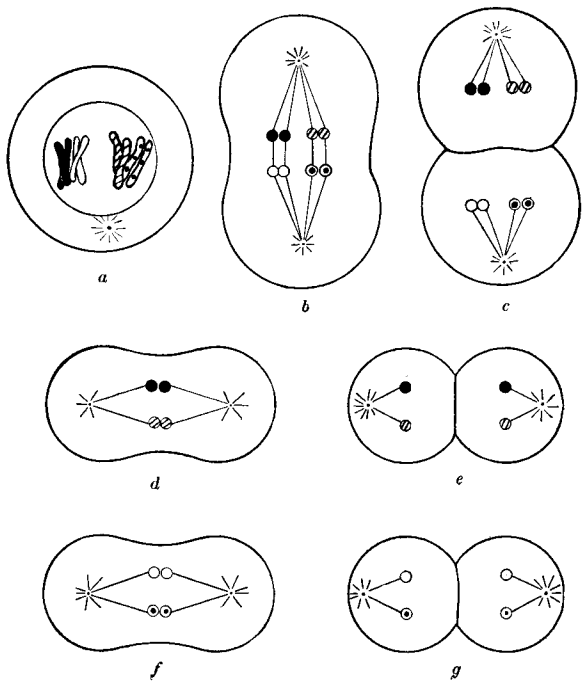

FIG. 45.—Diagram to illustrate the two reduction divisions of spermatogonial cells. *a*, first spermatocyte with two tetrads; *b* and *c*, division of last; *d* and *f*, division of two cells of *c*; *e* and *g*, completion of second division.

maturation spindle each half of the double chromosome splits lengthwise so that four parallel strands are present (Figs. 45 and 46); such a group of strands is known as a tetrad. It is usually held, although there

is some dissent, that the first longitudinal split that appears in the thick thread (pachytene stage) lies between the two chromosomes that had previously come together, such a separation of the members of a pair of chromosomes being known as a reductional split. The second lengthwise split is supposed to separate like halves of the same chromosomes. It is called an equational split.

These two splits are in preparation for the two maturation divisions that usually take place in rapid succession, without an intervening resting stage. It is customary therefore to look upon the second lengthwise split as a precocious split in the chromosomes preparatory to the second division. If the reduction in the number of the chromosomes to half of the original number were the sole object of the reduction divisions, one division would suffice to separate the two chromosomes of a pair that had united and it is not apparent why there should be a second division at all.

The two maturation divisions with tetrad formation are typically illustrated in the changes that take place in the spermatogenesis and oögenesis of Ascaris, the thread worm of the horse, as worked out by van Beneden, Brauer, O. Hertwig and others. In one variety four chromosomes occur which become reduced to two; hence there are only two tetrads present (Fig. 45, *a*). At the first division two halves of each thread move to one pole and two to the other as in *b* and *c*. At the second division the separation of the two remaining threads takes place,

d and *f*. At the end of the process there are two chromosomes remaining in each of the four cells, *e* and *g*. Each cell becomes a spermatozoon. Here as in most cases there is nothing to show whether the first division is reductional and the second equational, or the reverse. There is much divergence of opinion on this point for different species. The end

