



DEVELOPMENT OF THE FROG'S EGG

CHAPTER I

THE FORMATION OF THE SEX-CELLS

THE development of the sex-cells is generally divided into three periods: 1) a multiplication-period, during which the primitive germ-cells pass through a large number of divisions; 2) a growth-period, in which the primitive germ-cells, that have become reduced in size through repeated division, grow larger; 3) a maturation-period, when only two divisions take place, between which the nucleus does not pass into a resting-stage. At the end of this last division the male germ-cells undergo histological changes by which they become transformed into spermatozoa.¹

SPERMATOGENESIS

The changes that take place in the testes of the frog have not been so fully worked out as in some other animals; we may therefore follow, first, the method of development of the

¹ This is a modification of the terminology of v. la Valette St. George, whose nomenclature of spermatogenesis is still often used. La Valette's classification is as follows: —

The primordial germ-cells give rise to spermatogonia, which cease to divide after a time and increase in size. Each spermatogonium is thus converted into a primary spermatocyte. Each primary spermatocyte divides into two cells, the spermatocytes of the second order, and each of these divides once more, without a resting-period, to form two spermatids. In this way four spermatids are formed from each primary spermatocyte. Each spermatid is then changed directly into a spermatozoön.

spermatozoon in two forms in which the process is better known, and then consider the special case of the frog.

The development of the spermatozoa of *Gryllotalpa*, the mole-cricket, has been described by vom Rath ('92, '95). As the process of spermatogenesis is relatively simple in this form, and as it is, according to vom Rath, much like the process that takes place in the frog, we may therefore first briefly consider the changes in *Gryllotalpa*.

First Period. A cell in the resting-stage at this time shows a large nucleus with a distinct membrane enclosing a network of fine chromatin (Fig. 1, A). The beginning of the cleavage is indicated by the withdrawal of the chromatin from the nuclear membrane and the thickening of the fibres of the

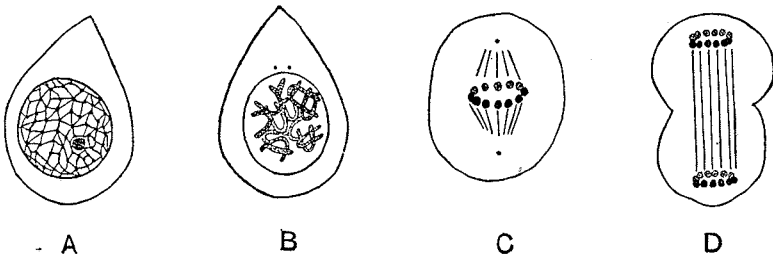


FIG. 1. — Division of sperm-mother-cells in *Gryllotalpa*. (After vom Rath.)

chromatic network. The tangled mass of threads, or network, then takes a somewhat excentric position. This thread seems to consist of linin, on which chromatin-granules are arranged. Sometimes the thread can be seen to be split along its length into two parts. The halves of the thread remain, however, closely sticking to each other. The double thread then breaks up by *cross-division* into twelve equal segments, or chromosomes (Fig. 1, B). The chromosomes next become shorter, and finally spherical, and come to lie in an equatorial plate (Fig. 1, C). When the chromatin is still in the skein-stage, two minute bodies are seen in the protoplasm just outside of the nuclear membrane (Fig. 1, B). These are the two centrosomes, which separate more and more from each other, and finally come to lie on opposite sides of the nucleus. A protoplasmic spindle develops between the two centrosomes (Fig. 1, C) and the fibres of the spindle become fixed to the

chromatin-granules of the equatorial plate. Each of the twelve chromatin-granules divides into two equal parts and the halves migrate toward one or the other of the centrosomes (Fig. 1, D). The cell-protoplasm next divides into two parts, so that two new cells are formed. Each cell contains twelve chromosomes. In this way the primitive sperm-cells continue to increase in number by a series of cell-divisions, all like that just described.

