III. ORIGIN, GROWTH AND DIFFERENTIATION OF THE GERM-CELLS

A. General Outline

1. Introductory, Terminology

The origin of the germ-cells during ontogeny, and their processes of growth and differentiation, have long occupied a central position of interest for obvious reasons. Attention was concentrated upon their mode of origin, particularly because of Nussbaum's contention ('80) that the germ-cells are not, strictly speaking, produced by the parental body (as was explicitly or tacitly assumed by earlier writers), but only have a common origin with it from a preceding germ-cell.[1] As extended and developed by Weismann, this conception made the multicellular individual appear to us as a kind of dual organism [2] in which germ-cells and somatic cells lead quasi-independent lives. The far-reaching influence of this conception on the modern study of heredity is evident (p. 12).

As Weismann himself recognized, no hard and fast line can in many cases be drawn between germ-cells and somatic cells: hence his theory of a continuity of germ-*plasm* rather than of germ-*cells*, which has so long been a subject of controversy. The distinction between germ-cells and somatic cells, like that between "germ-plasm" and "somatoplasm," was however too sharply drawn by Weismann and his followers, and led to an opposition to his views in which the fundamental truth which they expressed often seemed to be lost sight of. A large body of evidence has accumulated in favor of the view that fundamentally any cell may be tototipotent (*i. e.*, contain the heritage of the species) and that the limitations of potency that it may display are due to secondary inhibitory conditions (p. 1078). Nevertheless the fact is patent that heredity is effected by germ-cells which transmit a specifically organized protoplasmic system (*i. e.*, "germ-plasm") from generation to generation, and the general principle of genetic continuity as applied to both germ-cells and germ-plasm is so obviously true as to have survived all criticism.

In most higher animals the germ-cells seem to arise in the "germinal epithelium," which occupies a localized region of the peritoneal or cœlomic epithelium.[3] By Waldeyer ('70) and many later observers down to the

[1] A review of the history of this conception, with an account of the earlier views of Owen, Galton and Jäger is given in Weismann's book on the *Germ-plasm* (1892).

[2] *Cf.* Waldeyer, '06.

[3] This fact is in harmony with the so-called gonocoele theory, according to which the cœlome of the higher forms represents the enlarged gonad-cavity of the platodes or related ancestral types. See especially Lang ('04).

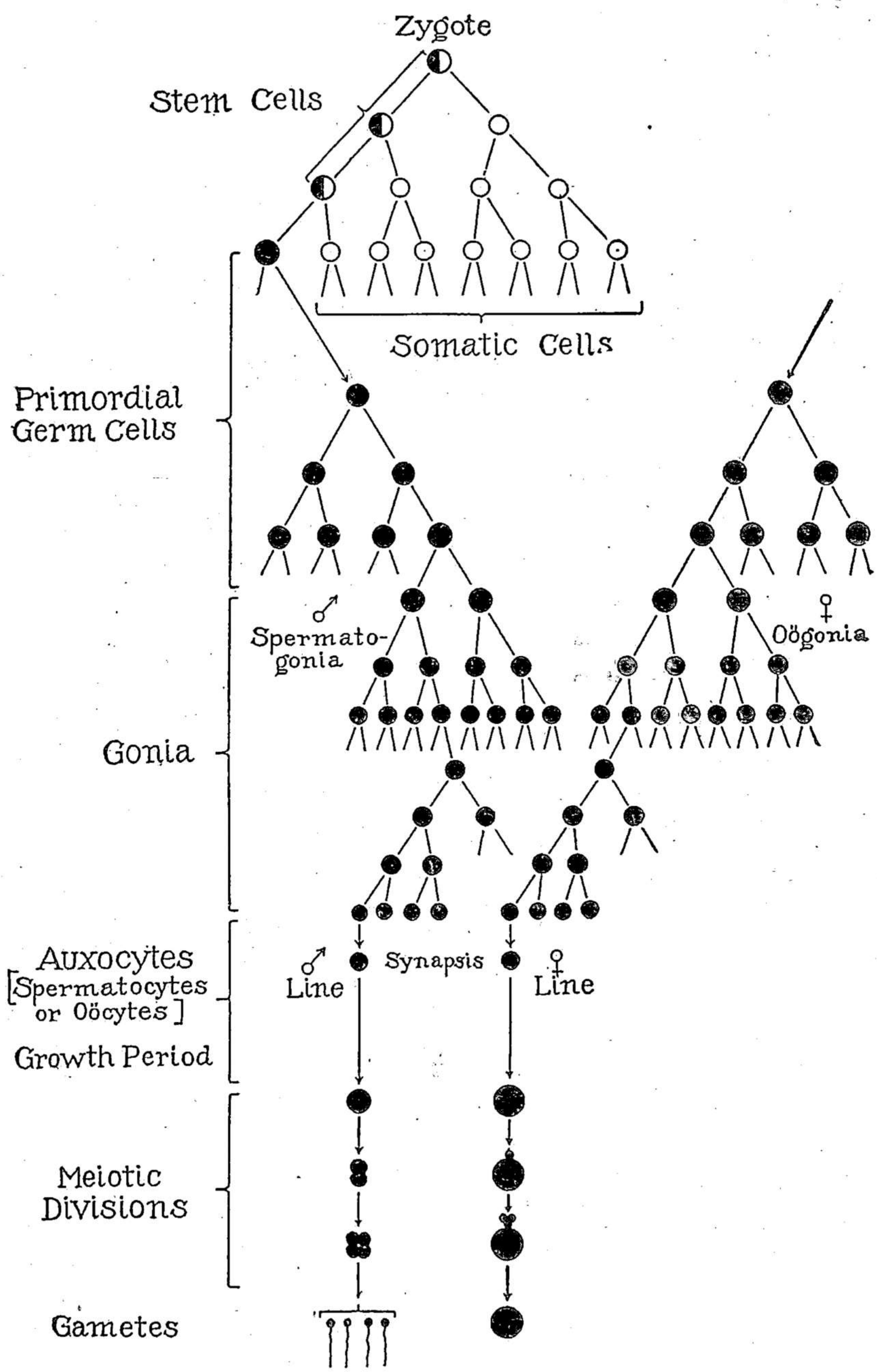

Fig. 135.—General diagram of the germ-line in animals, showing parallelism between the male line (left) and the female (right). The number of divisions (except in meiosis) is actually much larger than here shown.

present time the germ-cells were believed to arise from the germinal epithelium itself.[1] This may indeed correspond to the facts in some cases (p. 318); nevertheless it is certain that in some animals the germ-cells are set aside from the somatic cells at a very early period in the ontogeny, so that we can actually trace their line of ontogenic descent or *germ-line*

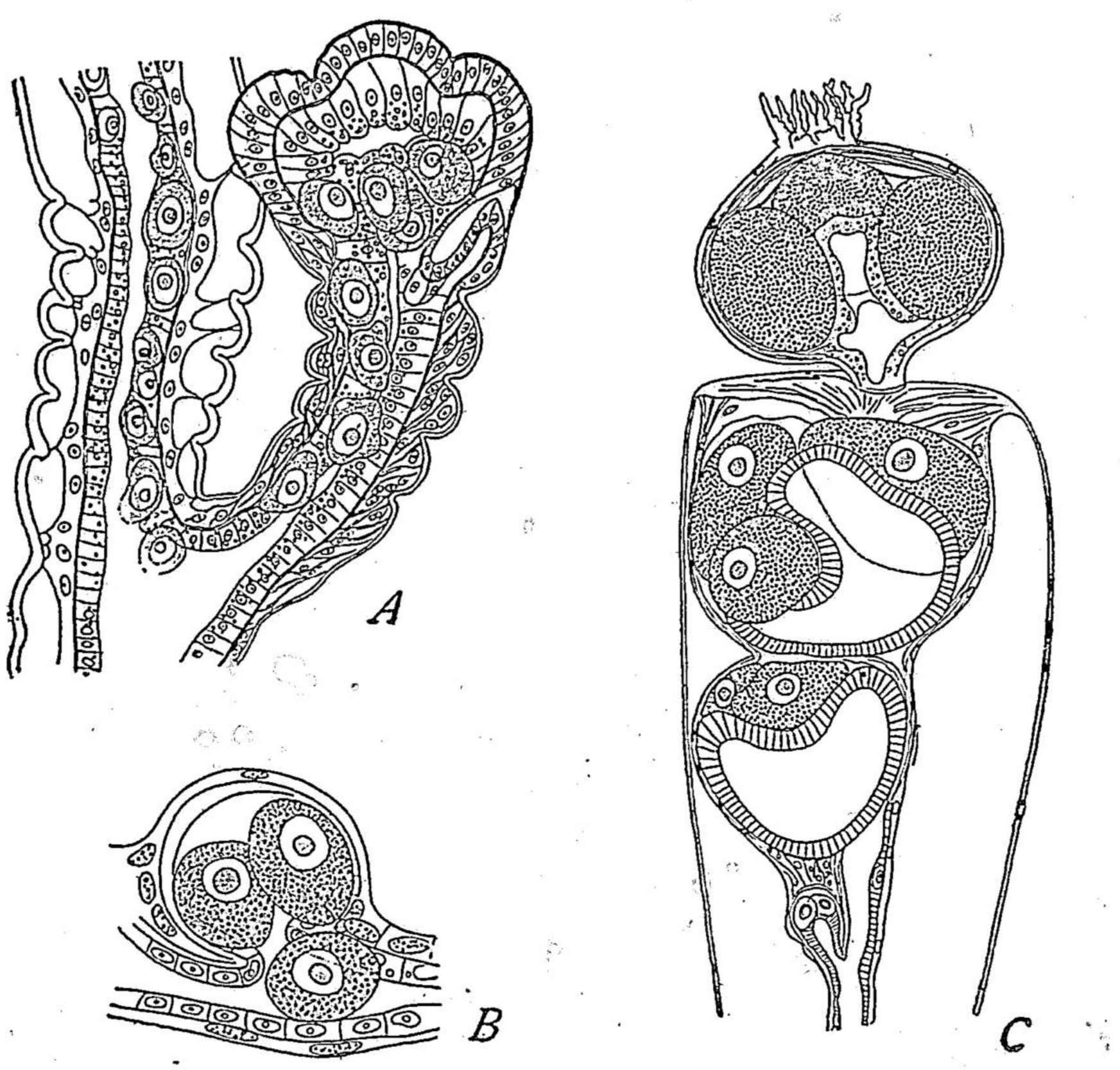

Fig. 136.—Early history of the germ-cells in *Gonothyræa Loveni* (WEISMANN).

A, female blastostyle at an early period, showing eggs passing into it from the main stem of the hydroid at the left; *B*, early stage in gonophore budding from the blastostyle with three eggs passing into it; *C*, terminal portion of blastostyle surrounded by the gonotheca with 3 young female gonophores, each with eggs, and a terminal mature gonophore (sessile medusoid) with ripe eggs.

backwards to early stages of development, sometimes even to the initial cleavages of the egg. In such cases the primordial germ-cells seem to lead an independent life within the soma, almost as if they were parasitic or symbiotic organisms. This fact should not be unduly emphasized. Germ-cells, like somatic cells, result from a process of histogenetic differentiation, and the somatic line of ontogenetic descent is not less real than the germ-line.[2] Nevertheless, the germ-line has an especial interest of its own, since it represents the actual as distinguished from the theoretically possible line of heredity from one generation to another.

[1] For a general review see Waldeyer ('03) and Gutherz ('18). *Cf.* Allen ('23).
[2] See Eigenmann ('97); O. Hertwig ('16).

Terminology. The germ-cells of animals are originally derived from *stem-cells* (Fig. 135), which give rise by division to both germ-cells and somatic cells, and in certain striking cases may be clearly distinguished as early as the 2-cell stage of development (*Ascaris*, *Cyclops*, pp. 315, 323). From the stem-cells arise the *primordial germ-cells*, which after a certain

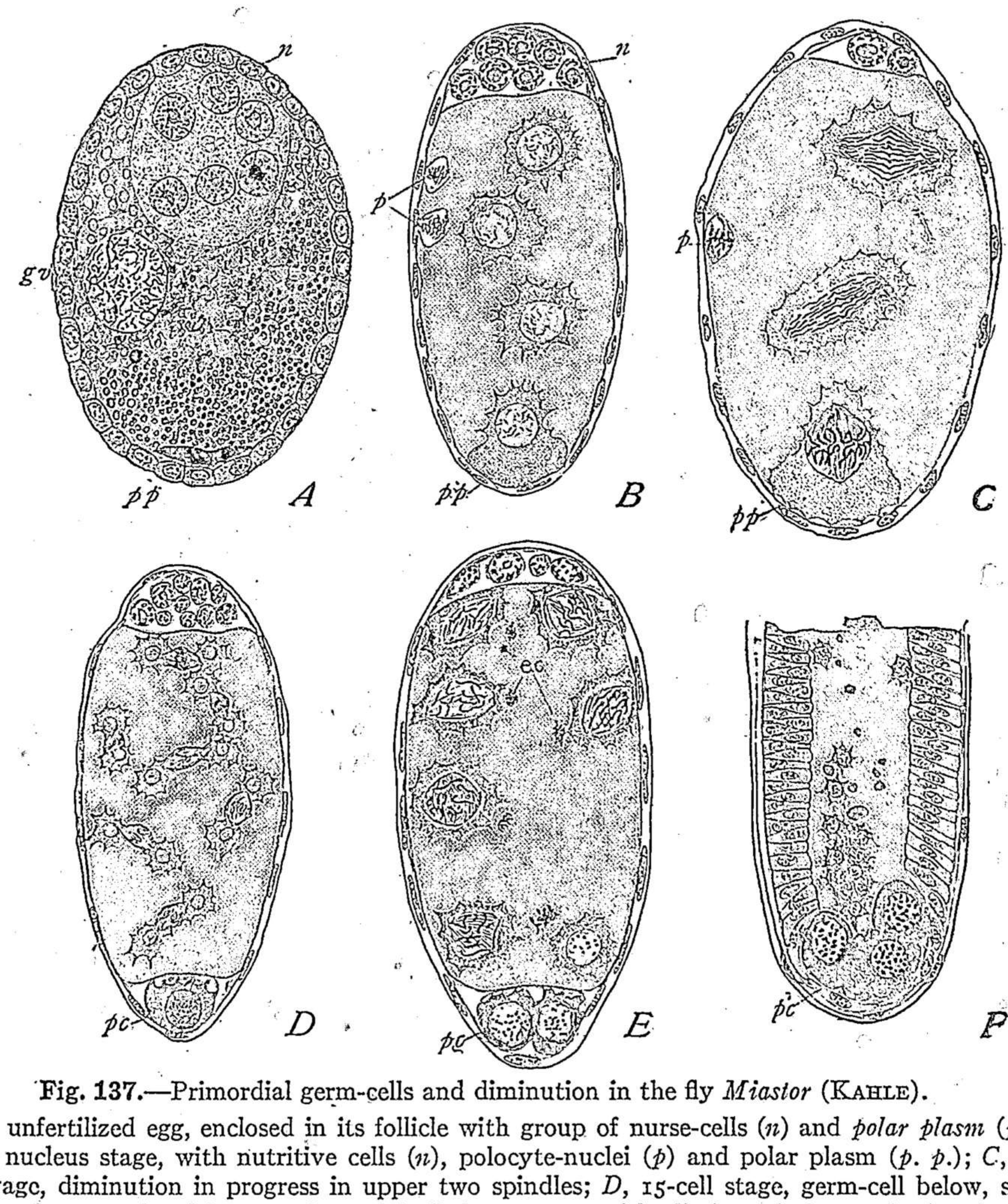

Fig. 137.—Primordial germ-cells and diminution in the fly *Miastor* (KAHLE).

A, unfertilized egg, enclosed in its follicle with group of nurse-cells (*n*) and *polar plasm* (*p. p.*); *B*, 4 nucleus stage, with nutritive cells (*n*), polocyte-nuclei (*p*) and polar plasm (*p. p.*); *C*, third cleavage, diminution in progress in upper two spindles; *D*, 15-cell stage, germ-cell below, second diminution completed in somatic nuclei; *E*, later stage, with eliminated chromatin (*e. c.*); *F*, inwandering of primordial germ-cells at posterior pole.

number of divisions enter upon a quiescent period of considerable duration. They are then converted into the *gonia*, which most frequently aggregate to form localized *gonads* or "germ-glands." These cells show the same general characters in both sexes, but are sooner or later distinguishable by the eye as primary *oögonia* or *spermatogonia*. Resuming the process of active division, they give rise to secondary oögonia or spermatogonia

and also, in many cases, to accessory cells such as nurse-cells in the ovary or Sertoli-cells in the testis. These cells lose the power of further development and are devoted to nutritive or supporting functions tributary to the growth and differentiation of the functional germ-cells. At the end of a certain period division of the gonia ceases and they enter upon a *growth-period*, much more prolonged in the female, characterized by a series of phenomena that differ widely from those in preceding stages. The cells of this period are accordingly called *auxocytes* (*oöcytes* or *spermatocytes*), from which the gametes are finally produced by two divisions known as the *maturation* or *meiotic* divisions. In the male these divisions give rise typically to four sperms, in the female to the mature ovum and three "polar bodies" or *polocytes*.

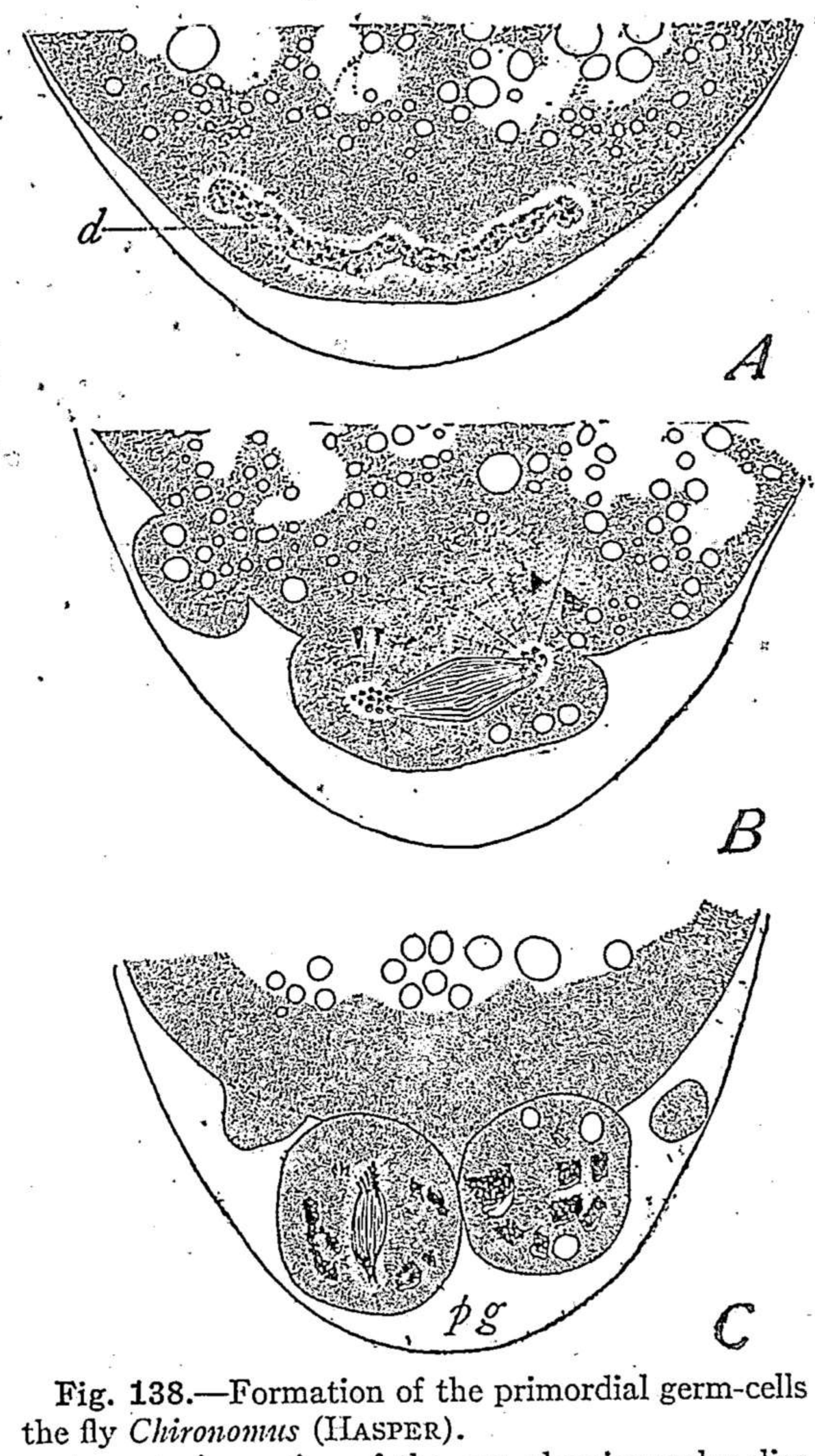

Fig. 138.—Formation of the primordial germ-cells in the fly *Chironomus* (HASPER).

A, posterior region of the egg, showing polar disc or "germ-cell determinant"; *B*, first mitosis in this region; *C*, two resulting primordial germ-cells.

2. The Germ-line and the Primordial Germ-cells

Striking examples of early segregation of the germ-cells are offered by certain of the hydomedusæ in which Kleinenberg, Weismann and others found the primordial germ-cells present already in the "asexual" hydroid, where they lead a quasi-independent life, wandering in the tissues like *Amœbæ*, and in some cases creeping through the mesogloea from one layer to another. In some species, usually characterized by a great reduction of the gonophore or sexual generation (as in *Eudendrium*), the germ-cells are according to Weismann distinguishable several bud-generations before the appearance of the gonophore, passing successively from hydranth to hydranth as new buds form, then into the blastostyle, and at last into the gonophore where they finally aggregate to form localized gonads

(Fig. 136).[1] No case so striking as this is elsewhere known; but in a considerable number of the higher invertebrates the primordial germ-cells, or the stem-cells from which they arise, can be identified with certainty in the early cleavage and can be traced thence onwards by means of cytological peculiarities of the nucleus, of the cytoplasm, or of both. The most certainly determined of these cases occur in insects, crustaceans, nematodes and chætognaths, where they have been the objects of numerous studies.[2]

Of these cases we can here consider only a few examples. The first of them to be described were found in the Diptera (*Miastor*, *Chironomus*, *Musca*) where the primordial germ-cells are budded forth from the posterior pole of the egg at a very early period, and hence were called by Weismann ('63) the *pole*-cells. By Metschnikoff ('55, '66) and Leuckart ('65) these were identified as germ-cells and Metschnikoff actually traced them into the gonads of the larva, a result confirmed by many later observers who have described primordial germ-cells in the early stages especially of the Diptera, Coleoptera and Hymenoptera.[3] In some of these cases all the primordial germ-cells are traceable to a single pole-cell, formed at the posterior pole of the ovum in one of the early cleavages (Fig. 137). In other cases, two or more pole-cells seem to be extruded from the egg, simultaneously or successively (Fig. 138),so that no single complete pole-cell can be taken as ancestral to all the others. In *Calliphora* Noack ('01) found several pole-cells to be separately extruded from the egg: and the same has been found to be the case in beetles by Hegner ('14a) and in *Drosophila* by Huettner ('21).

Striking examples of the early differentiation of primordial germ-cells have been described in the Entomostraca; where the facts were early observed by Grobben ('79) in *Möina* and latter by other observers in a number of daphnids and copepods,[4] in some of which the stem-cells are distinguishable as such from the first cleavage onwards (Fig. 139). Even more remarkable are the similar cases found among nematodes, as first made known by Boveri ('87) in *Ascaris megalocephala*, and later studied by him in a masterly manner, both by observation and experiment. In the hermaphroditic form *Sagitta*, R. Hertwig ('80) discovered four primordial germ-cells,

[1] See Weismann's remarkable memoir of 1887, in which many variants of this process are described. Many of his more detailed results are disputed in the later works of Götte ('07) and G. T. Hargitt ('13–'18). These observers nevertheless confirm Weismann and his predecessors on the main point, namely, the production of the germ-cells in some cases by the "asexual" hydroid long before the appearance of the sexual zooids or gonophores.

[2] See especially Hegner 14a, 14b.

[3] See especially Balbiani ('82), Heymons ('91), Ritter ('90), Noack ('01), Hasper ('11), Silvestri ('06, '08, '09, 14), Kahle ('08), Hegner (08, '09, '12, '14, '15), Gatenby ('19, '20), Martin ('14), etc.

[4] Weismann and Ishikawa ('89), Haecker ('97), Amma ('11), Kühn ('11, '13) and others.

distinguishable already in the early gastrula and (as later determined by Elpatiewsky, '09) derived from a single original cell. Of these four, two are said to give rise to the testes and two to the ovaries, so that the segregation of male and female germ-cells would here seem to be accomplished by a single cell-division (Fig. 143).

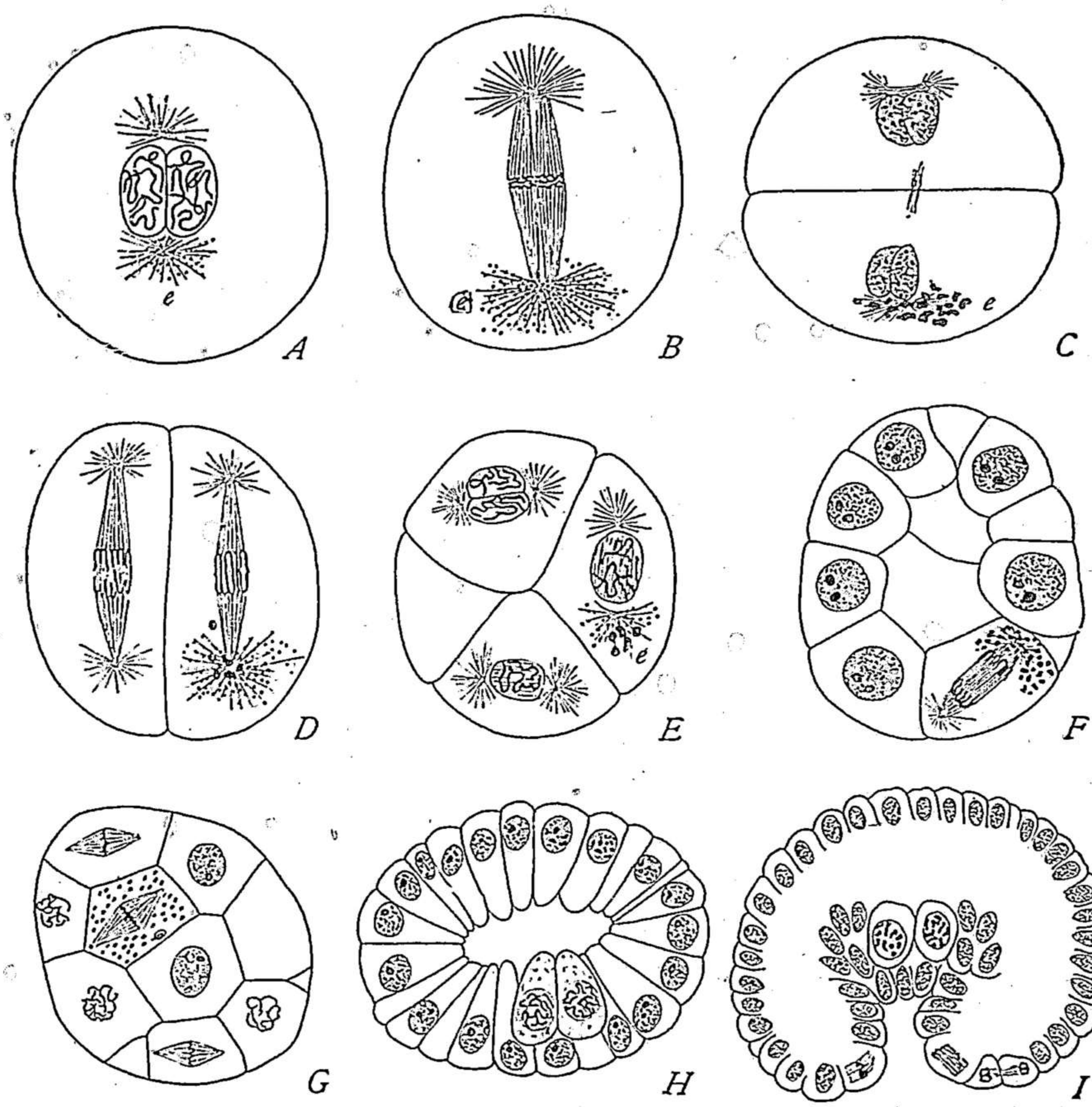

Fig. 139.—The germ-line and "germ-cell determinants" in the copepod *Cyclops* (AMMA).

e, the ectosomes or "germ-cell determinants"; *g*, primordial germ-cells; *s*, stem-cells.

A, conjugation of gamete-nuclei; *B–D*, first and second cleavages; *E*, from 12-cell stage, gonomery seen above; 15-cell stage, in optical section; *G*, sixth cleavage in progress, division of stem-cell; *H*, *I*, earlier and later gastrulas, primordial germ-cells.

In the vertebrates the case is still uncertain. Views on this subject were long dominated by the conclusion of Waldeyer, put forward in his classical work *Eierstock und Ei* (1870), that the germ-cells arise relatively late in the ontogeny by direct transformation of epithelial cells in the "germinal epithelium" of the young embryo; but a considerable group of later observers found that the primordial germ-cells arise at a much earlier period widely scattered in other regions of the germ to wander thence through the embryonic tissues to their final destination in the germinal

epithelium.[1] In most of these cases the primordial germ-cells, so called, are scattered either in the entoblast, in the splanchnic mesoblast, or between these layers (Fig. 140) and appear to pass from this position, as development advances, towards the axial region, and finally into the germinal epithelium of the genital ridge; but in some instances they are distinguishable still earlier and still further away from their final destination. In the tortoise, Allen found them in the entoblast near the junction of the

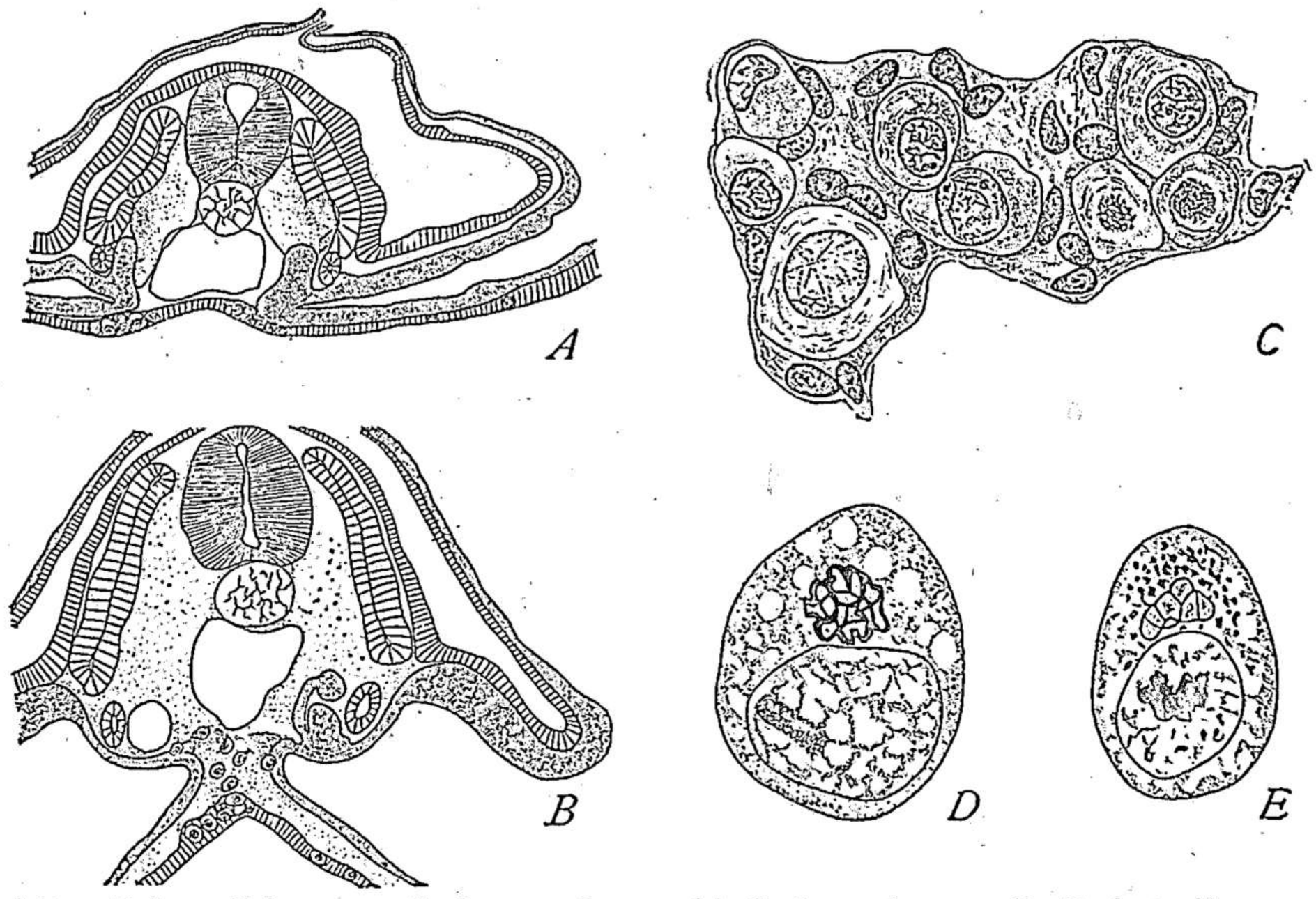

Fig. 140.—Primordial germ-cells in vertebrates (*A–C*, from ALLEN; *D*, *E*, from BEHRENBERG-GOSSLER).

A, cross-section of early embryo turtle (*Chrysemys*) germ-cells in the entoblast; *B*, later stage, germ-cells migrating into mesentery; *C*, group of young oöcytes from cortex of ovary; still in the peritoneal epithelium; *D*, primordial germ-cell of duck-embryo (110 hrs.), showing Golgi-apparatus; *E*, the same from chick of 60–72 hrs.; Golgi-apparatus and chondriosomes.

pellucid and the vascular areas, lateral to the embryo; while in the birds, Swift found them already in the stage of the primitive streak, lying in the entoblast where it joins the germinal wall, and quite outside the embryonic area.

More recently doubts have arisen in regard to the subject. Some observers have found that many of these cells may, as it were, lose their way and remain outside the ovary;[2] or that they may enter the blood-vessels

[1] Among the earlier advocates of this conclusion may be named especially Jungerson ('87) and Eigenmann ('92, '97) in the case of teleosts; Hofmann ('93) and Nussbaum ('01) in birds; Beard ('00–'04) and Woods ('03) in elasmobranchs. Prominent among later works are those of Rubaschkin ('07), Berenberg-Gossler ('12–'14), and Swift ('13) on birds; Allen ('06, '09) on reptiles and fishes; Jarvis ('08) on reptiles; Dodds ('10) on teleosts, and Rubaschkin ('07, '10) and Fuss ('11) on mammals, including man. Literature in the papers especially of Berenberg-Gossler, Allen, Dodds, Swift, and Hegner. *Cf.* Allen, '23.

[2] See Jarvis, '08, Firket, '14, Berenberg-Gossler, '14–'15.

and thus be carried to all parts of the embryo and vascular area, later passing out of the vessels into the tissues, degenerating, or giving rise to ordinary mesoblast-cells.[1] Winiwarter and Saintmont, in a careful study of the histogenesis of the ovary of the cat ('09), found that the large, rounded cells seen in early stages of the ovary do not in fact give rise to ova but degenerate; and the same is true of a second group of similar cells formed by proliferation from the germinal epithelium. The definitive germ-cells

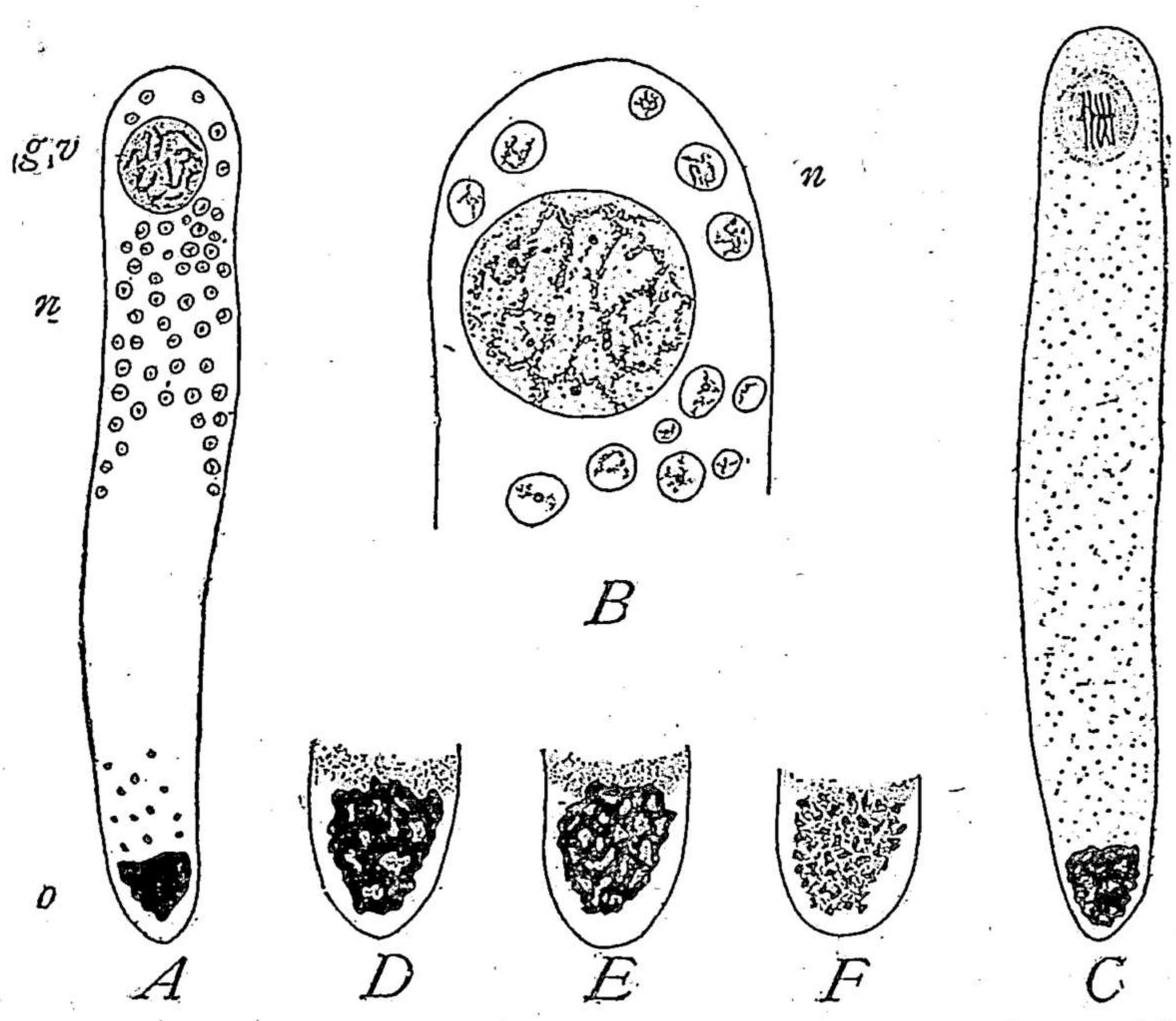

Fig. 141.—Oöcyte of the hymenopter *Apanteles glomeratus* showing secondary nuclei and "germ-cell determinant" or oösome (HEGNER).