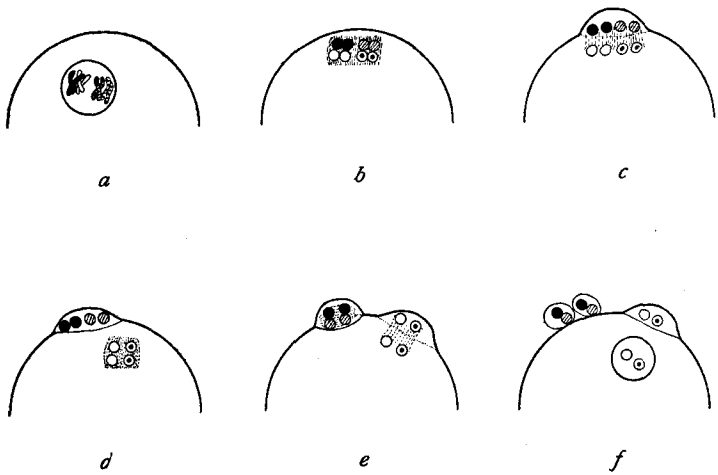

FIG. 46.—Diagram to show the extrusion of the two polar bodies. Two tetrads are represented in *a*. The two succeeding divisions *b-c*, *d-e*, show the separation of the members of the tetrads with the result that one of each kind is left in the egg.

result, however, is the same so far as the genetic problem is concerned, the sequence being ordinarily a matter of no significance.

In the egg (Fig. 46) the process is identical with that in the sperm, except that one of the two cells formed is much smaller than the other. The small cell is the polar body. At the first division the nucleus

sends out half of its chromatin into the first polar body (Fig. 46, *c*). Without a resting stage a new spindle is formed around the chromosomes in the egg and a second polar body is thrown off, as in *e*. The first polar body may also divide. The three polar bodies and the egg, *f*, are comparable to the four spermatozoa. All four spermatozoa are functional, but only one product of the two divisions of the egg is functional. Unless the tetrad is specifically oriented upon the polar spindle of the egg the chance is equally good that any one of the four threads that make up the tetrad will be the one that remains in the egg.

Crossing Over

If the preceding account of the maturation of the egg and of the sperm were accepted as covering the entire behavior of the chromosomes during this period, there would be no possibility for an interchange between the members of a pair. But there are several stages in the ripening of the germ cells when an interchange between homologous chromosomes might possibly take place. For instance, when the thin threads are coming together (Fig. 43, *e*, *f*, *g*, *h*) several observers have described them as twisting around each other (synaptic twisting) as represented in these figures. If where the threads cross a part of one thread becomes continuous with the remainder of the other thread (Fig. 24) an interchange of pieces will have been accomplished. If, as shown in Fig. 24, *B*, the chromosomes are represented as a linear

series of beads (chromomeres), then, when the conjugating chromosomes twist around each other, whole sections of one chain will come to lie, now on one side, now on the other side, in the double chromosome. If, when the two series of beads come to separate from each other, all of the segments that lie on the same side tend to go to one pole, and all of those on the opposite side to the other pole, each series must, in order to separate, break apart between the beads at the crossing point. Moreover, since the essential part of the process is that homologous beads go to opposite poles it follows that the break between the beads of two chains must always be at identical levels. It is not necessary to assume that crossing over takes place at every node, but only that it may sometimes take place. In fact, our work on Drosophila shows for the sex chromosome in the female that crossing over takes place in only about half of the cells, and double crossing over is a rather rare event.

There is a later stage also at which crossing over might be supposed to take place. After the thin threads have conjugated to form the thick threads, and these have shortened and split lengthwise, four strands are present (Fig. 47). If two of the strands fuse at the crossing place (the pieces of one strand uniting endwise with the pieces of the other) crossing over is brought about. It is this type in particular that Janssens named chiasmatype. In support of this method of crossing over are Janssens' observations on Batracoseps, where he concludes from the

method by which the strands are found joined at the time when they draw apart, that cross union of the threads must have previously taken place.

If crossing over be supposed to take place between two single threads (Fig. 24) all four gametes that ultimately result from such a cell will be crossover gametes. On the other hand, if crossing over takes

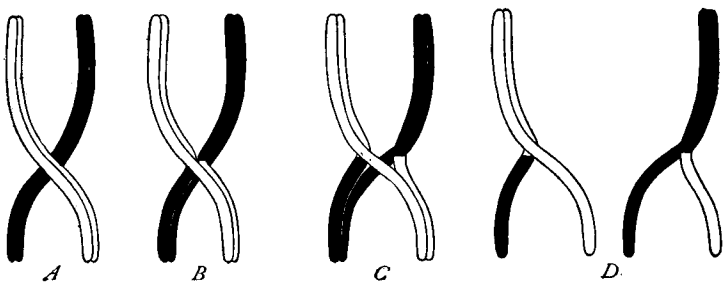

FIG. 47.—Four stages in crossing over, according to the "typical" chiasma type of Janssens. The white rod and the black rod are each split lengthwise; crossing over takes place only between two of the four strands.

