

CHAPTER IV

FERTILIZATION OF THE OVUM

“It is conceivable, and indeed probable, that every part of the adult contains molecules derived both from the male and from the female parent; and that, regarded as a mass of molecules, the entire organism may be compared to a web of which the warp is derived from the female and the woof from the male.”
HUXLEY.¹

IN mitototic cell-division we have become acquainted with the means by which, in all higher forms at least, not only the continuity of life, but also the maintenance of the species, is effected; for through this beautiful mechanism the cell hands on to its descendants an exact duplicate of the idioplasm by which its own organization is determined. As far as we can see from an *a priori* point of view, there is no reason why, barring accident, cell-division should not follow cell-division in endless succession in the stream of life. It is possible, indeed probable, that such may be the fact in some of the lower and simpler forms of life where no form of sexual reproduction is known to occur. In the vast majority of living forms, however, the series of cell-divisions tends to run in cycles in each of which the energy of division finally comes to an end and is only *restored by an admixture of living matter derived from another cell*. This operation, known as *fertilization* or *fecundation*, is the essence of sexual reproduction; and in it we behold a process by which on the one hand the energy of division is restored, and by which on the other hand two independent lines of descent are blended into one. Why this dual process should take place we are as yet unable to say, nor do we know which of its two elements is to be regarded as the primary and essential one.

Harvey and many other of the early embryologists regarded fertilization as a stimulus, given by the spermatozoön, through which the ovum was “animated” and thus rendered capable of development. In its modern form this conception appears in the “dynamic” theories of Herbert Spencer, Bütschli, Hertwig, and others, which assume that protoplasm tends gradually to pass into a state of increasingly stable equilibrium in which its activity diminishes, and that fertilization restores it to a labile state, and hence to one of activity, through mixture with protoplasm that has been subjected to different conditions. Bütschli ('76) pointed out that the life-cycle of the metazoön is com-

¹ Evolution, in *Science and Culture*, p. 296, from *Enc. Brit.*, 1878.

parable to that of a protozoan race, a long series of cell-divisions being in each case followed by a mixture of protoplasts through conjugation; and he assumed that, in both cases, conjugation results in rejuvenescence through which the energy of growth and division is restored and a new cycle inaugurated. The same view has been advocated by Minot, Engelman, Hensen, and many others. Maupas ('88, '89), in his celebrated researches in the conjugation of Infusoria, attempted to test this conclusion by following out continuously the life-history of various species through the entire cycle of their existence. Though not yet adequately confirmed, and indeed opposed in some particulars by more recent work,¹ these researches have yielded very strong evidence that in these unicellular animals, even under normal conditions, the processes of growth and division sooner or later come to an end, undergoing a process of natural "senescence," which can only be counteracted by conjugation. That fertilization in higher plants and animals does in fact incite division and growth is a matter of undisputed observation. We know, however, that in parthenogenesis the egg may develop without fertilization, and we do not know whether the tendency to "senescence" and the need for fertilization are primary attributes of living matter.

The foregoing views may be classed together as the rejuvenescence theory. Parallel to that theory, and not necessarily opposed to or confirmatory of it, is the view that fertilization is in some way concerned with the process of variation. Long since suggested by Treviranus and more lately developed by Brooks² and Weismann³ is the hypothesis that fertilization is a source of variation — a conclusion suggested by the experience of practical breeders of plants and animals. Weismann brings forward strong arguments against the rejuvenescence-theory, and regards the need for fertilization as a secondary acquisition, the mixture of protoplasts to which it leads producing variations — or rather insuring their "mingling and persistent renewal"⁴ — which form the material on which selection operates. On the other hand, a considerable number of writers, including Darwin, Spencer, O. Hertwig, Hatschek, and others, believe that although crossing may lead to variability within certain limits, its effect in the long run tends to neutralize indefinite variability and thus to hold the species true to the type.

It is remarkable that we should still remain uncertain as to the physiological meaning of a process so general and one that has been the subject of such prolonged research. Both the foregoing general views are in harmony with the results of Darwin's work on variation and with the experience of practical breeders, which have shown that

¹ Cf. Joukowsky, '99.

² *The Law of Heredity*, 1883.

³ *Amphimixis*, 1891.

⁴ '99, p. 326.

crossing produces both greater vigour and greater variability. In view of all the facts, however, we are constrained to the admission that the essential nature of sexual reproduction must remain undetermined until the subject shall have been far more thoroughly investigated, especially in the unicellular forms, where the key to the ultimate problem is undoubtedly to be sought.

A. PRELIMINARY GENERAL SKETCH

Among the unicellular plants and animals, fertilization is effected by means of *conjugation*, a process in which two individuals either fuse together permanently or unite temporarily and effect an exchange

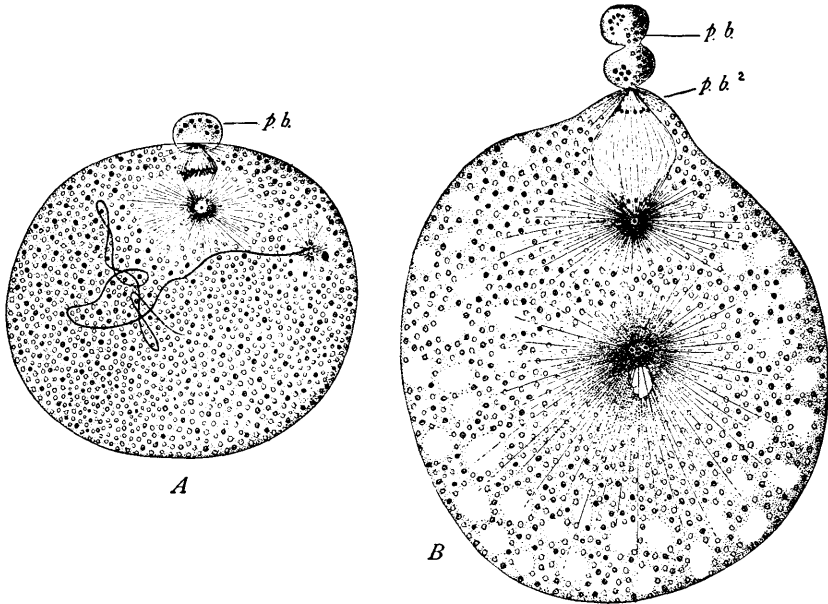


Fig. 89. — Fertilization of the egg of the snail, *Physa*. [KOSTANECKI and WIERZEJSKI.]

A. The entire spermatozoön lies in the egg, its nucleus at the right, flagellum at the left, while the minute sperm-amphaster occupies the position of the middle-piece. The first polar body has been formed, the second is forming. *B.* The enlarged sperm-nucleus and sperm-amphaster lie near the centre; second polar body forming and the first dividing. The egg-centrosomes and asters afterward disappear, their place being taken by those of the spermatozoön.

of nuclear matter, after which they separate. *In all the higher forms fertilization consists in the permanent fusion of two germ-cells, one of paternal and one of maternal origin.* We may first consider the fertilization of the animal egg, which appears to take place in essentially the same manner throughout the animal kingdom, and to be closely paralleled by the corresponding process in plants.

Leeuwenhoek, whose pupil Hamm discovered the spermatozoa (1677), put forth the conjecture that the spermatozoön must penetrate into the egg; and the classical experiments of Spallanzani on the frog's egg (1786) proved that the fertilizing element must be the spermatozoa and not the liquid in which they swim. The penetration of the ovum was, however, not actually seen until 1854, when Newport observed it in the case of the frog's egg; and it was described by Pringsheim a year later in one of the lower plants, *Ædigionium*. The first adequate description of the process was given by Hermann Fol, in 1879,¹ though many earlier observers, from the time of Martin Barry ('43) onward, had seen the spermatozoön inside the egg-envelopes, or asserted its entrance into the egg.

In many cases the entire spermatozoön enters the egg (mollusks, insects, nematodes, some annelids, *Petromyzon*, axolotl, etc.), and in such cases the long flagellum may sometimes be seen coiled within the egg (Fig. 89). Only the nucleus and middle-piece, however, are concerned in the actual fertilization; and there are some cases (echinoderms) in which the tail is left outside the egg. At or near the time of fertilization, the egg successively segments off at the upper pole two minute cells, known as the *polar bodies* (Figs. 89, 90, 116) or directive corpuscles, which degenerate and take no part in the subsequent development. This phenomenon takes place, as a rule, immediately after entrance of the spermatozoön. It may, however, occur before the spermatozoön enters, and it forms no part of the process of fertilization proper. It is merely the final act in the process of *maturation*, by which the egg is prepared for fertilization, and we may defer its consideration to the following chapter.

1. *The Germ-nuclei in Fertilization*

The modern era in the study of fertilization may be said to begin with Oscar Hertwig's discovery, in 1875, of the fate of the spermatozoön within the egg. Earlier observers had, it is true, paved the way by showing that, at the time of fertilization, the egg contains *two nuclei* that fuse together or become closely associated before development begins. (Warneck, Bütschli, Auerbach, Van Beneden, Strasburger.) Hertwig discovered, in the egg of the sea-urchin (*Toxopneustes lividus*), that *one of these nuclei belongs to the egg, while the other is derived from the spermatozoön*. This result was speedily confirmed in a number of other animals, and has since been extended to every species that has been carefully investigated. The researches of Strasburger, De Bary, Schmitz, Guignard, and others have shown that the same is true of plants. *In every known case an*

¹ See *l'Hénogénie*, pp. 124 ff., for a full historical account.

essential phenomenon of fertilization is the union of a sperm-nucleus, of paternal origin, with an egg-nucleus, of maternal origin, to form the primary nucleus of the embryo. This nucleus, known as the cleavage- or segmentation-nucleus, gives rise by division to all the nuclei of the body, and hence every nucleus of the child may contain nuclear substance derived from both parents. And thus Hertwig was led to the conclusion ('84), independently reached at the same time by Strasburger, Kölliker, and Weismann, that the nucleus is the most essential element concerned in hereditary transmission.