A, entire oöcyte with germinal vesicle (*g. v.*), secondary nuclei (*n.*), and oösome (*o*) at lower pole; *B*, upper portion of same, enlarged; *C*, oöcyte with first polar spindle forming; *D–F*, successive stages of oösome.

are formed by a third proliferation in the kitten of 3–4 months; but the authors leave it undecided whether they arise from primordial germ-cells left over from the earlier generations (failing to degenerate) or from indifferent cells of the epithelium. These results are confirmed in their essentials, though with some variations of detail, by several later observers.[2]

A remarkable fact observed by Winiwarter and Saintmont is that before degeneration the primordial cells undergo some of the nuclear changes characteristic of auxocytes, passing through characteristic leptotene, pachytene and diplotene stages.[3] The same fact was described by Firket in the

[1] Dantschakoff, '08, Berengberg-Gossler, Swift (*op. cit.*).
[2] Rubaschkin ('12), Firket ('14), Kingery ('14, '17, '18), Kirkham ('16), Swingle ('21).
[3] *Cf.* the similar changes undergone by the nurse-cells of insect-eggs (p. 336).

chick, and by several observers in mammals. Kingery found in the mouse that the primordial germ-cells might even proceed to the formation of a polocyte and second polar spindle before their degeneration. More recently Swingle ('21) found in the male bull-frog that a first generation of germ-cells of the young tadpole pass though a partial maturation-cycle, showing characteristic tetrad-formation, up to the anaphase of the first division

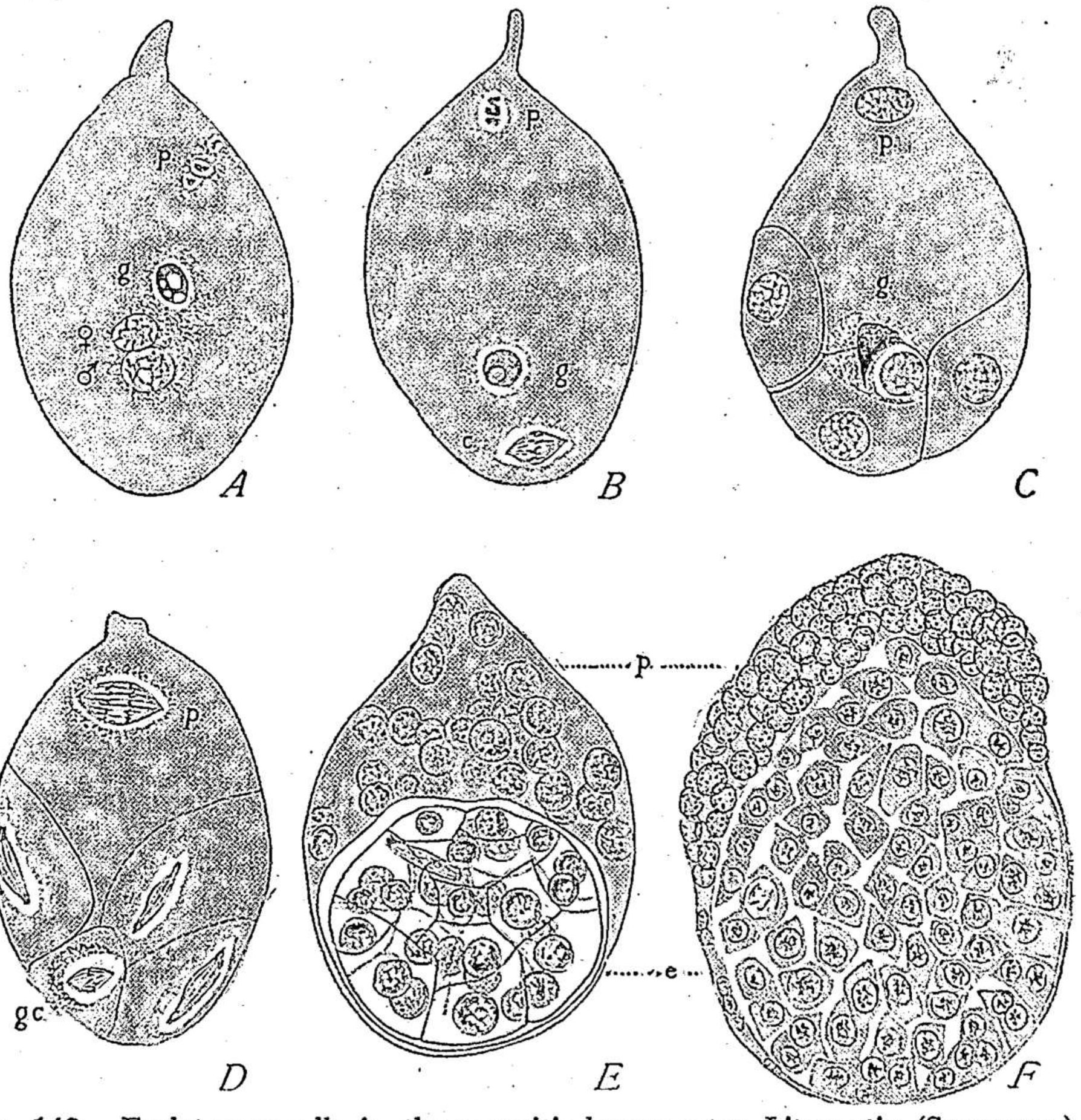

Fig. 142.—Early germ-cells in the parasitic hymenopter *Litomastix* (SYLVESTRI).

A, gamete-nuclei in conjugation, polar-nuclei above, "oösome" or "germ-cell determinant" at *g*; *B*, first cleavage-figure below, polar nuclei fusing; *C*, 4-cell stage, germ-cell at *g*, polar fusion-nucleus above; *D*, third cleavage in progress, polar fusion-nucleus dividing above; *E*, *F*, later stages, with embryonic mass below (*e*) and polar mass above.

but then degenerate, the definitive germ-cells arising from a second generation of uncertain origin.[1]

These curious facts seem to afford strong evidence that the "primordial germ-cells" of the vertebrates really are such. Why they should degenerate to be replaced by cells of later origin is an interesting puzzle: but we find in these facts further ground for the conclusion that the differentiation between primordial germ-cells and somatic cells is not a fixed or fundamental

[1] *Cf.* p. 310.

one, and that it varies greatly in respect to the time at which it appears and in the extent of the morphological changes that it involves (contrast *Ascaris* with the bird or mammal).

3. Differentiation of the Primordial Germ-cells

(*a*) *Cytoplasmic Characteristics.* "*Germ-cell Determinants.*" It has been experimentally demonstrated in the case of *Ascaris* and made very probable in other cases, that the nature of the early germ-cells is primarily determined by their cytoplasm, and that the pecularities of their nuclei (when such are distinguishable) are called forth by the region of the egg in which they lie (p. 1091). At this point we may examine certain visible characteristics of the cytoplasm of these cells that have by some writers been supposed, though on insufficient grounds, to determine their nature.[1] A number of the early observers (Weismann, Metschnikoff, Grobben, Ishikawa) observed the presence of deeply staining granules or other specific cytoplasmic inclusions in the early germ-cells of certain insects and Crustacea and their history was later traced in *Cyclops* by Haecker ('97, '03), whose observations were confirmed and extended by Amma ('11) in several other genera of copepods. In these forms the granules in question, known as *ectosomes* ("Aussenkörnchen"), collect at one pole of the spindle in the first cleavage of the ovum, and continue to be segregated in a single cell until the end of the fourth division; and the cells which then receive them, can later be identified (by their lagging mitosis) as the primordial germ-cells (Fig. 139). In insects the existence of such granules (as distinguished from yolk) was observed by Ritter ('90) in the egg of *Chironomus* before the pole-cells are formed. This account was confirmed by many later observers in several orders of insects, though the details vary more or less in different species. Hasper ('11) showed in *Chironomus* that at the 4-cell stage one nucleus migrates to the posterior pole and there divides, both products being extruded as pole-cells into which the granules pass (Fig. 138). In *Miastor* (Kahle, '08) the origin of the pole-cells is slightly different, since the fourth nucleus divides at the posterior pole to produce one pole-cell and one somatic cell (Fig. 137). The specific granules have not been described as such: but a definite mass of "polar plasm" enters the pole-cells, which are further distinguished by nuclear characters, as explained beyond. On the other hand, distinct polar granules in the polar plasm are described by Noack ('01) in *Calliphora*, and more recently by Huettner ('21) in *Drosophila*.

Analogous phenomena in Coleoptera and Hymenoptera have been carefully studied in an interesting series of papers by Hegner ('08, '14), Sylvestri

[1] A valuable historical and critical review of this subject is given by Hegner ('14). See also the review in Amma ('11).

('06, '08, '15) and Gatenby ('19 '20,). In the chrysomelid beetles (*Calligrapha, Lepintotarsa*) Hegner found a polar disc at the posterior pole of the unsegmented egg, composed of deeply staining granules, the so-called "germ-cell determinants," which pass into the primary pole-cells formed at the posterior pole. Hegner succeeded in killing with a hot needle the pos-

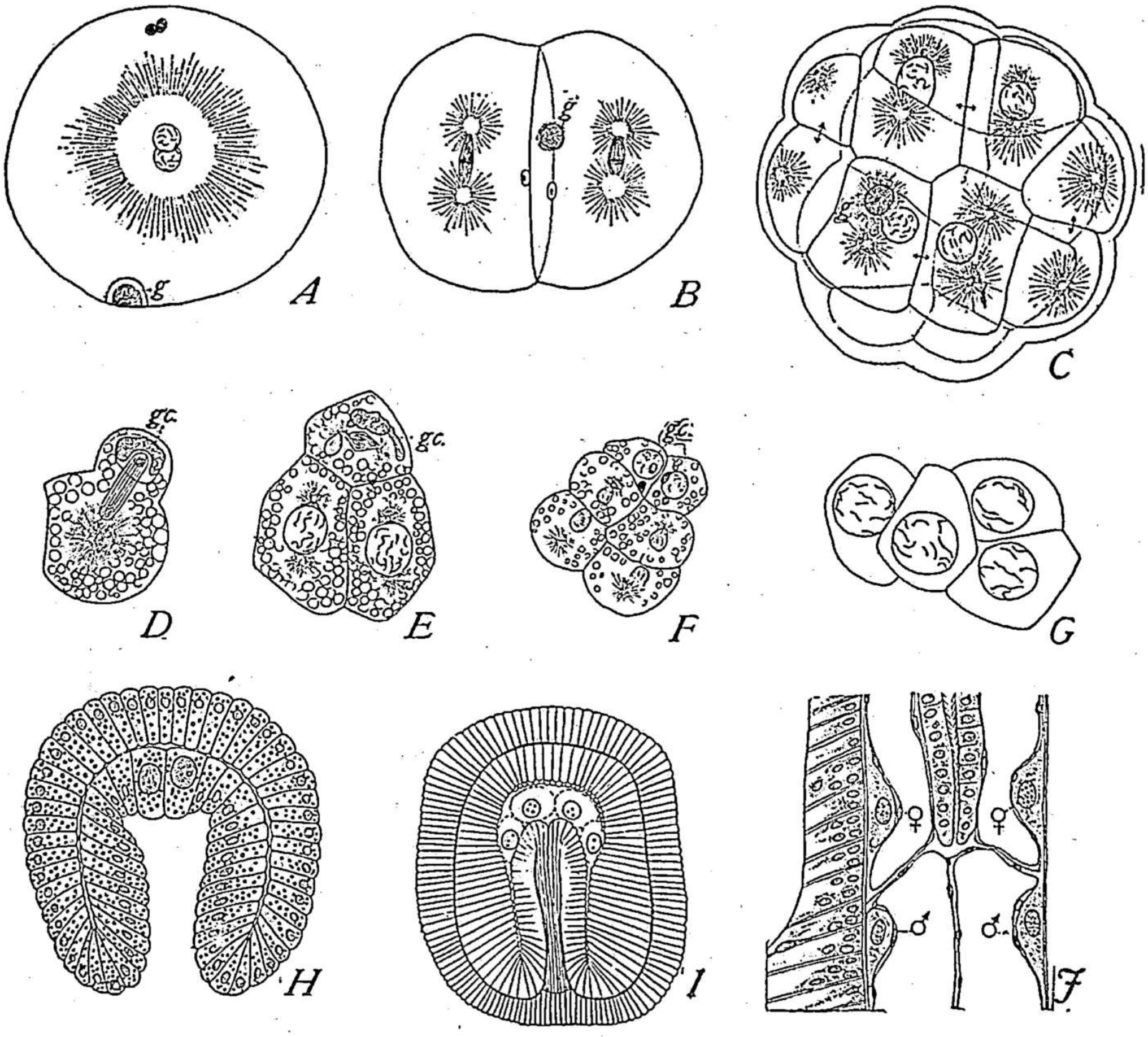

Fig. 143.—Primordial germ-cells in *Sagitta* (*A–G*, from ELPATIEWSKY, *H–J* from R. HERTWIG).

A, fertilized egg with "germ-cell determinant" at *g*; *B*, 2-cell stage; *C*, 16-cell stage; *D*, division of stem-cell during fifth cleavage to form primordial germ-cell *g. c.* in *E*; *F*, first division of *g. c.*; *G*, result of second division of *g. c.*; *H*, gastrula with two primordial germ-cells; *I*, later stage with four germ-cells; *J*, portion of much later embryo showing the two anterior germ-cells (primary oögonia) in front of transverse septum (*s*) and two posterior ones (primary spermatogonia) behind it.

terior region of the egg, containing the polar disc, before the pole-cells had been formed. Such eggs produced a normal blastoderm up to a certain stage, *but no trace of germ-cells was found*. Analogous phenomena occur in the Hymenoptera. In *Copidosoma* (*Litomastix*), a cytoplasmic spheroidal body, the "oösome" (Fig. 142), is present in the unsegmented egg, passes to the posterior end, and enters the posterior cell of the 4-cell stage (this form undergoes a total cleavage) where it breaks up into granules

which were assumed by Sylvestri to pass into the germ-cells.[1] In *Trichogramma* and *Apanteles* densely aggregated polar granules are present, more like those of the Coleoptera.[2]

The nature and origin of the cytoplasmic "germ-cell determinants" is still problematical. They have been supposed to come originally from the

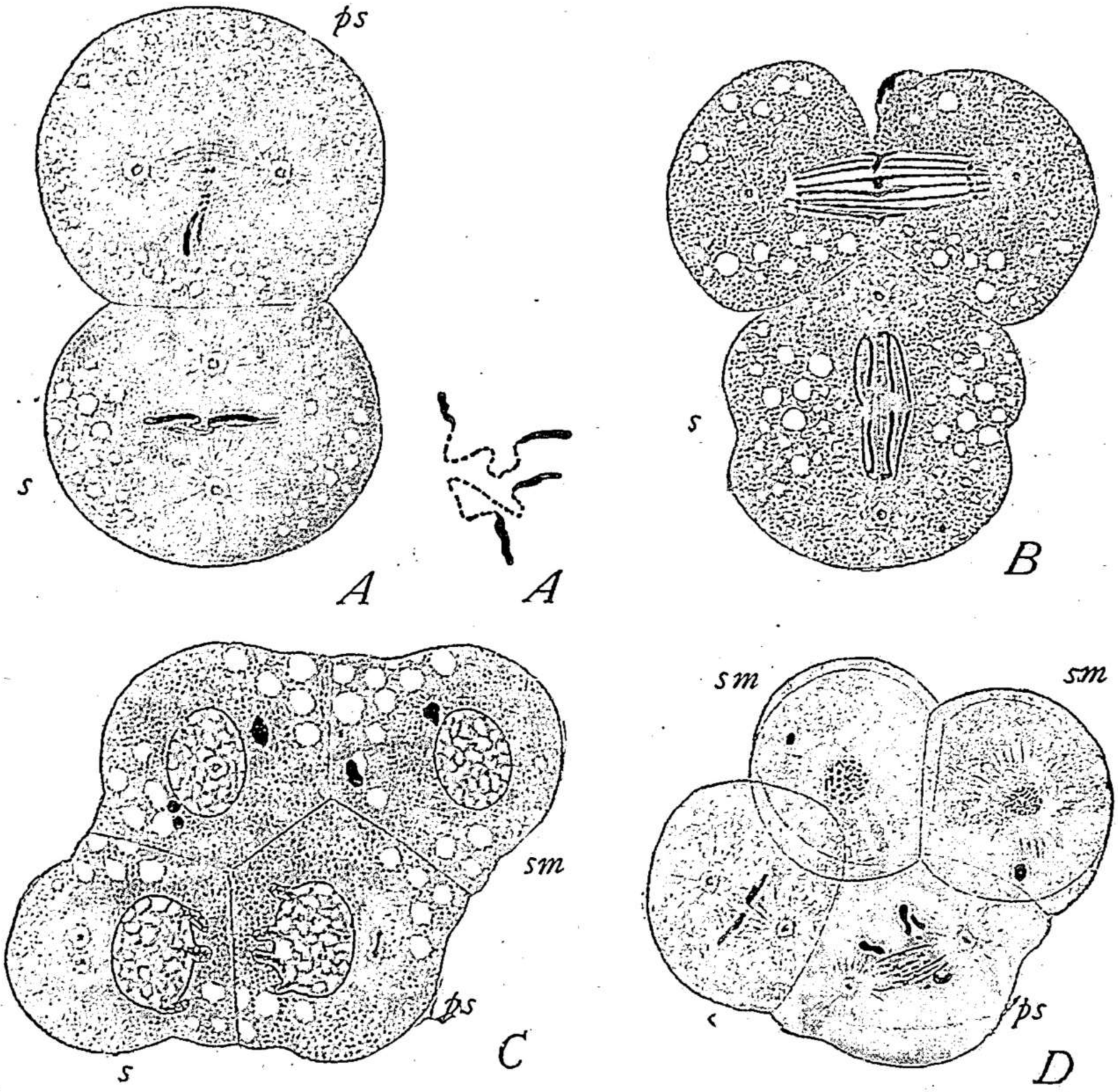

Fig. 144.—Stem-cells and primordial germ-cells in *Ascaris megalocephala*, early stages showing diminution (BOVERI).

p. s., primordial somatic cell; *s*, stem-cell.

A, second cleavage in progress; *A*[1], polar view of chromosomes of the upper cell (*p. s.*) to show diminution in progress; *B*, later stage, elimination-chromatin at equator of upper spindle (T-stage); *C*, 4-cell stage, showing eliminated chromatin in upper two cells; *D*, third cleavage in progress, second diminution at *p. s.* (later stages in Fig. 145).

nucleus (Haecker, Sylvestri, in their earlier work) or from chondriosomes (Hegner). In *Sagitta* (Fig. 143) a special cytoplasmic body is handed on from the unsegmented egg to a particular cell of the 32-cell stage, which is then recognizable as the first primordial germ-cell. Elpatiewsky ('09), to whom these observations are due, considered this body to be of nucleolar origin; Buchner ('10), on the other hand, regards it as a mass of chromidia

[1] A closely similar body was found in *Paracopidosomopsis* by Patterson ('21) who, however, was unable to find any direct evidence that it enters the germ-cells.

[2] Hegner ('15), Gatenby ('19, '20).

derived from an accessory cell that fuses with the egg. In the case of the daphnid *Polyphemus* the work of Kühn ('11, '13) seems to show that it arises from one or more accessory "nurse-cells" that migrate into the egg. The most recent studies on these bodies have produced no definite reason for identifying them with any of the other known types of formed elements.[1] Whether they can properly be called germ-cell "determinants" is equally problematical. So far as the facts at present show they may be no more than accompaniments or by-products of the true determining factors in the cytoplasm; and on the whole this seems more probable (pp. 1067, 1090).

(*b*) *Nuclear Characteristics of the early Germ-cells and the Process of Chromatin-diminution.* Foremost in interest among the cases here concerned is that of the nematode *Ascaris megalocephala*, the object of a masterly series of studies by Boveri ('87, '92, '99) which threw a clear light upon the lineage of the germ-cells in general, and opened a new field of cytological inquiry. Related phenomena have since been observed in the beetle *Dytiscus* (Giardina, '01), the fly *Miastor* (Kahle, '08, Hegner '12) and a few other cases.

In *Ascaris megalocephala* (Figs. 144, 145) the germ-line may be followed without a break back to a stem-cell that is distinguishable as such already in the 2-cell stage of the embryo, and in each succeeding cleavage. This cell differs from the somatic cells at every stage in the fact that it alone retains the sum-total of the nuclear substance, while every somatic nucleus has cast out a portion of its chromatin, having undergone the process of so-called *chromatin-diminution.* The somatic nuclei are in consequence both smaller and paler than those of the stem-cells and thus from the first readily distinguishable by the eye. More in detail, the process is as follows: In the first cleavage two long chromosomes are present, dividing in the usual manner. In the prophases of the second cleavage the two chromosomes reappear in each cell but differ in their behavior. In the stem-cell they undergo simple division, as before. In the sister-cell, destined to produce only somatic cells, the thickened ends of the chromosomes are cast off into the cytoplasm, where they ultimately degenerate, while the central portion segments into a large number of small chromosomes,[2] which split lengthwise and are distributed to the daughter-cells. In the ensuing division each of the diminished nuclei divides with numerous small chromosomes, and the same is true of all their descendants (which give rise only to somatic cells). The undiminished nuclei of the two stem-cells, on the other hand, repeat the process seen in the 2-cell stage, one of them undergoing diminu-

[1] Gatenby, '20, Huettner, '21.

[2] The observations of Geinitz ('15) indicate that the number is in the female 30, in the male either 22 or 30 (see p. 773).

tion and giving rise to somatic cells with numerous small chromosomes, the other dividing into two without diminution. This process takes place in each of the three succeeding cleavages (*i. e.*, four times in all, according to

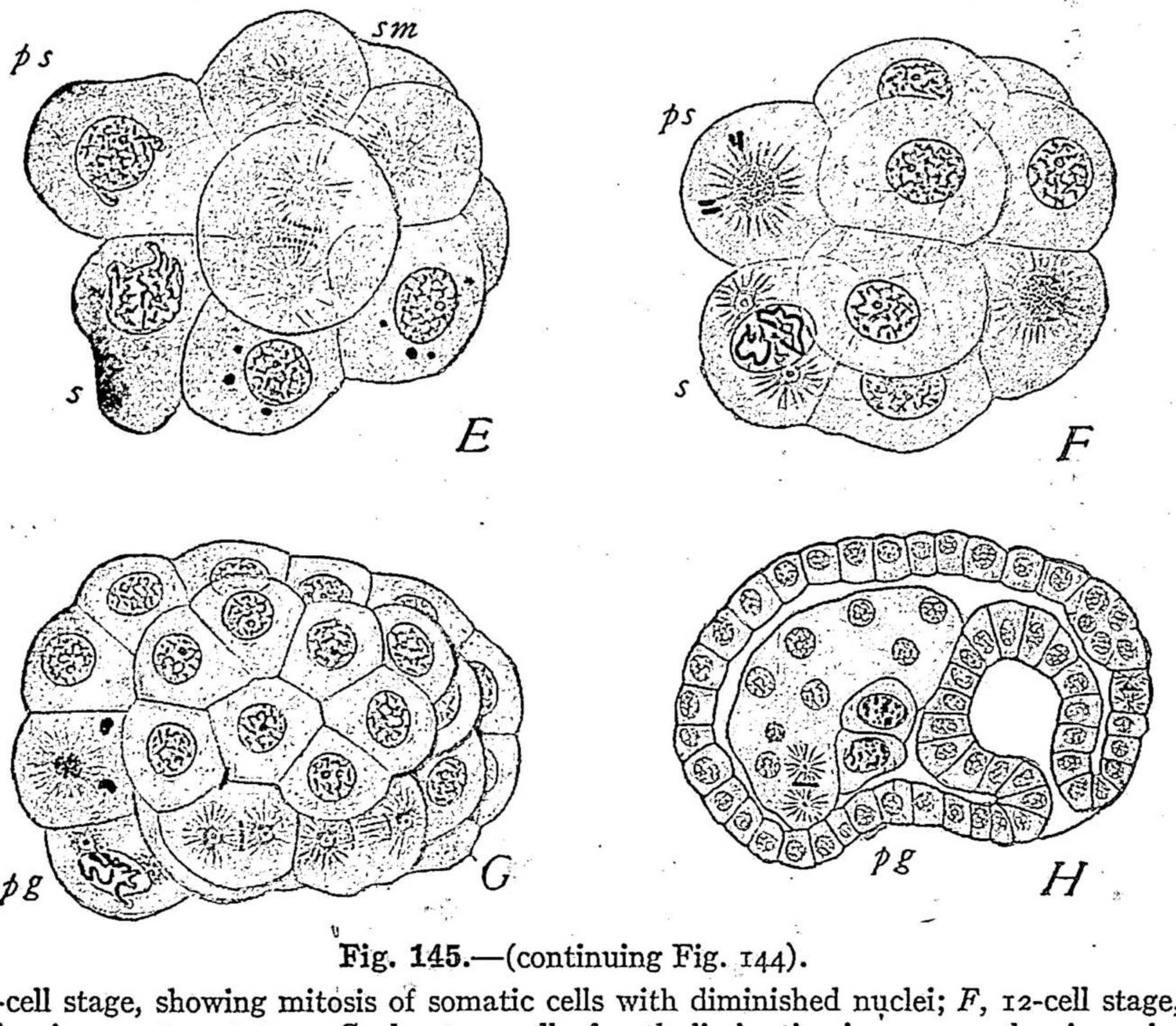

Fig. 145.—(continuing Fig. 144).

E, 10-cell stage, showing mitosis of somatic cells with diminished nuclei; *F*, 12-cell stage, third diminution in progress at *p. s.*; *G*, about 32 cells, fourth diminution in progress, leaving primordial germ-cell (in prophase) at *p. g.*; *H*, gastrula completed with two primordial germ-cells.

Boveri) after which the segregation of the somatic and the germ-cells is complete.

At the 16-cell stage two cells are left with undiminished nuclei one of which becomes the ancestor of all the germ-cells, undergoing no further diminution and giving rise to no further somatic cells. This cell, accordingly, is the first primordial germ-cell. It soon divides into two cells (32-cell stage) which later sink into the interior and multiply to form the primary cells of the gonads. It thus comes to pass that *only the germ-cells receive the sum-total of the chromatin present in the fertilized egg, while all of the somatic cells have lost a portion of their heritage.* "The original nuclear constitution of the fertilized egg is transmitted, as if by a law of primogeniture, only to one daughter-cell, and by this again to one, and so on; while in the other daughter-cell the chromatin in part degenerates, in part is transformed, so that all of the descendants of these side-branches receive small, reduced nuclei." [1] By an ingenious study of centrifuged and double-fertilized eggs

[1] Boveri, '91, p. 437.

Boveri was able to establish the fact that the process of diminution is not an autonomous act on the part of the chromosomes but is induced by their

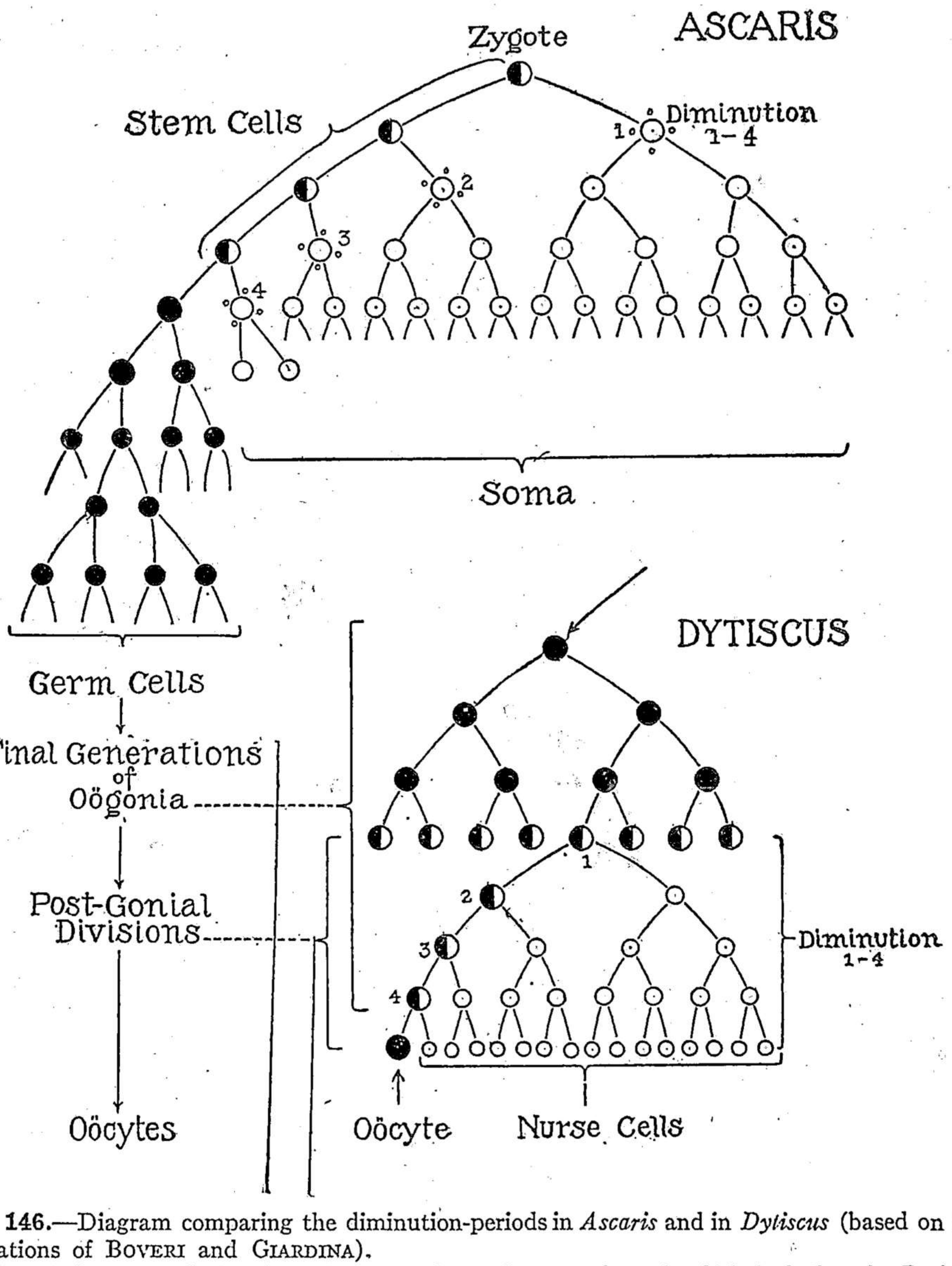

Fig. 146.—Diagram comparing the diminution-periods in *Ascaris* and in *Dytiscus* (based on the observations of BOVERI and GIARDINA).

In *Ascaris* it occurs during four cleavages, from the second to the fifth inclusive; in *Dytiscus* during the last four cleavages of the oögonia.

cytoplasmic surroundings in the egg, a conclusion of fundamental importance for our general conceptions of development.[1]

A somewhat analogous process of diminution was found by Meyer ('95) in three other species of *Ascaris*, and this is confirmed by Bonnevie ('01) in *A. lumbricoides;* but in these species the diminution is not accompanied by

[1] See p. 1091 for further account.

any increase in the number of separate chromosomes. Another well-determined case seems to be that of the fly *Miastor* (Kahle, '08; Hegner '12, '14), in which the diminution takes place in the course of the third and fourth cleavages, a large part of the chromosomes (apparently their ends, as in *Ascaris*) being left behind at the equator and ultimately degenerating (Fig. 137). In the third cleavage the fourth (posterior) nucleus divides without diminution, one of its products passing into a polar plasm at the lower pole of the egg with which it is cut off to form the primordial germ-cell or "pole-cell." From the 15-cell stage onwards, therefore, only the primordial germ-cell and its descendants contain the whole complement of chromatin.

In these cases, evidently, the process of diminution is somehow connected with the segregation of germ-cells from somatic cells; but it is a puzzling fact that diminution is sometimes deferred until a much later period of the ontogeny. In the beetle *Dytiscus* (Giardina) it occurs in the course of the last four divisions of the germ-line (Fig. 146) from which result the primary oöcyte and fifteen smaller nutritive or nurse-cells by which it is accompanied, the oöcyte alone retaining the whole complement of chromatin, as follows: In preparation for the first of these divisions the chromatin of the oögonium segregates into sharply distinct portions, one of which gives rise to the chromosomes, which enter the equatorial plate as usual, while the other forms a conspicuous deeply staining ring that passes to one pole of the spindle (Fig. 147). The ensuing division is unequal, and the ring passes into the larger cell, while the chromosomes are equally divided. This process is repeated in the three following divisions of the larger cell, while the smaller cells divide equally and with no further process of diminution, the ring finally passing into the germinal vesicle of the oöcyte, where for a time it is still distinguishable [1] as a voluminous nucleolus-like body which ultimately fragments into smaller "nucleoli." The observations of Debaisieux indicate that these take no part in the formation or transformation of the chromosomes.

In certain Lepidoptera, the diminution is still further deferred until the formation of the first polocyte during the maturation of the egg. This is well shown in *Phragmatobia*, *Orgyia* and *Limantria* (Seiler, '14), where a very considerable amount of basichromatin is cast off from the chromosomes during the metaphase. In *Orgyia* and *Limantria* this chromatin is seen during the anaphases as a double, plate-like structure which at first closely resembles a metaphase equatorial chromosome-plate (Fig. 148), and later

[1] All the essential features of Giardina's account are confirmed by Debaisieux ('09). A somewhat similar process is also described by Buchner ('00), in *Gryllus* and by Günthert ('10) in the beetle *Colymbetes*.

gives rise to a rounded mass in some cases closely resembling a nucleus, which ultimately disappears.

Taken together, the foregoing facts make it more than doubtful whether the process of diminution can be regarded as a primary cause of the dif-

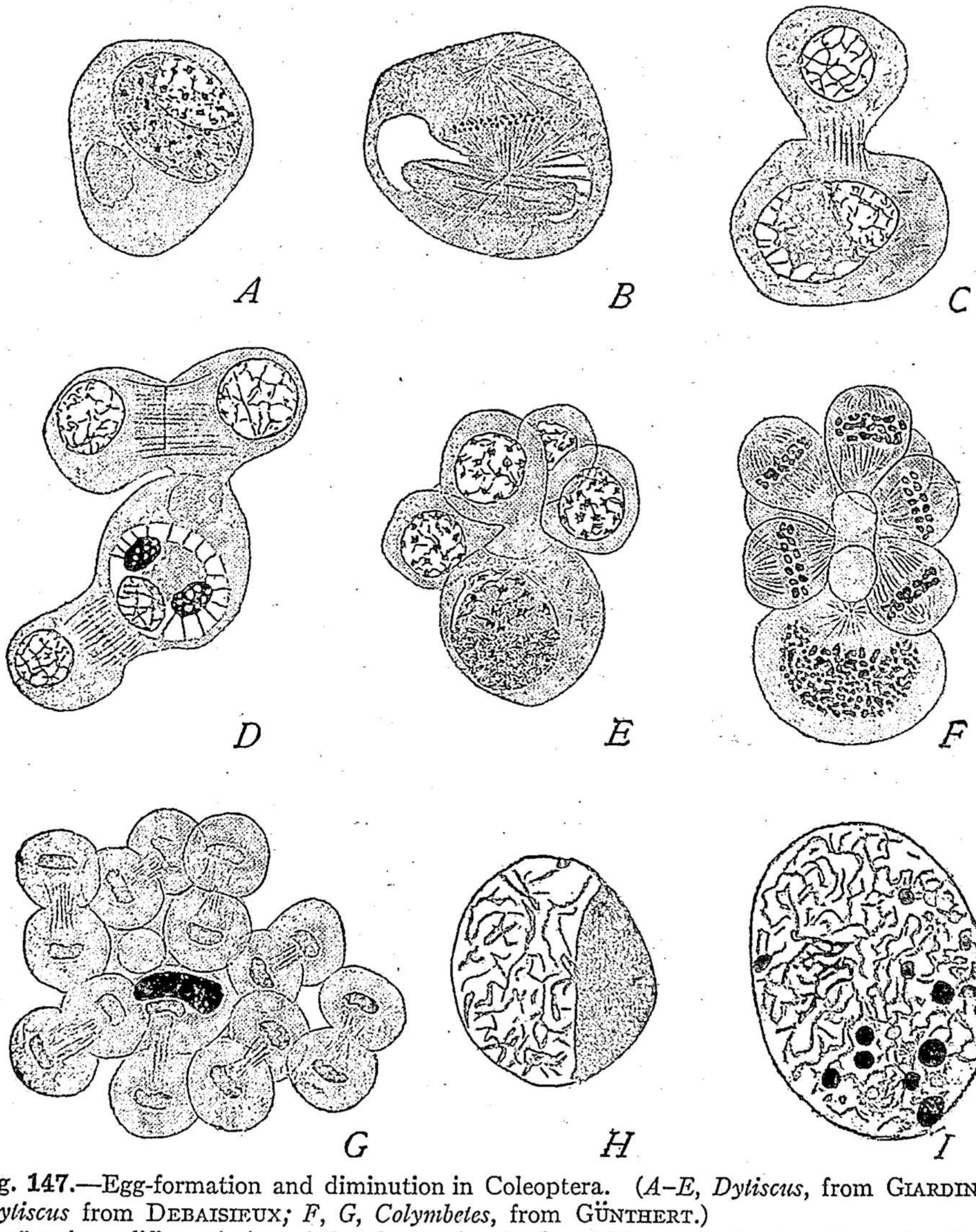

Fig. 147.—Egg-formation and diminution in Coleoptera. (*A–E*, *Dytiscus*, from GIARDINA; *H*, *I*, *Dytiscus* from DEBAISIEUX; *F*, *G*, *Colymbetes*, from GÜNTHERT.)

A, *oögonium*, differentiation of the chromatin; *B*, first differential division; *C*, result of last; *D*, second differential division; *E*, result of third division (only five of the 8 cells shown); *F*, the fourth differential division (2 cells not shown); *G*, telophase of same, oöcyte near center: *H*, nucleus of the final oöcyte, diminution-chromatin at right; *I*, later stage, diminution-chromatin fragmented to form nucleolus-like bodies.

ferentiation of the germ-line from the somatic line. It would seem rather that the eliminated material may have played some definite part in the earlier history of the germ-cells and has become superfluous. Such a con-

clusion would be akin to Weismann's early conception of an "öogenetic" plasma that is cast out of the egg in one of the polocytes (p. 498); and also to those dualistic conceptions of the nuclear substance which postulate the existence of a nutritive *trophochromatin* and a generative *idiochromatin* (p. 725). All the facts would fall into line under the assumption that the

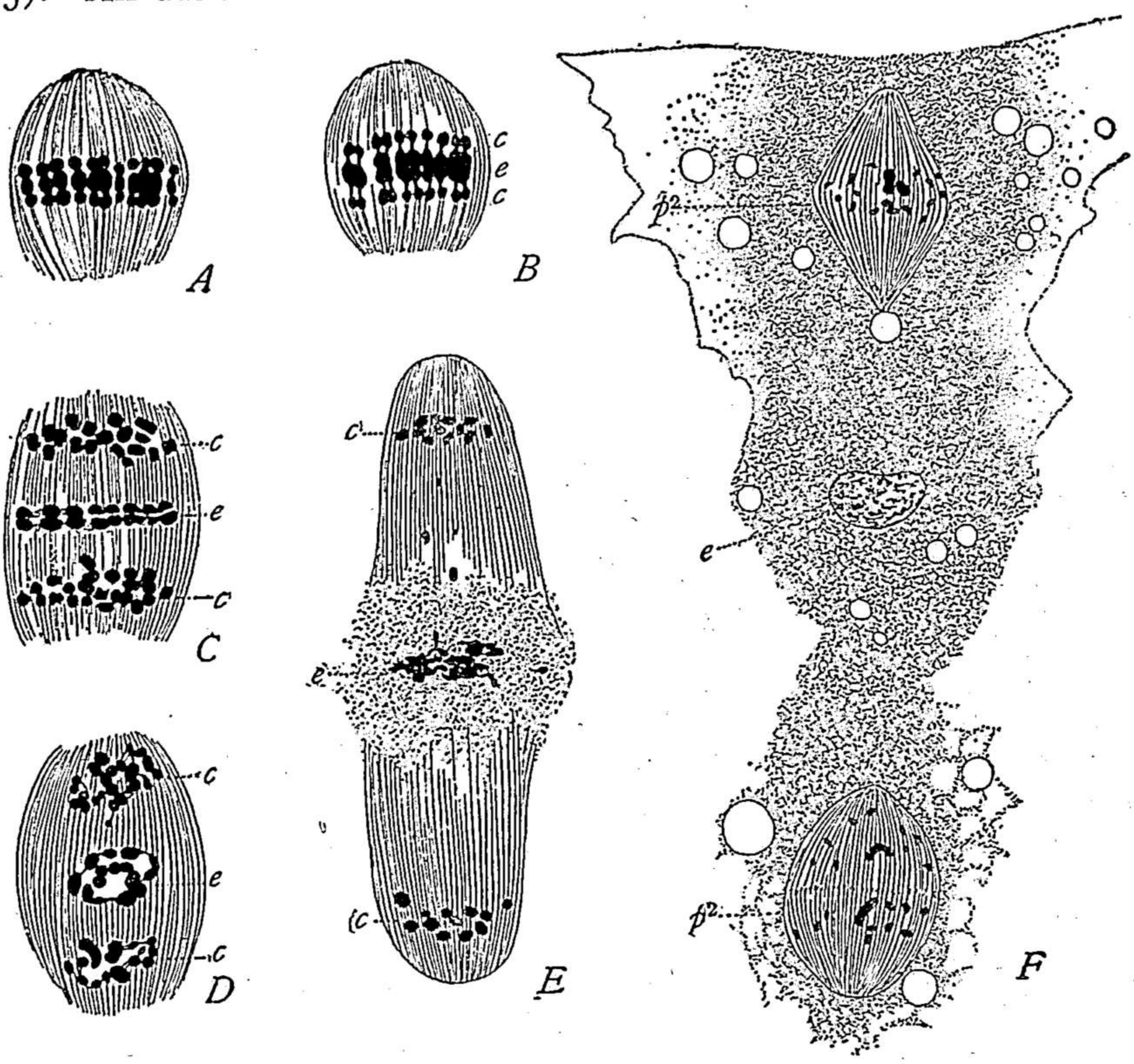

Fig. 148.—Diminution in the polar mitoses of *Lepidoptera* (SEILER); *e*, in each case the eliminated chromatin; *c*, the chromosomes.