place by means of the chiasmatype (Fig. 47) only two of the resulting four cells will be crossover gametes, the other two being non-crossover gametes.[1]

Looked at from the point of view of the total output, there would be no way in which to tell whether one or the other of the above processes has taken place; although the formation of a given number of crossover gametes involves only half as many participating cells in the case of the single thread type as in the case of the double thread type.

[1] If, after the thick threads have split, crossing over involving *both* strands of each chromosome should take place, instead of only one strand as in the chiasmatype, sensu strictu, the four gametes that result would be crossover gametes.

At present it seems better not to attempt to commit the theory of crossing over to one rather than to another of these stages; for, whether the process occurs at the leptotene thread stage as suggested above, or, as Janssens believes, at a later stage (strepsinema), the genetic result is the same. What we wish to point out is that in the phases through which the chromosomes pass at the maturation stages there is given an opportunity for an interchange of parts. The genetic evidence shows very clearly that interchanges do take place, as is best illustrated in the case of the sex chromosomes, whose history can be traced with some assurance from one generation to the next.

What we wish especially to insist upon and emphasize is that the evidence from linkage in Drosophila has shown beyond any doubt that crossing over is not a process that involves only a particular factor in relation to its allelomorph. Our work has shown positively that there is a tendency for large sections of the chromosomes to interchange whenever crossing over occurs.

Another idea that is likely to suggest itself in this connection has also been disproven by the evidence from Drosophila. It might be supposed that at a resting stage the chromosomes go to pieces and the fragments come together again before the next division period. Linkage might then mean the likelihood of fragments remaining intact, etc. But if the chromosomes broke up completely into their constituent elements at each resting period then

there is no explanation as to why the factors in a group remain together in sections as explained on page 66. If it is supposed that the chromosomes break only once or twice, and that linkage represents the holding together of the pieces, then one is forced to assume that the breaking up is the same in both members of a pair, yet entirely inconstant in different cells; for otherwise the reunion of the fragments would lead to duplication or loss of whole sections of the chromosomes, and all order would soon be lost. A large amount of data relating to sex linked characters has shown that the sex chromosomes must remain intact as often as they break apart, and even when they break apart this takes place, as a rule, at only one place.

Cytoplasmic Inheritance

The interpretation of Mendelian inheritance on a chromosomal basis by no means excludes the possibility that there may be other forms of inheritance depending on other cell materials. Although the cytoplasm is essential for the development of the organism, and is transmitted by the egg to each new generation, its materials do not perpetuate themselves unchanged as do the chromosomes, and are therefore really not hereditary. There are, however, certain bodies carried by the protoplasm, such as plastids (possibly also chondriosomes), which like the chromatin are able to grow and divide, and hence might have the power to perpetuate themselves unchanged

indefinitely. Such bodies might not only produce passive products, like starch or pigment, but even active enzymes, which, interacting with other products of development, might determine the characteristics of the race.

Structures like the shell and the yolk of eggs are purely maternal in origin, but since they do not have the power of growth and division, they are not able to perpetuate themselves indefinitely, nevertheless they may determine certain characteristics of the embryo, and to this extent may appear to influence the hereditary characters of the generation to which the embryo belongs. For instance, the females of certain races of silkworm moths have white eggs, because the shell is white. If such eggs are fertilized by sperm of another race, that has eggs with a dominant green colored shell, the shells are nevertheless white. Conversely when the green eggs of a female moth of the green egg race are fertilized by the sperm of a male of a white egg race, the color remains green. When the moths develop from either of these two kinds of hybrid eggs, one white, one green, they lay only green eggs, because in the hybrid the factor for green dominates and determines the color of the shell that is produced in the new eggs. These green eggs give rise to moths, three of which lay eggs that are green to one that lays eggs that are white, showing that here there is only the ordinary case of Mendelian inheritance, which is obscured, however, when the characters of the young embryo are considered, because, as has been shown, these

characters are due to peculiarities of the eggs before they are laid.