This conclusion received a strong support in the year 1883, through the splendid discoveries of Van Beneden on the fertilization of the thread-worm, *Ascaris megalocephala*, the egg of which has since ranked with that of the echinoderm as a classical object for the study of cell-problems. Van Beneden's researches especially elucidated the structure and transformations of the germ-nuclei, and carried the analysis of fertilization far beyond that of Hertwig. In *Ascaris*, as in all other animals, the sperm-nucleus is extremely minute, so that at first sight a marked inequality between the two sexes appears to exist in this respect. Van Beneden showed not only that the inequality in size totally disappears during fertilization, but that the two nuclei undergo a parallel series of structural changes which demonstrate their precise morphological equivalence down to the minutest detail; and here, again, later researches, foremost among them those of Boveri, Strasburger, and Guignard, have shown that, essentially, the same is true of the germ-cells of other animals and of plants. The facts in *Ascaris* (variety *bivalens*) are essentially as follows (Fig. 90): After the entrance of the spermatozoön, and during the formation of the polar bodies, the sperm-nucleus rapidly enlarges and finally forms a typical nucleus exactly similar to the egg-nucleus. The chromatin in each nucleus now resolves itself into two long, worm-like chromosomes, which are exactly similar in form, size, and staining-reaction in the two nuclei. Next, the nuclear membrane fades away, and the four chromosomes lie naked in the egg-substance. Every trace of sexual difference has now disappeared, and it is impossible to distinguish the paternal from the maternal chromosomes (Fig. 90, *D, E*). Meanwhile an amphiaster has been developed which, with the four chromosomes, forms the mitotic figure for the first cleavage of the ovum, *the chromatic portion of which has been synthetically formed by the union of two equal germ-nuclei.* The later phases follow the usual course of mitosis. Each chromosome splits lengthwise into equal halves, the daughter-chromosomes are transported to the spindle-poles, and here they give rise, in the usual manner, to the nuclei of the two-celled stage. *Each of these nuclei, therefore, receives exactly equal amounts of paternal and maternal chromatin.*

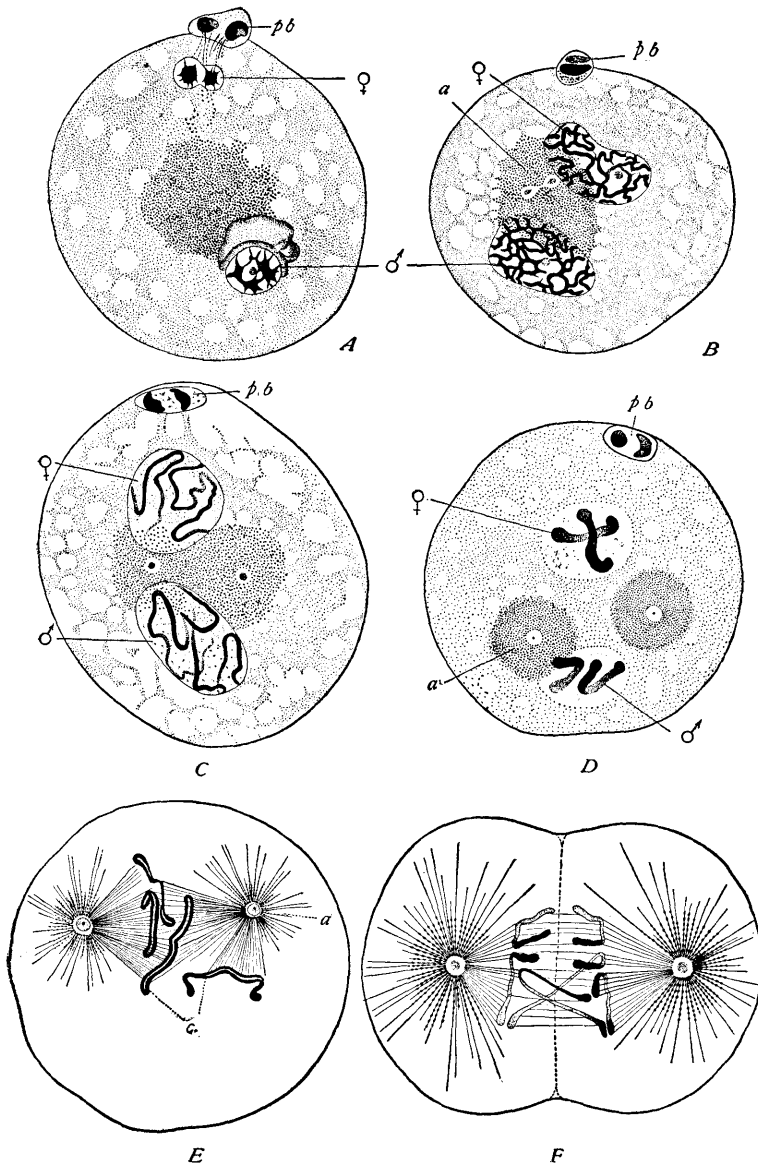


Fig. 90. — Fertilization of the egg of *Ascaris megalcephala*, var. *bivalens*. [BOVERI.] (For later stages see Figs. 31, 145.)

A. The spermatozoön has entered the egg, its nucleus is shown at ♂; beside it lies the granular mass of "archoplasm" (attraction-sphere); above are the closing phases in the formation of the second polar body (two chromosomes in each nucleus). *B.* Germ-nuclei (♀, ♂) in the reticular stage; the attraction-sphere (*a*) contains the dividing centrosome. *C.* Chromosomes forming in the germ-nuclei; the centrosome divided. *D.* Each germ-nucleus resolved into two chromosomes; attraction-sphere (*a*) double. *E.* Mitotic figure forming for the first cleavage; the chromosomes (*c*) already split. *F.* First cleavage in progress, showing divergence of the daughter-chromosomes toward the spindle-poles (only three chromosomes shown).

These discoveries were confirmed and extended in the case of *Ascaris* by Boveri and by Van Beneden himself in 1887 and 1888 and in several other nematodes by Carnoy in 1887. Carnoy found the number of chromosomes derived from each sex to be in *Coronilla* 4, in *Ophiostomum* 6, and in *Filaroides* 8. A little later Boveri ('90) showed that the law of numerical equality of the paternal and maternal chromosomes held good for other groups of animals, being in the sea-urchin *Echinus* 9, in the worm *Sagitta* 9, in the medusa *Tiara* 14, and in the mollusk *Pterotrachea* 16 from each sex. Similar results were obtained in other animals and in plants, as first shown by Guignard in the lily ('91), where each sex contributes 12 chromosomes.

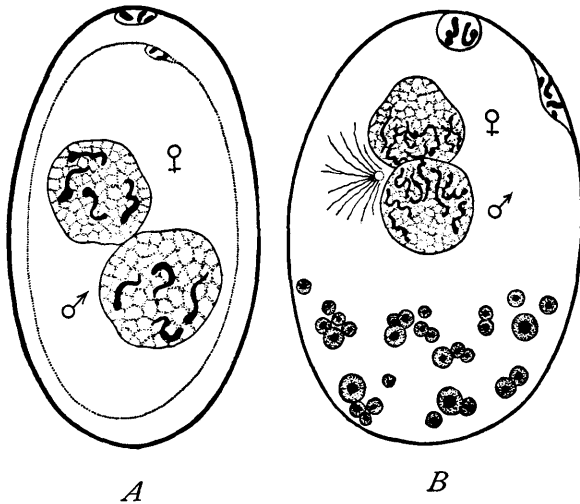


Fig. 91.— Germ-nuclei and chromosomes in the eggs of nematodes. [CARNOY.]

A. Egg of nematode parasitic in *Scyllium*; the two germ-nuclei in apposition, each containing four chromosomes; the two polar bodies above. B. Egg of *Filaroides*; each germ-nucleus with eight chromosomes; polar bodies above, deutoplasm-spheres below.

In the onion the number is 8 (Strasburger); in the annelid *Ophryotrocha* it is only 2 from each sex (Korschelt). In all these cases the number contributed by each is one-half the number characteristic of the body-cells. The union of two germ-cells thus restores the normal number, and here we find the explanation of the remarkable fact commented on at page 67 that the number of chromosomes in sexually produced organisms is always even.¹

These remarkable facts demonstrate the two germ-nuclei to be in a morphological sense precisely equivalent, and they not only lend very strong support to Hertwig's identification of the nucleus as the bearer of hereditary qualities, but indicate further that these qualities

¹ Cf. p. 67.

must be carried by the chromosomes; for their precise equivalence in number, shape, and size is the physical correlative of the fact that the two sexes play, on the whole, equal parts in hereditary transmission.

