CHAPTER VII

REPRODUCTION AND SEXUALITY IN LOWER ORGANISMS

GENEALOGICAL CONSIDERATIONS ON SYNGAMY AND MEIOSIS

"There is in Protozoa only one kind of reproduction, namely, cell-division."

R. HERTWIG.[1]

We are accustomed to think of sexuality as forming an integral part of reproduction; and such, in fact, it has become in all higher animals. But already in the higher invertebrates, such as insects and crustaceans, and in almost all plants, we find asexual forms of reproduction as well as sexual; while in the *Protista* sexuality and reproduction appear as quite distinct, and in some respects opposite processes. The unicellular protistan has but one mode of multiplication, cell-division, in itself a purely asexual process; and the immediate effect of syngamy, obviously, is not to increase but to decrease the number of cells. The most that can be said is that syngamy in *Protista*, as in higher forms, may initiate a new cycle of growth and cell-division. The widespread occurrence of syngamy in unicellular organisms, and its evident similarity to that seen in higher forms, might be expected to throw light on the sexual processes of higher forms; and to a certain extent this is true. In practice, however, the process in Protista offers many difficulties, which often compel us to interpret the observed phenomena in a measure deductively in the light of our knowledge of higher forms.

In the Bacteria, Cyanophyceæ, and certain other low forms no sexual process has thus far been made known; but most of the main groups of Protista exhibit a process of syngamy effected by a union of gametes that show many degrees of differentiation and in some cases are as unlike as in higher plants and animals. The correlative process of meiosis likewise seems, in some Protista at least, to parallel very closely that seen in higher groups (p. 597). Finally, many of the Protista exhibit an alternation of sexual and asexual cycles analogous to the alternation of generations seen in higher organisms, though it is still unknown whether it is of the antithetic type, i. e., an alternation between haploid and diploid forms.

The Protista have undergone innumerable modifications due to parasitism, degeneration, special adaptation and the like; and it is as difficult here to

[1] Arch. Protistenk., I.

distinguish between primitive and highly modified conditions as in higher forms. Nevertheless some of the sexual phenomena in Protista are evidently of simpler and more primitive type than those seen in higher forms, and in some measure help us towards a better understanding of the latter. Only a few illustrative facts can here be indicated; a full account of them would require a large volume.[1]

I. GENERAL SURVEY. TERMINOLOGY

Among unicellular organisms syngamy is accomplished by a process of *conjugation* in the course of which two cells unite, their nuclei fusing to form the nucleus of a zygote which may be considered as the starting point for a new cycle of growth and cell-division, as in multicellular forms. In the latter the cells belonging to each cycle combine to form a multicellular body; in the Protista they remain separate (though they may temporarily form colonies) each to lead an independent life as a physiological individual. *Morphologically*, therefore, the multicellular body may be compared to a succession or cycle of individual Protista. Physiologically the single protozoan is as truly an individual as the metazoan; but morphologically it is comparable both in structure and mode of origin to a single tissue-cell, or a single germ-cell, of a metazoan (pp. 101, 238).[2]

1. Types of Conjugation

In most Protista union of the gametes is complete and permanent, nuclear fusion or *karyogamy* being accompanied by complete protoplasmic fusion or *plastogamy*. In the ciliates, however, complete plastogamy does not take place, the two conjugants merely becoming temporarily united and undergoing an exchange of nuclei. Following the terminology of Bütschli these two types of syngamy are commonly designated respectively as "copulation" and "conjugation"; but it seems preferable to employ the self-explanatory terms *total* and *partial* conjugation. Conjugation is often exogamous, *i. e.*, takes place only between gametes of different ancestry; but there are many exceptions to this, and it is probable that strict exogamy is only one extreme of a series of conditions leading to *endogamy* and finally to *autogamy*. In *Paramœcium aurelia* Calkins ('02) found that descendants of the same cell not more than eight or nine divisions removed would con-

---

[1] See especially the general treatises of Calkins ('01, '09), Minchin ('12), Doflein ('16), West ('16), Oltmanns ('22, '23).

[2] This comparison, due primarily to Siebold (1845), was first clearly stated with reference to the reproductive cycle by Bütschli in 1876 (*cf.* p. 238). It has been widely accepted by modern students of protistology, for instance, by R. Hertwig, Schaudinn, Lang, Doflein, Calkins, Minchin and others, but has also encountered energetic opposition on the part of some writers, *e. g.*, A. Sedgwick ('94), Awerinzew ('10), Franz ('11), Dobell ('11, '14).

jugate (endogamy) and the offspring from such a zygote were able to live through a complete cycle of 379 bipartitions.[1] Carried to an extreme, endogamy becomes *autogamy*, in which the gametes are sister-cells, resulting from the division of a single mother-cell (*e. g.*, in *Actinophrys* or *Actinosphærium*, Fig. 282). In such cases the sister-cells (or nuclei) are known in some cases to undergo a process of meiosis before their reunion (p. 597); the zygote-nucleus, therefore, is not identical with the original mother-nucleus, having undergone a process of reorganization presumably involving a recombination of nuclear elements. As Hartmann has indicated ('09) autogamous conjugation is probably a secondary mode of union, possibly even a degenerate one, the primitive type having been exogamous.