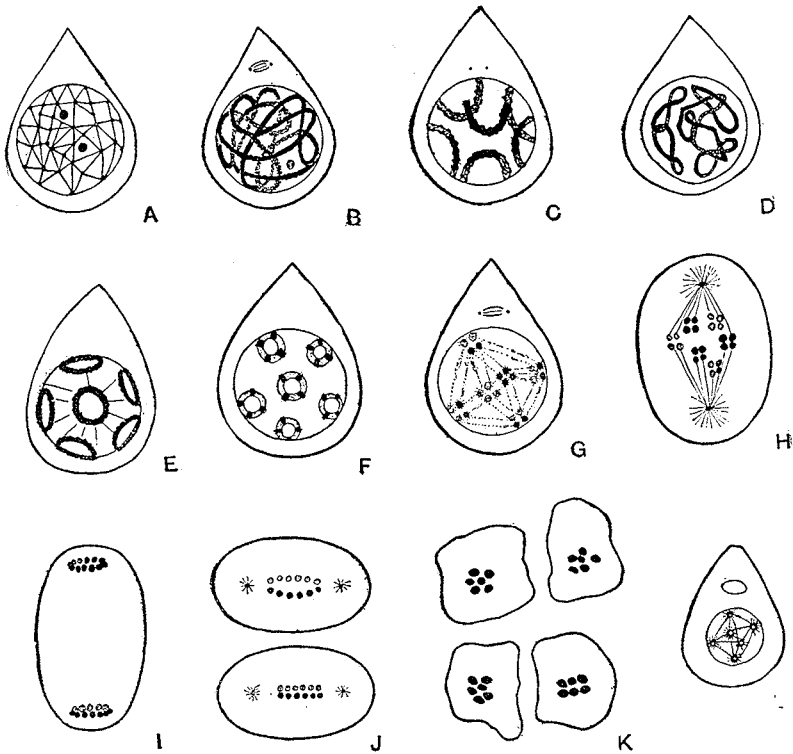


FIG. 2.—The formation of spermatozoa in *Gryllotalpa*. The two maturation-divisions. (After vom Rath.)

Second Period. A period of rest then follows, during which the cells grow larger. During this time the chromatin is again arranged in a fine network.

Third Period. Two successive and most peculiar cell-divisions now take place. The chromatin-network becomes thicker, and forms a tangled skein of threads (Fig. 2, A, B).

Each thread is split longitudinally into two parts. Two centrosomes again appear. The chromatin-thread next breaks up into *six* bent rods or chromosomes (Fig. 2, C). There is some doubt as to the way in which the next change is brought about. The account of vom Rath, which we follow here, seems to be in harmony with the process that is known to take place in some other forms during this period of development of the germ-cells. It appears that the halves of each of the six bent rods begin to separate from each other except at the ends of the rods, where the halves remain united. Each rod is in this way converted into a ring (Fig. 2, D). These rings are often so bent on themselves that they form a loop. The six chromatin-rings lie close to the periphery of the nucleus. The rings contract and become smaller and thicker (Fig. 2, E). This stage lasts but a short time and is succeeded by a stage shown in Fig. 2, F, G. Out of each ring four star-like granules are formed, the tetrad or "Vierer-gruppe." The four granules of each set are closely held together by clear linin threads. If each granule be counted as a distinct chromosome, then there are present at this time six groups of four chromosomes each, or twenty-four chromosomes. These twenty-four chromosomes become attached to the fibres of the achromatic spindle (Fig. 2, H) and arrange themselves into an equatorial double plate. Then twelve of these granules united in pairs wander toward one pole of the cell and twelve toward the other pole, and the division of the cell takes place (Fig. 2, I). This process is spoken of as the *first maturation-division*. Without passing into a *resting-stage*, a second division of each cell follows (Fig. 2, J). A new karyokinetic spindle is formed and the twelve chromosomes are separated into two plates of six chromosomes each, which go to their respective poles. Each of the two new cells contains therefore only six chromosomes (Fig. 2, K). The number of chromosomes is now *reduced to half the normal number present in the other cells of the body of the animal*. Each of the four cells formed by these two consecutive divisions of the sperm-mother-cell then differentiates into a spermatozoön (Fig. 2, L). Each spermatozoön consists of three parts,—a head, a middle piece, and a tail. The head is formed almost entirely out of the nucleus of the parent-cell of the spermatozoön, as

seen in Fig. 7, A, B, C. It is probable that a very thin layer of cytoplasm covers the outer surface of the head. The chromatin is densely packed into the head-piece, and cannot be resolved into its component chromosomes. The middle piece lies just back of the head.¹ In some animals this middle piece is known to enter the egg with the spermatozoon and a part of it becomes the centrosome, which then divides into two centrosomes and around these arise the achromatic rays of the dividing egg. The tail of the spermatozoon is generally described as coming from the cytoplasm of the cell.