2. The Achromatic Structures in Fertilization

It is generally agreed that the amphiaster of the primary mitotic figure of the fertilized ovum arises from the egg-substance precisely

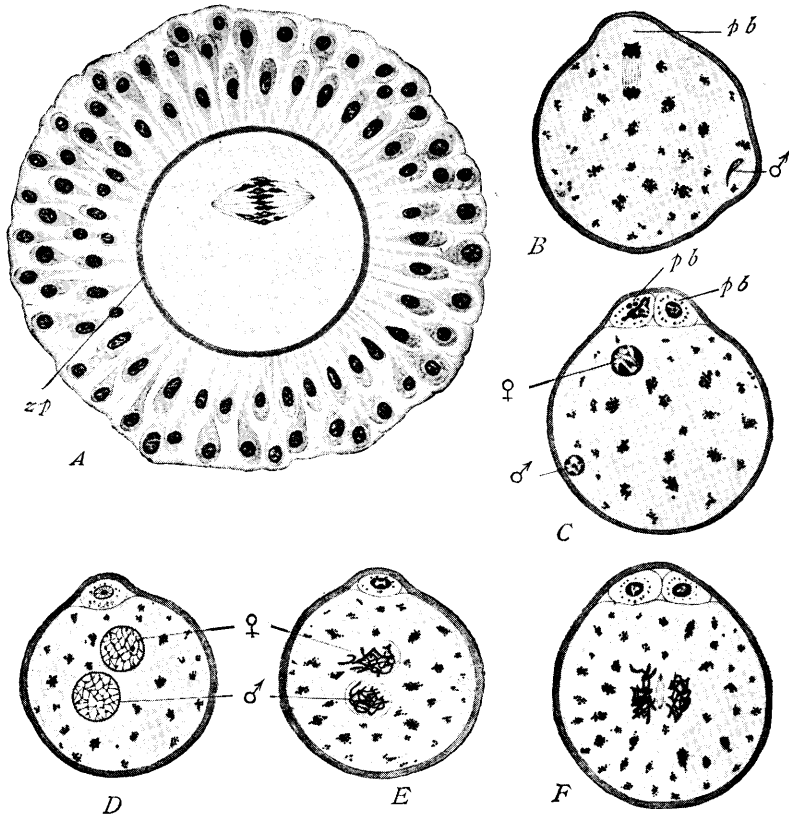


Fig. 92. — Maturation and fertilization of the egg of the mouse. [SOBOTTA.]

A. The ovarian egg still surrounded by the follicle-cells and the membrane (*z.p.* zona pellucida); the polar spindle formed. *B.* Egg immediately after entrance of the spermatozoon (sperm-nucleus at ♂). *C.* The two germ-nuclei (♂, ♀) still unequal; polar bodies above. *D.* Germ-nuclei approaching, of equal size. *E.* The chromosomes forming. *F.* The minute cleavage-spindle in the centre; on either side the paternal and maternal groups of chromosomes.

as in the ordinary mitosis of tissue-cells, and its mode of origin therefore involves the same questions as those already discussed at page 72. It is quite otherwise with the centrosomes at the astral centres, the

origin of which still remains one of the most difficult, as it is one of the most interesting, problems relating to fertilization.

After the formation of the polar bodies, the egg-nucleus is reconstituted near the upper pole of the egg, and the entire polar mitotic apparatus disappears. In the meantime a new astral system (sperm-

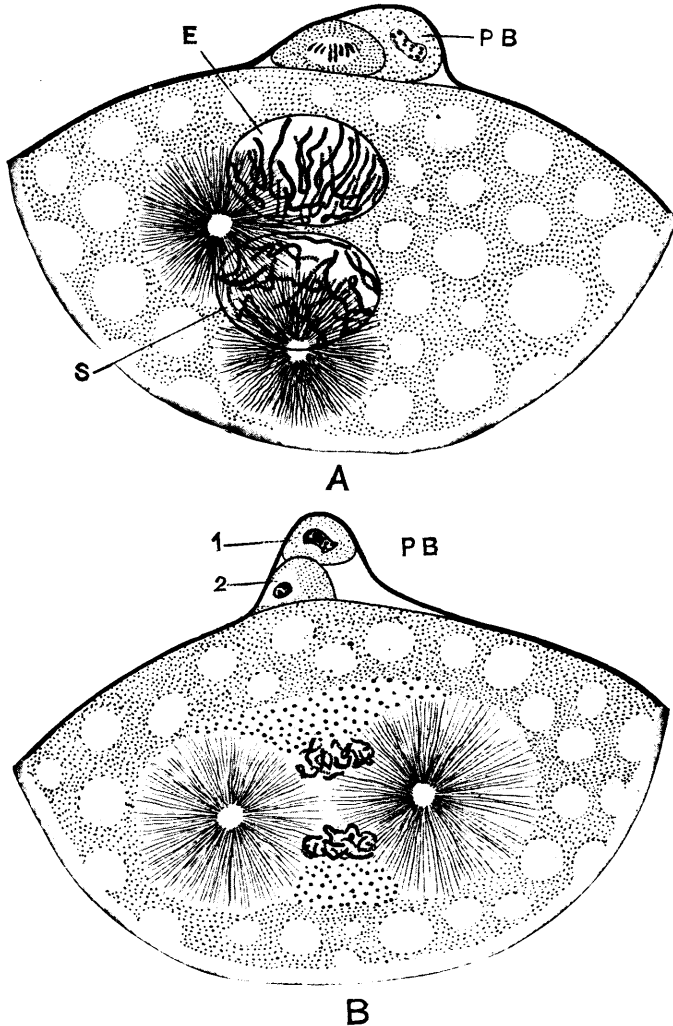


Fig. 93. — Fertilization of the egg of the gasteropod, *Pterotrachea*. [BOVERI.]

A. The egg-nucleus (*E*) and sperm-nucleus (*S*) approaching after formation of the polar bodies; the latter shown above (*P. B.*); each germ-nucleus contains sixteen chromosomes; the sperm-amphiaser fully developed. B. The mitotic figure for the first cleavage nearly established; the nuclear membranes have disappeared, leaving the maternal group of chromosomes above the spindle, the paternal below it.

aster or amphiaster) is developed in the neighbourhood of the sperm-nucleus, and this in a large number of cases gives rise or is definitely related to the cleavage-amphiaster (coelenterates, flat-worms, echinoderms, nematodes, annelids, arthropods, mollusks, tunicates, vertebrates). In many of these cases the sperm-aster, which by division gives rise to the amphiaster, has been found to arise in intimate relation with the middle-piece of the spermatozoön; *e.g.* in echinoderms (Flemming, Hertwig, Boveri, Wilson, Mathews, Hill, etc.), in the axolotl (Fick) and salamander (Michælis), in the tunicates (Hill), annelids (Foot, Vejdovsky), insects (Henking), nematodes (Meyer, Erlanger), and mollusks (Henking, Kostanecki, and Wierzejski). The agreement between forms so diverse is very strong evidence that this is a very general phenomenon, and it is one of great interest, owing to the fact that the middle-piece is itself derived from or contains the centrosome of the spermatid.¹

The facts may be illustrated by a brief description of the phe-

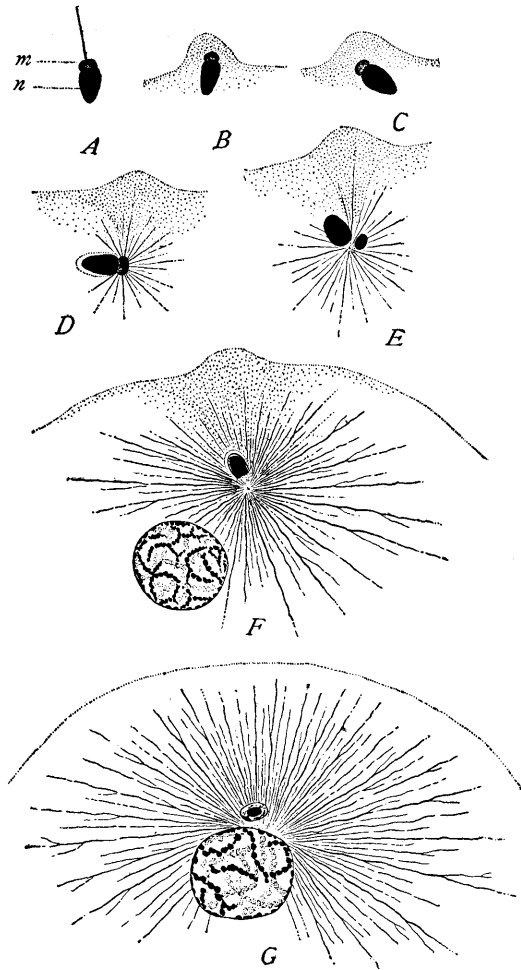


Fig. 94. — Entrance and rotation of the sperm-head and formation of the sperm-aster in the sea-urchin, *Toxopneustes* (A-F, $\times 1600$; G, H, $\times 800$).

A. Sperm-head before entrance; *n.* nucleus; *m.* middle-piece and part of the flagellum. B. C. Immediately after entrance, showing entrance-cone. D. Rotation of the sperm-head, formation of the sperm-aster about the middle-piece. E. Casting off of middle-piece; centrosome at focus of the rays (*cf.* Fig. 12). The changes figured occupy about eight minutes. F. G. Approach of the germ-nuclei; growth of the aster.

¹ *Cf.* p. 170.

nomena in the sea-urchin *Toxopneustes* (Fig. 94). As described at page 197, the tail is in this case left outside, and only the head and middle-piece enter the egg. Within a few minutes after its entrance, and while still very near the periphery, the lance-shaped sperm-head, carrying the middle-piece at its base, rotates through nearly or quite 180° , so that the pointed end is directed outward and the middle-piece is turned inward (Fig. 94, A-F).¹ During or shortly after the rotation appears a minute aster centring in or very near the middle-piece. As it enlarges, the middle-piece itself is thrown to one side (Fig. 12), where it soon degenerates, while in the centre of the aster a minute intensely staining centrosome may be seen. Both sperm-nucleus and aster now rapidly advance toward the centre of the egg, the aster leading the way and its rays extending far out into the cytoplasm and finally traversing nearly an entire hemisphere. The central mass of the aster comes in contact with the egg-nucleus, divides into two, and the daughter-asters pass to opposite poles of the egg-nucleus, while the sperm-nucleus flattens against the latter and assumes the form of a biconvex lens (Fig. 95). The nuclei now fuse to form the cleavage-nucleus. Shortly afterward the nuclear membrane fades away, a spindle is developed between the asters, and a group of chromosomes arises from the cleavage-nucleus. These are 36 or 38 in number; and although their relation to the paternal and maternal chromatin cannot in this case be accurately traced, owing to the apparent fusion of the nuclei, there can be no doubt on general grounds that one-half have been derived from each germ-nucleus. The egg then divides into two, four, etc., by ordinary mitosis (Figs. 4, 52).