A, *B*, first polar spindle of *Limantria*, showing equatorial accumulation of elimination-chromatin; *C*, *D*, anaphase and telophase, *Orgyeia; E*, late anaphase of *Phragmatobia; F*, same, outer and inner second polar spindles (p^2, p^2) with elimination-nucleus between them.

evolution of the egg requires a certain amount of "trophochromatin" which, sooner or later after its function has been performed, is eliminated by the diminution-process. In harmony with this is the suggestion of Bonnevie ('05) that the process of elimination may be comparable to the extensive casting out of residual substance from the germinal vesicle during the prophases of the maturation of the egg as recorded by many observers (p. 355), and which is itself only a more conspicuous form of that which takes place in the somatic divisions generally. A broad field of inquiry is here offered, relating on the one hand to the general relations between nucleus and protoplasm, on the other to dualistic conceptions of the cell substance in general (p. 725).

B. The Auxocytes

The starting point for the final differentiation of the gametes is given by the auxocytes (oöcytes or spermatocytes) which in their earliest stages are closely similar in the two sexes and contain the same components. The marked differences which they later display are due in part to the prodigious growth of the oöcyte as compared with the spermatocyte, in part to a different type of transformation in both cytosome and nucleus.[1] The auxocytes are of especial interest because in them take place the preliminary operations of meiosis or maturation that result in the reduction of the chromosomes; and also because in them preëxist the specific formed cytoplasmic materials for the building of the gametes.

In the very young auxocytes of both sexes the nucleus is a relatively very large vesicular body which has at first a reticulated structure ("resting stage"), and later assumes a more or less spireme-like structure (leptotene, pachytene, etc.). These changes are for the most part undergone while the auxocytes are still small and before they have entered upon the more active processes of growth. At this time the nucleus is probably always more or less eccentric, the investing layer of cytoplasm being therefore thicker on one side. In this thickened region, and usually close to the nucleus, lies a rounded cytoplasmic body within which are one or two centrioles (Fig. 149), and which is sometimes surrounded by cytoplasmic radiations to form an aster-like body. This body is now generally known as the *idiozome*.[2] This body is composed of a clear substance which we shall speak of as *sphere-substance*. In some cases (spermatocytes of mammals) it contains a considerable number of distinct "pro-acrosomic granules," which are believed to persist and by their aggregation to produce the acrosome of the sperm; but as a rule the specific acrosome-forming material is first seen at a later period (p. 361).

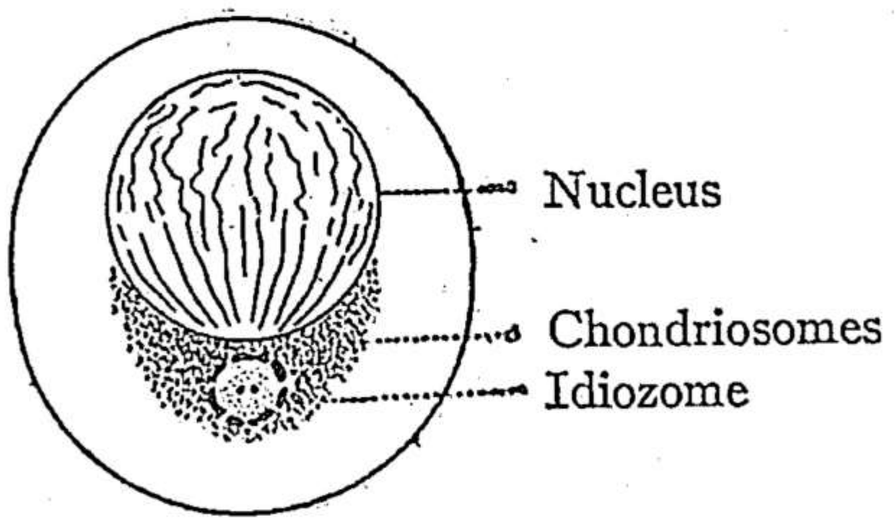

Fig. 149.—Diagram of early auxocyte.

[1] In some cases the spermatocytes are of two or more sizes (p. 304) and they are sometimes so large as to take on more or less the aspect of oöcytes. This is characteristic of all or most of the spermatocytes in some animals, *e. g.*, in the myriapods (Blackman, '05, '07), and the hemipteran forms *Galgulus* (Payne, '08) and *Notonecta* (Pantel and Sinéty '06, Browne, '13). In other cases such large spermatocytes appear only as occasional variations (as in scorpions, p. 820), or as temporary stages in the development of a "pro-testis" destined to degenerate and disappear (frogs) or to form a special "Bidder's organ" (toads). By a considerable group of observers these cells have been regarded as actual oöcytes and the gonad, accordingly, as primitively hermaphroditic; but this view involves many difficulties (p. 820).

[2] See especially Meves ('99) who emphasized the fact, that the idiozome does not persist as such but sooner or later breaks up (Fig. 48), thus setting free the centrioles. The term *centrotheca*, was later proposed Meves ('02) as a substitute. Regaud ('10) proposed the form *idiosome* (early employed in a different sense, see Glossary) as the equivalent of idiozome. It seems preferable, however, to use Meves's earlier and equally non-committal term.

The idiozome is typically surrounded by Golgi-bodies ("batonettes," "platelets," etc.), sometimes so closely aggregated as to give the appearance of a network, or even of a continuous shell (Fig. 150) but

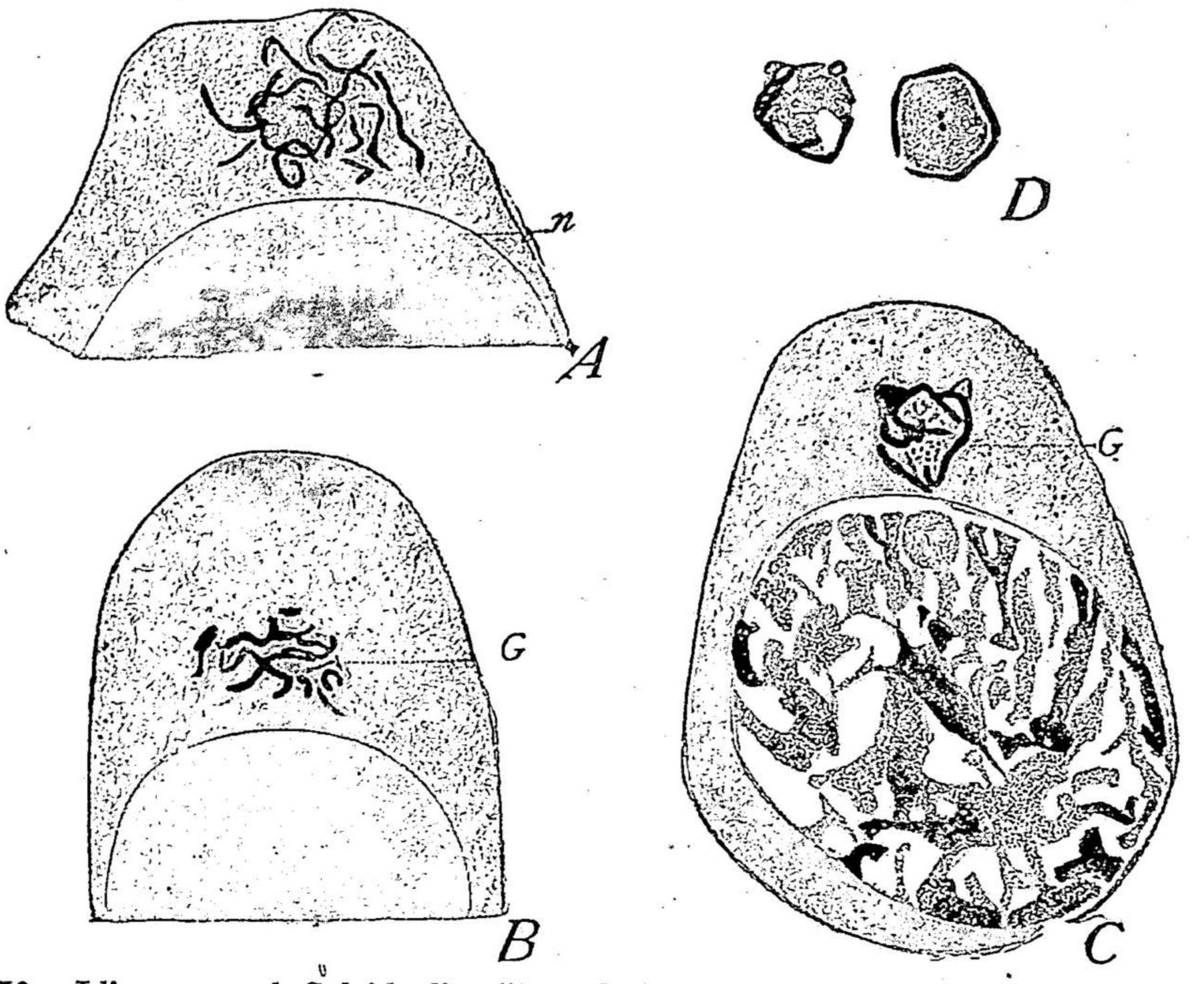

Fig. 150.—Idiozome and Golgi-bodies ("pseudochromosomes, chondriomites") in the spermatocytes of *Proteus* (HEIDENHAIN).

Both structures shown in *A*, the Golgi-bodies alone in *B* and *C; D*, two views of the "central capsule" formed by the Golgi-bodies, enclosing the idiozome and two central bodies.

both these latter appearances may be, wholly or in part, due to the technique.[1]

Lastly, the auxocytes always contain numerous chondriosomes in the form either of small mitochondria, rods, chondrioconts or chondriospheres. In earlier stages these are often more or less massed around the idiozome outside the Golgi-bodies and sometimes almost wholly confined to this region. They thus tend to form a cap on the nuclear wall (Figs. 157, 158) and may even extend completely around the nucleus to form a perinuclear zone, a condition especially common in the oöcyte.

[1] By earlier observers the Golgi-bodies associated with the idiozome were variously called "archoplasmic loops" (Hermann '91, '97) "pseudo-chromosomes" and "central capsule" (Heidenhain '00), or "peri-idiosomatic" bodies (Terni '12). Kuschekewitsch ('13) proposed to call the whole structure thus formed the *statosphere*, the Golgi-bodies or "spherosomes" forming a "sphærotheca" (central capsule) enclosing the idiozome and centrioles. The identification of the Golgi-apparatus in the auxocytes was first clearly indicated by Sjöval ('06) and has been confirmed by many later observers. Their subsequent dispersal through the cytosome is shown with especial clearness by the work of Weigl ('12) on insects, of Gatenby ('17–'18) on insects and pulmonates, of Hirschler ('19) on ascidians, and of Bowen ('20, '21) on insects.

C. Oögenesis. Growth and Differentiation of the Oöcyte

The phenomena of oögenesis offer numerous interesting problems, of which the most important are as follows:

(1) The morphological and physiological relations of the growing oöcyte to the accessory cells usually associated with it.

(2) The growth and transformation of the oöplasm, including the history of the central bodies, mitochondrial formations and Golgi-elements and the formation of yolk, pigment and other secondary structures.

(3) The history of the nucleus or germinal vesicle, in relation to the intense constructive processes of the oöplasm.

(4) The history of the chromosomes with reference to synapsis and meiosis.

(5) The localizing processes which result in the appearance of polarity, bilaterality and other promorphological features of the ovum.

We are at this point concerned primarily with only the first three of these, deferring the fourth to Chapter VI, and the fifth to Chapters XIII and XIV.

1. The Egg and Its Accessory Cells

During its period of growth the oöcyte very commonly becomes intimately associated with accessory cells that play an important part in its nutrition, though they may be absent. The two cases are distinguished respectively by Korschelt and Heider [1] as *alimentary* and *solitary* types of growth, the alimentary type being further characterized as either *follicular*, in which case the accessory cells form a continuous layer (follicle) surrounding the egg, or *nutrimentary*, in which the egg is accompanied by one or more nutritive *nurse-cells*, locally attached to it in various ways. These relations show many variations of which a few examples must here suffice. In the solitary type (*e. g.*, in pelecypods, or some echinoderms) the oöcyte, as it begins to enlarge, projects from the epithelial wall of the ovary and is finally set free, either into the cavity of the ovary or into the general cœlome or the genital duct. In some cases of this type it is set free at a very early stage and undergoes the greater part of its growth while floating in the cœlomic fluid (annelids); but in these cases the egg is often accompanied by nurse-cells (Fig. 151). More commonly the egg remains attached until nearly or full grown, often assuming a pear-shape, the narrow end forming a pedicel of attachment; and it has been shown in several such cases that the pedicel is situated at one pole of the egg and that the micropyle corresponds to the point at which it is finally withdrawn (p. 1023). The pedicel may be very long, *e. g.*, in the mollusk *Scrobicularia* (Fig. 488),

[1] '02, in which an extended review of the subject is given.

and still more so in the hemipteran insects, where it extends from the terminal nutritive end-chamber through a considerable part of the ovary to the growing egg (Fig. 152). It seems to have been proved in some of these cases that granular protoplasmic material (mitochondria?) actually flows from the other nutritive ovarian cells through the pedicel into the egg.[1]

In the follicular type, which is the most frequent, the egg is completely surrounded by a conspicuous follicle that often persists up to a late stage in its growth, or even until after its discharge. A good example of the latter case is offered by the ascidian egg. In many cases, however, the follicle

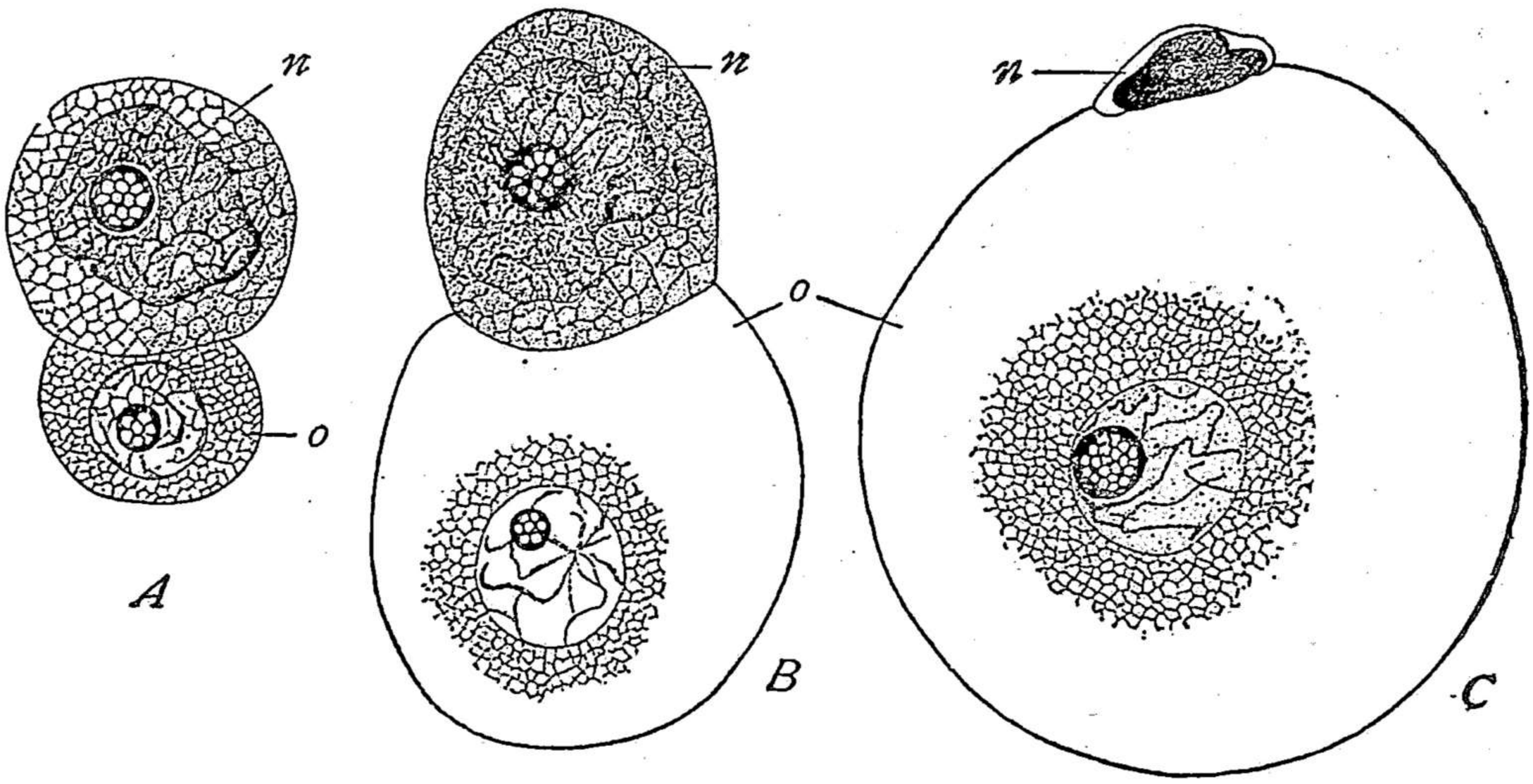

Fig. 151.—Oöcyte and nurse-cell in the annelid, *Ophryotrocha* (KORSCHELT).

A, young stage, the nurse-cell (*n*) larger than the oöcyte (*o*); *B*, growth of the oöcyte; *C*, late stage, the nurse-cell degenerating.

finally disappears, often after having secreted about the egg a resistant secondary envelope or chorion, as is typically seen in the insects. In other cases the follicle is ruptured and thrown off at the time the egg escapes from the ovary (mammals). The follicle-cells show wide differences of structure in different cases varying from an inconspicuous flattened epithelium up to high columnar forms, sometimes of remarkable type (ascidians) or to thick stratified epithelia (mammals). Their structural connections with the egg are referred to beyond (p. 335).

The follicular type of growth is connected by various intermediate conditions with the "nutrimentary," in which the egg is accompanied by a certain number of nurse-cells, which, however, do not completely surround

[1] This was suspected by some of the earlier observers, and seems to have been clearly established by more recent workers. See Jhering ('74), Stauffacher ('94), Wieman ('10), Dederer ('15), Nusbaum-Hilarowicz ('17).

it. In the simplest case there is but a single nurse-cell attached to the side of the oöcyte (*Sacculina*, *Ophryotrocha*). In the annelid *Ophryotrocha* (Korschelt, '93) the oöcyte and nurse-cell float in the cœlomic fluid, the nurse-cell being at first much larger than the oöcyte and containing a nucleus of wholly different type, being large, irregular and rich in basichromatin (Fig. 151). As the oöcyte grows the nurse-cell diminishes, being finally reduced to a mere rudiment, but without fusing with the egg. In *Myzostoma* (Wheeler, '97) the oöcyte is accompanied by two nurse-cells at opposite sides which fuse with the oöcyte at an early period, though their nuclei long persist at opposite poles. Owing to this fact Wheeler was able to show that the axis thus marked out persists as that of the mature egg, one nucleus lying in the region of the vacuolated cytoplasm at the animal pole, the other in that of the granular cytoplasm of the vegetative pole. In the annelid *Diopatra* Andrews ('91) found that the very young oöcyte lies between two long strings of nurse-cells attached to it on opposite sides; and here, too, there appears to be a constant relation between the nurse-cells and the egg (though a different one from that seen in *Myzostoma*).

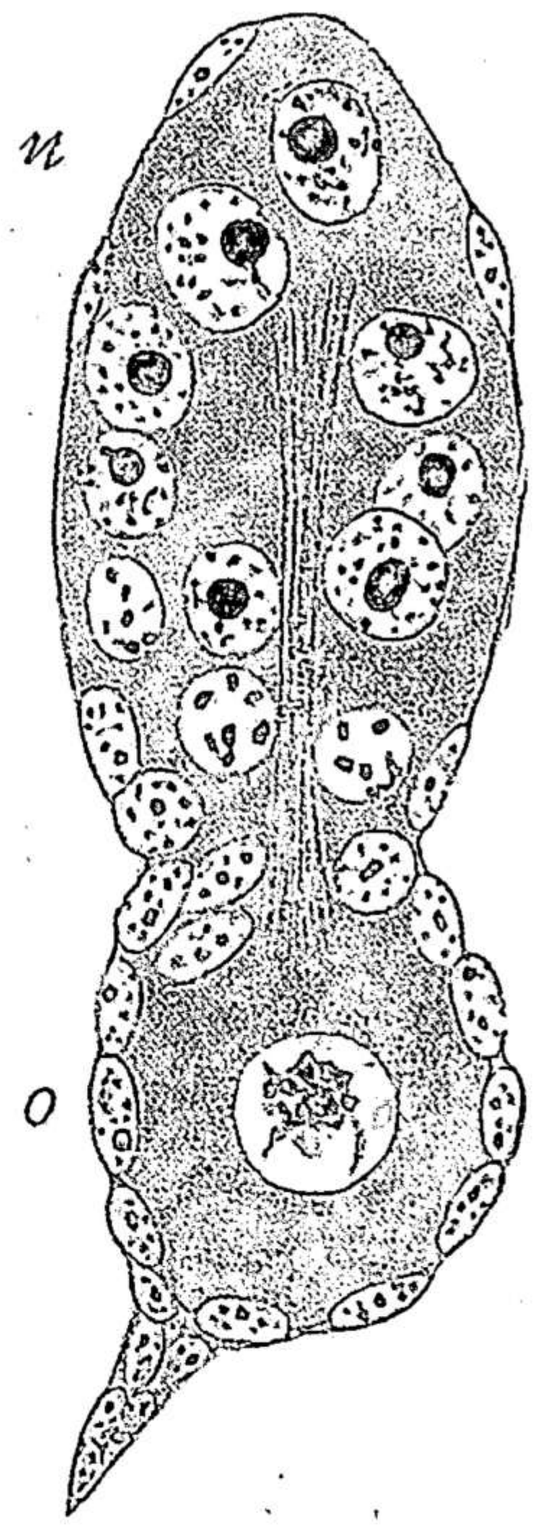

Fig. 152.—Ovary of parthenogenetic female of *Aphis rosæ*, showing mass of nurse-cells above and oöcyte below, connected with the former by nutritive root (DE BAEHR).

In a more frequent and rather widely distributed type the nurse-cells are aggregated in a coherent group at one side of the egg. In the annelid *Tomopteris elegans* (Chun) the germ-cells (oögonia) are set free from the ovary in groups of eight, one of which becomes the oöcyte, growing at the expense of the other seven which remain attached until a late stage at one side of the egg. The conditions are similar, but more extreme in the leech (*Pisciola* Jörgenssen, '10). Here the oögonia are set free from the solid portion of the ovary into the lumen in groups of four or five, which increase by division to the number of about fifty. Of these only one, as a rule, becomes an oöcyte, while all the others remain small and finally degenerate; two or even three of the cells may produce ova; and it is an interesting fact that *all of them, nurse-cells and oöcytes alike, may pass through the synaptic stages* (bouquet, etc., p. 543). There seems, therefore, to be no doubt in this case that the *nurse-cells are abortive eggs* which, by a physiological division of labor, sacrifice their own future to that of the functional egg (p. 336). This is confirmed by the conditions seen in various arthro-

pods, particularly in the insects, which have been the object of numerous important investigations, prominent among them those of Korschelt ('86, '89), from which many of the following facts have been drawn.

In these animals the eggs and nurse-cells lie serially in the ovarian tubules and the eggs are usually surrounded by a follicle. In the grasshoppers the eggs typically lie in a single series, surrounded and separated by follicle-cells, but without other nutritive cells. In many other insects the eggs lie in a series, alternating with nutritive chambers in which lie one or more nurse-cells. In *Forficula* each nutritive chamber is occupied by a single

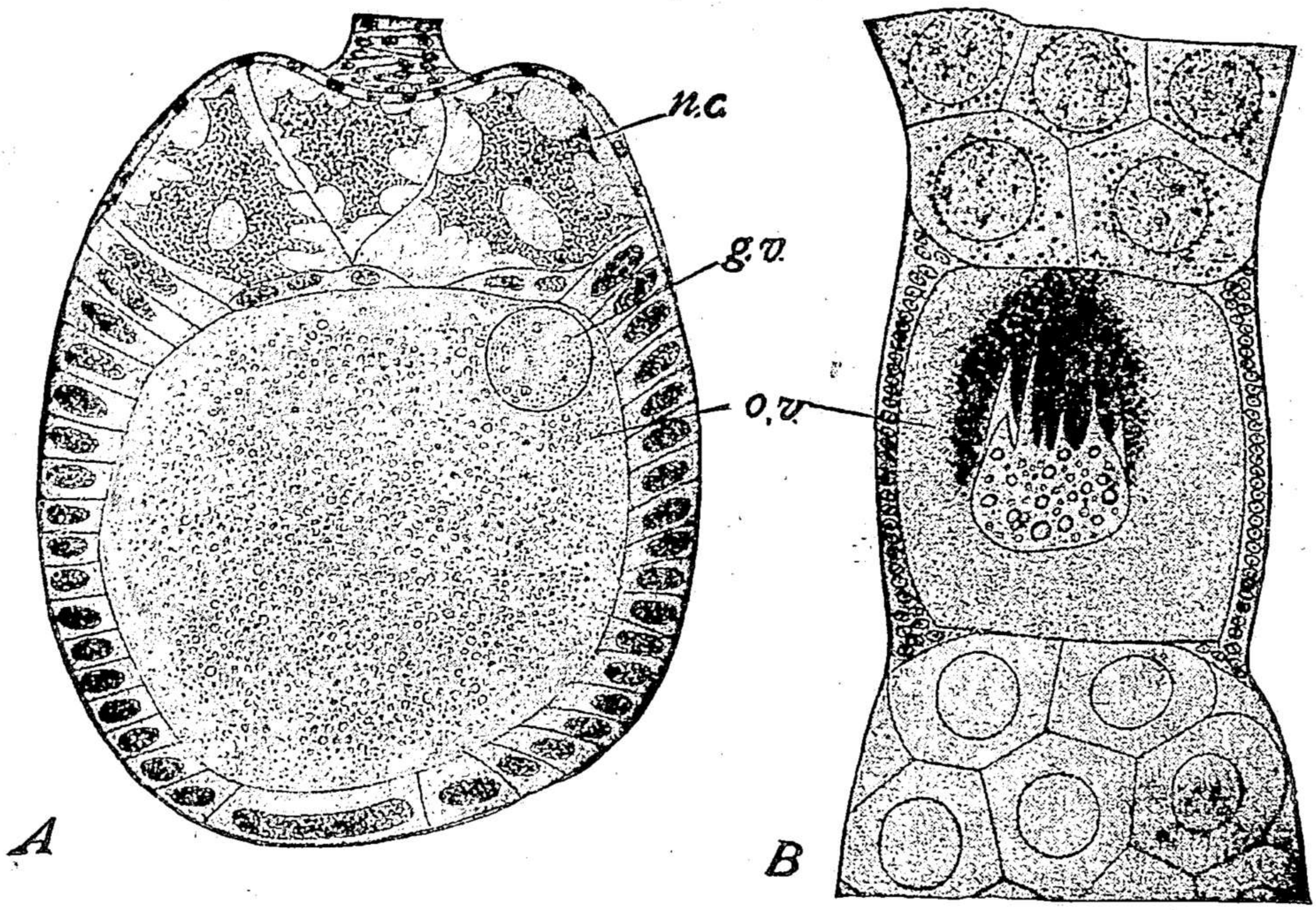

Fig. 153.—Ovarian eggs of insects (KORSCHELT).

A, egg of the butterfly, *Vanessa*, surrounded by its follicle; above, three nurse-cells (*n. c.*) with branching nuclei; *g. v.*, germinal vesicle; *B*, egg of water-beetle, *Dytiscus*, living; the egg (*o. v.*) lies between two groups of nutritive cells; the germinal vesicle sends amœboid processes into the dark mass of food-granules (chondriosomes?).

nurse-cell of great size and having in its fully developed state a very large branching nucleus (Fig. 320). In the coleopteran type the nutritive chamber contains a considerable number of closely packed nurse-cells (Figs. 153, 155), which in *Dytiscus* are 15 in number. The lepidopteran type is in some respects intermediate between the coleopteran type and that seen in *Forficula*, the nurse-cells lying (in *Vanessa*, Fig. 153) in a single layer so as to appear like enlarged follicle cells with very large branching nuclei.

In some cases an actual fusion takes place between the egg and the accompanying nutritive cells. In the tunicates, for example, follicle-cells

migrate into the egg in considerable numbers to form the "test-cells," long a morphological puzzle.[1] Nearly related with this are the phenomena in various cœlenterates (*Hydra*) and mollusks (*Helix*) where the egg fuses with or engulfs certain of the neighboring nutritive cells or follicle-cells (Fig. 154)[2] and the same occurs in certain cœlenterates (*Hydra*) and

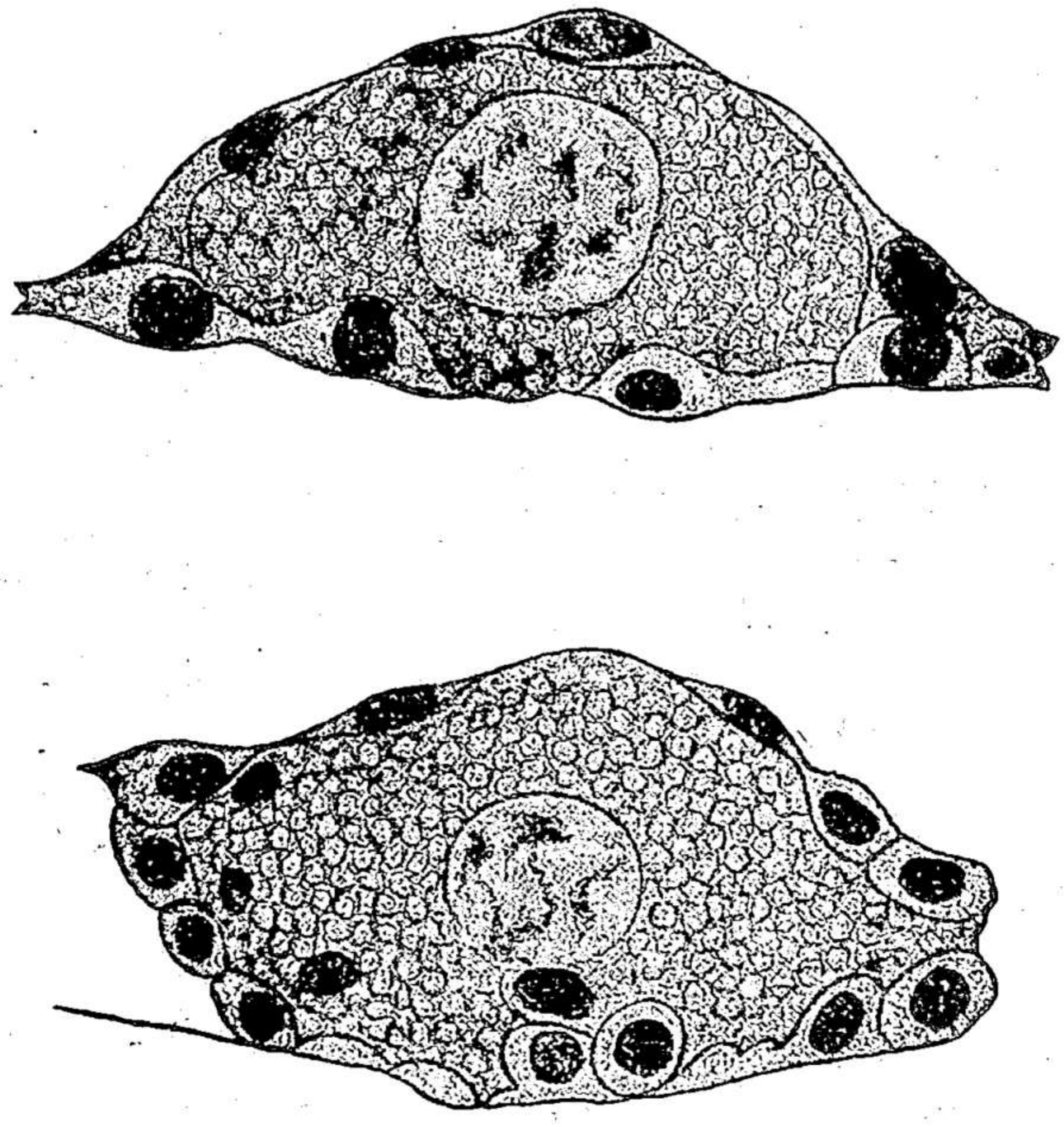

Fig. 154.—Ovarian eggs of *Helix* (OBST).

A, earlier stage, surrounded by follicle; *B*, later stage, showing inward migration and absorption of follicle-cells.

mollusks (*Helix*). In other cases the perinuclear zone of the oöcyte is connected directly with the nurse-cells by conspicuous protoplasmic bridges, through which the mitochondrial contents of the nurse-cells are said finally to pass inwards to the egg (Fig. 155). A similar function, perhaps, is performed by the more numerous and delicate protoplasmic cell-bridges between the oöcyte and its surrounding follicle-cells, which have been described by many observers from an early period. They have recently been carefully studied by Retzius ('12) who has given a valuable review of the litterature and has published many remarkable figures showing how in all the main groups of vertebrates the follicle cells give off from their bases conspicuous protoplasmic processes or filaments which penetrate the so-called *zona radiata* (egg-envelope) and enter the cortical layer of the oöcyte (Fig. 156). Similar cell-bridges have also been demonstrated connecting the eggs of plants with the surrounding cells.[3] It is not known whether

[1] See Floderus, '96, Bancroft '99, etc. [2] See Doflein ('97), Floderus ('95), Obst ('99), etc.
[3] See Goroschankin, '83, Ikeno, '98, A. Meyer, '96, etc.

these bridges are lines of actual protoplasmic flow, but it can hardly be doubted in view of all the facts, that they are paths through which nutrient substances are in some form passed into the egg.

The morphological relationship of the nutritive or accessory cells to the oöcyte has long been a subject of controversy. In cases where a single nurse-cell or group of such cells is associated with the egg, it is beyond doubt that these cells have a common origin with the oöcyte and should be regarded as abortive or rudimentary eggs that have been specialized for

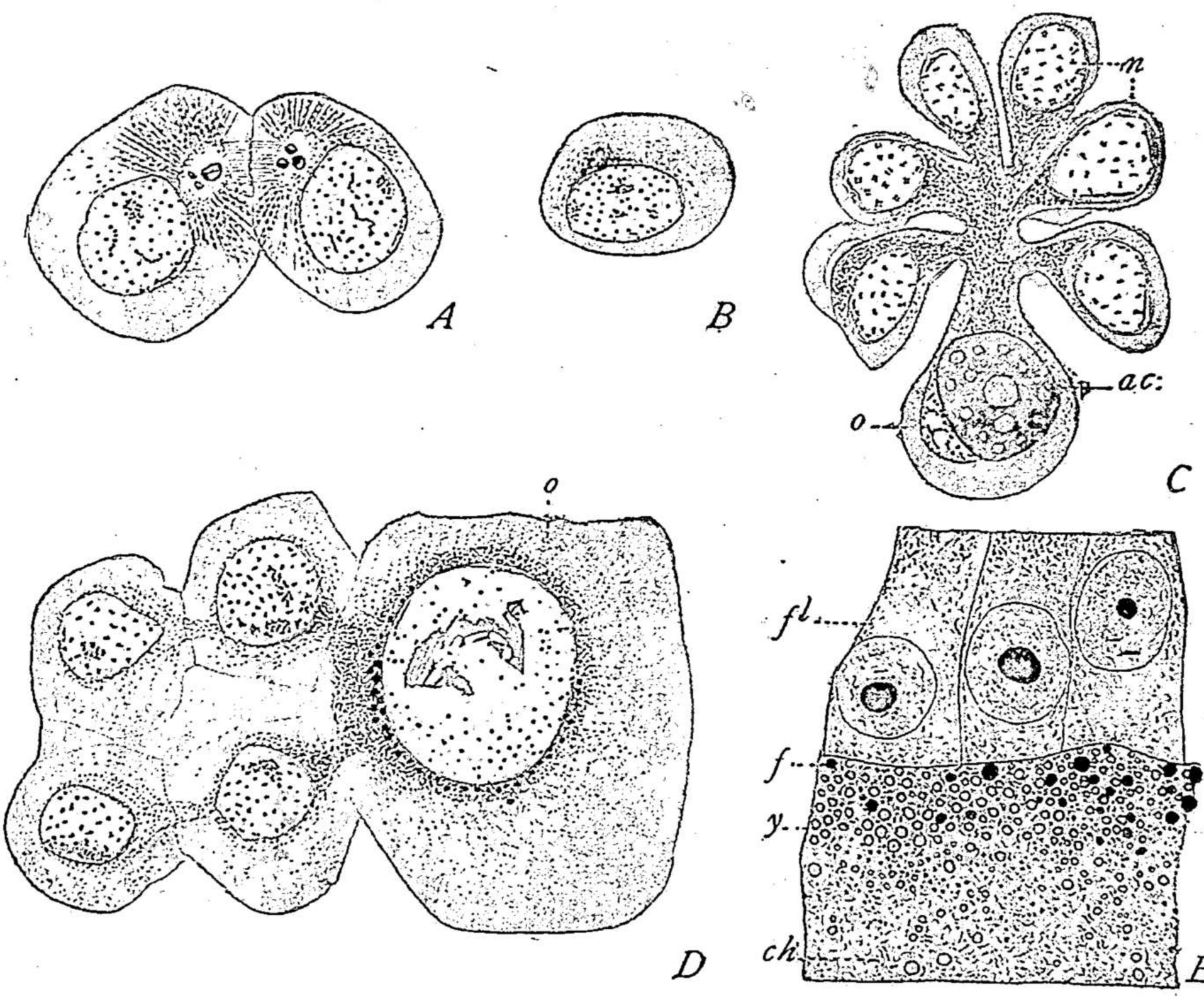

Fig. 155.—Relations of egg and nutritive cells in *Dytiscus* (NUSSBAUM-HILAROWICZ).

A, *B*, oögonia, in division and in repose; *C*, oöcyte (*o*) showing six of the 15 nurse-cells (*n*) and the accessory body, *ac*, Giardina's ring; *D*, later stage, showing streams of mitochondria; *E*, peripheral region of much older oöcyte; *ch*, chondriosomes; *f*, fat-drops; *fl*, follicle-cells; *y*, developing yolk-spheres. (*Cf.* Fig. 147.)

the elaboration of food-materials at the expense of which the functional ovum grows. This is demonstrated, for instance, by Giardina's observations on *Dytiscus* (p. 326), or those of Jörgenssen ('13*b*) on *Pisciola*; and it is very probably true of many other cases of this type (*Orphryotrocha*, *Myzostoma*, *Diopatra*, etc.). A confirmation of this is given by the above mentioned remarkable fact (p. 333) that *the nuclei of the nurse-cells may form tetrads* closely similar to those seen in the oöcyte-nucleus,[1] and even may

[1] Woltereck ('98) in the ostracodes, Giardina ('02) in *Dytiscus*, Grünberg ('03) in *Pieris*, Marshall ('07b) in *Platyplax*, and others.

pass through characteristic leptotene, synizesis and pachytene-diplotene stages (p. 537), in the course of which they undergo pseudo-reduction.[1] In some of these cases, according to the observations of Dederer and of

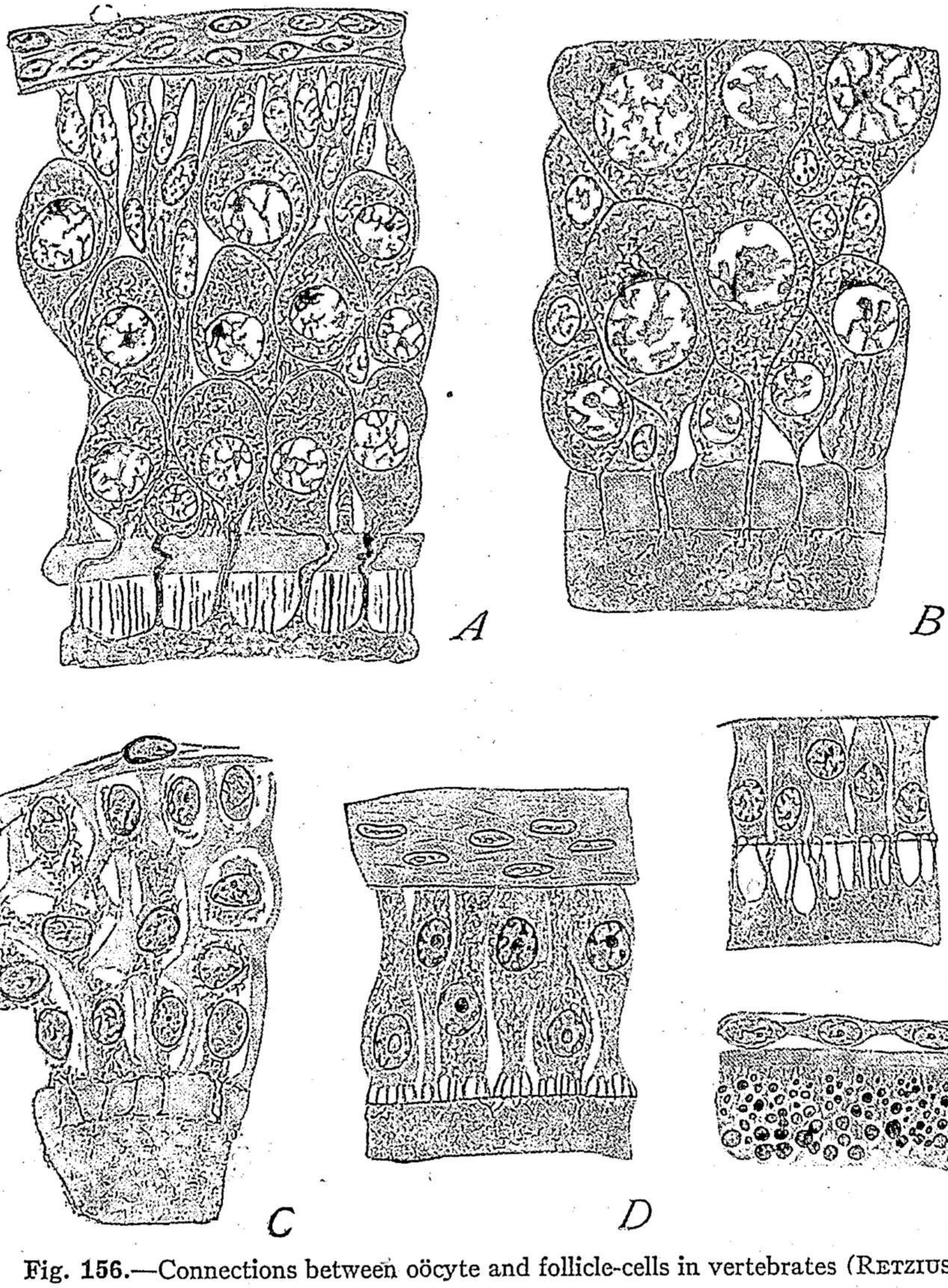

Fig. 156.—Connections between oöcyte and follicle-cells in vertebrates (RETZIUS).