The development of the spermatozoon in the salamander has been carefully studied by Flemming ('87), vom Rath ('93), Meves ('96), and others. There are certain remarkable processes that take place in the spermatogenesis of these Amphibia that seem to occur also in the frog, but as they have not been as carefully worked out in the latter form we may examine first the changes that take place in the salamander. Each year after the male has lost its supply of sperm, new spermatozoa begin to develop. The epithelial cells lining the cavities of the testes divide at first after a type of cleavage called, by Flemming, homœotypic. This first period of activity produces the first generation of spermatocytes, which divide according to another type, the heterotypic.

The cells of the second generation of spermatocytes also divide in the same way, but with an occasional homœotypic cleavage. Finally, in the third generation of spermatocytes, both types of cleavage occur. The products of the third generation transform directly into spermatozoa. In the *heterotypic division* the process is as follows. The chromatin is at first arranged in a thick thread, having a definite arrangement. The skein-stage follows, and a longitudinal splitting of the chromatin-thread is apparent. A thickening of the thread then takes place, and it breaks up into twelve chromosomes (only *half* the number present in other cells of the body) (Fig. 3, A, B). At the free ends of the bent chromosomes, each of which is split longitudinally, the halves fuse together (Fig. 3, B), but elsewhere the

¹ Its origin in the frog has not been definitely made out. It is probably cytoplasmic in origin (Fig. 7, A, B, C).

halves of the chromosomes separate from each other along the longitudinal line of division. The process is similar to the ring-formation of *Grylotalpa*. In this way twelve loops are formed from the twelve chromosomes. The bent ends of the new loops or rings correspond to the middle portions of the earlier rods or chromosomes (see the + and - signs in Fig. 3, A, C). Meanwhile the achromatic spindle between the centrosomes has developed, and the loops of chromatin are arranged on the threads of the spindle, as seen in Fig. 3, B. At the next stage each loop

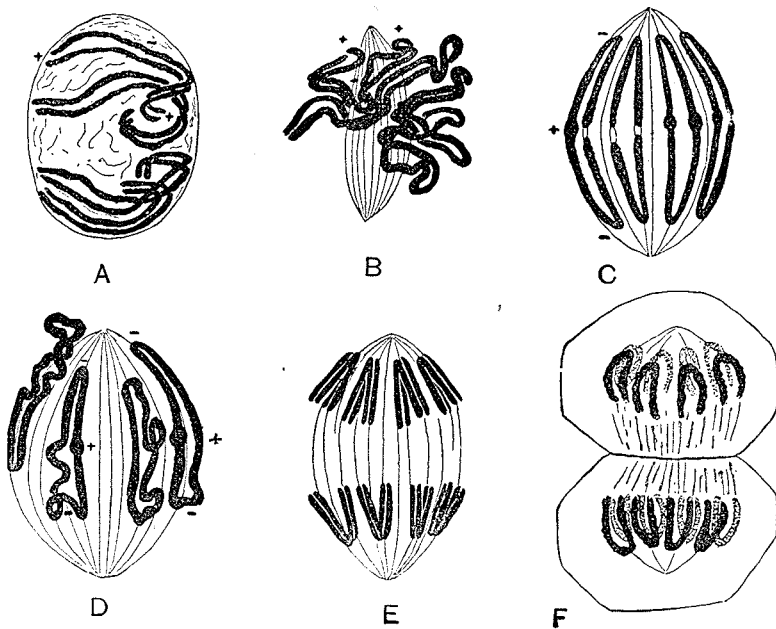


FIG. 3. — Heterotypic type of nuclear division in *Salamandra*. (After Flemming.)

breaks at the equator, *i.e.* at the point where the ends of the rods fused at an earlier period, and begins to migrate toward its centrosome (Fig 3, D). While this migration of the twelve bent chromosomes is taking place, each chromosome may be seen again to split longitudinally, although the two halves remain in contact (Fig. 3, E). The cell then passes into a resting-stage.