In the type of fertilization just described, the polar bodies are formed long before the entrance of the spermatozoön and the germ-nuclei conjugate immediately upon entrance of the spermatozoön, fusing to form a true cleavage-nucleus. In a second and more frequent type (*Ascaris*, Fig. 90; *Physa*, Fig. 89; *Nereis*, Fig. 97; *Cyclops*, Fig. 98) the sperm-nucleus penetrates for a certain distance, often to the centre of the egg, and then pauses while the polar bodies are formed. It then conjugates with the re-formed egg-nucleus. In this case the sperm-aster always divides to form an amphiaser before conjugation of the nuclei, while in the first case the aster may be still undivided at the time of union. This difference is doubtless due merely to a difference in the time elapsing between entrance of the spermatozoön and conjugation of the nuclei, the amphiaser having, in the second case, time to

¹ The first, as far as I know, to observe the rotation of the sperm-head was Flemming in the echinoderm-egg ('81, pp. 17-19). It has since been clearly observed in several other cases, and is probably a phenomenon of very general occurrence.

form during extrusion of the polar bodies. The two types just described (Fig. 96) are connected by various gradations. Thus, in the lamprey, the frog, the rabbit, and in *Amphioxus*, one polar body is expelled before, and one after, the entrance of the spermatozoön; in the annelid *Ophryotrocha*, entrance takes place when the first polar spindle is in the stage of the equatorial plate;

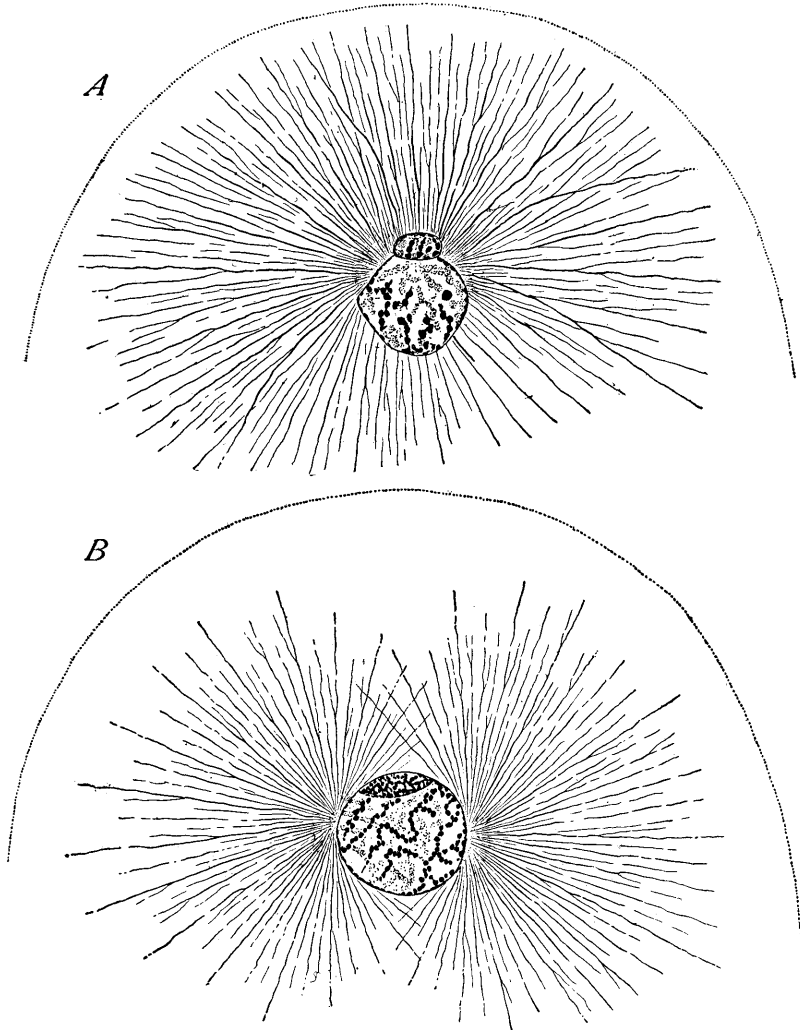


Fig. 95.—Conjugation of the germ-nuclei and division of the sperm-aster in the sea-urchin *Toxopneustes*, $\times 1000$. (For later stages see Fig. 52.)

A. Union of the nuclei; extension of the aster. B. Flattening of the sperm-nucleus against the egg-nucleus; division of the aster.

while in *Chætopterus* and *Pieris* the first polar spindle has advanced into the anaphase.¹

It is an interesting and significant fact that the aster or amphiaster always leads the way in the march toward the egg-nucleus; and in many cases it may be far in advance of the sperm-nucleus.² Boveri ('87, 1) has observed in sea-urchins that the sperm-nucleus may indeed be left entirely behind, the aster alone conjugating with the egg-

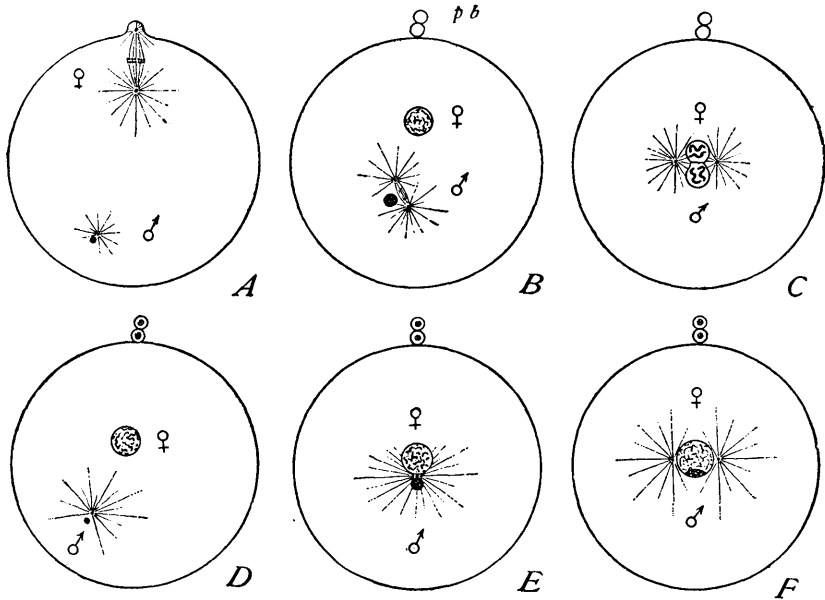


Fig. 96. — Diagrams of two principal types of fertilization. *I.* Polar bodies formed after the entrance of the spermatozoa (annelids, mollusks, flat-worms). *II.* Polar bodies formed before entrance (echinoderms).

A. Sperm-nucleus and centrosome at ♂; first polar body forming at ♀. *B.* Polar bodies formed; approach of the nuclei. *C.* Union of the nuclei. *D.* Approach of the nuclei. *E.* Union of the nuclei. *F.* Cleavage-nucleus.

nucleus and causing division of the egg *without union of the germ-nuclei*, though the sperm-nucleus afterward conjugates with one of the nuclei of the two-cell stage. This process, known as "partial fertilization," is undoubtedly to be regarded as abnormal. It affords, however, a beautiful illustration of the view that *it is the centrosome alone that incites division of the egg, and is therefore the fertilizing element proper* (Boveri, '87, 2).

The foregoing facts lead us to a consideration of Boveri's theory of fertilization, which has for several years formed a central point of discussion. The ground for this theory had been prepared by Oscar

¹ Cf. p. 181.

² Cf. Kostanecki and Wierzejski, '96.

Hertwig and Fol. The latter ('73) early reached the conclusion that the asters represented "centres of attraction" lying outside and independent of the nucleus. Oscar Hertwig showed, in 1875, that

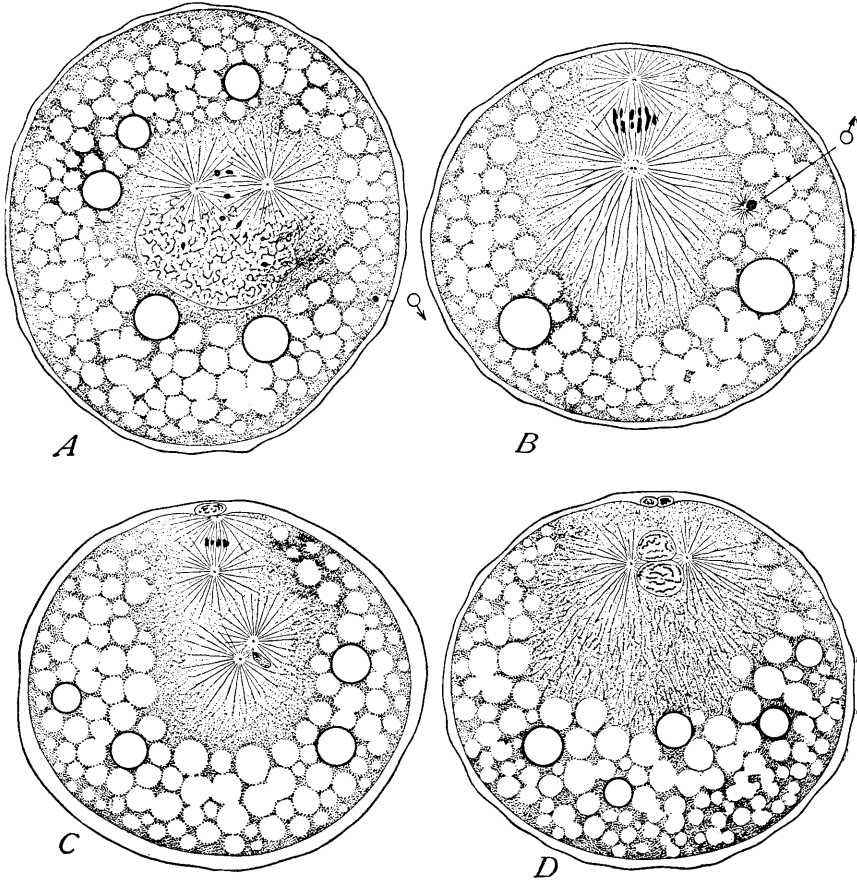


Fig. 97.—Fertilization of the egg of *Nereis*, from sections. ($\times 400$.)