The serosa on the other hand is a cellular membrane that develops around the embryo and produces pigment. The pigment seen through the shell gives the embryo a definite color, which in the hybrid embryo is characteristic of the maternal race. Since the serosa pigment is not present in the egg, but develops after fertilization the inheritance here appears to be determined by the character of the egg and not by the sperm. But the genetic history of this character of the embryo is apparently the same as that of the color of the shell or of the yolk. It can, therefore, be interpreted in the same way. There must, then, be present in the egg some substance that is at first uncolored, and later this substance when carried into the serosa produces pigment, presumably by interacting with something else there. In the next generation, however, the influence of the father comes to light when the F_2 embryo produces its serosa material; for now the nucleus of the P_1 male has had opportunity to determine what this material may be, and should the paternal factor be the dominant one it determines the kind of material that the eggs will contain and hence the color of the serosa of this new generation.

A case of cytoplasmic inheritance has been described by Correns in the four-o'clock, Mirabilis jalapa. There is a race whose leaves are checkered with green and white, but some branches may have leaves entirely green, other branches may have only

white leaves. If the flowers of the green branches are self-fertilized, the young plants are green. If the flowers of the white branches are self-fertilized, the offspring have white leaves and these plants perish for want of chlorophyll. From the checkered branches the offspring may be green, or checkered, or white.

When a cross is made between the flowers borne by branches that are unlike, the inheritance is purely maternal. For example, if the pistil of a white branch is fertilized with pollen from a pure green plant, only white leaved offspring are produced. The reciprocal cross, the pistil from a green branch fertilized with pollen from a white branch, gives only green offspring, and these remain green through all subsequent generations.

Correns points out that these results can be interpreted if the whitening is due to a sort of disease that is carried by the cytoplasm. The egg cytoplasm carries over the disease to the next generation. As the pollen does not bring in any cytoplasm the disease is not transmitted through the male side.

Baur points out that in several other plants in which varieties with leaves marked with white exist, as in Melandrium, Antirrhinum, etc., the inheritance is strictly Mendelian, for the F_1 generation is green and the F_2 generation is made up of three greens to one marked with white. In these cases the color may depend on a chromosomal factor. But there is a case in Pelargonium that Baur thinks can not be explained in either of the foregoing ways. Here

again there are mosaic branches, white branches, and also green branches. Flowers on green branches crossed with flowers on white branches give mosaic plants, irrespective of which way the cross is made. A self-fertilized flower from a green branch gives rise to a plant with purely green leaves. If a flower from a checkered branch is self-fertilized it produces a checkered plant. If a flower from a white branch is self-fertilized it gives rise to a white plant.

Baur suggests tentatively, the following hypothesis to explain the case of Pelargonium. The green color of this plant, like that of all flowering plants, is due to chlorophyll grains and these grains multiply, supplying all the cells in generations that subsequently arise with their quota of grains. In the white parts these grains are defective in the sense that they fail to produce the green color, but retain their power of multiplying. If now it is assumed that the pollen as well as the egg may transmit some chlorophyll grains the results can be explained. For, in the division of the cells that contain both green (normal) and white (abnormal) grains there will arise at times an unequal distribution of the grains, and in extreme cases two kinds of branches may arise, one with green and the other with white grains. The hypothesis calls for transmission through the cytoplasm of the pollen as well as through that of the egg cell. Baur states that until this fact can be established the interpretation must be uncertain.