CHAPTER V

FERTILIZATION AND PARTHENOGENESIS

"Fertilization is not alone a physico-chemical process, as physiologists so often assume, but also a morphological phenomenon." O. HERTWIG.[1]

"It is conceivable, and indeed probable, that every part of the adult contains molecules derived 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.[2]

Fertilization comprises two closely associated but experimentally separable events. One of these, primarily of interest from a physiological point of view, is the *activation of the egg by the sperm*. The second, of especial importance for the study of cytology and genetics, is *a union or association of corresponding maternal and paternal elements*, which results in biparental heredity.[3] Both these results are brought about by a union and fusion of two germ-cells or *gametes* to form a single cell, the *zygote*—a process which finds its prototype in the conjugation of unicellular organisms.

It was the first of these effects alone that was commonly designated as "fertilization" or "fecundation" ("Befruchtung," "fécondation") by early embryologists who had not the least notion of the real nature of the process. The word has often been employed in the same sense by modern writers; and this usage is often convenient. Some writers, however, have sometimes almost seemed to forget that fertilization comprises a second and not less important series of events of which the most conspicuous is *karyogamy*, *i. e.*, the union of the gamete-nuclei or pronuclei to form the cleavage-nucleus or primary nucleus of the embryo. The fact of central interest here is the *union and close association of two corresponding groups of chromosomes* derived from the two respective pronuclei and hence respectively of maternal and paternal origin. Fertilization commonly involves also a process of plastogamy or union of gamete-cytoplasms; but in higher forms generally the amount of paternal cytoplasm introduced into the egg is both absolutely and relatively very small, and we still know little of its history in the zygote.

By O. Hertwig ('75) and some other early writers the activation of the

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[1] *Jenaische Zeitschrift*, XVIII, p. 291.

[2] *Evolution*, in *Science and Culture*, p. 296, from Enc. Britt., 1878.

[3] "Fertilization is the incitement of the egg to development together with the conveyance of paternal qualities to the egg." (Roux, '12, p. 48). See also Giard ('01), Fick ('05), O. Hertwig ('05), Boveri ('07).

egg in fertilization was ascribed to karyogamy, or union of the gamete-nuclei, but this was disputed by Boveri ('87, '91) on cytological grounds and is evidently inconsistent with the phenomena of parthenogenesis, of partial fertilization (p. 458), merogony (p. 465) and gynogenesis (p. 460) in none of which does activation involve a process of karyogamy.

For the rest, the problems of fertilization are intimately bound up with those of cell-division. Superficially regarded, the two processes seem to be opposites; for fertilization involves the fusion of two cells into one, while mitosis results in the division of one cell into two. Fundamentally, however, the same cytological elements are involved in both, and the same end-result sooner or later follows, namely, the formation of a mitotic figure and the resulting process of cleavage. This is still clearer when we regard parthenogenesis or the activation of the egg by some agency other than the sperm, a process which, as will later be shown, is connected with true fertilization by intermediate gradations (p. 458). The analogy of fertilization to cell-division is thus made perfectly clear. Fertilization and parthenogenesis possess, however, a far-reaching interest of their own, for they set in motion the mechanism not alone of cell-division, but also of development; and here cytology merges with embryology and genetics.

In their essentials the phenomena of fertilization are closely similar in animals and plants, but show many minor differences which often involve a wide divergence of external aspect. For purposes of description, therefore, it is convenient to separate the two groups.

I. FERTILIZATION OF THE ANIMAL EGG

A. General Sketch

In outlining the main features of fertilization in animals we may take as a basis of reference the phenomena as seen in the eggs of sea-urchins and of *Ascaris megalocephala* (the parasitic threadworm of the horse) the study of which between 1873 and 1890, laid the main basis of our knowledge both of fertilization and maturation and in large measure also of mitosis. Both objects, made classical by these pioneer researches, have repeatedly been investigated by more recent observers and experimenters. They differ materially in respect to certain details of the process and hence offer a broader view when considered together.

In animals generally, the entire sperm enters the egg (Fig. 183); but in the sea-urchin and the starfish several observers have found that at least a part of the tail is left outside the egg. The sperm thus carries into the egg all of the components that have entered into its formation, in particular a nucleus, a central body or its derivatives, a certain quantity of chondriosome-material, an acrosome (a product of the Golgi-bodies) and a small amount of general cytoplasm. The fate of these components is completely known only in case of the nucleus, though interesting facts have been made known concerning the central bodies and the chondriosomes.

Almost immediately after its entrance the sperm-head rotates through an angle of 180°, so that the middle-piece or basal region of the nucleus is directed inwards; and at the same time, or a little later, a single *sperm-aster* appears in the region of the middle-piece or actually centering in it (Figs. 184, 207). The sperm-nucleus now slowly enlarges, approaches the egg-nucleus and sooner or later unites with it (Figs. 185, 186). In the sea-urchin the two pronuclei completely fuse to form a *fusion-nucleus* or *cleavage-nucleus* in which all traces of the original maternal and paternal constituents are temporarily lost to view. In *Ascaris* (Fig. 187), the two nuclei place themselves side by side in close contact but do not actually fuse. In either case the synkaryon thus formed sooner or later undergoes a typical mitotic division, thus producing duplicates of itself in the nuclei of the first

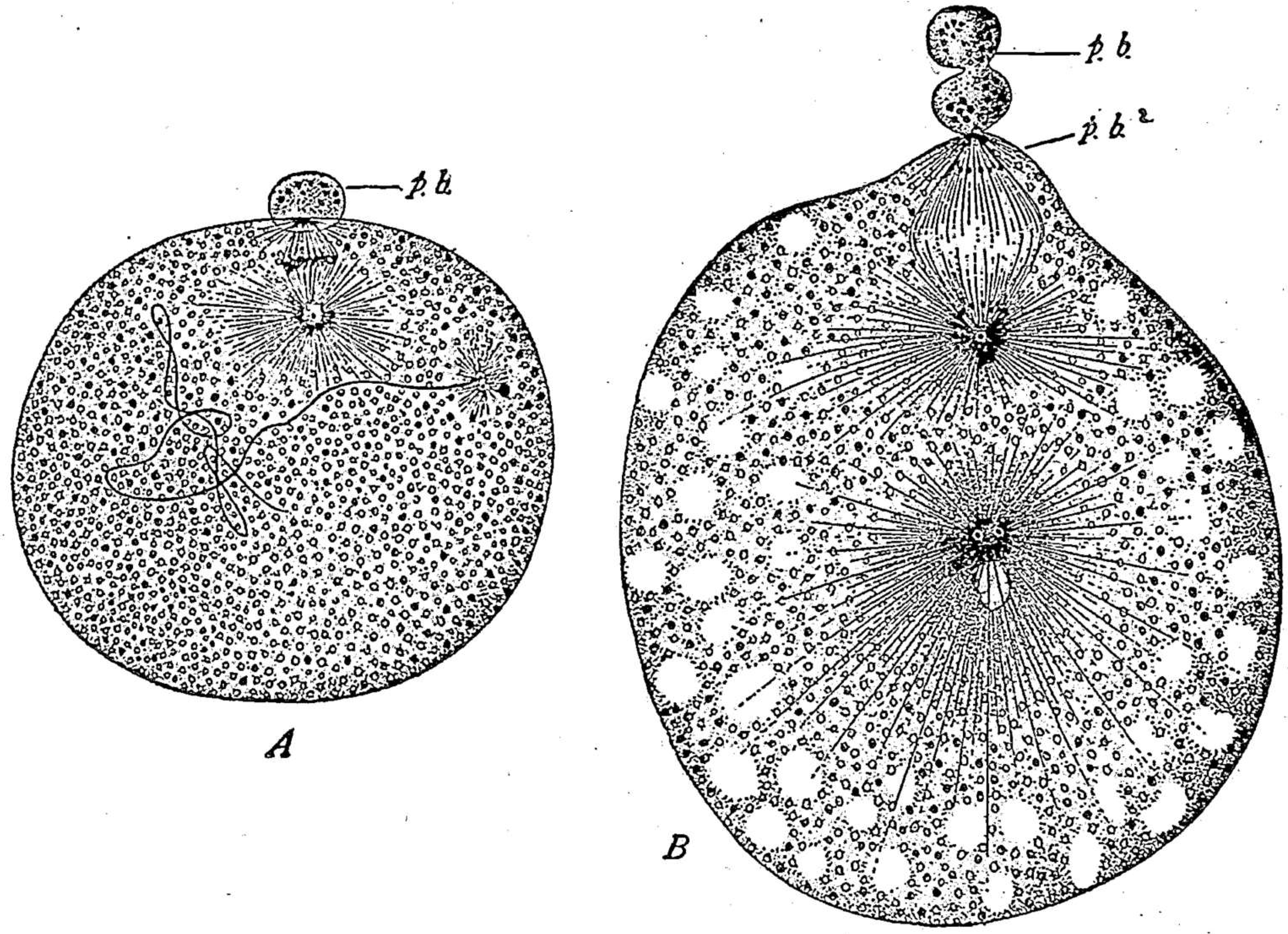

Fig. 183.—Fertilization of the egg of the snail *Physa* (Kostanecki and Wierzejski).

A, the entire sperm lies in the egg, the first polocyte has been formed, the second is forming; *B*, the enlarged sperm-nucleus and sperm-aster lie near the center; second polar body forming and the first dividing. The egg-centers and asters afterwards disappear, their place being taken by the sperm-centers.

two cells of the embryo. During this process the daughter-nuclei receive maternal and paternal chromosomes, usually in equal number; and from them, by continued division arise all the nuclei of the resulting organism. In

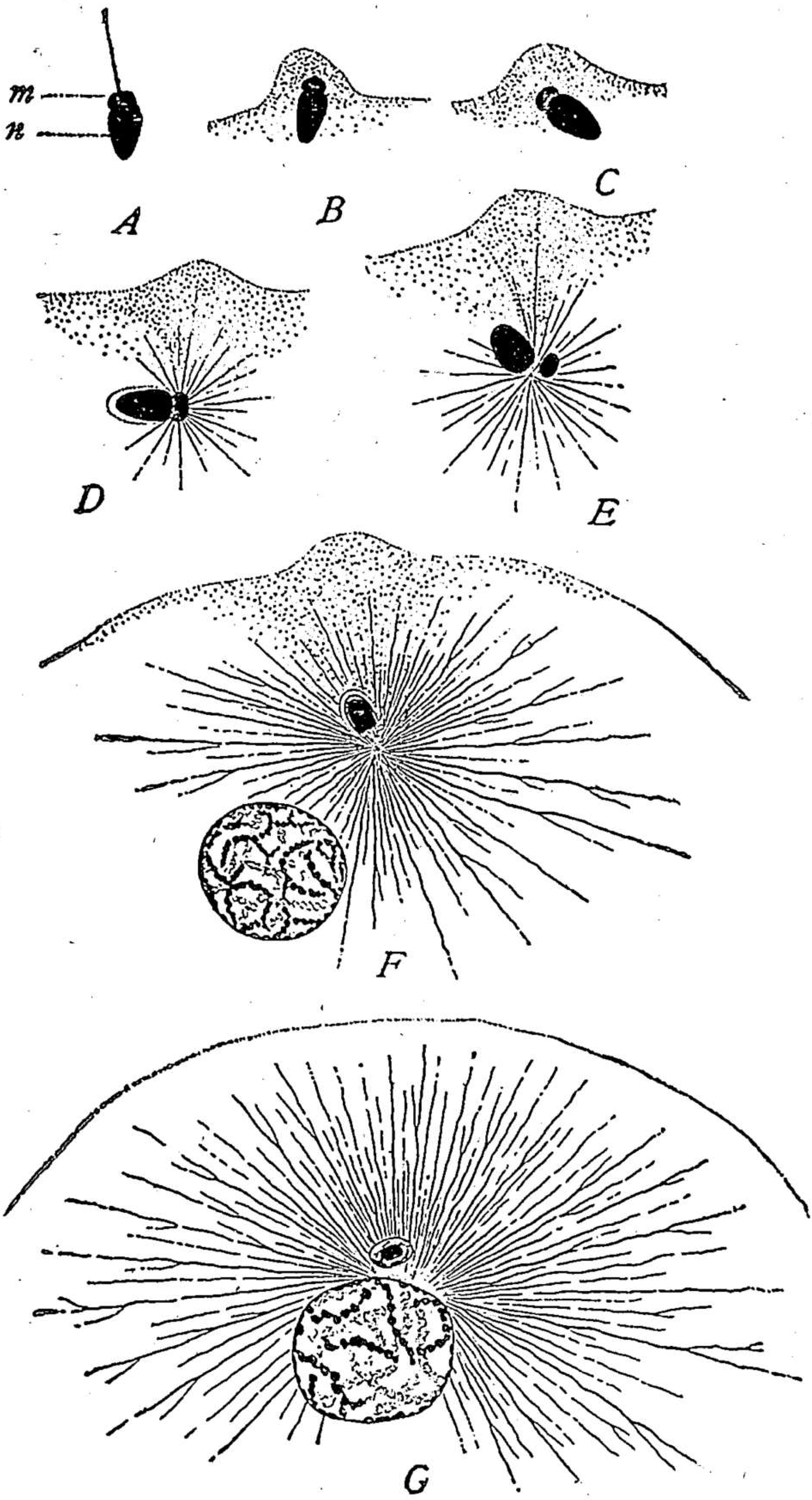

Fig. 184.—Entrance and rotation of the sperm-head and formation of the sperm-aster in the sea-urchin *Toxopneustes* (*A–E*, × 1600; *F*, *G*, × 800).

these phenomena we see the physical counterpart of biparental heredity, an effect that may appear in any portion of the offspring.

Relations between Fertilization and Maturation of the Egg. It is necessary to consider at this point certain relations between fertilization and the "ripening" or maturation of the egg. The latter process is accomplished in the animal ovum by means of two successive "polar divisions," in the course of which the egg buds forth two small and often extremely minute cells at or near its upper pole (Figs. 183, 233). These cells, called *polar bodies* or *polocytes* because of their position, have nothing to do with the process of fertilization proper and were a puzzle to the earlier observers.[1] Ultimately they were shown by Mark, Van Beneden and Boveri to belong to the final act in the maturation of the egg, a process during which is effected the reduction of the chromosomes from the diploid to the haploid number. We are here concerned only with the wide differences shown by different species of animals in respect to the time at which the sperm enters the egg with respect to the polar divisions.[2] At one extreme is the comparatively rare case illustrated by the sea-urchin egg (Figs. 186, 189) which cannot be fertilized until both polar divisions have taken place. The polocytes are in this case extruded in the ovary long before the sperm has access to the egg. The sperms will, it is true, readily penetrate eggs which have not formed their polar bodies; but in this cases development does not ensue (p. 405). The sea-urchin type seems to occur also in certain cœlenterates, possibly in some of the tunicates [3] and is paralleled in a way by the eggs of higher plants in which meiosis occurs during the spore-forming divisions, so that no polocytes are formed by the egg (p. 452).

At the opposite extreme is the type represented by *Ascaris* and various other nematodes, platodes, annelids, mollusks, and crustaceans,[4] in which the sperm normally enters the egg before either polocyte has been extruded, and in some cases before the germinal vesicle has broken down (Figs. 187, 197). Here, therefore, the later stages of maturation are overlapped by the earlier stages of fertilization; and this is true, in lesser degree, of many cases intermediate between the two extremes just outlined. In this fact lies a source of great diversity in the general aspect of fertilization in different animals; and to it also is due the greater complexity of the phenomena in animals generally, as compared with plants, where maturation and fertilization are most commonly widely separated (p. 491). Eggs of the two types are shown together in Fig. 188.

All these cases have certain features in common. In all cases the

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[1] The polocytes were first observed by Carus (1824) in the gasteropods. The most diverse views concerning their origin and nature were held by other early observers. They were supposed to arise by the extrusion of the nucleolus or even of the germinal vesicle, wholly or in part (Van Beneden, Bütschli, etc.), but were regarded by O. Hertwig ('76), and Giard ('77) as products of two successive unequal divisions of the egg—a conclusion fully confirmed by all later observers.

[2] For further details see Korschelt-Heider, '03, p. 630 ff.

[3] See Boveri, '90 (*Tiara*), Morgenstern, '01, Wulfert, '02 (*Cordylophora*), Hill, '96 (tunicates); see, however, Conklin, '05.

[4] See, for instance, Boveri, '87 (*Ascaris*), Wheeler, '95 (*Myzostoma*), Rückert, '95 (*Cyclops*), Haecker, '95 (copepods), Van Name, '99 (*Turbellaria*) Griffin, '99 (*Thalassema*), Kostanecki, '02 (*Mactra*), Lillie, '01 (Unio), and '12 (*Nereis*), Goldschmidt, '02 (*Polystomum*). etc.

sperm-nucleus is unable to conjugate with the egg-nucleus until after the polar divisions have been accomplished. In all cases the polar divisions are initiated by the breaking down of the germinal vesicle (as in ordinary mitosis), and the formation of chromosomes and of an achromatic figure. In all cases the re-formed egg-nucleus, after the completion of both divisions, is much smaller than the original germinal vesicle; and in almost all cases

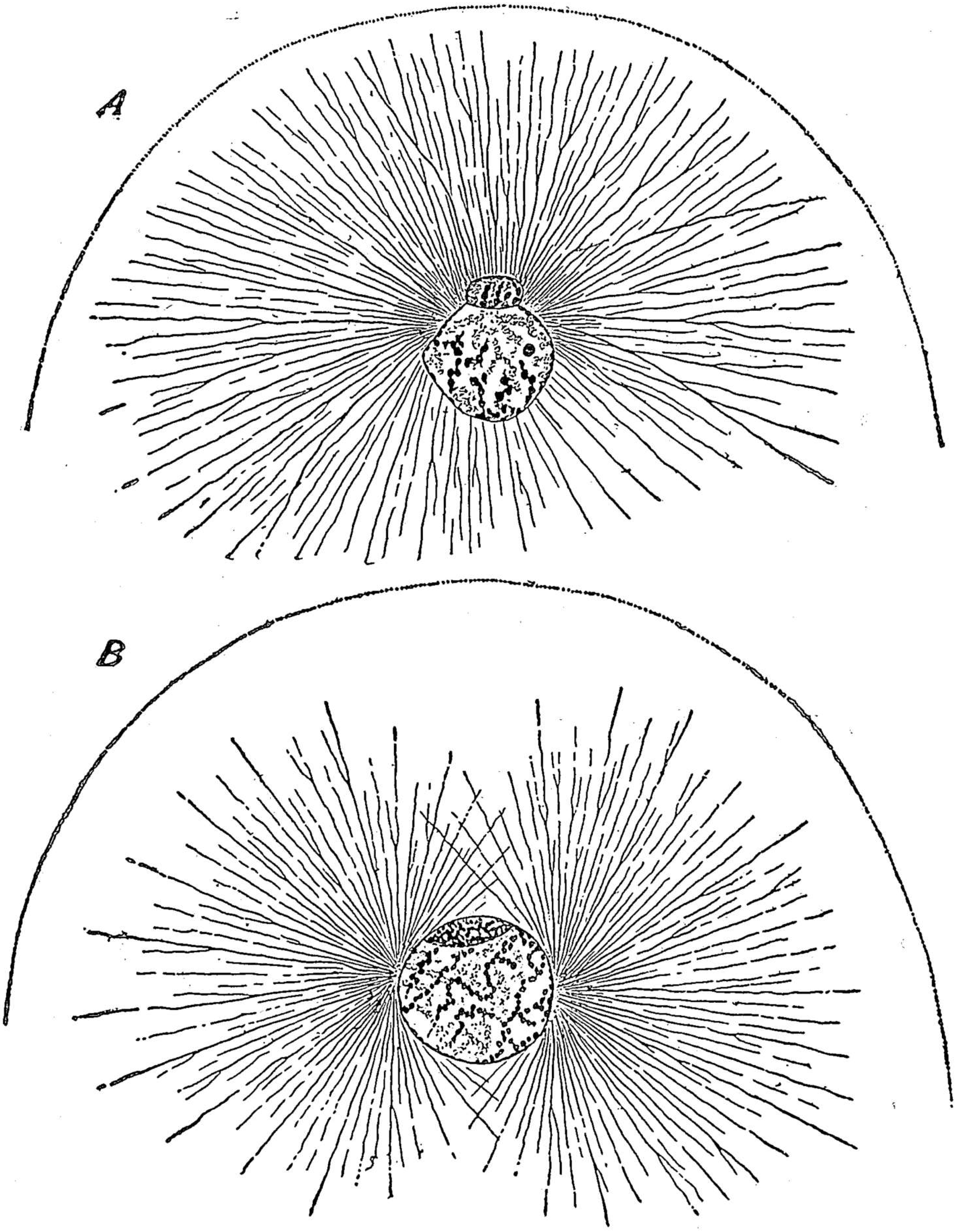

Fig. 185.—Conjugation of the gamete-nuclei and division of the sperm-aster in the sea-urchin *Toxopneustes*, × 1000. (For later stages see Fig. 58.)

the sperm-nucleus, as it advances within the egg, is typically preceded by a *sperm-aster* which sooner or later divides to form an *amphiaster* that is the forerunner of the *cleavage-amphiaster*. In other respects the two extreme types display the following conspicuous differences:

(1) In the sea-urchin type the pronuclei conjugate immediately after entrance of the sperm and apparently fuse completely to form a *fusion-nucleus* (Figs. 185, 186). Owing to the short time elapsing between entrance of the sperm and nuclear union the pronuclei are as a rule, still very unequal at the time of union, while the sperm-aster does not ordinarily divide until after the nuclei have united, though the central body is already double (Boveri, 95) from an earlier period (Fig. 186, A–C). Fusion of the pronuclei in most of these cases appears to the eye to be complete, so that the maternal and paternal elements cannot be distinguished as such either in the fusion-nucleus or in the resulting division-figure. This type of fertilization, rather infrequent in animals, is (so far as the nuclei are concerned) common in plants (*Œdogonium*, *Fucus*, bryophytes, pteridophytes, and many seed-plants (p. 453).

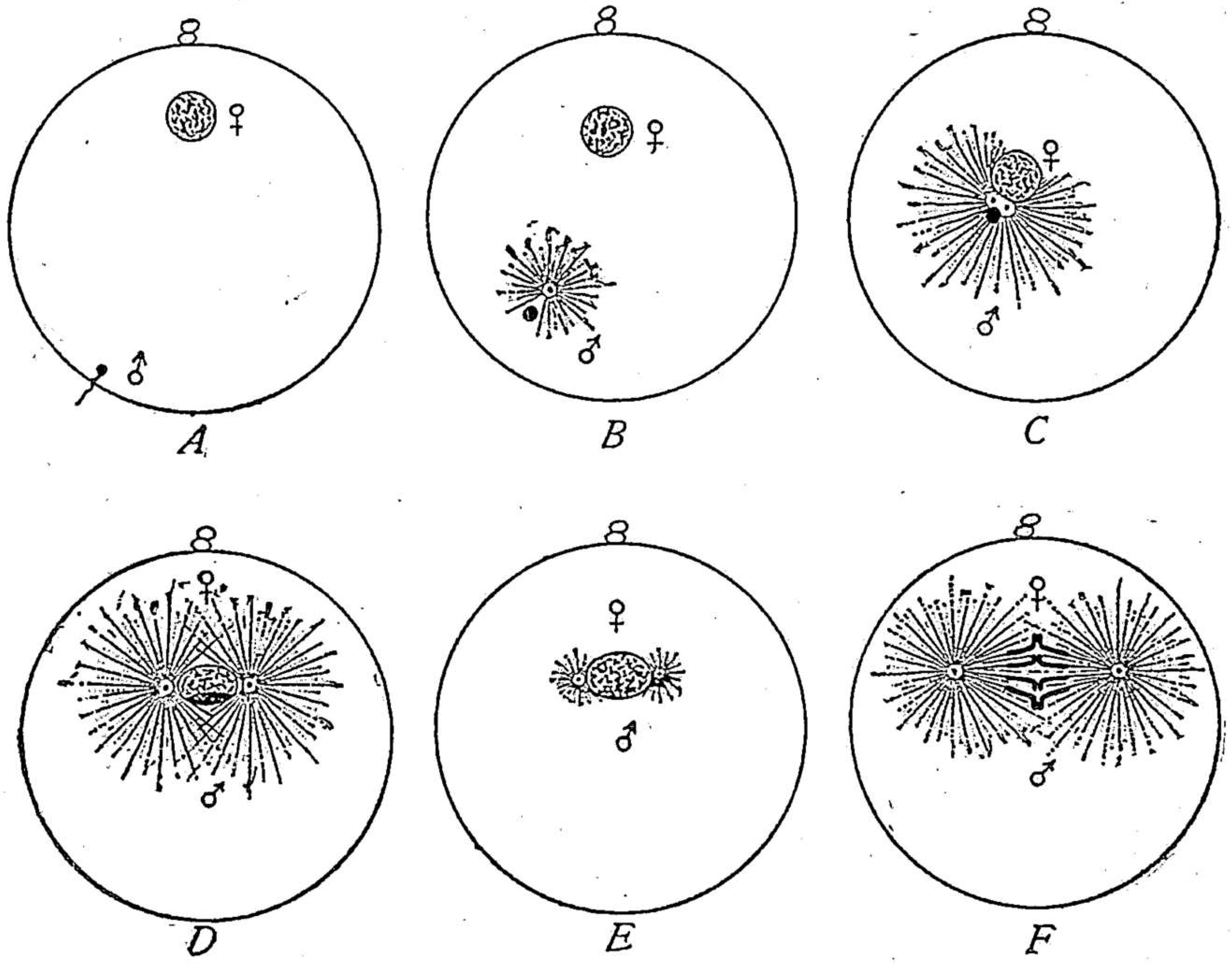

Fig. 186.—Diagram of sea-urchin type of fertilization.

A, matured egg, entrance of sperm; *B*, *C*, approach of pronuclei, division of sperm-center; *D*, sperm-aster divided, fusion of pronuclei; *E*, fusion-nucleus in the "pause," reduction of asters; *F*, first cleavage figure.

(2) In the second or *Ascaris* type (Fig. 187, D–F) the sperm-nucleus pauses within the egg until the polar divisions have been accomplished, with

the three following consequences: (1) During the pause the sperm-nucleus enlarges, assumes a vesicular structure, and finally may become as large as the egg-nucleus, and indistinguishable from it, except by its position, the pronuclei being exactly alike at the time of union (Figs. 197, 198, 200). (2) In many cases each pronuclus at the time of union has already given rise to a group of chromosomes for the ensuing division; hence no true fusion-nucleus is formed and the maternal and paternal nuclear elements remain in distinct groups that lie side by side throughout the whole process.

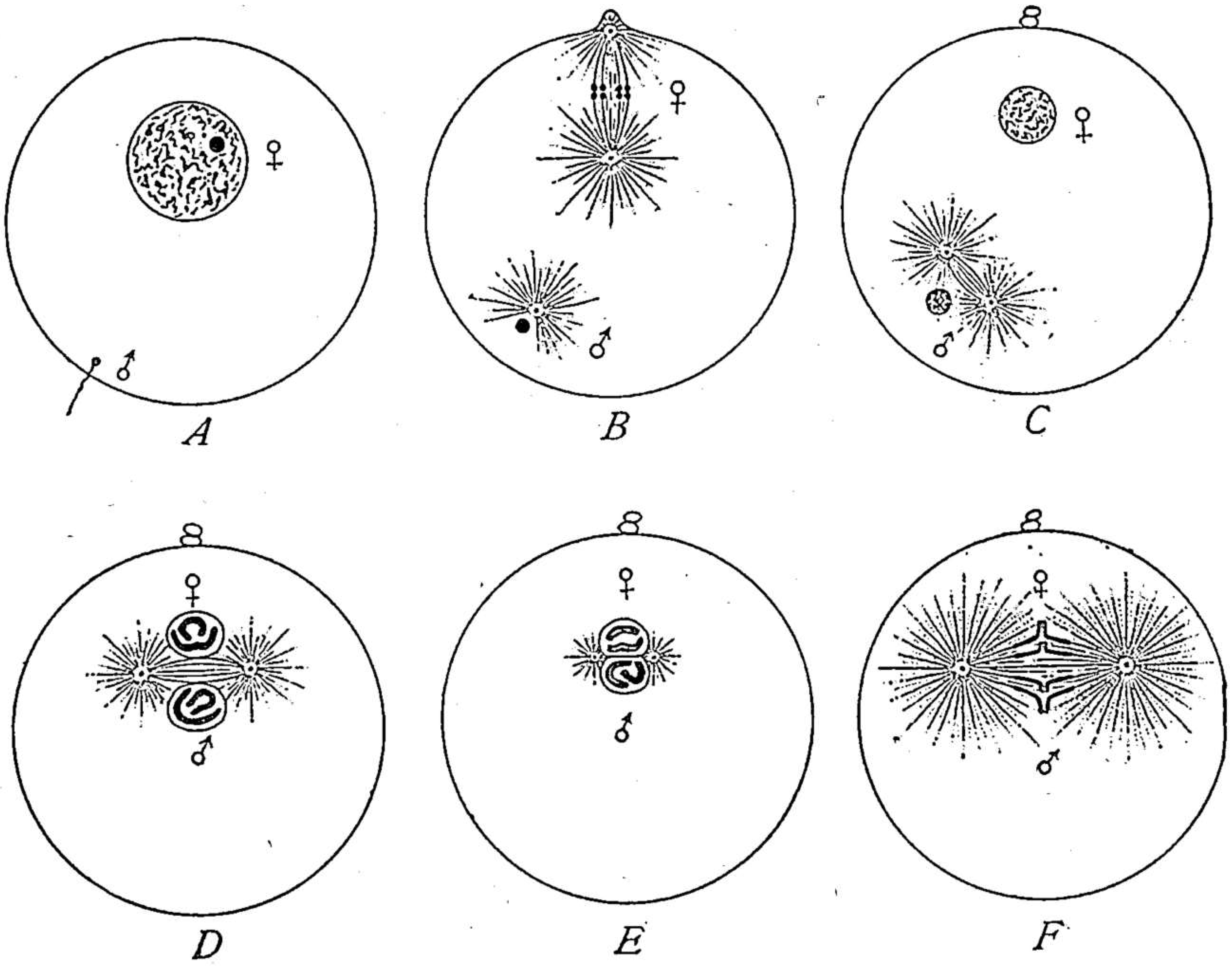

Fig. 187.—Diagram of *Ascaris* type of fertilization.

A, unmatured egg, entrance of sperm; *B*, sperm-aster and first polar spindle; *C*, sperm-amphiaster, polar divisions completed; *D*, union of the pronuclei; *E*, ensuing "pause," reduction of asters; *F*, first cleavage-figure.

Ascaris megalocephala, first worked out by Van Beneden in 1883–84, offers the classical example of this; but many other similar cases have since been described by other observers. (3) The sperm-aster typically divides to form an amphiaster before karyogamy has taken place (Figs. 187, 200, etc.).

The difference between the two foregoing types is determined mainly by the time-element, *i. e.*, the length of the interval between entrance of the sperm and conjugation of the pronuclei; for it has been shown experimentally (Wilson, '01b) that by artificially prolonging this pause by slight etherization of eggs of the first type (*Toxopneustes*) the phenomena of fertilization take on more or less completely the character of the second type

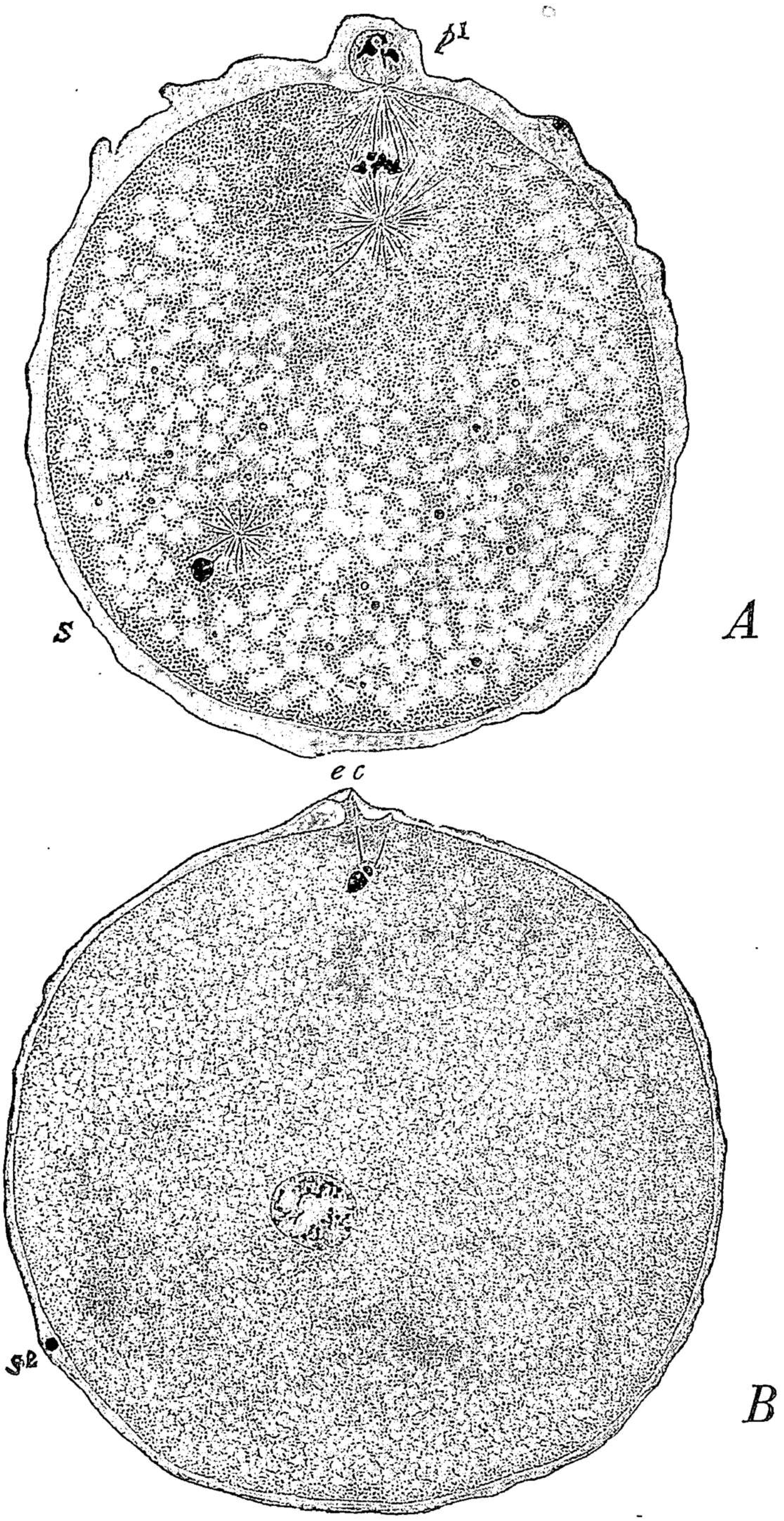

Fig. 188.—The *Ascaris* type and the sea-urchin type of fertilization compared.

A, egg of the worm *Thalassema* (*Ascaris* type) soon after insemination, surrounded by the fertilization-membrane; at p^1 the first polocyte and the second polar spindle. Near *s* the sperm-nucleus and sperm-aster, already completely rotated. Pseudoalveolar structure of the cytoplasm.

B, egg of the sea-urchin *Toxopneustes* about two minutes after insemination surrounded by the fertilization-membrane. Both polocytes have earlier been extruded and cast off. Below, the vesicular egg-nucleus; above the entrance-cone (*ec*) and below it the sperm-head and middle-piece beginning their rotation, with the first indications of the sperm-aster. At s^2 a second sperm that has not entered the egg.

(Fig. 209). It is, therefore, not surprising to find the two extremes connected in nature by a graded series of intermediate forms, due to the varying relation between the time of polocyte-formation and entrance of the sperm.[1] In certain extreme cases of this type the sperm appears to enter the egg at a much earlier period, even in the immature ovarian egg (oöcyte), there to remain in a quiescent state until the latter has completed its growth.[2]

Among the series of intermediate stages between the two extremes there are many forms in which the first polar spindle is fully formed and advances as far as the metaphase and then pauses until the sperm enters the egg, whereupon the polar divisions immediately proceed. This condition seems to be of rather widespread occurrence among invertebrates, typical examples being offered by the nemertine, *Cerebratulus*, the annelid *Chætopterus*, the mollusk *Dentalium* (Wilson) and various insects (Fig. 189).[3] In the annelid *Ophryotrocha* the first polar spindle may advance into the anaphase before entrance. In the next stage entrance of the sperm normally does not take place until after the first polocyte has been extruded from the egg. This condition has been found in some invertebrates and is widespread among the chordates.[4] In many of these cases (frog, mouse) the second spindle forms and proceeds to the metaphase before the sperm enters. In others this spindle may advance to the anaphase, as in *Siredon* (Fig. 189) or in the bat [5] but goes no further unless the sperm enters. One step more brings us to Type A, in which both polocytes must be extruded before fertilization, as in the sea-urchins, and in a number of cœlenterates (Boveri, Wulfert, Morgenstern). These various intermediate stages show at the time of karyogamy varying conditions of the gamete-nuclei and astral systems which in a general way run parallel to the relations between maturation and fertilization, though the correlation is not very exact.

That the eggs of different animals should display these various time-adjustments is an unexplained and curious fact; but they involve no essential differences in the main phenomena.[6]

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[1] For review of these and other cases, see Korschelt-Heider, '03, pp. 630 ff.

[2] Examples of this have been described in certain platodes (*Otomesostoma*, v. Hofsten, '09, *Brachycœlium*, Kemmitz, '13) and in the annelid *Saccocirrus*, Buchner, '14. Further study of these cases seems desirable.

[3] See Henking, '90, '91 (insects), Mead, '98, Lillie, '99 (*Unio*.), '06 (*Chætopterus*), Coe, '99, Wilson, '04, Yatsu, '09 (*Cerebratulus*), Wilson, '03 (*Dentalium*), etc.

[4] See Maas, '99 (sponges), Bigelow, '02 (barnacles), Sobotta, '95 (*Amphioxus*), Fick, '93 (Axolotl), O. Schultze, '87 (frog, salamander), Sobotta, '97 (mouse), etc.

[5] Schultze, '87 (Siredon), Van der Stricht, '02 (bat).

[6] As might be expected these various intermediate conditions seem in certain cases to vary somewhat in the same species. See Schultze ('87), Korschelt ('95), Bigelow ('07), In *Asterias*, O. Hertwig ('78) long ago found that when the sperm enters prior to the formation of the first polar spindle the fertilization approximates to Type B, the pronuclei being of equal size at the time of union, but if entrance be deferred until after the polar bodies are formed the pronuclei are very unequal, as in the first type. This is evidently in harmony with the results of etherization.

The physiological and cytological aspects of fertilization offer a series of problems of which the most important are: (1) the general conditions of fertilization; (2) the union of the gametes and the fertilization-reaction; (3) the history of the gamete-nuclei, and the origin of the chromosomes; (4) the origin and history of the sperm-center, cleavage-centers and the associated cytoplasmic structures, and (5) history of the mitochondrial formations.

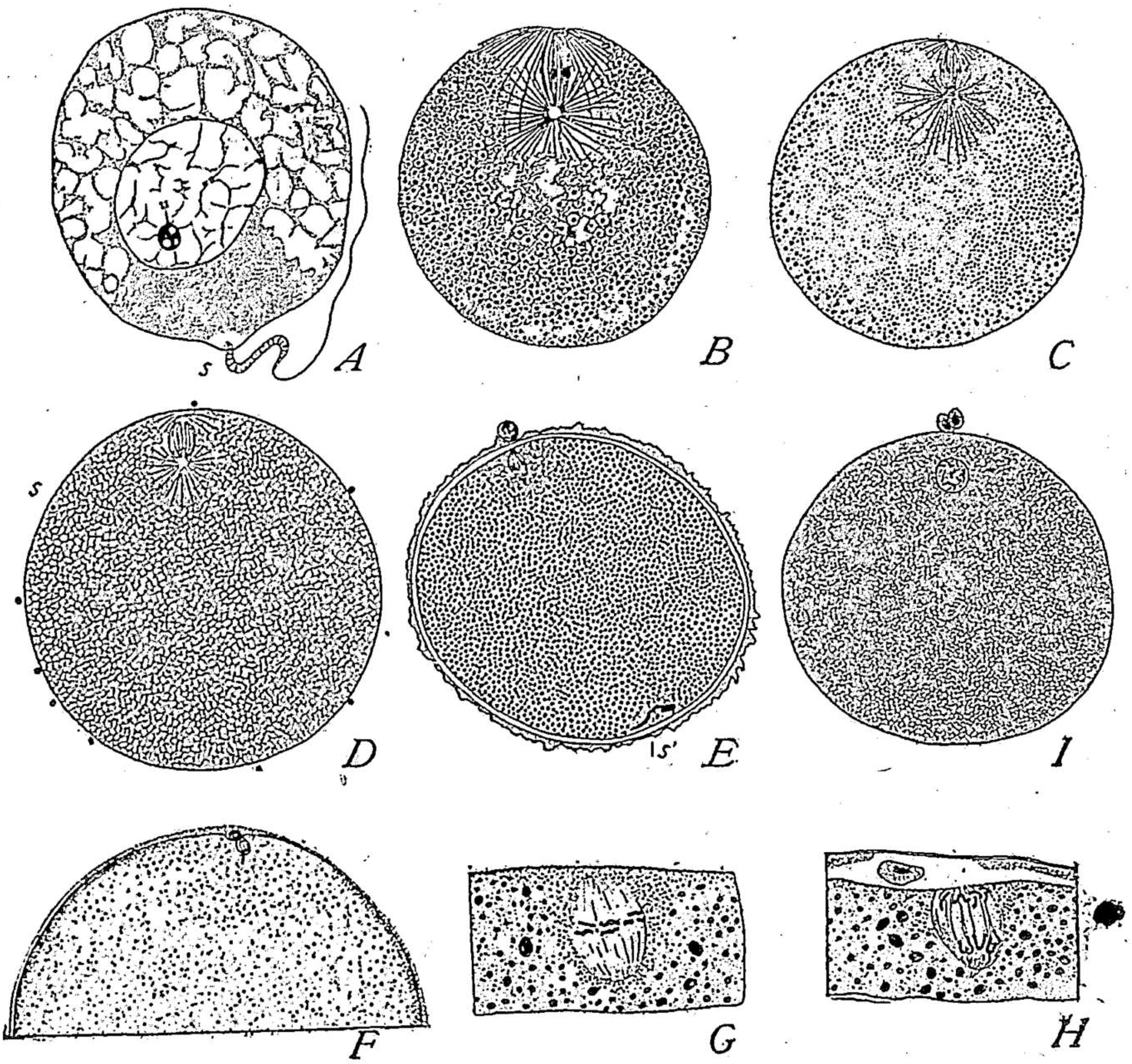

Fig. 189.—Different conditions of the egg when ready for fertilization.

A, the annelid *Myzostoma*, germinal vesicle intact, the sperm just entering egg (WHEELER); *B*, the annelid *Chætopterus*, first polar spindle in metaphase (LILLIE); *C*, the nemertine *Cerebratulus* (COE); *D*, the annelid *Ophryotrocha* (KORSCHELT); *E*, *Amphioxus* (SOBOTTA); *F*, the urodele *Siredon*; *G*, *H*, second polar spindles of same, enlarged, to show variations (O. SCHULTZE); *I*, the sea-urchin *Toxopneustes* (WILSON).