2. Relation of the Gametes to the Vegetative Cells

In considering the structure of the gametes and their relation to the vegetative cells we find it necessary to enlarge the meaning of the term "gamete" as heretofore employed. In case of higher organisms generally we are accustomed to restrict this term to the germ-cells after completion of the meiotic or maturation process; but even here the sperm often enters before the egg has completed the process of meiosis and while it is technically a *gametocyte* (oöcyte). In the Protista, and even in some higher forms, the same is often true of both conjugants (*e. g.*, in ciliates), syngamy and meiosis being more closely associated than in higher forms. If then, we insist on restricting the term "gamete" to haploid conjugants we are driven to the conclusion that in some of the most familiar examples of conjugation true gametes do not exist. In this dilemma it seems preferable to adopt a more elastic usage, designating the conjugating cells as "gametes" whenever we find it convenient to do so, even at some cost of consistency. The difficulty, however, may be avoided by employing the word "conjugant."

In respect to the character of the conjugants, three main types may conveniently be distinguished among Protista:

a. *Hologamy* or *Macrogamy*. In this case the conjugants are of the same structural type as the original vegetative cells, and do not differ markedly from them in size or external appearance. This condition is seen in various flagellates, rhizopods, ciliates, diatoms, desmids and the Conjugatæ generally. Like the vegetative cells the conjugants may be flagellated, ciliated, amœboid or non-motile, and are often indistinguishable in appearance from the corresponding vegetative cells. In most of these cases, the conjugants are isogamous (*e. g.*, in most diatoms, desmids, Zygnemaceæ, ciliate Infusoria), in others more or less unequal in size (anisogamy) though still

[1] Enriques ('08), states that ex-conjugants that have not yet divided may immediately proceed to conjugate again.

structurally alike. This condition is illustrated by certain species of *Spirogyra* (Fig. 283), or by the vorticellid Infusoria. In the latter case different species show anisogamy in various degrees, the microgamete or "male" being a smaller, free-swimming cell of the same general type as the larger attached macrogamete or "female," save that the stalk is absent (Fig. 304). Even when hologamous gametes are alike or closely similar in appearance
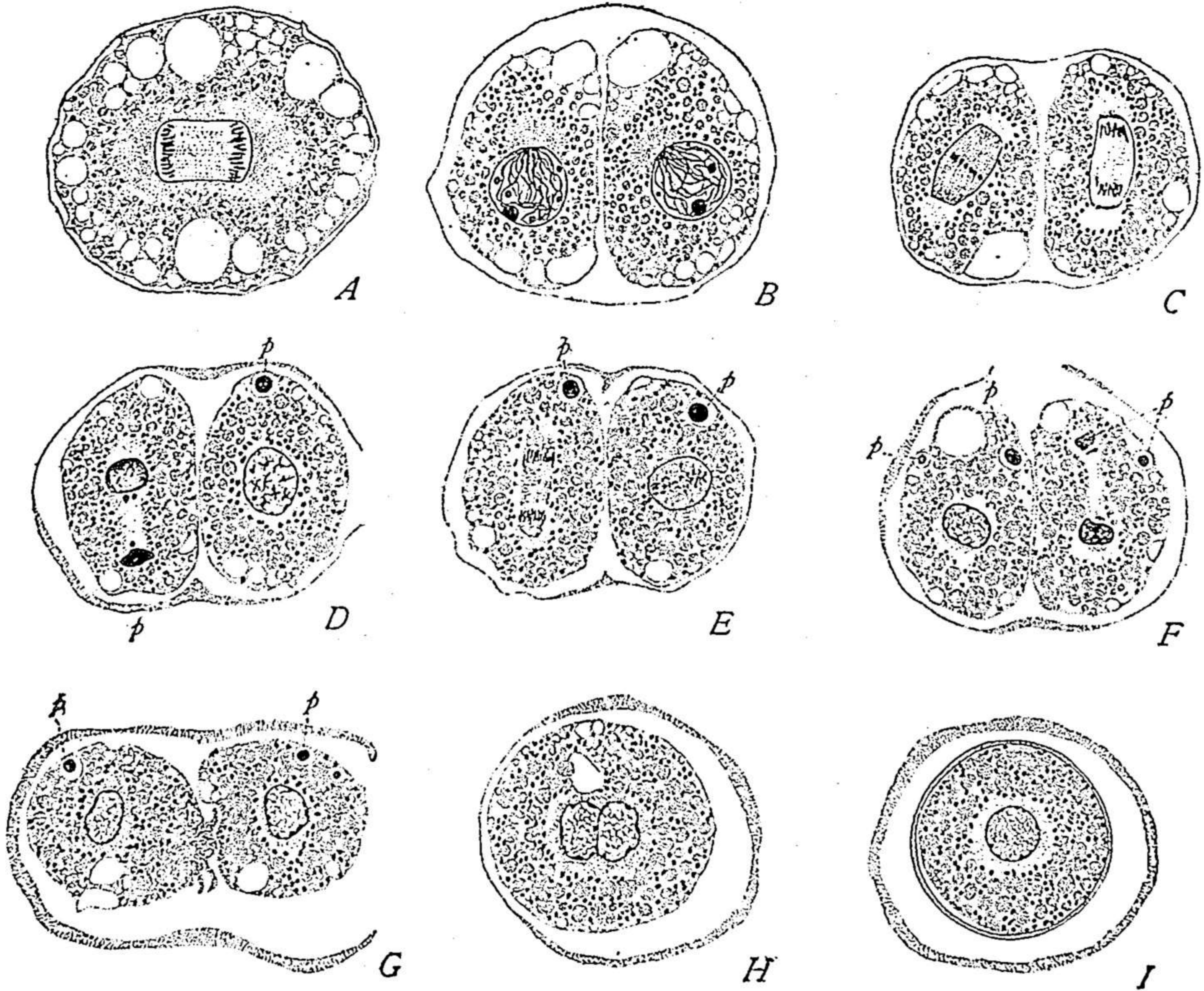