In the *homœotypic division* the first phase, the spireme, is simi-

lar to the last, *i.e.* it is a skein (Fig. 4, A) with longitudinally split thread. Twelve bent rods appear and become shorter than the bent rods of the heterotypic type. These rods then arrange themselves about the middle of the achromatic spindle (Fig. 4, B). The twelve bent rods divide each into two by separation along a longitudinal line, and twenty-four rods are present. Immediately twelve of these migrate toward one pole, and twelve toward the other, and the cell-division follows (Fig. 4, C, D, E, F). The cells then come to rest.

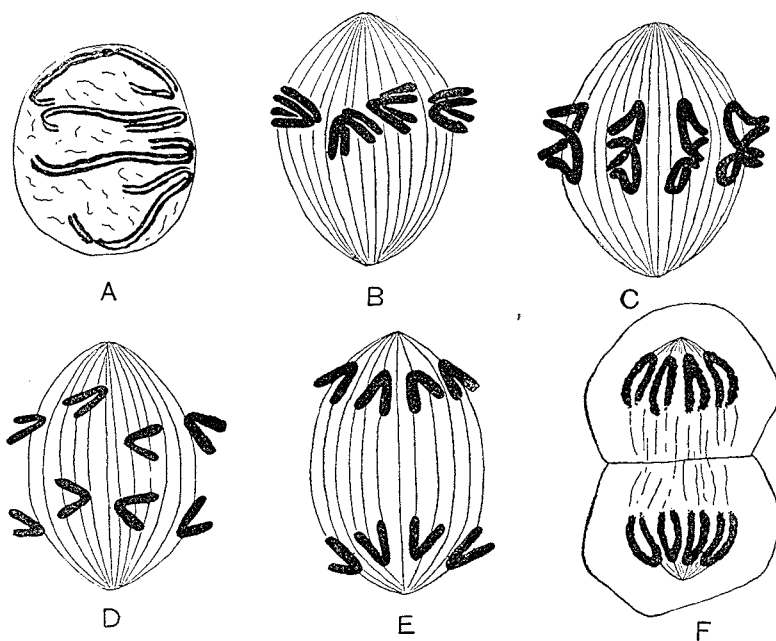


FIG. 4. — Homœotypic type of nuclear division in *Salamandra*. (After Flemming.)

The end result in the two types of cleavage is the same, but the details are, as described, different. It is important to note that the number of chromosomes is half that of the number of chromosomes in the other cells of the body.

Vom Rath maintains that a fourth generation of cells appears in the development of the spermatozoa of the salamander. Flemming supposed that at the end of the third generation of cells, described above, the differentiation into spermatozoa began, but

vom Rath has found that at the end of the third generation large cells appear with huge nuclei (Fig. 5, B), in which there are twelve groups of chromosomes. Each group or tetrad is composed of four granules. There are, therefore, present forty-eight spherical chromosomes united in groups of four. These tetrads arose from a heterotypic spindle, and in the following way. As the twelve loops (which are now double, making twenty-four loops) passed toward one pole they became much thicker (Fig. 5, A). The middle point of union of each of the twenty-four loops broke (Fig. 5, B), and the portions rounded up, so that there were present forty-eight chromosomes arranged in twelve groups of four chromosomes each (Fig. 5, B). Immediately after the formation of the tetrads the groups of chromosomes arranged themselves along the rays of the achromatic

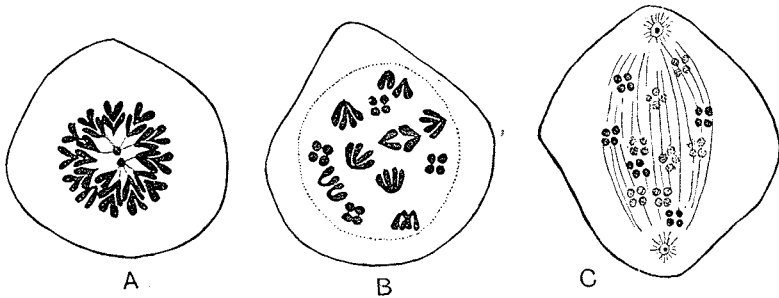


FIG. 5. — Formation of tetrads in testis of Salamandra. (After vom Rath.)