B. UNION OF THE GAMETES

1. General Conditions of Fertilization

Fertile union of the gametes only takes place when both have attained a certain physiological state of "maturity," (not to be confused with the result of "maturation" or meiosis). If insemination occurs at a period earlier than the normal the sperm either does not enter the egg or fails to initiate development. In the latter case many sperms may enter (*e. g.*, in the sea-urchin), but the nuclei do not penetrate far below the surface, do not enlarge, and sperm-asters do not make their appearance (O. and R. Hertwig, '87). Experiments especially on the eggs of echinoderms and nemertines have clearly proved that a condition of "cytoplasmic maturity" is necessary for the normal transformation of the sperm within the egg, and they indicate that this condition results from the setting free of certain substances from the nucleus. This conclusion was established by Delage ('99, '01) by cutting the individual eggs of sea-urchins and starfishes in two with the scalpel and fertilizing the fragments. When this operation is performed after breaking down of the germinal vesicle, both fragments of the egg, the nucleated and the non-nucleated, may be fertilized and undergo development. When the operation is performed before dissolution of the germinal vesicle the non-nucleated fragment cannot be fertilized. Similar results were subsequently obtained in the nemertine *Cerebratulus* (Wilson, '03). When the egg is cut in two before breaking down of the germinal vesicle and then fertilized, only the nucleated fragment develops (having first formed the polocytes); when the egg is cut after disappearance of the germinal vesicle both fragments develop, the cytoplasmic substance having become fertile.

This is in harmony with the results both of normal fertilization and of artificial parthenogenesis. Previous to maturation the sea-urchin egg is not only incapable of normal fertilization, but, as was shown by Morgan ('96, '99), it is also incapable of forming asters when chemically treated, a result which explains the failure of later observers to cause the immature eggs to undergo artificial parthenogenesis. This was experimentally tested in *Cerebratulus*, by Yatsu ('04, '05) who found that cytaster-formation is readily induced in enucleated fragments by treatment with $CaCl_2$ solution *if the egg be cut after fading of the germinal vesicle*, but in no case at an earlier period. There is reason to believe that this change affects especially the cortical layer of the egg; for egg-fragments or extra-ovates which lack this layer are incapable of fertilization.[1]

2. Approach of Egg and Sperm

We need not here 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 sperm are as a rule discharged into the water, where fertilization and development take place. Both egg and sperm may

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[1] Chambers, '19, '21; Just, '23.

live for a long time before discharge and for a considerable time afterwards without losing their capacity for fertilization; but this differs widely in different species. As a rule the sperms are motionless while within the testis and only begin to swim when acted upon by secretions of the ducts or their accessory glands, or when set free into the water. Their movement is ordinarily produced by whip-like lashings of the flagellum which drive the sperm onwards, head foremost, and usually in a spiral course. To this latter circumstance, probably, as shown by Ballowitz ('90) and by Buller ('02) is due the fact that when the sperm comes in contact with a solid surface it remains in contact with it, rotating rapidly in a constant direction.[1] It is noteworthy that *Nereis*, the insects and the sea-urchins should agree in the fact that the direction of rotation is anti-clockwise.

What brings the sperm and egg together is still imperfectly known. In plants it seems certain, both in case of the motile sperms of bryophytes and pteridophytes and the growing pollen-tubes of the gymnosperms and angiosperms, that the approach of the germ-cells is conditioned by chemical stimuli. Pfeffer's classical studies ('84) demonstrated that the free-swimming sperms (antherozoids) of ferns and mosses react positively to weak solutions of certain chemical substances, crowding about and entering the ends of capillary glass tubes containing such solutions as they do about the opening of the archegonia in nature; and this has been fully confirmed by later observers. In the case of ferns Pfeffer, followed by Buller ('00), found the most active substances to be the salts of malic acid (*e. g.*, sodium malate), but a number of other salts, both organic and inorganic, produce the same effect, though none are as active as the malates. Most of these substances (tartrates, oxalates, phosphates, nitrates, chlorides, etc.) are of common occurrence in the cell-sap of plants. Shibata ('05) reached similar results with the sperms of *Isoëtes*. On the other hand, the sperms of mosses were shown by Pfeffer to be indifferent to the compounds of malic acid, but react positively to solutions of cane-sugar. In *Fucus*, Strasburger believed he had evidence of chemotactic attraction; but this is not sustained by the more recent work of Bordet ('94) and Robbins ('16). In the seed-plants there is a considerable body of evidence to show that the direction of growth of the pollen-tube is a chemotropic phenomena, determined by substances set free by various tissues of the pistil and ovules.[2]

The facts thus briefly reviewed have been generally taken as proof of a positive chemotaxis between the egg and sperm; but the question remains whether this may not be interpreted as a form of "trap-action." In the

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[1] This phenomenon was discovered by Dewitz ('86) and since studied by Ballowitz, Buller, Lillie ('13) and others.

[2] Molisch, '89, '93, Miyoshi, '94, Lidforss, '95, Buller, '00, '02.

case of animal sperms, the evidence is conflicting. Most of the experiments on the question have seemed to give a negative result. Neither von Dungern ('01, '02) nor Buller ('00, '03), after careful studies, were able to find any satisfactory evidence of a directive reaction between egg and sperm, though von Dungern proved that the movements of the sperm may be accelerated or depressed by various substances. Yatsu ('09) found in *Cerebratulus* that the sperm, after completely traversing the thick membrane, may sometines be distinctly seen in the large peri-vitelline space, again entering the membrane from the inside instead of passing into the egg. Additional evidence was produced by Morgan ('04, '10), in his studies on cross-and self-fertilization in ascidians; and the conclusions of all these observers are in harmony with the earlier ones of Dewitz ('86) and Massart ('88, '89) which showed that the sperms of insects and frogs come into contact with the egg-envelope by accident, but having once done so remain in contact with it.

These results indicate that the sperm and egg are not brought together by an actual "attraction" between them as was once assumed. More recently the question has been reëxamined by De Meyer and F. R. Lillie. De Meyer found that the sperms of sea-urchins actively enter capillary tubes filled with egg-extract, while few or none enter similar tubes containing only sea-water. The extended experiments of Lillie ('13, '14, '15), were carried out by an adaptation of the method of Jennings in his well-known studies of chemotaxis among Protozoa. Drops of the substance to be tested were introduced by means of a capillary pipette into water containing numerous sperms in suspension. Drops of egg-extract, under these conditions, become surrounded by a multitude of sperms which penetrate the drop and form a double ring just within its margin, while such aggregation fails to occur in a drop of ordinary sea-water. A similar though less vigorous effect is produced by drops of weak solutions of CO_2 and of various acids (acetic, nitric, hydrochloric, sulphuric), while the sperms are indifferent to alkalis (KOH, NaOH) which have the same agglutinative effects upon them as have egg-extracts.

The facts in these experiments are perfectly clear; but they seem to offer nothing that is not explicable as a result of "trap-action," *i. e.*, that the sperms which accidentally enter the drop are unable to leave it. It seems probable, therefore, that the approach of egg and sperm is accidental and that no "attraction" between them exists. Sperms that enter the egg-envelopes or come into contact with the egg-periphery remain there; and as a rule no reaction between egg and sperm seems to take place save upon actual contact.[1] An exception to this is, however, offered by the starfish

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[1] This subject is closely connected with the block to self-fertilization in hermaphrodites and to the entrance of additional sperm after fertilization has taken place (p. 421).

(*Asterias*), as was long ago described by Fol ('79) and recently confirmed by Chambers ('23). Here the sperm first attaches itself to the outer surface of the thick gelatinous envelope of the egg, and passes thence slowly inward. Fol figured the cone, some time before contact with the sperm, drawing out into an almost filamentous form to meet the latter. Chambers finds the cone present when the sperm is still at the periphery of the jelly-layer, the two already connected by a long protoplasmic thread that appears to be spun out from the cone to meet the sperm, and later drags the latter inwards to the egg-periphery. Further observation will be necessary to determine the origin of the thread and the nature of its activities.

In many cases (*e. g.*, insects, fishes, cephalopods) the sperm reaches the egg through a definite micropyle, the point of entrance being thus predetermined or limited. When no micropyle is present, the sperm passes bodily through the substance of the membrane, and it is a singular fact that even in the presence of a micropyle the sperm may enter by traversing the membrane at some other point. This fact has been clearly established in the sea-urchin by Boveri ('01), where the gelatinous envelope of the egg is perforated by a very definite funnel-shaped micropyle at the upper pole (Fig. 512), yet the sperm may enter at any other point. Another striking example is the egg of the nemertine *Cerebratulus*, which is surrounded by a thick membrane drawn out at the lower pole into a hollow projection, in some cases at least open at the tip. The sperm does not, however, enter through the opening, but penetrates the membrane at any point (Coe, '99, C. B. Wilson, '99, E. B. Wilson, '03, Yatsu, '09). This fact is less anomalous than at first appears, for the micropyle-lobe of *Cerebratulus*, is merely the remains of a stalk of attachment in the ovary, as is also the case with the micropyle in the sea-urchin and in pelecypods and other mollusks; and such micropyles may never have played more than an accidental part in fertilization. The perforation of the egg-membrane by the sperm is a widespread phenomenon and probably represents a more primitive condition.

How the sperm penetrates the membrane is not clearly known. It was formerly supposed that the sperm, once attached, bores its way actively through the envelope; and Dewitz has suggested, in case of the insect egg, that the rotary movements of the sperm on the surface may enable the egg to find its way to the micropyle by which it enters. It is probable, however, that another, and more important factor lies in some physical action that causes the sperm to be drawn passively into and through the membrane, somewhat as is the case with the entrance of the sperm into the egg, as described beyond. Bataillon ('19) has observed that naked frogs' eggs, whether from the body-cavity or after dissolution of the jelly-envelope by the action of potassium cyanide, are incapable of fertilization by the sperm, though they may readily be activated to parthenogenetic development by the puncture-inoculation method (p. 474). Somehow, therefore, the envelope of the egg is in this case necessary for entrance of the sperm.[1]

The point at which the sperm enters varies widely in different cases. When entrance is effected through a micropyle the point is to that extent predetermined, but it must be borne in mind that several micropyles may be present. When single the micropyle is most commonly at or near one pole of the egg, *e. g.*, at the upper pole in the cephalopod (Fig. 113), in various fishes (Fig. 114), and in most insects (Fig. 112), at the lower pole in the pelecypods (Fig. 488). When the micropyle is absent or is not used for entrance the point of entrance may be indeterminate (*e. g.*, in sea-urchins), but more commonly is more or less definitely localized. In telolecithal eggs with a large amount of yolk (*e. g.*, birds, reptiles, elasmobranchs) entrance commonly takes place in the region of the upper pole; in smaller ova, such as those of platodes, mollusks, tunicates, or *Amphioxus*, the sperm more commonly enters in the lower hemisphere and often near the lower pole.[2] The relation is, however, too variable to be brought under any general rule.

It is not wholly certain whether the point of entrance (when constant) is predetermined by special structures in the cortical layer. A specially modified "receptive spot" in the neighborhood of the micropyle, or at the upper pole of the egg, has been described in many forms, *e. g.*, in insects (Henking, '91), and in *Petromyzon* (Herford, '96); and various receptive structures have been described as preëxisting at this point, such as the "attraction cone" of *Asterias* or the funnel-shaped depression described by Metschnikoff ('86) in *Mitrocoma*. Many of these cases need reëxamination, for it is now certainly extremely doubtful whether the entrance-cone is ever formed before attachment of the sperm to the egg or its envelope.

3. Reaction of the Egg. Entrance of the Sperm [3]

Contact of the sperm calls forth a powerful and almost instantaneous reaction by the egg that is responsible not only for entrance of the sperm, but also for many other changes in the oöplasm. This reaction first appears in the peripheral or cortical layer of the egg, which thus plays an essential

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[1] *Cf.* p. 421.

[2] See Wheeler ('97), Conklin ('05), Sobotta ('97).

[3] For modern reviews of the literature of this subject see especially Loeb ('13), Lillie ('19), Hyman ('23), Just ('12–'23).

part in fertilization; and in its absence the egg is unfertilizable.[1] The most obvious features of the reaction are: (1) the sudden throwing off of a *fertilization-membrane or activation-membrane*, which entirely surrounds the egg and separates from it to a considerable distance; and (2) by the sudden formation at the point of contact of a protoplasmic prominence, the *entrance cone* or *fertilization-cone* (Fig. 190), by which the sperm is swallowed up. These two events are so closely connected that they can hardly be separated in the description. They are often accompanied or followed by changes in the viscosity of the oöplasm and by active movements of its substance (p. 415).

a. The Cortical Changes. Loeb ('04, '13, etc.) has brought forward a large body of evidence to show that the cortical change is primarily a cytolytic or destructive process which is regarded by him as the first essential step in the activation of the egg, whether by the sperm or in artificial parthenogenesis, through its effect upon the oxidative-processes of the egg (p. 475). This particular conception of the cortical change has, however, met with considerable opposition on the part of some observers.[2] R. S. Lillie ('09, '15, etc.) has especially championed the view that its most im-

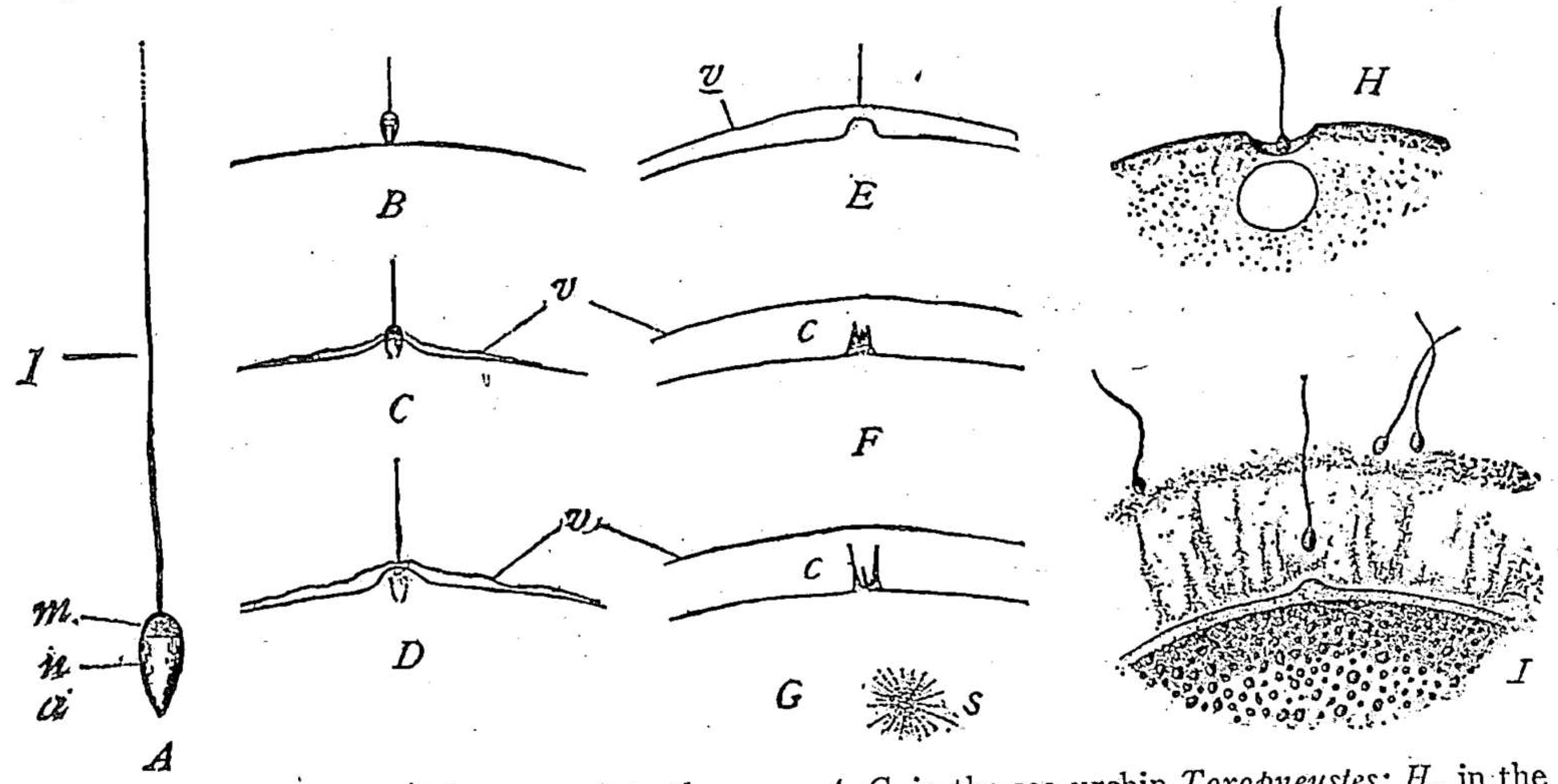

Fig. 190.—Entrance of the sperm into the egg. *A–G*, in the sea-urchin *Toxopneustes; H*, in the medusa *Mitrocoma* (Metschnikoff); *I*, in the star-fish *Asterias* (Fol).

A, sperm of *Toxopneustes*, × 2000; *a*, the acrosome; *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 fertilization membrane; *E, F*, later stages; *G*, appearance of the sperm-aster (*s*) about 3–5 minutes after contact; entrance-cone breaking up; *H*, entrance of the sperm into a preformed depression; *I*, approach of the sperm, showing a supposedly preformed entrance-cone.

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[1] See Chambers ('21a, 21b), Just ('23). By tearing the egg-cortex the endoplasm may flow out to form an extravate which may contain the nucleus and assume a spheroidal form. Such fragments are often devoid of a cortical layer, and are then unfertilizable. If, however, cortical material be present such a fragment may be fertilized and undergo cleavage.

[2] See especially F. R. Lillie ('19), Just ('20).

portant physiological effect is an increase in the permeability of the egg, thus altering the electrical equilibrium within the egg and initiating the process of mitosis (p. 191). That such a change of permeability occurs at the time of fertilization has been experimentally demonstrated by many observers, but we are here concerned more particularly with the cytological changes.

Whether the fertilization-membrane preëxists as such before fertilization is a long debated question concerning which the evidence now seems to weigh in favor of the affirmative. O. and R. Hertwig ('87) denied its preexistence, for the reason that if ripe eggs be shaken to pieces and sperm be added the egg-fragments, like entire eggs, throw off membranes on fertilization by sperm. More recently the same conclusion was reached by Harvey ('10, '14) and by Loeb, who on the basis of a series of studies (summed up in '13) believed the membrane to be formed immediately before it is thrown off as a result of a cytolysis of the cortical layer. On the other hand, Fol ('79) considered the membrane to preëxist in the form of a delicate "couche enveloppante"; and the same result was reached by Theel ('92) and by Herbst ('93) who found that by crushing the sea-urchin egg the contents might be pressed out, leaving a delicate membrane-like surface film behind. More recently Kite ('12, '13) found that the fertilization-membrane preëxists in the unfertilized egg as a surface-film of firm consistency forming a vitelline membrane which may be dissected off from the egg by the micro-dissection needle. A similar conclusion was reached on various grounds by F. R. Lillie ('11, '14), Glaser ('13), Heilbrunn ('15) and Chambers ('21).[1]

Though the foregoing observations show considerable differences of detail, it is now generally agreed that the fertilization-membrane is not a wholly new formation produced at the moment of fertilization, but probably is derived from a preëxisting vitelline membrane which itself arises by a direct transformation of the plasma-membrane or modified surface-layer of the oöplasm. It is usually thrown off very suddenly, and there is now a general agreement that its separation from the egg is due to a sudden accumulation of liquid between them (Fol '79). Some observers have ascribed the result to a sudden contraction of the egg by which liquid is extruded from it.[2] Were this the only explanation, however, the diameter of

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[1] Some observers have believed the fertilization-membrane to consist of two layers. Fol believed that in the sea-urchin only the peripheral layer is thrown off while the inner one remains to form the ectoplasmic layer (p. 413). A somewhat similar account is given by Brachet ('14). In the immature egg of *Amphioxus*, Sobotta ('97) described a delicate preëxisting vitelline membrane beneath which, upon discharge into the water, is formed a second and thicker membrane by direct transformation of the cortical oöplasm. If the egg remains unfertilized both membranes remain close to the egg; but immediately upon fertilization they spring out from the egg, fusing together to form a double "fertilization-membrane."

[2] See especially Bataillon, '00, '12, '14.

the unfertilized egg should be equal to that of the fertilized egg plus the perivitelline space; but as was long ago determined by Fol, Theel, Herbst and more recently by many others, such is not the case, the diameter of the sphere bounded by the fertilization-membrane being always greater and sometimes much greater than that of the unfertilized egg.[1] Clearly, therefore, the egg does not merely shrink away from the membrane but actually "throws it off." Fol considered this to result from a sudden imbibition of sea-water by a colloidal substance formed by the egg below the membrane; and such is also the conclusion of Loeb ('08, '13), who has demonstrated its correctness by interesting experiments.[2] Somewhat different from this is the process made known by F. R. Lillie ('11) in a careful study of the cortical changes in *Nereis*. Here, too, the unfertilized egg is surrounded by a delicate vitelline membrane, and within this is a "zona radiata," formed as a cortical alveolar layer with large, radially disposed alveoli. Immediately after attachment of the sperm the liquid contents of the cortical alveoli are discharged to the exterior, passing through the vitelline membrane, and swelling up outside it to form a thick layer of homogeneous jelly surrounding the egg. The zona radiata thus almost disappears as such, its remains, filled with liquid, now constituting a perivitelline space bounded peripherally by a delicate plasma-membrane lying just within the vitelline membrane (Fig. 191).

Considerable discussion has arisen, as to the behavior of the membrane as it separates from the egg. Fol ('77, '79) maintained that it rises from the surface of the egg in wave-like progress from the point of contact progressively around the egg (Fig. 190), and a similar account is given by many later observers.[3] Other observers, however, have failed to observe this and believe that the membrane rises simultaneously at all points, or at a number of separate points, around the periphery.[4] It is possible that both accounts are correct, or that the wave-like progress of elevation may, in some cases be too rapid to be readily observed. Loeb (*op. cit.*) has produced convincing evidence in favor of the second view by cooling the eggs (of sea-urchins) by which the process may be greatly slowed down for observation. It may then readily be seen that the membrane separates from the egg at

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[1] In *Sphærechinus granulosus* Herbst found the diameter of the egg itself, whether fertilized or unfertilized, to be 0.88–0.96 mm., while that of the fertilized egg, membrane included, is 1.20–1.28.

[2] Whether the egg undergoes a change of volume upon fertilization is still a disputed question. That the egg shrinks after fertilization has been maintained by Bataillon as above, by Glaser ('13) in *Arbacia* and *Paracentrotus*, and by some others, especially by Okkelberg ('14) whose measurements on the lamprey seem convincing. Many observers, however, have found little or no evidence of such shrinkage, *e. g.*, Calberla ('77), Loeb ('08), McClendon ('10) and Chambers ('21). Perhaps different species differ in this respect.

[3] See Calberla ('78), Theel ('92), Herbst ('93), Wilson ('96); and more recently Ries ('09), Okkelberg ('14), Just ('19), etc.

[4] Harvey ('10), Heilbrunn ('15), Loeb ('08, '13).

many points, thus forming vesicles or blister-like projections from the egg around its whole periphery. By fusion of these vesicles the egg becomes surrounded by a continuous perivitelline space, filled with liquid and bounded externally by the continuous and evenly rounded fertilization-membrane. This space is often very extensive, yet the egg does not (at least in the early stages of development) move freely within it. It is probable, therefore, as was long since concluded by Fol ('79), that the liquid within it is of jelly-like consistency and does not consist merely of water.

The throwing off of the membrane is followed, sometimes after a considerable interval, by the appearance of the hyaline or ectoplasmic layer immediately around the periphery of the egg (p. 261). Most later observers have considered it as a separate product of the cortical oöplasm, formed either as a jelly-like secretion or by a direct transformation of the peripheral oöplasm which first becomes free of granules and then more sharply marked off from the egg.[1] The part played by this layer in cell-division indicates that in any case it should be regarded as a peripheral zone of active cytoplasm rather than a true membrane.

b. The Entrance-cone. The formation of the entrance-cone, long since described by Fol ('79) in starfish and sea-urchins, has often been studied by later observers. In the sea-urchins it is formed very rapidly by a sudden rush of the peripheral protoplasm towards the point of contact, generally forming a conical prominence into which the head of the sperm almost instantly passes (Fig. 190). After the sperm has entered, the cone persists for a short time, then assumes a ragged flame-shape and finally breaks up. In the sea-urchins the entrance-cone certainly is not formed until the sperm touches the egg; but in *Asterias*, as above indicated (p. 408), the evidence seems to show that it is formed while the sperm is still outside the jelly-envelope at a considerable distance from the egg-periphery.

In *Nereis* (Lillie, '11), which offers peculiar advantages for the study of the process, the conspicuous entrance-cone is formed essentially in the same manner as in the sea-urchin and extends outwards through the cortical "zona radiata" until it comes in contact with the membrane at the point where the sperm is attached (Fig. 191). Within a few minutes the entrance cone is retracted, drawing the membrane down to form a depression in which the sperm-head lies. When the cortical zone narrows by discharge of the jelly-forming substance (p. 412) the entrance-cone is again prominent externally and so remains for 10–15 minutes, when it rather suddenly disappears into the egg.

c. Entrance of the Sperm. The sperm was formerly supposed to bore its way actively into the egg by lashing movements of the flagellum; but this

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[1] See especially Goldschmidt and Popoff ('08), Loeb ('08, '13), Painter ('18).

explanation, obviously inadmissible in the case of non-flagellated sperms, was undoubtedly erroneous. It is now generally agreed that movements of the flagellum quickly cease after attachment of the sperm to the egg, and that the egg plays an active part in the process of entrance by its instantaneous formation of the entrance-cone into which the head is as it were engulfed,[1] drawing the middle-piece and flagellum after it. An unrivaled

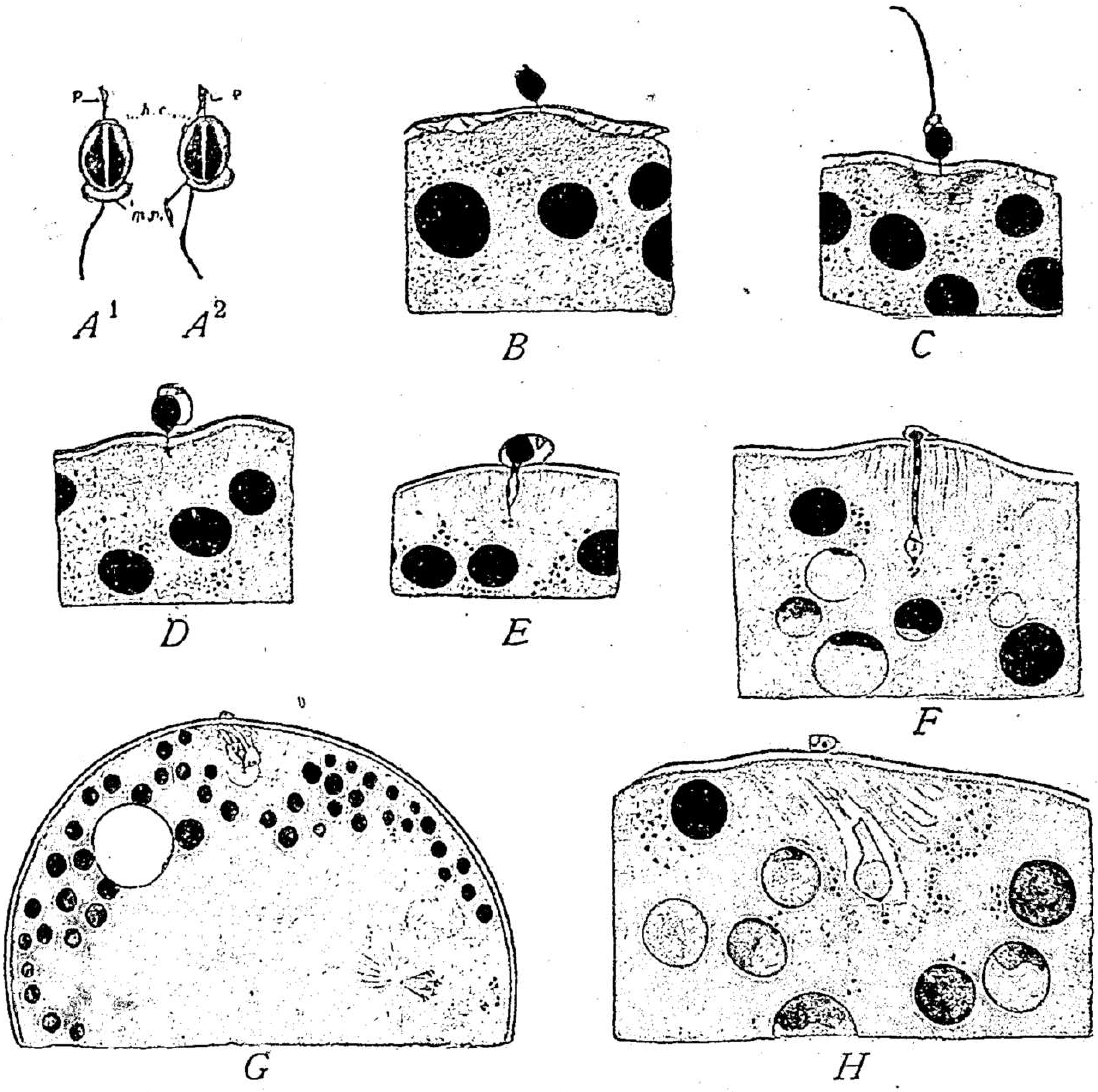

Fig. 191.—Fertilization in the annelid *Nereis* (F. Lillie).

A^1, A^2, sperm-heads, the latter from the side to show asymmetrical attachment of flagellum (*h, c,* "head-cap," *m, p,* middle-piece, *p,* acrosome); *B*, sperm-head attached to egg-periphery; *C–H*, successive stages of entrance of the head, leaving the middle-piece and flagellum outside. The elapsed time between *B* and *H* is from 40 to 50 minutes.

opportunity for study of this process is offered by the egg of *Nereis* where the sperm-head is not completely taken into the egg until forty or fifty minutes after its attachment. Lillie's important studies (*op. cit.*) showed that the sperm is fixed to the egg-periphery by the tip of the long and spike-like acrosome, which penetrates the plasma-membrane and in about fifteen minutes has entered the cortical layer of the egg, leaving the nucleus outside (Fig. 191, *B–D*), to enter the egg later. Throughout these and the

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[1] Kupfer and Benecke on the lamprey ('78). Fol on echinoderms ('79), etc.

later stages the sperm remains motionless and passively anchored to the entrance-cone. The head is now drawn into the egg in the form of a thick thread which perforates the vitelline membrane, and after its entrance enlarges to form a vesicle within the egg (Fig. 191), while *both the middle-piece and the flagellum are left outside.* In this respect *Nereis* at present remains a solitary exception. Certainly in most cases both middle-piece and flagellum are drawn into the egg after the head; for the entire sperm may often be seen after its entrance lying within the egg (Fig. 183). Another exception has been described in the sea-urchins, in which the earlier observers believed the flagellum, or its distal portion, to be left outside or to disintegrate in the remains of the entrance-cone;[1] it has more recently been asserted, however, that the flagellum enters the egg (Ries, '09). The case of *Nereis* conclusively demonstrates, however, that the flagellum is not necessary for the fertilization of the egg. More remarkable is the case described in the crab *Menippe* by Binford ('13), who could find no direct evidence that the nucleus enters the egg. He suggests, however, that the chromatin may go into solution, pass into the cytoplasmic capsule, and thus be carried into the egg. F. R. Lillie made the remarkable discovery that the sperm is still capable of fertilizing the egg after not only the tail and middle-piece but a portion of the head has been removed by centrifuging the eggs before the head has completely entered (p. 445). Still more remarkable is the result of Just ('22) who found that the egg may actually be fertilized by sperm that has been boiled[2] in oxalated sea-water (!) but the explanation of this is not yet apparent.

d. Movements and other Changes of the Oöplasm. The reaction of the egg to the sperm is shown in many other ways. The egg sometimes undergoes wave-like changes of form and the physical consistency of the oöplasm often alters. Active streaming movements of the oöplasm often take place, particularly in the cortical layer, and the relative distribution of protoplasm and yolk may undergo marked changes. For example, in pelagic fish-eggs a rush of the cortical oöplasm takes place towards the point where the sperm has entered, near the upper pole, and this gives rise to the germinal disc in which the blastoderm is formed (Agassiz and Whitman, '84). In the tunicate *Styela* (Fig. 524) the sperm, entering near the lower pole, calls forth a sudden down-flow of the cortical substance toward the entrance-point, and later a movement of this substance towards the posterior side of the egg (Conklin, '05). An interesting phenomenon is the formation of *polar rings*, described by Whitman ('78) in the eggs of leeches (*Clepsine*), by Vejdovský ('87) and by Foot ('96) in the naids (*Rhynchelmis*) and earth-

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[1] Fol, '79, Wilson, '95, etc.

[2] *Cf.* artificial parthenogenesis by puncture of the egg with a glass stylet (p. 474); also the phenomena of gynogenesis (p. 460).

worms (*Allolobophora*). In the first two of these cases the polar rings are formed as thickenings of the cortical layer in the polar regions which contract about the poles as they thicken, the polocytes being formed at the center of the upper ring (Fig. 192). Vejdovský's work has shown that the material of these rings has a definite prospective value in the development, giving rise in part to the teloblasts from which arises the main basis of the germ-bands (p. 1005). In *Allolobophora* the polar rings are believed by Foot to arise from a specific "archoplasm," originally scattered through the oöplasm. These movements of the oöplasm play a definite and essential part in the morphogenic process and are comparable to, perhaps are not separable from, those which take place as a consequence of maturation (p. 1005). From this point of view they are of the first importance in relation to the fundamental problems of prelocalization in the egg (p. 1062).

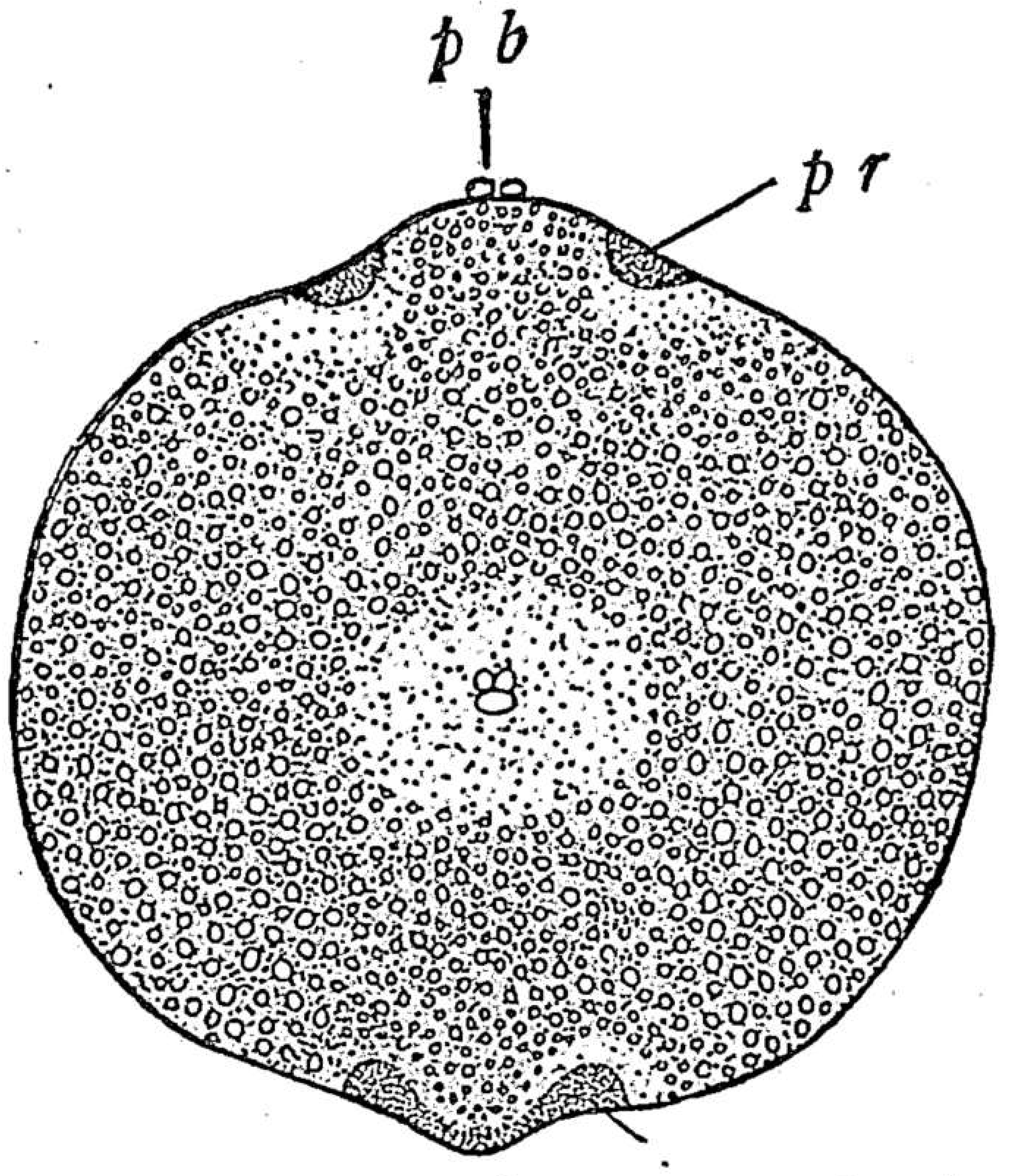

Fig. 192.—Egg of the leech *Clepsine*, during fertilization (WHITMAN).
p, *b*, polar bodies; *p*, *r*, polar rings; cleavage-nucleus near the center.

4. Monospermy, Dispermy, Polyspermy

Normal fertilization seems always to be accomplished by a single sperm, though more than one sperm may enter the egg. In this respect two well-marked types of eggs are to be distinguished. In the more frequent case fertilization is monospermic, the egg being normally adjusted for the entrance of a single sperm (echinoderms, nematodes, platodes, mollusks or mammals). The entrance of more than one sperm is in this case abnormal (*pathological polyspermy*) and nearly always leads to pathological or monstrous development. In a second type, mainly confined to eggs heavily laden with yolk (insects, elasmobranchs, amphibians, reptiles, birds), the egg is normally entered by several sperms (*physiological polyspermy*) [1] of which, however, only one is concerned in the further operations of syngamy.

Remarkable differences between these two types exist in respect to both the earlier and the later stages of development. In physiological polyspermy but one sperm-nucleus conjugates with the egg-nucleus (Figs. 194, 195) the

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[1] For review of the earlier literature see Rückert, '99. See Kupffer, '70 (urodeles, anura), Kupffer, and Benecke, '78 (*Petromyzon*), Fick, '92 (axolotl), Braus, '95 (*Triton*), Oppel '91–'92 (reptiles), Blochmann, '87, '89, Henking, '92 (insects), Rückert, '90, '91. Beard, '96, Sobotta, '96 (elasmobranchs), Harper, '04 (birds).

others sooner or later degenerating without conjugating either with the egg-nucleus or with one another. In some cases, the degeneration takes place very early (urodeles, insects). In others, as was first demonstrated by Rückert ('90, '92, '99) in the elasmobranchs, the supernumerary sperm-nuclei may enlarge, assume a vesicular form and divide repeatedly by mitosis, thus giving rise to numerous small nuclei (*merocytes*) that lie around the periphery of the early blastoderm and may there even imitate a kind of accessory cleavage, *e. g.*, in the pigeon's egg (Harper). In both these cases these nuclei divide with the reduced or haploid number of chromosomes, and seem ultimately to degenerate, without making any direct contribution to the embryo. Very different is the result of polyspermy in the normally monospermic egg. In these cases dispermy or polyspermy is sometimes merely accidental, owing to the fact that two or more sperms strike the egg at the

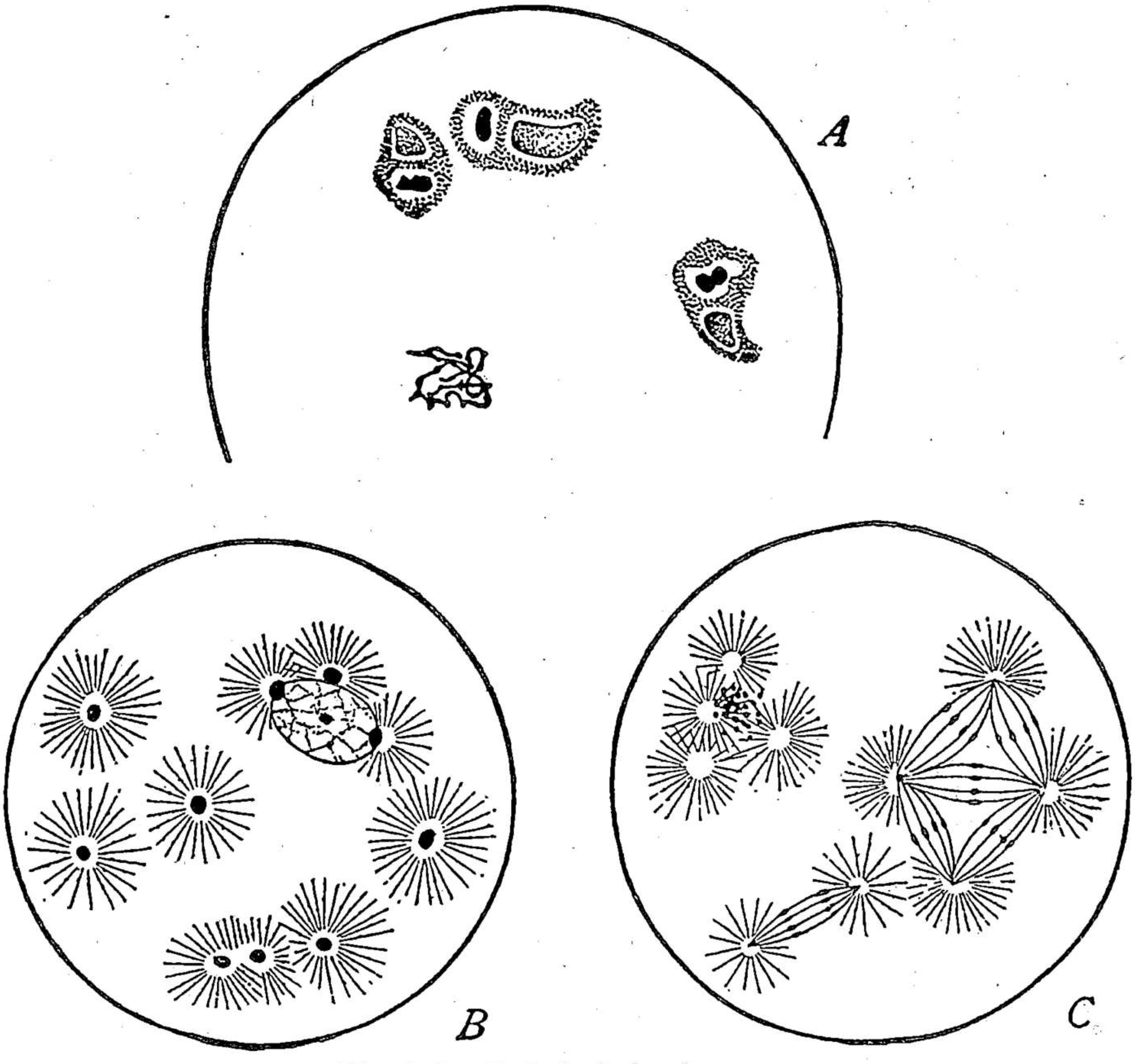

Fig. 193.—Pathological polyspermy.

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 amphiasters (O. and R. Hertwig).

same instant, a conclusion justified by the fact that dispermy and polyspermy is often readily produced experimentally merely by adding a very large excess of sperms to the eggs (sea-urchins, frogs). The same effect may, however, be produced as O. and R. Hertwig ('87) long ago showed in sea-urchin eggs, by subjecting them to the action of small quantities of nicotine, strychnine or morphine, to abnormally high temperature (31° C.), by allowing them to stand long in sea-water before sperm is added, or otherwise weakening their vitality.