A. Soon after the entrance of the spermatozoön, showing the minute sperm-nucleus at σ , the germinal vesicle disappearing, and the first polar mitotic figure forming. The empty spaces represent deutoplasm-spheres (slightly swollen by the reagents), the firm circles oil-drops. B. Sperm-nucleus (σ) advancing, a minute amphiaster in front of it; first polar mitotic figure established; polar concentration of the protoplasm. C. Later stage; second polar body forming. D. The polar bodies formed; conjugation of the germ-nuclei; the egg-centrosomes and asters have disappeared, leaving only the sperm-amphiaster (cf. Fig. 60).

in the sea-urchin egg, the amphiaster arises by the division of a single aster that first appears near the sperm-nucleus and accompanies it in its progress toward the egg-nucleus. A similar observation was soon afterward made by Fol ('79) in the eggs of *Asterias* and *Sagitta*, and in the latter case he determined the fact that the astral

rays do not centre in the nucleus, as Hertwig described, *but at a point in advance of it*—a fact afterward confirmed by Hertwig himself and by Boveri ('88, 1). Hertwig and Fol afterward found that in cases of polyspermy, when several spermatozoa enter the egg, each sperm-nucleus is accompanied by an aster, and Hertwig proved that each of these might give rise to an amphiaster (Fig. 101). In 1886–87 Vejdovsky brought forward strong evidence to show that in the fresh-water annelid *Rhynchelmis* the cleavage-amphiaster arises directly from the sperm-amphiaster, itself derived by the division of a "periplast" (attraction-sphere) imported into the egg by the spermatozoön, while the polar amphiaster entirely disappears. It was Boveri ('87, 2) who first carefully studied the facts with reference to the centrosome, reaching the conclusion (in the case of *Ascaris* and the sea-urchin) that a single centrosome is brought in by the spermatozoön, and that it divides to form two centres about which are developed the two asters of the cleavage-figure. He was thus led to the following conclusion, which has received the support of many later investigators: *The ripe egg possesses all of the organs and qualities necessary for division excepting the centrosome, by which division is initiated. The spermatozoön, on the other hand, is provided with a centrosome, but lacks the substance in which this organ of division may exert its activity. Through the union of the two cells in fertilization, all of the essential organs necessary for division are brought together; the egg now contains a centrosome which by its own division leads the way in the embryonic development.*¹ Very numerous observations, supporting this conclusion, have been made by later observers. Böhm could find in *Petromyzon* ('88) and the trout ('91) no radiations near the egg-nucleus after the formation of the polar-bodies, while a beautiful sperm-aster is developed near the sperm-nucleus and divides to form the amphiaster. Platner ('86) had already made similar observations in the snail *Arion*, and the same result was soon afterward reached by Brauer ('92) in the case of *Branchipus*, and by Julin ('93) in *Styleopsis*. Fick's careful study of fertilization of the axolotl ('93) proved in a very convincing manner not only that the amphiaster is a product of the sperm-aster, but also that the latter is developed about the *middle-piece* as a centre. The same result was indicated by Foot's observations on the earthworm ('94), and it was soon afterward conclusively demonstrated in echinoderms through the independent and nearly simultaneous researches of myself on the egg of *Toxopneustes*, of Mathews on *Arbacia*, and of Boveri on *Echinus*. Nearly at the same time a careful study was made by Mead ('95, '98, 1) of the annelid *Chaetopterus*, and of the starfish *Asterias* by Mathews,

¹ '87, 2, p. 155.

both observers independently showing that the polar spindle contains distinct centrosomes, which, however, degenerate after the formation of the polar bodies, their place being taken by the sperm-centrosome, which divides to form an amphiaster before union of the nuclei, as in *Rhynchelmis*. Exactly the same result has since been reached by Hill ('95) and Reinke ('95) in *Sphaerechinus*, by Hill in the tunicate *Phallusia*, by Kostanecki and Wierzejski ('96) in *Physa* (Fig. 89), and by Van der Stricht ('98) in *Thysanozoön*; and in all of these the centrosome is likewise shown to arise from the middle-piece or in its immediate neighbourhood. Among others who have produced

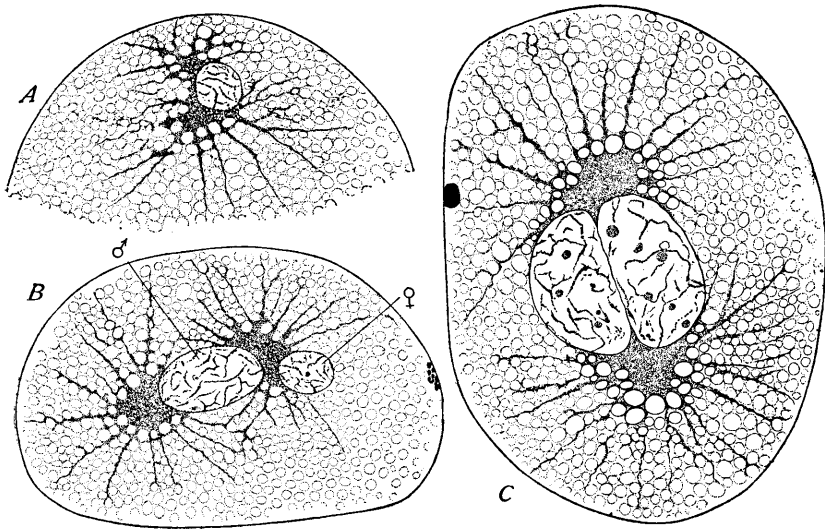


Fig. 98. — Fertilization of the egg in the copepod, *Cyclops strenuus*. [RÜCKERT.]

A. Sperm-nucleus soon after entrance, the sperm-aster dividing. B. The germ-nuclei approaching; ♂, the enlarged sperm-nucleus with a large aster at each pole; ♀, the egg-nucleus re-formed after formation of the second polar body, shown at the right. C. The apposed reticular germ-nuclei, now of equal size; the spindle is immediately afterward developed between the two enormous sperm-asters; polar body at the left.

evidence that the cleavage-centrosome stands in definite relation to the spermatozoön, may be mentioned Oppel ('92) in reptiles, Brauer ('92) in *Branchipus*, Henking ('92) in insects, Rückert ('95, 2) in *Cyclops*, Sobotta ('95) in the mouse and ('98) *Amphioxus*, Ziegler ('95) in *Diplogaster* and *Rhabditis*, Castle ('96) in *Ciona*, Korschelt ('95) in *Ophryotrocha*, Meyer ('95) in *Strongylus*, Griffin ('96, '99) in *Thalassema*, and Coe ('98) in *Cerebratulus*.

Beside the foregoing evidence may be placed the following additional data based on experiment and the study of pathological fertilization. (1) In the case of sea-urchin eggs, Hertwig, Boveri, and

several later observers have shown that egg-fragments, obtained by shaking eggs to pieces, are readily penetrated by the spermatozoa, and that such fragments, though containing no nuclear matter from the egg, may segment and give rise to perfect larvæ.¹ (2) Boveri ('88) has observed that in ordinary fertilization the sperm-aster may separate from the sperm-nucleus, travel through the cytoplasm to the egg-nucleus and cause cleavage, the sperm-nucleus afterward fusing with one of the nuclei of the two-cell stage ("partial fertilization"). (3) Most remarkable of all, Boveri, confirmed by Ziegler ('98), has recently observed that during the first cleavage the whole of the chromatin may pass to one pole, so that upon division one of the halves of the egg receives only a centrosome without a nucleus. In the nucleated half cleavage proceeds as usual. In the enucleated half the centrosomes and asters continue for a considerable period to multiply at the same rate as the cleavage of the nucleated half, though the cell-body does not itself divide.² Putting these facts together we must conclude (1) that something is introduced into the egg by the middle-piece of each spermatozoon entering it that is either a centrosome or has the power to incite the formation of one; (2) that the centrosome thus arising is structurally independent of both nuclei and may divide independently of them; (3) that independently of the division of the nucleus or cell-body there is some kind of historical continuity between the centrosomes of successive generations.

In the case of echinoderm-eggs this continuity is not yet known to be effected by actual persistence of the centrosomes.³ There are, however, a number of cases in which the division of the primary cleavage-centrosomes and the persistence of their descendants as those of the daughter-cells seem to have been conclusively shown — for example on *Ascaris* (Van Beneden, Boveri, Kostanecki, and Siedlecki), in the trout (Henneguy, '96), in *Thalassema* (Griffin, '96, '99), in *Chaetopterus* (Mead, '95, '98), in *Physa* (Kostanecki and Wierzejski, '96), in *Cerebratulus* (Coe, '98), and in *Rhynchelmis* (Vejdovsky and Mrazek, '98). In *Thalassema* and *Cerebratulus* (Figs. 99, 155) the centrosome is a minute granule at the focus of the sperm-aster, which divides to form an amphiaser soon after the entrance of the spermatozoon. During the early anaphase of the first cleavage, each centrosome divides into two, passes to the outer periphery of the centrosphere, and there forms a minute amphiaser for the second

¹ Cf. p. 353.

² Cf. p. 108.