spindle (Fig. 5, C). The tetrads next passed toward the equator of the spindle, and there they divided, so that two of each of the four chromosomes passed toward one pole of the cell (as in *Grylotalpa*). In this way two new cells are formed with twenty-four chromosomes each. A second division succeeds without an intervening resting-stage, and the number of chromosomes is reduced, so that each cell has twelve chromosomes. The cells resulting from the last division, having each twelve chromosomes, differentiate each into a spermatozoön.

The second division, according to some workers (Boveri, Hertwig, and Brauer), is the result of a second *longitudinal division*. But vom Rath holds that this second division in the Amphibia and in *Grylotalpa* is the result of a *cross-division* of

the threads. According to Boveri, the meaning of the formation of the tetrad is only the precocious separation of the chromatin-threads for two rapidly succeeding divisions (without an intermediate resting-stage). The doubling of the chromosomes previous to division has, he thinks, no further significance than the preparation for two quickly succeeding divisions. It is not obvious, however, in the *development of the spermatozoön* why this rapid division should take place at this time and at no other in the life of the cell.

Meves ('96) has most recently reëxamined the development of the spermatozoön in the salamander. His results differ in several respects from the earlier results of Flemming, and in one essential respect from the work of vom Rath. According to Meves, the germ-cells undergo many divisions in the upper part of the testis. The chromatic figure is that of the usual type of division; and *twenty-four chromosomes* are present. As a result of the division, the cells become smaller, and each cell becomes surrounded by a layer of connective tissue. Each of these cells then divides many times according to the usual type of division, so that clusters of cells are produced surrounded by a common wall of connective tissue. Then follows the resting-period, in which the cells enlarge. After this the maturation-divisions take place. Meves thinks that most probably each cell divides only twice during this period, as in other forms. The first division is heterotypic, and now for the first time the number of *chromosomes is reduced to twelve*. Without a resting-period each cell again divides, the twelve chromosomes splitting longitudinally. This second division is homœotypic. Each cell, containing twelve chromosomes, then transforms directly into a spermatozoön.

Meves shows therefore that Flemming was mistaken in regard to the number of cell-generations that are present in the spermatogenesis of the salamander, and further that Flemming failed to make out the real sequence of the generations and the number of chromosomes present in each. More important is Meves' statement that, *normally*, there is not a formation of tetrads as vom Rath had affirmed. At present it is impossible to decide between the divergent accounts of Meves and vom

Rath, and we must suspend judgment until further work throws more light upon the question.

The spermatogenesis of the frog has not been worked out in the same detail as that of the salamander, yet vom Rath ('95) has made certain important statements in regard to it. The prophase of the mitoses, before the ripening period, has in the frog a close resemblance to the skein-stage of the heterotypic and homœotypic variations in the salamander. But, on the other hand, in the metakinetic stage the peculiarities of the homœotypic and heterotypic forms, as described by Flemming,

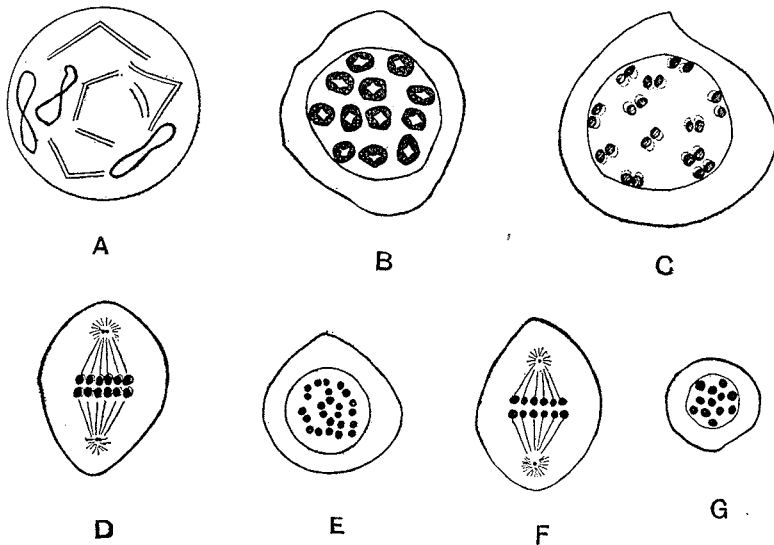


FIG. 6. — Stages in the last maturation-division of sperm-cells of Frog.
(After vom Rath.) (Figs. C, D, F slightly modified.)