In respect to the internal phenomena two cases are to be distinguished. In one of these, exemplified by the ascidian (Conklin, '00) or the frog

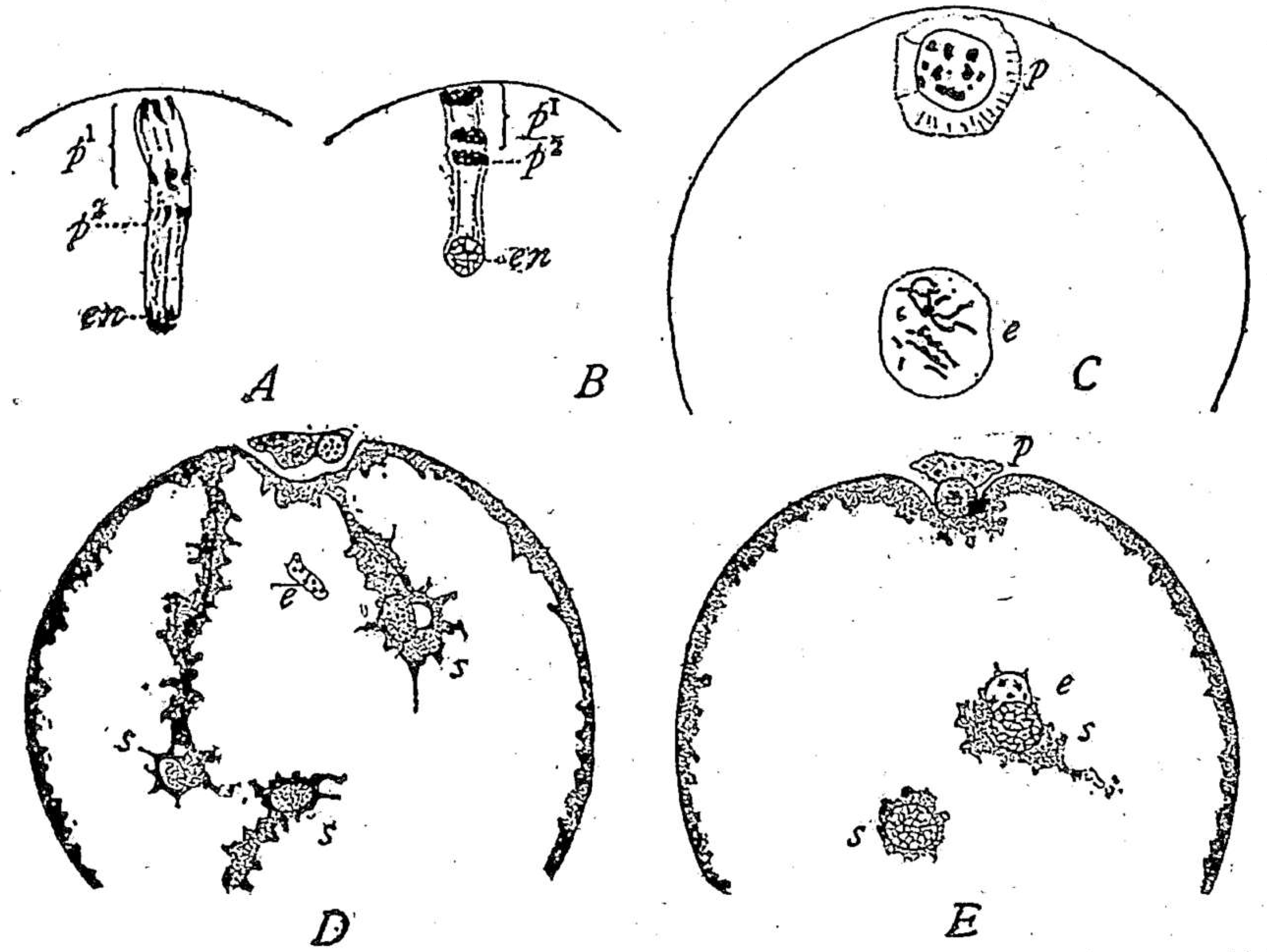

Fig. 194.—Formation of the polar nuclei and physiological polyspermy in insects; *A–C*, in the gall-fly *Rhodites*; *D*, *E*, in the bug *Pyrrchoris* (HENKING).

A, the second polar division in progress, in the egg; *B*, reconstruction of the egg-nucleus (*e*, *n*), three polar nuclei at p^1, p^2; *C*, polar fusion-nucleus (*p*) and egg-nucleus below; *D*, three sperms (*s*) in the egg, each lying in a granular sperm-track; egg-nucleus at *e*; *E*, conjugation of one sperm-nucleus with the egg-nucleus.

(Brachet, '10, Herlant, '11) but one sperm-nucleus conjugates with the egg-nucleus; in the second case two or more as in the sea-urchin (Fol, Hertwig, Boveri). In both cases development is abnormal for different reasons. In the ascidian dispermic or polyspermic eggs do not develop at all (Conklin, '05a). In the frog, as shown by Brachet and Herlant, a progressive cleavage may take place, each nucleus (including the fusion-nucleus) with ts attendant astral system giving rise to a separate bipolar mitotic figure in a manner that will be made clear from Fig. 195. The cleavage thus produced is more or less irregular, according to the number of sperms that have entered the egg, and many of the resulting blastomeres are binucleate. Owing, however, to the fact that the mitotic figures remain separate, each nucleus divides regularly and symmetrically with a bipolar spindle. A normal distribution of the chromosomes is effected at each mitosis; hence the resulting embryos contain nuclei of two kinds, diploid ones descended from the original fusion-nucleus, and haploid ones derived from the supernumerary sperm-nuclei. In this condition, no doubt, lies the explanation of the fact that the resulting embryos or larvæ are abnormal in various degrees and incapable of complete normal development. Herlant nevertheless determined the surprising fact that dispermic and even trispermic eggs may exhibit a development which up to a certain point is outwardly nearly normal. The dispermic egg develops its normal plane of symmetry, gastrulates and gives rise to a tadpole, one of which lived for three months; but all such larvæ, without exception, perish before reaching maturity.

In a third type of pathological polyspermy, exemplified by the sea-urchin egg, two or more sperm-nuclei conjugate with the egg-nucleus to form a triploid or polyploid fusion-nucleus, while other sperm-nuclei may remain separate in the protoplasm (as in the frog, the sperm-nuclei do not conjugate with one another). Such eggs undergo a multipolar cleavage, the nature of which is best studied in dispermic eggs, where both sperm-nuclei conjugate with the egg-nucleus. Such eggs usually produce a quadripolar or tripolar spindle (Fig. 430) and divide at once into four or three cells. When a large number of sperms enter the egg the resulting spindles do not remain separate (as in the frog) but unite to form all kinds of multipolar spindles (Fig. 193). In all these cases the chromosomes are distributed at random, and almost always irregularly, to the three, four or more poles. The resulting nuclei, therefore, receive varying numbers or combinations of chromosomes. After the initial tripolar or quadripolar division, the dispermic eggs continue to segment by bipolar mitosis, and often produce free-swimming larvæ; but in the great majority of cases development sooner or later becomes abnormal or monstrous and death ensues. Boveri has demonstrated by a brilliant experimental analysis ('02, '07) that the pathological effect is here due not to the abnormal chromosome-numbers but to their *false combinations*. Decisive proof is thus given of the qualitative differences of the chromosomes (p. 916).

It is an interesting question how the entrance of supernumerary spermatozoa is prevented in normal monospermic fertilization. In the case of echinoderm eggs, Fol suggested that it is accomplished mechanically by means of the vitelline membrane formed instantly after the first spermatozoön touches the egg. Immature eggs, before the formation of the polar bodies, have no power to form a vitelline membrane, and the spermatozoa always enter them in considerable numbers. O. and R. Hertwig found that in polyspermy induced by poisons the vitelline membrane is only slowly formed, so that several spermatozoa have time to enter. The fertilization-membrane seems in fact to be impermeable to the sperms; but it is now certain that this is not the only block to penetration of the sperm. It was long since shown by Driesch that the fertilization-membranes may be removed by shaking the fertilized eggs before cleavage; and such eggs cannot again be fertilized, *i. e.*, cannot be rendered dispermic or polyspermic. The writer found ('03) that if the egg of *Cerebratulus* be cut in two shortly after the entrance of the sperm and additional sperm be added, only one fragment develops (presumably that which contains the sperm-nucleus) while the other extrudes the polocytes but is unable to develop. If the same experiment be performed prior to fertilization both fragments, as earlier stated, develop in case the germinal vesicle has previously broken down, while only one develops if the germinal vesicle be still intact (p. 405). In all these cases alike the fragment lacks the protection of a fertilization-membrane (since in the first case the fragments have fresh-cut surfaces). Hence the failure of the fertilized fragment to development is not due to such protection but to some change in the oöplasm due to fertilization. To restate the facts in this case: the egg passes through two critical periods, the first

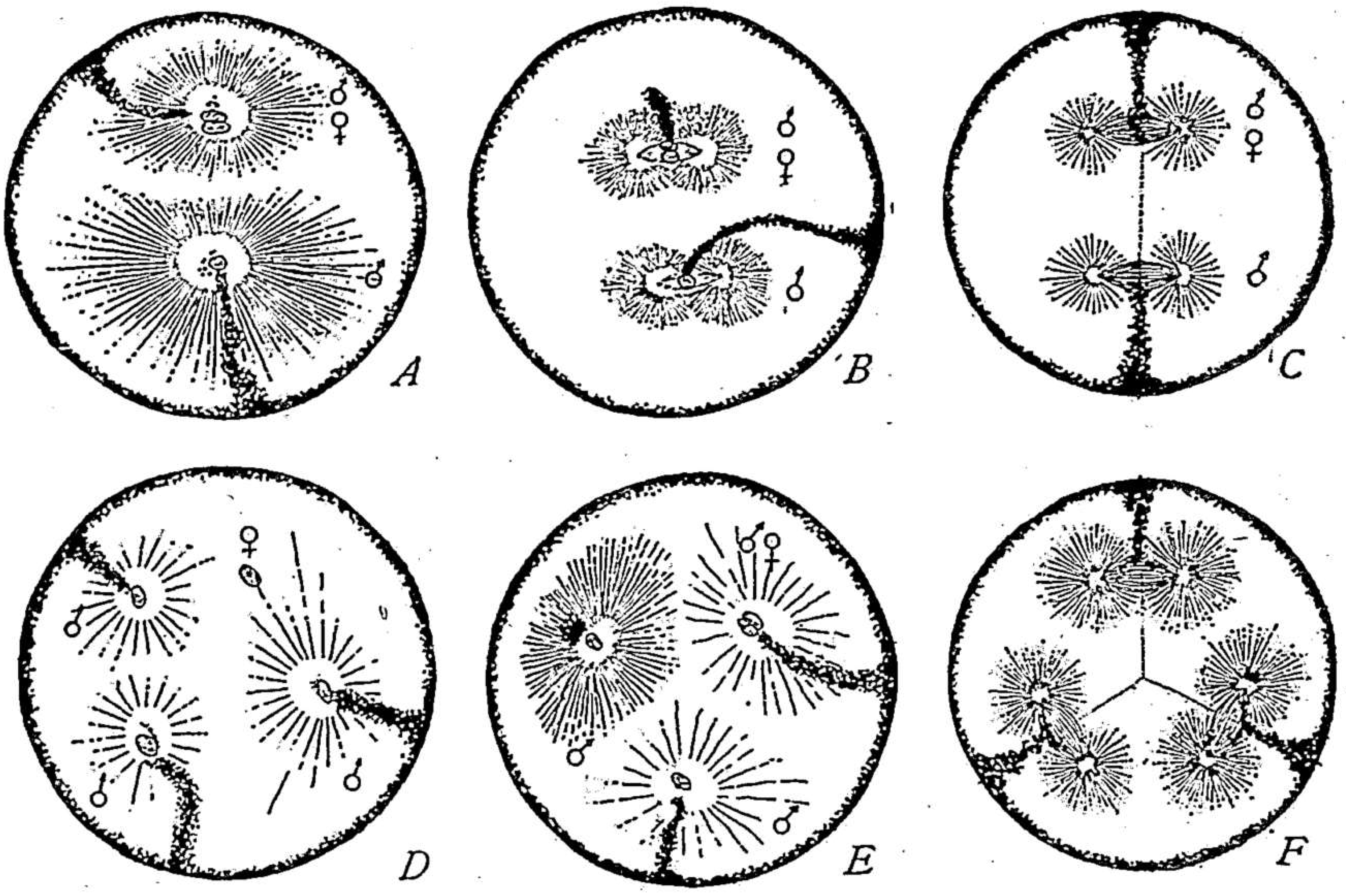

Fig. 195.—Dispermic and trispermic frog's eggs, from reconstruction of sections. The lines of black granules show the paths of the sperms in the egg (Herlant).

A, *B*, *C*, dispermic eggs, in *A* and *B* one sperm-nucleus conjugating with the egg-nucleus above; *D*, *E*, *F* trispermic eggs; in *D* egg-nucleus at ♀; *E*, *F*, in each case one sperm-nucleus only conjugates with the egg-nucleus.

marked by the breakdown of the germinal vesicle and the consequent liberation of some substance into the oöplasm, the second by the entrance of the sperm. The first of these events renders the oöplasm fertilizable, the second establishes some kind of block that renders it once more infertilizable.[1]

An interesting further demonstration of this is offered by Just ('19) who found in *Echinarachnius* that as soon as the sperm-head begins to enter the egg the oöplasm becomes impermeable to other sperms even at points where the raising of the membrane has not yet taken place, a "wave of negativity" sweeping around the egg-periphery from the point of entrance towards the opposite pole in advance of the membrane-formation. A similar, though less simple result is obtained by causing the egg to throw off an activation-membrane by treatment with butyric acid or similar agents (p. 475), then rupturing or removing the membranes by shaking the eggs, and finally adding sperm. Such eggs, as shown especially by Moore ('16) and Just ('20), *if the butyric activation has been of a certain optimum degree*, have wholly lost the capacity for fertilization by the sperms. The latter may indeed enter the egg-periphery, but remain inert, "like foreign bodies" (Moore). On the other hand, eggs either underexposed or overexposed to the membrane-producing agent are still capable of fertilization by sperm and of subsequent development, though often abnormal, particularly in the case of overexposure.[2] The reaction thus shows a quantitative relation, its optimum being represented by normal fertilization, which causes total immunity of the oöplasm to action of the sperm.[3]

F. R. Lillie [4] has devoted an interesting series of works to establish the conclusion that the fertilizable condition is due to the presence of a soluble colloidal substance, *fertilizin*, which in some sense forms a chemical link between the egg and sperm. This substance is characterized by its agglutinating effect upon the sperm, and may thus readily be detected in the sea-water. By means of experimental tests thus made possible it has been shown that fertilizin is first produced at the time the germinal vesicle breaks down; that its formation proceeds actively for some time after maturation; and that it wholly ceases with fertilization or parthenogenetic activation.[5]

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[1] Wilson, '03, p. 419.

[2] Thus, apparently, are explained the earlier results of Herbst ('06, '12) and of Loeb ('13) who found that complete development might take place in eggs treated as above. The work of Moore makes it probable that in these cases the eggs did not receive the optimum exposure.

[3] An interesting nearly related question is why in many hermaphroditic organisms the egg cannot be fertilized by sperm from the same individual, though perfectly fertilizable by sperms from other individuals. Morgan ('23) has recently shown that in ascidians the block to self-fertilization is removed if the egg be freed from its membranes; apparently, therefore, it must lie in the membrane or structures associated with it (follicle-cells, test-cells, or their products). *Cf.* p. 586.

[4] Lillie, '13, '14, '15, '19, etc. See also in support of these conclusions Just, '15, '19, etc.

[5] Lillie found also that in *Nereis* the egg-exudate poured forth upon contact of the first sperm paralyzes the movements of the sperm and thus prevents their approach to the egg.

The substances set free into the oöplasm at the first critical period may therefore include fertilizin or its chemical antecedents. Since fertilizin may be extracted by sea-water an explanation is here offered of the fact noted by many observers that the fertilizing capacity of the egg, commonly diminishes progressively, and sometimes very rapidly, if the eggs lie unfertilized in the water.[1] The total disappearance of fertilizin at the second critical period (upon activation) results, as plausibly assumed by Lillie, from a neutralization or binding of the fertilizin by some other substance brought in or activated by the sperm. These conclusions should, perhaps, not be taken too literally; but they have the great merit of opening the way to exact experimental studies of the problem on its physiological side.[2]

History of the Pronuclei

1. General

From the standpoint of heredity the central fact of syngamy is the *equivalence* of the gamete-nuclei. This does not mean that these nuclei are absolutely identical; both cytology and genetics have proved that such is often not the case. It means only that they, or their products, play approximately *corresponding* parts in development, as appears from the fact that in the main the offspring inherit equally from both parents. Morphologically, this equivalence is made visible to us in the fact that during the process of fertilization the gamete-nuclei undergo parallel changes, which in the end renders them nearly or quite indistinguishable. As a final result of these changes they give rise to corresponding, and in most cases almost identical, groups of chromosomes, the number of each being haploid or half that of the diploid (somatic) number characteristic of the tissue-cells. This remarkable fact (p. 426) has been designated as Van Beneden's Law (O. Hertwig). The only known exceptions to it (except in hybrids) are given by the sex-chromosomes and supernumerary chromosomes, and these are of such a nature as only to give additional weight to the conclusion.

2. Movements and Paths of the Pronuclei

Since the time of Van Beneden's early work on fertilization in the rabbit (1875) the sperm-nucleus and egg-nucleus within the fertilized egg have commonly been designated as "male" and "female" "pronuclei" respectively,[3] though by many authors they have been called *germ-nuclei* (O. Hertwig), or simply sperm-nucleus and egg-nucleus.

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[1] See Lillie, '19, Just, '15, with further references.

[2] For more critical discussions see Loeb ('13) and especially Lillie ('19) with literature-lists.

[3] These nuclei are not "male" and "female" but paternal and maternal; and they differ from the somatic nuclei only in the haploid number of chromosomes.

After extrusion of the second polocyte a haploid group of chromosomes is left in the egg from which the egg-nucleus is built up. This commonly takes place by the formation of a group of chromosomal vesicles or karyomeres, as was long since observed by Bütschli ('70) in the eggs of gasteropods and nematodes and has since been described in a great variety of animals. In nearly all cases these fuse progressively to form a single vesicular nucleus with which the sperm-nucleus conjugates; but in a few cases (*e. g.*, in certain trematodes) karyogamy occurs before these vesicles have completely fused. The history of the sperm-nucleus in the egg differs from that of the egg-nucleus in that its starting-point is a greatly condensed and almost homogeneous solid mass; but in many cases this, too, gives rise to a vesicular nucleus of the ordinary type before union. As already indicated (p. 401) in the *Ascaris* type of fertilization this nucleus finally becomes precisely similar in appearance to the egg-nucleus, and is indistinguishable from it except by its position.

The rotation of the sperm-head, already referred to (p. 396), is a very widespread if not universal phenomenon. It begins almost immediately after entrance of the sperm and is quickly completed as the sperm advances —in *Toxopneustes* the whole process is completed within two or three minutes from the time of insemination. Nothing is known as to the causes or physiological significance of this process. Its effect is to reverse the original position of the sperm-head and the middle-piece, the nucleus thus coming to lie with its base turned inward with the middle-piece lying in front of it. As a result of this the sperm-aster, which is sooner or later developed in the neighborhood of the middle-piece (sometimes already during the rotation) also typically lies in front of the sperm-nucleus and leads the way in the march towards the egg-nucleus (Figs. 184, 207).[1]

Both pronuclei now move through the protoplasm to their meeting-point, the direction and the extent of the movements varying widely in different species and within certain limits even in the same species. In general, the movement of the egg-nucleus is less extensive than that of the sperm-nucleus; but the paths of both pronuclei vary both with the point of entrance of the sperm and the position of the egg-nucleus at the time of insemination. The egg-nucleus, it is true, often lies eccentrically towards, and sometimes very near to, the upper pole; but Boveri's studies ('02) on *Paracentrotus* prove that in this sea-urchin at least the egg-nucleus, after formation of the polocytes, may wander to almost any point in the egg.

The first accurate study of the paths of the pronuclei was made by Roux ('87, '85) in case of the frog, where the course of the sperm is marked

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[1] The rotation of the sperm-head seems first to have been definitely described by Flemming ('81) and has since been studied by many observers.

by a trail of pigment-granules carried in from the periphery as the sperm advances (Fig. 195). This study, which in its essential results has been confirmed by many subsequent studies on other animals, showed that the track of the sperm-nucleus is typically curved, and may be resolved into

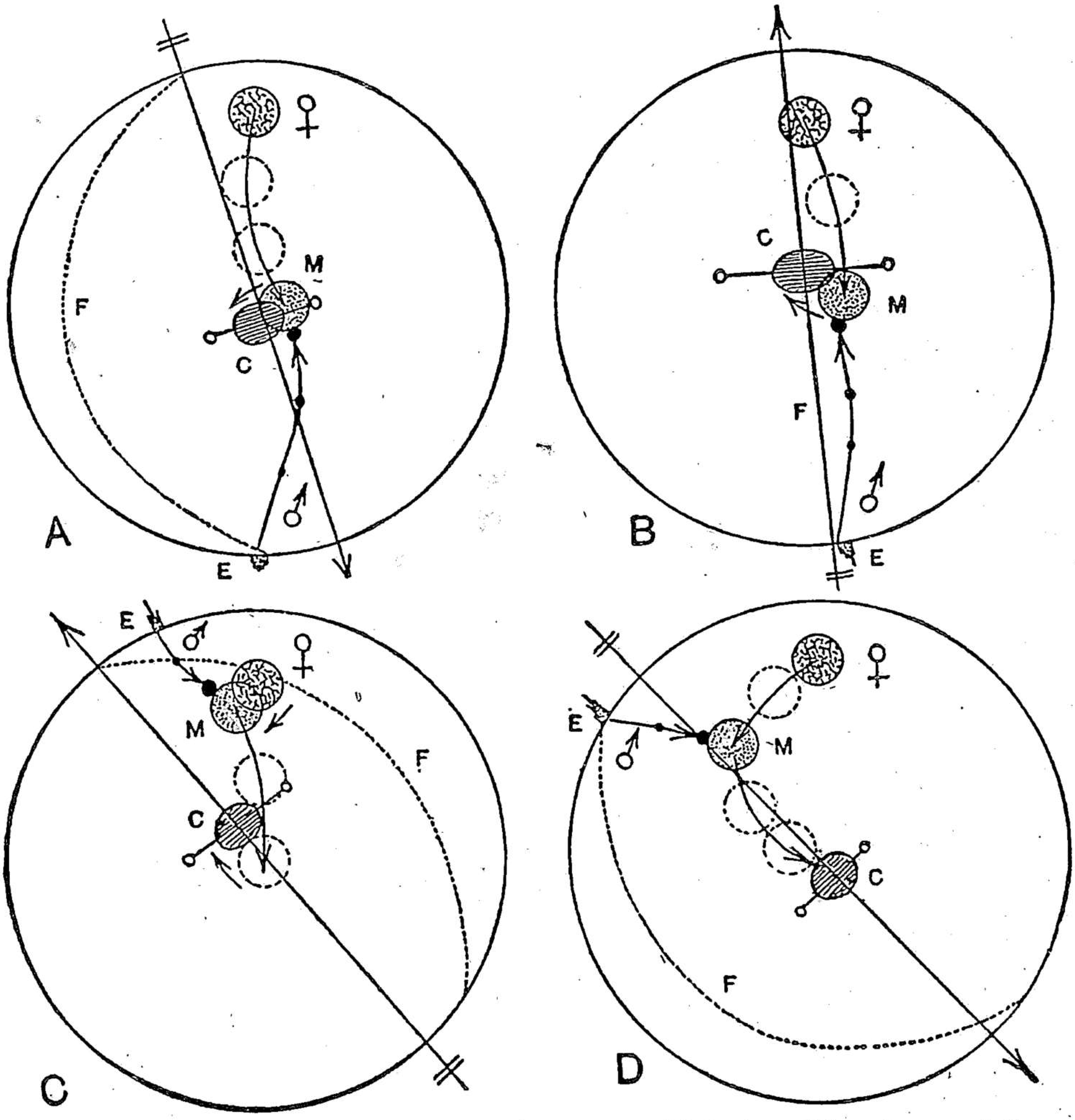

Fig. 196.—Diagrams showing the paths of the pronuclei in four different eggs of the sea-urchin *Toxopneustes*. From camera drawings of the transparent living eggs.

In all the figures the original position of the egg-nucleus (reticulated) is shown at ♀; the point where the sperm 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 towards the upper pole. Plane of first cleavage, passing near the entrance-point, shown by the curved dotted line.

two components, a *penetration-path,* nearly vertical to the surface, and a *copulation-path* along which the sperm-nucleus moves towards the point of union with the egg-nucleus. These two paths often form a considerable angle. To these may be added a third, the *cleavage-path,* along which the united nuclei move together to the definitive position of the first cleavage-nucleus. These facts are well illustrated in the sea-urchin egg (Fig. 196), where the egg-nucleus at first occupies an eccentric position near the point at which the polar bodies are formed but later (before fertilization) may wander to any position. Entering 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 center of the egg. After penetrating a 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 amœboid changes of form as it advances through the cytoplasm. From the meeting-point the apposed nuclei move slowly along the cleavage-path to their final position, which is always in the egg-axis and slightly eccentric towards the animal pole. In this respect the sea-urchin is typical of a large number of animals, though the eccentricity is often much greater than in the sea-urchin.

In the tunicate *Styela*, where Conklin ('05) has carefully studied the phenomena, the egg-nucleus typically lies at first near the upper pole and the sperm enters near the lower pole. After its penetration the sperm-nucleus moves along nearly parallel to the surface towards the posterior side of the egg, while the egg-nucleus moves towards the same region. Their union takes place in the posterior region of the egg, not far from the equator, after which the two move in conjunction to the usual position in the egg-axis, not far from the upper pole (Fig. 524).

The causes that determine the movements of the pronuclei during fertilization of the egg are unknown. It was assumed by some of the earlier observers that approach and union of the nuclei were determined by some kind of attraction between them;[1] but this assumption is very insecurely based and the same may be said of the assumption that they are passively drawn together by the rays of the sperm-aster or by protoplasmic currents in the oöplasm (Conklin, '94, '99). Lillie has suggested that "as both sperm-nucleus and egg-nucleus are in physiological relation to the same mass of cytoplasm which is preparing to divide, they must reach the same position of equilibrium within the cell, and hence of necessity meet."[2] This, however, is contrary to the observed facts in the sea-urchin egg[3] where it is easy to see that the meeting-point of the pronuclei rarely if ever coincides with the

[1] See Wilson, '00, p. 204.

[2] '19, p. 65.

[3] See Wilson, in Wilson and Mathews, '95.

ultimate position of the fusion-nucleus and the cleavage-amphiaster and is sometimes far from it. It is readily seen, also, that the paths show no fixed predetermination but vary with the relation between the entrance-point and the position of the egg-nucleus. It seems clear also that the penetration-path is not affected by the position of the egg-nucleus, but is due to some relation between the sperm and the oöplasm—a conclusion further supported by the fact that in merogony (p. 465) the sperm penetrates an enucleated egg or egg-fragment.

The copulation-path, on the other hand, is clearly influenced by some relation between the pronuclei (Fig. 196 D). This is indicated by the fact that in cases of dispermy in sea-urchin eggs if one of the sperm-nuclei is the first to meet the egg-nucleus the movement of the second is immediately retarded;[1] and of similar significance is the fact that in cases of physiological polyspermy but one sperm-nucleus, as a rule, conjugates with the egg-nucleus. The nature of that relation is unknown; and nothing seems to be gained by the assumption of an actual attraction between the pronuclei. On the other hand, the movement of the fusion-nucleus to its final position (cleavage-path) evidently accomplishes somehow an equilibrium in the cell-system; but the nature of this, too, is unknown. The fact that the cleavage-nucleus takes up a definite position with respect to the egg-axis and the poles of the egg proves that the movements of this nucleus are accurately correlated with the organization of the egg as a whole; for the polarity of the egg, as Boveri clearly proved, is predetermined before fertilization and cleavage (p. 1067).

3. Conjugation of the Pronuclei. General History of the Chromosomes.

a. Karyogamy and the Chromosomes. Van Beneden's Law. As early as 1881, Mark clearly showed that in the slug *Limax*, the two pronuclei merely come into contact without actual fusing. Van Beneden in his epoch-making work on *Ascaris* ('83–'84) demonstrated that the pronuclei not only fail to fuse but give rise to two separate groups of chromosomes which enter the equatorial plate and there independently divide, their products passing separately into the daughter-nuclei (Fig. 197). Van Beneden thus first established the fact that the number of chromosomes contributed by each pronucleus is the haploid number—in this particular case two (*Ascaris megalocephla bivalens*) or one (*A. megalocephla univalens*).[2] This result was soon after extended by Carnoy ('87) to other nematodes, and the subject was more broadly studied in a striking paper by Boveri ('90) who showed

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[1] Wilson, '96.

[2] This case is atypical in that these chromosomes are compound bodies which later break up into a larger number of small chromosomes. *Cf.* p. 323.

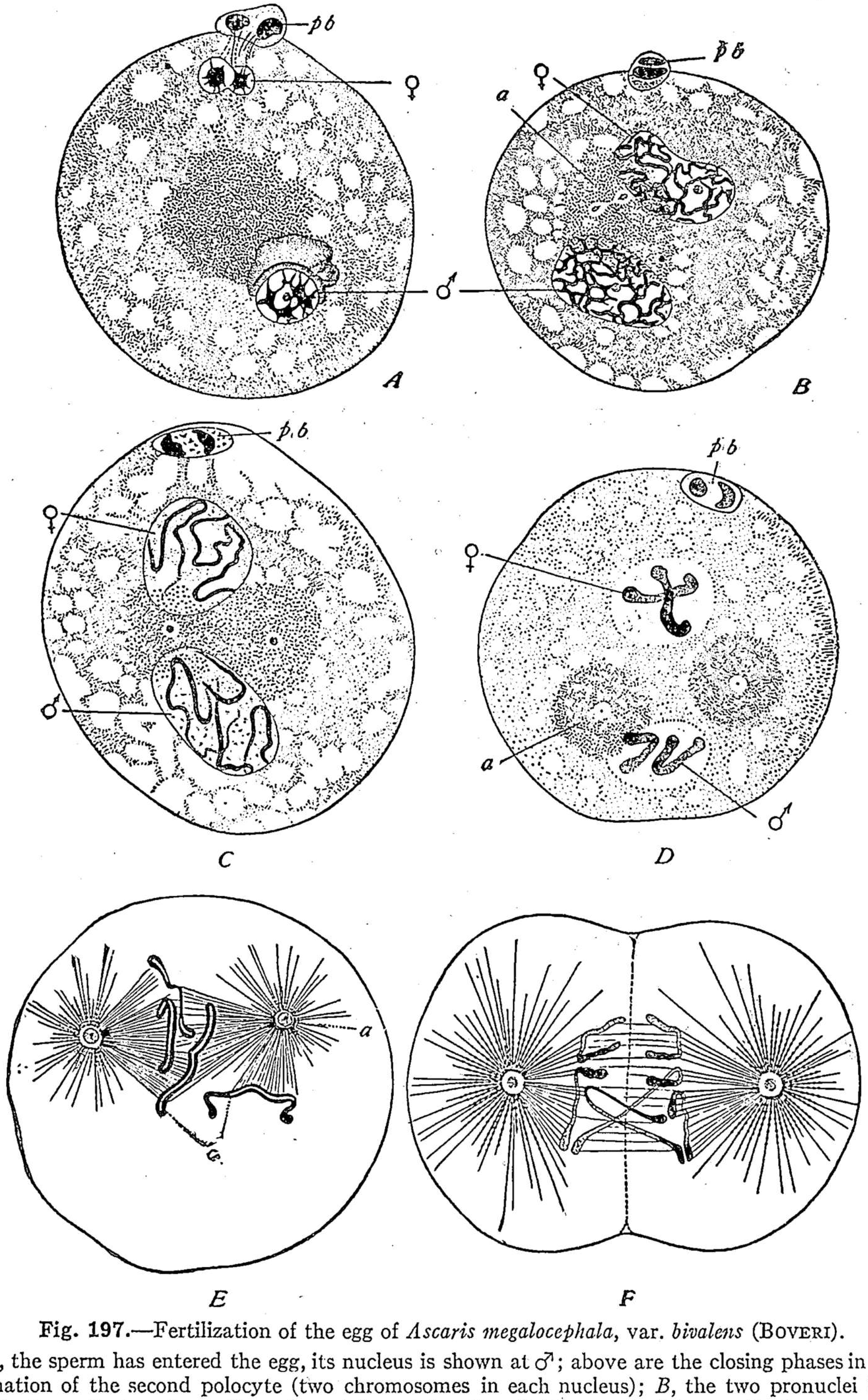

Fig. 197.—Fertilization of the egg of *Ascaris megalocephala*, var. *bivalens* (BOVERI).

A, the sperm has entered the egg, its nucleus is shown at ♂; above are the closing phases in the formation of the second polocyte (two chromosomes in each nucleus); *B*, the two pronuclei (♀, ♂) in the reticular stage; the sphere (*a*) contains the dividing central body; *C*, chromosomes forming in the pronuclei; the central body divided; *D*, each pronucleus resolved into two chromosomes; 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).

that the number from each pronucleus is 9 in the sea-urchin, *Echinus*, the worm *Sagitta* and the tunicate *Ascidia;* 14 in the medusa *Tiara;* and 16 in

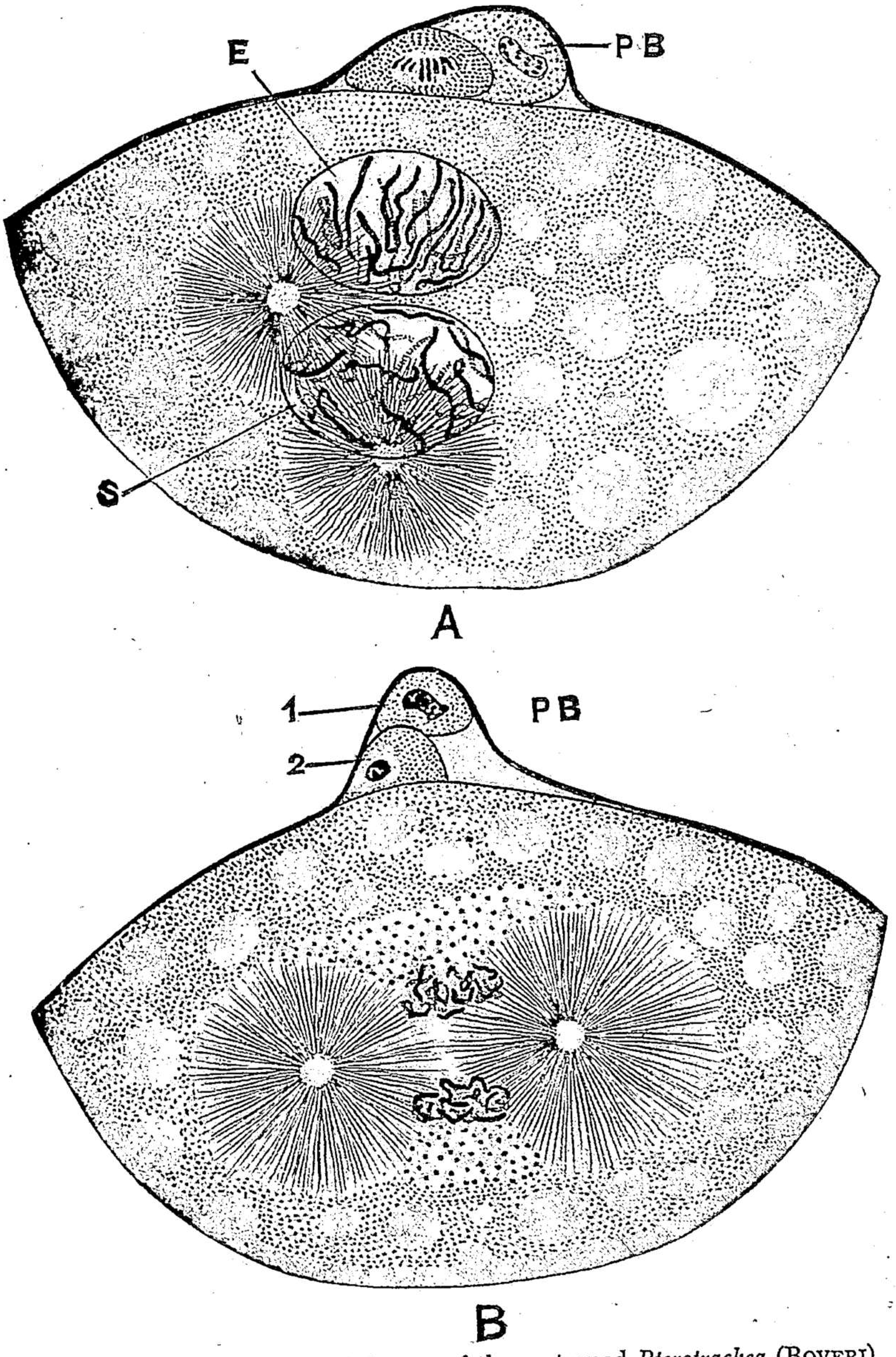

Fig. 198.—Fertilization of the egg of the gastropod *Pterotrachea* (Boveri).

A, the egg-nucleus *E*, and sperm-nucleus *S*, approaching after formation of the polocytes; the latter shown above (*P*, *B*); each pronucleus contains sixteen chromosomes; the sperm-amphiaster fully developed; *B*, later prophase of the first cleavage.

the gastropods *Pterotrachea* (Fig. 198), *Carinaria* and *Phyllirhoë*. Similar results have since been found in a large number of animals and plants.

Even when the pronuclei seem to fuse into a single cleavage-nucleus before formation of the chromosomes the validity of Van Beneden's law is established by numerous experimental and other data. Before reviewing this evidence we may briefly recall the various conditions observed in the gamete-nuclei at the time of their approach and union.

In the *Ascaris* type, as already described (p. 401) the pronuclei are at this time large, vesicular nuclei of the ordinary type, indistinguishable from each other save by their position. This condition, described by many of the early students of the subject, was first fully analyzed in detail in *Ascaris* by Van Beneden ('84, '87), whose epoch-making discoveries were confirmed and further extended by Boveri's early work on the same object ('88). It is now known to be of very wide occurrence. In some of these cases the pronuclei fuse while still in the reticular condition to form a single synkaryon (fusion-nucleus or cleavage-nucleus); and this may occur exceptionally even in *Ascaris*. On the other hand, such fusion is the typical process in many animals. This condition graduates almost insensibly into one in which no actual fusion takes place. In some forms the nuclei come together when in the spireme stage (*Triton*, *Pristiurus*); in others the chromosomes are still in the form of rather long threads (*Pterotrachea*, *Sagitta*); in still others they have nearly assumed their final form (*Ascaris*, *Ciona*). In some of the trematodes (*Polystomum*) the germ-nuclei at or shortly before the time of union are in the form of separate chromosomal vesicles or *karyomerites*.[1] Here the karyomerites formed after expulsion of the second polocytes, persist as such, while the sperm-nucleus is transformed into a corresponding group. As already indicated (p. 423), this is a common mode of formation of the egg-nucleus, and a more or less similar transformation of the sperm-nucleus has been observed in a number of cases;[2] but in most cases this condition is followed by the vesicular one.

That Van Beneden's law holds for all these various cases is demonstrated by a large body of evidence drawn from many sources, some of which may briefly be summarized as follows:

(1) The chromosome-number that appears in the first cleavage of the zygote is always the sum of the haploid numbers received by the gamete-nuclei in meiosis.

(2) In hybrids between forms having different chromosome-numbers the zygotic number is always (with certain exceptions that emphasize the rule) equal to the sum of the two parental haploid numbers (p. 841).

(3) An egg deprived of its nucleus, or an enucleated egg-fragment fer-

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[1] See Goldschmidt, '02.

[2] *E. g.*, in *Physa* (Kostanecki and Wierzejski, '96), *Prosthecereus* (Klinckowström, '97), *Thysanozoön* (Van der Stricht, '98), *Allolobophora* (Foot and Strobell, '00).

tilized by a sperm (*merogony*), segments with the haploid number of chromosomes (p. 465).

(4) Conversely, an egg activated by some other agent than the sperm develops with the haploid number of chromosomes (haploid parthenogenesis) unless the number be increased by a compensatory process (p. 477).

(5) A dispermic egg (in sperms normally monospermic) begins its development with the triploid number of chromosomes (p. 920).

(6) If the pronuclei be prevented from union by any cause, as in "partial fertilization" (p. 458), after etherization (p. 447) or in physiological polyspermy (p. 417), each produces the haploid number of chromosomes.

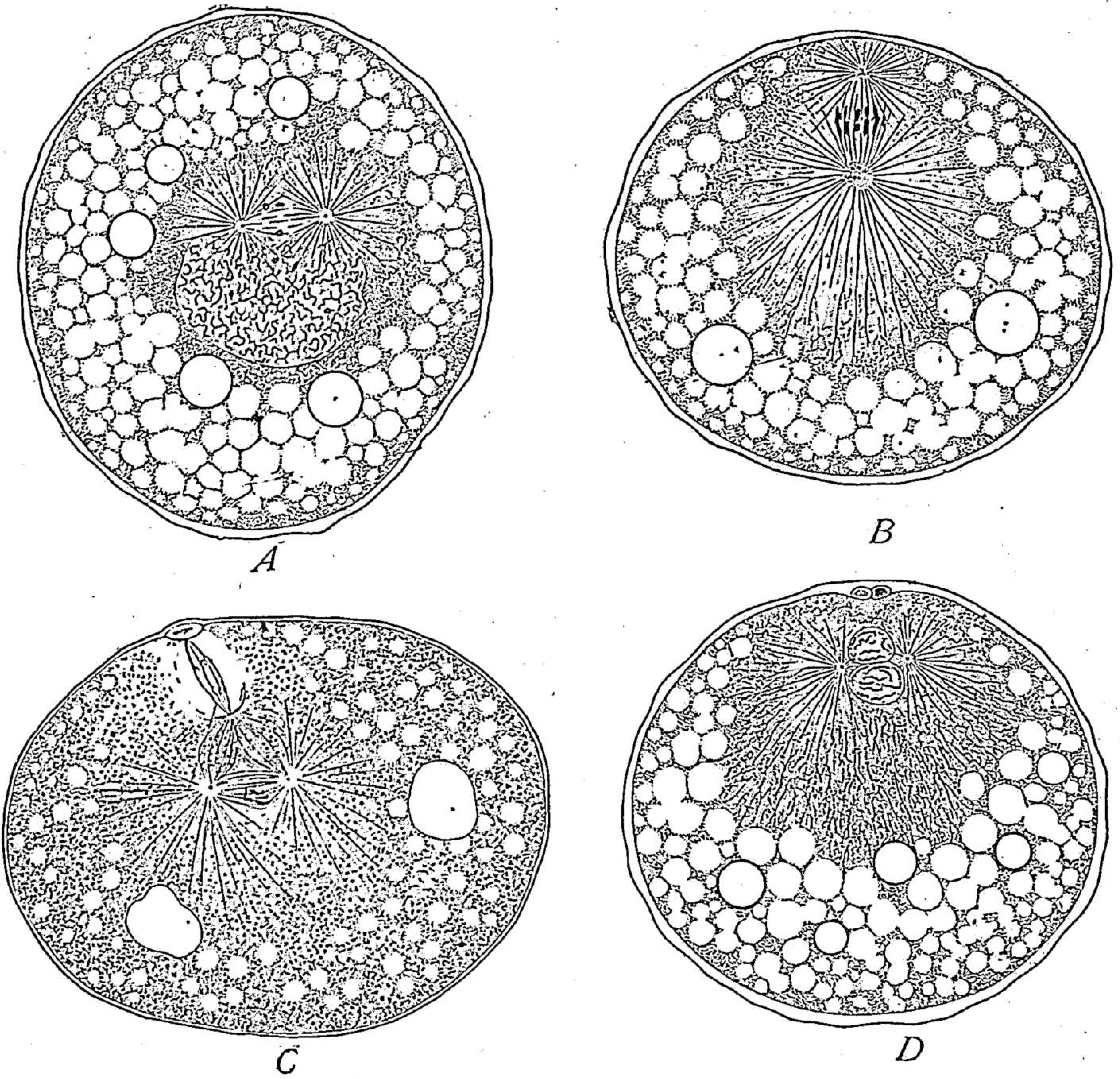

Fig. 199.—Fertilization in *Nereis* (*C* from LILLIE).