³ Erlanger's statement ('98) that the centrosomes persist through the first cleavage in echinoderm-eggs is not supported by his figures; and I am convinced from my own long-continued studies of these eggs, as well as by an examination of Erlanger's preparations, kindly placed in my hands by Professor Bütschli, that these difficult objects are very unfavourable for a decision of the question.

cleavage before the first cleavage takes place. The minute centrosomes of the second cleavage are therefore the direct descendants of the sperm-centrosome; and there is good reason to believe that the continuity is not broken in later stages. The facts are nearly similar

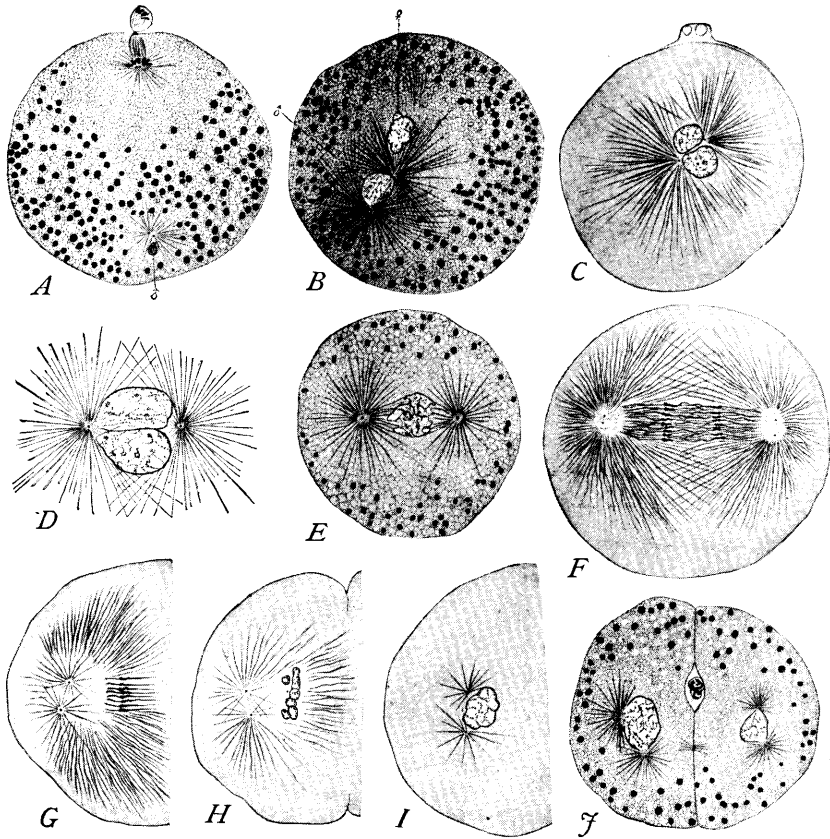


Fig. 99. — Fertilization in an annelid (armed Gephyrean), *Thalassema*. [GRIFFIN.]

A. Second polar body forming; sperm-nucleus and centrosome below. B. Approach of the egg-nucleus and sperm-nucleus, the latter accompanied by the sperm-amphiaster. C. Union of the nuclei. D. Later stage of last. E. Prophase of cleavage-spindle. F. Anaphase of the same; centrosome divided. G. H. I. Successive stages in the nuclear reconstitution and formation of the daughter-amphiasters for the second cleavage. J. Two-cell stage.

in the trout, in *Chætopterus*, and in *Physa*. In *Ascaris* division of the centrosome first occurs at a somewhat later period (Figs. 90, 176). If now the centrosomes were indeed permanent cell-organs, we should thus reach the following result: *During cleavage the cytoplasm of the blastomeres is derived from that of the egg, the centrosomes from*

the spermatozoön, while the nuclei (chromatin) are equally derived from both germ-cells.

There is very strong reason to accept the first part of this conclusion (applying to nucleus and cytoplasm), but the question of the centrosomes remains an open one. The array of evidence given above, derived from the study of so many diverse groups, seems to place Boveri's lucid and enticing hypothesis upon a strong foundation. Two essential points still remain, however, to be determined: first, whether the facts observed in *Ascaris*, Echinoderms, *Physa*, *Thalassema*, and the like, are typical of all forms of fertilization; and, second, whether, if so, the primary cleavage-centrosome is actually imported into the egg by the spermatozoön or is only formed under its influence out of the egg-substance. Both these questions have been raised by recent investigators, apparently on good evidence, and some of this evidence is directly opposed to both of the principal assumptions of Boveri's theory. Thus, Wheeler ('97) has found that in *Myzostoma* both centrosomes are derived from the egg; Carnoy and Le Brun ('97) maintain that in *Ascaris* one centrosome is derived from each of the germ-nuclei; in some mollusks, according to MacFarland ('97) and Lillie ('97), both egg-centrosomes and sperm-centrosomes disappear, to be replaced by two centrosomes of unknown origin; while recent botanical workers are unable to find any centrosomes in fertilization. These and other divergent results will be critically considered beyond (p. 208) in connection with a more detailed examination of the general subject. It may be pointed out here, however, that recent researches on spermatogenesis (p. 170) render it nearly certain that the centrosome of the sperm-aster cannot be the unmodified centrosome of the spermatid, since the latter, in some cases, enlarges to form a "middle-piece" or analogous structure that is far larger than the sperm-centrosome.

B. UNION OF THE GERM-CELLS

It does not lie within the scope of this work to consider the innumerable modes by which the germ-cells are brought together, further than to recall the fact that their union may take place inside the body of the mother or outside, and that in the latter case both eggs and spermatozoa are as a rule discharged into the water, where fertilization and development take place. The spermatozoa may live for a long period, either before or after their discharge, without losing their fertilizing power, and their movements may continue throughout this period. In many cases they are motionless when first discharged, and only begin their characteristic swimming movements after coming in contact with the water. There is clear evi-

dence of a definite attraction between the germ-cells, which is in some cases so marked (for example in the polyp *Renilla*) that when spermatozoa and ova are mixed in a small vessel, each ovum becomes in a few moments surrounded by a dense fringe of spermatozoa attached to its periphery by their heads and by their movements actually causing the ovum to move about. The nature of the attraction is not positively known, but Pfeffer's researches on the spermatozoids of plants leave little doubt that it is of a chemical nature, since he found the spermatozoids of ferns and of *Selaginella* to be as actively attracted by solutions of malic acid or malates (contained in capillary tubes) as by the substance extruded from the

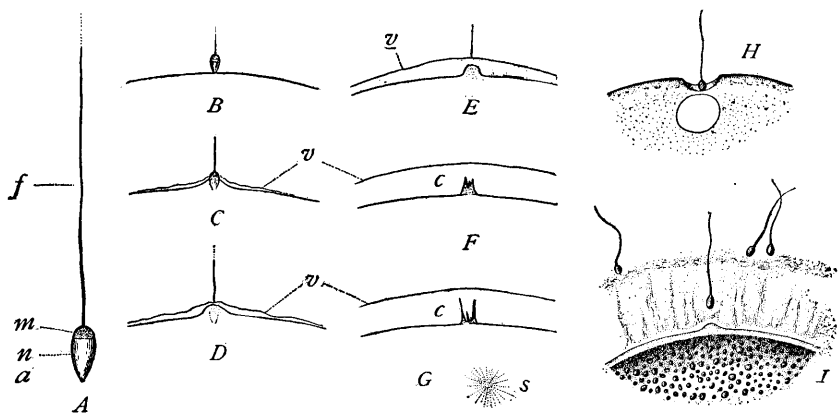


Fig. 100. — Entrance of the spermatozoon into the egg. A-G. In the sea-urchin, *Toxopneustes*. H. In the medusa, *Mitrocoma*. [METSCHNIKOFF.] I. In the star-fish *Asterias*. [FOL.]

A. Spermatozoon of *Toxopneustes*, $\times 2000$; a. the apical body, n. nucleus, m. middle-piece, f. flagellum. B. Contact with the egg-periphery. C. D. Entrance of the head, formation of the entrance-cone and of the vitelline membrane (v), leaving the tail outside. E. F. Later stages. G. Appearance of the sperm-aster (s) about 3-5 minutes after first contact; entrance-cone breaking up. H. Entrance of the spermatozoon into a preformed depression. I. Approach of the spermatozoon, showing the preformed attraction-cone.

neck of the archegonium. Those of mosses, on the other hand, are indifferent to malic acid, but are attracted by cane-sugar. These experiments indicate that the specific attraction between the germ-cells of the same species is owing to the presence of specific chemical substances in each case. There is clear evidence, furthermore, that the attractive force is not exerted by the egg-nucleus alone, but by the egg-cytoplasm; for, as the Hertwigs and others have shown, spermatozoa will readily enter egg-fragments entirely devoid of a nucleus.

In naked eggs, such as those of some echinoderms, and cœlenterates, the spermatozoon may enter at any point; but there are some cases in which the point of entrance is predetermined by the

presence of special structures through which the spermatozoön enters (Fig. 100). Thus, the starfish-egg, according to Fol, possesses before fertilization a peculiar protoplasmic "attraction-cone" to which the head of the spermatozoön becomes attached, and through which it enters the egg. In some of the hydromedusæ, on the other hand, the entrance point is marked by a funnel-shaped depression at the egg-periphery (Metschnikoff). When no preformed attraction-cone is present, an "entrance-cone" is sometimes formed by a rush of protoplasm toward the point at which the spermatozoön strikes the egg and there forming a conical elevation into which the sperm-head passes. In the sea-urchin (Fig. 100) this structure persists only a short time after the spermatozoön enters, soon assuming a ragged flame-shape and breaking up into slender rays. In some cases the egg remains naked, even after fertilization, as appears to be the case in many coelenterates. More commonly a vitelline membrane is quickly formed after contact of the spermatozoön, — *e.g.* in *Amphioxus*, in the echinoderms, and in many plants, — and by means of this the entrance of other spermatozoa is prevented. In eggs surrounded by a membrane before fertilization, the spermatozoön either bores its way through the membrane at any point, as is probably the case with mammals and Amphibia, or may make its entrance through a micropyle.