are absent. The formation of the chromatin-rings and tetrad-groups in the frog (Fig. 6, A, B, C) differs from that of the salamander and is much more like that of *Gryllotalpa*. The rings, owing to the strong contraction of the segments, are relatively small in the frog, but proportionately thick (Fig. 6, B). From each ring arise the four spherical chromosomes of each tetrad-group (Fig. 6, C). The ring-stage lasts quite long in *Rana*, judging from the frequency of its presence. The rings lie at the periphery of the nucleus.

The shape of the spermatozoön is very different in different species of frogs. In some species the head of the spermatozoön is drawn out into a fine point (Fig. 7, A, E); in other forms it ends bluntly (Fig. 7, D). The middle piece is easily found in some spermatozoa, but in others only by the application of special reagents. In the European toad (Fig. 7, A) the tail of

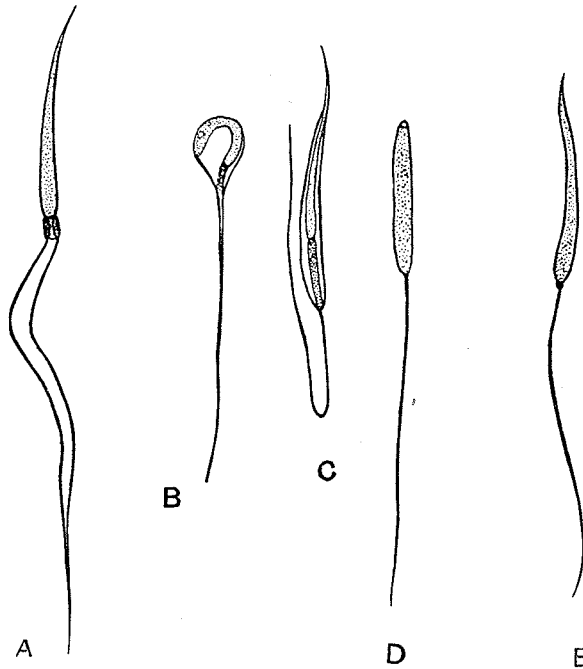


FIG. 7. — Spermatozoa. A. *Bufo cinereus*. B-C. Two stages in development of spermatozoön; D. Fully formed spermatozoön of *Hyla arborea*. E. Spermatozoön of *Rana esculenta* (the tail is too short). (After v. la Valette St. George.)

the spermatozoön is formed by a flat membrane with a thickened border. In *Hyla arborea* and *Rana esculenta* (Fig. 7, D, E) the tail of the spermatozoön is like a long lash or thread. In *Rana esculenta* the head measures .015-.021 mm. in length and the tail .04 mm. in length.

“DIRECT” DIVISION OF THE GERM-CELLS

In the testes of the frog and of other Amphibia are often found germ-cells whose nuclei have very irregular outlines.

Centrosomes can generally be demonstrated in these resting-cells. Other cells have the nuclei broken up into a number of smaller spheres. Still other nuclei may have a deep depression on one side, as though the nucleus were dividing into two by constriction. These nuclei have often been described as dividing by amitotic or direct division, *i.e.* without the characteristic mitotic division. Meves ('91) has stated that in the testes of the salamander amitotic division occurs regularly, and he believes that such cells will later form spermatozoa. Other authors (Bellonci and vom Rath), admitting that such a division may take place, affirm that such cells are in process of degeneration, and never subsequently form spermatozoa. Vom Rath declares that cells that have once divided by amitosis can never again divide by karyokinesis (mitosis), and that such cells degenerate later, and do not ever develop into sex-cells.