A, egg shortly after entrance of sperm, in early prophase of the first polar mitosis; *B*, metaphase of first polar mitosis; *C*, anaphase of second polar mitosis; below, sperm-nucleus and amphiaster, the latter connected with inner polar center; *D*, karyogamy.

(7) If one of the pronuclei be destroyed or rendered impotent, the remaining one gives rise to the haploid number of chromosomes, and development may proceed with that number, as in gynogenesis (p. 460) or androgenesis (p. 464).

The foregoing cumulative evidence affords a complete demonstration of Van Beneden's law and likewise contributes to the proof that the chromosomes behave as if they were independent individuals which do not lose their identity from generation to generation but conform to the general law of genetic continuity by division. To this important subject we shall return in Chapter XI.

b. Cleavage and the Chromosomes. The Theory of Gonomery. Since the chromosomes of the fertilized egg are derived equally from the two gamete-nuclei, they are traceable equally to the two parents; and since each of these chromosomes (as in any other mitosis) splits into identical halves that are transported to opposite poles of the spindle during the first cleavage,[1] it follows that the primary diploid nucleus of the fertilized egg is exactly duplicated in the two daughter-nuclei. The latter therefore are built up from two haploid chromosome-groups that are respectively of maternal and paternal origin.

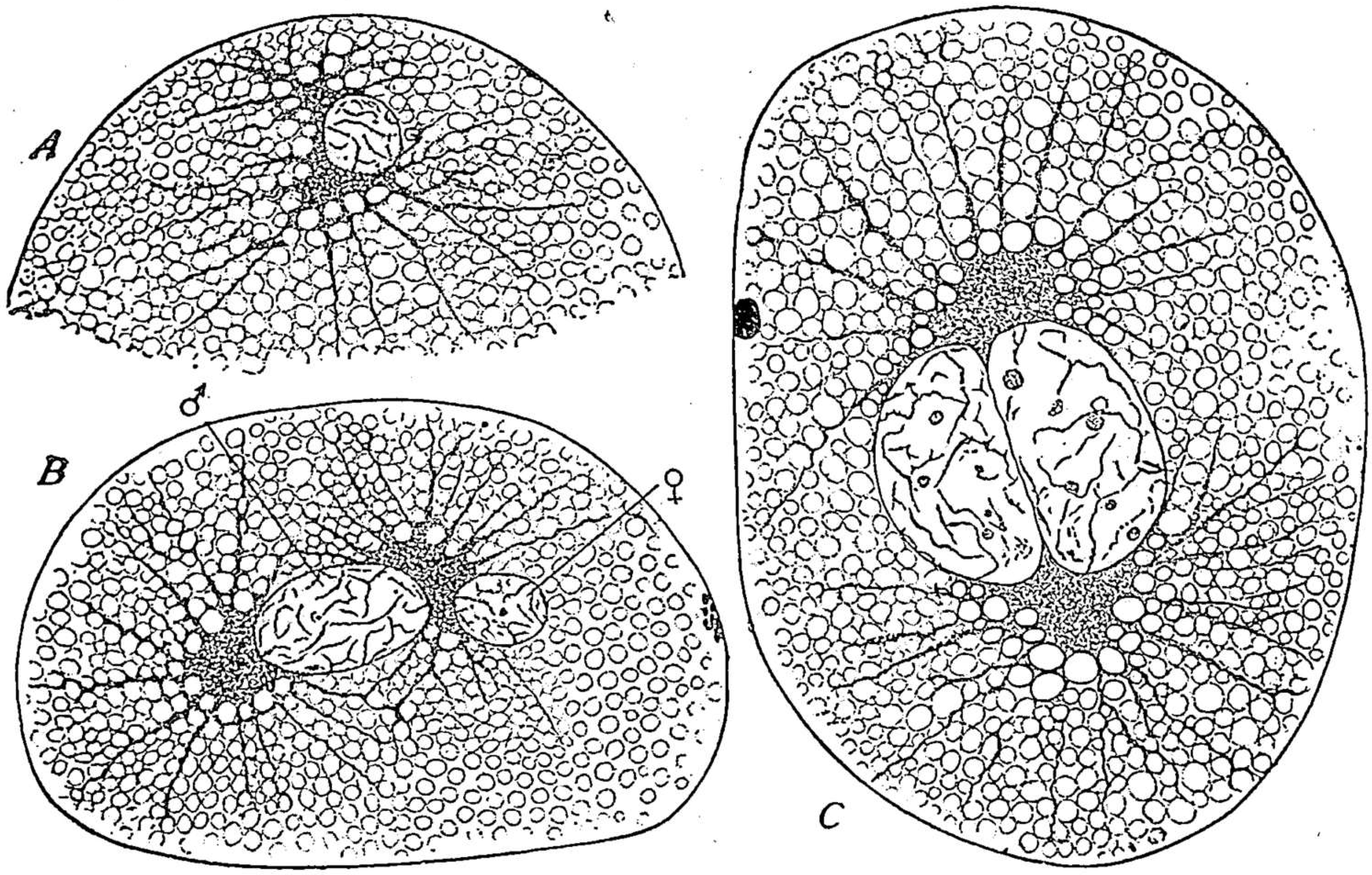

Fig. 200.—Fertilization of the egg in the copepod *Cyclops strenuus* (Rückert). *A*, sperm-nucleus soon after entrance, the sperm-aster dividing; *B*, the gamete-nuclei approaching; ♂, the enlarged sperm-nucleus with a large aster at each pole; ♀, the egg-nucleus reformed after formation of the second polar body, shown at the right; *C*, the apposed reticular pronuclei, now of equal size; the spindle is immediately afterwards developed between the two enormous sperm-asters; polar body at the left.

From the genetic standpoint, evidently, this fact constitutes the central

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[1] That the chromosomes are already longitudinally split in the pronuclei at the time of their union, was first demonstrated in *Ascaris*, by Van Beneden ('83–'84), whose results were confirmed by Boveri and later observers.

phenomenon in fertilization. Since the result of every normal mitosis is an exact duplication of the diploid mother-nucleus in the daughter-nuclei, it comes to pass that every nucleus of the embryo and finally of the adult is a dual structure to which the two gamete-nuclei have made equal contributions;[1] and here, evidently, is offered the basis for a physical explanation of the fact that no detail in any part of the offspring is too small to be beyond the influence of both original germ-cells.

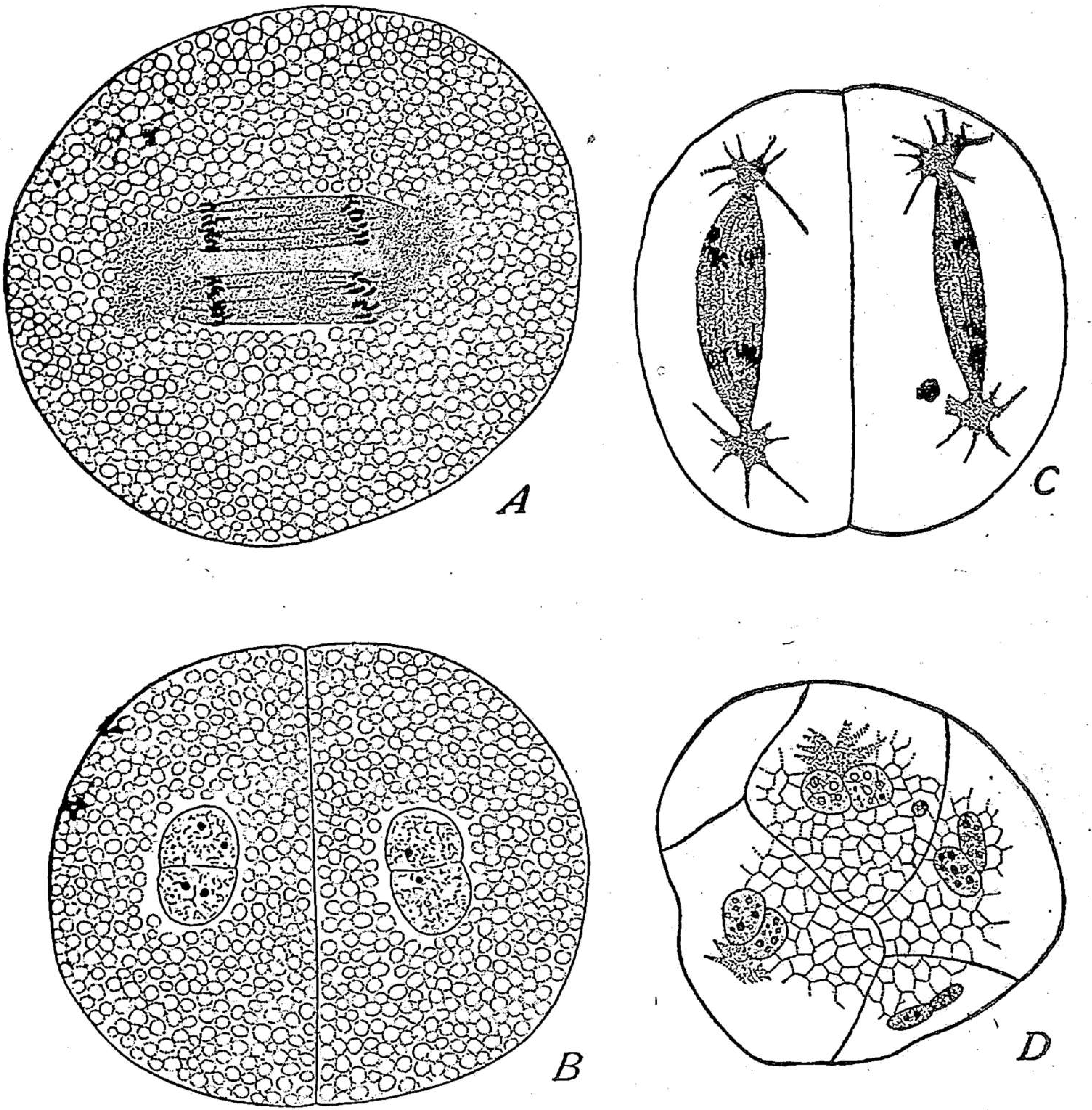

Fig. 201.—Independence of paternal and maternal chromatin in the segmenting eggs of *Cyclops* (*A–C*, from Rückert; *D*, from Häcker).

A, first cleavage-figure in *C. strenuus;* complete independence of paternal and maternal chromosomes; *B*, resulting two-cell stage with double nuclei; *C*, second cleavage; chromosomes still in double groups; *D*, blastomeres with double nuclei from the eight-cell stage of *C. brevicornis.*

Van Beneden's demonstration of the independence of these two groups during the whole of the first cleavage in *Ascaris* soon led to attempts to

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[1] Here again a reservation must be made in respect to the sex-chromosomes (p. 751).

trace them as such into later stages. It was shown by Haecker ('92b) and by Rückert ('95c,) that in certain copepods of the genus *Cyclops* the two groups not only remain distinct during the first cleavage, but give rise to *double nuclei* consisting of two distinct though closely united components. These observers also found, that from these double or bilobed nuclei arise in each cell double spiremes and double chromosome-groups which again divide separately and give rise to double nuclei in the 4-cell and 8-cell stages; double nuclei were often observed in later cleavage stages even up to the time when the germ-layers were being formed (Fig. 201).

Out of these observations grew the conception of *gonomery, i. e.*, that the maternal and paternal chromosomes might remain in separate groups throughout life, even though inclosed in a common nuclear membrane. Haecker and Rückert believed that distinct evidences of such a duality could be discerned even in the germinal vesicle of the young ovum, indicating that the original gonomery had been maintained throughout the whole cycle of individual life, from egg to egg. Some evidence in favor of this conception has in fact been obtained. Conklin ('01, '04) found in the early development of the gasteropod *Crepidula* conditions closely analogous to those described in the copepods. The double character of the nuclei is here most clearly seen in the telophases and is usually partially lost in the resting-stage, though still indicated by a groove on one side of the nucleus in which the central spindle for the following mitosis appears. It is also indicated by the presence of two nucleoli in the "resting-nuclei," while each gamete-nucleus has but one (Fig. 202). By these indications Conklin traced the double character of the nuclei in all the cleavages up to the 29-cell stage, and in some cases to the 60-cell stage. Beard ('02) likewise found double nuclei in the embryonic stages of *Raja batis;* and more recently Bertram Smith ('19) has found in *Cryptobranchus* that all the nuclei are distinctly double from the 2-cell stage throughout the cleavage, and are still recognizable as late as the gastrula.

It has been conclusively demonstrated that the theory of gonomery is at best of limited application, double nuclei appearing only in certain cases, and only in the earlier stages of development. When the chromosomes show conspicuous and constant differences of size (a common condition in both plants and animals), they do not, in vastly the greater number of cases, show any trace of gonomeric grouping, nor are the nuclei externally double (see, for instance, Figs. 394, 395, 396). Conclusive proof of this is offered by hybrids in which the parental types of chromosomes can be distinguished by the eye. In *Menidia-Fundulus* hybrids (Fig. 400) Moenkhaus clearly showed that the two parental *groups* remain distinct as such only during the first two or three cleavages, the two kinds of chromosomes being thereafter intermingled indiscriminately. Substantially the same result is given by the later work of Morris ('14) on the fish-hybrids *Fundulus* × *Ctenolabrus*, and that of Harrison and Doncaster ('14) on moth-hybrids (Fig. 405). Again, in the Diptera the work of Stevens ('08), Metz ('14–'22), and others has conclusively established the fact that from an early stage in the ontogeny all or most of the chromosomes are arranged in pairs, each one of which consists recognizably of a maternal and a paternal descendant (Fig. 396). This, obviously, is quite incompatible with gonomery; and the chromosomes show traces of such a paired arrangement in a number of other animals and plants (p. 837).

It appears, therefore, that gonomery, though occasionally to some extent recognizable, represents no more than a tendency on the part of the chromosomes to remain in separate maternal and paternal groups during a part of the earlier development; but this seems to be of somewhat exceptional occurrence, and is in any case soon lost, chromosomes of maternal and paternal ancestry becoming intermingled as development proceeds.[1] This only throws into stronger relief the fact that the maternal and paternal *chromosomes*, individually considered, do not lose their identity, but conform to the principle of genetic continuity throughout the ontogeny.

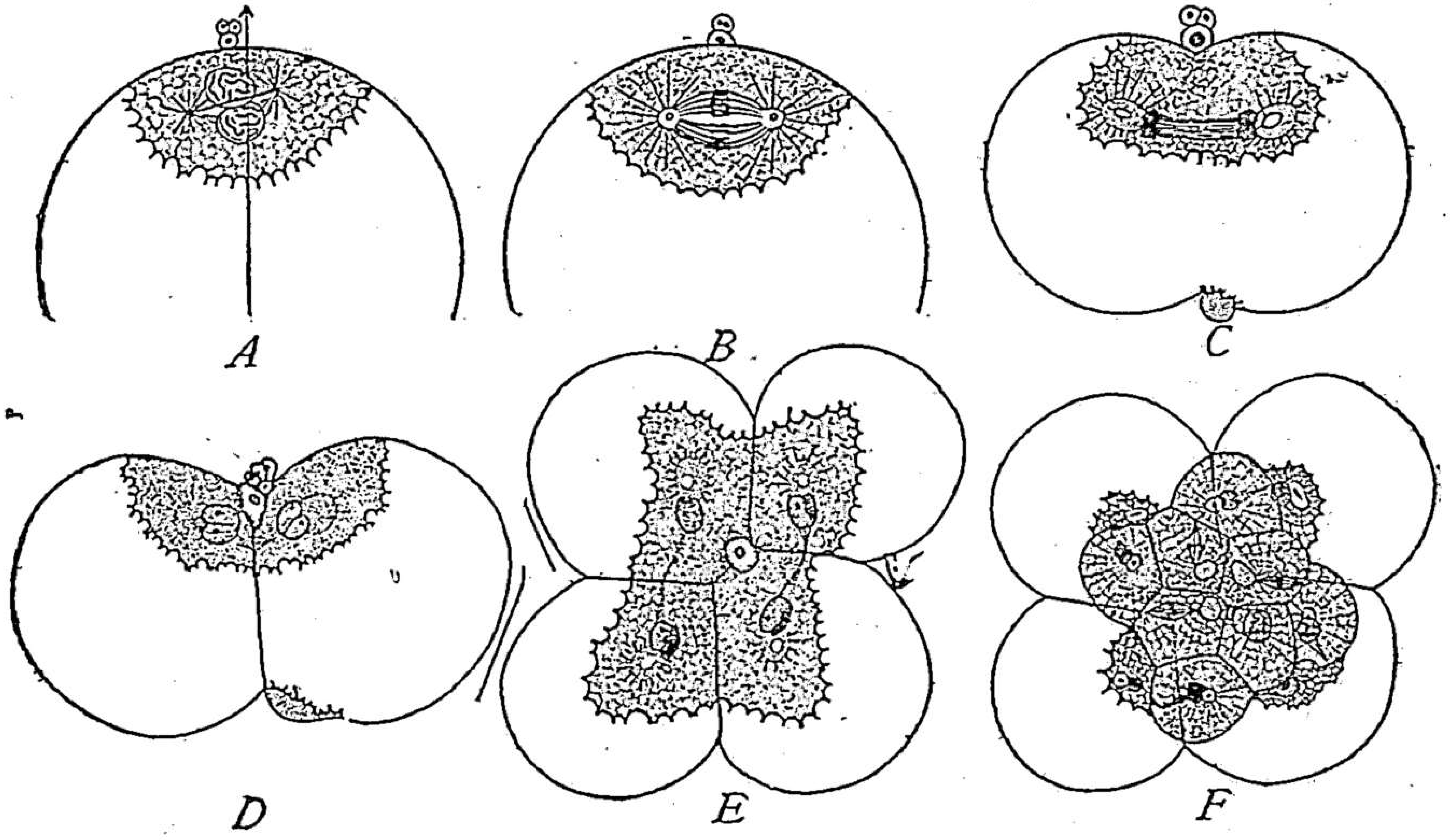

Fig. 202.—Gonomery in the early cleavage of the gastropod *Crepidula* (CONKLIN). *A*, conjugation of the gamete-nuclei; *B*, spindle, with separate maternal and paternal chromosome-groups *C–F*, later stages, showing at each stage double nature of the nuclei.

D. THE CYTOPLASMIC STRUCTURES

The history of the cytoplasmic structures in fertilization offers many problems that are still urgently in need of further investigation. The most

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[1] See the figures of cleavage-nuclei and chromosomes in Hemiptera by Morrill ('10) and Hoy ('16), and in Diptera by Metz (*op. cit.*).

obvious of these relate to the central bodies, the chondriosomes and the Golgi-bodies or their products (acrosome); but it is probable that other cytoplasmic elements may be concerned in fertilization. After entrance of the sperm into the egg the acrosome, middle-piece and flagellum sooner or later disappear as individualized bodies, though some of their products may in some cases persist during the earlier stages of cleavage. In the bat, for example, Van der Stricht ('09) found that the sperm-tail (including the middle-piece) may still clearly be seen in one of the first two blastomeres.[1] In the sea-urchin Meves has shown [2] that the middle-piece may be traced with little change, at least up to the end of the fifth cleavage, being clearly distinguishable in one blastomere of the 32-cell stage. It seems clear, therefore, that neither middle-piece nor flagellum as such plays any direct part in the operations of fertilization. Nevertheless, the possibility remains that one or both these regions may bring into the egg certain structural elements that play such a part. From the region of the neck and middle-piece the egg receives two important structural elements, one of which, at least, is in fact believed to play an important part in fertilization. These are the chondriosomes and the central bodies, the history of which has raised many interesting questions.

1. The Acrosome

The acrosome has long been treated with rather scanty respect, often being regarded as no more than an organ of attachment to, or penetration into, the egg. Since, however, it seems always to be a product of the Golgi-bodies (p. 381) and since it enters the egg, we may suspect that it plays a more important rôle, though nothing is yet certainly known of this (p. 716). Lillie ('12) has made a careful study of its history in *Nereis*, showing that after entering the egg with the nucleus it passes inwards with the remains of the entrance-cone (Fig. 191) and breaks up into a small group of deeply staining granules which persist near the apex of the sperm-head until after the latter has completed its rotation and assumed a vesicular form, and after the sperm-aster has appeared. Finally, still connected with the remains of the entrance-cone, it separates from the sperm-nucleus and disappears from view. Bowen ('23), urging the analogy between the formation of the acrosome and that of the secretory granules (p. 716) has suggested the interesting possibility that it may be the bearer of some substance (?enzyme) that plays a part in the activation of the egg; but this is wholly hypothetical.

2. The Chondriosomes

Benda predicted that mitochondria would be found to be brought into the egg by the sperm and might play a definite rôle in fertilization.[3] The

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[1] See also Lams ('10) and Levi ('15).

[2] '11, '12a, '12b, '14.

[3] '03, p. 781.

first part of this prediction has been realized, especially through a series of studies by Meves, which conclusively demonstrate that mitochondria are actually brought into the egg by the middle-piece, and perhaps also by the flagellum.[1] This was first observed in *Ascaris* (Meves, '11, Held, '12, '17), where the sperm-mitochondria are at first much larger and much less numerous than those of the egg (Fig. 204); and are thus distinguishable for some time after their escape from the disintegrating sperm-cytosome. As they scatter through the egg they fragment into smaller bodies indistinguishable in size from the egg-mitochondria with which they are now mingled. The mitochondria are now distributed by cleavage with approximate equality to the resulting blastomeres. Meves assumed on theoretical grounds that sooner or later a conjugation must take place between the maternal and the paternal mitochondria (as also surmised by Benda); but no satisfactory support of this has been found.[2] Held endeavored to show that the paternal

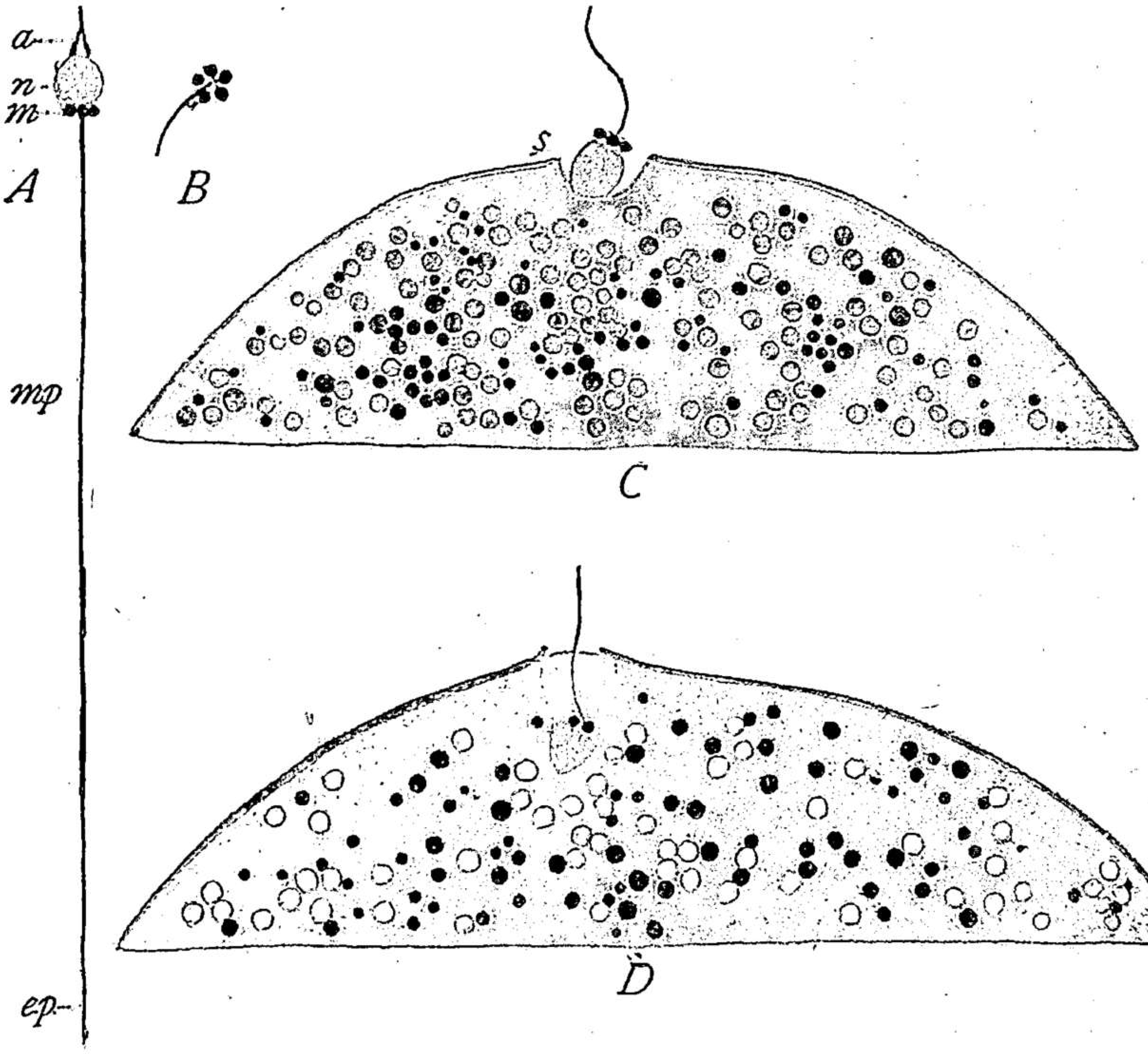

Fig. 203.—Entrance of the sperm, with chondriosomes, in the pelecypod *Mytilus* (Meves).

A, mature sperm; *B*, middle-piece, to show chondriospheres; *C*, *D*, entrance of sperm into the egg. The black bodies are chondriosomes, the paler ones yolk-spheres.

a, acrosome; *ep*, end-piece; *m*, middle-piece; *n*, nucleus; *s*, sperm-head.

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[1] This fact, as Meves has pointed out, had earlier been briefly noted in the case of *Ascaris* by the brothers Zoja ('91), the mitochondria being described as "plastidules."

[2] See Retzius ('11), Vejdovský ('11–'12), Held (*op. cit.*), Kemnitz ('12), Romeis ('13). For a rejoinder to these criticisms see especially Meves ('13 and '18).

and maternal mitochondria remain quite distinct and may be distinguished from one another at every stage by certain characteristic differences of staining-reaction, even after the sperm-mitochondria have become as small as those of the egg. Like Meves, he found that mitochondria from both sources are passed on bodily to the cells of the early embryo.

In subsequent papers Meves extended his conclusions to other forms, showing that mitochondria are brought by the sperm into the egg in the sea-urchin ('12, '14) in the tunicate *Phallusia* ('13) in the nematode *Filaria* ('15) and in the mollusk *Mytilus* (Fig. 203, '15b). In *Filaria* the phenomena are similar to those in *Ascaris*, a number of larger chondriosomes being set free from the sperm soon after its entrance and fragmenting into smaller mitochondria indistinguishable from those of the egg before the first cleavage. In later stages of cleavage the numerous minute mitochondria originally

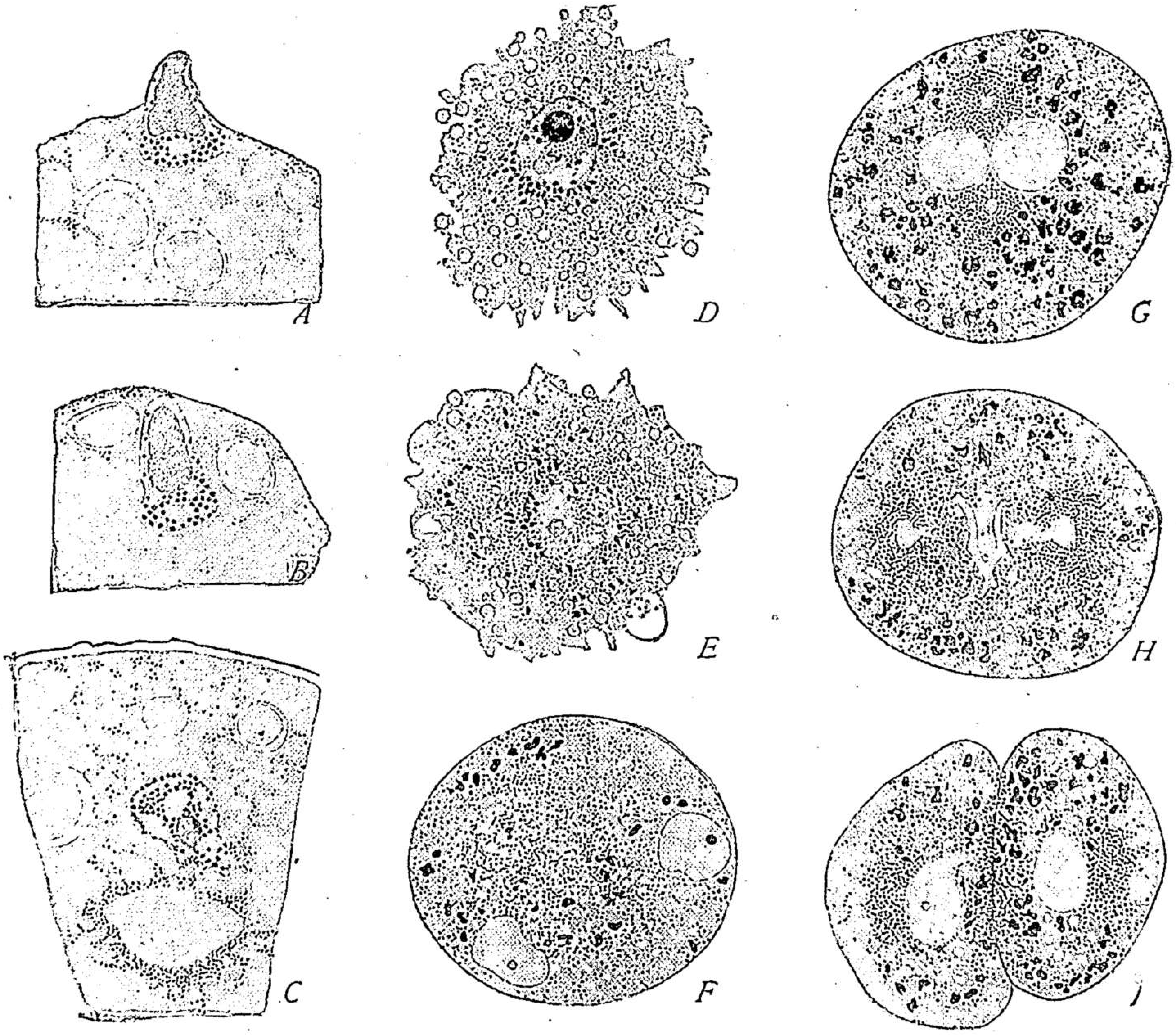

Fig. 204.—Mitochondria in the fertilization of *Ascaris megalocephala* (*D*, *E*, from Held, the others from Meves.

A–C, entrance of the sperm containing mitochondria, much larger than those of the egg; *D*, *E*, the sperm-cytosome disintegrating, setting free the sperm-mitochondria, which mingle with those of the egg; *F*, *G*, the sperm- and egg-mitochondria, now intermingled and indistinguishable in size; *H*, *I*, first cleavage, distribution of the mitochondria to the daughter-cells. The larger black bodies in Figs. *F–I* are yolk.

present have been replaced by a much smaller number of thick, short chondriomites which Meves assumed to arise by the linear alignment of both maternal and paternal mitochondria, which thus unite to form a common product. In *Echinus*, *Phallusia* and *Mytilus* the sperm-chondriosomes are less numerous and form a more or less compact mass of granules or short rods. In *Phallusia* and *Mytilus* their fate could not be traced; but in *Echinus*, as already stated, they could be followed in the cleavage as far as one cell of the 32-cell type. In order to save his hypothesis in this case Meves is driven into the assumption that in the course of the later development the permanent portions of the larva, which pass over into the young sea-urchins, are derived only or mainly from cells which receive derivatives of the middle-piece (*i. e.*, of the sperm-mitochondria), while the perishable larval structures arise from those cells that receive only maternal chondriosomes(!) Meves attempts, finally, to bring this into relation with the fact that in the mammals (bat, guinea pig) the tail and connecting-piece of the sperm, with their mitochondrial investment, pass into one of the first two cells, the assumption here being that the blastomere which receives the tail produces the embryo proper, the other the trophoblast of Hubrecht (an accessory nutritive organ).[1]

It will be seen from the foregoing that the assumption that maternal and paternal mitochondria conjugate or fuse, or even that they play any part in fertilization, still remains a purely theoretical postulate. Meves has permitted himself speculations in this direction which not only far outrun the observed facts but necessitate the most improbable subsidiary assumptions in order to make them fit. Nevertheless, it is difficult to believe that the sperm-mitochondria are wholly functionless in the egg or that Benda's surmise was an absolutely empty guess. We must, however, await further light on the general functional significance of the chondriosomes before a profitable attack can be made upon the problem of their relation to fertilization.

3. The Central Bodies and the Cleavage-Amphiaster [2]

The source of the cleavage-centers of the fertilized egg has been a subject of prolonged study, at first largely dominated by the general belief in the persistence of the division-center ("centrosome") as a permanent organ of the cell. Van Beneden somewhat doubtfully suggested that the cleavage-centers are derived from the egg alone; and a similar conclusion was afterwards adopted by Wheeler ('97) in the case of *Myzostoma*, and more

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[1] For further development of these conclusions, see Meves, '18.

[2] For fuller reviews of the literature, see especially Korschelt and Heider ('03), Kostanecki ('96, '02, '04, '06), Boveri, ('07), Nekrassoff, ('09).

recently by a few other observers (p. 442). Rabl ('89) urged, on the other hand, that if the doctrine of the persistence of the central body be well founded, we should expect fertilization to involve a conjugation not alone of nuclei but of central bodies; and in fact Fol soon afterwards published a remarkable paper entitled the "Quadrille of the Centers" ('91), in which he described precisely such a phenomenon in the sea-urchin egg. This result seemed at first to be confirmed by certain observations, but was later overthrown and, considered as a general theory of fertilization, is now regarded as obsolete.

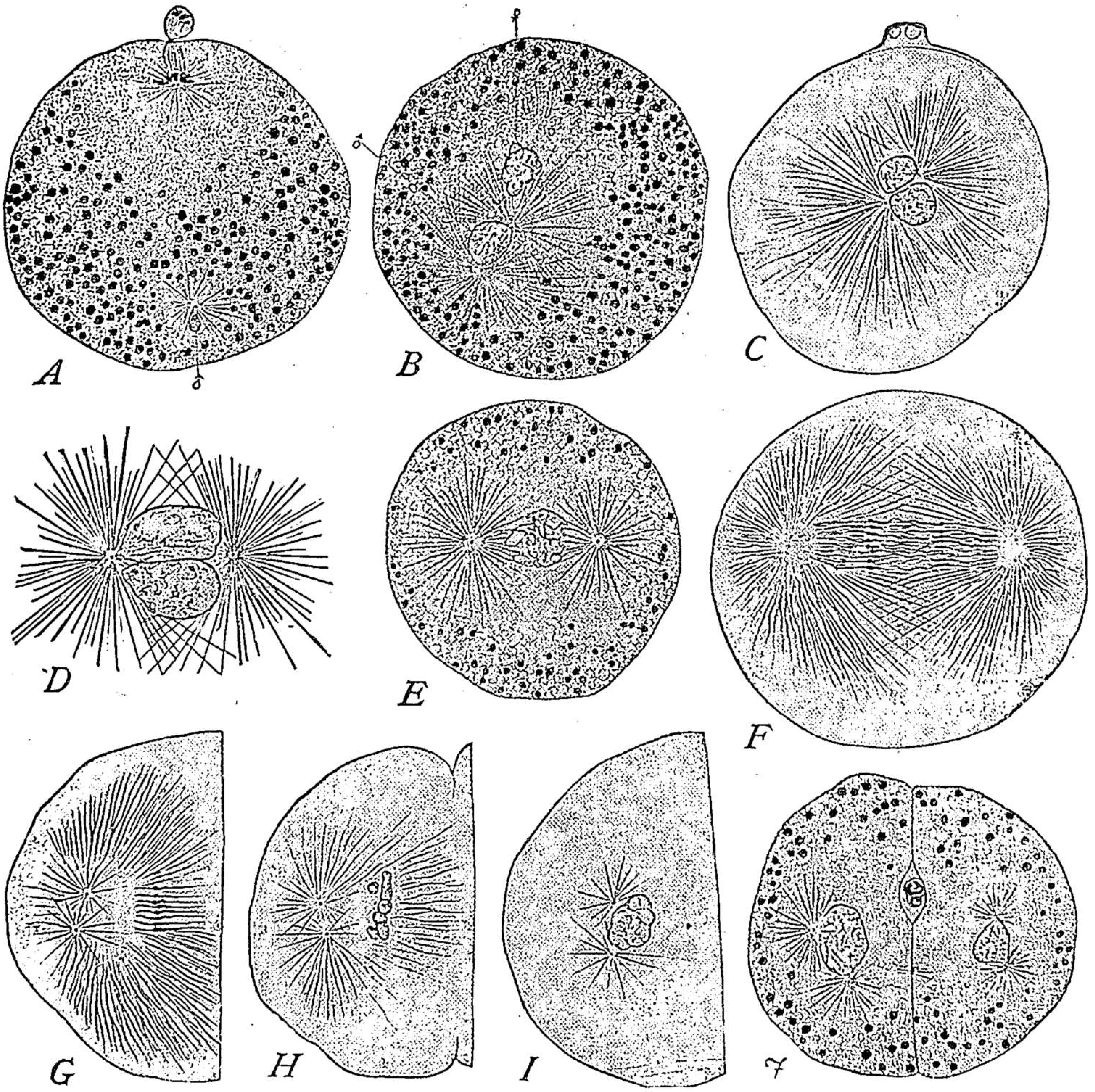

Fig. 205.—Fertilization in an annelid (armed Gephyrean) *Thalassema* (Griffin).

A, second polocyte forming; sperm-nucleus and centrioles below; *B*, approach of the egg-nucleus and sperm-nucleus, the latter accompanied by the sperm-amphiaster; *C*, union of the pronuclei; *D*, later stage of last; *E*, prophase of cleavage-spindle; *F*, anaphase of the same, centriole divided; *G*, *H*, *I*, successive stages in the nuclear reconstitution and formation of the daughter-amphiasters for the second cleavage; *J*, two-cell stage.

From the first it stood in opposition to very specific earlier observations by a number of excellent observers,[1] which showed that after extrusion of the polocytes the aster remaining in the egg, together with its central body, disappears without discoverable relation to the cleavage-amphiaster. The latter seems to arise by the division of a single sperm-aster first seen close to the sperm-nucleus and preceding the latter in its march towards the egg-nucleus. Hertwig and Fol established the important fact that in pathological dispermy and polyspermy each sperm-nucleus is accompanied by a single sperm-aster which in each case may divide to form an amphiaster. On these facts primarily Boveri based his celebrated theory of fertilization,[2] which may form a convenient point of departure for a survey of the subject.

4. Boveri's Theory of Fertilization

The essential postulates of this theory were (1) that the central body ("centrosome") is the fertilizing element proper; (2) that it is actually imported into the egg by the sperm; (3) that the cleavage-centers arise directly by division of this sperm-center.[3] As outlined by Boveri the theory took the following form: During the somatic divisions the center is continuously handed on by division from generation to generation of cells. This process comes to an end in the mature egg after extrusion of the second polocyte, when the egg-center degenerates or becomes physiologically ineffective; further cell-division is thus inhibited and the occurrence of parthenogenesis avoided. *The ripe egg possesses all of the elements necessary for development save an active division-center. The sperm, on the other hand, possesses such a center but lacks the protoplasmic substratum in which to operate. In this respect the egg and sperm are complementary structures;* their union in syngamy thus *restores to each the missing element necessary to further development.*[4] Accepting this it follows that the nuclei of the embryo are derived equally from the two parents; the central bodies are purely of paternal origin; and to this it might be added that the general cytoplasm of the embryo seems to be almost wholly of maternal origin. Unfortunately this simple and clear conception can no longer be accepted in its original form, as Boveri ('14) himself finally admitted. Nevertheless, in the author's opinion, to reject it *in toto* would leave unexplained many striking facts.

When Boveri's theory was first put forwards nothing was definitely known concerning the history of the central body in the formation of the

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[1] Fol ('79), O. Hertwig ('84), Vejdovský ('87, '89), Boveri, (87a, 88b, etc.).

[2] Boveri, '87b, '88a, '92, '95, '02a, '05, '07, '14.

[3] A nearly similar conclusion was independently reached by Vejdovský ('88).

[4] '87b. p. 155.

sperm. Observations by Platner ('89) and others seemed to show that this body passes into the apical region. Later observations demonstrated that the central body, or a derivative from it, all but invariably lies behind the nucleus in the region of the neck or middle-piece (p. 283). In harmony with the theory, therefore, was the fact, determined by numerous later observers, that the sperm-aster in the egg is typically focused at a point near the base of the nucleus, often very near to, or actually within the middle-piece—a relation now known to exist in most of the principal groups of animals.[1] A strong case is thus established in favor of the conclusion that a direct genetic relation of some sort exists between the central bodies of the spermatid, and those of the sperm-aster and cleavage-amphiaster in the egg.

In addition to the conditions observed in dispermic and polyspermic eggs (p. 416), Boveri's theory further fits with the following well-determined facts: In merogony (p. 465) non-nucleated egg-fragments are readily penetrated by the spermatozoa, and may segment and give rise to perfect larvæ. In "partial fertilization" as shown by Boveri ('88) the sperm-center and 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 a later stage (p. 458). Still more remarkable was the discovery of Boveri, earlier cited (p. 176), that during the first cleavage one of the resulting cells may receive only the division-center without a nucleus, and that this center may continue for a considerable period to multiply at the same rate as in the nucleated cell, though the nucleus is absent. Clearly therefore, (1) something is introduced into the egg by the middle-piece of the sperm that either is a central body or has the power to incite the formation of one; (2) this body is structurally independent of both nuclei and may divide independently of them; (3) independently of the division of the nucleus or cell-body some kind of specific genetic relation exists between the central bodies of successive generations. In harmony with Boveri's theory, also, are those numerous cases (nematodes, arthropods, chordates) in which the central bodies seem not to take any part in the polar mitoses (p. 508). This fact is particularly striking in cases, such as the ascidians (Hill, '95, Conklin, '12) in which the sperm-asters and the polar spindles may be seen lying side by side in the same section, the former with conspicuous asters and central bodies, the latter without trace of such structures. In this particular case Conklin (always

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[1] *E. g.*, in echinoderms (Flemming, '81, O. and R. Hertwig, '86, Wilson and Mathews, '95, Boveri, '95, etc.), nematodes (Meyer, '95), insects (Henking, '91), annelids (Korschelt, '95, Foot, '97), gastropods (Kostanecki and Wierzejski, '96, Linville, '00), platodes (Van der Stricht, '98, Francotte, '98), tunicates (Hill, '95, Golski, '99, Conklin, '05), amphibians (Fick, '93), elasmobranchs (Rückert, '99), etc.

skeptical concerning the universality of Boveri's theory) long since admitted that "there could not possibly be a clearer case of the origin of the cleavage centrosomes from the middle-piece of the spermatozoön." [1]

On the other hand, Boveri's theory was weakened by accumulating evidence that the central bodies seem in some cases to arise *de novo* (p. 684). It lost further ground with the final demonstration, that no central bodies are concerned in the fertilization of higher seed-plants, and that the cleavage-spindles are here devoid both of such bodies and of asters (p. 150). Even in the fertilization of those plants (*e. g.*, the cycads) where the whole sperm, including the blepharoplast (a derivative of a central body) enters the egg, no one has yet shown that this latter body plays any direct part in fertilization. Strasburger ('97) suggested that the essential fact in fertilization is the importation into the egg of a certain quantity of "kinoplasm," whether in the form of a definite central body (animals, generally) or in more diffused state (higher plants); but this still remains quite hypothetical. A third difficulty is offered by artificial parthenogenesis in such forms as sea-urchins, where the egg at the time of activation has long since extruded the polyctes, and all traces of central apparatus have disappeared from view. These eggs, evidently, must either form a new center or rejuvenate a preëxisting one.