Fig. 282.—Gamete-formation, meiosis and autogamous conjugation in the rhizopod *Actinophrys* (Bĕlař).

A, the progamic mitosis, producing two diploid conjugants; *B*, the latter in the polarized synaptic stage; *C*, the first meiotic division, producing in each conjugant (*D*) the first polar nucleus (*p*); *E*, second division; *F*, the two resulting haploid gametes each with two polar nuclei; *G*, *H*, conjugation; *J*, resulting zygote.

they often display definite physiological differences that become evident at the time of conjugation (pp. 585, 586).

b. Merogamy or *Microgamy.* In this type, widely distributed among unicellular organisms and in lower plants, the gametes are much smaller than the vegetative cells, more or less widely different from them in structural type, and commonly motile. They arise from cells of the ordinary vegetative type by a rapid series of divisions, the size of the merogametes thus produced varying with the number of divisions. For instance, in the flagellate *Polytoma uvella* but two such divisions typically take place, giving

rise to four relatively large gametes (Dangeard); in *Sphærella* the mother-cell produces 32–64 much smaller gametes; while in such rhizopods as *Poly-stomella* or *Trichosphærium* the number of the division is still larger, and the gametes correspondingly more minute [1] (Fig. 284). In some cases they are amœboid (*Arcella, Centropyxis*, some *Sporozoa*); more commonly they are flagellated and actively free-swimming, a condition seen in Foraminifera (*Polystomella*), Radiolaria (*Collozoum*), and various other rhizopods; in certain flagellates (*Mastigella*); and in many of the green algæ (*Ulothrix, Cladophora, Pandorina*, etc.). Many of these cases seem to be perfectly isogamous (*e. g.*, in Foraminifera, *Ulothrix, Ulva*), but here, too, often are
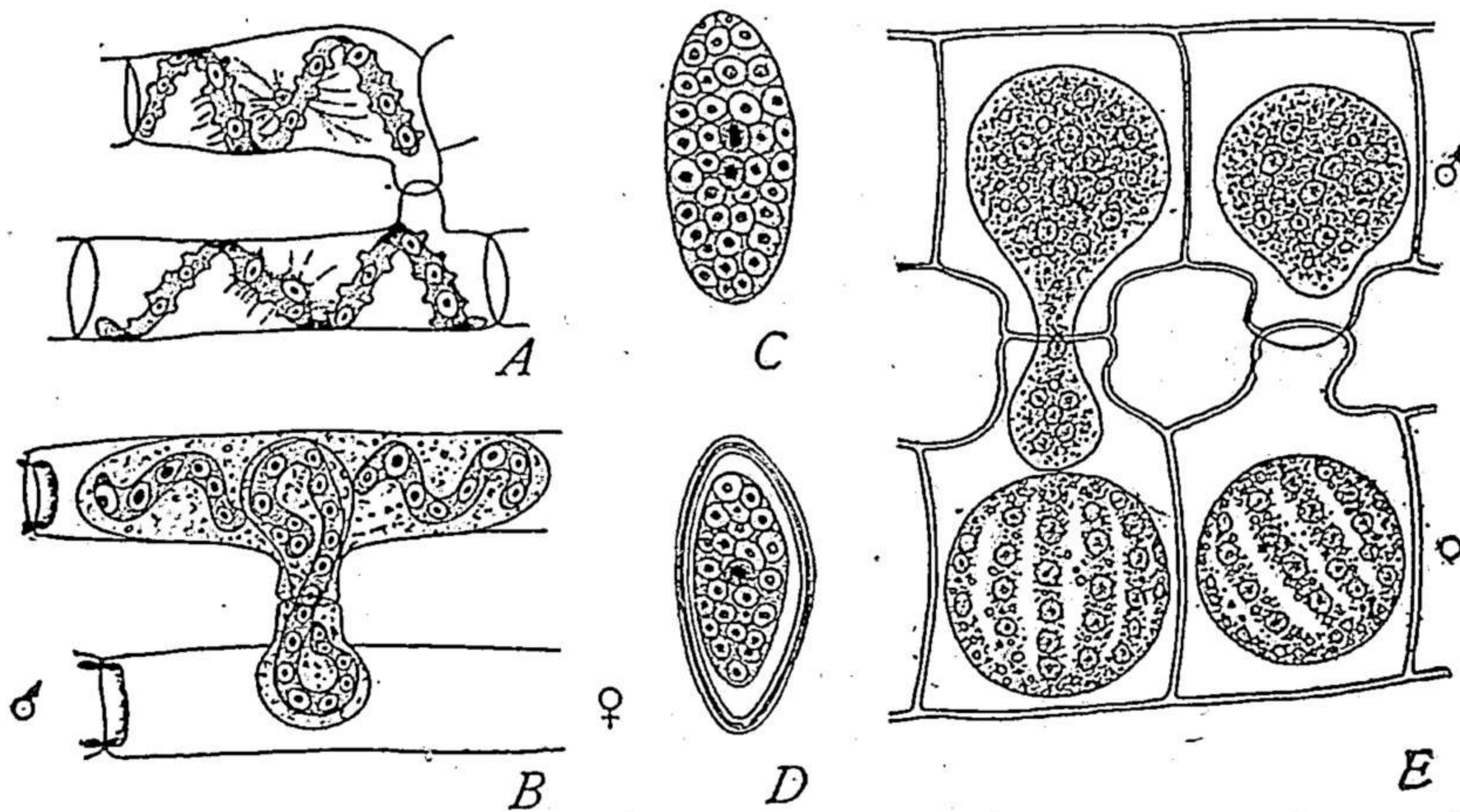