A, in *Chimæra; B*, *Raja; C*, the rabbit; *D*, the pigeon; *E*, the domestic fowl. The foregoing from earlier stages of the oöcyte; *F*, late stage in *Lacerta*.

Hogben, these changes are passed through by all the cells before the nurse-cells can be distinguished from the functional oöcytes, all starting out along the same pathway, but many of them being arrested to form nurse-

[1] Doncaster ('12) in *Pieris*, Dederer ('15) in *Phyllosamia*, Hogben ('20a) in *Rhodites*. See also Paulcke ('00), McGill ('06).

cells before completing their development. Undoubtedly, therefore, the nurse-cells should in such cases be regarded as abortive ova (oöcytes) that are sacrificed for the benefit of their more fortunate brethren.

The case is by no means so clear in respect to the follicle-cells. In the insects the important investigations of Heymons ('95) and others seemed to demonstrate that the follicle-cells and terminal filament of the ovary were derived from mesoblastic cells not arising from primordial germ-cells and only secondarily associated with the latter. On the other hand, Marshall ('07) found in Hymenoptera and in the phryganids that both kinds of cells had a common origin; and a similar result has been reached by Vejdovský ('11, '12) in the orthopter *Diastrammena*; by Dederer ('17) in Lepidoptera, and by Hogben ('20a) in Hymenoptera. In the vertebrates a strong case has been made out for the common origin of ova and follicle-cells. Winiwarter and Saintmont, as above stated, leave this question in doubt. Bühler ('94) long since described the origin of germ-cells (presumably oögonia) by tangential division of columnar epithelial cells in the germinal epithelium of the mammals; and this is confirmed by the recent studies of Gutherz ('18) who also described the origin of oögonia by a direct metamorphosis of the epithelial cells without division. For the present, however, it seems necessary to suspend judgment on this difficult question. It is, after all, a question of detail, relating as it does only to the relative time at which the germ-cells are finally set apart from the somatic cells, in respect to which we know that very wide variations occur (p. 312). We should not permit such variations to obscure our view of the large fact, emphasized by Nussbaum, that sooner or later in the ontogeny the descendants of the original egg become differentiated into two groups, germinal and somatic; and the significance of this is not lessened by the fact (if, as the writer believes, it is a fact) that in theory any cell of the body many contain the potentiality of the whole.

2. General History of the Oöplasmic Components

The enormous increase in the cytoplasmic or oöplasmic substance during the growth of the oöcyte leads to the production of the largest known forms of cells (p. 98). In large and heavily yolk-laden eggs this enlargement is mainly due to the loading of the cytosome with passive reserve-materials (yolk or deutoplasm); but apart from this it seems clear that the ovum also contains an exceptionally large amount of active protoplasm. The growing oöcyte therefore forms a very advantageous object for study of the cytological changes; while, more broadly viewed, it offers problems of fundamental interest for the analysis of the problems of localization and differentiation. These considerations have naturally at-

tracted the attention of cytologists to this subject from an early period, and have made it the center of an evergrowing mass of literature. Unfortunately, it offers difficulties that have not yet been overcome; and it must be admitted that in many respects we are still far from an adequate understanding of the phenomena.

In the young oöcyte, the cytoplasm is at first both very small in amount and simple in structure, often seeming to consist almost wholly of optically homogeneous hyaloplasm, which in the living object shows only a few scattered granules. As growth proceeds the structure becomes more complicated by the appearance of an alveolar or pseudo-alveolar structure, due to the formation of true alveolar spheres or of yolk-spherules (p. 73).[1] Since nothing is known of the manner in which the hyaloplasm increases, our study of cytoplasmic growth and differentiation thus reduces itself largely to the development of the yolk and other formed elements.

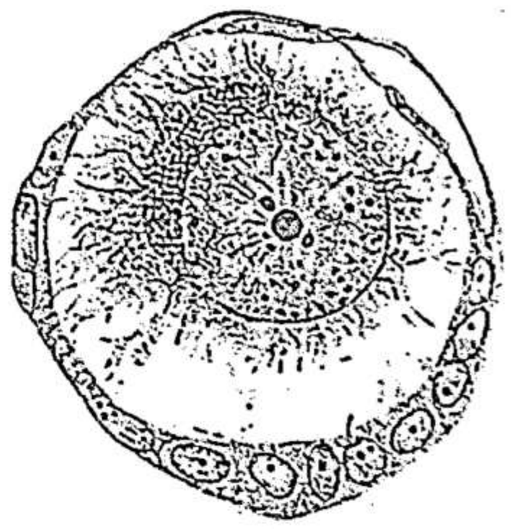

Fig. 157.—Young human oöcyte, surrounded by its follicle, showing the crescentic vitellogenous layer applied to the nucleus (VAN DER STRICHT).

In spite of numerous researches on the yolk-formation, extending over a period of more than fifty years, the subject still remains in so confused a state that all statements in regard to it must be made with considerable reserve. Even in the recent literature we find the origin of the yolk-spherules ascribed to chondriosomes, to Golgi-bodies, to chromidia, extruded nucleoli or nucleolar fragments; while some observers consider that the yolk arises *de novo* in the cytoplasmic substance without discoverable relation to other formed elements. We must, therefore, conclude either that there is no general uniformity in the mode of yolk-formation, or that many of the existing accounts of the subject are erroneous.

a. The Yolk-nucleus or Vitelline Body. (*Dotterkern, corps vitellin*). By these terms is designated a cytoplasmic body, first observed in the oöcytes of spiders by Wittich ('45) and called the "yolk-nucleus" by Carus ('50), and undoubtedly concerned in some manner with the yolk-formation.[2] The nature of this body has been a subject of long continued controversy.[3] In the greater number of cases it is a single body lying near the nucleus; but the same name has been applied to several or many cytoplasmic bodies scattered through the cytosome or in its peripheral layer. These latter in-

[1] *Cf.* Wilson, '99.

[2] The extended literature on this subject is reviewed in the works of Jordan ('93), Mertens ('93), Henneguy ('93, '96), Van Bambeke ('98), Crampton ('99), Korschelt-Heider ('02), Waldeyer ('06), Fauré-Fremiet ('10), Munson ('12), and others.

[3] The early view of Will ('11), Henneguy ('93), Balbiani ('83, '93) and a few others that the yolk-nucleus arises from the germinal vesicle, either by a process of budding or by the extrusion of nucleolar elements, has not been sustained by more recent studies.

clude amorphous masses of material that have been described by some observers as "archoplasm," "ergastoplasm," etc., and seem to be of varied nature. All these will here be left out of account except in so far as they may represent material arising by the fragmentation of one original yolk-nucleus or of material associated with it.

Wittich found the yolk-nucleus as a rounded body lying near the germinal vesicle, later enlarging markedly and acquiring a concentric fibrillated or laminated structure; and as a spheroidal body it was later described by a number of the earlier observers (Fig. 158).[1] Others found it as an elongated body, composed of finely granular material, either spread out like a crescentic cap at one side of the nucleus or extending completely around it to form a *perinuclear zone* closely applied to the nuclear wall (Fig. 157).[2]

Within the latter, in many cases, lies a well-defined, rounded body that corresponds to Wittich's yolk-nucleus, as was shown to be the case in Wittich's own object, the spider (*Tegenaria*) by Van der Stricht ('98) and later by Fauré-Fremiet ('10). As a matter of historical priority, therefore, it seems clear that the term "yolk-nucleus" should be restricted to the smaller spheroidal body within the crescent or ring. The latter has received various names.[3] We here make use of Bambeke's convenient and non-committal term *pallial layer* or pallial substance, employing the term *yolk-nucleus-complex* to the double structure formed by the yolk-nucleus together with the pallial substance.

(1) In many cases the yolk-nucleus has been found to contain one or two central granules closely similar to centrioles, thus suggesting its identity with the idiozome of the early spermatocytes.[4] It is in some cases surrounded by conspicuous astral rays but this seems exceptional.[5] A number of observers have failed to find any definite body surrounding the centriole; others have failed to observe either yolk-nucleus or central granules, but in all such cases it is doubtful whether the technical treatment has revealed the complete, normal structure. The identity of the yolk-nucleus with the idiozome of the early oöcyte or spermatocytes, accepted by most observers of the French and Belgian schools, receives strong support from the relation of the yolk-nucleus to the Golgi-bodies and the chondriosomes.

[1] *E. g.*, by Balbiani ('93) and Henneguy ('93).

[2] *E. g.*, by Holl ('90) in the hen, O. Schultze ('87) in the frog, Nemec ('97) in the myriapods, or Bambeke ('98) in *Pholcus*.

[3] *E. g.*, the *pallial layer* or *mantle-layer* (Van Bambeke); *vitellogenous layer* or *vitellogenous mass* (Van der Stricht); *yolk-matrix* (Crampton); *Dotterkernlager* of German writers.

[4] Balbiani ('93) first definitely urged this comparison, maintaining that the yolk-nucleus is equivalent to an "attraction-sphere plus a centrosome"; and the same conclusion was adopted by Van der Stricht ('98), Munson ('98, '04, '12), Lams ('07), Fauré-Fremiet ('10), Loyez ('11), Sonnenbrodt ('08) and many others. Meves ('98) adopted a similar view, recognizing the yolk-nucleus as an idiozome in the same sense as the "sphere" and central bodies of the spermatocytes.

[5] See Balbiani (*op. cit.*), Munson ('98, '04, '12), Lams ('07).

(2) The nature of the pallial layer is still but partially cleared up. It seems to include both chondriosomes and Golgi-bodies and according to some observers also extruded nucleolar material. That the pallial substance consists largely of chondriosomes has been maintained by many observers.[1] Nussbaum-Hilarowicz ('17) considers that in *Dytiscus* it is in large part derived from the nurse-cells associated with the oöcyte (Fig. 155). The Golgi-bodies of the pallial layer were recognized and figured by Holmgren ('00) and by Sjövall ('06) in the oöcytes of mammals and have recently been studied more carefully by Hirschler ('18) in the öocytes of ascidians and mollusks and by Gatenby ('19) in those of mollusks. In the latter case (*Paludina*) they show the typical relations, being aggregated about the idiozome with the mitochondria outside them. In the ascidian they are considerably modified, as will presently be indicated.

Although the data are still rather scanty, the conclusion seems on the whole justified that the yolk-nucleus-complex shows a close analogy to the idiozome-complex of the spermatocytes and that the two may be regarded as homologous formations. Some observers have, however, found a central body and aster lying quite outside the pallial mass or "yolk-nucleus," *e. g.*, in the myriapod *Polyzonium*, according to Nemec ('97). In other cases the mitochondria show but little tendency to aggregate about the yolk-nucleus, while the Golgi-bodies may be quite separate from it, *e. g.*, in the ascidian (Hirschler, '19) which also differs from the usual type in the fact that two yolk-nuclei are often present, in neither of which could central bodies be detected (Fig. 348). It seems probable, therefore, that the association between yolk-nucleus (idiozome) and the mitochondrial and Golgi-elements, though a very common one, is not essential.

Lastly, there is some reason to conclude that the pallial substance may also contain nucleoli or nucleolar fragments extruded from the germinal vesicle; but no general agreement has yet been reached concerning this point, which involves great difficulties of observation (p. 345); and the same may be said, with added emphasis, concerning the supposed extrusion of chromidia from the nuclear network described by Schaxel, Buchner, Hargitt and other observers (p. 700).

b. Formation of the Yolk. The pallial layer is found only in the earlier stages of the oöcytes and does not long persist as such. Sooner or later it separates from the germinal vesicle, moves outward toward the periphery, breaking up meanwhile into smaller and smaller fragments that are finally dispersed in a finely divided state through the cytosome; and this is followed by a rapid formation of yolk-spherules while the oöcyte enters upon an

[1] Van der Stricht ('05, '09, etc.), Lams and Doorme ('07), in mammals; Loyez ('09), in ascidians; Fauré-Fremiet ('10) in myriapods and arachnids, etc.

active process of growth. At the same time the yolk-nucleus commonly disappears from view and in most cases the central bodies cannot be distinguished until the period of maturation approaches.[1]

The details of these various processes vary widely in different species. In some cases, of which a remarkable example is offered by the spider

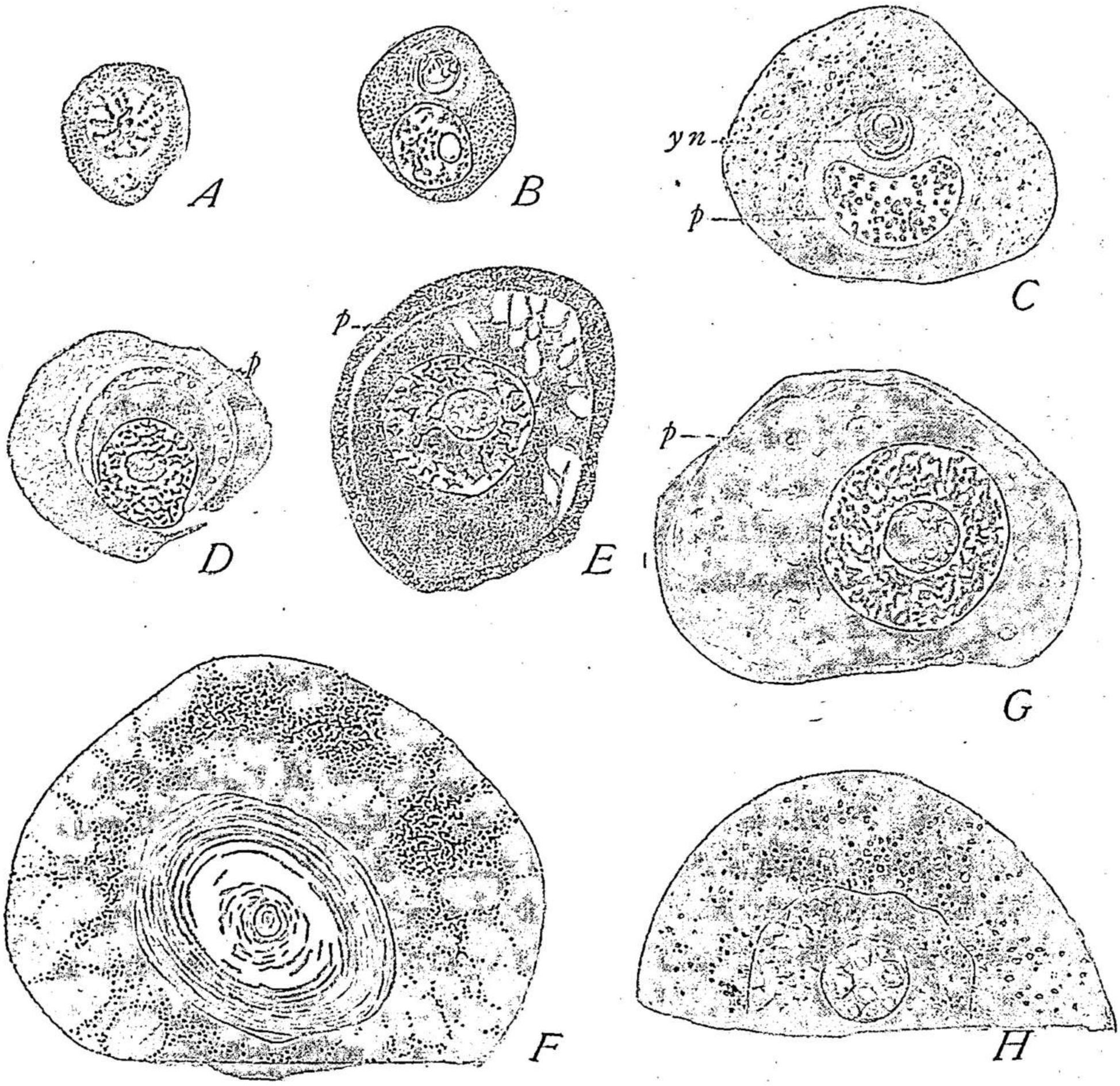

Fig. 158.—Yolk-nucleus, pallial substance and yolk-formation in spiders. (*A–C*, *F*, *Tegenaria*, from VAN DER STRICHT; *D*, *E*, *G*, *H*, *Pholcus*, from VAN BAMBEKE).

A, *B*, *C*, early stages, showing idiozome (yolk-nucleus) and pallial or vitellogeneous layer; in *B* the Golgi-bodies (pseudochromosomes) are shown; in *C* the pallial layer (*p*) completely surrounds the nucleus and yolk-nucleus; in *D* the pallial substance is separating from the nucleus in the form of a crescent; in *E* in the form of a ring; in *G* it is breaking up; *F*, yolk-nucleus and mitochondrial granules; in *H* the pallial substance is broken up and the deutoplasm is appearing; yolk-spherules pale, fat-drops black.

Pholcus (Van Bamkeke, '98) the pallial layer separates from the germinal vesicle, usually in the form of a very definite horse-shoe shaped cap which may nearly encircle the nucleus, or of a ring which moves outward nearly to the cell-periphery where it fragments at first into irregular masses

[1] In the spiders the yolk-nucleus persists throughout a large part of the growth-period; and in some species, as long since shown by Wittich, it remains nearly unchanged in the embryo throughout nearly the whole of its development.

(Fig. 158) and finally into very minute granules dispersed through the whole egg. A similar ring, having a similar history is described in the myriapod *Julus* by Fauré-Fremiet ('10) (Fig. 159). The process of disintegration and dispersal may take place while the pallial mass is still in contact with the germinal vesicle, for instance in the earthworm or the tunicate (Fig. 346). The process in the latter case was carefully examined by differential staining in *Molgula* by Crampton ('99), and later in *Ascidia* by Hirschler ('18) who employed modern methods for the differentiation of the mitochondria and Golgi-bodies. By the aid of these staining-reactions it is easy to follow the progressive breaking up of the pallial mass, its dissemination through the cytoplasm and the ensuing appearance of yolk-spherules.

The general type of transformation described above is of widespread occurrence, but shows many variations of detail. An interesting example of the latter appears in the amphibian oöcyte, which has been carefully examined by Lams ('07) in *Rana* and by Gajewska ('17, '19) in *Triton*. In both these cases the pallial substance forms in the early stages the usual cap-like mass within which Lams demonstrates the yolk-nucleus (idiozome) with one or two central bodies. In both a perinuclear ring or pallial layer may be formed, but this appears to be a regular process in *Triton* and only an occasional one in *Rana*. In both, the pallial substance spreads out through the oöplasm in a deeply staining and more or less net-like condition; and in *Triton* tends to accumulate near the periphery to form an "exoplasmic layer." Ultimately, as in other cases, this substance becomes finely disseminated through the oöplasm.

During or subsequent to the foregoing changes the deutoplasm begins to appear in the form of yolk-spherules, fat-drops or both, at first scattered and very minute but later rapidly enlarging and commonly becoming crowded so as to produce a "pseudo-alveolar" structure. The precise origin of these bodies is a question which the researches and controversies of fifty years have left still unsettled. Modern studies have indeed made it nearly certain that they are somehow connected with the pallial substance (hence the term "vitellogenous substance" or "vitellogeneous layer") but beyond this point the existing accounts are still widely divergent.

Concerning the relation of the original formed elements to the definitive yolk-bodies two divergent views have been held from an early period down to the present time. It has been held, on the one hand, that the original formed elements (dispersed fragments of the pallial substance or its derivatives) are directly transformed into yolk-spherules or fatty bodies; on the other, that most of the original formed elements are completely absorbed by the protoplasm and disappear while the deutoplasmic elements are formed *de novo*. Perhaps both views are correct, for the term yolk or

deutoplasm designates a variety of storage materials in the egg, and these may differ in mode of origin, so far at least as the visible phenomena go. This possibility was clearly placed in evidence by the notable work on the spider's egg of Van Bambeke ('98), who concluded that there are two principal types of deutoplasm, namely, fatty granules which blacken greatly in osmic acid and are readily soluble in xylol, and ordinary yolk-spherules which show the opposite qualities and are presumably albuminous in nature. The former Van Bambeke believed to be directly derived

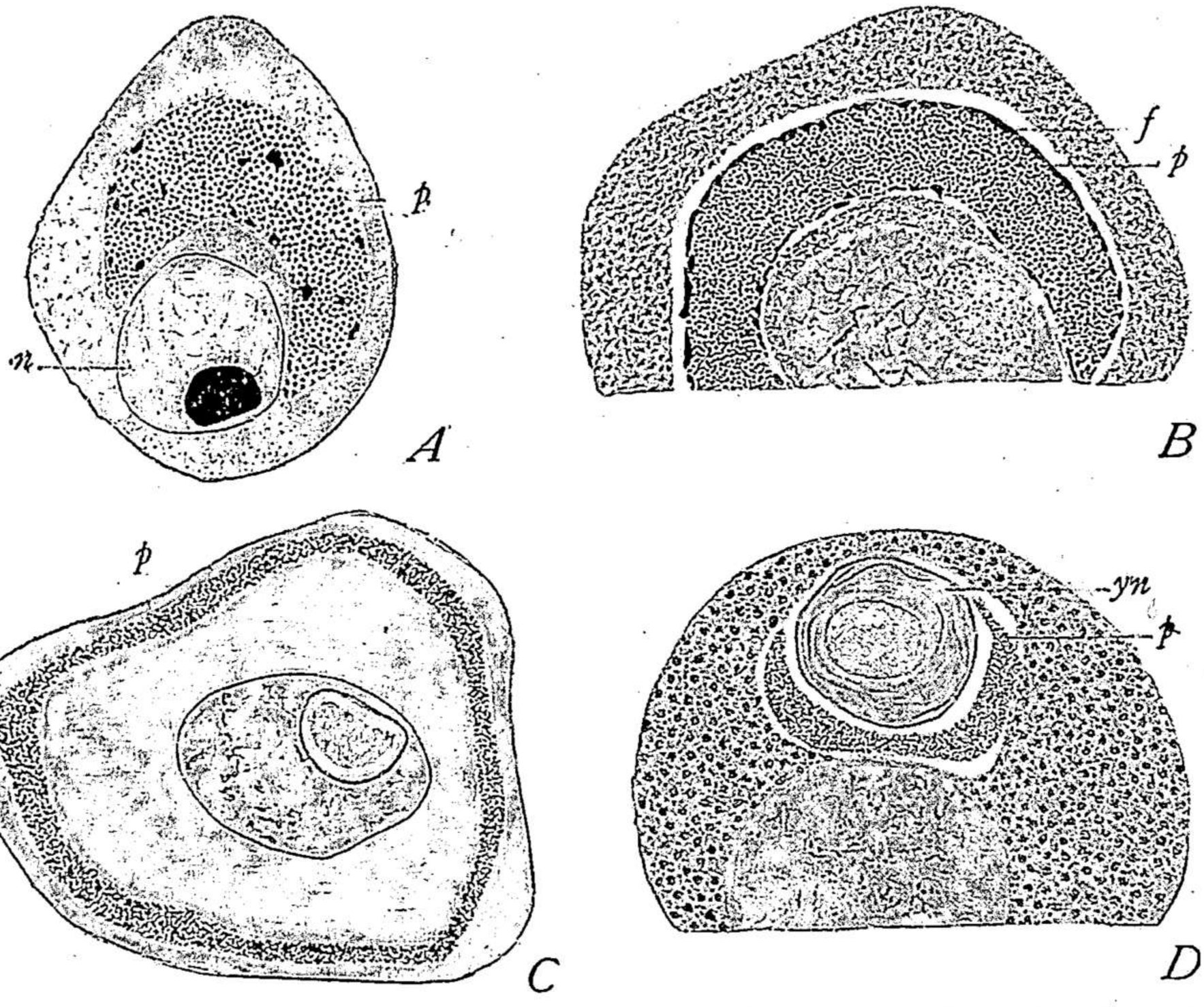

Fig. 159.—Yolk-nucleus, pallial substance and yolk in the oöcytes of myriapods and spiders (FAURÉ-FREMIET).

A–C, Julus; D, Tegenaria.

A, earlier stage pallial substance (*p*) separating from nucleus; *B*, later stage; in both these cases fatty deutoplasmic elements (black) developing in the pallial substance; *C*, the pallial substance has separated in the form of a ring. In *D*, the yolk-nucleus, *yn* (idiozome) is partly surrounded by the pallial layer and by fibrillar differentiations (chondrioconts?).

from finely divided pallial substance, the latter to be formed *de novo* in the cytoplasm; and this is substantially confirmed by the important later work of Fauré-Fremiet ('10) who brings forward evidence in the case especially of myriapods (*Julus*, *Lithobius*) and of the ascidian *Ciona*, that the fatty bodies are in some cases actually formed within the pallial layer (Fig. 159). It is, however, still doubtful in what measure this distinction holds for the yolk-formation generally. The close connection between chondriosomes and the yolk-formation has been urged especially by Van der Stricht and his followers. Van der Stricht himself ('05, '09) was inclined to the view

that the mitochondria and chondrioconts (in mammals) are not actually converted into yolk but become clumped to form mitochondrial aggregates which give rise on the one hand to yolk-bodies, on the other to the scattered chondriosomes of the oöplasm. The results of Lams and Doorme ('07) on mammals likewise demonstrated a very close connection between the mitochondria and the yolk. Loyez ('09) found in some species of ascidians (*Ciona, Molgula, Ascidia*) a direct transformation of mitochondria into yolk-bodies; and Hirschler ('16) has recently confirmed this account in *Ascidia*. In an earlier work ('13) the latter author follows out very circumstantially the direct transformation of mitochondria into yolk-spherules by enlargement accompanied by differentiation of a central clearer substance, which no longer takes the crystal violet stain by Benda's method, and a peripheral one that at every stage is intensely stained by that dye. This seems to be the best evidence thus far produced of a direct origin of yolk-spherules from mitochondria. A similar possibility is indicated by the works of Gatenby ('20a, '20b) on the pulmonates and some others, though this observer seems inclined to a somewhat negative attitude concerning such a mode of yolk-formation.

The most recent addition to the list of supposed yolk-forming bodies is given by the Golgi-bodies. Hirschler believes that in the ascidian egg the Golgi-elements as they enlarge and spread through the oöplasm unite secondarily with enlarged mitochondria to produce the yolk-spherules, which are accordingly said to have a double origin; in *Ascaris*, on the other hand, Hirschler found no connection between the yolk and the Golgi-bodies; Gatenby ('20, etc.) also produces evidence that the Golgi-bodies may take part in the yolk-formation in the pulmonate mollusks and in *Patella*. In the sponge *Grantia*, however ('20c), he believes the yolk-spherules to form independently of other preëxisting formed elements in the cytoplasm.

The origin of yolk from extruded nucleoli or their fragments has been advocated by some of the latest as well as a number of the early observers, but has also met with contradiction in many cases.[1] The best evidence seems to be offered in Hogben's remarkable accounts of the yolk-formation in insects ('20a, in Hymenoptera and especially 20b in the cockroach). Here the yolk is said to arise from granules ("deutosomes") that are formed in vacuoles inside the nucleoli *while still inclosed in the nucleus*, and are later cast out into the cytoplasm, when they migrate towards the periphery

[1] An extrusion of nucleoli or nucleolar fragments into the cytoplasm has been maintained (among others) by Will ('84), Henneguy ('93), Kohlbrügge ('01), Loyez ('06) and Gajewska ('17, '20) in the case of vertebrates; by Will, Woltereck ('98), Vejdovský ('11–'12), Gatenby ('20), and Hogben (20a '20b) in arthropods; by Montgomery ('98) and Jörgenssen ('13) in leeches; Hempelmann ('06) and Buchner ('14) in chætopods; Gelei ('13) in platodes, G. T. Hargitt ('13) in hydomedusæ, and by Denby ('14–'15) and Gatenby ('20) in sponges; but some of these, *e. g.*, Gatenby and Gajewska, have been unable to find any connection between the nucleoli and the yolk.

and there enlarge to form the yolk-spherules. Interesting possibilities are raised by these various observations which, broadly speaking, are in line with those of earlier observers who believed the zymogen-granules of the pancreatic and other glandular cells to be likewise derived from extruded nucleolar fragments;[1] and also with the more recent work of Schreiner on the origin of fat and mucin (p. 705).

Nevertheless, in view of the contradictions even in the most recent literature of the subject the time has hardly yet arrived for a judgment upon the matter.

Summary. Existing accounts of the formation of the yolk and growth of the egg still show many apparent contradictions of detail, but nevertheless are practically unanimous in regard to two outstanding facts. First, an important part in the yolk-formation, and perhaps also in the general growth of the oöplasm, is played by cytoplasmic formed elements (chondriosomes, perhaps also Golgi-bodies) that are known to be handed on from one cell-generation to another by mitosis and possibly may have the power of multiplication by growth and division. Not less striking, secondly, is a close association of these formed elements with the egg-nucleus prior to the growth of the cytosome and the production of yolk. The meaning of this is unknown, but we may conjecture that it is an indication of a nuclear activity that plays a part in the later history of the oöcyte. One might imagine, for instance, that during this association the formed elements receive from the nucleus certain substances (enzymes or other chemical messengers) of which they become the carriers, transporting them to regions of the cytosome where they play their specific part. It is important to bear in mind the rapid increase in the number of the chondriosomes (and of Golgi-bodies?) that takes place during the growth of the egg. Only a part of them are used up in the production of other formed elements. The greater number persist, possibly to play some rôle in the fertilization of the egg (p. 434), in any case to be handed on to the embryonic cells by cleavage. Perhaps we catch here glimpses of a mechanism concerned not merely with the yolk-formation but with the general processes of determination, localization and heredity. To the same mechanism may perhaps belong also the extrusion of formed elements from the nucleus; but the occurrence of such a process does not yet seem sufficiently demonstrated.

The Secondary or Accessory Nuclei. We have lastly to consider the so-called secondary or accessory nuclei that appear in the oöcytes of Hymenoptera and other insects during the middle or later growth-period, and have

[1] See for instance Ogata ('83), Platner ('89), Melissinos and Nicolaides ('90), Galeotti ('95), Laguesse ('99, '00), *Cf.* Saguchi ('20).

exactly the appearance of small nuclei, but *which do not arise by division of the egg-nucleus*. They may be aggregated about the germinal vesicle, or may be quite separate from the latter, either scattered through the cytosome or more or less aggregated in a peripheral zone (Figs. 141, 160).[1] Buchner has found that the secondary nuclei of different species show characteristic differences similar in type to those existing between the main nuclei or germinal vesicles. Buchner has described some conditions in these nuclei which he considers as stages in budding or amitotic division, but the evidence is inconclusive. They have never been observed to divide by mitosis and finally disappear entirely before the period of maturation.

The history of these bodies prominently raises the question of the origin of nuclei *de novo* and has therefore been the subject of investigation by many observers. Blochmann considered them to arise from the germinal vesicle by a process of budding; and this has been supported by Marshall ('07) and a few others; Korschelt believed them to be derived from the surrounding follicle-cells, as is the case with the "test-cells" of ascidians (p. 334). Many observers, on the other hand, have produced evidence that they arise from minute granules extruded from the germinal vesicle (perhaps in some cases, according to Büchner, from the nuclei of the surrounding follicle-cells) which gradually grow into smaller nuclei. This view, advanced by Loyez ('08), has received support from the work especially of Hegner ('15), Buchner ('18), Gatenby ('20) and Hogben ('20a), all of whom have studied the phenomena especially in Hymenoptera of various genera, including ants, bees, and gall-flies. The process may take place close to the germinal vesicle, in which case (as in *Camponotus* or *Rhyssa*, Fig. 141) the germinal vesicle becomes surrounded by a nest of smaller nuclei. In other forms the secondary nuclei never show as such any discoverable relation to the germinal vesicle, first appearing scattered through the cytosome, or around the periphery of the egg (*Apanteles*, Fig. 141). In such cases it is assumed that the chromatin-granules from which they arise are extruded from the germinal vesicle at an earlier period and spread through the oöplasm before giving rise to secondary nuclei.

All recent researches seem to show that these bodies finally disappear completely. Their significance is unknown. Gatenby ('20) makes the plausible suggestion that they may be concerned with certain vegetative functions in the growing oöcyte, the egg-nucleus having become partly decentralized by the separation and migration of a kind of "trophochromatin" (p. 725) specialized for this purpose. If the foregoing results be well founded these structures would seem to be nuclei that are not built

[1] For a history of observations on this subject see especially Hegner ('15), Buchner ('18).

up from chromosomes—a condition unparalleled among Metazoa—and to this may perhaps be due their incapacity for division. We know, especially from Boveri's observations (p. 729), that perfect nuclei, capable of mitotic division, may be built up of a group of chromosomes far less numerous than the normal haploid group; that even single chromosomes that go astray

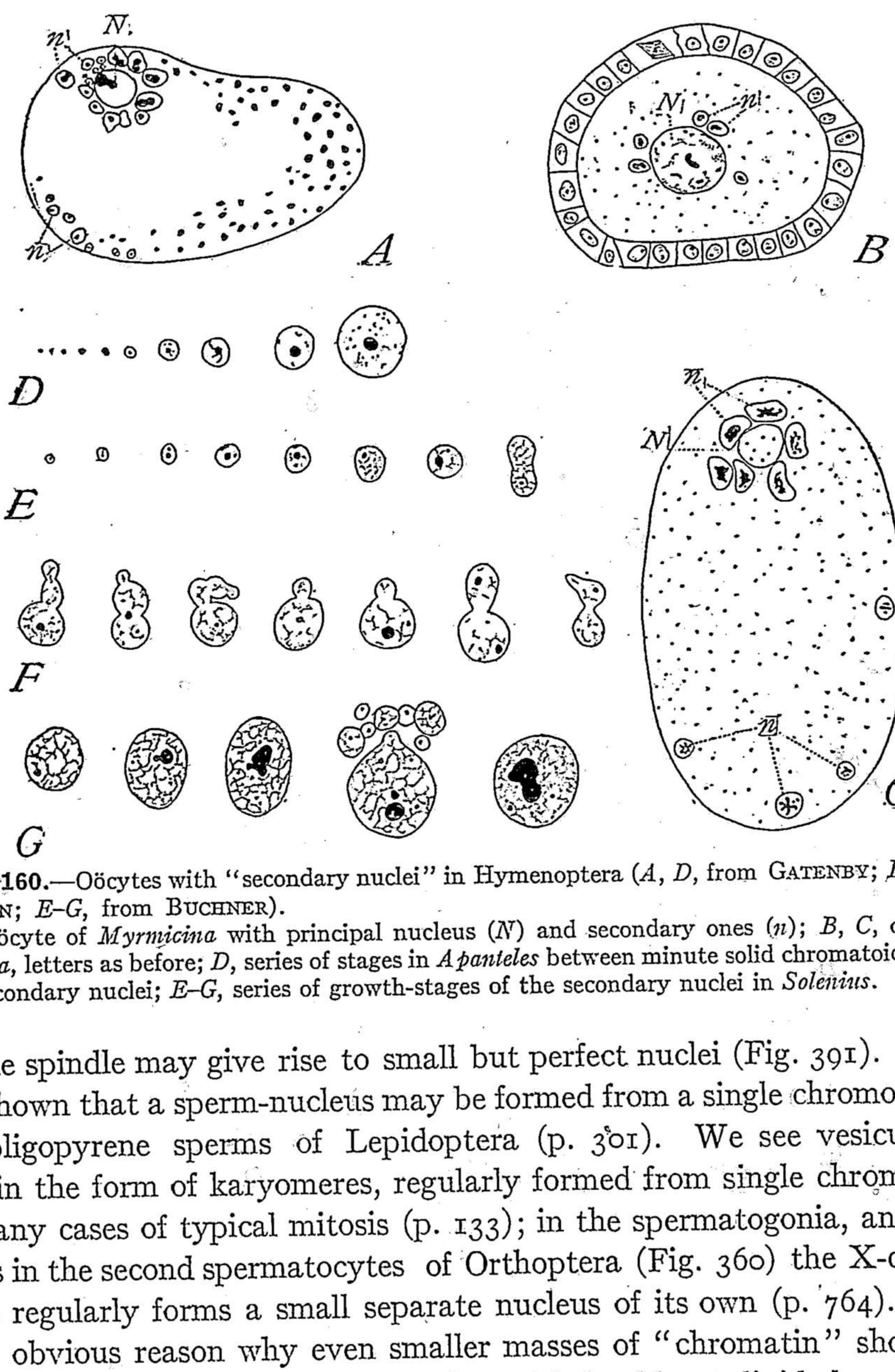

Fig. 160.—Oöcytes with "secondary nuclei" in Hymenoptera (*A*, *D*, from GATENBY; *B*, *C*, from HOGBEN; *E–G*, from BUCHNER).

A, oöcyte of *Myrmicina* with principal nucleus (*N*) and secondary ones (*n*); *B*, *C*, oöcytes of *Formica*, letters as before; *D*, series of stages in *Apanteles* between minute solid chromatoid granules and secondary nuclei; *E–G*, series of growth-stages of the secondary nuclei in *Solenius*.

on the spindle may give rise to small but perfect nuclei (Fig. 391). Meves has shown that a sperm-nucleus may be formed from a single chromosome in the oligopyrene sperms of Lepidoptera (p. 301). We see vesicular nuclei, in the form of karyomeres, regularly formed from single chromosomes in many cases of typical mitosis (p. 133); in the spermatogonia, and sometimes in the second spermatocytes of Orthoptera (Fig. 360) the X-chromosome regularly forms a small separate nucleus of its own (p. 764). There is no obvious reason why even smaller masses of "chromatin" should not likewise form nuclei or even why such nuclei should not divide by a kind of mitosis. We may, however, feel reasonably sure that such nuclei are strictly

limited in potency; that they are not equivalent to those of the tissue-cells.