A more specific difficulty appears in the fact that in a few cases no sperm-center or aster is found at any time associated with the sperm-nucleus. An early described case of this was that of *Myzostoma* (Wheeler, '95, '97), and more recently the same condition is described by Nekrassoff ('09) in the pteropod *Cymbulia;* in a number of the trematodes; [2] in the spider *Theridium* (Montgomery, '07); and in the mouse (Lams and Doorme, '07). Some of these cases are no doubt owing to a delayed development of the sperm-aster; and Kostanecki ('06) has proved such to be the case in *Myzostoma*, demonstrating that if union of the germ-nuclei be delayed by treating the eggs with hypertonic sea-water a sperm-aster and amphiaster develop as usual in close association with the sperm-nucleus. Nekrassoff found in *Cymbulia* a conspicuous egg-aster and center at every stage up to the period of karyogamy, though it finally disappears. Gille, on the other hand ('14), believes the egg-center to persist and give rise to the cleavage-centers (in trematodes). Again, in case of the honey-bee Nachtsheim ('13) believes the cleavage-centers to be derived from the egg, supporting this by the observation that in the mitosis of supernumerary sperms in the egg the spindles are of the anastral type, like those of the polar mitoses, while conspicuous

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[1] '05, p. 23, '12, p. 545. For a full review of the earlier literature see Kostanecki, '04 (on *Mactra*) and '06 (on *Myzostoma*).

[2] Goldschmidt ('05), Katheriner ('04), Henneguy ('06), Schellenberg ('11), Gille ('14).

centers appear in the cleavage. This conclusion though accepted by Boveri ('14), does not seem very strongly grounded.

Still another, and apparently better founded, conclusion is reached by Conklin [1] in the case of the gastropod *Crepidula*, in which appears a division-center in connection with each pronucleus and persists up to the time of union or karyogamy (Fig. 206). These centers seem to disappear before cleavage; nevertheless the facts indicate that the two cleavage-centers which subsequently appear are still associated, one with each pronucleus, as before.

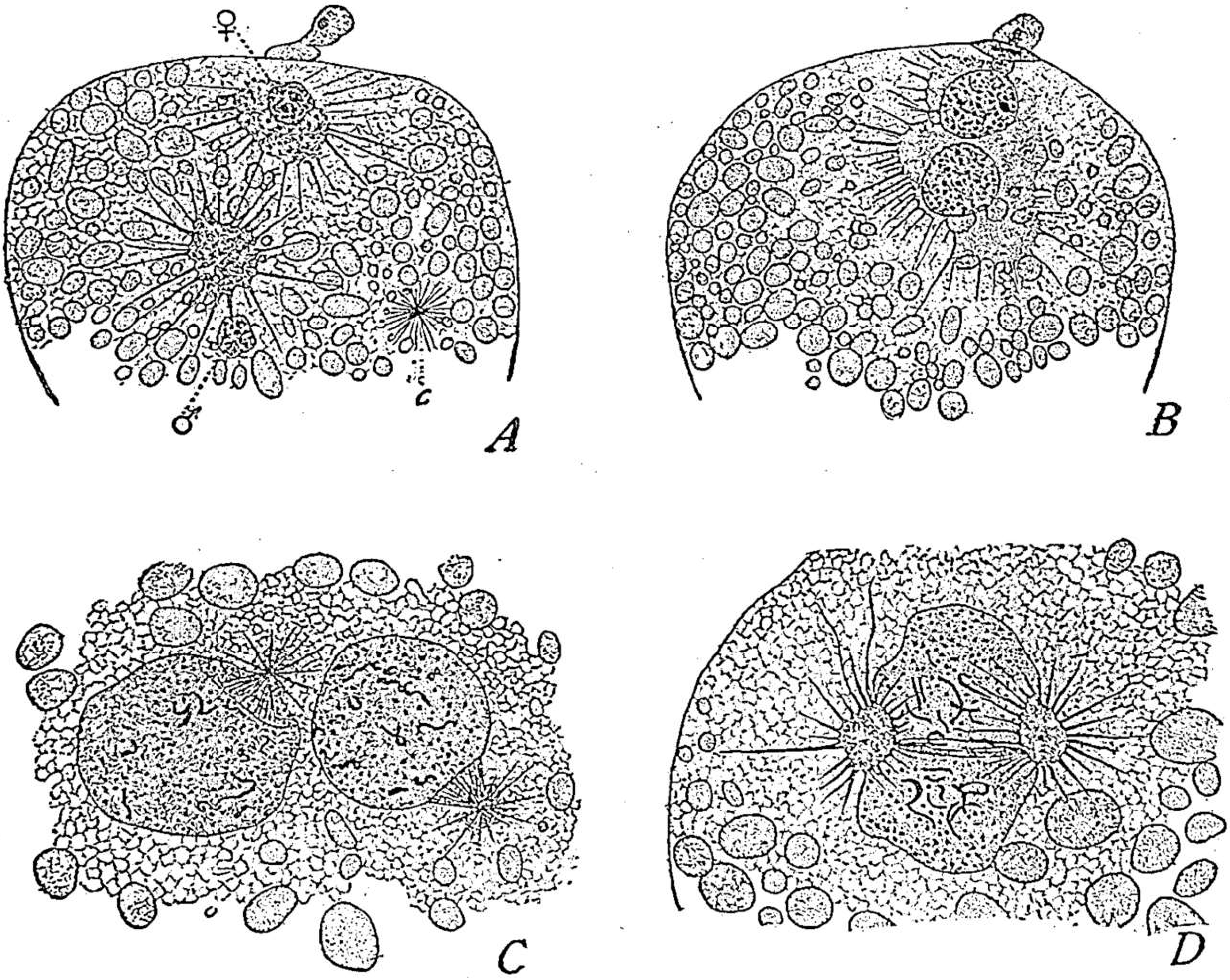

Fig. 206.—Fertilization in the gastropod *Crepidula* (Conklin).

A, approach of sperm-nucleus (♂) and aster to those of the egg (♀); at *c*, a cytaster or accessory aster; *B*, pronuclei nearly in contact; *C*, enlarged view of pronuclei somewhat later, a central body, aster and half-spindle in connection with each; *D*, late prophase.

Even in the typical case (*e. g.*, in the sea-urchin, tunicate, or nematode) two difficult questions still remain, namely, whether the cleavage-centers are actually derived from the sperm-center, and whether the latter is actually brought into the egg by the sperm. (1) Doubts concerning the first of these questions arise from the fact that conjugation of the gamete-nuclei is always followed by a "pause" during which the sperm-asters, in some cases also the centers, become more or less reduced and may even wholly disappear from view. Examples of this occur in nearly all groups,[2] showing many degrees in the reduction of the asters at this time. These more extreme

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[1] '03, '04, '12.

[2] On this point, see especially Coe, '98.

cases led to the opinion expressed by several writers [1] that the cleavage-centers are not directly connected with those of the sperm-amphiaster but are formed *de novo*. To establish such a conclusion is evidently difficult; for the centrioles are bodies of such extreme minuteness that if not surrounded by astral rays they might readily be lost to view among the protoplasmic granules of the egg or they may even become reduced to ultra-microscopical dimensions. Full weight must also be given to the fact, urged by several good observers, that the cleavage-centers arise at or very near the points at which the sperm-asters disappear from view.[2] A remarkable demonstration of this was found by Lillie ('12) in *Nereis*, where the sperm-amphiaster is from the beginning heteropolar, both aster and center being larger at one pole than the other and thus foreshadowing the unequal cleavage (Fig. 475). During the pause following union of the germ-nuclei the larger aster and center are always present while the smaller one disappears from view, but only to reappear at a later stage. It is here highly probable that the disappearance is only seeming, and that the cleavage centers are really identical with the sperm-centers.

Of the same type as the foregoing is the question whether the sperm-center is actually brought into the egg as a preformed structure in the neck or middle-piece of the sperm near which it so often lies (p. 396). The earlier opinion that the entire middle-piece is converted into the sperm-center was long since proved erroneous, by the observation that this structure is left behind near the egg-periphery and degenerates.[3] It is, however, a matter of great difficulty actually to trace the centriole of the sperm-aster to the neck or middle-piece of the sperm in the egg, though direct evidence of such an origin has been produced by several observers.[4] In the nemertines a minute, intensely staining granule (probably an "end knob") is readily seen within the globular middle-piece before entrance of the sperm. Within the egg the aster forms about the middle-piece but the outer portion of the latter is soon cast off, leaving the central granule as the sperm-center, which early divides into two to form the amphiaster (Fig. 207). This is probably the nearest approach yet made to a demonstration of the actual derivation of the sperm-center from a preformed structural element within the middle-piece.

An interesting argument against such a derivation has been based by F. R. Lillie ('11, '12) on the fact in *Nereis*, that the middle-piece, like the

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[1] Lillie, '97, Foot, '97, Child, '97, etc.

[2] See especially Kostanecki and Wierzejsky ('96) on *Physa*, Coe ('98), Kostanecki ('02), and Yatsu ('09) on *Cerebratulus*.

[3] Field, '95, Wilson, '97, Meves, '12, '14, etc.

[4] See Hill, '95, Kostanecki and Wierjeski, '96 (*Physa*), Boveri, '00 (sea-urchins), Conklin, '05 (tunicates) and especially Yatsu '07, '09 (nemertines).

tail, is left outside the egg (p. 415), and that a sperm-aster containing a central body appears *when only a fragment of the nucleus enters the egg.* This observation is made possible by the fact, already mentioned (p. 415), that as the sperm-nucleus enters the egg it is drawn out into an elongate rod-like form, so that at this time the portion left outside the egg may readily be removed by centrifuging the eggs. Here there cannot be the least doubt that both the middle-piece and the basal region of the nucleus are left outside the egg. Nevertheless, the partial sperm-nucleus (which may be larger or smaller) rotates as usual after its entrance and *a sperm-aster of correspondingly diminished size is developed in relation to its most basal point* (Fig. 208). Such asters divide to form amphiasters and possibly may lead to cleavage, though this could not be determined with certainty.

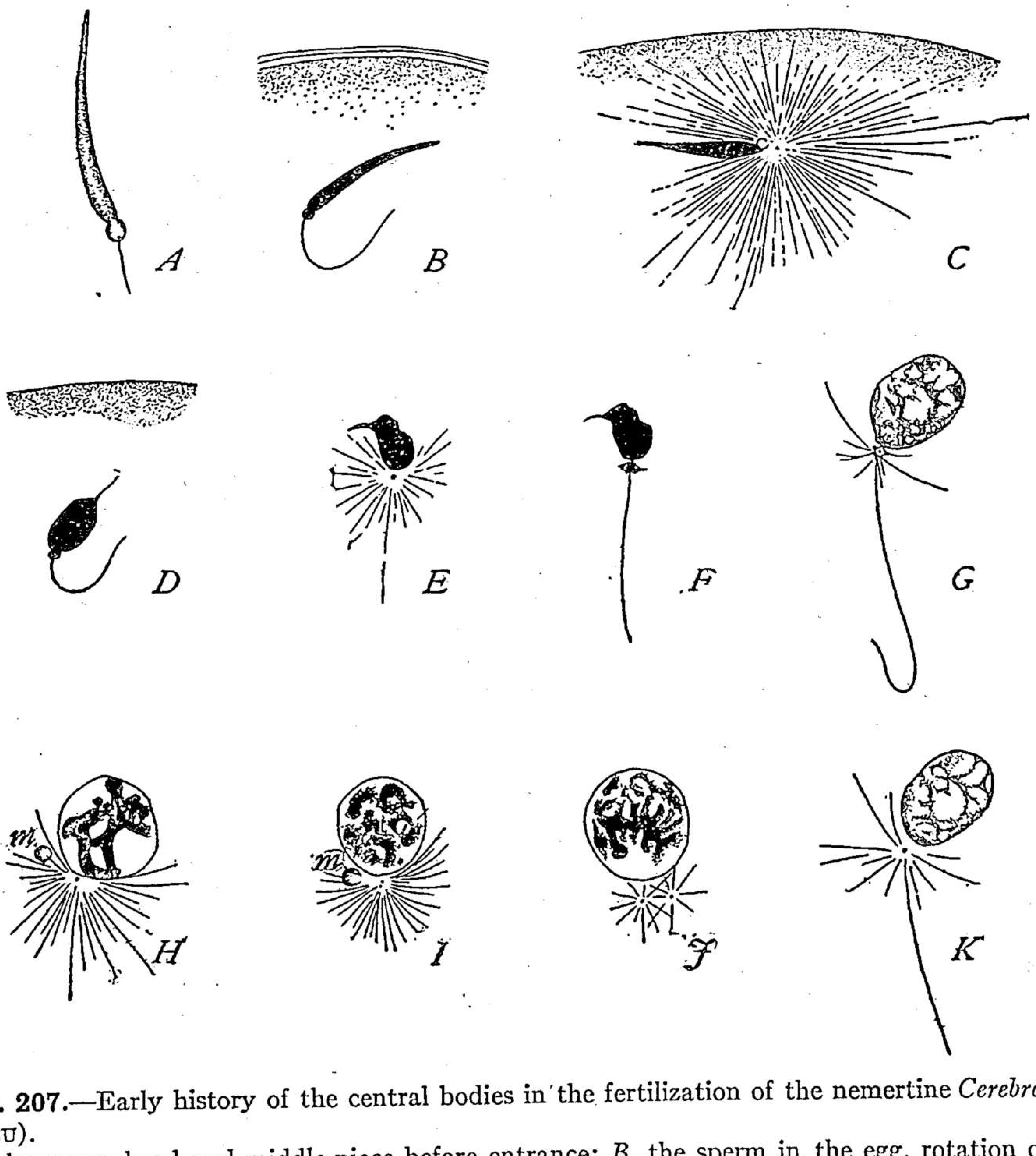

Fig. 207.—Early history of the central bodies in the fertilization of the nemertine *Cerebratulus* (Yatsu).

A, the sperm-head and middle-piece before entrance; *B*, the sperm in the egg, rotation of the head; *C*, precocious development of sperm-aster; *D*, *E*, *F*, rotation completed, rounding of head; *G*, nucleus vesicular; *H–K*, later stages, showing cast-off middle-piece at *m*.

Lillie considered that these results gave crucial evidence against the existence of any genetic relation between the sperm-center within the egg and that of the sperm or spermatid, and concluded that "the centrosome and aster owe their existence to an interaction between nucleus and cytoplasm, and not to any third element"; further, that the position in which they arise is "a function of polarity of the sperm-nucleus." The theory that a centrosome introduced by the sperm is necessary for such formation is therefore shown to be incorrect."[1]

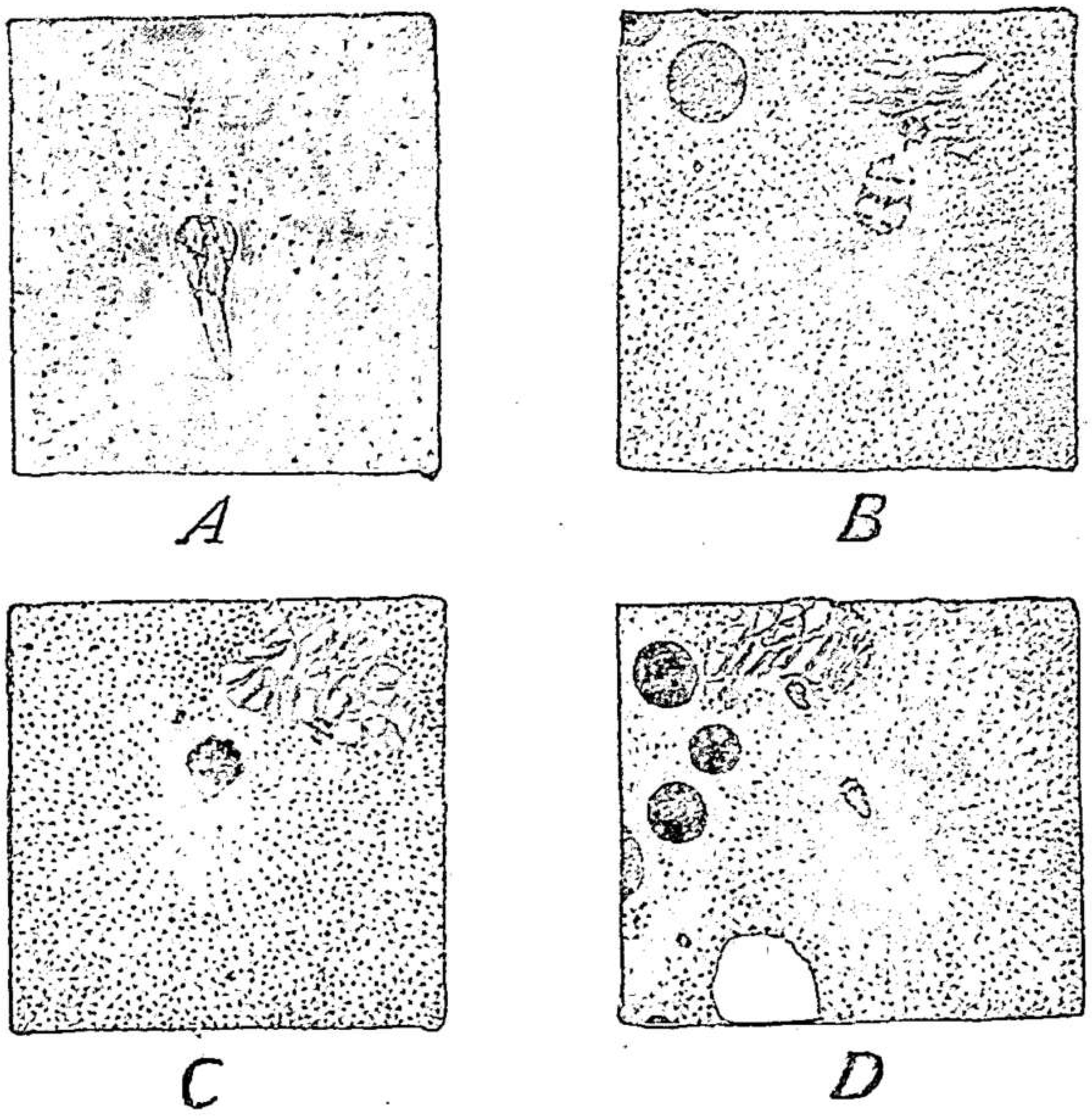

Fig. 208.—Fractional fertilization in *Nereis* (Lillie).
A, entire sperm-nucleus after entrance and rotation, with aster below and entrance-cone and acrosome above; *B*, *C*, *D*, three nuclear fragments, progressively smaller in size, with accompanying asters.

This conclusion, however, is not justified by the facts, for Koltzoff ('09) has shown that the sperm-head of *Nereis* (Fig. 119) is traversed by a cytoplasmic axial rod extending throughout its whole length from middle-piece to acrosome, as is also the case in sperms of certain other animals (p. 281), and in some of these cases the rod is known to arise as a forward growth from the proximal centriole[2] seen in the middle-piece of *Helix*, or in the basal part of the nucleus in *Paludina* (p. 380). If such be the case in *Nereis* all the phenomena are simply explained by assuming the sperm-aster to be developed about the exposed end of this body; and the case falls into line with what is shown in other forms.

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[1] '12, pp. 437–8, 445.

[2] In *Murex* (Stepan, '03), *Aporrhais* (Schitz, '20), *Fasciolaria* (Hyman, '23, with lit.).

Boveri's theory, evidently, must not be taken in too narrow or mechanical a sense; but there are facts still to be considered that tend to support it, in principle if not exactly in form. In the artificial parthenogenesis of sea-urchins, an egg that is normally adjusted to fertilization, and in which the whole mitotic apparatus seems to have disappeared, is still able, upon purely physico-chemical activation, to give rise to a normal cleavage-amphiaster. Further studies have shown that this egg is likewise able to produce (or rejuvenate) an active division-center as a result even when activated *by the sperm.* This was long since shown by O. and R. Hertwig ('87) by treating the eggs just after fertilization with solution of chloral hydrate or sulphate of quinine, or by subjecting them to abnormal temperatures. By these agents the union of the gamete-nuclei is often prevented, yet both nuclei may separately undergo the mitotic transformation accompanied by the development of a central body and aster. Ziegler ('98) attained the same result by constricting the egg with a fiber of cotton in such a way as to keep the gamete-nuclei apart. The writer ('01b) found the same phenomenon in eggs slightly etherized so as to retard or prevent the union of the gamete-nuclei; and Conklin ('04) reached a similar result in *Crepidula* by treating the eggs with hypertonic solutions.

In all these cases an aster or division-center arises in the neighborhood of each nucleus; but it is a significant fact that in the sea-urchin the sperm-center and aster typically divide to form an amphiaster, while the egg center, typically *gives rise to a monaster*, which rarely if ever divides to form an amphiaster (Fig. 209). This is exactly comparable to the production of a monaster in cases of incomplete activation in artificial parthenogenesis (p. 484).[1] These facts and those observed in etherized or chloralized eggs (as above) demonstrate that "*in these eggs the egg-center and sperm-center, under the same conditions, differ in potency, or in susceptibility to the activating agent;* and this supports Boveri's contention that in normal fertilization the spermatozoön imports into the egg an active central body which replaces one that has disappeared or become relatively ineffective."[2] "In normal fertilization the egg receives through the entrance of the sperm a strongly localized stimulus which affects the entire egg (as shown by the cortical reaction, etc.) and at the same time induces a local activity about the middle-piece which is transferred to the cleavage-nucleus by copulation of the germ-nuclei. This activity takes the place—or perhaps, to speak more accurately, becomes a

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[1] On this point the observations of the Hertwigs, of Ziegler and the writer are in agreement, except that the Hertwigs also observed abnormal "pseudo-tetrasters." In Ziegler's case (a single egg only) the egg-nucleus passed through three successive mitotic cycles without division (p. 168). In the writer's material one case was observed in which the egg-nucleus produced a tetraster at the second cleavage. See also Painter ('18).

[2] Wilson, '01, p. 364.

part—of an activity on the part of the egg-nucleus *that would have ensued even had the germ-nuclei not united.*" [1]

From the point of view thus gained it is not difficult to interpret the observed departures from the more usual case formulated in Boveri's theory. In *Crepidula*, where both egg-center and sperm-center are present during fertilization (p. 443), Conklin ('04) observed that in eggs fertilized in hypertonic sea-water the gamete-nuclei often fail to unite, in which case a division-center appears near each of them and both centers may divide to form amphiasters (Fig. 210). In this case, therefore, the egg-center and sperm-center seem to be nearly equally balanced and if kept separate go through the same changes. In the normal fertilization the two become

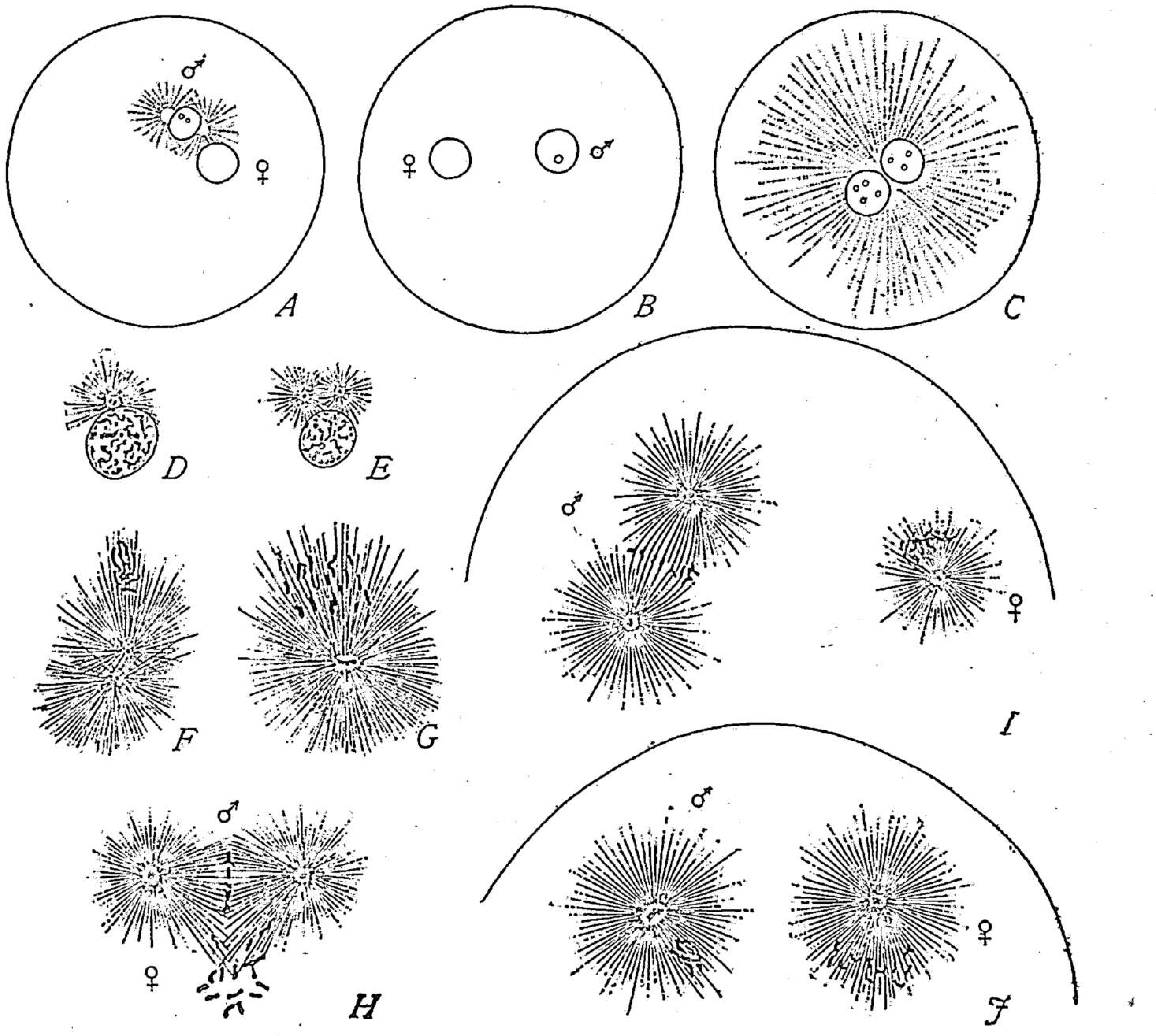

Fig. 209.—Fertilization of etherized eggs in *Toxopneustes*.

A–C, from living eggs, showing various observed conditions; *D–J*, from sections; *D–F*, sperm-nucleus and aster, showing division of latter; *G*, sperm-monaster; *H*, metaphase, sperm-amphiaster and chromosomes above, egg-chromosomes below; *I*, at the left, sperm-amphiaster in metaphase, at the right egg-nucleus with monaster; *J*, both sperm-nucleus and egg-nucleus have given rise to monasters.

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[1] Wilson, '01a, p. 582.

associated to form a single amphiaster, the division of both being thus for the time inhibited (p. 443). In the bee, on the other hand (assuming the correctness of Nachtsheim's observations), we might assume the egg-center to be readily capable of complete activation, while that of the sperm has become relatively, or perhaps completely, inactive. Such an interpretation is hardly more than a restatement of the facts as actually observed; but it takes into account an essential element of truth in Boveri's theory which should not be ignored.

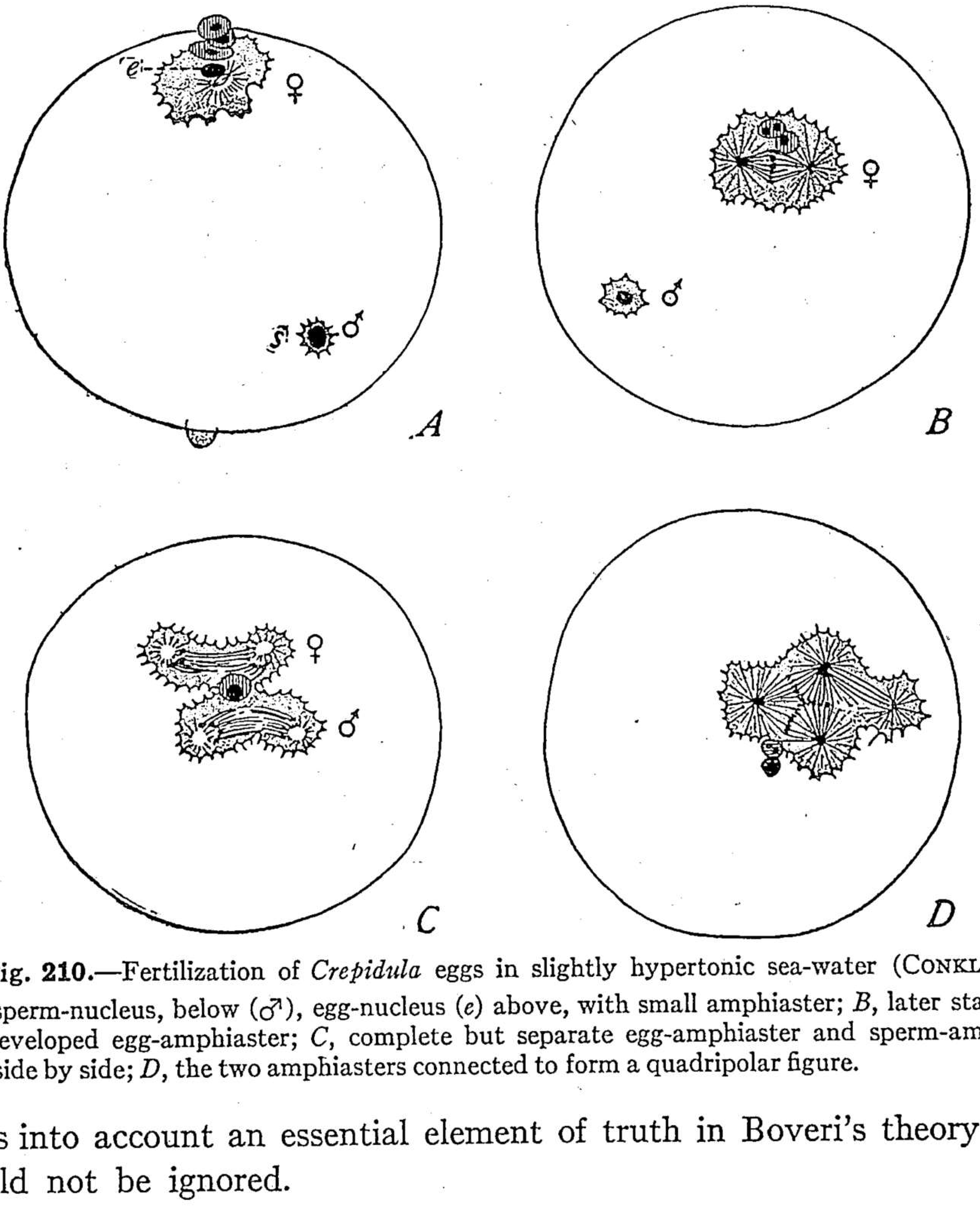

Fig. 210.—Fertilization of *Crepidula* eggs in slightly hypertonic sea-water (CONKLIN). *A*, sperm-nucleus, below (♂), egg-nucleus (*e*) above, with small amphiaster; *B*, later stage, with well-developed egg-amphiaster; *C*, complete but separate egg-amphiaster and sperm-amphiaster lying side by side; *D*, the two amphiasters connected to form a quadripolar figure.

II. FERTILIZATION IN PLANTS[1]

So far as its broader features are concerned the problem of fertilization in plants, both in its morphological and its physiological aspects, is identical with that offered in animals, though its details are different. Neither in

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[1] In the brief sketch here offered free use has been made of the valuable reviews of Mottier ('04) B. M. Davis ('05), Campbell ('05), of the works of Coulter and Chamberlain on the *Morphology of Angiosperms* ('03), and the *Morphology of Gymnosperms* ('17) and of Sharp's *Cytology* ('21).

higher nor in lower plants are individualized central bodies yet known to play any definite part in the process, which in this respect wears a somewhat simpler aspect than in animals; and in no plants above the thallophtyes is there an overlapping of the process of fertilization with that of meiosis such as is so commonly seen in animals (p. 398). Externally, on the other hand, the phenomena in plants seem more varied and intricate because of complications introduced by the antithetic alternation of generations (p. 496) and its many modifications. Only a few of these can here be considered, and we shall confine ourselves for the most part to higher plants or cormophytes and to such features as are necessary for an understanding of the essential relations between plants and animals.[1]

The nearest approach to the conditions seen in animals is found among lower plants, such as some of the algæ (*Œdogonium*, *Fucus*, etc.), and the bryophytes and pteridophytes generally, in which a large quiescent egg-cell is fertilized by a minute free-swimming sperm-cell. In the seed-plants, ciliated sperms have been retained only in certain of the gymnosperms, and here lead no free-swimming life, being inclosed in the pollen-tube and discharged thence directly into the ovum.[2] In the lower archegoniates the en-

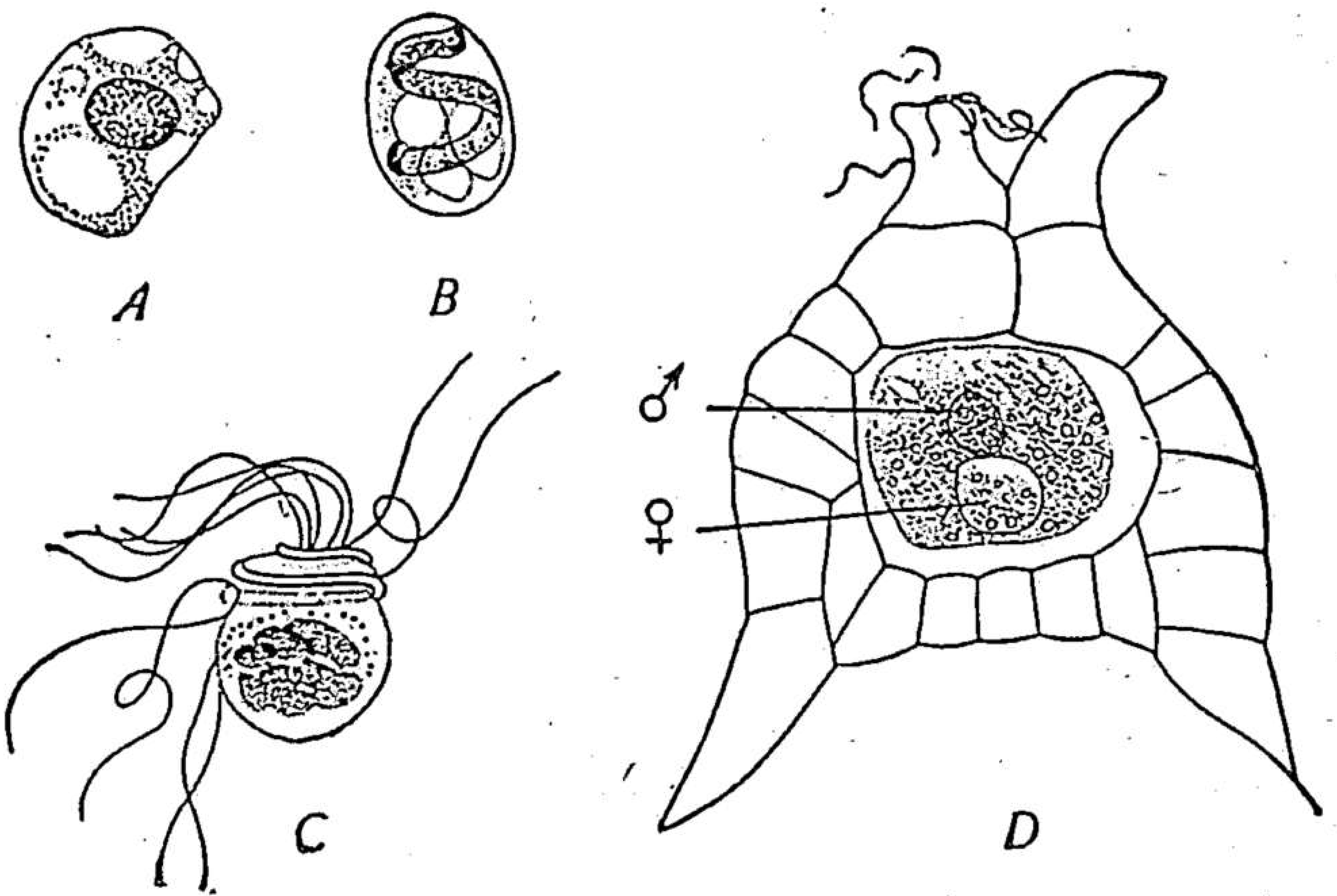

Fig. **211**.—Fertilization in *Pilularia* (Campbell).

A, *B*, early stages in the formation of the sperm; *C*, the mature sperm; *D*, archegonium during fertilization. In the center the ovum containing the apposed pronuclei (♂, ♀).

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[1] A few of the lower forms are considered in Chapter VII.

[2] The formation of the pollen-tube, and its growth down through the tissue of the pistil to the ovule, was observed by Amici ('23), Brongniart (26), and Robert Brown ('31); and in 1833–34 Corda asserted the entrance of its tip into the ovule. The botanists of the eighteenth century engaged in the same fantastic controversy regarding the origin of the embryo as that of the zoölogists of the time. Moreland (1703), followed by Étienne François Geoffrey, Needham, and others, placed himself on the side of Leeuwenhoek and the spermatists, maintaining that the pollen supplied the embryo which entered the ovule through the micropyle (the latter had been described by Grew in 1672)· and even Schleiden adopted a similar view. On the other hand, Adanson (1763) and others maintained that the ovule contained the germ which was excited to development by an aura or vapor

tire sperm enters the egg, as in animals generally; and in the pteridophytes, the entire sperm seems even to enter the egg-nucleus (*Nephrodium*, Fig. 212).[1] In these forms more than one sperm may enter, but only one of them unites with the egg-nucleus (Mottier). As in many animals, entrance of the sperm is followed by the formation of a fertilization-membrane.

In the cycads the sperms are discharged from the tip of the pollen-tube into the archegonial chamber and there swim in a liquid discharged from the pollen-tube. Several sperms may thus reach the same egg, but only one normally enters it, passing in with its spiral apex in advance. All observers have found that the entire sperm enters; but, as was first shown by Webber in *Zamia* and Ikeno in *Cycas*, both the spiral blepharoplast, with its cilia, and the protoplasmic body of the sperm, are left behind near the periphery (Fig. 213), while the nucleus "slips out of its cytoplasmic sheath and passes on alone from this point to the egg-nucleus " (Webber).

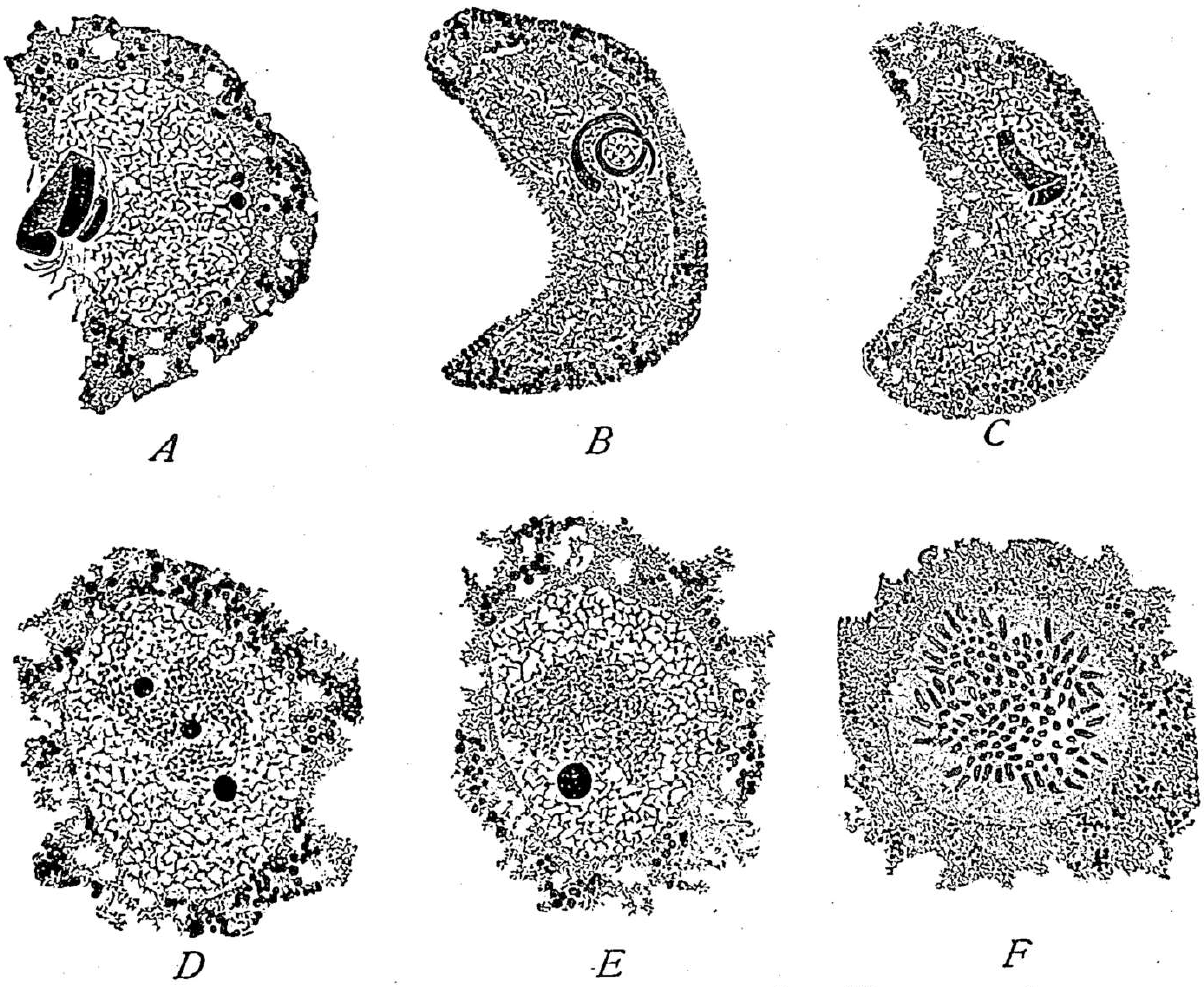

Fig. 212.—Fertilization in the fern *Nephrodium* (Yamanouchi).

A, sperm entering the egg-nucleus; *B*, *C*, the sperm lying within the egg-nucleus (two successive sections); *D*, *E*, disintegration of the sperm inside the egg-nucleus; *F*, metaphase-group of the first cleavage of the zygote, 128 chromosomes.

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emanating from the pollen and entering through the tracheæ of the pistil. For a further account of the history of this subject see Coulter and Chamberlain ('03, Chap. VII).

[1] Yamanouchi, '08.

In all higher gymnosperms, and in angiosperms, the tip of the pollen-tube actually penetrates the upper pole of the embryo-sac and discharges into it both generative nuclei (*cf.* p. 309) and also, in higher gymnosperms one or two sterile or vegetative nuclei (the "tube-nucleus" and "stalk-cell-nucleus"). In the gymnosperms only one of the generative nuclei conjugates with the egg-nucleus, the other degenerating, as do also the two vegetative cells (Fig. 214). In the angiosperms also, but one generative nucleus conjugates with the egg-nucleus; but here the second is also functional, taking part in the formation of the endosperm-nucleus, as explained beyond.