In some forms only one spermatozoön normally enters the ovum, as in echinoderms, mammals, many annelids, etc., while in others several may enter (insects, elasmobranchs, reptiles, the earthworm, *Petromyzon*, etc.). In the former case more than one spermatozoön may accidentally enter (pathological polyspermy), but development is then always abnormal. In such cases each sperm-centrosome gives rise to an amphiaster, and the asters may then unite to form the most complex polyasters, the nodes of which are formed by the centrosomes (Fig. 101). Such eggs either do not divide at all or undergo an irregular multiple cleavage and soon perish. If, however, only two spermatozoa enter, the egg may develop for a time. Thus Driesch has determined the interesting fact, which I have confirmed, that sea-urchin eggs into which two spermatozoa have accidentally entered undergo a double cleavage, dividing into four at the first cleavage, and forming eight instead of four micromeres at the fourth cleavage. Such embryos develop as far as the blastula stage, but never form a gastrula.¹ In cases where several spermatozoa normally enter the egg (physiological polyspermy), only one of the sperm-nuclei normally unites with the egg-nucleus, the supernumerary sperm-nuclei either degenerating, or in rare cases — *e.g.* in elasmobranchs and reptiles — living for a time and even dividing to form

¹ For an account of the internal changes, see p. 355.

“merocytes” or accessory nuclei. The fate of the latter is still in doubt; but they certainly take no part in fertilization.

It is an interesting question how the entrance of supernumerary spermatozoa is prevented in normal monospermic fertilization. In the case of echinoderm-eggs Fol advanced the view that this is mechanically effected by means of the vitelline membrane formed instantly after the first spermatozoon touches the egg. This is indicated by the following facts. Immature eggs, before the formation

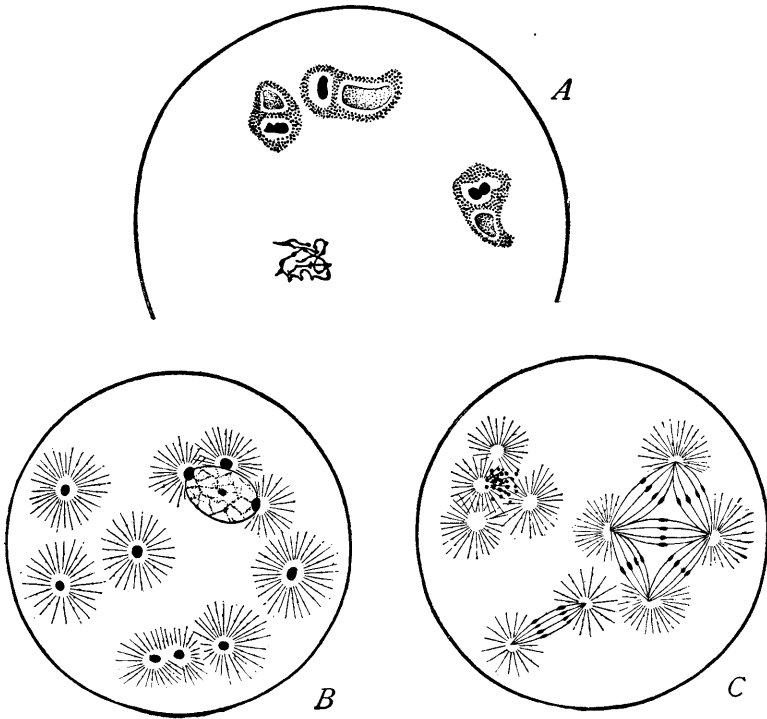


Fig. 101. — Pathological polyspermy.

A. Polyspermy in the egg of *Ascaris*; below, the egg-nucleus; above, three entire spermatozoa within the egg. [SALA.]

B. Polyspermy in sea-urchin egg treated with 0.005% nicotine solution; ten sperm-nuclei shown, three of which have conjugated with the egg-nucleus. C. Later stage of an egg similarly treated, showing polyasters formed by union of the sperm-amphiatsters. [O. and R. HERTWIG.]

of the polar bodies, have no power to form a vitelline membrane, and the spermatozoa always enter them in considerable numbers. Polyspermy also takes place, as O. and R. Hertwig's beautiful experiments showed ('87), in ripe eggs whose vitality has been diminished by the action of dilute poisons, such as nicotine, strychnine, and morphine, or by subjection to an abnormally high temperature

(31° C.); and in these cases the vitelline membrane is only slowly formed, so that several spermatozoa have time to enter.¹ Similar mechanical explanations have been given in various other cases. Thus Hoffman believes that in teleosts the micropyle is blocked by the polar bodies after the entrance of the first spermatozoön; and Calberla suggested (*Petromyzon*) that the same result might be caused by the tail of the entering spermatozoön. It is, however, far from certain whether such rude mechanical explanations are adequate; and there is considerable reason to believe that the egg may possess a physiological power of exclusion called forth by the first spermatozoön. Thus Driesch found that spermatozoa did not enter fertilized sea-urchin eggs from which the membranes had been removed by shaking.² In some cases no membrane is formed (some coelenterates), in others several spermatozoa are found inside the membrane (nemertines), in others the spermatozoön may penetrate the membrane at any point (mammals), yet monospermy is the rule.

1. *Immediate Results of Union*

The union of the germ-cells calls forth profound changes in both.

(a) *The Spermatozoön.*—Almost immediately after contact the tail ceases its movements. In some cases the tail is left outside, being carried away on the outer side of the vitelline membrane, and only the head and middle-piece enter the egg (echinoderms, Fig. 100). In other cases the entire spermatozoön enters (amphibia, earthworm, insects, etc., Fig. 89), but the tail always degenerates within the ovum and takes no part in fertilization. Within the ovum the sperm-nucleus rapidly grows, and both its structure and staining-capacity rapidly change (*cf.* p. 182). The most important and significant result, however, is an *immediate resumption by the sperm-nucleus and sperm-centrosome of the power of division*, which has hitherto been suspended. This is not due to the union of the germ-nuclei; for, as the Hertwigs and others have shown, the supernumerary sperm-nuclei in polyspermic eggs may divide freely without copulation with the egg-nucleus, and they divide as freely after entering enucleated egg-fragments. The stimulus to division must therefore be given by the egg-cytoplasm. It is a very interesting fact that in some cases the cytoplasm has this effect on the sperm-nucleus

¹ The Hertwigs attribute this to a diminished irritability on the part of the egg-substance. Normally requiring the stimulus of only a single spermatozoön for the formation of the vitelline membrane, it here demands the more intense stimulus of two, three, or more before the membrane is formed. That the membrane is not present before fertilization is admitted by Hertwig on the ground stated at page 132.

² On the other hand, Morgan states ('95, 5, p. 270) that one or more spermatozoa will enter nucleated or enucleated egg-fragments whether obtained before or after fertilization.

only after formation of the polar bodies; for when in sea-urchins the spermatozoa enter immature eggs, as they freely do, they penetrate but a short distance, and no further change occurs.

(b) *The Ovum.*—The entrance of the spermatozoon produces an extraordinary effect on the egg, which extends to every part of its organization. The rapid formation of the vitelline membrane, already described, proves that the stimulus extends almost instantly throughout the whole ovum.¹ At the same time the physical consistency of the cytoplasm may greatly alter, as for instance in echinoderm eggs, where, as Morgan has observed, the cytoplasm assumes immediately after fertilization a peculiar viscid character which it afterward loses. In many cases the egg contracts, performs amœboid movements, or shows wave-like changes of form. Again, the egg-cytoplasm may show active streaming movements, as in the formation of the entrance-cone in echinoderms, or in the flow of peripheral protoplasm toward the region of entrance to form the germinal disc, as in many pelagic fish-eggs. An interesting phenomenon is the formation, behind the advancing sperm-nucleus, of a peculiar funnel-shaped mass of deeply staining material extending outward to the periphery. This has been carefully described by Foot ('94) in the earthworm, where it is very large and conspicuous, and I have since observed it also in the sea-urchin (Fig. 94).

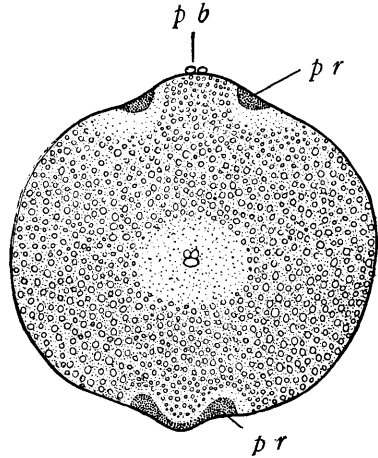


Fig. 102. — Egg of the leech *Clepsine* during fertilization. [WHITMAN.]

p.b. polar bodies; *p.r.* polar rings; cleavage-nucleus near the centre.

The most profound change in the ovum is, however, the migration of the germinal vesicle to the periphery and the formation of the polar bodies. In many cases either or both these processes may occur before contact with the spermatozoon (echinoderms, some vertebrates). In others, however, the egg awaits the entrance of the spermatozoon (annelids, gasteropods, etc.), which gives it the necessary stimulus. This is well illustrated by the egg of *Nereis*. In the newly discharged egg the germinal vesicle occupies a central position, the yolk, consisting of deutoplasm-spheres and oil-globules, is uniformly distributed, and at the periphery of the egg is a zone of clear perivitelline protoplasm (Fig. 60). Soon after entrance of the sperma-

¹ I have often observed that the formation of the membrane, in *Toxopneustes*, proceeds like a wave from the entrance-point around the periphery, but this is often irregular.

tozoön the germinal vesicle moves toward the periphery, its membrane fades away, and a radially directed mitotic figure appears, by means of which the first polar body is formed (Fig. 97). Meanwhile the protoplasm flows toward the upper pole, the peri-vitelline zone disappears, and the egg now shows a sharply marked polar differentiation. A remarkable phenomenon, described by Whitman in the leech ('78), and later by Foot in the earthworm ('94), is the formation of "polar rings," a process which follows the entrance of the spermatozoön and accompanies the formation of the polar bodies. These are two ring-shaped cytoplasmic masses which form at the periphery of the egg near either pole and advance thence toward the poles, the upper one surrounding the point at which the polar bodies are formed (Fig. 102). Their meaning is unknown, but Foot ('96) has made the interesting discovery that they are probably of the same nature as the yolk-nuclei (p. 156).