3. The History of the Nucleus

During the growth-period both the chromosomes and the nucleoli undergo a series of transformations analogous in a measure to those which may be observed in the tissue-cells, but in magnified form, so as to offer unusual opportunities for their study in detail. These phenomena show wide differences in different forms—sometimes even within the limits of

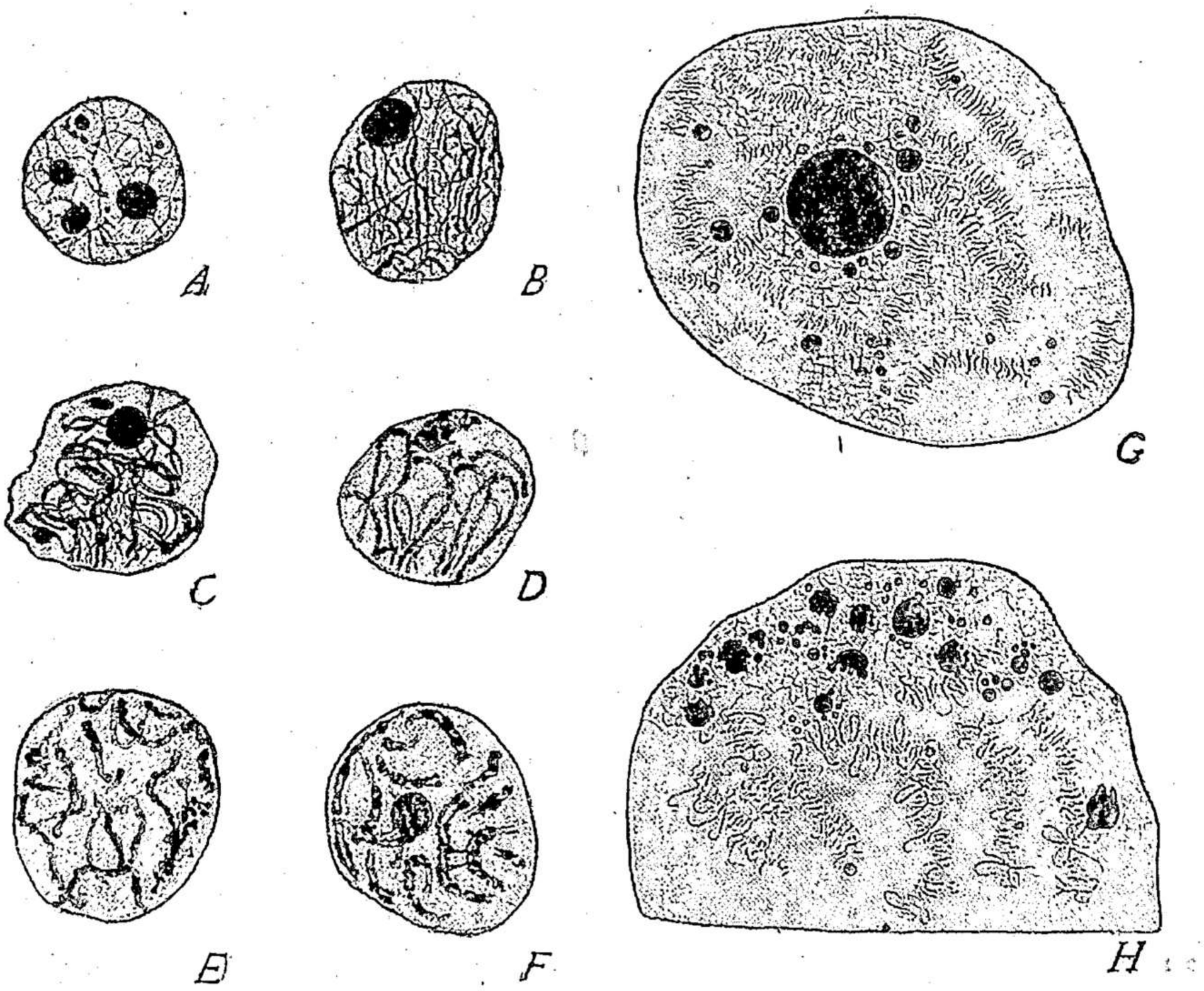

Fig. 161.—The germinal vesicle of the elasmobranch egg in the early and middle growth-period (MARECHAL).

(*A–F*, *Pristiurus*, *G*, *H*, *Scyllium*.)

A, presynaptic reticulum; *B*, leptotene; *C*, synapsis; *D*, polarized pachytene; *E*, later pachytene, non-polarized; *F*, diplotene; *G*, much later stage, loosening-up of the chromosomes; *H*, still later, "lamp-brush" chromosomes.

a single genus—and the conditions by which they are determined are still imperfectly understood.

a. The Chromosomes and the Nuclear Framework. In its earlier stages, the egg-nucleus is probably always netlike in character (*protobroch* stage of Winiwarter), and in many cases is nearly or quite oxyphilic while the single nucleolus is strongly basophilic. This is followed by the reappearance of the chromosomes in the form of fine, basophilic spireme-threads (*leptonema*) which pass through the synaptic stages and later become shorter,

thicker, strongly basophilic (*pachynema*) and longitudinally double (*diplonema*). These are the bivalent chromosomes (later tetrads), which are haploid in number and are destined to divide in the polar or meiotic divisions that take place at the end of the growth-period. These changes will be considered in Chapter VI.

In the following stages, while the egg is rapidly growing, the chromosomes always become in some degree looser in texture, less regular in contour and less strongly basophilic, while the nucleus recedes in various degrees towards the "resting" or reticular condition, in some cases to such a degree that the chromosomes as such are temporarily lost to view, as in an ordinary tissue-cell. During this process the nuclear cavity becomes filled with a lightly staining, often oxyphilic, netlike framework or reticulum in which the chromosomes are suspended. Some doubt still exists concerning the origin of this framework. It certainly arises in part from fine branches of the chromosomes; but it may also be formed in part by coagulation of the nuclear sap or enchylema. In respect to the later stages we may conveniently distinguish four general types, connected by various intergradations, but widely different in their extreme forms.

(1) In the simplest of these (which approaches most nearly to the conditions commonly seen in the spermatocytes), the deconcentration of the chromosomes is but slightly marked, and the chromosomes may readily be traced individually throughout the growth-period, always retaining in some degree their basic staining-capacity. Typical examples of this are seen in certain of the copopods (*Cyclops*) and Turbellaria [1] which represent a condition roughly analogous to that seen in the spermatocytes, for instance of Amphibia or the Orthoptera (p. 552), and it is possible that it may occur also in some of the mammals (see Newman, '12, armadillo). These nuclei usually have a single large basophilic nucleolus.

(2) In a second type the growth and deconcentration of chromosomes proceeds much further, and at the same time the longitudinal halves of the diplotene separate more or less widely, so that the chromosomes seem to be grouped in pairs, the members of which are often twisted about each other. In the elasmobranchs, where the deconcentration process can be studied to great advantage, the chromosomes become loose in texture and in outline and finally give off numerous lateral thread-like radiating branches or loops that lose themselves in the general network. Thus arise the very loose so-called "lamp-brush" chromosomes (Figs. 161, 162), characteristic of the middle growth-period in large, yolk-bearing eggs, such as those of vertebrates (fishes, amphibians, sauropsida), and also of various insects,

[1] See especially Schleip ('07), Gelei ('13, '21, '22).

crustacea, or *Sagitta* (Fig. 111). These chromosomes sooner or later become nearly or completely oxyphilic like the general framework in which they lie, so as to be distinguishable from the latter with difficulty; and at the same time they lose their resistance to peptic digestion,[1] which in their earlier basophilic state is very marked. In spite of these remarkable changes it appears to be certain that in some of these cases the chromosomes do not at any time disappear from view but persist without loss of their identity throughout the whole growth-period. One of the best determined of these cases is offered by the elasmobranch *Pristiurus* in which Rückert ('92, '95) was able to follow the chromosomes through the whole growth-period up to the late prophases, when they rapidly decrease in size, reconcentrate in structure, regain their basophilic character and pass upon the first polar spindle; and the same appears to be true in *Scyllium*. This conclusion was confirmed by the extended and accurate studies of Maréchal ('06) on these forms and on the teleost *Trigla;* and a similar result has been reached by several observers in other forms.[2] These facts afford conclusive proof that *the individuality and genetic continuity of chromosomes does not depend upon a persistence of "chromatin" in the older sense* (*i. e., basichromatin*). It is the expression of a morphological organization that is not destroyed by those chemical and physical transformations that lead to a netlike structure and a change from the basophilic to the oxyphilic condition (p. 652).

A third class includes those still more extreme cases in which the chromosomes are finally completely lost to view in the nuclear network (*dictyotic* stage of Winiwarter) and the whole structure becomes nearly or quite oxyphylic. The classical case of this is offered by the Amphibia, which have long been a center of controversy in this respect. Most observers are now in agreement that both in urodeles and anurans the deconcentration of the chromosomes finally reaches a point at which many or all of them become indistinguishable.[3] A similar conclusion has been reached in a number of other cases, belonging to various groups; but it is probable that a more accurate study will demonstrate the persistence of the chromosomes in some of these. In cases of this type the germinal vesicle shows only a fine, oxyphilic meshwork and one or more nucleoli in which the entire basophilic content of the nucleus is contained (Fig. 163, F). In the final stages the chromosomes reappear as localized areas in this meshwork, at first

[1] See Jörgenssen ('13) with references to the earlier literature. See also Lubosch ('13) whose results were somewhat different.

[2] See the critical reviews in the works of Loyez ('06), Lubosch ('13), Jörgenssen, ('13), and Stieve ('20).

[3] See, for instance, on Anura, Oscar Schultze ('87), Carnoy and Lebrun ('99), King ('08); and on Urodela, Born ('94), Carnoy and Lebrun ('98, '99), Schmidt ('05), Jörgenssen ('13) and Stieve ('20).

loose in texture, vague and irregular in outline, and oxyphilic or but slightly basophilic, as in class 2.

These facts at one time led a considerable number of observers to conclude that when the chromosomes disappear from view they go out of existence, to be re-formed from the nucleoli or otherwise at a later period; but there is very strong ground to doubt this. It is important to note that they

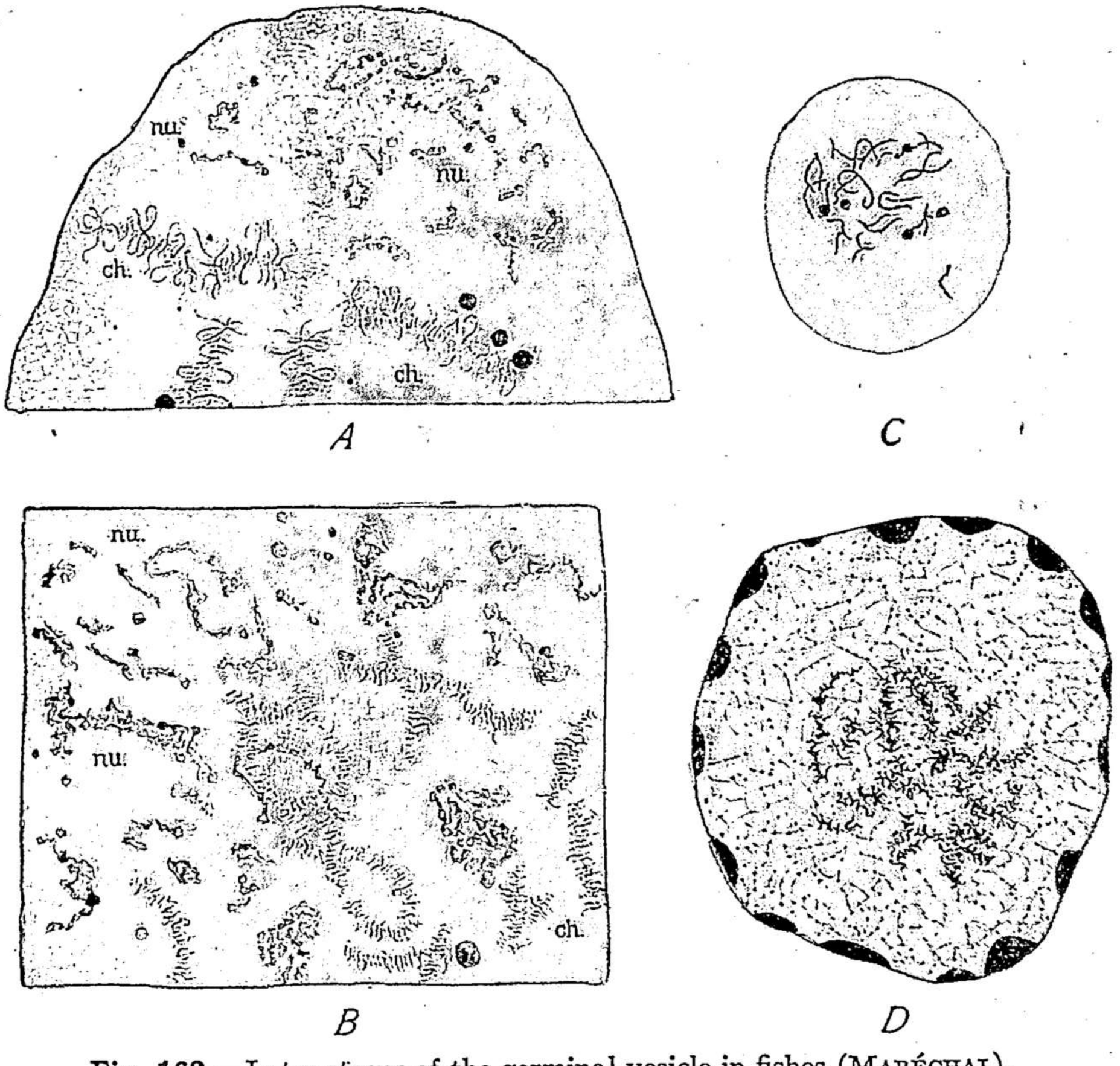

Fig. 162.—Later stages of the germinal vesicle in fishes (MARÉCHAL).

A–C, *Scyllium; D*, the teleost *Trigla*.

A, *B*, maximum stage of deconcentration of the lamp-brush chromosomes, spireme-like transformation of the nucleoli; *C*, the chromosomes near the end of the growth period, at the same enlargement; *D*, rather late growth-period, centripetal movement of the chromosomes.

do not disappear by breaking up into a structureless "magma" or mass of fine granules, as some observers have concluded; there is reason to believe that this account rests on faulty technique. As was early described by Rückert, in the elasmobranchs, the production of "lamp-brush" chromosomes takes place by the formation of lateral thread-like branches, radiating from a central axis which itself finally seems to disappear, leaving a thread-like framework in which the individual chromosomes *seem* to have been lost. Soon afterwards, however, they reappear by a condensation and convergence of the threads along an axial region, showing the same

type of structure as before, and likewise grouped in twisted pairs. All this plainly indicates that *the chromosomes have not lost their identity during the period when they are temporarily lost to sight and hence are not subsequently formed de novo*. The view that they are re-formed from the nucleoli, is now completely exploded (p. 354).

(4) In a fourth group may be placed those rather exceptional cases in which all the chromosomes are condensed into a karyosphere from which they escape in the prophases of the polar mitoses (p. 93). This condition, too, has been assumed by some writers to involve a total destruction of the original chromosomes and their formation *de novo* from the karyosphere [1] and thus to offer a fatal difficulty for the hypothesis of the individuality of genetic continuity of the chromosomes; but here again the facts now point to a different conclusion. Karyosphere-formation, as earlier indicated (Fig. 109), is foreshadowed in many forms by a tendency to aggregate towards the center of the germinal vesicle, and both Vejdovský (in the case of *Diastrammena* and *Gordius*) and Jörgenssen (in *Nephelis*) have followed the process of their aggregation to form the karyosphere. Vejdovský shows also that the chromosomes, though much condensed and closely aggregated in the karyosphere, do not actually disappear; and the same observation has been made by Browne ('13) in the karyosphere of the spermatocytes of *Notonecta*. The formation of a karyosphere therefore offers no contradiction to the general theory of the genetic continuity of the chromosomes, even though its physiological meaning remains obscure.

b. The Nucleoli. The transformations of the chromosomes described above are accompanied by more or less parallel changes in the nucleoli, of which the most striking is the intensely basophilic character commonly assumed by the nucleoli during the greater part of the growth-period and retained by them even in cases where the chromosomes and general nuclear framework have become completely oxyphilic. This is in general true whether one or many nucleoli be present. In the latter case the multiplication of the nucleoli seems to be effected either by a progressive fragmentation of the original principal nucleolus or by new formation (as appears to be the case in fishes, reptiles and birds). These intensely basophilic nucleoli are sometimes so closely crowded as almost to conceal the oxyphilic network in which they lie (*Scolopendra*, Fig. 111). More commonly they migrate towards the periphery, sometimes lying against the nuclear membrane outside the chromosomes, which at this time have usually become diffuse, irregular, or have assumed the "lamp-brush" condition, may even have disappeared from view, or have more or less completely

[1] See for example Lubosch ('13), p. 296.

lost the basophilic character. This condition is characteristic of the ova of most fishes, amphibians, birds and reptiles and occurs also in the yolk-laden eggs of some invertebrates, though in some cases the nucleoli may be scattered irregularly through the germinal vesicle.

A remarkable feature of these nucleoli is that in many cases during or subsequent to their period of multiplication, they become drawn out into more or less convoluted thread-like bodies (Figs. 162, 163) which, because of their basophilic staining-reactions may closely simulate spireme-threads or chromosomes. For such (in case of the oöcytes of amphibians, elasmobranchs, and other fishes), they were actually mistaken by Carnoy and Lebrun ('97–'99), whose conclusions were supposed to constitute a strong argument against the individuality of the chromosomes. All this, however, was rendered untenable by later observations, in particular those of Maréchal, Loyez and Jörgenssen, which revealed the persistence of the chromosomes as such during the very stages in which the nucleoli are unraveling, and demonstrated the complete morphological independence of the nucleoli.[1]

The meaning of these curious modifications is almost wholly unknown; but it now seems probable that the nucleoli are not merely accumulations of waste products but contain materials that play some definite part in the metabolism of the growing egg. The deconcentration and partial or complete loss of basophily by the chromosomes during the growth-period may probably be regarded as an exaggeration of the process seen in the nuclei of tissue-cells during their vegetative state, and as connected in some way with the constructive processes in the cytosome. The facts suggest that this involves a splitting off of the nucleic acid component of the basichromatin and its storage wholly or in part in the nucleoli. This conjecture is, however, somewhat hazardous because of the physical effects due to changes of density and the like in both nucleoli and chromosomes; and to this cause may probably be traced many recorded inconsistencies in the results of both staining and digestion-tests as applied to these bodies (see p. 644.).[2]

In the latter part of the growth-period the nucleoli commonly again assume a condensed and spheroidal form and fragment into smaller bodies. As above stated, a considerable group of observers have maintained that many of the nuclear fragments are extruded as such through the wall of the germinal vesicle into the oöplasm, and some believe that such fragments may contribute directly to the formation of the yolk. There is, however, no general agreement on this point. It is certain that in some cases the nucleolus persists until after the germinal vesicle breaks down

[1] See Maréchal ('05,–'07), Loyez ('06), Jörgenssen ('13), Stieve ('20), etc.
[2] See the above cited works; also Floderus ('96), Popoff ('07), Stauffacher ('11).

and the polar spindle forms, when it is cast off in the cytoplasm as a "metanucleolus" which finally disappears.[1] Nevertheless the extrusion of nucleolar fragments in earlier stages of the oöcyte has been described so

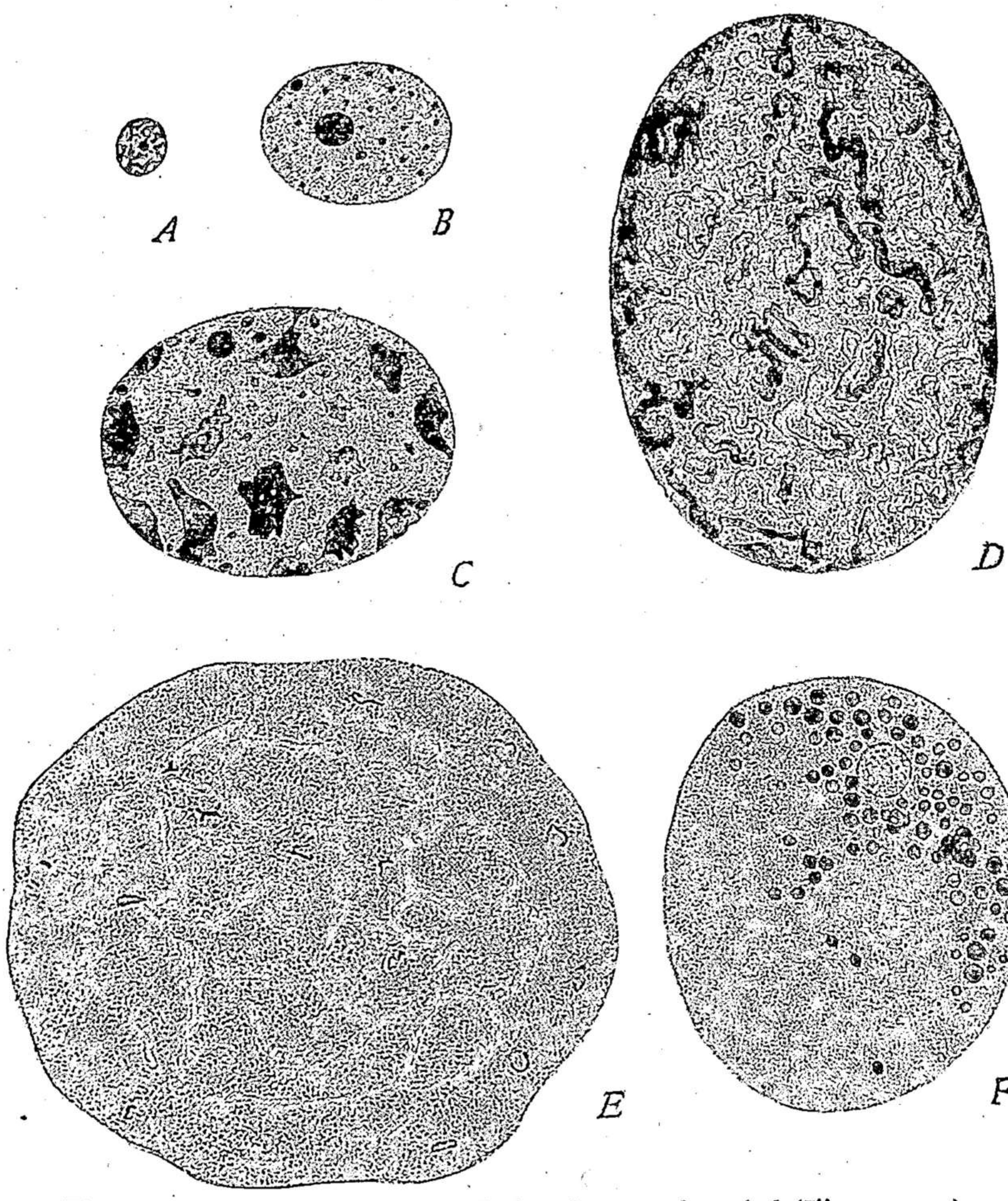

Fig. 163.—The germinal vesicle during the growth-period (JÖRGENSSEN).
(*A–E*, the teleost *Melamphaës*; *F*, the orthopter, *Gryllotalpa*.)
A, early stage, single basichromatic nucleolus; *B*, appearance of small marginal nucleoli; *C*, growth and transformation of the nucleoli; *D*, nucleoli converted into spireme-like threads; *E* (from a different species), much later stage, with deeply staining nucleoli, much reduced, and pale "lamp-brush" chromosomes; *F*, middle growth-period of the mole-cricket, after disappearance of the chromosomes, one oxychromatic nucleolus and many basichromatic.

circumstantially and by so many observers that the question must at least be held open (p. 345).

c. The Residual Substance. During the final stages of the germinal vesicle the chromosomes rapidly condense and decrease in size, until in the

[1] Long since observed by Haecker ('92) in the eggs of medusæ and in other cases by later observers.

final stages they constitute only a small fraction, sometimes a very minute fraction, of the total nuclear substance. At this time their grouping varies widely; in some cases they are scattered irregularly through the germinal vesicle; in others lie near the periphery; in still others are massed in a more or less compact group (Fig. 110). As the wall of the germinal vesicle breaks down and the chromosomes pass upon the spindle there is therefore left behind a large amount of "residual substance," comprising the remains of the nuclear framework and of the nucleoli, that mingles with the general cytoplasm of the egg (Fig. 199).[1] Both absolutely and relatively the amount of this substance varies widely in different cases, these variations corresponding, in some measure at least, to the varying degrees in which the chromatin of the germinal vesicle returns towards the net-like condition characteristic of the "resting" or vegetative state. Lillie ('06) has shown that in *Chætopterus* this material contains very numerous oxyphilic microsomes which, after their extrusion into the cytoplasm, quickly become basophilic like those of the cytoplasm generally, but seem not to lose their morphological identity. As will later be seen there are many reasons for the belief that this material is not merely waste but may play an important part in the phenomena of development (pp. 405, 1096).

Origin and Differentiation of the Sperm

The relation of the various components of the sperm to the cells from which it arises is of especial interest because we may here look for a basis of interpretation of the part played by the sperm in fertilization and heredity. The pioneer observations of Kölliker, Schweigger-Seidel and La Valette St. George early established the fact that the sperm is a cell, but it required a long series of subsequent researches by many observers to make known the whole course of the spermiogenesis. To La Valette is due the general terminology now universally adopted; it is indicated in the following main outlines, which hold true for animals generally.

The primary spermatocyte first divides to form two secondary *spermatocytes* or sperm-mother-cells, and each of these again divides—often without pausing and without the reconstruction of the daughter-nuclei—to form two *spermatids*. Each of the latter is then transformed into a single sperm, its nucleus becoming very small and compact, while from its cytoplasmic components arise the acrosome, middle-piece and flagellum. The basis

[1] This fact, emphasized by Rückert ('92), Van der Stricht ('95, '98), Gardiner ('98), Griffin ('99), and others has been described by many later observers and gave the first basis for various theories of nuclear dualism (*trophochromatin* and *idiochromatin*, etc.) (p. 725). In the platode *Polychærus* Gardiner calculated that the amount of residual substance thus cast out is not less than 500 times that of the basichromatin that forms the chromosomes; and Conklin ('12) reports a similar condition in ascidians, gasteropods and other forms. The importance of this material in development is elsewhere discussed (pp. 405, 1096).

of the two latter is formed by the *axial filament* which grows forth from one of the spermatid-centrioles which plays the part of a *blepharoplast*. The envelope or sheath of this filament is in part formed from the chondriosomes of the spermatid, while the acrosome is a product of the Golgi-apparatus. All these structures thus arise from corresponding formed bodies in the spermatocytes which seem not to lose their identity during the divisions.

1. Source of the Sperm-forming Materials. The Spermatocyte-divisions

a. The Central Bodies. Early in the growth-period as above described, the idiozome-complex breaks up more or less completely and its components ultimately scatter through the cytoplasm. During this process the centrioles are often lost to view; but it seems probable that they persist throughout the growth-period, hidden among the cytoplasmic granules or perhaps in some cases within the nucleus. In any case they reappear in the prophases of the first division,[1] and show the typical behavior. Their persistence during both divisions has been demonstrated by numerous observations which leave no doubt that the central bodies of the resulting four cells (spermatids or oötids) are direct descendants of those already present in the auxocytes.

The most striking demonstration of this is afforded by certain cases in which the axial filaments of the future sperms begin to grow forth from the centrioles of the spermatocytes *before the maturation-divisions have taken place*, persisting thenceforward through every stage up to the formation of the mature sperm, a phenomenon discovered by Meves ('97) and Henneguy ('98) in the Lepidoptera (*Bombyx*, *Pygæra*). In *Pygæra* the two original spermatocyte-centrioles lie near the cell-periphery and are V-shaped, with an axial filament extending outwards from the extremities of both V's, *i. e.*, four in all (Fig. 165). They divide at each pole in the first anaphase by breaking in two at the apex of the V, and the two rod-shaped products, each bearing a single flagellum, pass to the poles of the second division. A single rod-shaped centriole, bearing its flagellum, is thus delivered to each spermatid where it lies close against the nuclear membrane. Similar conditions have since been described in the Coleoptera, by Schäfer ('07) and Voïnov ('03), in birds and beetles by Korff ('01) and in Lepidoptera by Gatenby ('17a), and may no doubt be expected in other forms.[2] In *Bombyx* Henneguy found the centrioles as spherical granules rather than rods; and this is confirmed by Gatenby in *Smerinthus*

[1] An exception is offered by the anastral polar spindles of the oöcyte-divisions in many arthropods and vertebrates (p. 508). It is an interesting fact that in the sperm-producing divisions of many plants (*i. e.*, those having flagellated or ciliated sperms) central bodies (blepharoplasts) appear even in forms which are devoid of such bodies in the ordinary somatic divisions (p. 387).

[2] V-shaped centrioles are also described by Mottier ('98) in *Dictyota*.

and some other Lepidoptera. In most of these cases it would seem that the spermatid-centriole is at first single, though Gatenby figures it as double soon after the second division.

b. The Chondriosomes. The chondriosomes of the oöcyte seem for the most part to take little or no part in the polar divisions, remaining passively in the ovum. Those of the spermatocyte, on the contrary, undergo a conspicuous process of segregation or chondriokinesis, an account of which has earlier been given (p. 163). The general effect of this process is to distribute the spermatocyte-chondriosomes with almost exact equality to the four spermatids. It is interesting to compare the many variants of the process. In all cases the chondriosomes of the early spermatocytes are small, numerous, and commonly aggregated more or less definitely about the central bodies and idiozome (p. 329). In later stages they most commonly spread through the cytosome in the form of scattered mitochondria, spheres, rods or threads. In this condition they often remain during the divisions, being segregated, apparently passively, into four equal groups by the two divisions. In other cases they

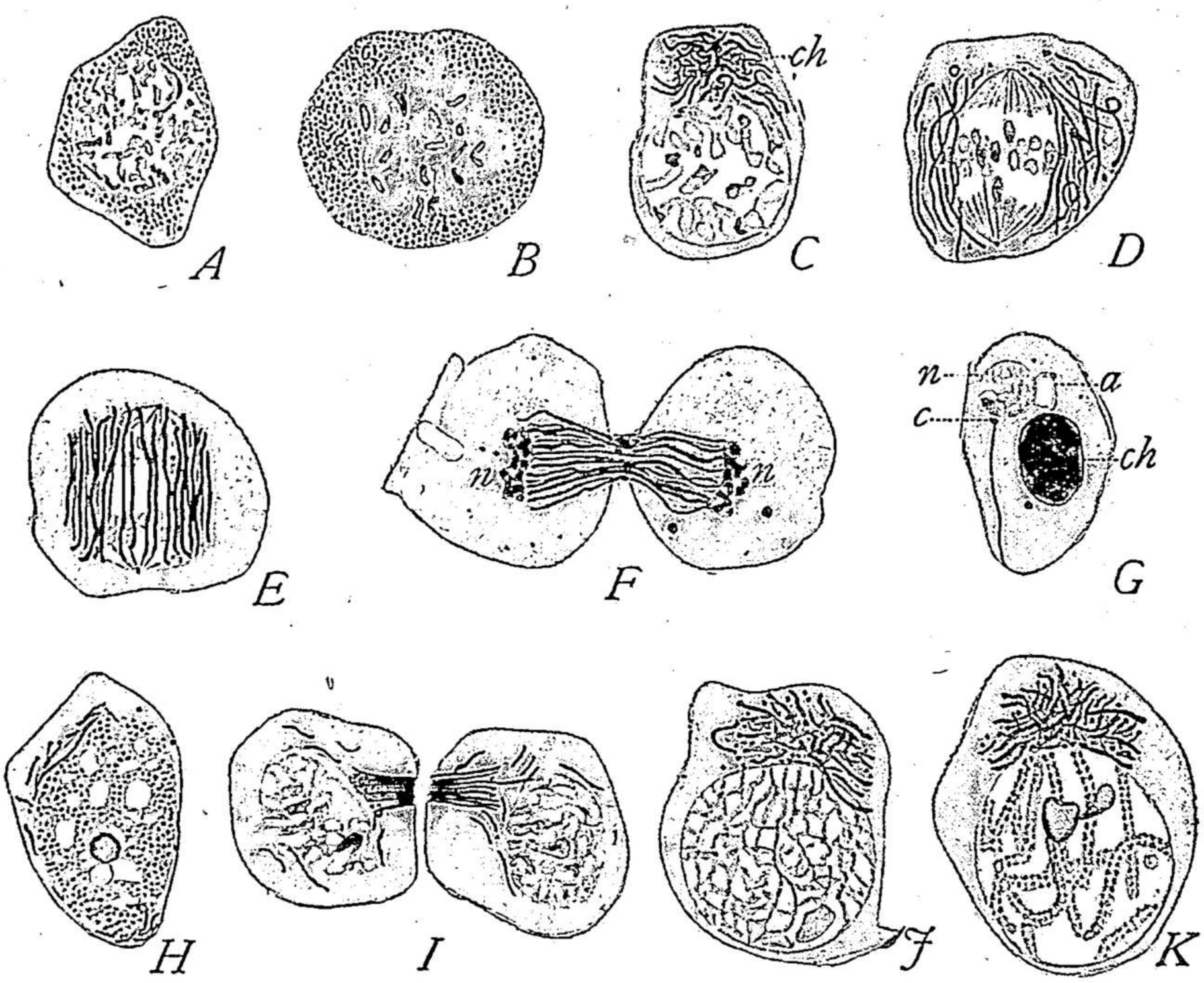

Fig. 164.—Chondriosomes and sperm-formation in insects (DUESBERG).

A–G in the beetle *Blaps; H–K* in the cockroach *Blatta.*

a, acrosome (acroblast); *c*, centriole; *ch*, chondriosomes; *n*, nucleus.

A, *B*, spermatogonia with granular mitochondria; *C*, early spermatocyte; *D*, first spermatocyte-metaphase; *E*, anaphase of same; *F*, teleophase; *G*, early spermatid, chondriosomes massed to form nebenkern or chondriosphere; *H*, *I*, spermatogonia; *J*, early growth-period; *K*, diplotene.

enlarge, or in some cases become closely aggregated, perhaps even fuse, to form larger bodies.

In the middle and late growth-period the larger chondriosomes are of two principal types, being either more or less elongated threads or rounded chondriospheres. The former condition is seen in Orthoptera

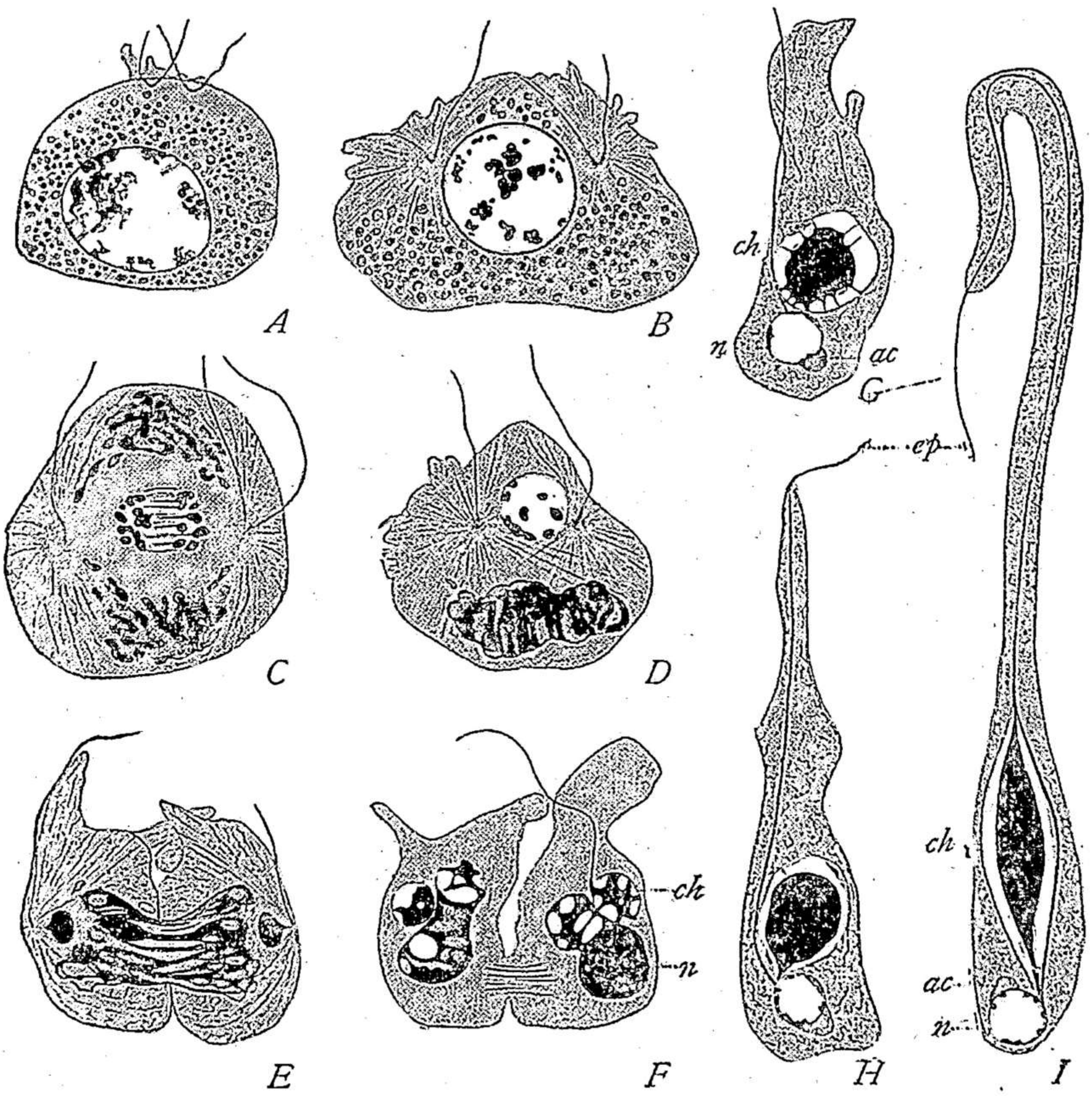

Fig. 165.—Spermatogenesis in the butterfly *Pygæra* (MEVES).

ac, acroblast; *c*, centriole; *ch*, chondriosomes, chondriosome-body, nebenkern; *cp*, end-piece; *n*, nucleus.

A, *B*, primary spermatocytes, V-shaped centrioles, axial filaments; *C*, first-division; *D*, interkinesis; *E*, *F*, telophases of second division; *G*, *H*, *I*, spermatids.

(Fig. 164), Coleoptera (Fig. 167), Hemiptera (Fig. 68), Hymenoptera and some gasteropods (*Paludina*), the latter in Lepidoptera and some of the scorpions. In the Lepidoptera, as shown by Meves ('00) and Gatenby, the chondriosomes enlarge to form numerous spheroidal chondriospheres which appear like vesicles owing to the differentiation of a lightly-staining or chromophobic central or medullary region. Enlarging still more, perhaps in part by fusion, these crowd about the first spermatocyte spindle and often form a kind of mantle that completely surrounds the latter. This draws out (Fig. 165) and divides into two in both divisions, the individual

chondriospheres apparently undergoing further fusion and often being drawn out almost into a thread-like form (Gatenby). Appearances indicate that these are to some extent cut across by the division, but considerable uncertainty exists on this point. The products fuse, or become closely aggregated in each spermatid to form a single condensed but more or less vacuolated body, the nebenkern or chondriosome-body. The chondriospheres of the scorpions have earlier been described, as have also those of the more frequent chondriocont type (p. 164).

The significant fact in all the foregoing cases is a distribution of the chondriosome-content by the spermatocyte-divisions with approximate equality

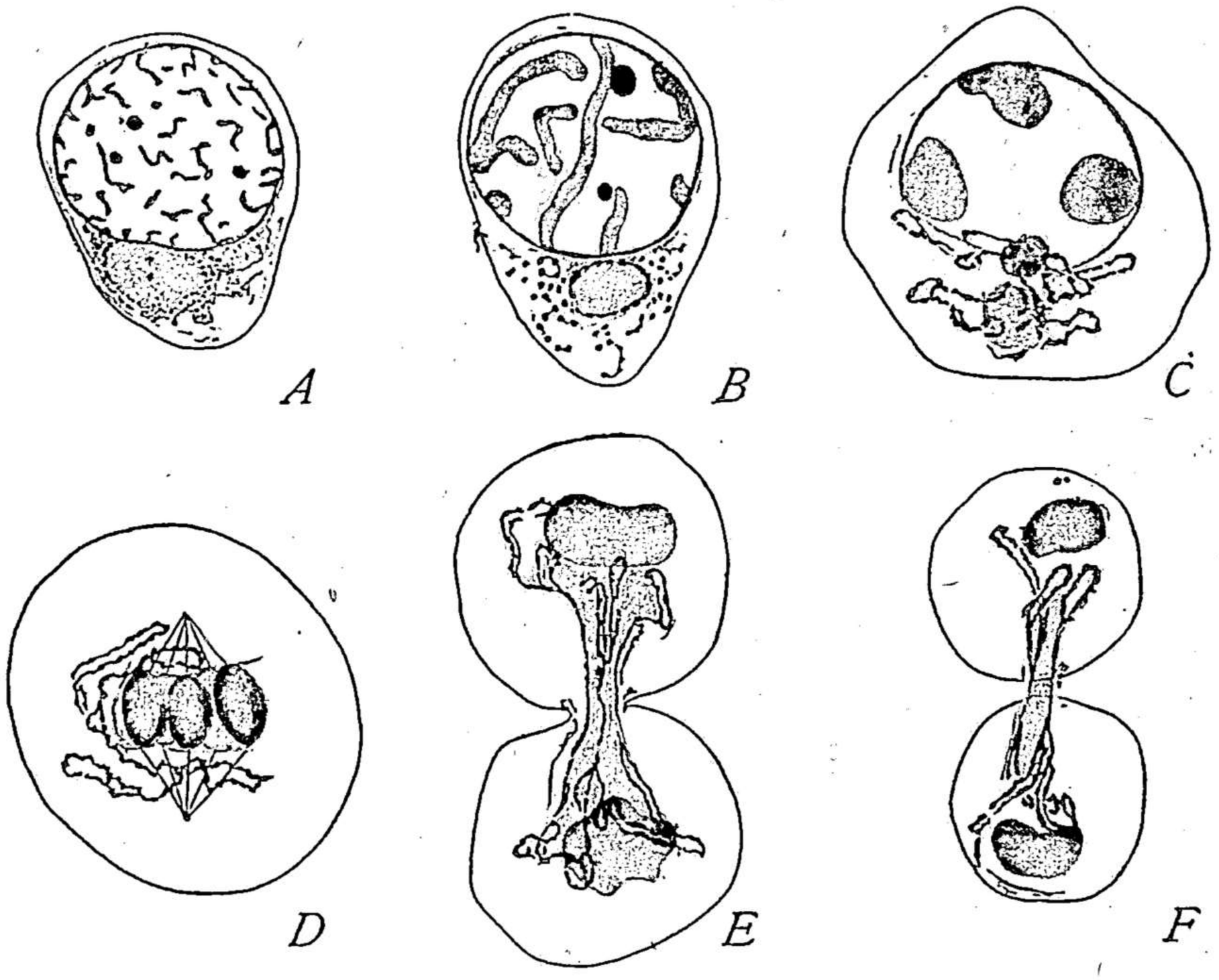

Fig. 166.—Spermatocytes of the snail *Paludina* (MEVES).