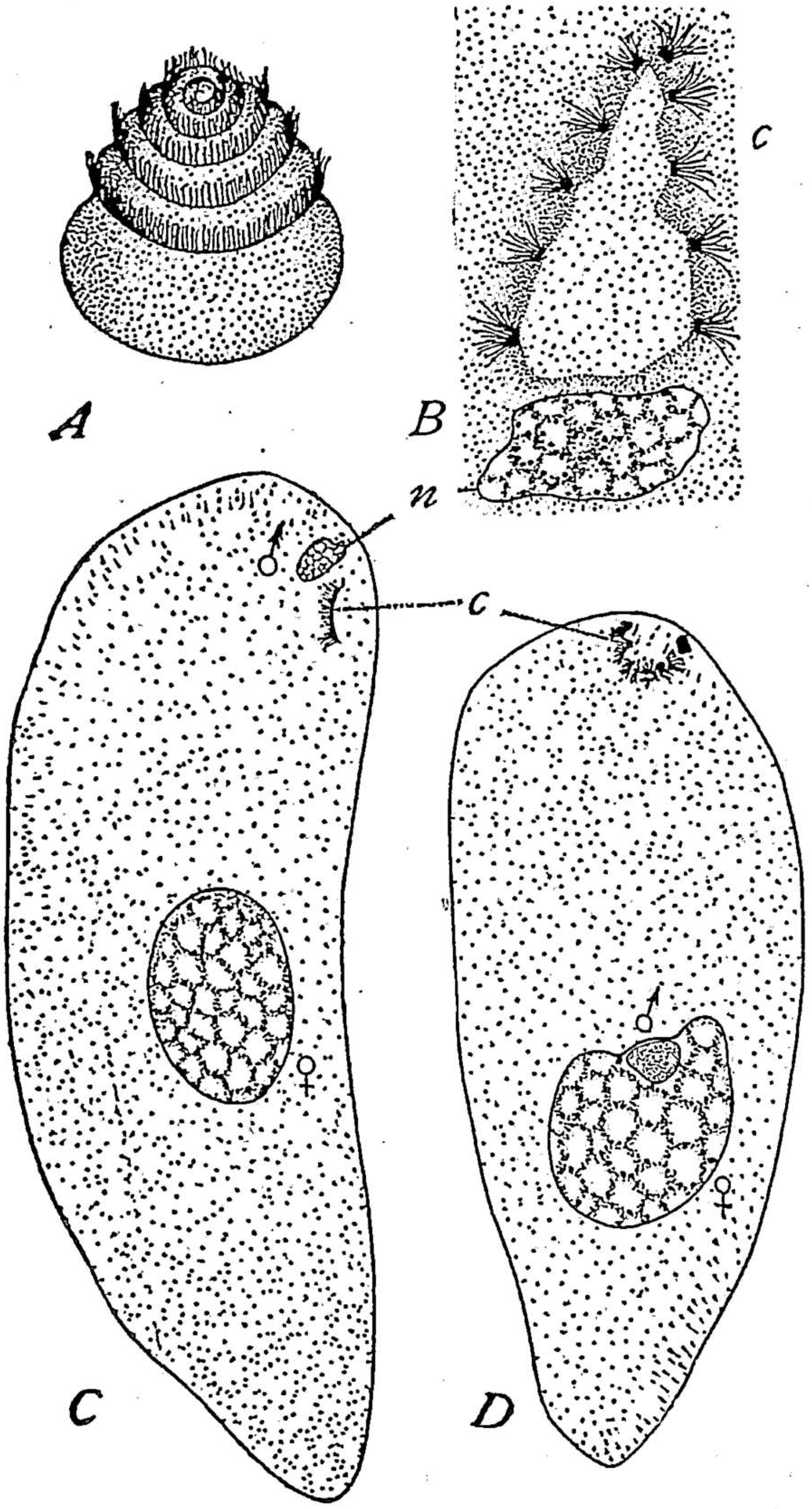

Fig. 213.—Fertilization in a cycad, *Zamia* (Webber). *A*, sperm; *B*, the same after entrance into the egg, showing nucleus (*n*) and cilia-bearing band (*c*); *C*, the ovum shortly after entrance of the sperm; *D*, union of the pronuclei, cilia-bearing band near periphery (*c*).

Since in all these cases the egg does not form polocytes or their homologues, we should expect to find fertilization similar in general type to that seen in the sea-urchin (p. 400), *i. e.*, with pronuclei differing in size and structure at the time of their union; and such is, in fact, often the case (Figs. 211, 213, 218), sometimes to an extreme degree, as in the fern (Fig. 212).

In angiosperms the sperm-nucleus is in many cases elongated and vermiform, and often has a sigmoid or spiral form. This fact, first noted in the lily by Mottier ('97), and more carefully studied by Nawaschin ('09) in several other angiosperms (*Fritillaria*, *Juglans*, *Helianthus*) led the last-named observer to conclude that the sperm-nucleus is motile, and makes its way through the embryo-sac to the egg-nucleus by its own worm-like activity. The same conclusion is reached in a more recent work on the lily by Blackman and Welsford ('13), but no evidence of this has been found in some cases [1] and probably a decisive result can only be reached by observations on living material.

In *Lilium*, according to the authors last named, both germ-nuclei at the time of their union are in a spireme-like condition (Fig. 218), but during the process of fusion (as earlier shown by Mottier) the sperm-nucleus gradually enlarges so as to become indistinguishable from the egg-nucleus (Fig. 217), and most observers have found that the paternal and maternal

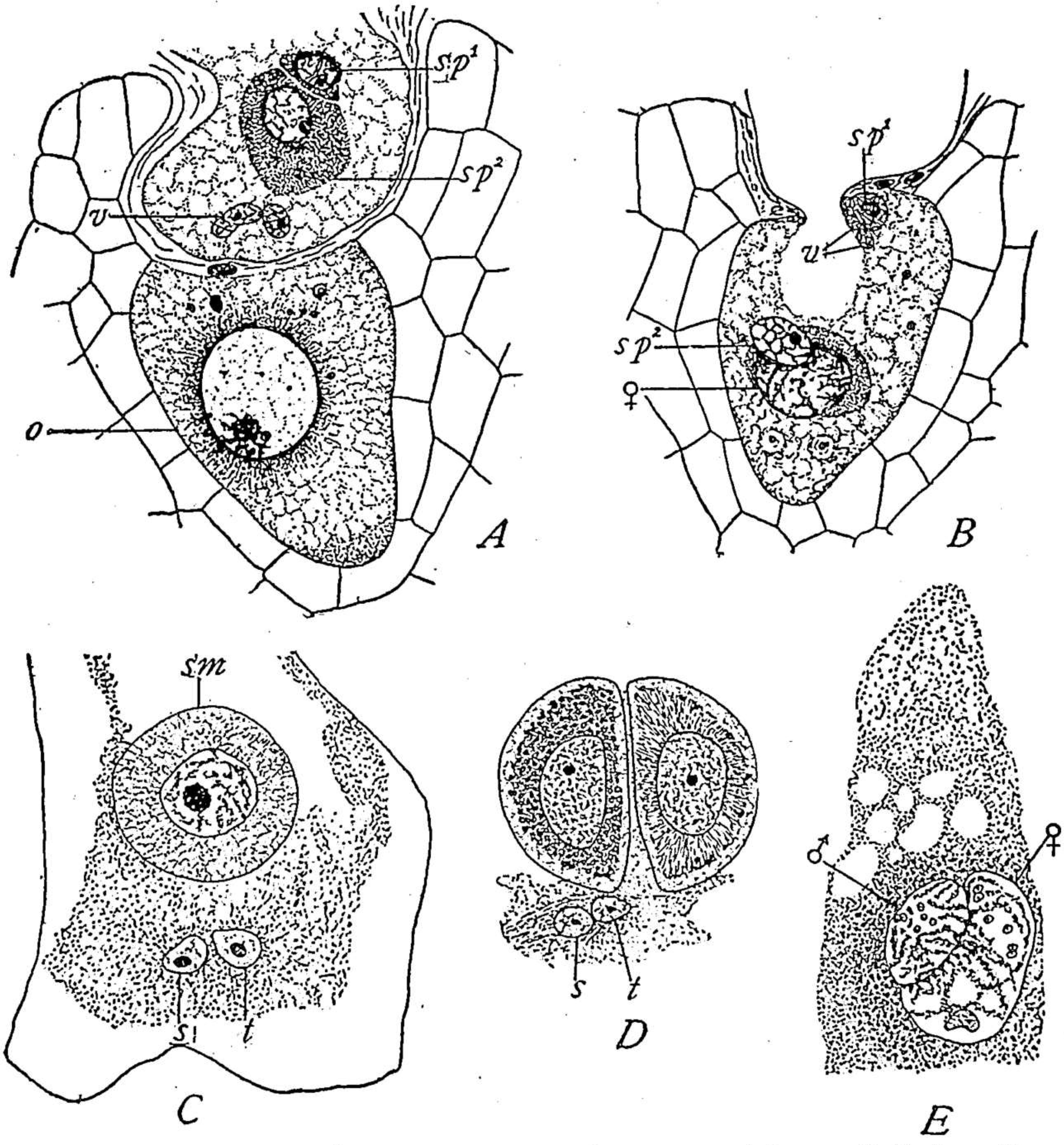

Fig. 214.—Fertilization in conifers (*A*, *B*, from Coulter and Land; *C–E*, from Coker). *A*, *Torreya*, the ovum (*o*) below, with the tip of the pollen-tube above, containing two unequal generative cells or sperm-cells (*sp*) and two vegetative cells (*v*); *B*, tip-contents discharged into the egg, conjugation of pronuclei below, second sperm-cell and vegetative cells above; *C*, *Taxodium*, tip of pollen-tube, sperm-mother-cell (*sm*) and vegetative nuclei (*s*, stalk-nucleus; *t*, tube-nucleus); *D*, two equal sperm-cells, one showing that dense sheath of starch-granules and "plastin-granules" surrounding the nucleus; *E*, conjugation of pronuclei, starch-bearing zone (from the sperm) extending around the nucleus.

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[1] *e. g.*, in *Vallisneria*, Wylie ('22).

spiremes soon become indistinguishable. Recently, however, it has been found by Nothnagel ('18) that in *Lilium martagon* and in *Trillium* the paternal and maternal spiremes remain separate and give rise to separate groups of chromosomes; and similar results were obtained by Weniger ('18). In gymnosperms there is a more marked tendency for the pronuclei to remain distinct, and in a few cases each gives rise to its own spireme before fusion;[1] such cases approximate to the condition seen in *Ascaris* or the copepods (p. 426). In *Pinus* the maternal and paternal spiremes are still distinguishable up to a rather later stage of the spindle-formation (Fig. 215); and Ferguson has found that the number of chromosomes from each nucleus may be determined as 12 before their union.

It will appear from the foregoing that in plants, as in animals, fertilization of the egg involves the union of a single sperm-nucleus with the egg-nucleus. The angiosperms are characterized by a remarkable secondary process of fertilization (if it can so be called), discovered by Nawaschin ('99) and Guignard ('99) in *Lilium*, *Fritillaria*, and *Endymion*, and other forms. It consists in a conjugation of the second generative nucleus with the two polar nuclei derived from the vegetative structures of the maternal prothallium (p. 621). This union takes place either before, during or after the fusion of the polar nuclei with each other (Figs. 217, 218); and the product constitutes the primary endosperm nucleus. Here, therefore, there is a "double fertilization" the embryo being (in Strasburger's terms) the product of a primary or "generative fertilization" of the egg; while the endosperm formerly reckoned as a purely maternal structure results from a secondary or "vegetative fertilization" of the polar nuclei. The physiological motive for the latter is unknown, though Strasburger conjectured that it might effect an activation of the endosperm, analogous to that received by the egg-cell by its union with the first generative nucleus. It is highly probable, as pointed out by DeVries ('99, '00), Webber ('00) and others, that the phenomena of xenia here find their cytological explanation.

The Cytoplasmic Structures. In plants none of the cytoplasmic components of the sperm are yet known to play any definite part in fertilization, in spite of the fact that in many of the higher forms, as in the lower ones, a considerable quantity of cytoplasm enters the ovum. This is clearly seen in cycads and Ginkgoales, where the whole sperm enters; and the same is true of those higher gymnosperms in which the sperm-nuclei are surrounded by definite cytosomes, as is shown with great clearness, for example, in *Taxodium* (Coker, '02) or *Torreya* (Robertson, '04, Coulter and Land, '05). In both these cases the generative nuclei are surrounded by well-defined

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[1] See especially Ferguson ('04) on *Pinus;* Noren ('07) and Nicholson ('10) on *Juniperus*, Miyake ('10) on *Cunninghamia*.

cytosomes, composed of finely granular protoplasm (Fig. 214). The whole contents of the terminal part of the pollen-tube are here forcibly discharged into the egg, including both generative cells, the tube-nucleus and stalk-cell-nucleus, and no doubt also part of the general protoplasm of the pollen-tube. The protoplasm of the generative cell is clearly visible after its entrance accompanies the nucleus in its advance towards the egg-nucleus, and spreads out to form an investing layer around both nuclei as they conjugate (Fig. 214, B, E); and according to Coulter and Land may still be distinguished in the 4-cell stage of the embryo. Coker shows in *Taxodium* that this material contains numerous minute starch-grains and "plastin-granules," the latter staining intensely with safranin. The question is here prominently raised whether the pollen-tube may not introduce into the egg plastids or chondriosomes, or both; thus offering another analogy to fertilization in animals (p. 435). A similar discharge of protoplasm into the egg takes place in *Pinus* (Ferguson) and probably in other gymnosperms, where the generative nucleus has no definite cytosome. In the case of angiosperms it still remains doubtful whether cytoplasm always accompanies the generative nuclei as they enter the egg, but it seems probable that such is the case. In *Vallisneria*, as recently described by Wylie ('22) both sperms are said to enter the egg in the form of complete cells, the cytosome of one of them being still present at the time the gamete-nuclei unite.

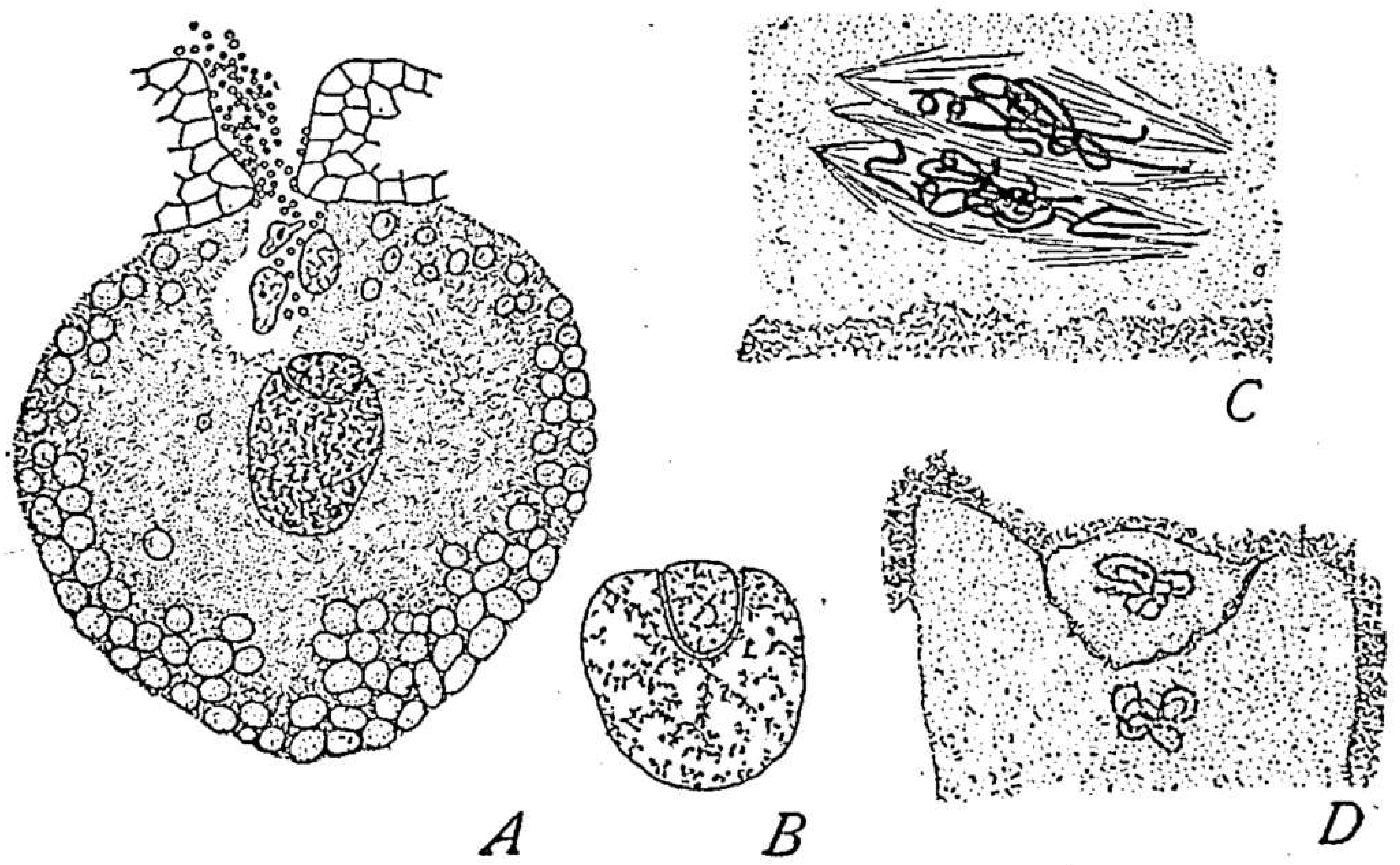

Fig. 215.—Fertilization in *Pinus* (FERGUSON).

A, the large ovum below, conjugation of the pronuclei near its center; the three small nuclei above are the second sperm-nucleus and two vegetative nuclei; *B*, more enlarged view of conjugating pronuclei; *C*, later stage of union, each pronucleus has formed its own spireme; *D*, first cleavage-spindle, maternal and paternal spiremes still distinct.

That the sperm-protoplasm as well as nucleus enters the ovum, in many higher plants, is thus well established; but little or nothing is known as to its functional significance. By analogy with fertilization in animals we are led to suspect that in plants likewise the egg is not activated by karyogamy but by some other factor; and many attempts were made by the earlier observers to trace this action to centrosomes or corresponding structures.[1] Later observations, in particular those of Mottier, Strasburger, and their followers, gradually established the conclusion that in the seed plants no centers or true asters are present in the mitotic figure at any stage of fertilization.[2] Strasburger assumed the ovum to be predominantly trophoplasmic, its main function being to manufacture and store formative material, while the sperm-cytosome is largely kinoplasmic. The relative deficiency of kinoplasm in the egg is responsible for the more or less complete inhibition of its capacity for division. The sperm overcomes this deficiency by bringing a fresh supply of kinoplasm into the egg, either in the form of individualized centers or of less definitely circumscribed kinoplasmic substance.[3] Stras-

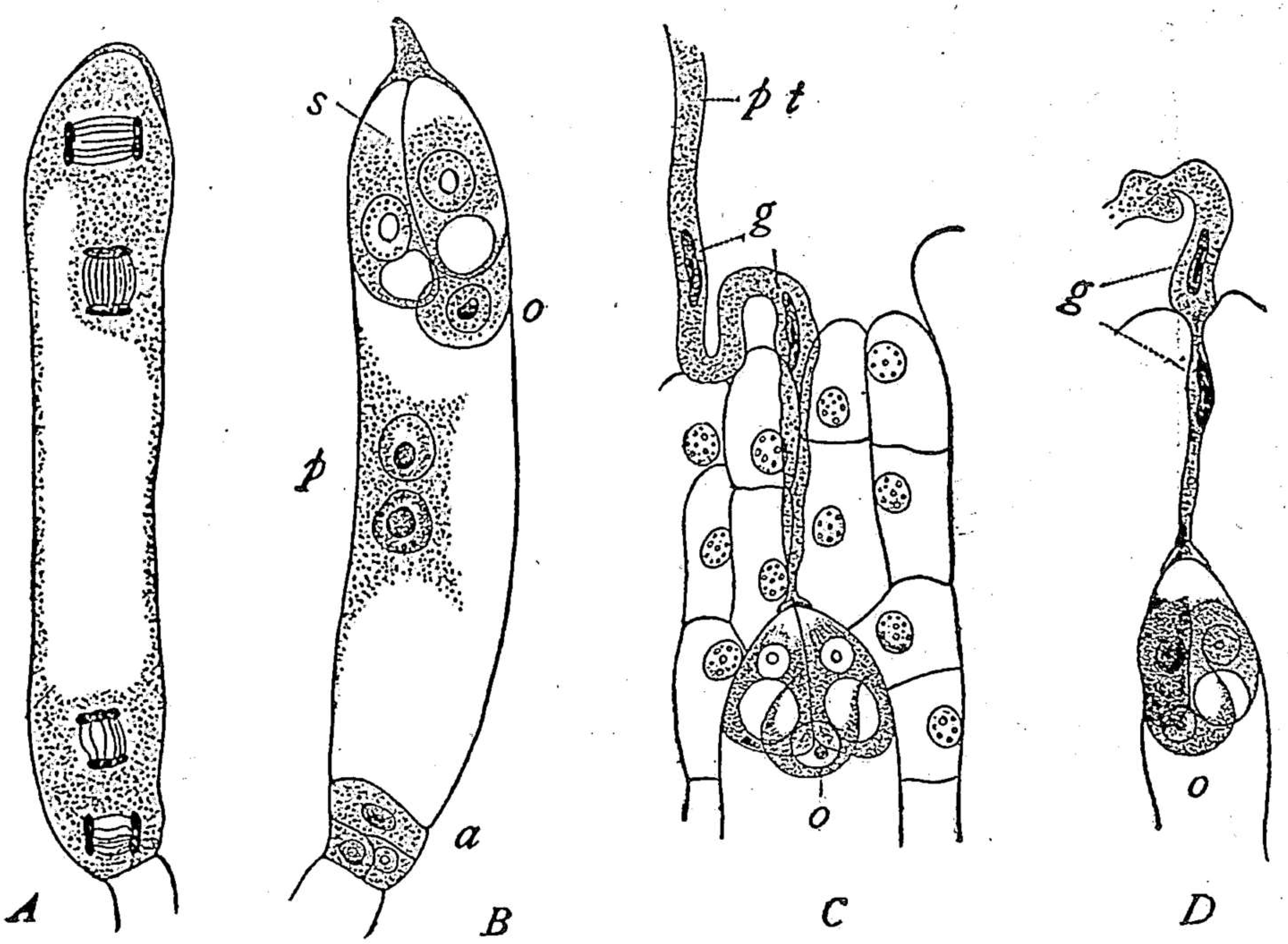

Fig. 216.—Formation of the ovum and penetration of the pollen-tube in angiosperms (STRASBURGER).

A, embryo-sac of *Monotropa*, showing the division that follows the two meiotic divisions and produces the upper and lower "tetrads"; *B*, the same, ready for fertilization, showing ovum (*o*), synergidæ (*s*), upper and lower polar cells (*p*), and antipodal cells (*a*); *C*, penetration of the pollen-tube (*p*, *t*) in *Orchis;* *o*, ovum, with synergidæ at either side, *g*, generative nuclei in the pollen-tube; *D*, slightly later stage with generative nuclei entering the micropyle.

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[1] See, for instance, Guignard ('91), who described in the lily a conjugation of paternal "centrosomes" with maternal closely agreeing with the "quadrille of centers" as depicted by Fol (p. 439).

[2] *Cf.* p. 150.

[3] '97, p. 420; see also '00, '01, etc.

burger thus sought to reconcile the apparent discrepancy between the higher plants and those forms in which definite central bodies are present (animals generally and various thallophytes such as *Fucus* or *Dictyota*). Strasburger and later Farmer and Williams ('98) showed that the fertilized egg of *Fucus* divides with well-developed asters and central bodies, and the same was found in *Dictyota* by Williams ('04) and in the bryophyte *Preissia* by Gra-

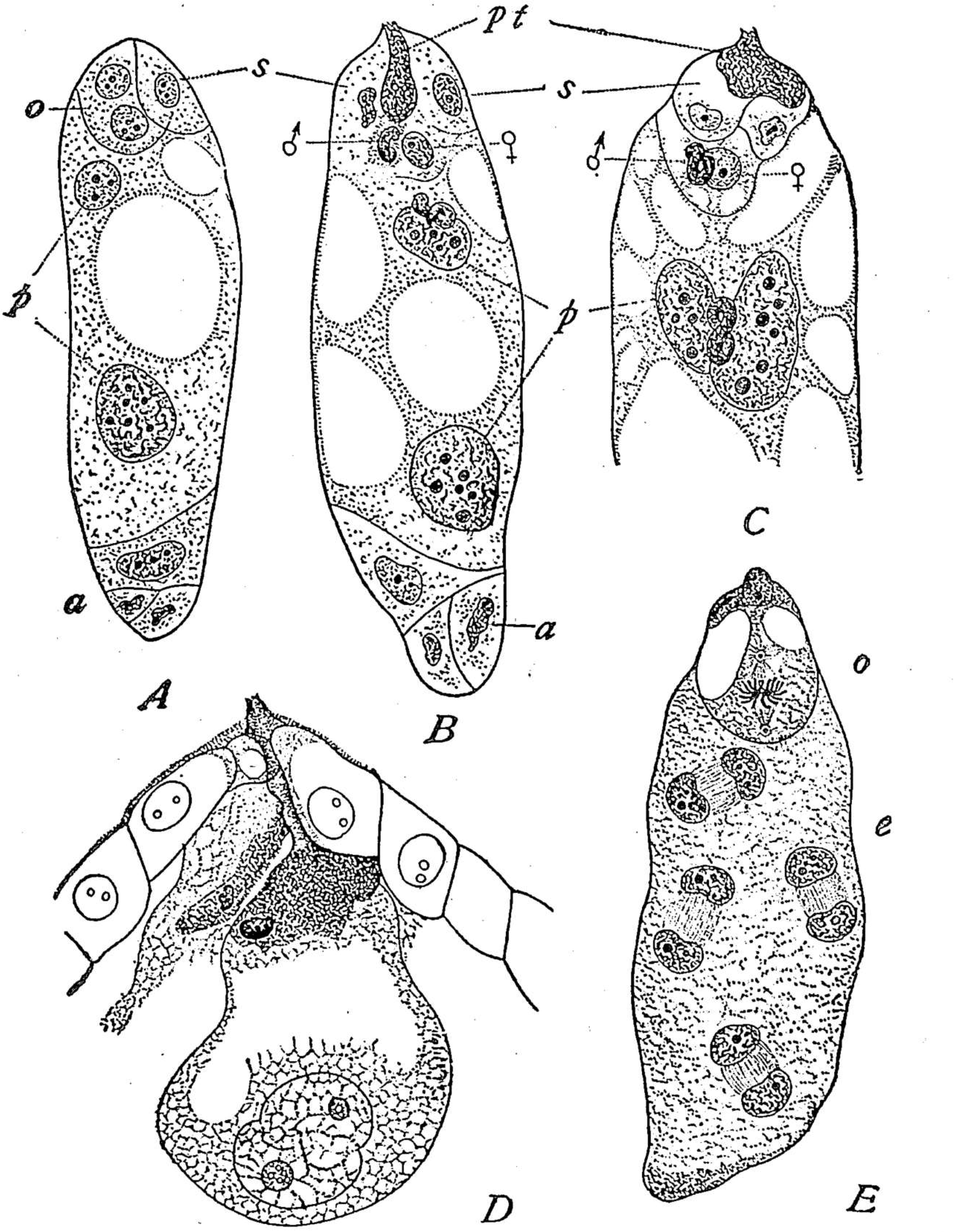

Fig. 217.—Fertilization in the lily (*D* from Mottier, the others from Guignard). *A*, embryo-sac, ready for fertilization; *B*, both generative nuclei have entered the embryo-sac; one is approaching the egg-nucleus, the other uniting with the upper polar nucleus; *C*, union of the pronuclei; below, union of the second generative nucleus and the two polar nuclei; *D*, the fertilized egg, showing fusion of the gamete-nuclei; *E*, the fertilized egg dividing; below, division of the endosperm-nuclei; *a*, antipodal-cells; *o*, the oösphere or ovum; *p*, polar nuclei; *p, t*, pollen-tube.

ham ('18). Williams found, further, that in *Dictyota* the parthenogenetic egg divides without asters or centers, the spindle being primarily multipolar and intra-nuclear, while the fertilized egg develops a single well-marked central body and aster at the side of the nucleus which (apparently) divides into two to form a bipolar figure. None of these observers, nevertheless, were able to trace any connection between this central body and the sperm; and in point of fact Farmer and Williams concluded that in both cases the centers are formed *de novo*. We are thus confronted with a situation not unlike that offered by the status of Boveri's theory in animals, save that the evidence in plants is less complete.

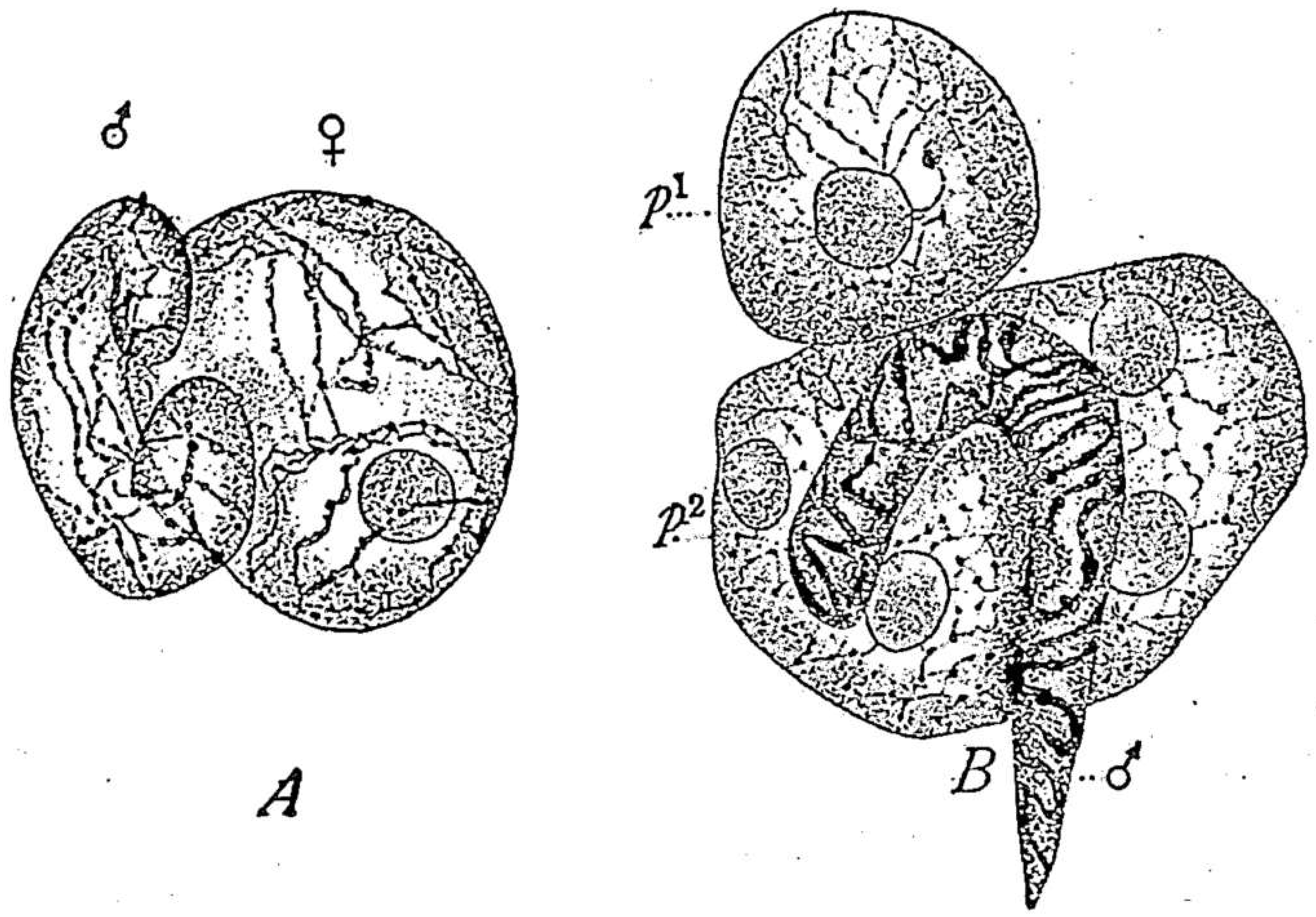

Fig. 218.—Double fertilization in the lily (Blackmann and Welsford).
A, conjugation of one sperm-nucleus (♂) with the egg-nucleus; *B*, conjugation of second sperm-nucleus (♂) with the two polar nuclei (p^1, p^2).

III. TRANSITIONAL CONDITIONS

We may here conveniently consider a group of phenomena which in respect to the activation of the egg by the sperm agree with fertilization, but in certain cytological respects approach the condition seen in parthenogensis.

1. Partial Fertilization

In normal fertilization, as earlier indicated (p. 396), the sperm-aster (or amphiaster) always leads the way in the march of the sperm-nucleus towards the egg-nucleus, sometimes far in advance. Boveri ('88a) discovered in sea-urchin eggs (*Echinus*) that occasionally the sperm-nucleus may be left entirely behind, the aster alone uniting with the egg-nucleus. In such cases the aster and the egg-nucleus divide normally, and the first cleavage of the egg takes place without participation of the sperm-nucleus

Karyogamy first takes place, as a rule, in the 2-cell stage, when the sperm-nucleus conjugates with the nucleus of one cell, and such eggs may continue their development at least as far as the blastula-stage. To this phenomenon (of course pathological in character) Boveri gave the name of *partial fertilization*, and saw in it fresh proof that fertilization of the eggs is not dependent on karyogamy, but on the sperm-center as the immediate agent of cleavage. Boveri found that in some cases karyogamy may be delayed until the 4-cell or 8-cell stage; and this is confirmed by Teichmann ('03), who has made a more extended study of the phenomenon (Fig. 219).

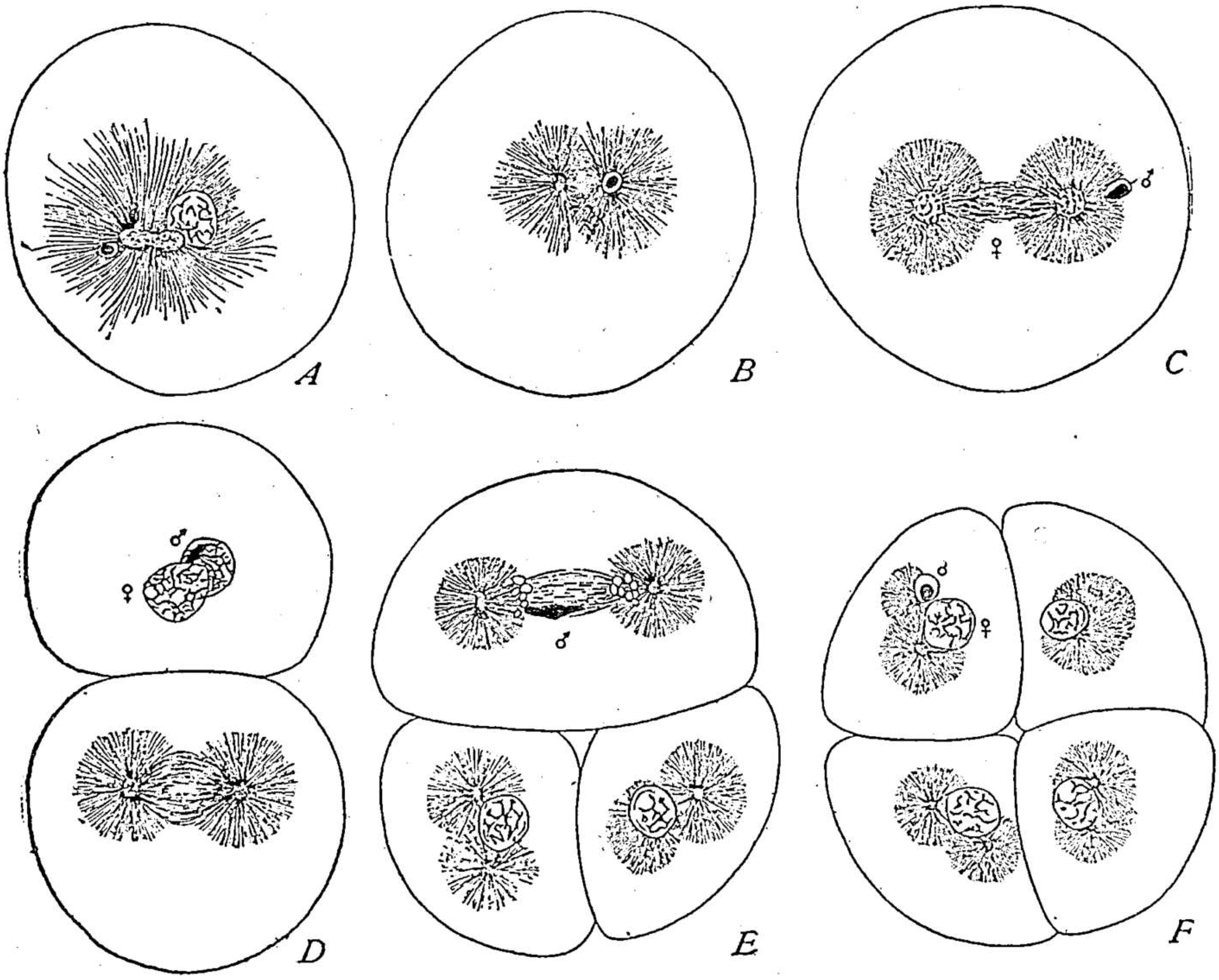

Fig. 219.—Partial fertilization in the sea-urchin *Echinus* after exposure of the sperms to weak KOH solution (Teichmann).

A, approach of pronuclei, sperm-aster dividing; *B*, egg-chromosomes below, sperm-nucleus and amphiaster above; *C*, anaphase, sperm-nucleus near one pole; *D*, 2-cell stage, conjugation of sperm-nucleus with cleavage-nucleus; *E*, late second cleavage, sperm-nucleus lagging on the upper side; *F*, 4-cell stage, with sperm-nucleus still separate, three cells with maternal nuclei only.

Since some of the nuclei in such embryos are purely maternal and others of biparental origin, we should expect to find the former of smaller size and dividing with the haploid number of chromosomes (9 in *Echinus*), the latter with the diploid or possibly a higher number; and such appears to be the case, though exact data are still lacking, in the pure-bred larvæ. In hybrids between *Sphærechinus* and *Paracentrotus* Herbst has observed a closely analogous case in which the nuclei are of two sizes and in some cases show small (maternal) nuclei on one side of the larva, and large (hybrid) nuclei on the other. This case, as will later be shown (p. 968), affords important evidence concerning the determinative action of the chromosomes in heredity.

2. Gynogenesis

In partial fertilization *sensu stricto* karyogamy still takes place, and the paternal nucleus takes part in development, though in more or less restricted degree. In a nearly related phenomenon, which may be called *gynogenesis,* the sperm penetrates (and in some cases activates) the egg but otherwise takes no part in the processes of development. Most such cases are pathological, but two remarkable cases have been made known in nematodes in which the phenomenon appears to be normal. In *Rhabdites aberrans* (Krüger, '13) the eggs are produced by individuals having the aspect of females, but producing sperms as well as eggs, and self-fertilizing; true males also exist, but are of extreme rarity. The eggs here seem to be set, as it were, for the diploid type of parthenogenesis, producing but one polar body without reduction of the chromosome-number (18). These eggs are *regularly penetrated by the sperm* which, however, takes no part in the development, degenerating *in situ* without any fusion with the egg-nucleus. In this case it is uncertain whether entrance of the sperm is necessary for activation of the egg, since in some cases no sperm-nucleus could be found.

In the closely similar case of *Rhabdites pellio,* on the other hand, *the eggs fail to develop unless penetrated by the sperm.* This species is normally diœcious, with males and females in nearly equal numbers. In a culture of this species P. Hertwig ('20) found a mutant that produced only females which, together with their offspring, showed the same cytological behavior as *R. aberrans* (Fig. 220), forming but one polocyte without reduction and developing with the diploid number (14). Here, however, development fails without entrance of the sperm. Both these cases are analogous to the parthenogenetic aphid or rotifer except that in one of them at least the eggs require activation by the sperm. The two cases thus offer, in the words of Brachet ('17) "a veritable bridge set up by nature between fertilization and natural parthenogenesis."

All other known cases of gynogenesis are pathological, occurring either in heterogeneous or incompatible crosses, or in cases where the sperm has been experimentally incapacitated to a certain extent by the application of external agents. An example of this is the activation of toads' eggs (*Pelodytes, Bufo*) with the sperms of a urodele (*Triton*) observed by Bataillon ('06, 09) in which the sperm traverses the envelopes and penetrates the egg-periphery but takes no other visible part in the development, and in *Pelodytes* remains near the surface without completely entering the egg. In neither case does the sperm-nucleus unite with the egg-nucleus, nor can a sperm-aster at any time be seen. Nevertheless, as a result of the action of the sperm the egg expels the second polocyte, the egg-nucleus re-forms, moves toward the center and there gives rise to the first cleavage-figure. Cleavage, in this particular case, is slow and irregular and soon comes to an end; but in others, presently to be described, may lead to the production of nearly or quite normal tadpoles.[1]

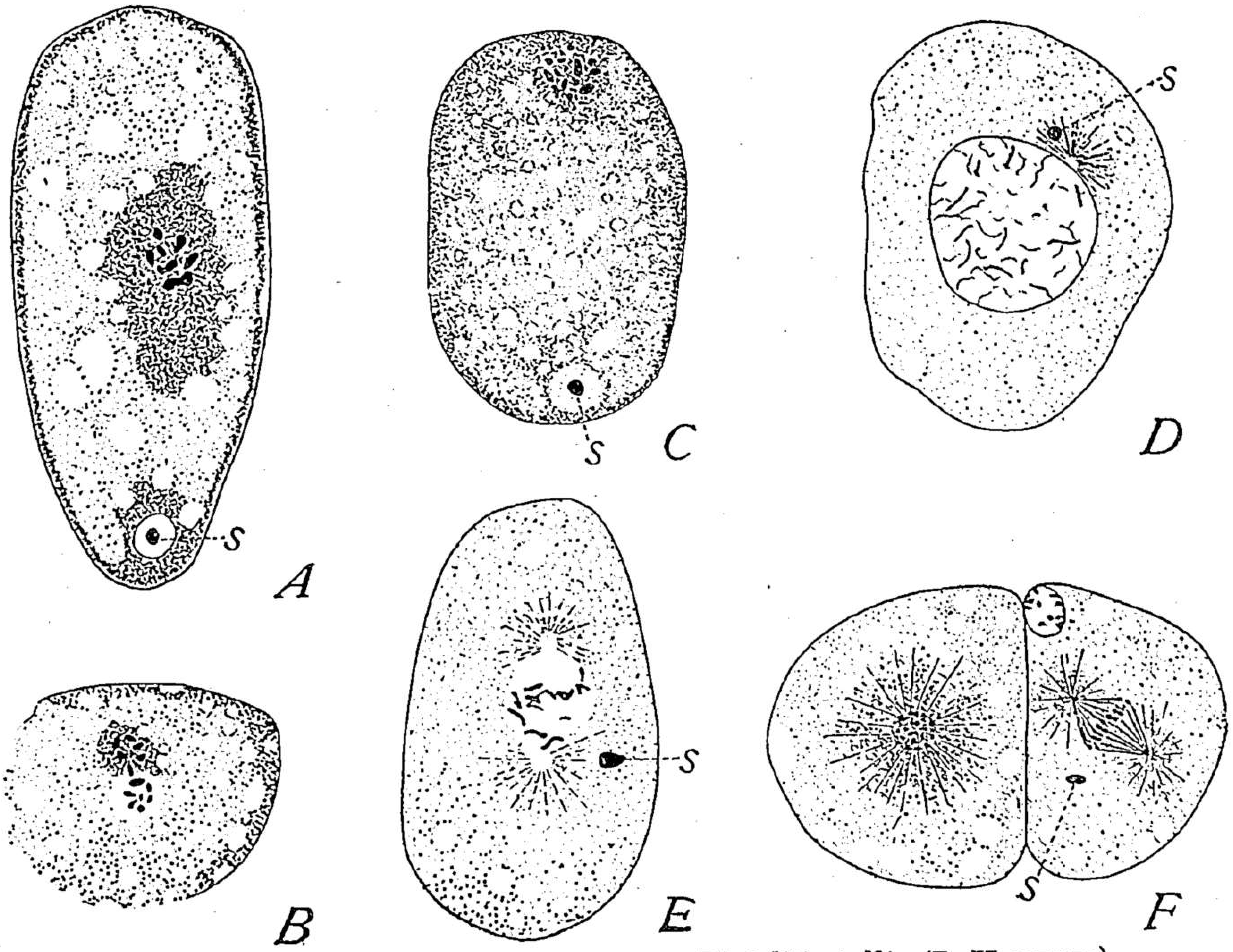

Fig. 220.—Gynogenesis in the nematode *Rhabditis pellio* (P. Hertwig).