2. Paths of the Germ-nuclei (*Pro-nuclei*)¹

After the entrance of the spermatozoön, both germ-nuclei move through the egg-cytoplasm and finally meet one another. The paths traversed by them vary widely in different forms. In general two classes are to be distinguished, according as the polar bodies are formed before or after entrance of the spermatozoön. In the former case (echinoderms) the germ-nuclei unite at once. In the latter case the sperm-nucleus advances a certain distance into the egg and then pauses while the germinal vesicle moves toward the periphery, and gives rise to the polar bodies (*Ascaris*, annelids, etc.). This significant fact proves that the attractive force between the two nuclei is only exerted after the formation of the polar bodies, and hence that the entrance-path of the sperm-nucleus is not determined by such attraction. A second important point, first pointed out by Roux, is that the path of the sperm-nucleus is *curved*, its "entrance-path" into the egg forming a considerable angle, with its "copulation-path" toward the egg-nucleus.

These facts are well illustrated in the sea-urchin egg (Fig. 103), where the egg-nucleus occupies an eccentric position near the point at which the polar bodies are formed (before fertilization). Entering

¹ The terms *female pro-nucleus*, *male pro-nucleus* (Van Beneden), are often applied to the germ-nuclei before their union. These should, I think, be rejected in favour of Hertwig's terms *egg-nucleus* and *sperm-nucleus*, on two grounds: (1) The germ-nuclei are true nuclei in every sense, differing from the somatic nuclei only in the reduced number of chromosomes. As the latter character has recently been shown to be true also of the somatic nuclei in the sexual generation of plants (p. 275), it cannot be made the ground for a special designation of the germ-nuclei. (2) The germ-nuclei are not male and female in any proper sense (p. 243).

the egg at any point, the sperm-nucleus first moves rapidly inward along an entrance-path that shows no constant relation to the position of the egg-nucleus and is approximately but never exactly radial, *i.e.* toward a point near the centre of the egg. After penetrating a

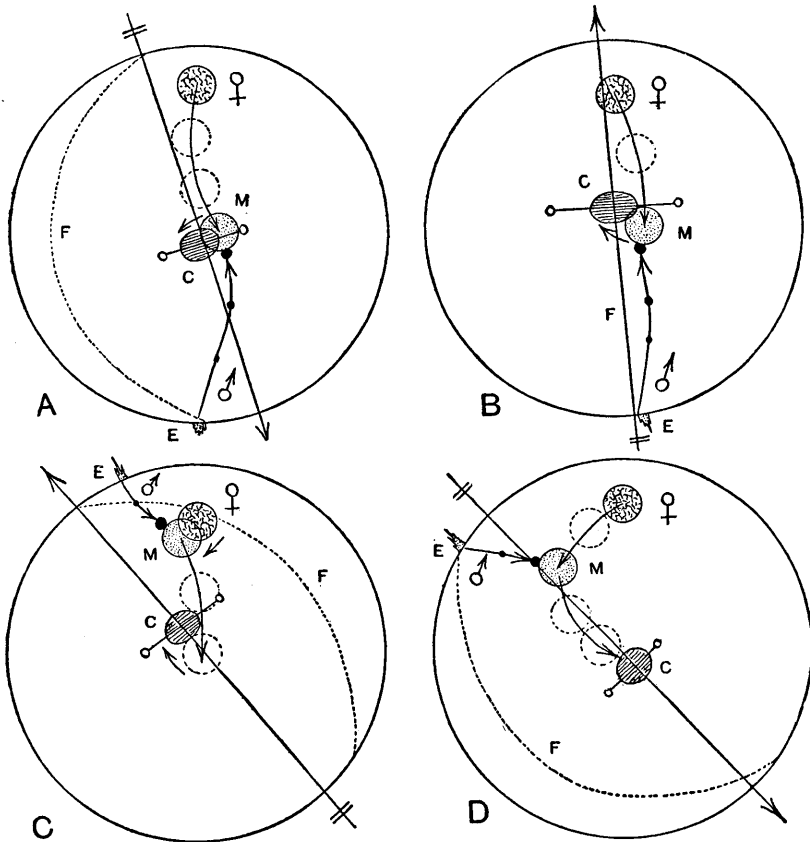


Fig. 103. — Diagrams showing the paths of the germ-nuclei in four different eggs of the sea-urchin, *Toxopneustes*. From camera drawings of the transparent living eggs.

In all the figures the original position of the egg-nucleus (reticulated) is shown at ♀; the point at which the spermatozoön enters at *E* (entrance-cone). Arrows indicate the paths traversed by the nuclei. At the meeting-point (*M*) the egg-nucleus is dotted. The cleavage-nucleus in its final position is ruled in parallel lines, and through it is drawn the axis of the resulting cleavage-figure. The axis of the egg is indicated by an arrow, the point of which is turned away from the micromere-pole. Plane of first cleavage, passing near the entrance-point, shown by the curved dotted line.

certain distance its direction changes slightly to that of the copulation-path, which, again, is directed not precisely toward the egg-nucleus, but toward a meeting-point where it comes in contact with the egg-nucleus. The latter does not begin to move until the

entrance-path of the sperm-nucleus changes to the copulation-path. It then begins to move slowly in a somewhat curved path toward the meeting-point, often showing slight amoeboid changes of form as it forces its way through the cytoplasm. From the meeting-point the apposed nuclei move slowly toward the point of final fusion, which in this case is near, but never precisely at, the centre of the egg.

These facts indicate that the paths of the germ-nuclei are determined by at least two different factors, one of which is an attraction or other dynamical relation between the nuclei and the cytoplasm, the other an attraction between the nuclei. The former determines the entrance-path of the sperm-nucleus, while both factors probably operate in the determination of the copulation-path along which it travels to meet the egg-nucleus. The real nature of neither factor is known.

Hertwig first called attention to the fact — which is easy to observe in the living sea-urchin egg — that the egg-nucleus does not begin to move until the sperm-nucleus has penetrated some distance into the egg and the sperm-aster has attained a considerable size; and Conklin ('94) has suggested that the nuclei are passively drawn together by the formation, attachment, and contraction of the astral rays. While this view has some facts in its favour, it is, I believe, untenable, for many reasons, among which may be mentioned the fact that neither the actual paths of the pro-nuclei nor the arrangement of the rays support the hypothesis: nor does it account for the conjugation of nuclei when no astral rays are developed (as in Protozoa or in plants). I have often observed in cases of dispermy in the sea-urchin, that both sperm-nuclei move at an equal pace toward the egg-nucleus; but if one of them meets the egg-nucleus first, the movement of the other is immediately retarded, and only conjugates with the egg-nucleus, if at all, after a considerable interval; and in polyspermy the egg-nucleus rarely conjugates with more than two sperm-nuclei. Probably, therefore, the nuclei are drawn together by an actual attraction which is neutralized by union, and their movements are not improbably of a chemotactic character. Conklin ('99) has recently suggested that the nuclei are drawn together by the agency of protoplasmic currents in the egg-substance.

3. *Union of the Germ-nuclei. The Chromosomes*

The earlier observers of fertilization, such as Auerbach, Strasburger, and Hertwig, described the germ-nuclei as undergoing a complete fusion to form the first embryonic nucleus, termed by Hertwig the *cleavage- or segmentation-nucleus*. As early as 1881, however, Mark clearly showed that in the slug *Limax* this is not the case, the two nuclei merely becoming apposed without actual fusion. Two years later appeared Van Beneden's epoch-making work on *Ascaris*, in which it was shown not only that the nuclei do not fuse, but that they give rise to two independent groups of chromosomes which separately enter the equatorial plate and whose descendants pass separately into the daughter-nuclei. Later observations have given the strongest reason to believe that, as far as the chromatin is con-

cerned, a true fusion of the nuclei never takes place during fertilization, and that the paternal and maternal chromatin *may* remain separate and distinct in the later stages of development — possibly throughout life (p. 299). In this regard two general classes may be distinguished. In one, exemplified by some echinoderms, by *Amphioxus*, *Phallusia*, and some other animals, the two nuclei meet each other when in the reticular form, and apparently fuse in such a manner that the chromatin of the resulting nucleus shows no visible distinction between the paternal and maternal moieties. In the other class, which includes most accurately known cases, and is typically represented by *Ascaris* (Fig. 90) and other nematodes, by *Cyclops* (Fig. 98), and by *Pterotrachea* (Fig. 93), the two nuclei do not fuse, but only place themselves side by side, and in this position give rise each to its own group of chromosomes. On general grounds we may confidently maintain that the distinction between the two classes is only apparent, and probably is due to corresponding differences in the rate of development of the nuclei, or in the time that elapses before their union.¹ If this time be very short, as in echinoderms, the nuclei unite before the chromosomes are formed. If it be more prolonged, as in *Ascaris*, the chromosome-formation takes place before union.

With a few exceptions, which are of such a character as not to militate against the rule, *the number of chromosomes arising from the germ-nuclei is always the same in both, and is one-half the number characteristic of the tissue-cells of the species. By their union, therefore, the germ-nuclei give rise to an equatorial plate containing the typical number of chromosomes.* This remarkable discovery was first made by Van Beneden in the case of *Ascaris*, where the number of chromosomes derived from each sex is either one or two. It has since been extended to a very large number of animals and plants, a partial list of which follows.

¹ Indeed, Boveri has found that in *Ascaris* both modes occur, though the fusion of the germ-nuclei is exceptional. (Cf. p. 296.)