A, early growth-period, idiozome, central bodies and scattered mitochondria; *B*, *C*, later stages, formation of chondriomites and rings; *D*, first spermatocyte-metaphase; *E*, telophase; *F*, second telophase (*cf.* Fig. 167).

among the four resulting spermatids and its application to the formation of the tail-envelopes. The accuracy of this distribution varies considerably in different cases. In *Centrurus* (Figs. 169, 170), it takes place with a precision comparable with that seen in the distribution of the chromosomes; in other scorpions it is demonstrably less exact, and we may perhaps infer that a similar lack of precision exists in many other cases. In this fact we find good reason to doubt whether the chondriosomes, even though they be regarded as agents in heredity, can play as definite a part as the chromosomes or one that can be directly concerned in the Mendelian phenomena.

During the foregoing changes the material of the chondriosomes undergoes a differentiation into a cortical intensely chromophilic substance, and a central chromophobic one, the latter being left nearly or quite unstained by crystal violet (Benda method), hæmatoxylin and some other dyes by which the cortical layer is deeply colored. This is shown with great clearness in the chondriospheres of Lepidoptera and scorpions and has been described also for the smaller mitochondrial granules and even for the rods or chondrioconts.[1] These two substances can still be distinguished in the larger bodies, such as the chondriospheres or the nebenkern, formed by aggregation of the smaller chondriosomes. In the spermatids they undergo a complicated series of transformations, which have recently been investigated in part by Gatenby and more completely by Bowen. These transformations, as will be seen, involve the appearance in the original chromophilic substance of a third "medullary substance" that is of quite different nature from either of the original ones and plays an important part in the formation of the tail-envelopes (p. 371).

The Golgi-apparatus and Sphere-substance. The Golgi-bodies or dictyosomes undergo a process of distribution to the spermatids (dictyokinesis) somewhat similar to that of the chondriosomes. In the early auxocytes, as before stated, the Golgi-bodies are often more or less closely aggregated about the clear central sphere of the idiozome within which, in certain cases, are contained the pro-acrosomic granules (p. 329).[2] Sooner or later in the growth-period the idiozome breaks up and the Golgi-bodies scatter through the cytosome. The details seem to vary rather widely. In some cases (mammals) the disaggregation of the Golgi-bodies seems not to take place until after the first spindle has been formed, the idiozome-complex having meanwhile divided into two. In others (insects) it occurs much earlier, the Golgi-bodies fragmenting into much smaller elements before the division. In either case a temporary reaggregation may occur during the interkinesis, followed by a second dispersal before the second division; but this is not invariable (insects). In certain of the Hemiptera, as described by Bowen ('20) the Golgi-bodies enlarge, and apparently become reduced in number after their dispersal in the spermatocytes (Fig. 69).

There is considerable reason to suspect that during the disaggregation each Golgi-body or dictyosome may carry with it a small mass of sphere-substance; and this is almost certainly the case in the developing oöcytes of mollusks (Fig. 347); but this point is uncertain.[3] In any case it seems

[1] See Meves ('00, '07), Wilson ('16), Gatenby ('17, '18), Bowen ('21, '22c.).

[2] By Moore ('94) these bodies were called "archosomes," by Kuschekewitsch ('13) "sphærosomes," by Stockard and Papanicolaou "idiogranulomes." They are in some way connected with, perhaps products of, the Golgi-bodies.

[3] See especially Gatenby and Woodger ('21), and Bowen ('22).

probable that the proacrosomic granules of the idiozome (when such are present) *do not lose their identity but are scattered through the cytosome and passed on intact during the divisions into the spermatids.*[1]

Each spermatid thus receives a group of Golgi-bodies, more or less scattered, or in some cases temporarily grouped about the nebenkern (*Brochymena*, Fig. 172); and also in some cases (mammals) a considerable number of proacrosomic granules. In nearly all known cases this is quickly followed by a reaggregation to form a single, much larger rounded *acroblast* (Figs.

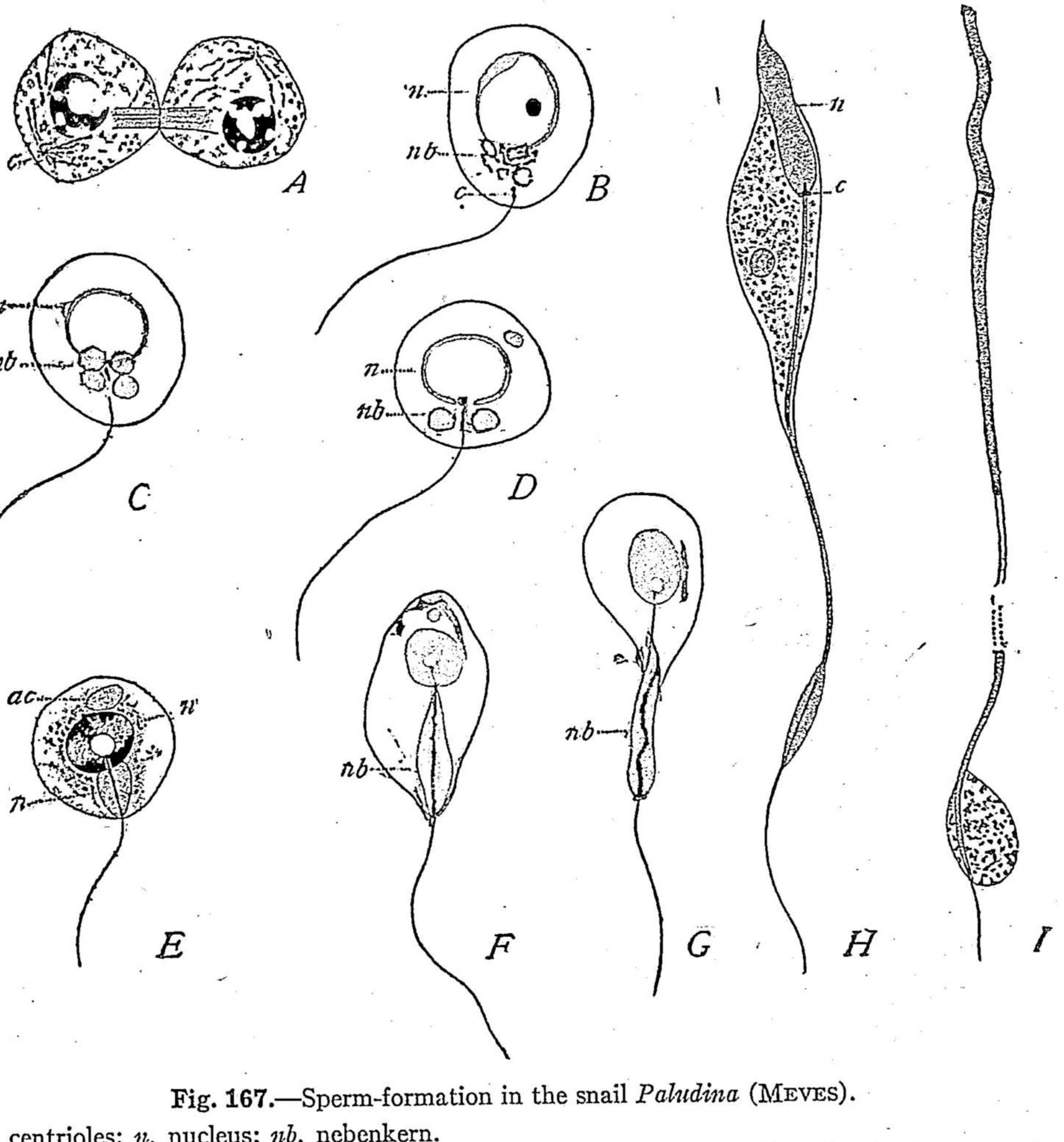

Fig. 167.—Sperm-formation in the snail *Paludina* (MEVES).

c, centrioles; *n*, nucleus; *nb*, nebenkern.

A, telophase of last division; *B*, early spermatid, with centrioles (*c*) and nebenkern forming; *C*, the quadripartite nebenkern in oblique view, and *D* from the side; *E*, *F*, *G*, later stages, elongation of distal centriole to form a rod, surrounded by the elongating nebenkern; *F*, *G*, later stages, to show centriolar apparatus in neck-region; *I*, terminal part of flagellum with residual cytoplasm.

[1] Niessing ('02), Stockard and Papanicolaou ('18), Gatenby and Woodger ('21).

172, 176, etc.), from which, by a complicated transformation is formed the acrosome of the mature sperm (p. 381).

2. Composition and General History of the Spermatid

The four spermatids resulting from the spermatocyte-divisions are rather large, rounded cells of the ordinary type, and with certain exceptions,[1] of equal size (Figs. 167, 172, 174, etc.). They consist of the following principal components.[2]

(1) The *cytosome*, consisting of undifferentiated cytoplasm in which are contained the following formed elements:

(2) The *nucleus*, at first relatively large, vesicular and lightly staining. This is built up from a haploid group of single chromosomes received from the second spermatocyte-division.

(3) The *central apparatus*, in the form of a pair of centrioles (or one which soon divides into two). These typically lie close to the periphery of the cell

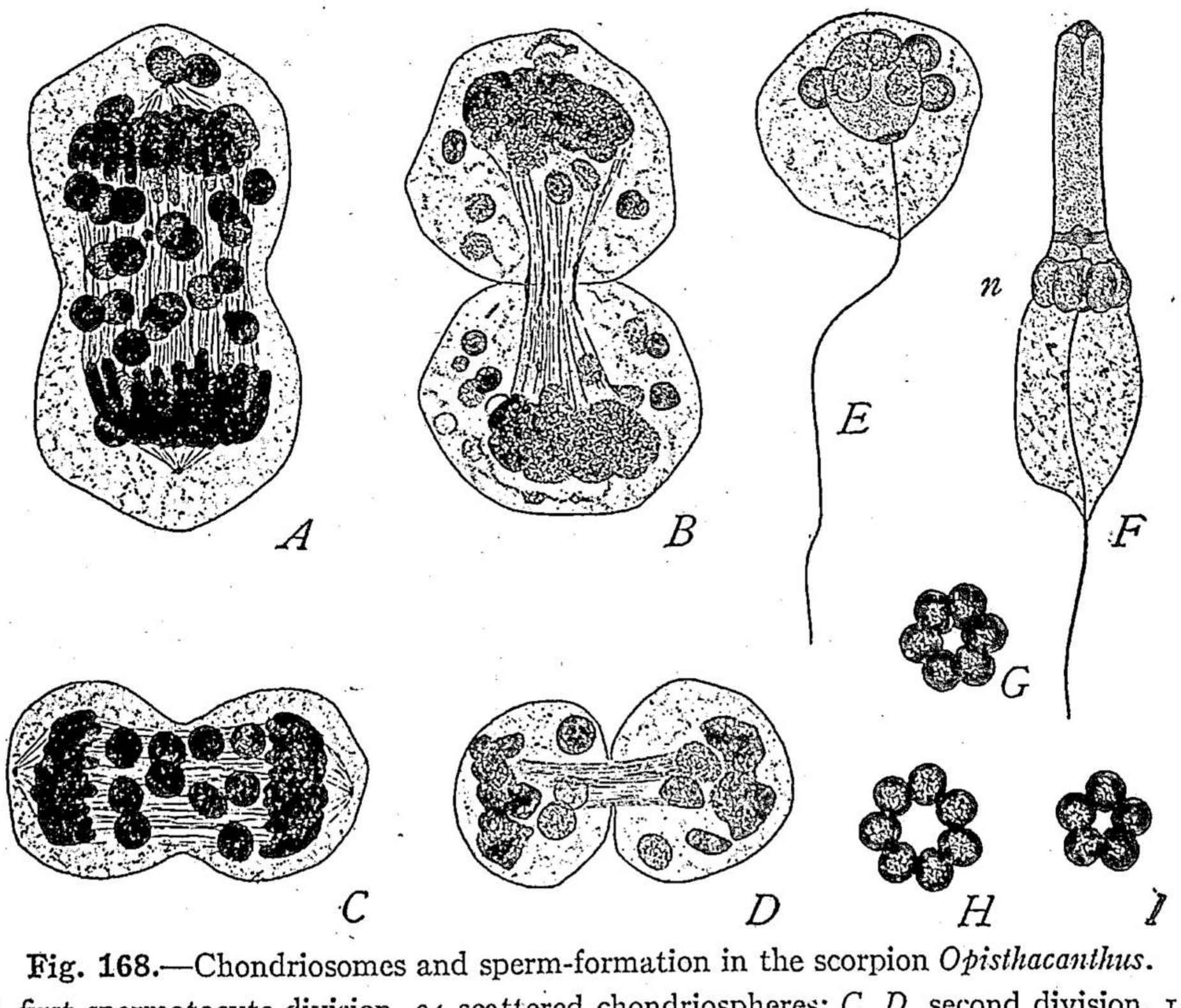

Fig. 168.—Chondriosomes and sperm-formation in the scorpion *Opisthacanthus*.

A, *B*, first spermatocyte-division, 24 scattered chondriospheres; *C*, *D*, second division, 12 chondriospheres; *E*, spermatid, sexpartite nebenkern; *F*, *G*, typical nebenkern; *H*, *I*, variations.

and are commonly surrounded by no special envelope, though a few observers have described them as inclosed in a "sphere" or "idiozome" supposed to be derived from a corresponding structure in the spermatocyte.

[1] In the spermatogenesis of bees, aphids, phylloxerans, and probably in rotifers and some other parthenogenetic animals (See pp. 797-799).

[2] For a detailed review of these phenomena as seen in insects, see Bowen, '22c.

The more peripheral or distal centriole plays the part of a *blepharoplast* or basal body from which grows forth the axial filament of the flagellum, while the proximal one, often also a portion of the distal, passes into the neck-region and in a few cases is pushed up into the base of the head (p. 380).

(4) The *chondrioma* or *chondriosome-apparatus*, which assumes many different forms in different animals. Among vertebrates it most commonly remains in a diffuse condition, consisting of small, scattered mitochondria or chondrioconts (Figs. 176, 175). On the other hand, in some of the invertebrates (characteristically in insects) the scattered chondriosomes sooner or later aggregate to form a single larger speroidal body, or in some cases several such (Figs. 171, 167, 172). When single (as in insects) this is called the *chondriosome-body* or *nebenkern*.[1] When two or more such bodies are formed (as in scorpions or some gasteropods) they may be called chondriosome-spheres or chondriosome-bodies, or collectively as the "nebenkern organ" (Retzius). These various forms are connected by many transitional conditions, and all have a similar origin in the small and often scattered chondriosomes of the early spermatocytes. In a single instance, the louse, the nebenkern is said to be present already in the spermatocytes (Doncaster and Cannon, '20).[2]

(5) The *acroblast*, typically a single rounded body (Figs. 176, 172, etc.), at first more or less lobulated, ultimately of rounded form. In structure it somewhat resembles the early idiozome of the spermatocytes, consisting of a clear sphere-substance, sometimes (mammals) containing the pro-acrosomic granules (p. 329), and bounded by an intensely chromophilic envelope composed of or derived from the Golgi-bodies.[3] For this reason it has been called by the same names (idiozome, idiosome, archoplasm-sphere, centrosphere, sphere, etc.). Morphologically, however, it is a new formation that has been rebuilt from the scattered remains of the original idiozome-complex, and it rarely if ever surrounds the centrioles. It seems preferable, therefore, when speaking of the spermatid, to employ the term acroblast [4] in place of "idiozome."

[1] The English equivalent of "nebenkern" should be *paranucleus*, but this form of the term has not come into general use.

[2] Platner ('89) described the nebenkern (in Lepidoptera) under the name "large mitosome," believing it to be derived from the spindle-fibers. Gatenby has recently revived the term in the form *macromitosome*. This seems inadmissible, both as a matter of priority and in reference to the structure of the nebenkern (p. 372).

[3] After fixation with reagents containing acetic acid this envelope may be wholly dissolved, so that only a pale sphere remains, as described by most observers until rather recently. With proper treatment the envelope may appear in the form of separate rodlets, of a net-like structure, or even of a continuous membrane.

[4] This word, due to King ('07) has been brought into more general use by Gatenby ('17) and Bowen ('20, '22a, '22b, etc.)

In rare cases (certain Lepidoptera and Orthoptera) the Golgi-bodies fail to aggregate completely, so that several or many acroblasts are formed. Even in this case, however, the end-result is a single acrosome, which seems to be of the same general nature as in the first case. These cases lend support to the conclusion that in the single or massive type of acroblast the sphere substance is formed by the progressive fusion of the clear spheres of idiozome-substance by which each Golgi-body is accompanied. The difference between the two types is thus owing merely to the fact that in the one the coalescence of the clear sphere-substance is accompanied by a close

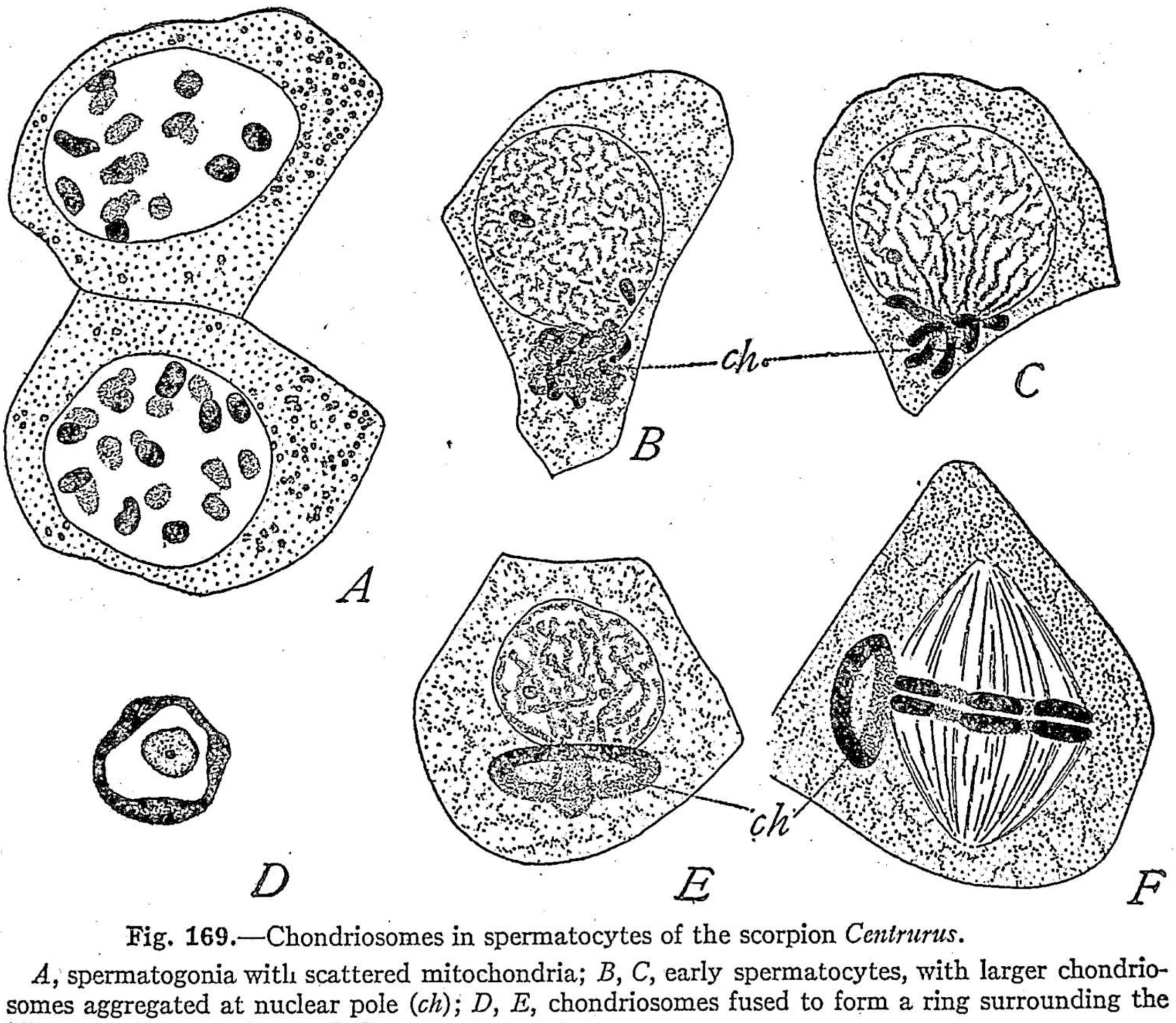

Fig. 169.—Chondriosomes in spermatocytes of the scorpion *Centrurus*.

A, spermatogonia with scattered mitochondria; *B*, *C*, early spermatocytes, with larger chondriosomes aggregated at nuclear pole (*ch*); *D*, *E*, chondriosomes fused to form a ring surrounding the idiozome; *F*, metaphase of first spermatocyte.

aggregation of the chromophilic Golgi-bodies or batonettes, while in the other these bodies always remain scattered (Bowen, *op. cit.*).

(6) The *chromatoid body*, a rather small, deeply-staining and highly refractive corpuscle (sometimes several such) of unknown significance. This structure (Fig. 178) is known in many cases to enter only certain of the spermatids and hence is probably a by-product which plays no direct part in the formation of the sperm (p. 382).

(7) The foregoing components are of widespread if not universal occurrence in the spermatids of animals. In addition to them other formed elements of more or less doubtful nature may be present. One of these is the *spindle-remnant*, sometimes called the "mitosome," a fibrillar or finely granular body, commonly lying near the periphery and derived from the spindle-fibers of the preceding mitosis. This body was believed by some earlier observers to play an important part in the sperm-formation, and was

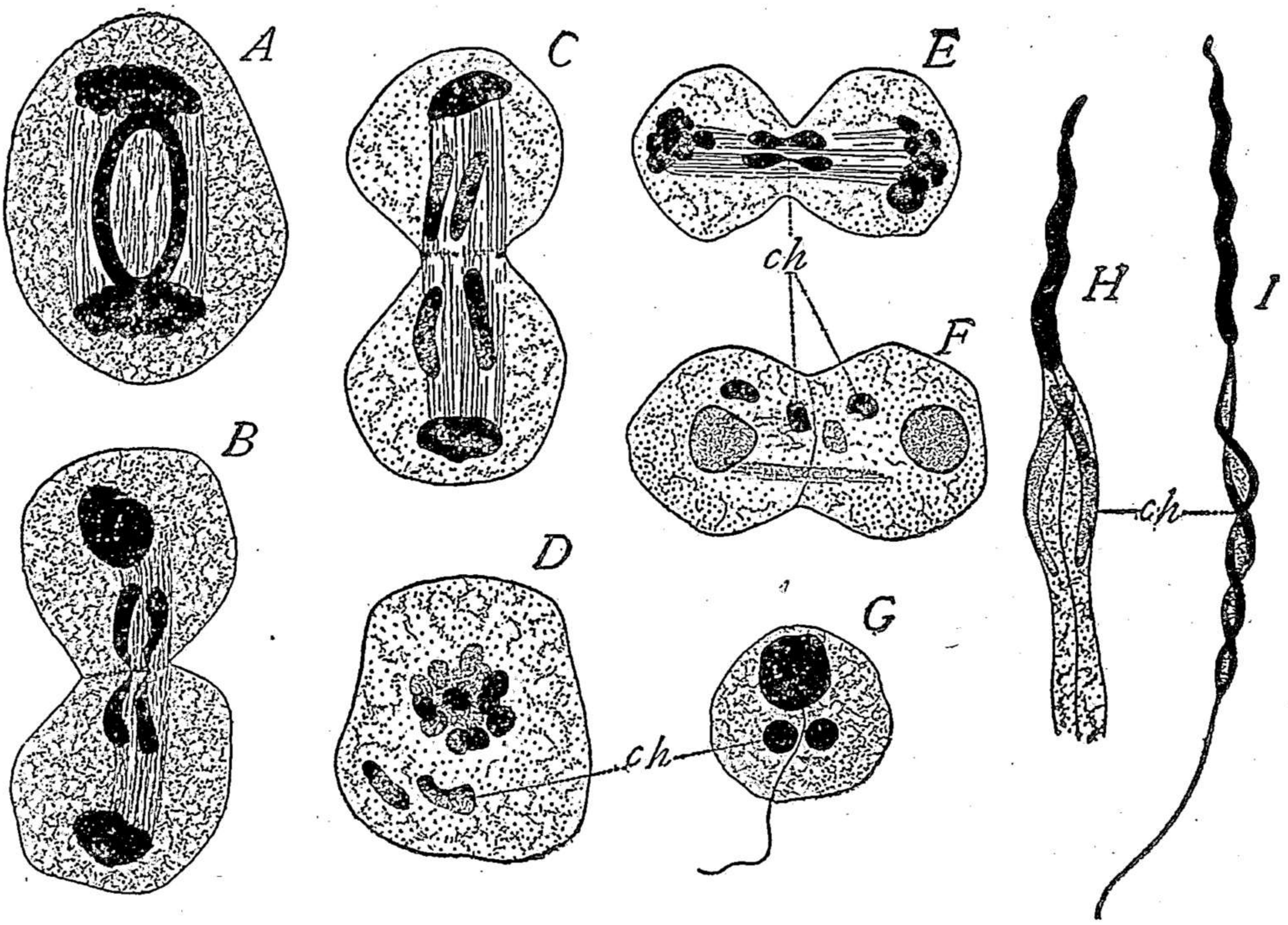

Fig. 170.—Chondriokinesis and sperm-formation in the scorpion *Centrurus*.

A–C, successive stages of first spermatocyte-division, division of the chondriosome ring; *D*, interkinesis; *E*, *F*, second division, completing division of original ring into eight parts; *G*, spermatid with double chondriosome-body (nebenkern); *H*, *I*, later spermatids, elongation and twisting of chondriosomes to form spiral tail-envelope.

confused with the nebenkern or with the acroblast; but later studies seem to show that it disappears without taking any definite part in the sperm-formation. Other obscure formations include deeply-staining granules of various kinds (not to be confused with mitochondria) and variously described as "seminal granules," "fat-droplets," "von Ebner's granules," or as products of the fragmentation of the chromatoid bodies. Little is known of their nature, and they are not known to play any definite part in the sperm-formation.

The later history of the spermatid may briefly be summarized as follows: The typical flagellate sperm assumes a filiform shape by an extensive

elongation in an axis marked by the nucleus and the centrioles, the latter always lying at or near the posterior pole of the nucleus on the side of the cytosome from which the tail afterwards grows out. The tail-formation is initiated by outgrowth of the axial filament from the distal or outer centriole (blepharoplast), at this time close to the periphery, the filament projecting freely outside the cytosome to form a naked flagellum (Figs. 176, 171, 167, 174, etc.). Meanwhile, both centrioles move inward towards the posterior pole of the nucleus, in some cases carried inwards by a deep infolding of the cell-periphery (urodeles), in others apparently by a movement of the centrioles themselves, thus lengthening the intra-cellular portion of the axial filament; and this is followed by a progressive drawing out of the whole posterior region of the spermatid to form the flagellum. The axial filament, which elongates *pari passu* with the surrounding cytoplasm, is thus provided with a cytoplasmic envelope, while its naked terminal or extra-cellular part forms the end-piece (Figs. 167, 175, etc.). During this process the chondriosome-formations are most commonly drawn out into the cytoplasmic envelope, of which ultimately they form a considerable and sometimes the principal part (p. 370), and often assuming a spiral structure (Fig. 175). The nucleus also often elongates, thus giving to the sperm-head the lance-shaped, cylindrical, rod-shaped or even filiform shapes found in many groups of animals.

Meanwhile the acrosome takes up its position typically at or near the anterior tip of the sperm-head, while the centrioles undergo various transformations and migrations which differ widely in different groups of animals. A remarkable feature of the spermiogenesis is the fact that only a portion of the spermatid is used in the formation of the sperm, a considerable mass of *residual protoplasm* being sloughed off late in the spermiogenesis and degenerating without taking any further part in the sperm-formation (Figs. 167, 175, 177). In this mass are included both the unused general cytoplasm, and certain remnants of the formed elements, including the chromatoid bodies, a remnant of the Golgi-apparatus or its products, and various other granules of uncertain nature.[1] A delicate investing peripheral layer of cytoplasm, by some observers regarded as the cell-wall, still surrounds the other structures. This certainly persists in the head-region and probably in all other parts of the sperm; and in or just below it are formed the peripheral spiral fibrillæ and other structures found in the head-region of many sperms (p. 283).

The casting off of the residual protoplasm takes place throughout a large series of both animals and plants and appears to be of general occurrence.

[1] It has been found in some insects that the remains of the residual protoplasm are ingested by the cells that form the walls of the cysts (Bowen, '22c).

In this respect the sperm-formation differs conspicuously from that of the egg which, with rare and doubtful exceptions retains the whole substance of the mother-cell. The sperm is nevertheless as truly a cell as the egg, the residual protoplasm doubtless representing only a portion of the cell-substance that has played its part in the nutrition and growth of the sperm and is then eliminated as needless ballast.

3. Further History of the Sperm-formation. Spermioteleosis.

A brief further account of the phenomena summarized in the preceding section is important for an understanding of the mature sperm.

a. The Telokinetic Movements. The second spermatocyte-division is followed by a series of telokinetic movements by which the polarized group-

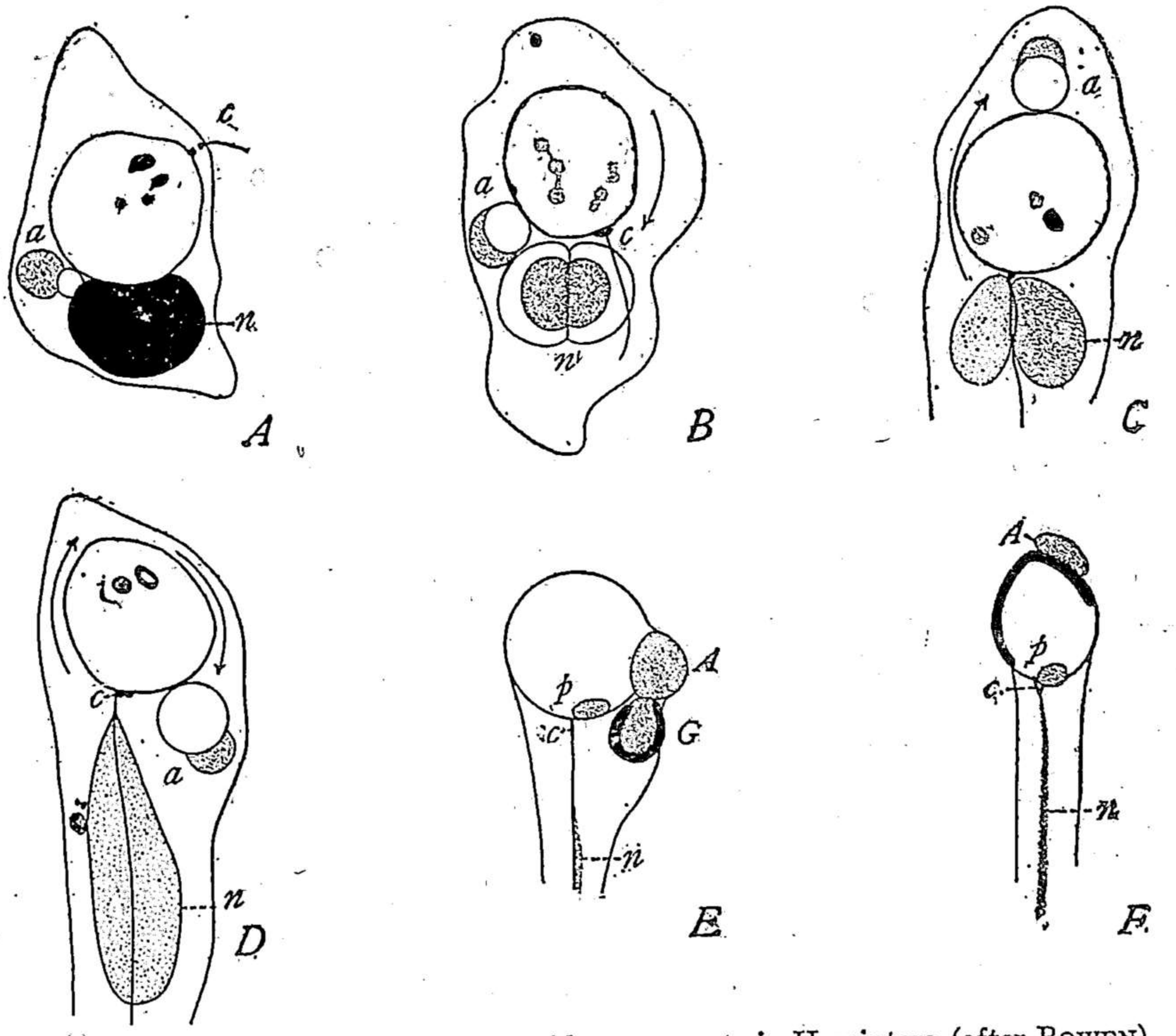

Fig. 171.—Movements of the spermatid-components in Hemiptera (after BOWEN).

E, from *Euschistus* the others from *Murgantia; a*, acroblast; *A*, acrosome; *c*, centrioles; *G*, Golgi-remnant; *n*, nebenkern; *p*, pseudoblepharoplast.

A, early stage, soon after formation of acroblast and nebenkern; *B*, backward migration of the centrioles, division of the nebenkern; *C*, forward migration of acroblast; *D*, completion of migration of acroblast; *E*, backward flow of cytoplasm, separation of acrosome, from Golgi-remnant; final forward migration of acrosome, Golgi-remnant has passed out into the tail; *F*, acrosome in final position.

ing of the spermatid-components is early effected.[1] These include (1) a

[1] See Meves, '97, '99 (vertebrates); Henking, '91, Meves, '00, Montgomery, '11, and especially Bowen, '22a (insects).

rotation of the daughter-chromosome-plates towards (usually) one side of the spindle so as to lie nearly parallel to the latter; (2) a movement of the centrioles (which may temporarily disappear from view) finally to the posterior or distal pole of the spermatid-nucleus; (3) a corresponding movement of the nebenkern (when present) to the same pole; (4), a movement of the acroblast towards the anterior pole of the nucleus (Fig. 171). In the Hemiptera, as shown especially by Bowen, the acroblast undergoes a further migration, passing almost completely around the nucleus on the side opposite to its original position so as to lie near the nebenkern and centrioles. Upon separation of the acrosome from the Golgi-remnant the former again passes to the apical pole, the latter backwards into the tail-region where it degenerates (Figs. 174, 172).

b. The Nucleus. With two exceptions the transformation of the spermatid nucleus, so far as known, offers comparatively little of interest as compared with the other structures. One of these, which seems to be widely distributed, includes the differentiation of the nuclear substance into two substances prior to its final condensation to form the dense and intensely basophilic sperm-nucleus, the other an elimination of nuclear substance into the surrounding cytoplasm that takes place at a certain period.[1] Both these processes have been most carefully examined in the sperms of insects, but nothing is yet known concerning their physiological meaning. In many animals the breaking up of the telophase-chromosomes is followed by a progressive accumulation of the basichromatin to form a rather thin peripheral layer, while the central region is occupied by a nearly homogeneous and lightly staining substance (oxychromatin, Figs. 167, 172). A little later, before the nucleus elongates, the peripheral layer thins away and apparently dissolves on the side towards the flagellum; and this is followed by the extrusion into the cytoplasm of a drop-like mass of oxyphilic medullary substance (Montgomery, '11, Bowen, '22a). The nucleus then closes, elongates and undergoes a resegregation of its substance, the peripheral basophilic layer becomes thickened, vacuolated, and finally collapses towards the center of the nucleus where it ultimately forms a deeply staining axial core surrounded by a clear oxyphilic substance.[2] The relative positions of the basophilic and oxyphilic substances have thus been completely reversed. Ultimately the cortical layer disappears from view and the head appears as a solid mass (in this case a rod) of basichromatin.

c. The Chondriosome-apparatus. Wide differences are shown by the spermatids of different species in respect to the conditions of the chondriosomes in the earlier spermatids and their later behavior.

[1] See for instance Meves, '03 (*Paludina*), Montgomery, '11 (*Euschistus*), Duesberg ('18) fishes.
[2] See Bowen ('20).

In the simplest case (vertebrates generally, and in some invertebrates), the scattered mitochondria gradually concentrate around the axial filament in the elongating flagellum and finally aggregate to form one or more long threads which in many cases become spirally coiled around the axial filament (Figs. 175, etc). This process was first clearly followed out in mammals by Benda ('97, '06) and has been confirmed by many subsequent observers. This condition represents one extreme in a series of transitional forms leading to a condition in which all the chondriosomes, before drawing out to form

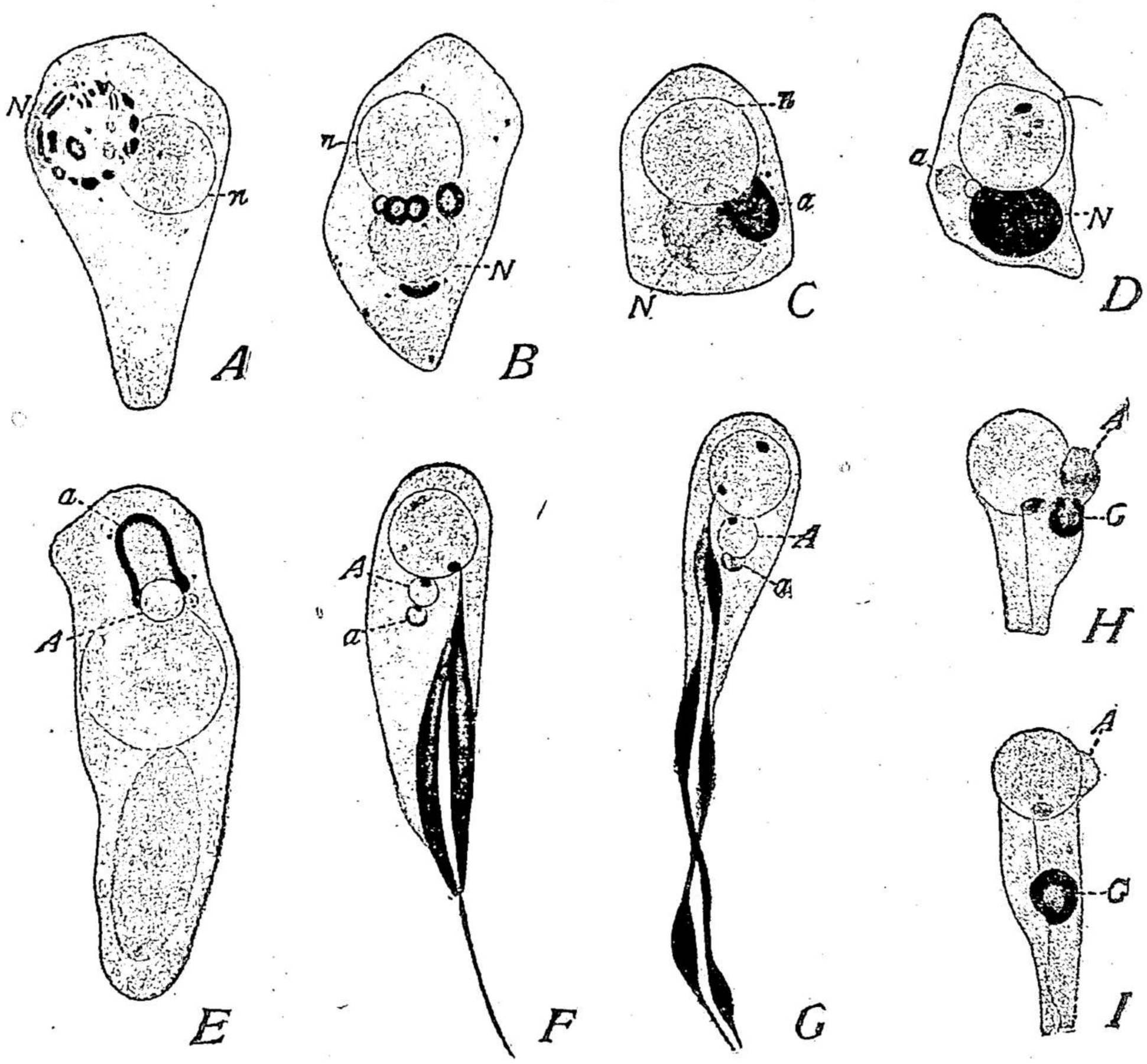

Fig. 172.—Spermiogenesis in Hemiptera (BOWEN).

a, acroblast; *A*, acrosome; *G*, Golgi-remnant = acroblast-remnant; *n*, nucleus; *N*, chondriosome-body or nebenkern.