A, normal sexual egg showing above the 1st polar metaphase with 7 bivalents (the haploid number), sperm-nucleus at *s*; *B*, anaphase of first polar mitosis; *C*, corresponding stage of gynogenetic diploid egg, showing above the 1st polar metaphase with 14 univalents; *D*, *E*, prophases of the first cleavage of such an egg; *F*, 2-cell stage of same, with sperm-nucleus at *s*.

Gynogenesis in various modifications has been observed in many other heterogeneous crosses (p. 970). Among such crosses have been found many transitions between gynogenesis and partial fertilization, the sperm-nucleus in some cases remaining wholly passive, in others conjugating with the egg-nucleus followed by a partial or complete elimination of the sperm-

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[1] Bataillon considered this phenomenon as a true process of parthenogenesis, and the same term is applied by P. and R. Hertwig to the analogous radium gynogenesis described below. If, however, we adhere to our definition of fertilization as the activation of the egg by the sperm (p. 394) it is evident that the term parthenogenesis cannot properly be applied to such cases.

chromatin at a later period of development. Such cases have afforded important genetic data bearing on the relations between heredity and the chromosomes (p. 965). Bataillon's initial observations on Amphibia have been much extended by the studies of the Hertwigs [1] on the so-called "radium parthenogenesis" in amphibians and echinoderms, and on hybrids in fishes and amphibians. O. Hertwig showed that when the sperms of frogs and toads have been exposed to radium emanations in a certain degree they may still be able to activate normal eggs, but development is more or less delayed or abnormal. The same observer, confirmed by G. Hertwig in the case of echinoderms, found the abnormality to be much less marked after prolonged exposure of the sperm to radium than after a much shorter exposure. This paradoxical result was explained by the assumption that after shorter exposures the sperm-nucleus, though injured by the radium emanations, is still able to conjugate with the egg-nucleus and to take part in cleavage and development; but abnormalities result because of the pathological influence of the affected sperm-chromatin. Longer exposures, on the other hand, kill the sperm-nucleus or render it incapable of taking part in the development. The egg, therefore, while still activated by the sperm, develops with only the normal maternal chromosomes. This explanation was borne out by cytological studies. In the sea-urchin G. Hertwig found that after prolonged treatment of the sperm the sperm-nucleus often fails

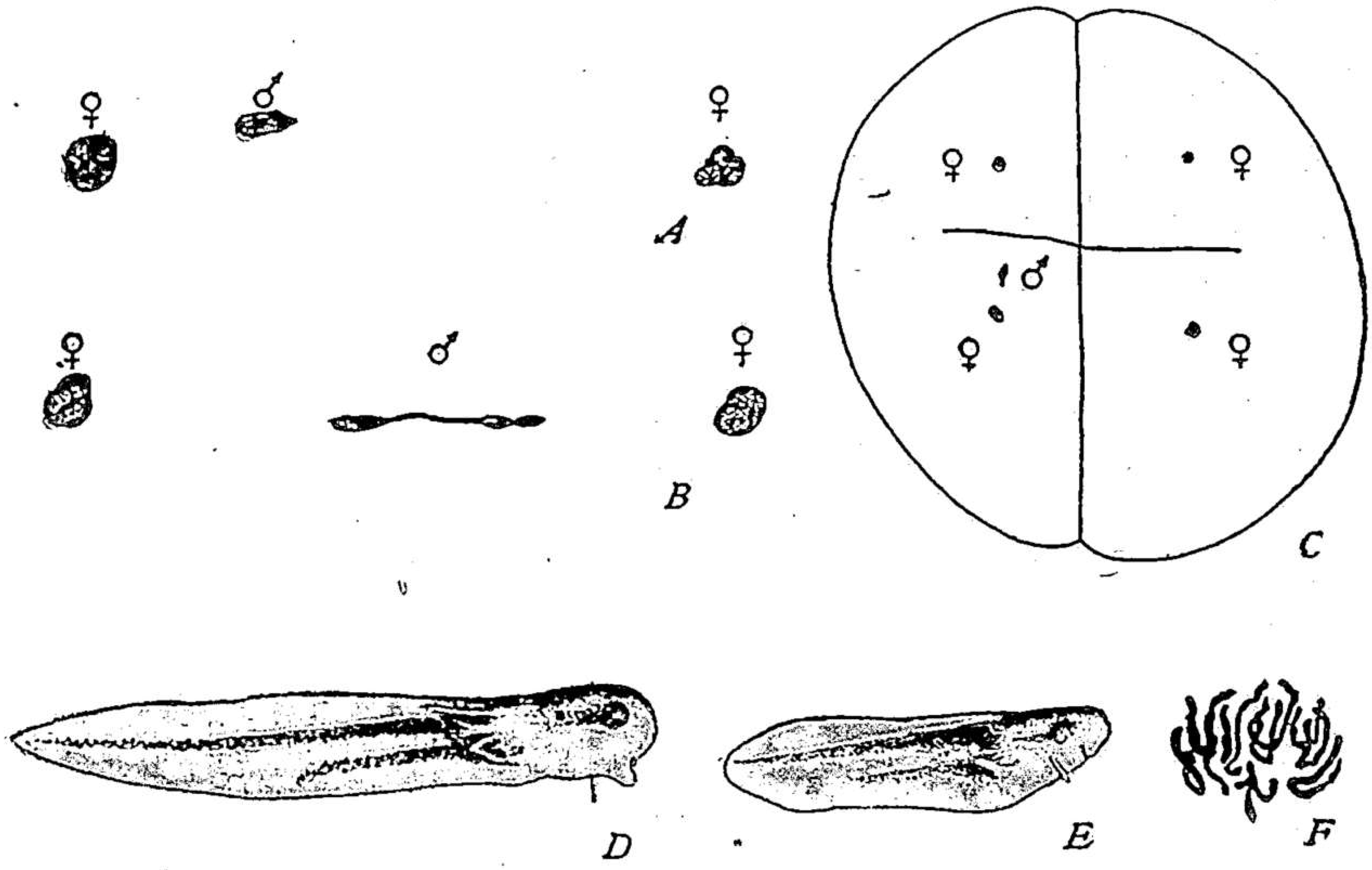

Fig. 221.—Gynogenesis in eggs of Amphibia fertilized by sperm exposed to radium (*A–C*, from P. Hertwig; *D–F*, O. Hertwig).

A, *B*, first cleavage telophases of frog, lagging sperm-chromatin at ♂; *C*, 4-cell telophase, sperm-chromatin at ♂; *D*, normal tadpole of *Triton*, 17 days; *E*, gynogenetic tadpole of same age; *F*, haploid chromosome-group from tail epidermis of such a larva (from a photograph).

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[1] O. Hertwig, '10, '11, '13; G. Hertwig, '11, '12, '13, '18; P. Hertwig, '13, '16, '17, '20.

to unite with the egg-nucleus, remaining passively in the protoplasm, sometimes near the equator, sometimes near one pole. In either case the egg-nucleus divides normally, with the haploid number of chromosomes. The sperm-nucleus often degenerates (gynogenesis in the strict sense), but there is evidence that in some cases it may later fuse with one of the cleavage-nuclei (partial fertilization). Many cases were also found in which the sperm-nucleus completely fuses with the egg-nucleus; but in such cases cleavage is of a very abnormal character.

In Amphibia the phenomena are of the same type. O. Hertwig ('13) showed that after intensive radiation of the sperms of *Triton* the fertilized eggs might give rise to nearly or quite normal tadpoles, in which, at the age of 24 days, the dividing epidermis-cells clearly showed the haploid number of chromosomes, 12 (Fig. 221). In the frog, similarly treated, P. Hertwig ('13) found that during the first cleavage only the egg-nucleus divides (presumably with the haploid number of chromosomes) while the sperm-nucleus is left behind near the equator and may be seen as a clump of chromatin in the 2- and 4-cell stages. No fusion with the egg-nucleus at any stage could be detected. "The entrance of the sperm here operates in the same manner as puncture by a fine needle in Bataillon's experiments." [1]

Further studies on hybrid amphibians and fishes,[2] in some cases preceded by radium treatment of the sperms, have yielded many other interesting results that can here be only briefly reported. Some of these crosses, such as *Rana arvalis* ♀ × *fusca* ♂, *Bufo communis* ♀ × *viridis* ♂, and *Triton tæniatus* ♀ × *cristatus* ♂, produce true hybrids as proved by the characters of the offspring; and G. Hertwig has shown in the first of these hybrids that the nuclei are as large as those of normal (pure-bred) larvæ and hence presumably diploid. In other crosses the nuclei are for the most part only approximately half the normal size and hence presumably haploid; examples of such crosses are *Bufo viridis* ♀ × *Hyla arborea* ♂ (with or without preceding radium treatment of the sperm) and *Bufo communis* ♀ × *Pelabates fusca* ♂. The larvæ thus produced are noteworthy both for their small nuclei and slow and often abnormal and dwarfed development. Others however—and this is the striking fact—are of normal size and development and have nuclei of normal size (hence presumably diploid). In some of these crosses the same result appears after intensive radiation of the sperm, a treatment which as shown by O. and P. Hertwig, the sperm-nucleus is killed. The conclusion seems probable, therefore, that even in such (presumably) diploid larvæ development is gynogenetic, the diploid number

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[1] P. Hertwig, p. 178.

[2] See especially O. Hertwig, '10, '11, '13; G. Hertwig, '13, '18; P. Hertwig, '11, '12, '13, '18; P Hertwig, '13, '16, '17, '20; G. and P. Hertwig, '14; Oppermann, '13.

having been restored by a doubling of the maternal haploid group. G. Hertwig's studies, especially of the cross *Rana esculenta* ♀ × *Bufo viridis* ♂ show that such diploid larvæ arise from eggs in which the first cleavage stages are notably delayed as compared with the haploid ones; this author finds reason herein for the conjecture that the diploid number may be restored by a monocentric mitosis (p. 168) occurring before cleavage begins.

The slow and often dwarfed development of the haploid larvæ is ascribed by G. and P. Hertwig to the disproportion between the nuclear and the cytoplasmic volume of their cells, raising the question whether such larvæ are capable of complete development unless the diploid number be restored by a compensatory doubling.[1] To this question we shall later return.

3. Androgenesis

By this term we may designate the activation of the egg by the sperm followed by development without the participation of the egg-nucleus.

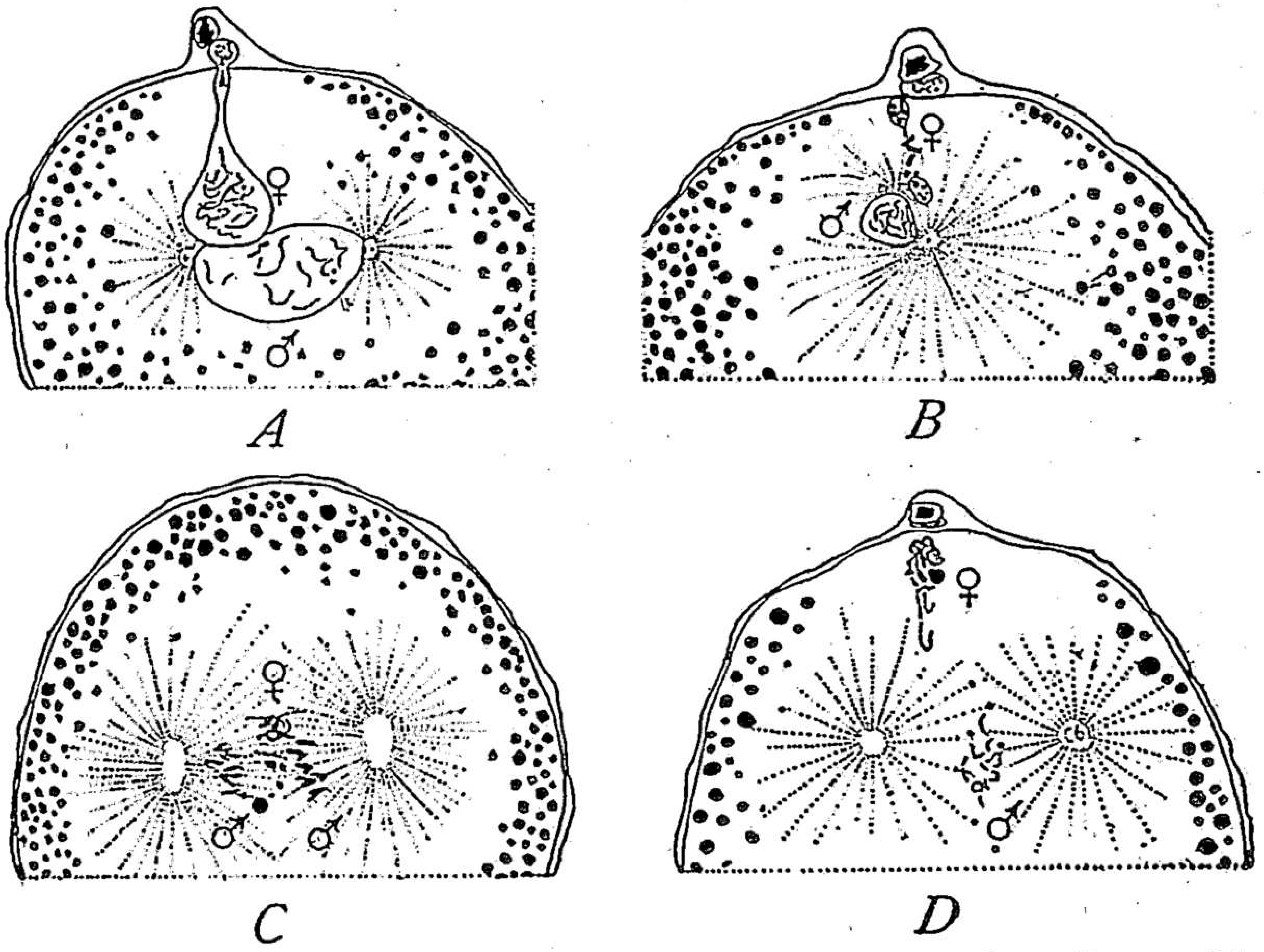

Fig. 222.—Androgenesis in *Chætopterus* after treatment of the eggs by radium and fertilization by normal sperm (Packard).

A, abnormal karyogamy in progress; *B*, defective formation of the egg-nucleus (♀) after second polar mitosis; *C*, maternal chromosomes (♀) lagging and degenerating on the spindle; *D*, maternal chromosomes wholly eliminated.

G. Hertwig ('11, '13) first demonstrated this phenomenon in the eggs of frogs or toads that were first treated by radium and then fertilized by normal frog-sperm. The result is closely similar to that of the converse experiment in which the normal egg is fertilized by the radiated sperm. As in the latter

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[1] See especially P. Hertwig, '20, Nachtsheim, '21.

case, development is more or less disturbed or abnormal, more so after shorter or less intensive treatment of the egg than after longer or more intensive; and in the latter case the nuclei of the resulting larvæ are markedly smaller than in the former. Hertwig therefore concluded, though without further cytological evidence, that in this case the egg-nucleus is incapacitated, and that the nuclei of the embryo are solely of paternal origin, *i. e.*, descendants of the sperm-nucleus alone. This process is designated as "androgenetic" or "merogonic" development ('13, p. 119); but as will presently be shown such a use of the word "merogonic" is hardly justifiable.

Analogous results were reached by Packard ('18) on radiated eggs of the annelid, *Chætopterus*, fertilized by normal sperm. Sections of these eggs show that already during the formation of the polocytes marked abnormalities occur in the history of the egg-chromatin and that the resulting egg-nucleus sooner or later degenerates without taking part in cleavage, while the sperm-nucleus and sperm-asters go through their normal transformations and form the first cleavage-figure and its successors (Fig. 222). The nuclei of the embryo, accordingly, are of purely paternal origin, and of haploid constitution, showing at each division 9 chromosomes instead of the diploid number, 18.[1] As in the foregoing case it is unknown whether such larvæ may develop into the adult stage.

4. Merogony

The climax is reached in the phenomena of *merogony* (Delage, '01) or the development of an egg-fragment *devoid of a nucleus* fertilized by a normal sperm.[2] This was discovered by O. and R. Hertwig ('87), who found that the eggs of sea-urchins may readily be shaken to pieces which quickly round up to a spheroidal form and may be fertilized by the sperm as if they were whole eggs. Some of the fragments are nucleated, others non-nucleated; but both kinds alike may be penetrated by the sperm, throw off fertilization membranes and undergo development. Boveri ('89, '95, etc.) and later observers demonstrated that such embryos may even develop into perfectly formed dwarf larvæ, in some cases not more than one-fourth the normal volume (Fig. 459). Boveri showed that non-nucleated egg-fragments of one species might be fertilized by the sperm of a different species, an experiment of remarkable interest to students of heredity. A possible source of error in some of these experiments, as shown in a posthumous paper by Boveri ('14) is the fact that in the operation of shaking

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[1] A nearly similar process was described by Goldschmidt ('12) in normal eggs of the evening primrose, *Œnothera biennis* when fertilized by pollen of *Œ. muricata;* but in a later work ('16) this conclusion is withdrawn.

[2] This name would seem to apply etymologically equally well to the fertilization of a nucleated egg-fragment, but the term has not been generally employed in this sense.

the eggs to pieces the egg-nucleus may collapse so as to become invisible in the living material, though sections show that it may still give rise to a group of chromosomes. Such an error is excluded if the eggs be cut singly with a knife into two halves and both fertilized. In nemertines, where this experiment may readily be performed, both the nucleated and the enucleated fragment may upon fertilization undergo development; and the same is true in *Dentalium* (pp. 405, 1065).[1] Merogony was demonstrated in the alga *Cytosira* by Winkler ('01); and more recently Spemann ('14) and Baltzer ('21) found it to be possible even in vertebrates (*Triton*).

Merogonic embryos or larvæ are remarkable for the fact that their cytoplasm is of maternal origin, their nuclei (and possibly also their central bodies) of paternal, in this respect agreeing with the androgenetic larvæ described in the preceding section. As in the latter case, we should expect them to develop with the haploid number of chromosomes and with much smaller nuclei than in case of normal larvæ; and in sea-urchins such is the fact, as shown especially by the work of Boveri ('95, '05), Morgan ('96) and other observers. Boveri showed that dwarf larvæ derived from nucleated fragments have at first nuclei as large as those from whole eggs, thus contrasting in a striking way with the merogonic larvæ from non-nucleated fragments. Later, however, this difference is equalized in the process of cleavage, by which the normal karyoplasmic relation is restored (p. 728).

In the newt (*Triton*) Spemann ('14, '19) ingeniously obtained merogonic larvæ by cutting the fertilized egg in two by means of a noose formed by a fine hair. This egg is normally polyspermic (p. 416), one sperm-nucleus conjugating with the egg-nucleus while the others remain apart and sooner or later degenerate. It thus becomes possible to cut in two the fertilized but still unsegmented egg in such a manner that one half contains the fusion-nucleus, the other a single sperm-nucleus.[2] Both fragments may develop into tadpole larvæ; and Baltzer ('22) was able to rear one such haploid merogonic larva to a period (100 days) when the metamorphosis was already beginning. The nuclei of this larva, as was to be expected, were found to be only half the size of corresponding diploid larvæ from nucleated fragments.

5. Summary

The various cases reviewed above clearly demonstrate that fertilization, *i. e.*, activation of the egg by the sperm, does not depend upon karyogamy or a union of gamete-nuclei but is a reaction between egg and sperm that

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[1] Wilson, '03, Yatsu, '04, '10, Zeleny, '04.

[2] The approximate position of the egg-nucleus in these eggs is indicated by the polar body, while the entrance points of the sperms are seen as dark spots on the surface of the egg. The plane of section can thus be determined during the operation.

may take place in the presence of only a single haploid nucleus. Genetically the facts have an important bearing on the chromosome theory of heredity, for they offer the probable explanation of "false hybrids" (Millardet) which show the characters of one parent only. As such, for example, G. Hertwig designates the gynogenetic larvæ from the cross *Rana esculenta* ♀ × *Bufo viridis* ♂, which show only the color of the mother.

In the same category belong those cases of hybrids in which some or many of the sperm-chromosomes degenerate without taking part in the latter development (*e. g.*, in sea-urchins, p. 843), and which in consequence show a more or less pronounced matriclinous heredity. Such cases should not be confused with those of simple Mendelian dominance; for the eliminated chromosomes represent lost characters which presumably cannot reappear in the offspring of such hybrids.[1]

IV. PARTHENOGENESIS

True parthenogenesis differs from all the foregoing cases in that activation of the egg is effected by some agent other than the sperm. It may conveniently (though not very logically) be divided into *natural parthenogenesis*, which forms a normal part of the life-history as it occurs in nature, and *artificial* or *experimental parthenogenesis* in which the egg is artificially activated by laboratory methods.

1. Natural Parthenogenesis

Natural parthenogenesis is of two clearly marked types, which differ in respect to the number of the chromosomes and may be designated respectively as *diploid* and *haploid*. Of these the diploid type is most frequent, being characteristic of aphids, phylloxerans, daphnids, ostracodes and some other animals, and occurring occasionally in many others, such as the phyllopods, orthopterans, lepidopterans, trematodes, echinoderms and nematodes. Haploid parthenogenesis is characteristic of many Hymenoptera (bees, ants, wasps) and is also found in some Hemiptera and arachnids; while both types occur in the rotifers and the gall-flies. The two types are connected by certain transitional cases in which development begins with the haploid number of chromosomes but later becomes diploid. The best known of these cases occur in the Lepidoptera and Hymenoptera (p. 803) and are paralleled by the conditions found in certain cases of artificial parthenogenesis (p. 476)

The haploid parthenogenetic egg is a sexual egg which undergoes complete reduction and is capable of fertilization, but may develop without any process of syngamy. Such eggs most commonly develop with the haploid

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[1] *Cf.* p. 965. See also Sutton ('03).

number of chromosomes, and in this case so far as known always produce males, which are themselves of haploid constitution (as in case of the drone bee, p. 794). In diploid parthenogenesis the egg typically undergoes no general reduction and hence develops with the diploid number of chromosomes (Fig. 223). These eggs, exemplified by the parthenogenetic broods of aphids, rotifers or daphnids, are in a certain sense asexual, and so far as known are incapable of fertilization; and even if fertilization took place the product would be a triploid and hence abnormal zygote.[1] The cytological evidence indicates that eggs of the diploid type were originally sexual eggs, capable of fertilization and of undergoing a process of complete reduction (p. 793); but the two types as they now exist have become widely different in nature and belong to different phases of the life-history. In the rotifers and gall-flies, the two types of eggs are produced by different generations of females, which in the gall-flies often differ in constitution, external structure and habits, so that a true alternation of generations exists. In the aphids and phylloxerans, likewise, the sexual forms often differ markedly from the preceding parthenogenetic females, but the sexual egg, so far as known, is here incapable of parthenogenesis.

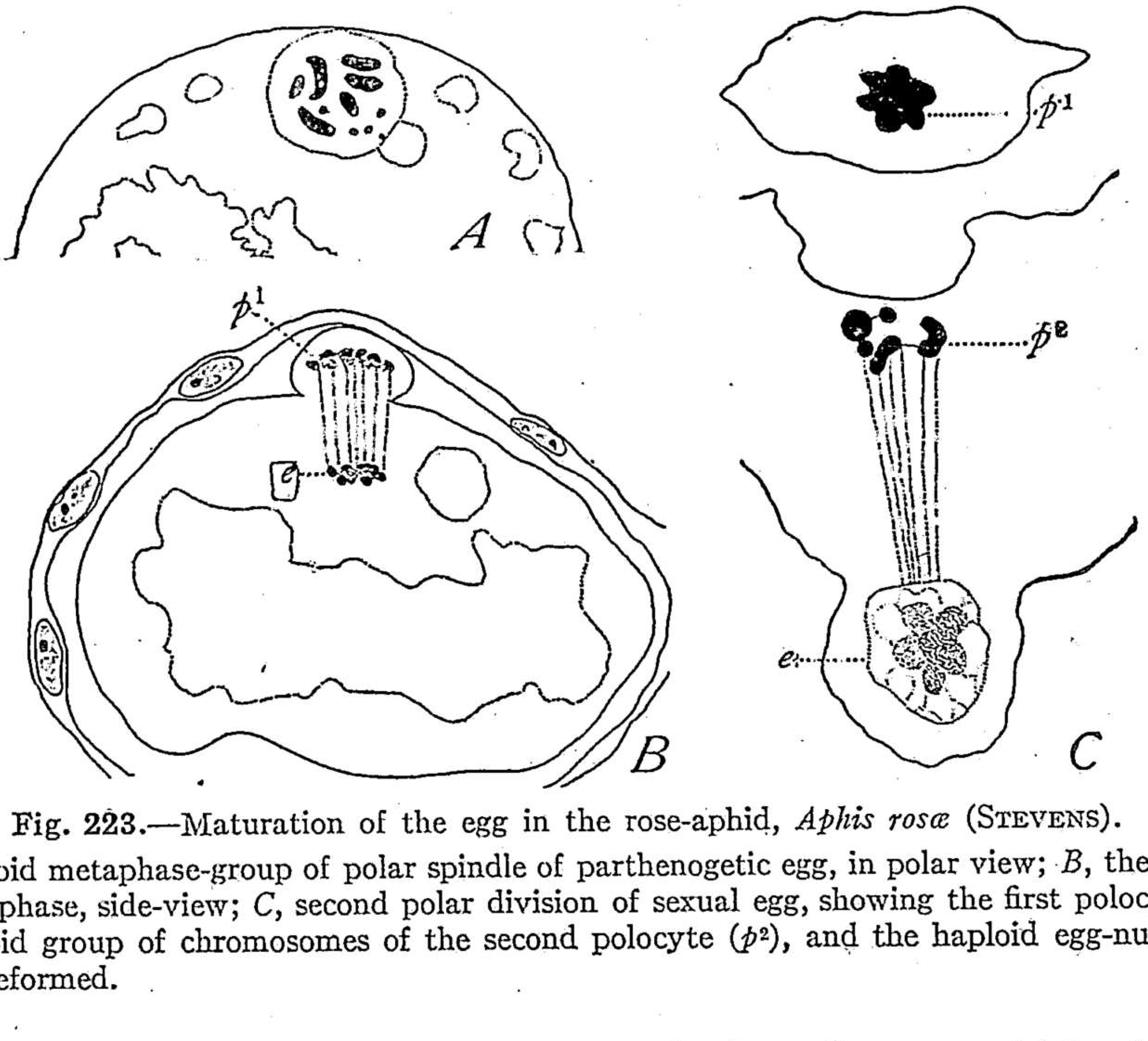

Fig. 223.—Maturation of the egg in the rose-aphid, *Aphis rosæ* (Stevens).

A, diploid metaphase-group of polar spindle of parthenogetic egg, in polar view; *B*, the same in early telophase, side-view; *C*, second polar division of sexual egg, showing the first polocyte (p^1), the haploid group of chromosomes of the second polocyte (p^2), and the haploid egg-nucleus (*e*) already reformed.

In animals the two types are generally distinguishable externally by the number of polocytes formed during maturation; eggs of the diploid type

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[1] See, however, the exceptional case of *Rhabditis*, p. 460.

typically produce but one polocyte, those of the haploid type, two. This difference was known long before the underlying internal differences were determined.[1] That no reduction in the number of chromosomes occurs where but one polocyte is formed was determined in the ostracodes by Woltereck ('98) and Schleip ('09), in the rotifers by Lenssen ('98) and especially by Whitney ('09), and in aphids, Stschelkanocew ('04), and Stevens ('05).[2] In all these cases the egg segments with the diploid number of chromosomes like a fertilized egg. In one or two somewhat doubtful cases diploid parthenogenesis is said to follow upon a suppression of both meiotic divisions, *e. g.*, in the gall-fly *Neuroterus* (Doncaster, '10, '11).

In higher plants parthenogenesis is not uncommon, but without careful study is often difficult to distinguish from the widespread process of vegetative apogamy.[3]

In all the seed-plants thus far accurately studied true parthenogenesis is of the diploid type; and as in animal diploid parthenogenesis no reduction-division occurs, one or both of the spore-forming divisions of the primary macrosporocyte being suppressed. The female gametophyte (embryo-sac), including the egg-nucleus, is therefore diploid and produces a diploid embryo, quite as in the case of animals.[4] Recent studies on this subject have shown that in some plants reproducing only by apogamy (presumably vegetative) the meiotic divisions of the primary sporocytes in both sexes are subject to many interesting irregularities that are closely similar to those of hybrids (p. 845). This question is thus prominently raised whether

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[1] The fact that parthenogenetic eggs of the diploid type form but one polocyte was observed by Balbiani ('69–'72) in aphids, by Weismann ('86) in the daphnids (*Polyphemus*), later by Weismann and Ishikawa ('88) in ostracodes and rotifers. At the same time Blochmann ('88, '89) determined the fact that in the aphids the parthenogenetic eggs forms but one polocyte, the sexual (fertilized) eggs two, while in the bee (*Apis*) the parthenogenetic egg forms both polocytes and is indistinguishable from the sexual egg. This observation was subsequently confirmed by Paulcke, Weismann, Petrunkewitsch ('01), Phillips ('03), Hewitt ('06), Schleip ('09), Nachtsheim ('13), and others. A similar type of parthenogenesis was also described in Lepidoptera by Platner ('89) and Henking ('92), later in rotifers by Erlanger and Lauterborn ('97), Mrazek ('97), and Whitney ('09).

[2] Also in the pædogenetic fly *Miastor* (Kahle, '08); in the phyllopod *Artemia* (Brauer, '94), Petrunkewitsch, '02, Fries, '10, Artom, '12); in daphnids (Kuhn, '08, Chambers, '12); in trematodes (Cary, '08) and in nematodes (Krüger, '13, P. Hertwig, '19).

[3] Considerable difference of opinion still exists among botanists concerning the use of the terms parthenogenesis and apogamy. (See Winkler, '08, '20, Ernst, '18, Strasburger, '09, etc., Vines, '11, Sharp, '21.) The term parthenogenesis is here applied, as in the case of animals, to the development of an egg or oösphere without fertilization (oöapogamy). Strasburger considered that this term should be restricted to the haploid type; but this position seems untenable in view of the fact that the word was first applied to diploid parthenogenesis (in arthropods). Some botanists, on the other hand, would restrict the term "apogamy" to the so-called vegetative type of this process (*e. g.*, to the development of a sporophyte, whether haploid or diploid, from the vegetative tissues of the gametophyte.

[4] This was first made known in *Antennaria* by Juel ('00) and subsequently in many other forms, *e. g.*, in *Thalictrum* (Overton, '02). In *Taraxacum* (Murbeck, '04), *Hieracium* (Rosenberg, '06), *Burmannia* (Ernst, '09, '18) in some of the ferns (Farmer, '07) and *Marsilia* (Strasburger, '07).

apogamous species may not have arisen as hybrids.[1] True haploid parthenogenesis possibly may take place in certain lower plants, *e. g.*, in the case of the parthenospores of the Zygnemaceæ. There seems to be no *a priori* reason why it should not occur in higher plants; for haploid sporophytes have been described in several cases of vegetative apogamy in ferns; [2] and Blakeslee and Belling have recently found haploid mutant sporophytes in the seed-plant *Datura* which were reared to full maturity (p. 572).

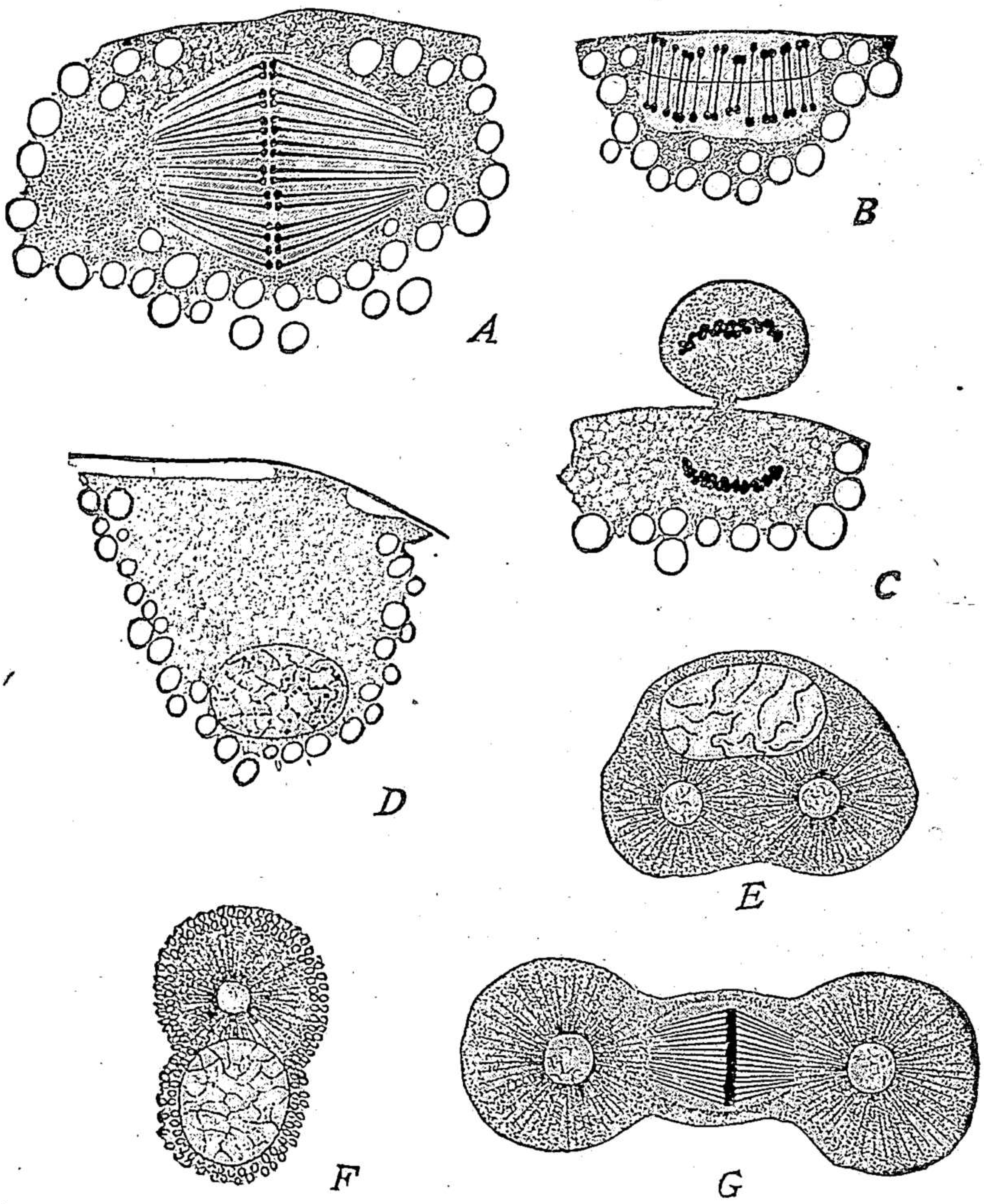

Fig. 224.—First type of maturation in the parthenogetic egg of *Artemia* (Brauer). *A*, the first polar spindle; the equatorial plate contains 84 tetrads; *B*, *C*, formation of the first polocyte; 84 dyads remain in the egg, and these give rise to the egg-nucleus, shown in *D; F*, appearance of the egg-centrosome and aster; *E*, *G*, division of the aster and formation of the cleavage-figure; the equatorial plate consists of 84 supposedly bivalent chromosomes.

Certain exceptions to the foregoing general statements (some of them to be considered at a later point) are important both for the theory of ferti-

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[1] See Winge, '17, Rosenberg, '17, Ernst, '18, Holmgren, '19, Täckholm, '22.

[2] In *Lastræa* (Farmer and Digby, '07), and *Nephrodium* (Yamanouchi, '08), (Steil, '19).

lization and because they demonstrate the possibility of diploid parthenogenesis even in the sexual egg after the completion of reduction. One of these is a reunion of the second polocyte (or polar nucleus) with the egg-nucleus subsequent to maturation, a process first described by O. Hertwig ('90) in the natural parthenogenesis of the starfish, *Astropecten*,[1] and more fully by Brauer ('94) in the phyllopod, *Artemia salina*. In the latter case two modes of parthenogenesis were discovered, the egg in one case undergoing but one polar division (Fig. 224), in the other, two. In the second case the second division produces a polar nucleus which subsequently reunites with the egg-nucleus to form the cleavage-nucleus (Fig. 225), quite

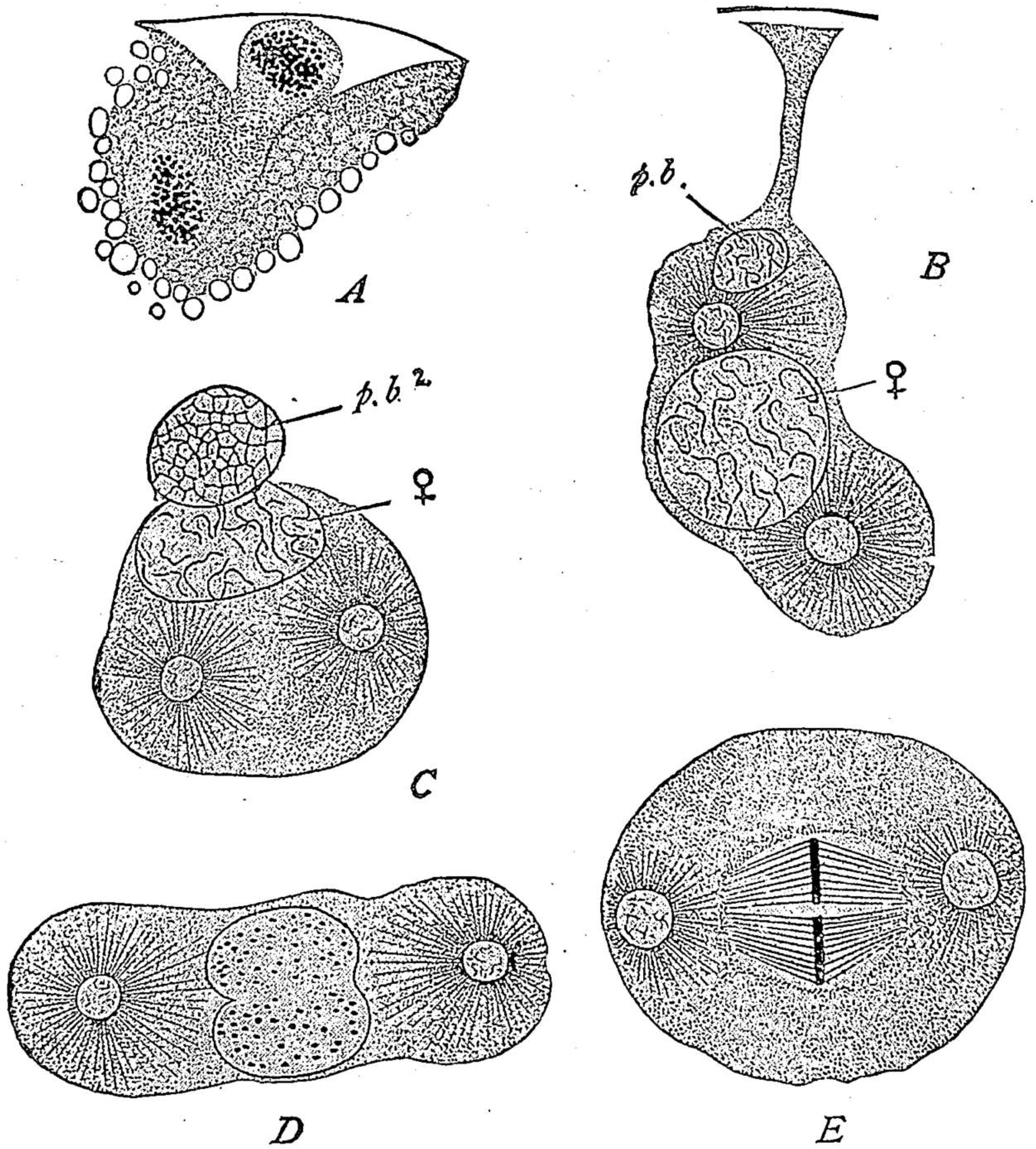

Fig. 225.—Second type of maturation in the parthenogenetic egg of *Artemia* (BRAUER). *A*, formation of second polocyte; *B*, return of the second polar nucleus (*p. b.*²) into the egg; development of the egg-amphiaster; *C*, union of the egg-nucleus (♀) with the second polar nucleus (*p. b.*²); *D*, cleavage-nucleus and amphiaster; *E*, first cleavage-figure with equatorial plate containing 168 chromosomes in two groups of 84 each.

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[1] A similar process was later observed in the artificial parthenogenesis of echinoderms, annelids, and mollusks (p. 4[illegible]

as in *Astropecten.* In view of this fact we should expect these eggs to develop with double the haploid number of chromosomes, and such Brauer found to be actually the case. Later observations, especially those of Artom ('21, etc.) have led to the suggestion that Brauer's results were based on pathological conditions;[1] but judging by the conditions found by Seiler in Lepidoptera (p. 805) it seems possible that Brauer's material was from a distinct race, tetraploid as compared with that of the other forms (p. 870). There seems to be no doubt that a doubling of the haploid number due to such a fusion between egg-nucleus and second polar nucleus actually occurs in some forms of parthenogenesis (p. 477). Possible modes of doubling by monocentric mitoses or by the occurrence of a second equation-division, are considered at another place.[2]

Concerning the history of the cytoplasmic structures in natural parthenogenesis little is positively known. It has been assumed that after the formation of the polocytes the egg-center may persist and give rise directly to the cleavage-centers; but this seems never to have been actually demonstrated. The problem here involved is essentially the same as that presented by artificial parthenogenesis which likewise still remains somewhat unsettled (p. 481).

2. Artificial Parthenogenesis[3]

The fact that mitotic activities may be incited in the unfertilized egg by artificial means was observed by a number of observers prior to the actual discovery of artificial parthenogenesis. R. Hertwig ('96) observed that the unfertilized egg of the sea-urchin, treated by weak solutions of strychnine, might give rise in a bipolar mitotic figure, and even divide irregularly. Morgan ('96, '99, '00) found that under the action of salt-solutions such eggs may segment more or less regularly, and previous to division often develop numerous small asters or "artificial astrospheres" (cytasters) containing central bodies. Mead ('98) found that in the normal development of the annelid *Chætopterus* the discharge of the egg into the sea-water leads to the appearance of numerous minute asters, two of which persist to form the first polar amphiaster while the others disappear. The mitotic figure thus formed normally pauses at the metaphase until entrance of the sperm, when the polar divisions proceed. If, however, the egg, without being fertilized, is placed in sea-water to which a small quantity of KCl has been added, it at once proceeds to form the polocytes

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[1] Petrunkewitsch ('01) and Fries ('10) found in this species only ordinary diploid parthenogenesis with a single polocyte and 84 chromosomes; and the same result was reached by Artom (p. 231).

[2] Further details concerning the chromosomes of parthenogenetic eggs will be found in Chapter X, p. 787.

[3] For recent general reviews of the subject see Bataillon ('12), Loeb ('13, '19), Delage and Goldsmith ('13), Brachet ('17), Herlant ('17, '18, '19), F. R. Lillie ('19), Just ('19, '22).

and undergoes the concomitant changes of form characteristic of the fertilized egg.