OÖGENESIS

The origin of the egg in the ovary of the frog has been studied by Schultze ('87, e), but many important details are still unknown. The egg

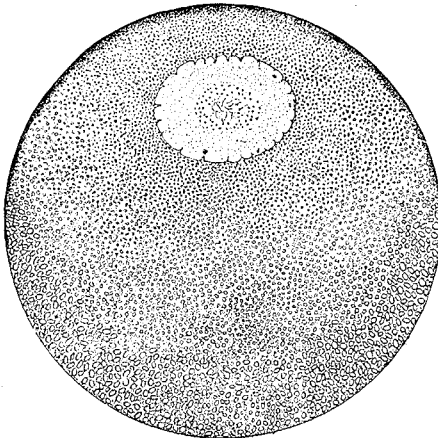


FIG. 8. — Ovarian egg of *Rana*.

derived from one of the cells of the outer layer of the ovary is surrounded by a large number of follicle-cells. The nucleus of the egg consists of fine chromatic threads, of a nuclear sap, and of scattered nucleoli. As the egg enlarges the nucleus also enlarges, and the chromatin stains more faintly and appears in the form of scattered threads.

The nucleoli stain well and become larger and more numerous as the egg enlarges, and are found generally around the periphery of the nucleus. Certain portions of the protoplasm now begin to stain dif-

ferently from the rest, and are spoken of as the yolk-nuclei. They seem, in some way not fully understood, to be connected with the development of the yolk-granules.¹ The yolk-granules, at first small and scattered, grow larger and become more numerous (Fig. 8). Before the egg leaves the ovary, the nucleus wanders toward the periphery and places itself under the black pole of the egg (Fig. 9). When the surface of the egg is examined, it shows a lighter area, owing to the displacement of the pigment-granules in the region occupied by the nucleus. The nucleoli at this time migrate toward the centre of the nucleus,² there disintegrate, and finally disappear. The chromatin-material draws together at this time into threads which stain more deeply, the nuclear membrane disappears, an achromatic spindle develops, and the egg is ready to extrude the first polar body.

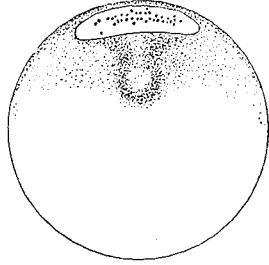


FIG. 9.—Section of ripe ovarian egg. (After Hertwig.)

COMPARISON OF SPERMATOGENESIS WITH OÖGENESIS

The method of extrusion of the polar bodies is described in the next chapter, but we may anticipate this account in order to consider here a remarkable parallel that has been discovered between the formation of the polar bodies and the formation of the spermatozoa. In the latter, as we have seen, two successive divisions follow each other during the maturation-period *without an intervening resting-stage*. The tetrad-groups are present at the beginning of the process. After the two maturation-divisions the number of chromosomes is reduced to half the number characteristic for the species.³ The same phenomena appear when the polar bodies are extruded from the egg. After the extrusion of the first polar body, the spindle for the second

¹ Will ('84) describes the yolk-nuclei as arising from constrictions of the nucleus set free with their nucleoli into the protoplasm.

² Schultze affirms that the later chromatin comes from these nucleoli, but Born has corrected this statement.

³ The reduction in number of the chromosomes seems in some forms to take place before the tetrad-period.

polar body forms immediately *without a resting-period*. Further, it is found, after the extrusion of the second polar body, that the number of chromosomes in the egg is reduced to half the number characteristic for the somatic cells. Tetrads have also been described as occurring just before the extrusion of the polar bodies. In many cases the first polar body divides into two, so that three polar bodies are present. These three polar bodies and the egg seem to correspond to the four spermatozoa from each spermatocyte. All four spermatozoa are functional, but only the egg (and not its three polar bodies) is capable of development. Weismann has utilized the discovery of the reduction of the number of chromosomes to build up an elaborate and highly speculative theory of heredity. The reduction division is, according to Weismann, not simply a quantitative division of the chromatic thread, but is at one stage at least a qualitative division. The reduction of the chromosomes to half the number present in the other cells of the body seems, according to Weismann and others, to be a preparation for fertilization. Since the spermatozoön brings into the egg only half the number of chromosomes found in the somatic cells of the animal, and since the egg-nucleus supplies the other half, the number of chromosomes will thus remain constant for the species from generation to generation.