A, *Brochymena*, aggregation of Golgi-bodies around the nebenkern to form the acroblast; *B*, *C*, fusion of the Golgi-bodies; *D*, *Euschistus*, differentiation of the acroblast; *E*, *Murgantia*, later stage, acroblast and acrosome at anterior pole; *F*, *G*, *H*, later stages of same; *H*, same, separation of the acroblast into the acrosome (*A*) and the Golgi-remnant (*G*); *I*, *Brochymena*, Golgi-remnant passing into the tail-region.

the tail-sheath, become concentrated into a single, large massive *nebenkern* or *chondriosome-body*.

A first step is seen in the pulmonates where the mitochondria, rather large and numerous and of two sizes, become concentrated in the posterior

region of the cytosome and surround the axial filament, but without aggregating closely to form larger bodies.[1]

In the scorpions appear several further steps which culminate in *Centrurus*. In certain species, as previously mentioned (p. 364) the spermatids receive a rather small number of large separate chondriospheres (5–7 in *Opisthacanthus* or *Hadrurus*, 4–6 in *Vejovis*) which place themselves in a ring surrounding the axial filament and are then drawn out to form its sheath but in this case without twisting (Fig. 168, Wilson, '16). These spermatids show a remarkable similarity to mature sperms of the first type, as seen in various nemertines, annelids, mollusks and other animals (pp. 287, 373) the ring of chondriospheres forming a "nebenkern-organ" that is here only a transient stage in the histogenesis. In the snail *Paludina* there are four symmetrical chondriospheres which surround the axial filament, and draw out to form the tail envelopes, without twisting (Fig. 167). A step beyond this is the scorpion *Centrurus* in which there are from the first but two chondriosome-bodies, received as such from the last division. Later these draw out to form the tail-envelopes, but in this case twist to form a close double spiral, ultimately of such fineness as to be invisible as such (Fig. 170, Wilson, '16).

We are thus brought finally to cases in which at the close of the second spermatocyte-division the chondriosomes at once aggregate to form a single *nebenkern* or chondriosome-body, as is typical in the insects where the nebenkern was first discovered.[2] Meves ('00) showed that it does not arise from spindle-fibers, as supposed by some earlier observers, but from numerous, crowded large mitochondria or small chondriospheres which form a dense sheath to the spindle. At the close of the division this slips off and quickly aggregates to form an irregularly spheroidal, more or less vacuolated body, which typically divides into two symmetrical and closely applied halves, between or beside which runs the axial filament, and which ultimately draw out along the latter to form the sheath of the flagellum.[3]

In the course of the foregoing changes the chondriosomes sooner or later differentiate into a central or medullary lightly staining "chromophobic" substance, and a peripheral "chromophilic" one that is deeply stained by the usual chondriosome dyes (crystal violet, hæmatoxylin) (p. 47).

[1] See Gatenby, '17, '18. In these spermatids the Golgi-bodies closely surround a large spheroidal body which doubtless represents the acroblast (or the sphere-remnant) of other forms. By earlier observers, as Gatenby points out, this body was called the "nebenkern"; but obviously this was a misnomer.

[2] By La Valette St. George ('67) who called it the "Nebenkörper"; the name "nebenkern" is due to Bütschli ('71). This has been called by many other names, and the term nebenkern has often been misapplied to other structures, such as the acroblast of the pulmonates (Fauré-Fremiet, '09, Weigl, '12, Gatenby, '18, '19), the secondary nuclei of the oöcytes (p. 346), the massed fibrillas of the pancreas-cell (Fig. 13), etc. The confusion in its use was first cleared up by Meves, ('00).

[3] See Benda, '98, Duesberg, '09.

When the smaller chondriosomes unite to form a single nebenkern, this body shows a multiple or mulberry-like appearance, the original chromophobic substance forming vacuole-like areas separated by partitions of chromophilic substance. Later the partitions partially break down, then offering the appearance of a framework which, as seen in sections has often been compared to the layers of a bisected onion.[1] In many cases this structure offers a spireme-like appearance, as if consisting of a convoluted thread; and as such it has been described (in Lepidoptera)[2] by Gatenby ('17a, '18); hence his proposed name *macromitosome* (p. 364). Bowen ('22b, '22d), however, has produced demonstrative evidence that in both Hemiptera and Lepidoptera the spireme-like appearance is given by optical section of a plate-work, forming the partition-walls between enlarged cavities closely pressed together. In later stages the chromophilic substance continually diminishes and finally disappears from view about the time the chondriosome-body divides into two. The chromophilic plate-work or thread-work is thus lost, while the chromophobic substance has come to occupy the main bulk of the nebenkern, now double and elongating to form the tail-envelope.

Meanwhile a third and new substance makes its appearance in the form of beaded cords running lengthwise in each half of the nebenkern.[3]

Their fate is still problematical. By some observers (Holmgren, Vejdovský) they were supposed to disappear entirely, leaving no trace of mitochondrial substance, at least in the flagellum. Bowen has, however, shown that the halves of the nebenkern (including both the cortical and the medullary substances) become drawn out into very thin threads which show at intervals conspicuous swellings or blebs (Fig. 172) which still later disappear. The same observer demonstrates very clearly that in some of the insects the axial filament is not actually surrounded by the two halves of the nebenkern but lies on one side of them in one of the bays or indentations where they meet.[4]

d. Central Apparatus and Blepharoplast. During the telokinetic movements of the centrioles (p. 368) or earlier the axial filament grows out from the *distal centriole* or *blepharoplast* to form the first foundation of the flagel-

[1] La Valette St. George ('86a, '87), Platner ('89), Henking ('91), Henneguy ('96), Wilke ('07), Stevens ('05), Gross ('07), Wassilieff ('07), Boring ('07), Doncaster and Cannon('20), Shaffer ('17), Bowen ('23), etc.

[2] See also the earlier figures of Giglio-Tos and Granata ('08), Baumgartner ('02).

[3] These structures were seen by several of the earlier observers, such as Paulmier ('99), Holmgren ('02), Boring ('07), Vejdovský ('12), but by most of them were erroneously considered as a product of the chromophilic substance. Their genesis was first fully worked out by Bowen ('22b) in the Hemiptera.

[4] In the case of *Peripatus* Montgomery ('12) held that the entire chondriosome-content of the spermatid is cast out with the residual protoplasm, making no direct contribution to the sperm-formation. This case, thus far unique, should be reëxamined.

lum. That the blepharoplast is identical with a centriole has been questioned in case of the plant-sperm (p. 388); and even in animals the blepharoplast has actually been traced to the spindle-pole of the preceding mitosis only in a few cases, for in many forms there is a brief period in the telophase when it is usually lost sight of. The identity of the blepharoplast with a centriole is, however, placed beyond doubt by the fact earlier described (p. 357) that in some cases the axial filament is present already in the spermatocytes and may be traced through both divisions in direct connection with the centrioles at the spindle-poles. The later history of the centriole shows a diversity so great that only a single feature can be regarded as of constant occurrence, namely, *that the anterior or proximal one, or its products, passes into the neck region of the sperm.* Beyond this point any attempt to give a summary treatment of the phenomena must at present be hampered by the deficiencies of our knowledge on the comparative side of the subject, nevertheless it seems necessary to offer some sort of provisional grouping of the facts. For this purpose we may distinguish at least five principal types (Fig. 173), here designated by numbers in order to avoid terms that involve doubtful questions of fact.

(1) In a first group may be placed those interesting forms of sperms, perhaps representing the most primitive type, in which the mitochondrial formations do not draw out to form an elongated sheath of the axial filament but remain near the base of the head to form a very short "middle-piece" or "nebenkern-organ" (p. 285). Though these sperms are insufficiently known, the evidence indicates that both centrioles may here remain near the base of the nucleus, *i. e.*, in the neck-region of the sperm, as in Fig. 173. The recent observations of Ballowitz ('15, '17) on teleost fishes seem clearly to show that such is the case in the trout *Salmo* (Fig. 121), and indicate that the same may be true also in several other fishes. In invertebrate sperms of this type the facts are less definitely known but point to the same conclusion. Boveri ('95, '01) describes two centrioles in the middle-piece of *Echinus* both before and after entrance of the sperm into the egg, and Yatsu ('09) likewise shows with great clearness a basal body (possibly double) in the middle-piece. (Fig. 207.)

(2) In a second group, may be placed such sperms as those of certain insects, and probably also those of some anuran Amphibia. Here, too, with an important exception presently to be noted, both centrioles remain near the base of the nucleus, in the region of the neck, but the mitochondrial formations (chondriospheres or mitochondria) are drawn out to form a more or less elongated tail-sheath. These forms commonly do not show externally any distinct middle-piece, though a definite neck-region containing derivatives of the centrioles may be revealed by staining (Figs. 173, 165, 122).

Most of the earlier observers of the insect sperm believed the entire centriolar apparatus to remain permanently in the neck-region, either as a single body [1] or dividing into two or more bodies that remain in the neck-region; [2] and this has been confirmed by some of the most recent work. In

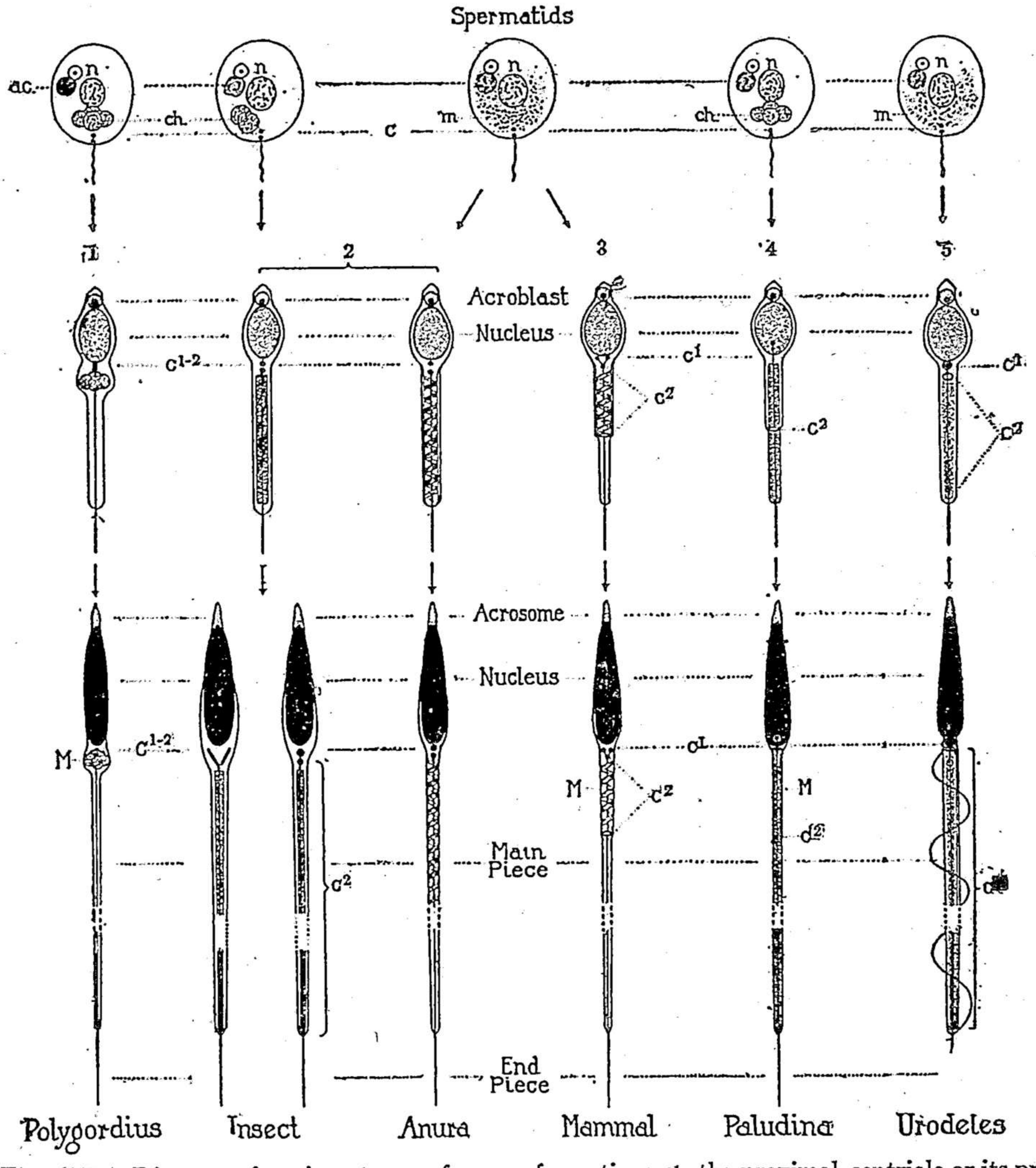

Fig. 173.—Diagram of various types of sperm-formation; c^1, the proximal centriole or its products, c^2, the distal.

Pygæra, as described by Meves ('00), in the Lepidoptera, the centriole seems clearly to be single as it enters the spermatid (Fig. 165) and Meves found no evidence of its division in the spermatid, though it elongates to form a short rod anterior to the chondriosphere, as is clearly seen in the apyrene

[1] *E. g.*, Paulmier ('99) in *Anasa*, Pantel and Sinéty ('06) in *Notonecta*, Montgomery ('11) in *Euschistus*, Meves ('00) in *Pygæra*.

[2] *E. g.*, in *Caloptenus* (Henneguy, '04), *Forficula* (Zweiger, '07), (Davis, '08), *Hydrometra* (Wilke, '07).

sperm (Fig. 131). In the grasshopper *Chorthippus* Lewis and Robertson ('16) in living cultures *in vitro* observed a pair of centrioles which were seen to pass into the neck-region and there to give rise, without separating, to the neck or "middle-piece," while the chondriosphere elongated. More recently a very careful study of the facts in Hemiptera (*Murgantia*, *Arvelius*, etc.) has been made by Bowen ('20, '22a), who clearly demonstrates that both centrioles, after outgrowth of the axial filament, assume the form of

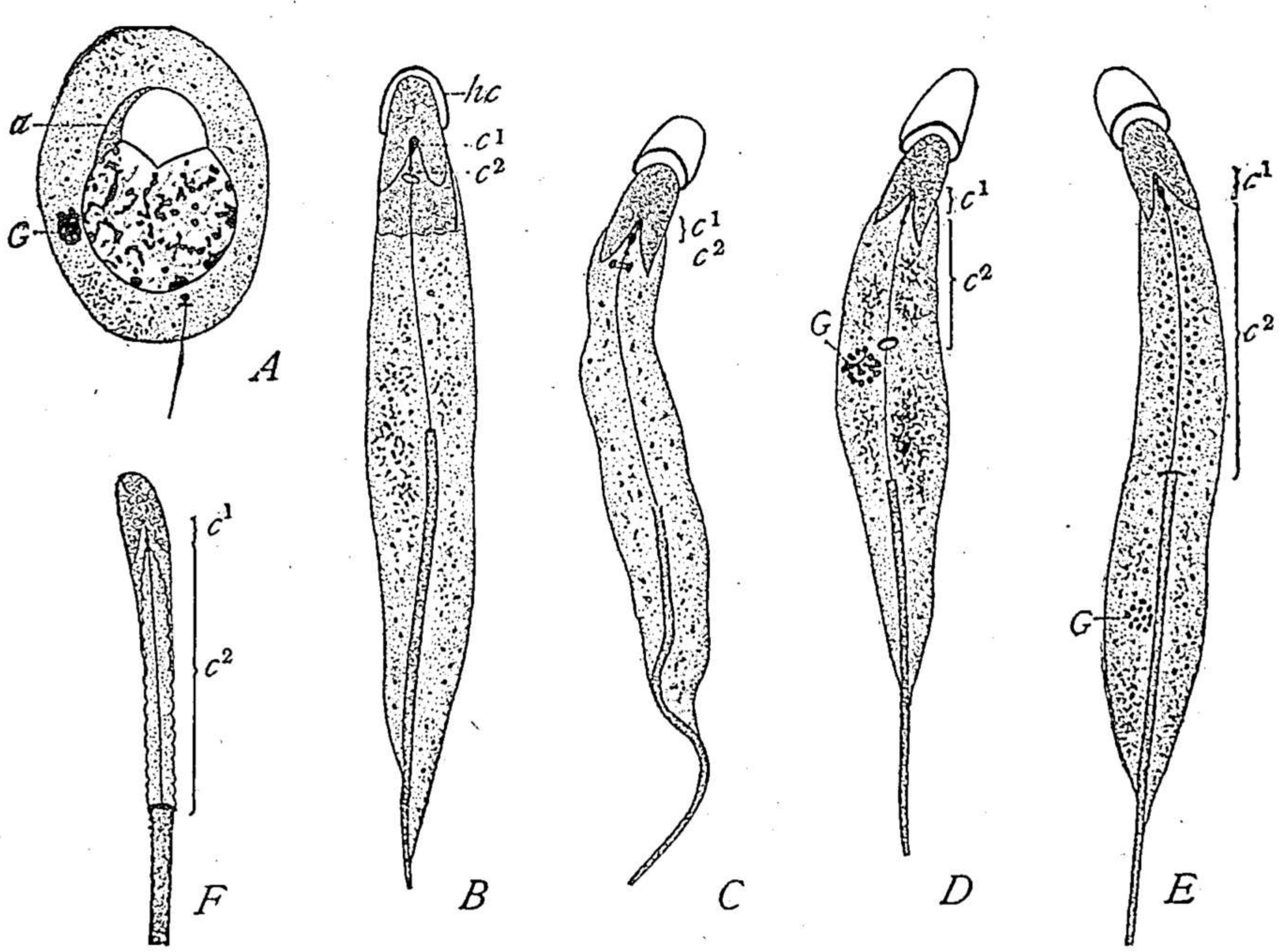

Fig. 174.—Sperm-formation in *Phalangista* (KORFF).

a, acrosome; c^1, c^2, proximal and distal centrioles; *G*, Golgi-remnant; *h*, *c*, head-cap.

A, early spermatid; *B*, later stage; *C*, division of proximal centriole; *D*, backward migration of ring; *E*, ring at final position, posterior limit of middle-piece; *F*. later stage after casting off of head-cap and residual protoplasm.

rods which lie side by side in the neck-region (at certain stages in *Murgantia*, approximating somewhat posteriorly so as to give a V-shaped figure), only one of them being connected with the axial filament (Fig. 173). These sperms accordingly are asymmetrical in respect to these structures.[1]

A curious feature in the history of the centrioles in some insects (Hemiptera, Orthoptera) is a temporary concentration of the chromatin on the inside of the nuclear membrane close to the centrioles (Figs. 171, 172) so as to give the appearance of a much larger single body or "pseudo-blepharoplast."[2] At a later period, however, according to Bowen, the chromatin-component

[1] In *Pediculus*, as described by Doncaster and Cannon ('20) both centrioles likewise persist in the neck-region, and each is said to give rise to an axial filament which traverses the tail-region.

[2] Bowen. '22a

of this body disintegrates, when the rod-shaped centrioles again come into view and in this form pass into the mature sperm.

In the *Anura* the most satisfactory observations are those of Broman on *Bombinator* ('00), *Pelobates* ('01) and especially on *Rana* ('00, '07). In all these, even in the highly modified sperms of *Bombinator*, the mature sperm shows two very distinct basal bodies in the neck-region close to the nucleus; and both in *Bombinator* and *Rana* (*fusca*) Broman shows that these are derivatives, respectively, of the two original centrioles (Figs. 122, 124). Meanwhile the mitochondrial granules are drawn out to form a sheath which assumes a spiral structure, and from this is formed a rather long and fairly distinct "middle-piece," which, in *Rana*, tapers gradually into the flagellum. In *R. esculenta*, *mugiens* and *arvalis* the two granules are likewise found in the neck-region, but the "middle-piece" is much shorter and more rounded. If this account is correct the anuran sperm is evidently rather similar in mode of formation to that of the insect and the so-called middle-piece seems to be no more than a thickening of the basal region of the flagellum.

(3) In this type and the following ones the history of the centriolar apparatus is complicated by a movement of the distal centriole, or one of its products distally for a certain distance along the axial filament. The point at which it remains marks the posterior limit of the true "middle-piece" or "connecting-piece" as defined by Waldeyer (*e. g.*, in mammals, birds or reptiles); but it must be borne in mind that in a few forms (urodeles) this point lies nearly at the junction between the main-piece and the end-piece, so that Waldeyer's terms lose their meaning (p. 378).

This backward movement is initiated already in some of the Anura and insects. In *Hyla*, for instance, Retzius found that the two centrioles separate somewhat, so as to mark off a short middle-piece; and a somewhat similar process seems to take place also in the myriapod *Lithobius* according to Tönniges (Korschelt-Heider, '02). In some of the insects the movement is more extensive, a derivative of the distal centriole passing out nearly to the tip of the tail. The earlier observers believed this migration to be performed by the whole distal centriole (which is here somewhat smaller than the proximal) [1] but such a peripheral movement of the whole distal centriole is to say the least anomalous. In point of fact observations on the sperm-formation in the orthopter *Œcanthus* made in my laboratory place the facts in a different light. Preparations by C. R. Driver and H. H. Johnson (Johnson, '22) clearly show that the distal centriole divides into two parts, *one of which remains in the neck-region with the proximal centriole, while the distal*

[1] See Prowazek ('01 on the beetle *Orycytes*) whose results seemed to be confirmed by those of Holmgren ('02) on *Silpha*, Buchner on *Pyrrhocoris* and *Syromastes* ('06), and *Œdipoda* ('15), and by those of Otte ('07) on *Locusta*. Something similar is indicated by the observations of Vejdovský ('11) on *Locusta* and of Gatenby ('17) on Lepidoptera.

part alone performs the outward migration (Fig. 173). This case thus becomes quite comparable with those of the mammal, bird or urodele, and for the first time we are able to connect the latter types of sperms with the simpler insect-type of Class 2.

(4) A fourth type is exemplified by the mammals, and is probably characteristic also of birds, reptiles and perhaps of other groups. The sperm-

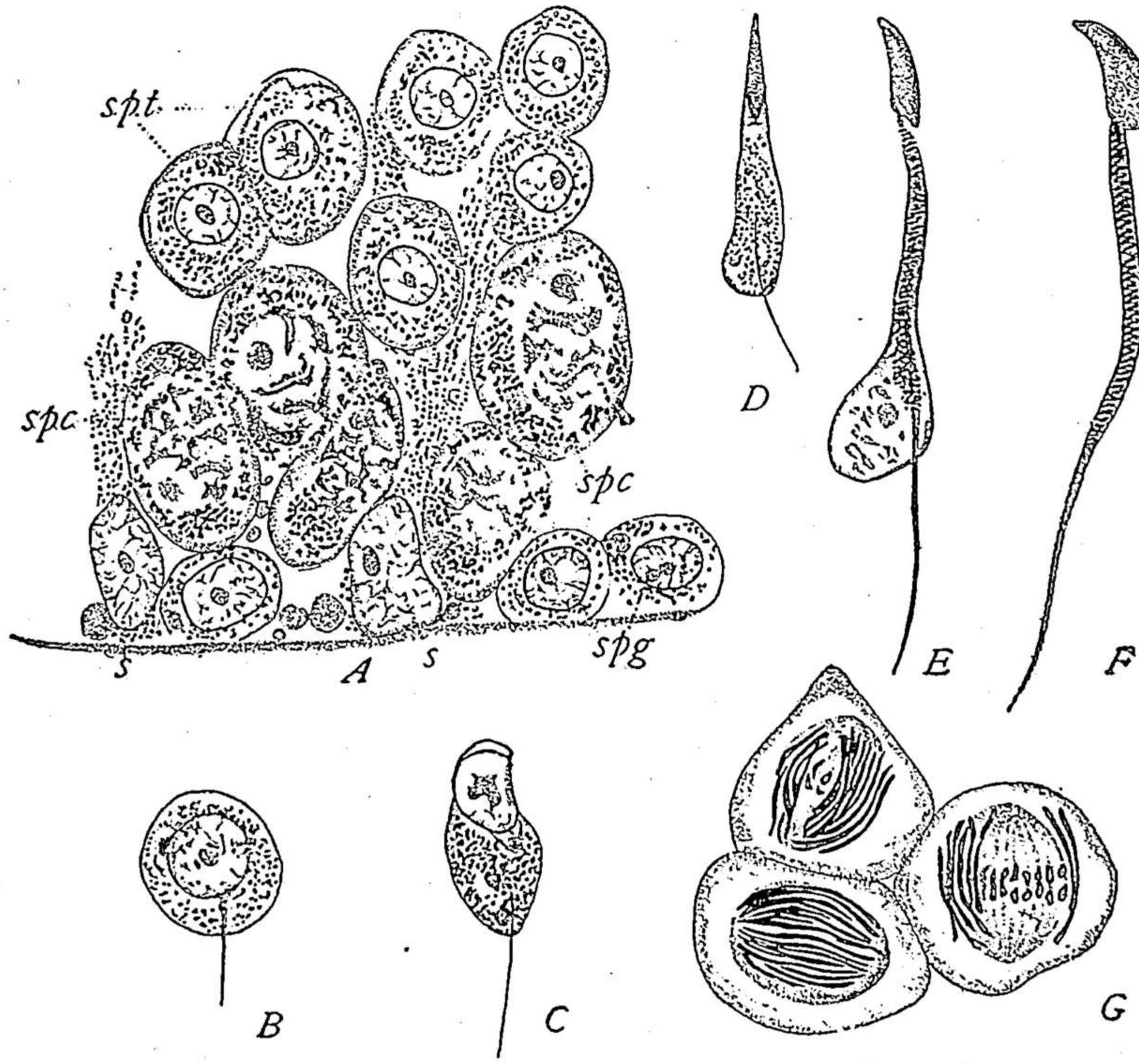

Fig. 175.—Mitochondria in sperm-formation (BENDA).

A, wall of testis-tubule in the mouse; *s*, Sertoli-cells; *spc.*, spermatocytes; *spg.*, spermatogonia; *spt*, spermatids, all with scattered mitochondria; *B–F*, stages of spermiogenesis, drawing out of mitochondria to form the spiral sheath; *G*, spermatocyte-divisions in the beetle *Blaps*, chondriosomes.

formation of mammals has been examined by many observers [1] but only a limited number of cases have been thoroughly studied. These (man, rat, guinea-pig and a few others) show a fair degree of uniformity on the main points. Apparently in all cases the distal centriole, after giving off the axial filament, assumes a disc-like shape and then separates into a small central body, to which the axial filament is attached, and a peripheral ring (Figs. 174, 177). The former constitutes a basal body (with which the axial filament maintains its attachment) while the ring, surrounding the axial filament,

[1] Especially (to mention only a few) Niessing ('96), Lenhossék ('98), Meves ('98, '99), Benda ('97, '06, etc.), Korff ('02), Duesberg ('08, '20), Jordan ('11), Oliver ('13), Stockard and Papanicolaou ('18). Gatenby and Woodger ('21), etc.

migrates backward to a position *at the junction between the middle-piece and the flagellum*. The basal body meanwhile moves forwards or remains near the proximal centriole at the base of the nucleus and there forms a part of the centriolar apparatus of the neck. The middle-piece thus becomes definitely marked off, extending from the original basal body, which remains in the neck-region, to the ring. In respect to the details the sperms of different species differ considerably in the history of these structures.[1] In the rat according to Meves and Duesberg the proximal centriole becomes flattened against the base of the nucleus and there remains, while the basal body or end-knob divides to form two centriole-like bodies. In man Meves finds the facts to be similar. In the guinea-pig they are complicated by the fact that both the proximal centriole and the original basal body break up into smaller basal bodies, the former into three, the latter into two or more (Fig. 177). In the marsupials the facts appear to be somewhat simpler (Fig. 174), there being three granules in the neck-region [2] of which two are supposed to arise by division of the proximal centriole, while the third is the distal basal body.

In spite of certain contradictions in the literature the broad fact now seems well established that in all cases the proximal centriole remains at or near the base of the nucleus as a basal body, or group of such bodies, while the principal part of the distal one passes as a ring to the distal limit of the middle-piece. Such a type of sperm might readily be derived from either of the first two described above.

Attention may here be directed to the *caudal sheath* ("Schwanzmanchette"), a structure characteristic of the mammalian spermatid but of unknown significance. This is a delicate tubular or funnel-shaped cytoplasmic structure encircling the base of the nucleus and the centrioles and extending a short distance backward in the rather early spermatid (Fig. 176, E).[3] This structure, originally formed from cytoplasmic fibrillæ (Meves), seems to disappear completely without contributing directly to the formed components of the sperm, though it has been supposed to form the peripheral part of the middle-piece (Oliver, '13).

(5) In a fifth group we may place the sperms of the urodele amphibia, which may be thought of as an extreme development of the preceding type, with which it seems to be connected by certain intermediate conditions; in the sperms of the pigeon, for example, according to Retzius, the middle-piece is greatly elongated, so as to occupy a large region of the flagellum (Fig. 125). In the urodele this region is still further extended. The proxi-

[1] A critical review is given by Meves ('00). See also Duesberg ('08, '20), Oliver ('13).
[2] Korff, '02, in *Phalangista*, Duesberg, '20, in *Didelphys*.
[3] See Meves ('99), Duesberg ('08, '20).

mal centriole, as usual, passes to the base of the nucleus, remains undivided, and undergoes great enlargement to form a conspicuous rounded, ovoidal or, in some species, rod-shaped body. This is the so-called "middle-piece" (properly the *neck*) which is closely applied to the base of the nucleus, and

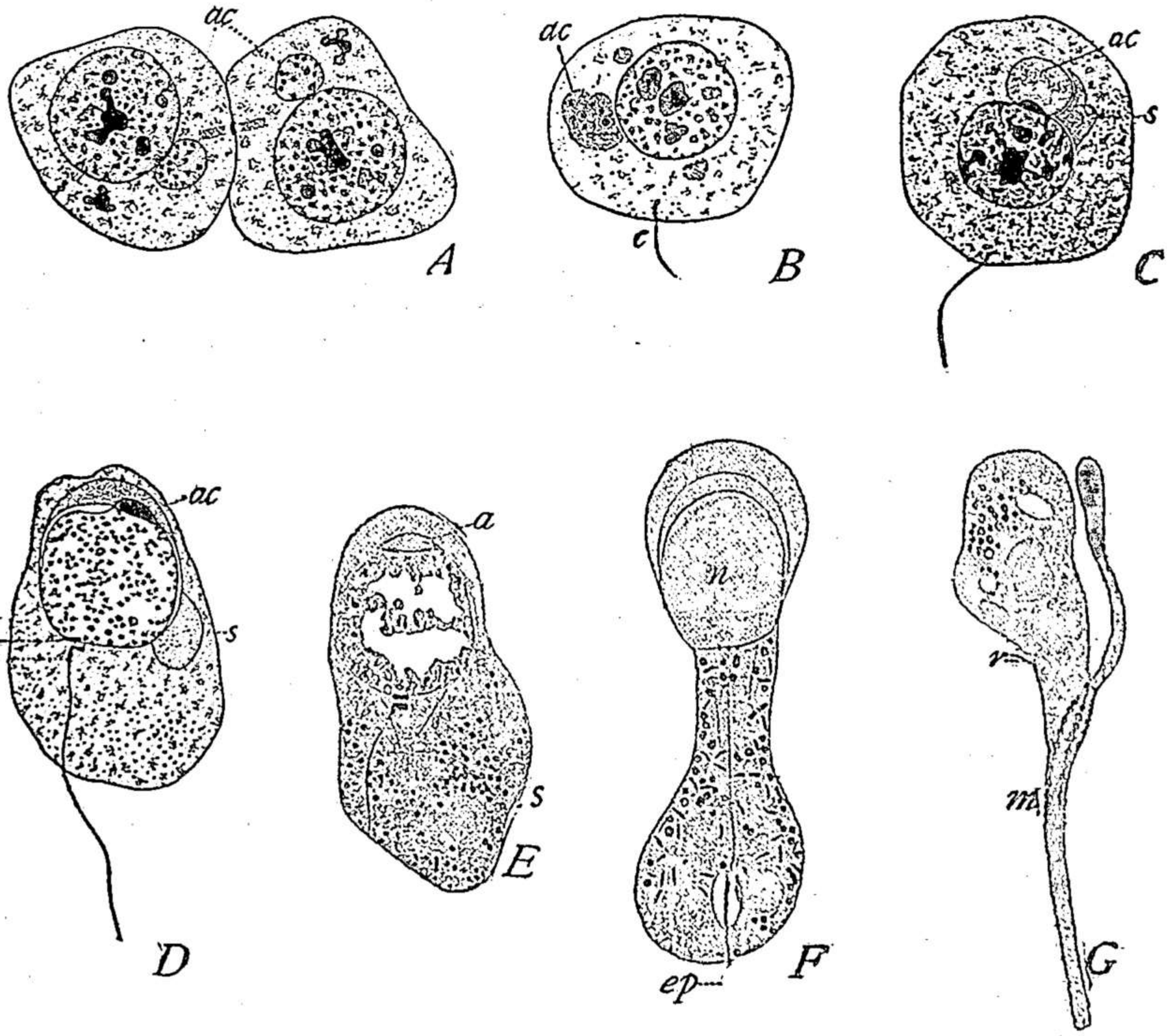

Fig. 176.—Earlier stages of sperm-formation in the guinea-pig. History of acroblast (*A–D*, from MEVES, *E–G*, from DUESBERG).

ac, acroblast, acrosome; c^1, c^2, centrioles; *ep*, end-piece; *n*, nucleus; *r*, residual cytoplasm; *s*, "sphere" (= Golgi-remnant).

A, young sister-spermatids, *B*, slightly later stage; both showing acroblast with proacrosomic granules; *C*, *D*, middle stages; *E–G*, later stages, *G*, in side-view.

by some observers is described as actually lying inside it. (Meves, McGregor).[1]

After giving off the axial filament, the distal centriole, as in the mammals, is converted into a ring which elongates, assumes a pessary-shape, and finally draws out along the axial filament, until it extends through the whole length of the main-piece. Its proximal or anterior moiety gives rise (in *Salamandra*) to a deeply-staining plate-like body just behind

[1] Meves ('97). McGregor ('99) believed the "middle-piece" (in *Amphiuma*) to arise from the remains of the "sphere" (idiozome), while the proximal centriole lies inside it as a much smaller body; but this seems to have been an error. Bowen ('22) has shown that the smaller body in question (in *Plethodon*) is formed by division of the proximal centriole into two parts, one of which enlarges to form the "middle-piece" while the second may be a blepharoplast from which grows forth the marginal filament of the fin-membrane.

the neck-region while its distal half finally passes to the limit of the main-piece of the flagellum at the beginning of the short end-piece, while the elongate narrow middle part becomes closely applied to the axial filament (Fig. 173).

(6) In a sixth group might perhaps be placed forms in which one or the other of the centrioles is stated to elongate bodily to form a long filament extending out to the base of the end-piece. In *Paludina*, for example, it is the distal centriole which thus elongates (Figs. 166, 167), while the proximal one is pushed up into the nucleus, having close behind it a ring-shaped

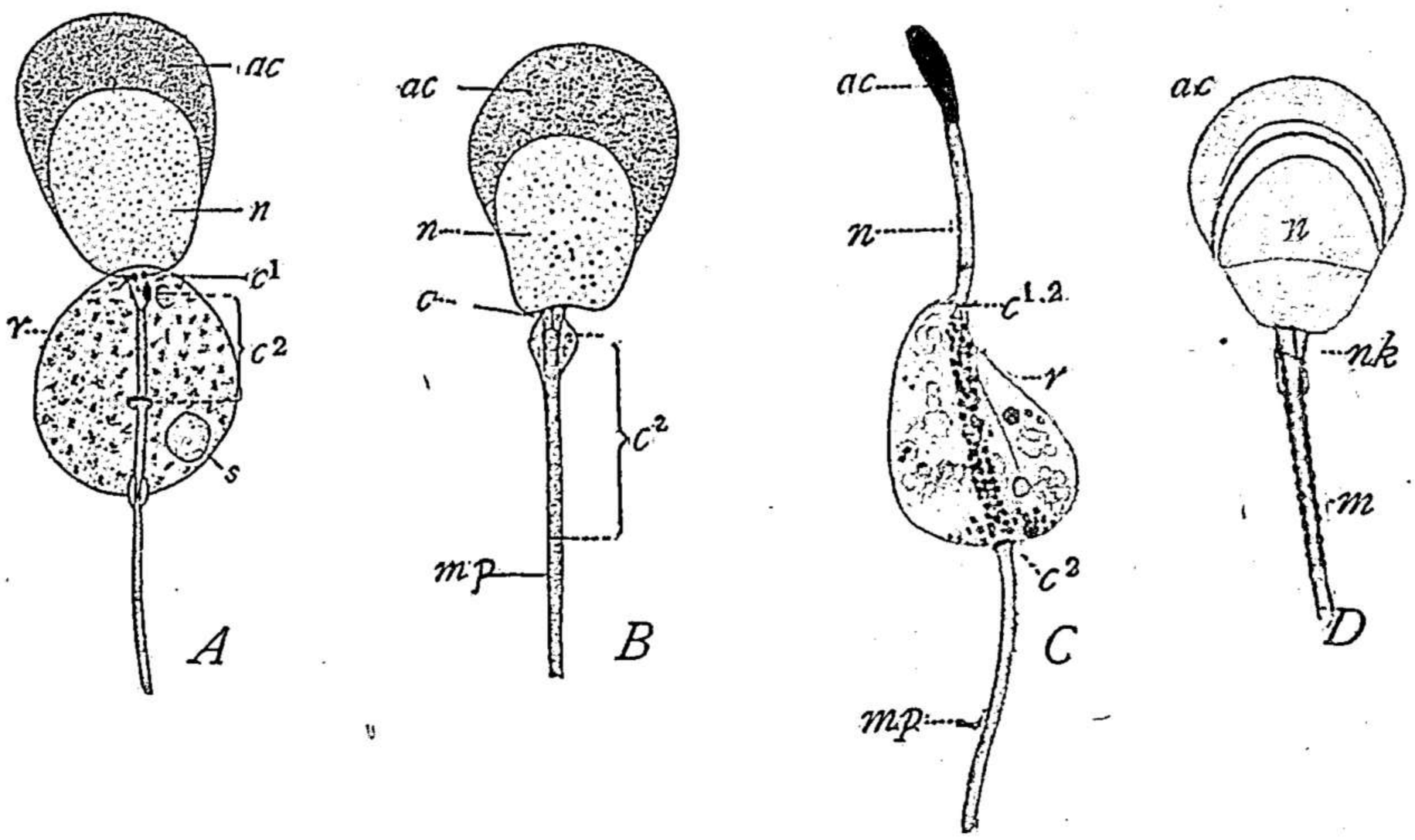

Fig. 177.—Later stages of sperm-formation in the guinea-pig. (*A*, *B*, from MEVES, *C*, *D*, from DUESBERG.)

ac, acrosome; c^1, c^2, proximal and distal centriole-derivatives; *m*, middle-piece; *mp*, main piece of flagellum; *n*, nucleus; *nk*, neck; *r*, residual cytoplasm; *s*, "sphere" (= Golgi-remnant).

A, centrioles, early acrosome, Golgi-remnant; *B*, later stage; *C*, side-view, residual cytoplasm; *D*, nearly mature sperm.

centriole-derivative of unknown origin (Meves, '03). On the other hand, in the elasmobranch (Suzuki, '98) and in *Helix* (Korff, '99) it is the proximal centriole which thus elongates and the ring, here said to be derived from the distal centriole, is carried out to the junction of the main-piece and the end-piece.