These observations prepared the way for the brilliant discovery by Loeb, in 1899, that after appropriate treatment of the egg of the sea-urchin the initial mitotic activities may be followed by complete parthenogenetic development and the production of normal larvæ, a discovery soon confirmed and extended by the work of Delage, the writer, Bataillon and many others, and by a long series of investigations by Loeb himself. By these works it was shown that artificial parthenogenesis may be incited in the eggs of various animals among which may be mentioned especially the sea-urchins, starfish, annelids, mollusks and frogs. Delage ('04 '08, '12, etc.) succeeded in rearing a few artificially parthenogenetic larvæ both of sea-urchins and of starfish through the metamorphosis; and this was accomplished on a somewhat larger scale by Shearer and Lloyd ('13) in the case of *Echinus*. Loeb later succeeded in rearing up to the period of metamorphosis more than eighty tadpoles from parthenogenetic eggs treated by the puncture-method (p. 474) and of these more than twenty passed successfully through the metamorphosis into the adult stage.[1] Only in case of the frog has it thus far been possible to rear larvæ from artificially parthenogenetic eggs up to sexual maturity (p. 806).

Artificial parthenogenesis in plants has been accomplished in only a few cases. An example of this is offered by *Fucus*, in which Overton ('13) induced development by the use of hypertonic sea-water; and under the same head, perhaps, may be classed the artificially induced production of parthenospores in a number of green algæ by Klebs ('96, etc.) and more recently by Faber ('12) in *Spirogyra* and by Ernst ('17) in *Chara*.

It is beyond the scope of this work to enter far into the methods for producing artificial parthenogenesis and the complicated physiological problems that it involves. These have been fully set forth in the general works of Loeb, R. S. Lillie, F. R. Lillie, Bataillon, Brachet, Herlant, Just, and many others. Nevertheless a brief outline of this side of the subject is necessary for an account of the cytological phenomena involved. Loeb's original method for sea-urchins consisted in exposure of the eggs to hypertonic sea-water; but later ('05, etc.) this procedure was greatly improved by development of the so-called double method, presently to be described. In the meantime, and subsequently, many other agents were found to be effective, including chemical, physical or even merely mechanical ones.[2] The chemical agents include such substances as neutral salts (KCl, etc.),

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[1] Loeb, '18, '21; Parmenter, '20.

[2] Since most of these experiments have been made on marine animals ordinary sea-water may be taken as the normal medium unless otherwise stated.

CO_2, and weak acids or bases added to the water in small quantities without noticeably raising its concentration. The physical agents include increase of osmotic pressure, either by adding to the sea-water neutral salts, sugar, urea and the like, or by simple evaporation of the sea-water (Hunter, Kostanecki); thermal changes (Delage, Greely, R. Lillie), or electrical stimulus (Delage, Bataillon, Schücking, McClendon). The mechanical agents include agitation of the eggs by shaking, or (in frogs) puncture of the egg-periphery with a fine needle (Guyer, Bataillon, Loeb), thus producing "traumatic parthenogenesis."

The eggs of different species display a marked specificity in their reaction to these various activators, those highly effective for a particular species being often useless for other species. For instance, the eggs of starfish (Delage) or of the annelid *Thalassema* (Lefevre) are readily activated by simple treatment with CO_2, but this agent is ineffective with sea-urchin eggs. Again, the eggs of the American leopard frog (*Rana palustris*) may be completely activated by simple puncture (Loeb), whereas in *R. fusca* and other European frogs Bataillon ('10, '12, etc.) obtained complete activation only when the puncture is accompanied with or followed by an inoculation of blood or lymph into the egg.[1]

Treatment of the eggs by agents unsuited to their physiological idiosyncrasy often leads to a variety of interesting pathological phenomena including irregular and multipolar cleavages, fusion of blastomeres, and especially irregular division of the nuclei without protoplasmic cleavage, often followed by more or less extensive fusion of the nuclei. The fact of greatest interest is that this process may give rise to syncytial embryos, unsegmented but containing a variable number of nuclei, of different sizes, or not infrequently a single giant nucleus. Such embryos, as F. R. Lillie ('02, '06) discovered in *Chæopterus*, may develop into ciliated, actively free-swimming larvæ (Fig. 521), which may show a considerable degree of resemblance to normal larvæ, not only in external form but also in the distribution of internal materials. These larvæ, though obviously pathological, are of great interest for the general problem of development, and will later be more carefully described.[2]

Loeb and other earlier observers found that sea-urchin eggs activated by hypertonic sea-water alone are in general characterized by

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[1] The discovery that the unfertilized frog's egg may be activated by puncturing with a fine-pointed capillary tube filled with blood or lymph is due to Guyer ('07) and this has been confirmed by a number of later observers, including besides Bataillon: Dehorne ('10), Henneguy ('11), Brachet ('11), and McClendon ('11).

[2] See p. 1083. This phenomenon has been observed also by Treadwell ('02), in *Podarke*, Fischer ('02, '03) in *Amphitrite* and *Nereis*, Scott ('06) in *Amphitrite*, Allyn ('13) in *Chaetopterus*, by Lefevre ('07) in the eggs of *Thalassema* activated by weak acids, and (for the early stages) by Kostanecki ('02, '04), in *Mactra*.

defective formation of the fertilization-membrane and by the frequent occurrence of multipolar cleavage leading to abnormal or pathological larvæ. On the other hand, there are certain agents which cause the mature but unfertilized sea-urchin egg to throw off a fertilization-membrane indistinguishable from that produced by the fertilized egg but ordinarily without inducing a subsequent cleavage or development.[1] If, however, the membrane-producing agent be followed by an appropriate second agent normal cleavage and development follow in a large percentage of cases. Loeb's improved or double method (especially applicable to sea-urchins) consists accordingly in treatment of the eggs first with a fatty acid such as butyric and then by sea-water made rather strongly hypertonic by the addition of NaCl or $MgCl_2$,—a procedure often followed by a percentage of normal development nearly or quite as high as when the eggs are fertilized.

From these results Loeb concluded that complete parthenogenetic activation of the egg involves two phases or series of activities (which need not follow each other in the same order). One is a destructive or cytolytic process, affecting the cortex, which leads to a sudden and very marked increase in the oxidative processes of the egg [2] (a process proved by experiment to take place in both artificial parthenogenesis and normal fertilization).[3] The primary effect is in itself, however, inadequate to produce complete development and actually leads to destruction of the egg by the excess of oxidative processes if its life is not saved by a corrective action (in this case hypertonic sea-water) in the course of which the chemical equilibrium of the egg is restored. Loeb ('13) even extended this conception to normal fertilization, suggesting that the sperm may bring to the egg a lysin which initiates the cortical change, and a second substance which plays a part in the regulation of oxidation similar to that of the hypertonic solution.

There are many reasons for doubting the validity of this ingenious hypothesis. Loeb himself showed that in sea-urchins the usual order of treatment might be reversed, *i. e.*, that perfect activation may be effected by employing the hypertonic sea-water first and the cytolytic agent afterwards, which obviously necessitates considerable modification of the interpreta-

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[1] O. and R. Hertwig ('87) observed this phenomenon in unfertilized eggs exposed to chloroform; Herbst ('93) found that the same effect may be produced by benzol, xylol, creosote and certain other substances. Many other such agents were subsequently made known, many of which, as Loeb especially has emphasized, have a destructive or cytolytic action on protoplasm. Without further treatment such eggs soon die and disintegrate, while control eggs, under normal conditions, live much longer. *Cf.* p. 484.

[2] Warburg ('08) showed that after fertilization the egg of *Arbacia* consumes six to seven times as much oxygen as before. See also Loeb and Wasteneys ('08).

[3] R. S. Lillie ('09, '11, '12) has suggestively urged the view that the cortical change involves an increase of ionic permeability in the plasma-membrane (or peripheral cortical region) and a consequent electrical disturbance by which the mitotic phenomena are set in motion in the egg. *Cf.* p. 191.

tion. More serious, secondly, is the fact, recently demonstrated by Just ('22) that perfect activation of the sea-urchin egg may be effected by hypertonic sea-water alone without use of the cytolytic agent, *provided the solution be of the proper concentration.* Thirdly, in very many other cases, also, a single agent is completely effective, such as CO_2, weak acids, mechanical agitation, heat or (in the case of the American frogs, cited above), simple puncture by a needle. All these facts point to the conclusion that the necessity of two agents in certain cases is due to the fact that one of them alone produces incomplete activation, and that the second agent merely supplements the first. As will later be shown, this conclusion is borne out in a striking manner by the cytological facts (p. 484). Valuable as Loeb's method is in practice, and for purposes of experimental analysis in particular cases, it may be doubted whether it can be taken as the basis of a general interpretation of fertilization or even of artificial parthenogenesis.[1]

a. History of the Nucleus. Artificial parthenogenesis, like natural, may be of either the haploid or the diploid type, or exceptionally may take place with higher chromosome-numbers, these differences being primarily dependent on the condition of the egg with respect to maturation at the time when the activation of the egg takes place. When (as in the sea-urchin), the egg is treated subsequently to the extrusion of both polocytes the egg undoubtedly begins its development with the haploid number of chromosomes (Fig. 228);[2] and this number (18 in *Toxopneustes* and *Strongylocentrotus*) is known to be retained at least through the metamorphosis (Shearer and Lloyd, '13). Whether the haploid number persists through the entire development is not known. Delage ('01) found what he believed to be the diploid number (18) in parthenogenetic larvæ of *Paracentrotus* (*Strongylocentrotus*) *lividus*, but did not sufficiently examine normal controls. As pointed out by Boveri, however ('02, '04), the diploid number is 36 in this species, which is therefore in conformity with the others cited.

A more complicated problem is offered by such eggs as those of starfish, nemertines, mollusks, annelids and frogs, in which the egg may be activated prior to the completion of maturation. In such cases, as a number of observers have found,[3] the maturation of the egg is subject to wide variations, one or both maturation-divisions often being suppressed or variously modified. The cytological phenomena in these cases are not yet sufficiently known and are complicated by frequent abnormalities of development. It is certain, however, that the number of chromosomes varies, being some-

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[1] For an interesting special argument against Loeb's theory as applied to normal fertilization, see Conklin ('17) on the giant polocytes of *Crepidula* (p. 494).

[2] Wilson, '01, Hindle, '10.

[3] *E. g.*, in the starfish (Delage, '02), in *Mactra* (Kostanecki, '04, '11), *Podarke* (Treadwell, '02), *Amphitrite* (Scott, '06), *Thalassema* (Lefevre, '07) etc., *Chætopterus* (Lillie, '06), Allyn, '13.

times haploid, sometimes diploid, and in some cases variable. These variations are undoubtedly traceable in part to corresponding variations in the maturation-process; but perhaps, also, in part to irregularities in cleavage.

In the simplest case both polocytes are extruded and the egg is said to develop with the haploid number of chromosomes. This is described by Lefevre ('07) in the annelid *Thalassema*, where the cleavage is of quite normal type and the haploid number (12) could be identified at least as late as the gastrula stage. Such embryos developed into normal trochophore larvæ. In the mollusk *Cumingia*, also, eggs that extrude both polocytes segment with the haploid number 18; but such eggs seem not to divide more than once or twice (Morris, '17). Eggs that have undergone complete meiosis may nevertheless restore the diploid number in at least three ways. In the mollusk *Mactra* it was found by Kostanecki ('04, '11) that the completion of maturation is commonly followed by a third mitosis of more or less suppressed type, in which the chromosomes divide and separate to form two nuclei (each receiving the haploid number of chromosomes, 12) which then fuse together to form a single cleavage-nucleus containing the diploid number. Such eggs, however, do not develop normally. A second possible mode by which the original haploid number may be doubled (as indicated by Boveri, '00) is by monocentric mitosis (p. 168) in the course of which each chromosome splits into two followed by the reconstruction of a single nucleus. This case, obviously, is nearly related to that described in *Mactra;* but, as Boveri showed experimentally, such eggs are capable (in the case of fertilized eggs) up to a certain point of normal development (p. 729).

A third case is seen when one or both polocytes fail to be extruded from the egg. When neither polocyte is formed the first polar spindle forms as usual and the chromosomes may have the usual tetrad structure (Lefevre, '07), but the spindle fails to take up its normal position, remaining "submerged" within the egg; and the same may be true of the second spindle after normal extrusion of the first polocyte. Lefevre believed that in either of these cases (in *Thalassema*) the submerged polar spindle may directly become the first cleavage-spindle, though satisfactory proof of this is lacking. More commonly either the first or the second "submerged" polar spindle produces two nuclei within the egg which then fuse together to form the cleavage-nucleus (Fig. 229), as also described in the natural parthenogenesis of *Astropecten* or *Artemia* (p. 471).[1] This process, like that described by Kostanecki for the first cleavage-spindle in *Mactra*, must obviously

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[1] This is described for the second polar spindle by Lefevre ('07) in the annelid *Thalassema*, by Buchner ('11) in *Asterias*, by Allyn ('13) in the annelid *Chætopterus*, and by Morris ('17) in the mollusk *Cumingia*. A similar fusion following the *first* polar mitosis is also described by Lefevre and by Morris.

double the number of chromosomes; but we cannot precisely state the expected result in either case, since the original chromosomes are quadruple chromosomes or tetrads, and it is not known whether the reduction-division takes place in these cases or not. In *Thalassema* Lefevre found clearly more than 12 (the haploid number) in the cleavage-spindle of eggs without polocytes, or with only one polocyte. In *Asterias*, after the second division, Buchner found the haploid number (18) in each nucleus, and the diploid number (36) in the division of the fusion-nucleus. On the other hand, in the eggs of *Cumingia* devoid of polocytes, Morris found in the cleavage 50 or 60 small chromosomes instead of the normal diploid number (36) of large ones. The explanation of this fact is not yet evident.

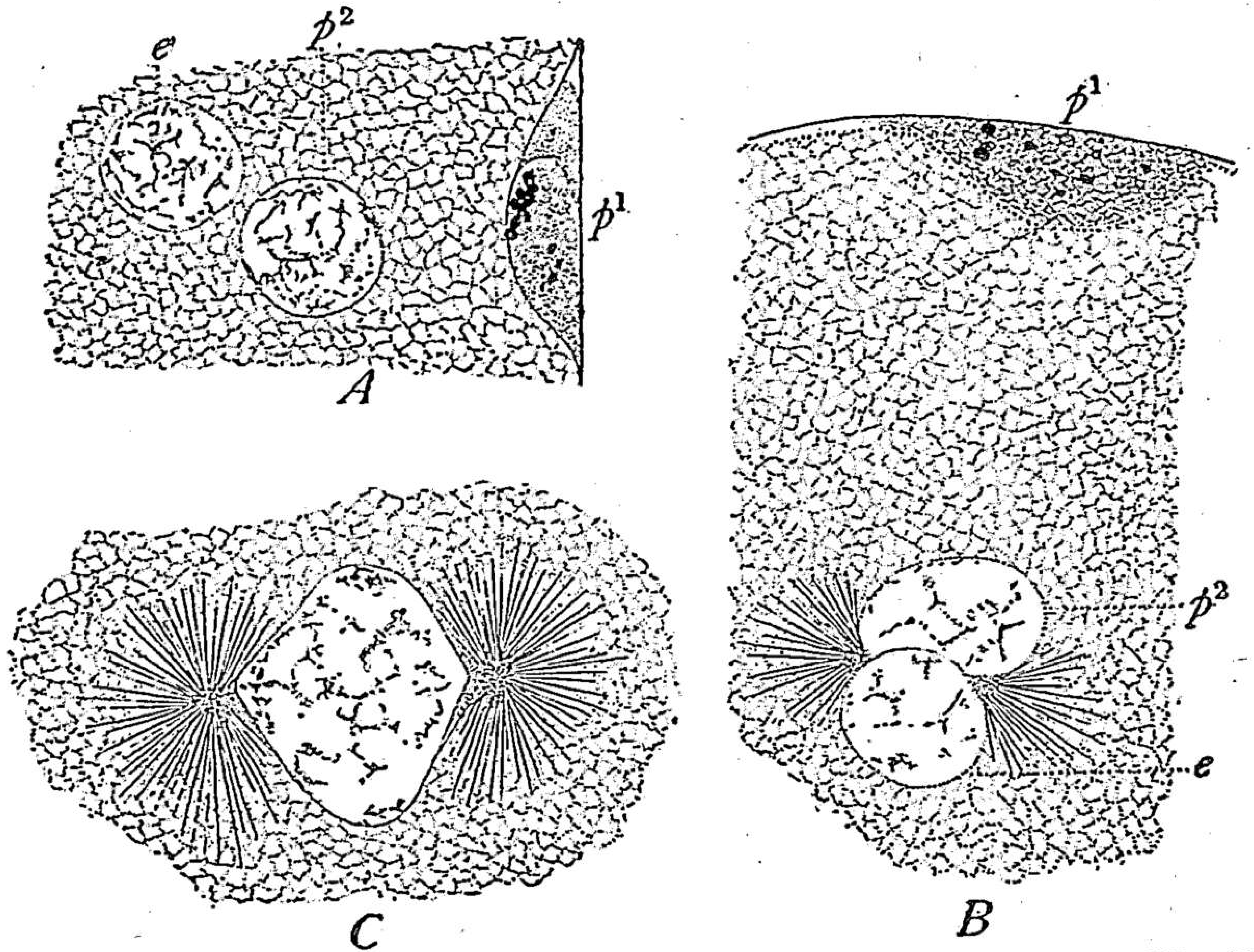

Fig. 226.—Artificial parthenogenesis in the star-fish *Asterias* after treatment by CO_2 (BUCHNER). *A*, first polocyte (p^1), second polar nucleus (p^2) and egg-nucleus (e) after completion of the two polar divisions; *B*, union of the two nuclei to produce *C*, the cleavage-nucleus.

An especial interest attaches to the case of the frog; for the parthenogenetic larvæ (puncture method) have in this case been successfully reared through the metamorphosis and up to sexually mature frogs more than a year old.[1] According to Bataillon ('10) the mature eggs at the time of activation by puncture have extruded the first polocyte and contain the second polar spindle in metaphase (as is the case prior to fertilization). Following puncture (in *R. fusca*) this division is completed and the second polocyte is thrown out. We should, therefore, expect the egg to begin its development with the haploid number; and Bataillon actually found this number (12)

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[1] Loeb, '16, '18.

during cleavage, up to the "morula" stage. On the other hand, Parmenter's work ('20) on Loeb's parthenogenetic sexually mature frogs (*R. palustris*) leaves no doubt that some of them, at least, were diploid (24–26 chromosomes), in agreement with earlier observations by Goldschmidt ('20, also in Loeb, '18), Henneguy ('11) and Brachet ('11). Additional more detailed studies will be necessary to reveal the explanation of this result, and the same may be said of the more recent ones of Hovasse ('22). This observer has found widely varying numbers in parthenogenetic tadpoles obtained by Bataillon's method, and has thus been led to reject the whole theory of the specific constancy of chromosome-numbers and of genetic continuity of the chromosomes. It is to be regretted that a conclusion so sweeping should be based upon data so inadequate. Hovasse records, however, two important observations which there seems to be no reason to doubt. First, the observed numbers, in spite of their great variability, show two principal mean values, one of which (8–14) is about half the other (22–27), presumably near the haploid and diploid numbers respectively. Secondly, the frequency of the haploid numbers is greatest in the early stages (7–24 hrs.) and in later stages decreases until in the oldest larvæ (18, 50, 64, and 84 days) only the diploid number is found. Hovasse insists that this is not due to differential mortality; but the data on which this is based do not seem convincing. A satisfactory explanation of these results must await the result of more definite information concerning the behavior of the egg-nucleus and the polar spindle, of the rôle of the cytasters during cleavage, and many other questions.

Résumé. It appears from the foregoing that the artificially parthenogenetic egg may develop up to a certain point with either the haploid or the diploid number of chromosomes. After extrusion of both polocytes the egg seems most commonly to develop with the haploid number of chromosomes and this number may be retained at least as late as the larval stages (sea-urchins). Whether such larvæ may develop into haploid adults is not yet certain. Since the size of the nucleus (*i. e.*, the number of chromosomes) is known to have an important effect on the rate and extent of growth (p. 654), we should expect haploid embryos and larvæ to be less vigorous in this respect than the normal diploid ones. In point of fact, as is the case of gynogenesis (p. 460) and merogony (p. 465), many observers have noted in the case of both sea-urchins and frogs, the slower rate of growth, greater number of abnormalities and higher mortality in artificially parthenogenetic larvæ as compared with the normal; and this becomes especially noteworthy as the time of metamorphosis approaches, so that comparatively few such larvæ have actually been reared to the adult condition. This difference is well shown by Fig. 227. It is perhaps due to the disproportion between protoplasmic mass and the number of chromosomes (nuclear mass). Herlant ('13) found that in parthenogenetic frogs' eggs, believed to be haploid, the cleavage-spindles are markedly smaller than in normally fertilized diploid eggs so as to be relatively ineffective in cleavage (*cf.* 484). It is possible, therefore, that in species not normally parthenogenetic the artificially parthenogenetic egg may often be unable to undergo complete development unless the number of chromosomes be doubled, as is assumed to be the case in androgenesis or gynogenesis by G. and P. Hertwig (p. 464). This process might take place by monocentric mitosis, by reunion of the second polar nucleus with the egg-nucleus, or in some other way; and it is also possible that when the egg has completed but one polar mitosis at the time of activation (as is the case with the frog) the oöcyte-nucleus may be directly converted into the cleavage-nucleus.

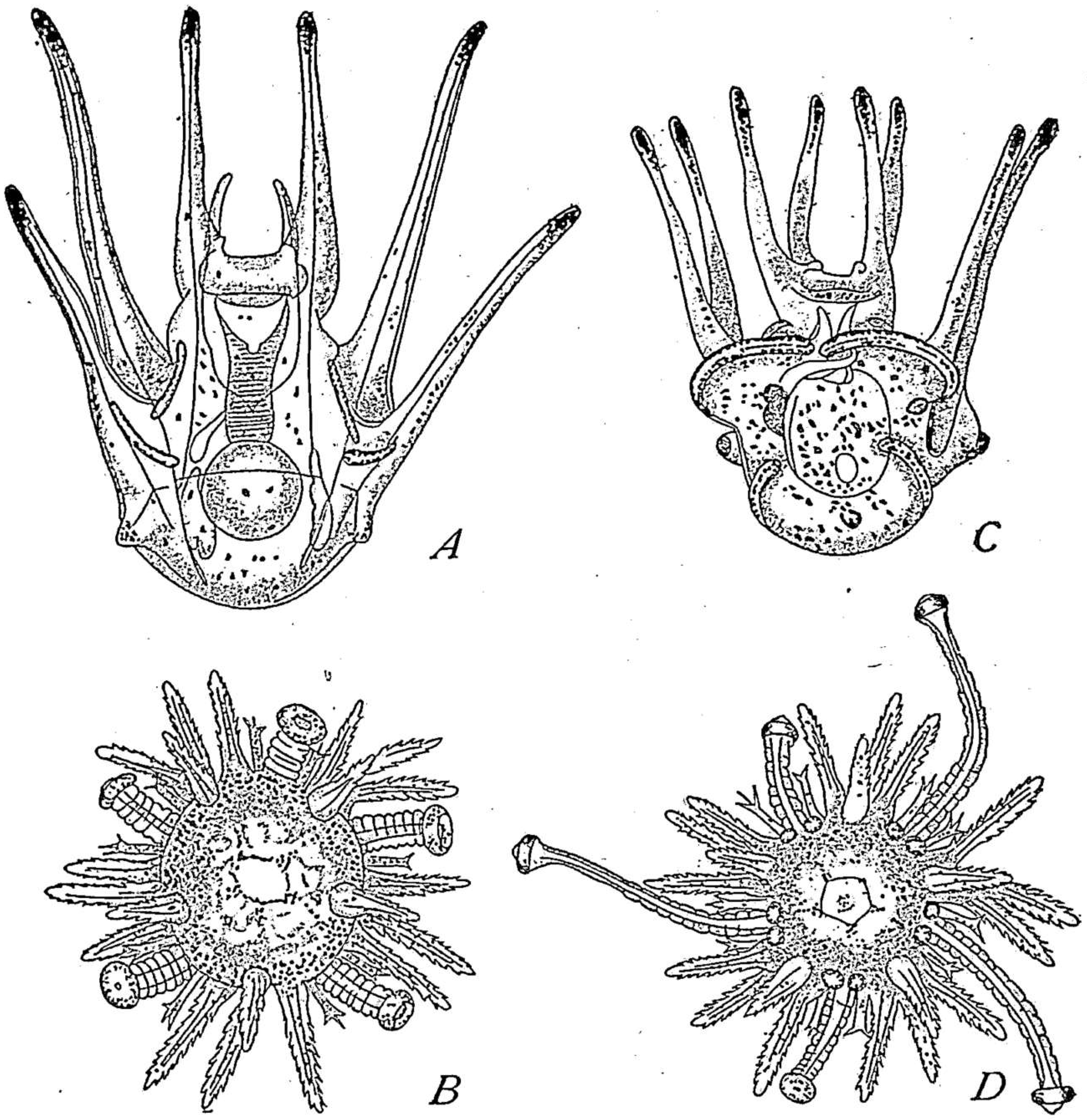

Fig. 227.—Comparison of young sea-urchins, *Echinus esculentus*, from artificially parthenogenetic eggs and fertilized eggs (Shearer and Lloyd).

A, parthenogenetic pluteus, 25 days (Loeb's method); *B*, young sea-urchin of same origin; *C*, pluteus from fertilized egg, 22 days; *D*, young sea-urchin from fertilized egg.

the dyads separating into univalent chromosomes before cleavage takes place. All these possibilities demand further examination.[1] It must not be forgotten, however, that natural haploid parthenogenesis may lead to complete development (p. 794) and that the same, beyond a doubt, is true of haploid mutants of *Datura* (p. 572) and of the haploid sporophytes observed in certain ferns.[2] There seems to be no *a priori* reason, therefore, why haploid larvæ may not in some cases be capable of complete development.

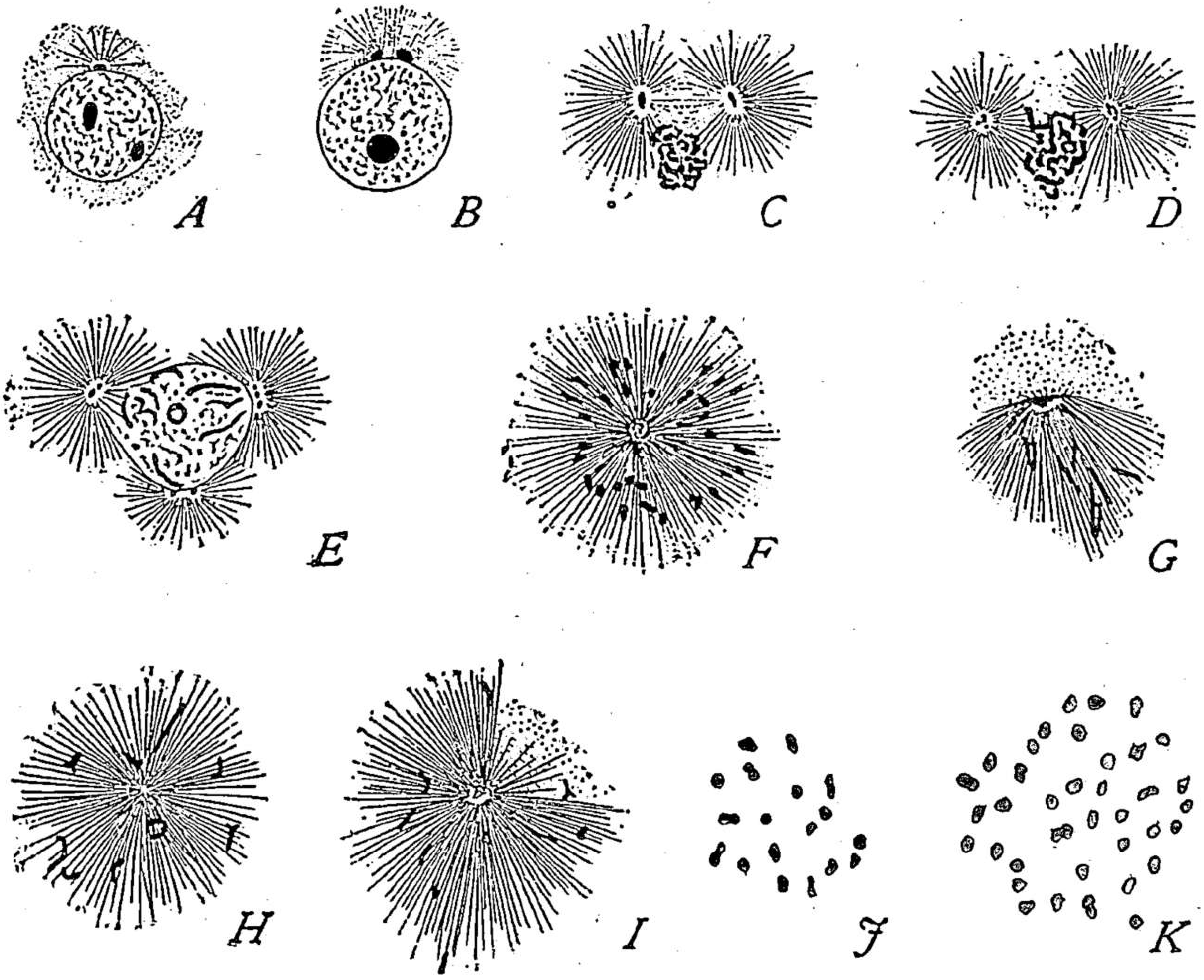

Fig. 228.—Artificial parthenogenesis (activation by hypertonic seawater) in the sea-urchin *Toxopneustes*.

A, *B*, egg-nucleus with aster and central bodies; *C*, *D*, prophases of first cleavage; *E*, early tripolar figure; *F*, monaster with about 36 chromosomes, probably from the second cycle; *G*, "fan-nucleus"; *H*, *I*, two sections through the same monaster from the first cycle, 18 chromosomes; *J*, haploid chromosome-group from early cleavage-stage; *K*, diploid group from fertilized egg.

b. Central Bodies and Asters. The history of the asters and cleavage spindle in artificial parthenogenesis offers many interesting phenomena of which the most striking is the fact that under certain conditions the egg not only gives rise to a bipolar cleavage-amphiaster but often also to accessory or supernumerary asters or *cytasters* scattered through the cyto-

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[1] In *Toxopneustes* (Wilson, '01) there appear to be two types of nuclear prophases in one of which the chromosomes arise from the general nuclear network, in the other from a massive karyosphere. The former process seems to occur after treatment by weaker solutions (of $MgCl_2$), the latter after stronger ones. This observation still remains without confirmation, but I have no reason to doubt its correctness.

[2] *Lastræa* (Farmer and Digby, '07), *Nephrodium* (Yamanouchi, '08, Steil, '19).

some, sometimes in great numbers, and often having no connection with the actual cleavage-figure (Figs. 331–333).[1] After the best methods of activation few or no cytasters make their appearance; the cleavage-figure is bipolar and cleavage proceeds nearly or quite normally. With less suitable methods—for instance in sea-urchin eggs treated by the earlier simple hypertonic solutions (Wilson, '01)—cytasters almost always appear, sometimes in great numbers so as to offer a most remarkable appearance.[2] The cytasters are often at every stage entirely separate from the cleavage-asters; but one or more of them may come into connection with the latter so as to form various kinds of multipolar figures. Such eggs undergo a multiple cleavage and most commonly give rise to abnormal or monstrous embryos.

The cytasters, at first scattered irregularly through the protoplasm, later show a marked tendency to migrate out towards the egg-periphery and here commonly *divide into two*, while at the same time cleavage-furrows are formed about them, as if the egg were undergoing multipolar division. In most cases, these furrows disappear without cutting completely through the egg, the only permanent division being across that spindle which is formed in connection with the nucleus. Sections through these stages show that the cytasters contain definite central bodies, and that their division is preceded by a doubling of the centers and the formation of a central spindle. Both in structure and in relation to cytoplasmic cleavage the cytasters show a very close analogy to normal cleavage-asters despite the fact that they do not ordinarily become centers of complete cleavage. In these facts we find strong ground for the conclusion that the central bodies of these asters are true division-centers, and that they are formed *de novo* (p. 684).

It is not yet known (and perhaps cannot directly be determined) whether the egg always contains a preformed central body (p. 259).

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[1] "Accessory asters," closely resembling those seen in artificial parthenogenesis, were described by Carnoy in the normal maturation-divisions in the egg of *Ascaris*. "Rien de plus curieux ou plus élégant tout á la fois que des œufs constellés. Quel travail que cela de la cinèse!" ('85, p. 47.) Accessary asters in normal development were also described by Reinke ('94), Watase ('95), Mottier ('97), Lillie ('97), Conklin ('98), Mead ('98), Griffin ('99), Smallwood ('01) and other observers.

[2] The cytasters are more numerous after longer exposure to hypertonic sea-water than after shorter, and also in higher concentrations (Wilson, '01, Chambers, '21, Just, '22). Herlant has shown that the number of cytasters formed after Loeb's double method (activation by butyric acid followed by hypertonic sea-water) is affected by the length of treatment by the first agent, though the cytaster-formation first takes place after transference of the eggs to the hypertonic solution. Thus, in a particular experiment the number of cytasters steadily increased after butyric treatment up to 30 minutes, diminished to nearly zero after 45–50 m., and again increased after longer exposures—a result which fits well with Moore's results ('15) on the rhythmical susceptibility of the eggs to hypertonic sea-water. Just ('22) has shown that at a certain optimum time or concentration of the sea-water no cytasters are formed and development proceeds normally. Under-exposure causes only the formation of a monaster, over-exposure the appearance of cytasters.

Most investigators have found that a single aster is first formed centering in a central body that lies upon or near the nuclear membrane and subsequently divides into two to initiate the formation of an amphiaster (Fig. 228), but the point is a difficult one to determine with certainty.[1] Those who accepted this view considered the cytaster-formation as a non-essential epiphenomenon,[2] due, as it were, to an outburst of the mitotic activities which may assume a pathological character or even prevent altogether the normal development of the egg. As will presently appear, however, later studies have raised the question whether the cytaster-formation may not play a definitive and perhaps essential part in development.

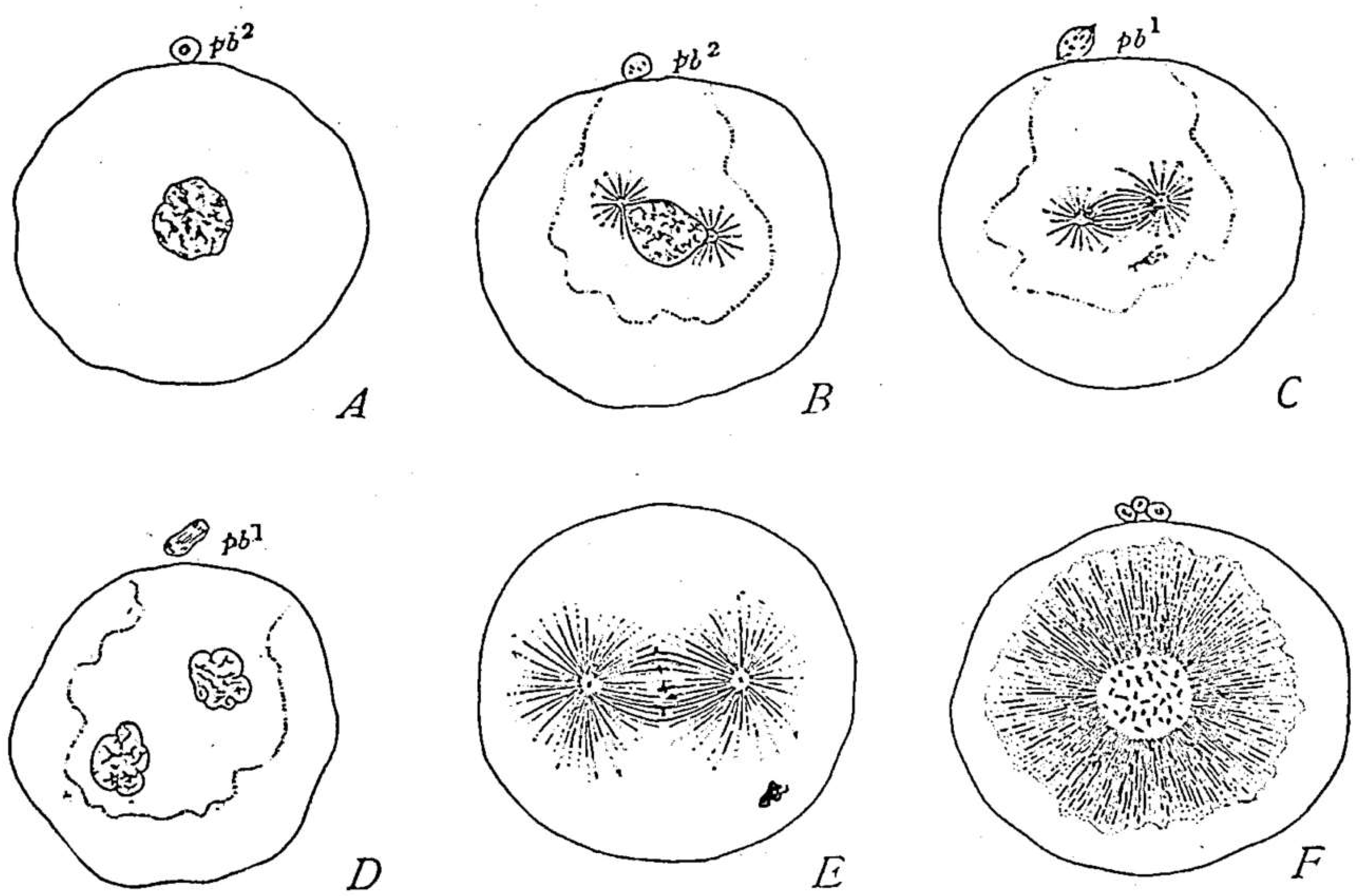

Fig. 229.—Artificial parthenogenesis in the annelid *Thalassema*, activation by dilute acid (LEFEVRE).

A, egg after extension of the second polocyte; *B*, "simultaneous appearance" of the cleavage-centers, on opposite sides of the nucleus; *C*, " submerged " second polar spindle; *D* products of preceding mitosis; *E*, cleavage-spindle, probably identical with first polar spindle; *F*, monaster, after maturation.

The physiological distinction between the primary activation of the egg and the actual achievement of cleavage (in the double process of activation), is in a measure paralleled by the cytological phenomena. By the first agent—*e. g.*, butyric acid in Loeb's method for sea-urchins, simple puncture or electric shock in Bataillon's method for frogs—is initiated the cortical change and the throwing off of an activation-membrane; also, in case of the frog's egg, the completion of the second polar mitosis, expulsion of the second polocyte, and the internal redistribution of material

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[1] Wilson ('01a), Kostanecki ('04), Hindle ('10) Chambers ('21). In *Asterias* Tennant and Hogue ('06) first find two centers close together which later pass to opposite poles of the nucleus. In *Thalassema* Lefevre ('07) found the two centers appearing independently at opposite poles of the nucleus.

[2] Wilson, *op. cit.*

that results in the rotation of the egg into its final position of orientation and the appearance of the gray crescent (p. 1069). In these cases the mitotic activities of the egg are set on foot, but do not ordinarily lead to cleavage and apparently never to complete development. Both in the sea-urchin and in the frog, *the egg thus activated ordinarily goes no further than the formation of a monaster* (p. 475), which is only rarely able to give rise to a bipolar figure and thus to cause cleavage.[1] The latter occurs only when the first agent is followed by a second (hypertonic sea-water in sea-urchins, inoculation in Bataillon's frogs). The most probable explanation of this fact is that the monaster-formation is due simply to incomplete activation, and that further stimulus is necessary to induce division of the center and amphiaster-formation. Such, in substance, is the interpretation offered by Bataillon ('12, etc.), who, in the case of frogs' eggs first activated by electric shock or by puncture, considered the second agent to be a catalyzer derived from the blood or lymph cells introduced by the stylet, presumably derived from the foreign nuclei. Its effect, in his view, is to heighten the mitotic activity of the egg-center to a point where it becomes capable of division and the production of an amphiaster, thus rendering the egg capable of cleavage. The complete development produced by puncture alone in Loeb's *Rana palustris*, or by single agents such as CO_2 in the case of other eggs, might, therefore, be explained merely as a result of a greater susceptibility of these eggs to the stimulus of one agent.

This conclusion harmonizes with those of Just ('22) on Loeb's double method (p. 476). On the other hand, Herlant ('14, '17) has developed a more complicated interpretation, which ascribes an essential rôle to the cytasters. Bataillon had found that inoculation of the frog's egg with blood or lymph causes the formation of a cluster of cytasters in the neighborhood of the puncture. According to Herlant, activation of the egg by simple puncture causes the egg nucleus to become surrounded by a monocentric radiation, constituting the "female energid," which then gives rise to a small dicentric figure or amphiaster, but protoplasmic cleavage fails in this case because of the insufficient size of the amphiaster. Cleavage is made possible by the cytasters in two ways, namely (1) by repelling the "female energid" so as to cause its approach to the surface, where it becomes more effective and (2) by placing themselves on either side of the future cleavage-plane and establishing between them a "diastem" or plane of least resistance along which the first cleavage-plane cuts through the egg. Complete cleavage

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[1] This fact, observed by a number of earlier workers, was first clearly placed in evidence in the case of sea-urchin eggs by Herbst ('07, '09), Hindle ('10) and Herlant ('17); and by Bataillon in the case of frogs' eggs activated by simple puncture. Herbst found in one culture of *Sphærechinus* that out of 800 eggs treated with valerianic acid 791 produced monasters and not one an amphiaster ('21, p. 2).

is thus made possible, despite the abnormally small size of the amphiaster. One cannot consider this interpretation without considerable scepticism; and serious objections to it have been pointed out by Bataillon ('19).

This scepticism grows in view of Herlant's interpretation of the phenomena in sea-urchin eggs activated by Loeb's double method, which ascribes a totally different rôle to the cytasters. Like his predecessors Herlant found that the monaster incited by the first agent (butyric acid) only rarely gives rise to a bipolar figure. In this respect these eggs show an evident analogy to fertilized eggs in which the gamete-nuclei have been prevented from union (p. 447). When, however, the butyric treatment is followed by hypertonic sea-water cytasters make their appearance, and *one of these is said to become associated with the monaster to form the first cleavage-amphiaster.* Herlant believed that he had followed this process step by step both in sections and in the transparent living eggs of *Paracentrotus* (*Strongylocentrotus*).

This account is not so anomalous as it first appears; for many cases have been described in which amphiasters are formed synthetically by the secondary union of asters previously separate, for instance, the first cleavage-spindle of *Crepidula* according to Conklin (p. 443), or the first polar spindle in eggs of some animals,[1] and even in ordinary mitosis of the second type the original spindle seems wholly to disappear, to be replaced later by a new spindle found between the separate centers (p. 148). Herlant's result would also explain the fact, which long puzzled the writer ('01), that the cytasters seem only to divide at the close of the first mitosis instead of at its beginning. Herlant's account of the phenomena in the sea-urchin is, however contradicted by Chambers's careful study of the transparent living eggs of *Echinarachinas* ('21) and is thus rendered still more improbable.

On the whole it seems most natural to assume that the cleavage-centers in all these cases arise by the division of a single original center closely associated with the nucleus and that the cytasters represent a kind of epiphenomena which may often modify the process of division but do not form an essential part of it. Every egg, we may assume, is capable of producing the complete apparatus of mitosis in response to adequate activation. In natural parthenogenesis and fertilization alike, this reaction is complete; but in the former case the egg plays the sole rôle, while in the latter the sperm takes a leading share, providing from the start a focus, as it were, in which the reaction centers and which inhibits in greater or less degree certain other activities *which would otherwise take place* in the egg (*e. g.*, the formation of an ovo-center and "egg-aster"). In artificial parthenogenesis we are dealing with activating agents which are admittedly of vary-

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[1] See Mead ('95, '98), Griffin ('99), Lams ('10).

ing degrees of efficiency and which call forth varying types of reaction by different species of eggs. Experimentally it has in some cases been found possible to supplement the defective activation of one agent by that of another and thus to bring the reaction of the egg more nearly to the normal. To the cytologist the processes called forth by fertilization or parthenogenetic activation offer the appearance of a single train of connected events, more or less plastic in each individual case and varying materially in its details from species to species.

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