Conclusion. It is impracticable to enter here into other modifications of the central apparatus of the sperm, most of which are still imperfectly known. The fact that at least one of the centrioles passes into the neck-region seems to be common to nearly all forms of sperms, and is unmistakably correlated with the appearance of the sperm-center during fertilization from, or in close association with, this region of the sperm in the egg (p. 441). A second point of general interest is the complete demonstration offered by the sperm-formation of animals that the centriole may play the part

not only of a division-center but also of a blepharoplast. In this fact, possibly, we may find a clue to the remarkable diversity of behavior on the part of the distal centriole during spermiogenesis. It seems possible that the all but invariable separation of this centriole into two parts means that it is a dual structure, being differentiated into two components, one concerned with division, the other with the flagellum-formation, as is known to be the case with the basal bodies of certain flagellates (p. 696). The backward movement of the ring or its representative in so many sperms may therefore represent the removal of the blepharoplast-component while the basal body represents the division-center. If this conjecture has any value, we might consider that the proximal centriole alone represents the persistent active center of the sperm, and that the puzzling variations of behavior on the part of the distal one have no particular physiological significance.

e. Acroblast and Acrosome. The most conspicuous feature in the history of the acroblast is its ultimate separation into two parts one of which moves to the anterior pole and there gives rise to the definitive acrosome, while the other and often larger portion constitutes the *acroblast-remnant* (idiozome-remnant) *containing the original Golgi-bodies* surrounding the sphere. This body passes backwards commonly into the tail region, disintegrates, and most of its substance appears to be finally eliminated with the residual protoplasm (Figs. 176, 172, 177).[1] By Gatenby and Woodger ('21) a portion of this body is believed to contribute to the formation of the middle-piece (in the guinea-pig).

The definitive acrosome seems always to be formed from or within a clear vacuole-like space that appears within that part of the acroblast that passes to the anterior nuclear pole. Within this space appears a small deeply staining spheroidal body which by some of the earlier observers was mistaken for a centriole; [2] and by its enlargement and differentiation (Figs. 172, 176) gives rise to the central or principal portion of the acroblast, undergoing a great variety of changes in different species. The most divergent extremes of these are on the one hand the greatly elongated and filiform acrosome of the Lepidoptera (Bowen, '22), and on

[1] This has been observed more or less fully by many investigators, including Henking ('91), Niessing ('97), Lenhossék ('98), Meves ('99), Voïnov ('03), Sjövall ('06) and more recently by Montgomery ('11), Schitz ('16), Schaffer ('17), Gatenby ('19), Bowen ('20) and Duesberg ('20). This process, or indications of it, has been seen in insects, arachnids, mollusks, and vertebrates and is undoubtedly of widespread occurrence.

[2] This granule was designated by Lenhossék ('97) as the "acrosome" the term being expanded by later writers so as to apply to the completed structure. By Stockard and Papanicolaou ('16) the clear vesicle is called the *idiosphærotheca*, the central granule (acrosome of Lenhossék) the *idiosphærosome*, the inner zone derived from the latter the *idiocryptosome*, the outer zone the *idiocalyptosome*, the head-cap the *spermiocalyptrotheca*, and the idiozome-remnant the *idiophthartosome*. This terminology, though etymologically excellent, seems too cumbersome. For other synonyms see Gatenby and Woodger ('21).

the other, the cap-like one of many mammals (guinea-pig, Fig. 177) which spreads out over the anterior part of the nucleus, while the clear substance gives rise to a thin outer zone. Both zones are surrounded by the so-called *head-cap* which is derived from the peripheral layer of the clear vesicle or outer zone.[1]

In the mammals the concordant results of many observers, beginning with Moore ('94) make it nearly certain that the acrosome-granule in question is formed by the fusion or aggregation of the proacrosomic granules (p. 329) already present within the spermatocyte-idiozome. These granules are set free in the cytosome when the idiozome breaks up, persist during the first division, reaggregate in the interior of the idiozome of the second spermatocytes, are again dispersed in the second division, and once more aggregate within the substance of the acroblast where they are surrounded by small vacuoles (Fig. 176, A, B, D). A progressive process of fusion now sets in [2] the vacuoles running together until they produce the usual large clear vesicle, while the granules progressively fuse until they form a single much larger body (Fig. 176, C, D). In many other cases, however (insects), no proacrosomic granules have been observed, the acrosome first appearing as a single intensely chromophilic granule within the acroblast-vesicle. It seems probable that the foregoing two modes of acrosome-formation differ only in degree, *i. e.*, in the earlier or later appearance of the granule-material. The fact that both vacuoles and granules are in the one case multiple, in the other single, clearly points to the conclusion that in the former case they are produced separately by elements that are themselves still separate, in the latter case as the single common product of these same elements intimately united or fused. In any case it seems certain that the Golgi-bodies are somehow concerned in the production of the acrosome. It seems incredible that so elaborate a process should be necessary for the formation of a structure whose only function lies in the attachment of the sperm to and penetration into the egg; and the process seems still more remarkable in view of the fact that the Golgi-apparatus, though it gives rise to the acroblast, seems for the most part to be itself cast out of the sperm. Interesting questions for further research are here raised (p. 716).

f. Chromatoid Bodies. Nearly all observers are thus far agreed that the bodies thus called do not directly give rise to any of the formed elements of the sperm. They first make their appearance in the protoplasm of the primary spermatocytes, usually lying near to the nucleus but not in contact

[1] In the marsupial *Phalangista* the head-cap is said by Korff ('02), to be cast off (Fig. 174).

[2] This was carefully described by Meves ('99) in the guinea pig, and more recently by Stockard and Papanicolaou ('16) and by Gatenby and Woodger ('21).

with it (Fig. 178). As a rule but one such body appears; but two or several may be present. The most diverse accounts have been given of its origin and later history, and it is far from certain whether all the bodies which go under this name are of the same nature.[1]

In insects [2] the chromatoid body is usually single, and stains intensely in hæmatoxylin, safranin and other dyes. With the Benda alizarin-violet

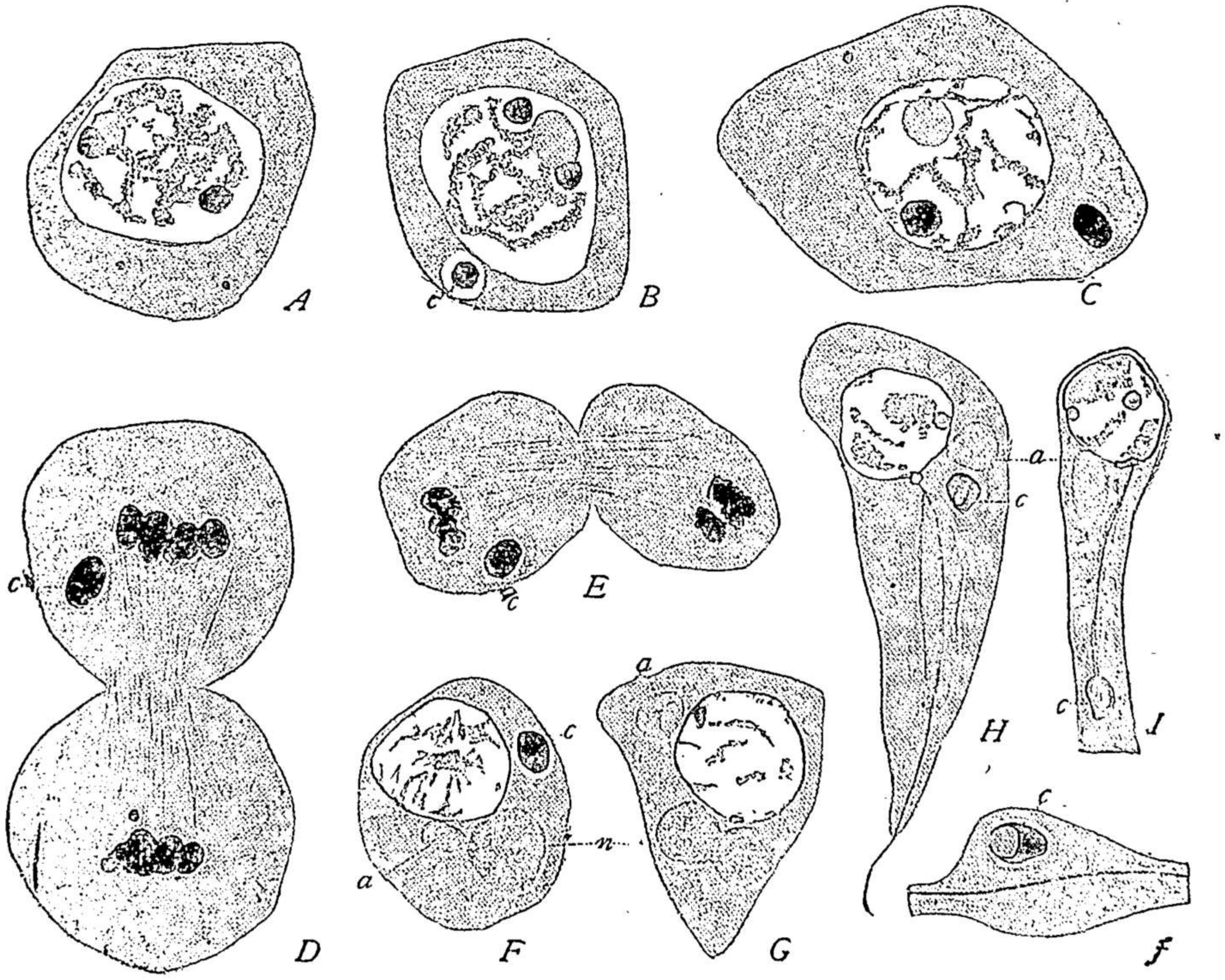

Fig. 178.—Spermiogenesis in the hemipter *Pentatoma senilis*.

A, early spermatocyte, chromatoid granules in cytoplasm; *B*, later stage, single chromatoid body, (*c*), plasmosome, two chromosome-nucleoli (*X* and *Y*); *C*, later, confused period; *D*, first spermatocyte division; *E*, second division; *F*, early spermatid, with chromatoid body, acroblast (*a*) and chondriosphere (*n*); *G*, later spermatid, with double chondriosphere, blepharophast, no chromatoid body; *H*, *I*, later spermatids, outwandering of chromatoid body; *J*, portion of tail-region with chromatoid body, shortly before casting off of residual protoplasm.

method it is stained bright purple; with Auerbach's rubin-methyl-green mixture it is red while the nuclei are green; with Altmann's mitochondrial stain bright red (Plough). It might be supposed from these reactions that this body is of mitochondrial origin; but Plough has shown that this

[1] This body, figured by some of the earlier observers in the mammals (Brunn, '76, Brown, '85), was more carefully studied by Benda ('91) who called it the "chromatoider Nebenkörper" or "chromatoider Körper"; subsequently by Niessing ('96), Lenhossék ('98), Meves ('99), Schoenfeld ('00)-Korff ('02), Van Molle ('06), Duesberg ('08) and other spermatologists. It was later observed in the fishes (*Myxine*, Schreiner, '05), in insects (Schaefer, '07, Wilson, '13), Crustacea (Fasten, '14, '18) and other invertebrates, and is probably of wide occurrence in animals generally.

[2] Wilson ('13), Lewis and Robertson ('16), Plough ('17).

body is not stained *intra vitam* by Janus green but is stained by neutral red. Its history in the hemipter *Pentatoma senilis*, where it is of large size, is as follows: [1]

It is first seen in the early spermatocytes in the form of several small, deeply staining granules, lying in the *cytoplasm*. Slightly later it typically becomes single and much larger. It persists during both spermatocyte-divisions, but fails to divide in either, passing over bodily first into one of the second spermatocytes and then into one of the spermatids (Fig. 178). It thus comes to pass that *this body enters only one spermatid out of four*—a result fully confirmed by counts of the spermatids. In the spermatid it is finally carried out into the outgrowing flagellum and sloughed off with the residual protoplasm in this region.

The facts are similar in almost every detail in various other Hemiptera and also (as Plough has shown) in the Orthoptera.[2] In the crayfish *Cambarus* (Fasten, '14) the facts are similar, except that two chromatoid bodies, of equal size are present. These likewise persist without division during the spermatocyte-divisions and most commonly pass together to one pole, more rarely to opposite poles. In the foregoing cases there can be no possibility of regarding the chromatoid body as an extruded nucleolus (as assumed by some earlier observers), for during the whole period of its formation and growth both the spermatocyte-nucleoli (plasmosome and chromosome-nucleolus) remain intact within the nucleus. Its physiological meaning remains completely in the dark, but the facts suggest that if it have any function it must be performed not later than the spermatocyte-divisions. It may, however, be no more than a by-product of other activities, and in this sense a functionless excretion-product.

In respect to the vertebrates, the existing accounts are still conflicting and confused in respect to almost every point in the history of the chromatoid. Most of the earlier writers, relying mainly on the staining-reactions, regarded it as probably of nuclear origin,[3] and several of them accepted the probability that it is an extruded nucleolus or is formed from a nucleolus (Lenhossék, Meves and particularly the Schreiners, who have made an extended study of the phenomena in *Myxine*). These observations, however, stand alone, all other observers having failed to observe the origin of the chromatoid body, or having relied wholly on indirect evidence. By Benda, Moore, Niessing, Lenhossék, and more recently Regaud ('10), it was observed already in the growth-period of the primary spermatocytes; on the other hand Ebner, ('99), and especially Duesberg ('08) were not able

[1] Wilson, '13.
[2] The passing of the chromatoid body into the tail is also described in *Dytiscus*, by Schäfer ('07).
[3] Benda ('91), Moore ('93), Lenhossék ('98), Meves ('99.)

to find it until the second spermatocyte-division, and Meves ('99) first describes it in the telophase of that division. Lenhossék asserted (in case of the rat and guinea-pig) the persistence of the chromatoid body (or bodies) during both divisions though it may temporarily break up into a number of granules; and he also asserts its presence, "one in every spermatid.[1] In later stages it fragments and disappears, as was also observed by Niessing. Duesberg ('08) concluded that in the rat the chromatoid body (first seen in the second spermatocytes) divides into two, so that every spermatid receives one. Regaud ('10), on the other hand, found in the same object that during the first division this body (already present in the first spermatocyte) disappears, or perhaps fragments into small granules, but is reconstituted in the second spermatocyte to form a single body which passes undivided to one pole. This account implies that but half the spermatids receive such a body. In *Myxine*, the Schreiners found that a single chromatoid body is present in every spermatid and in later stages *passes into the nucleus* to form an integral part of the sperm (!) In respect to this point the Schreiners' account differs from those of all other observers.

Conclusion. In surveying the complicated phenomena of sperm-formation we are impressed alike by their astonishing diversity in detail and by an underlying uniformity in respect to the most fundamental features of the process. In both respects the sperm-cell is comparable with the flagellated Protista, with which it shows so far-reaching an analogy. The fundamental uniformities of the sperm-formation have been sufficiently emphasized, particularly in respect to the nucleus, chondriosomes, central bodies, Golgi-bodies and the acrosome. Its diversities and their physiological meaning may serve to remind us of our ignorance of an immense field of inquiry in the exploitation of which cytology has made a mere beginning.

In a broad view of the phenomena, what most arrests our attention is the large measure in which the sperm-forming materials are already preformed in the spermatocytes to be distributed to the spermatids by the spermatocyte-divisions with at least approximate equality. This applies alike to the chromosomes, central bodies, chondriosomes and Golgi-bodies. When we consider how recent is our acquaintance with these facts, we cannot help suspecting that there may be still other elements of equal importance, as yet unseen, that may be similarly distributed. The possible larger significance of this will become apparent in the course of the discussion of cell-organization in general offered in Chapter IX.

[1] '98, 272.

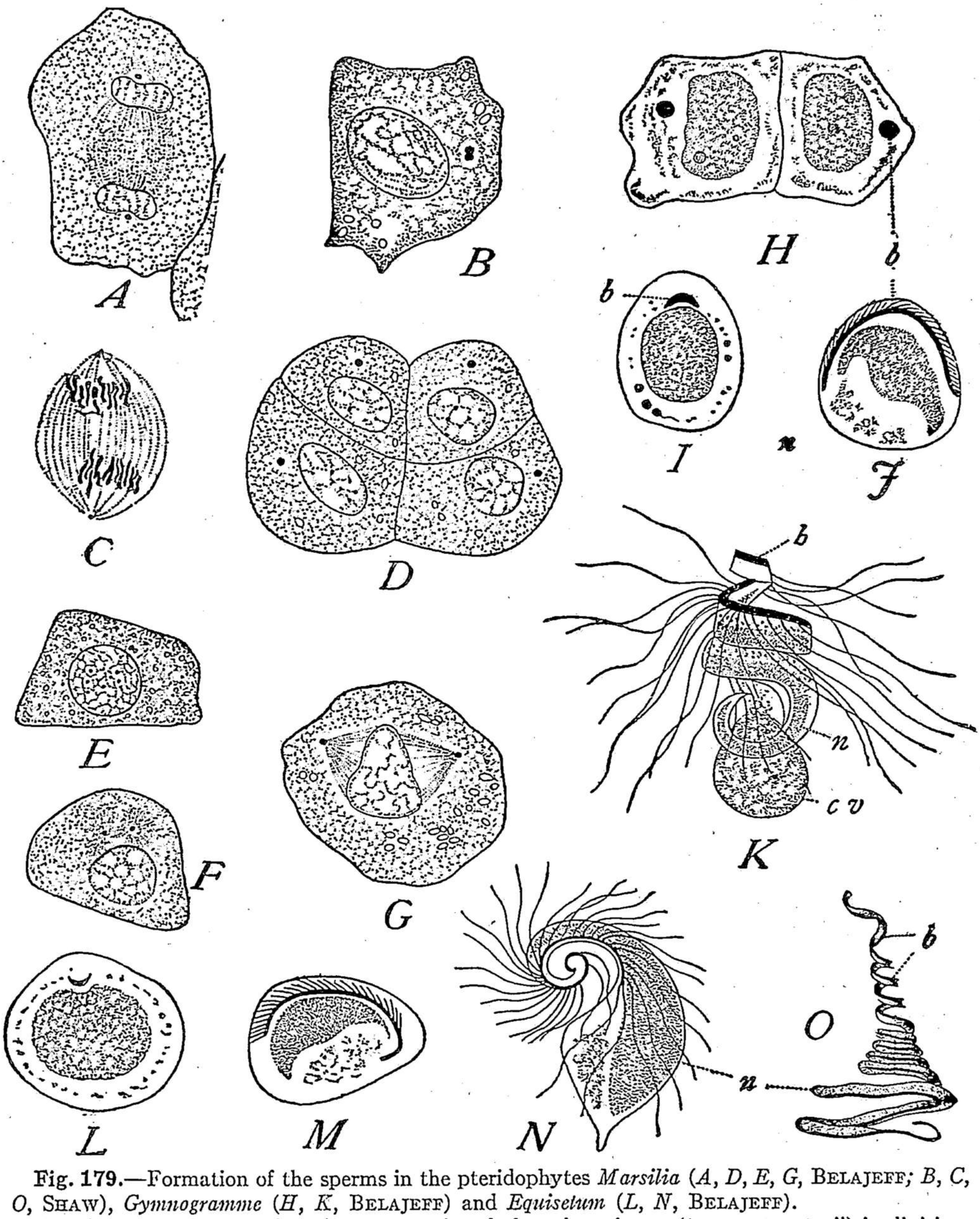

Fig. 179.—Formation of the sperms in the pteridophytes *Marsilia* (*A*, *D*, *E*, *G*, BELAJEFF; *B*, *C*, *O*, SHAW), *Gymnogramme* (*H*, *K*, BELAJEFF) and *Equisetum* (*L*, *N*, BELAJEFF).

A, primary spermatogonium (two generations before the primary "spermatocytes") in division, showing centrioles; *B*, primary spermatocyte with pair of "blepharoplastoids" (centrioles); *C*, spindle of primary spermatocyte (first maturation-division); *D*, four of the eight secondary "spermatocytes" with blepharoplast; *E–G*, prophase of second division; *H*, pair of spermatids (*Gymnogramme*) with blepharoplasts; *I–J*, formation of the ciliated band from the blepharoplast; *K*, nearly ripe sperm, showing ciliated band (*b*), nucleus, and "cytoplasmic vesicle" (the latter is ultimately cast off); *L*, *M*, spermatids of *Equisetum;* *N*, ripe spermatozoid from above, showing spiral ciliated band; *O*, ripe sperm of *Marsilia* with very long spiral ciliated band.

IV. THE GAMETE FORMATION IN PLANTS

As earlier indicated, the gamete-formation of higher plants offers a somewhat simpler problem than that of animals, because it is uncomplicated by the phenomena of meiosis which here take place at a different period of the life-history. Apart from this the process shows a fundamental agreement with that of animals, though the analysis of its details thus far remains much less complete. The phenomena can here be examined in only a cursory manner, especially with reference to the motile type of sperm-cells in higher forms.

As in animals the final generation of spermatogenous cells may be called spermatids, but spermatocytes, in the sense in which this term is employed

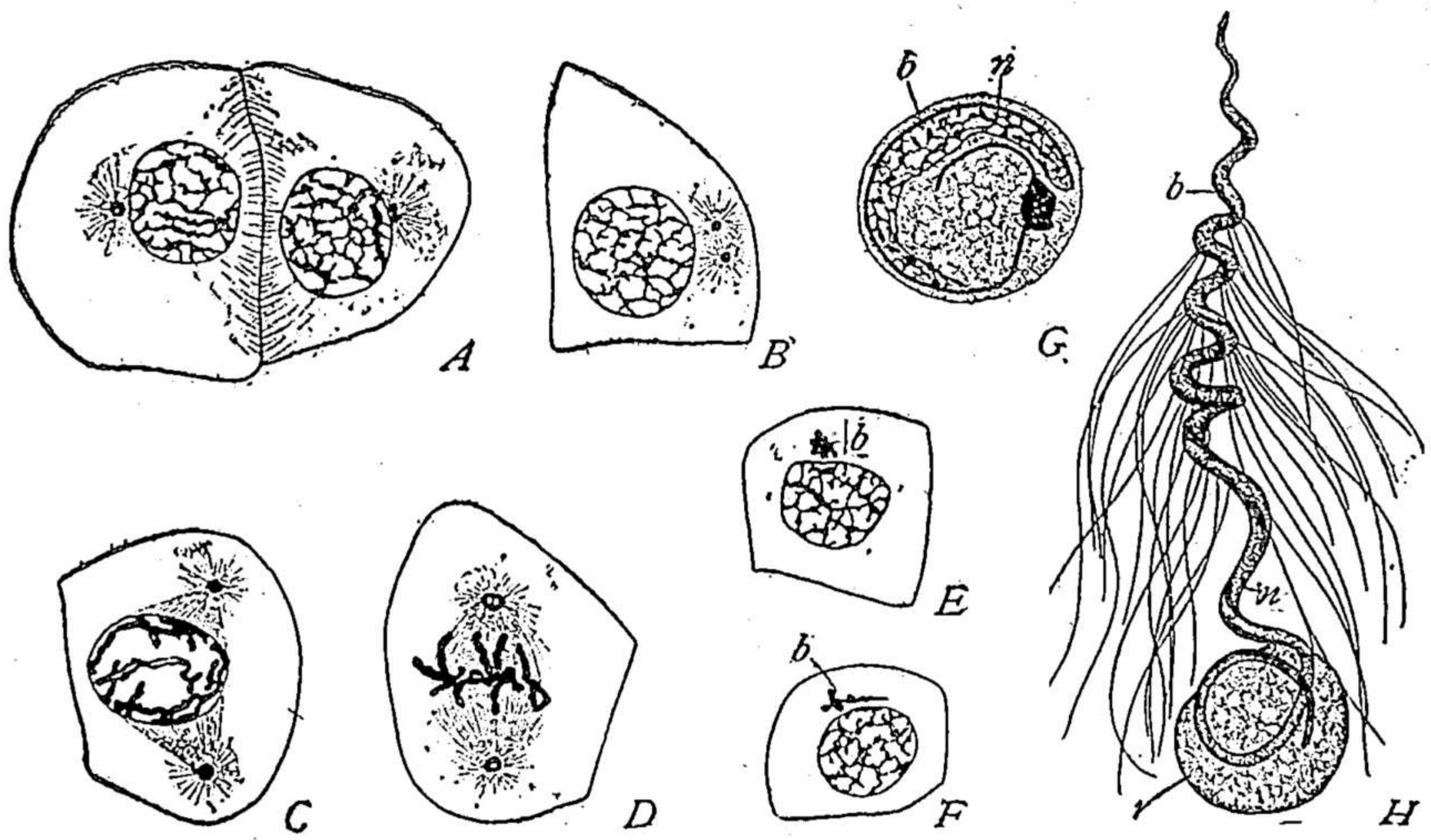

Fig. 180.—Spermatogenesis in *Marsilia* (SHARP).

A, products of third spermatogenous mitosis; *B*, *C*, *D*, stages of fourth and last spermatogenous mitosis; *E*, *F*, spermatids; *H*, mature sperm-cell, partly uncoiled, showing nucleus (*n*), much elongated blepharoplast (*b*), and residual protoplasm (*r*).

in animal spermatogenesis, do not occur in plants, being represented rather by the *sporocytes* which precede the formation of the microspores (p. 496).[1] In all the sperm-producing divisions, accordingly, the mitoses are of the ordinary somatic type so far as the chromosomes are concerned. It is an interesting fact that *in these mitoses the acromatic figure often shows at the spindle-poles distinct central bodies, sometimes surrounded by conspicuous asters, which ultimately play the part of blepharoplasts from which the flagella or cilia grow forth* (Figs. 179, 180, 182). Recent studies have further demonstrated, in a few cases, that in addition to the central bodies the spermatids and sperms of plants receive also chondriosomes and probably an

[1] By Allen '12, ('17) the spermatids as above defined are called *androcytes*.

acroblast closely analogous to that of the animal spermatid, though the Golgi-bodies have not yet actually been identified.

The relation of the blepharoplasts to the central bodies in plants, first indicated by the work of Belajeff, Shaw, Webber, and others, was clearly established by subsequent observations.[1] The blepharoplasts of the spermatids have been traced beyond a doubt to a position at or near the spindle-poles of the last division by many observers beginning with Shaw, Belajeff and Webber. Many botanists, including those just mentioned, have hesitated to recognize their homology with the central bodies in the animal spermatid, for the following reasons. In all the forms here in question the ordinary somatic mitoses, and often also the earlier spermatogenous, are of anastral type and devoid of central bodies (p. 150). The latter, as seen in the spermatid-producing division, must therefore be new formations to which the law of strict genetic continuity seems not to apply. In the cycads, as shown especially by Webber, they seem to play no actual part in the division, but merely lie opposite the spindle-poles without direct connection with them. In the cycads and some of the ferns, they seem to arise separately, either in the spermatids or in the preceding cell-generation, not by the division of a single body as is typically the case with true central bodies.

The force of these objections is much weakened by the discovery that in many cases the blepharoplasts are in fact produced by the division of a single body which behaves in all respects as a central body; further, that such bodies are sometimes present in earlier generations of the spermatogenous cells. Shaw ('98) found central bodies at the spindle-poles in the last two divisions of *Marsilia*, and followed their division in the telophases of the penultimate division to form the centers of the last division while Belajeff ('99) and Sharp ('14) found such bodies, surrounded by astral rays, at the spindle-poles in all of the four spermatogenous divisions excepting the first. Belajeff also believed that these centers divided after each mitosis to form the centers for the ensuing mitosis. Sharp ('12) demonstrated the division of the center in the prophase of the last spermatogenous mitosis in *Marsilia* and its behavior as a typical central body in that mitosis (Fig. 180); and the same was also shown by Allen ('12) in the moss *Polytrichum*. Ikeno ('03) found (in *Marchantia*) central bodies at the spindle-poles in each spermatogenous division, dividing into two at the close of each mitosis and after

[1] The initial work on this subject was done by Guignard ('89) on certain mosses, ferns and algæ, extended by that of Belajeff ('92, '94, '97, '99) on *Chara* and the pteridophytes *Gymnogramma*, *Equisetum* and *Marsilia*. To the same period belongs the important work of Shaw ('97, '98) on *Marsilia* and the ferns; of Webber ('97, '01) and Ikeno ('98) on cycads, and of Hirase ('94, '98) and Fujii ('98, '99, '00) on *Ginkgo*. See also Ikeno ('03, '04, '06, *Marchantia*, *Cycas*), Miyake ('06, *Ginkgo*), Escoyez ('07, *Marchantia*), Caldwell ('07, *Microcycas*), Yamanouchi ('08, *Nephrodium*), Chamberlain ('09, *Dioön*), Allen ('12, '17, *Polytrichum*), Sharp ('12, '14, '20, *Equisetum*, *Marsilia*, *Blasia*).

For literature see Wilson ('00), Prenant ('09), Allen ('12), Sharp ('12, '14, '21), Meves ('18).

the last one persisting to form the blepharoplasts. In view of these facts and the later history of these bodies it is impossible to doubt the correctness of the view, early urged especially by Belajeff and Ikeno, that the blepharoplasts of higher plants are of the same nature, morphologically and physiologically, as those of animal sperms. The only doubts that can arise relate

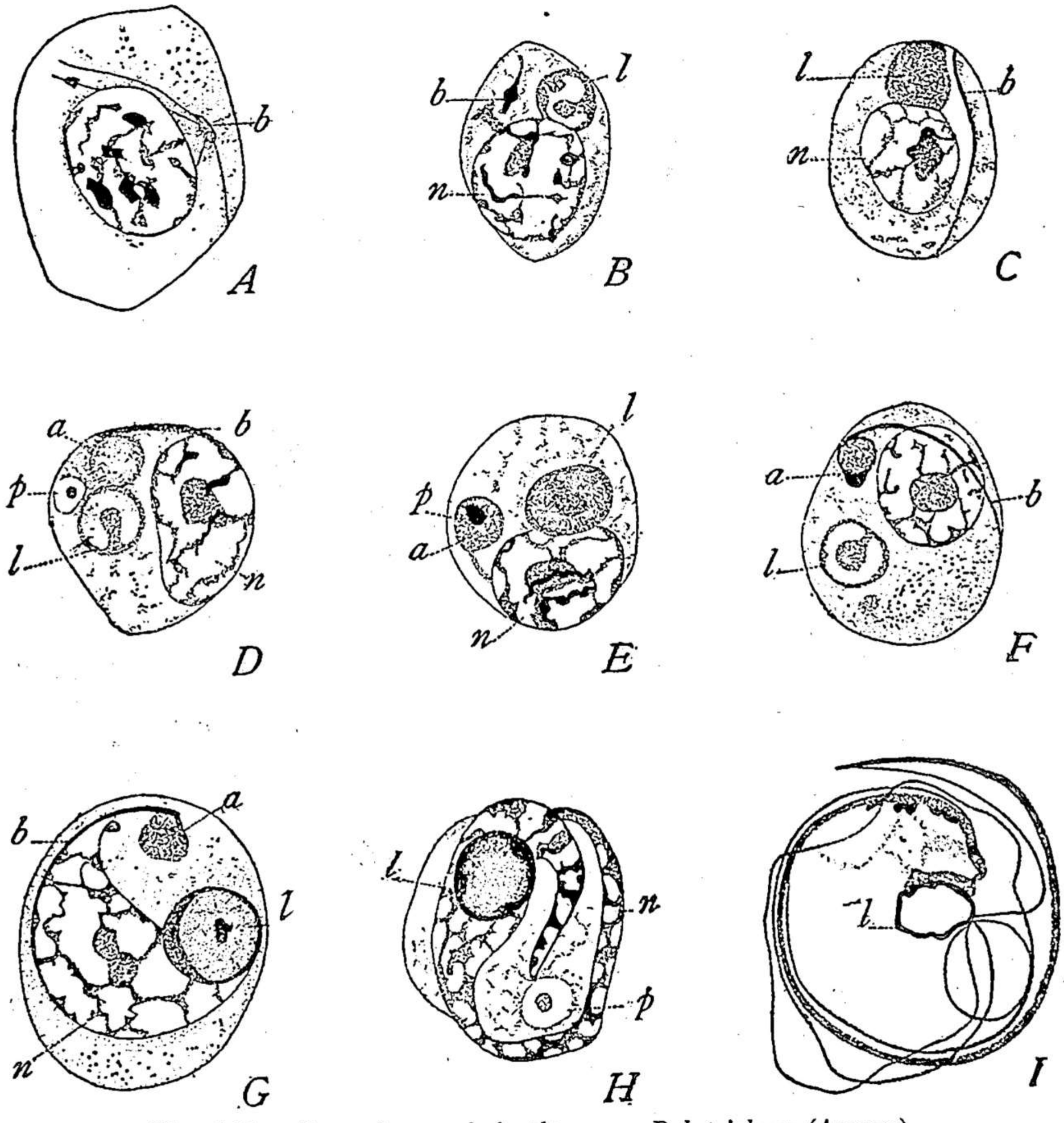

Fig. 181.—Spermiogenesis in the moss *Polytrichum* (ALLEN).

a, the apical body; *b*, blepharoplast; *l*, limosphere (probably the Golgi-apparatus or Golgi-remnant); *n*, nucleus; *p*, probably the percnosome, which may be equivalent to the acrosome-granule.

A, androcyte (spermatid) with nucleus and blepharoplast; *B*, appearance of the limosphere, elongation of blepharoplast; *C*, further elongation of blepharoplast; *D*, the apical body (*a*) has separated from the limosphere; *E*, *F*, slightly later; *G*, nucleus beginning to elongate; *H*, later stage (blepharoplast not shown); *I*, sperm fully formed but still coiled within its enclosing vesicle of residual cytoplasm, *l*, probably the limosphere.

to the nature of the central body itself and its proper definition in the light of these facts (p. 672).

In the early spermatid the blepharoplast is in most cases, as in animals, a small spheroidal granule; but in the cycads it is a much larger spheroidal body which has a complicated structure and metamorphosis, and is sur-

rounded by conspicuous astral rays (Fig. 182). In all the higher forms, the blepharoplast, or the group of granules produced by its fragmentation, grows out into an elongate flattened band or ribbon, at first coiled within the cell, from which grow forth the cilia or flagella (Figs. 179, 182). In later stages it uncoils more or less, but seems always to retain its spiral course in some degree, in general following the spiral coiling of the cytosome so con-

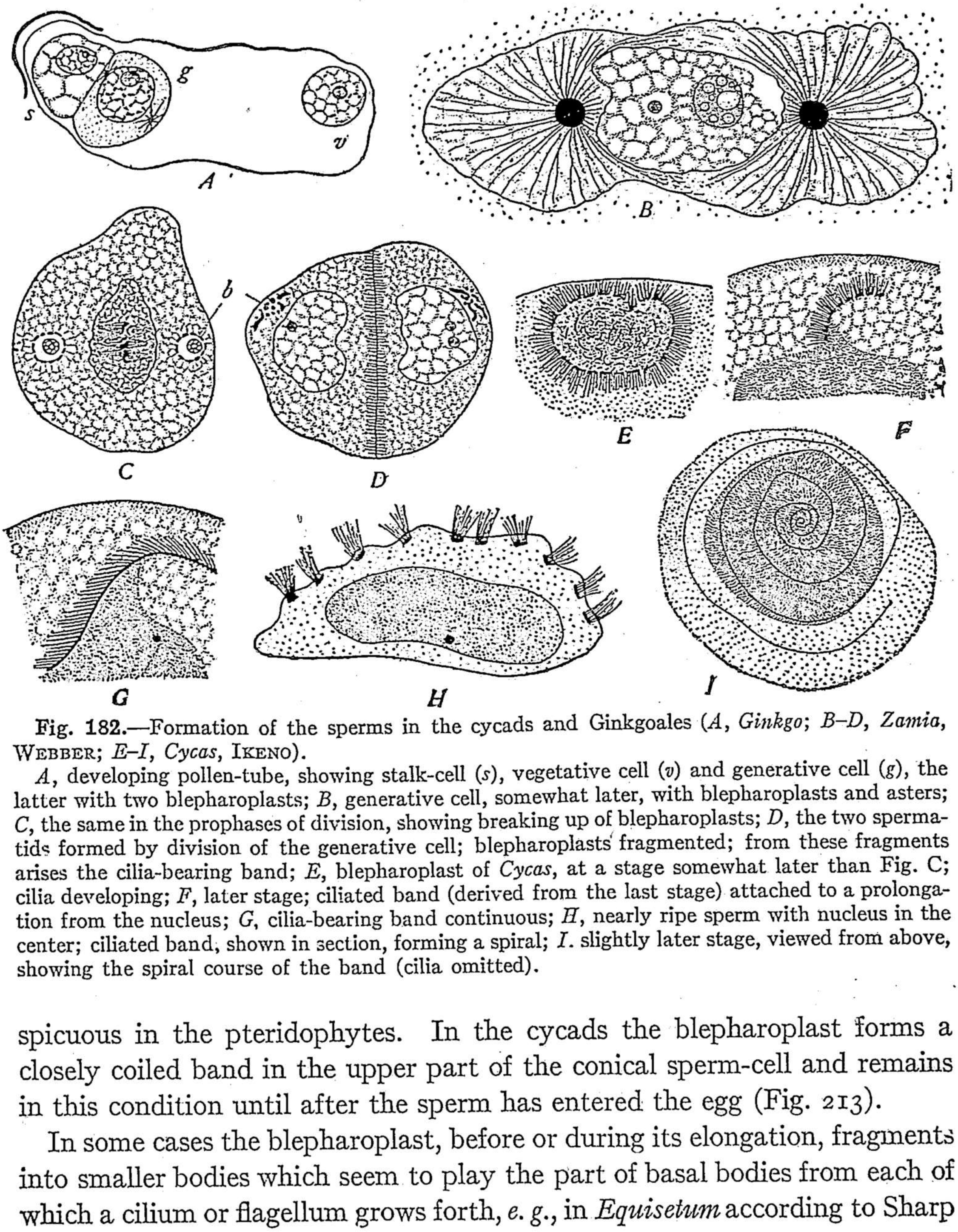

Fig. 182.—Formation of the sperms in the cycads and Ginkgoales (*A*, *Ginkgo*; *B–D*, *Zamia*, WEBBER; *E–I*, *Cycas*, IKENO).

A, developing pollen-tube, showing stalk-cell (*s*), vegetative cell (*v*) and generative cell (*g*), the latter with two blepharoplasts; *B*, generative cell, somewhat later, with blepharoplasts and asters; *C*, the same in the prophases of division, showing breaking up of blepharoplasts; *D*, the two spermatids formed by division of the generative cell; blepharoplasts fragmented; from these fragments arises the cilia-bearing band; *E*, blepharoplast of *Cycas*, at a stage somewhat later than Fig. C; cilia developing; *F*, later stage; ciliated band (derived from the last stage) attached to a prolongation from the nucleus; *G*, cilia-bearing band continuous; *H*, nearly ripe sperm with nucleus in the center; ciliated band, shown in section, forming a spiral; *I*. slightly later stage, viewed from above, showing the spiral course of the band (cilia omitted).

spicuous in the pteridophytes. In the cycads the blepharoplast forms a closely coiled band in the upper part of the conical sperm-cell and remains in this condition until after the sperm has entered the egg (Fig. 213).

In some cases the blepharoplast, before or during its elongation, fragments into smaller bodies which seem to play the part of basal bodies from each of which a cilium or flagellum grows forth, *e. g.*, in *Equisetum* according to Sharp

('12) and in the cycads according to Webber, Ikeno and other observers. In the bryophytes and certain pteridophytes, but two flagella are thus produced, though the blepharoplast is much elongated. In *Chara*, which has a similar type of sperm, each flagellum is attached basally to a short, rod-shaped body (Meves '20). In most pteridophytes and in cycads numerous and usually closely crowded cilia grow forth along the course of an elongated spiral blepharoplast (Figs. 179–182), which in many cases seems, at least in its later stages, to be quite homogeneous.

The remaining components of the sperm-cells have been very incompletely traced. Neither chondriosomes nor Golgi-bodies as such have been identified during the spermiogenesis, but, as earlier stated, Meves ('20) has found chondriosomes in the mature sperms of *Fucus* (p. 594); and in the gymnosperms the microgametes probably contain plastids which may be derivatives of chondriosomes (p. 455). In the spermiogenesis of mosses appears a body that seems almost certainly to be the acroblast, though the Golgi-bodies are not in evidence, probably because of inadequate technique. This is a rather large, spheroidal body, of uncertain origin, described by M. Wilson ('11) as the *limosphere* and more carefully examined by Allen ('12, '17). It lies near the anterior end of the elongate blepharoplast (Fig. 181), and divides into two unequal parts, of which the smaller gives rise to an "apical body" while the latter persists until a rather late stage when it disappears from view. It seems very probable that the two portions into which the limosphere divides represent the acroblast and the acrosome and acroblast-remnant respectively; but this cannot be positively asserted without further examination. Wilson described also a smaller cytoplasmic *percnosome*, which may be a chromatoid body; but this, too, is uncertain.[1]

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(See also V, VI, XI, XIII, XIV. For abbreviations see General Literature List.

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[1] Allen compares the limosphere to the "chromatoider Nebenkörper" (chromatoid body?) found by Ikeno ('03) in *Marchantia*.

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