Fig. 283.—Conjugation in *Spirogyra* (*A–D* from OVERTON, *E* from OLTMANS). *A, B*, union of the gametes in *S. communis; C, D*, the resulting zygote; *E*, the gametes in *S. heeriana*.

physiologically different (p. 585). Others are anisogamous in various degrees, and may even vary within the limits of a single species. These differences no doubt depend in part on the number of divisions in the mother-cell; but may in part be due to variation in the size of the latter.

c. Heterogamy. A third type, connected with the foregoing by intermediate conditions, is true *heterogamy*, analogous to that seen in the higher forms. The gametes are here widely different from each other and often also from the vegetative cells, the macrogamete (now called the ovum) being of the hologamic type, often larger than the vegetative cells and almost always non-motile, while the sperm is merogamic, and typically has the form of a minute actively motile flagellated cell. In such cases the macrogametes are often called "female," the microgametes "male," but these terms are misleading, since even in higher forms it is inadvisable to speak of the gametes as possessing sex (p. 818). This condition is ex-

[1] In the algæ the size of the asexual zoöspores shows similar variations, due to the same cause, sometimes within a single species (*Ulothrix*).

emplified by such forms as *Œdogonium* (Fig. 306), *Volvox* (Fig. 291), *Vaucheria* or *Fucus*. It is not impossible, as Minchin has indicated, that the heterogamic type has arisen from the merogamic by "progressive, and finally complete, inhibition of the divisions that produce the swarm-spores in one sex — possibly also with an enhanced tendency to such divisions in the other sex " ('12, p. 172).

3. Physiological Differences of the Gametes

It is a fact of fundamental interest for the theory of sex that in many lower organisms definite physiological differences often exist between gametes
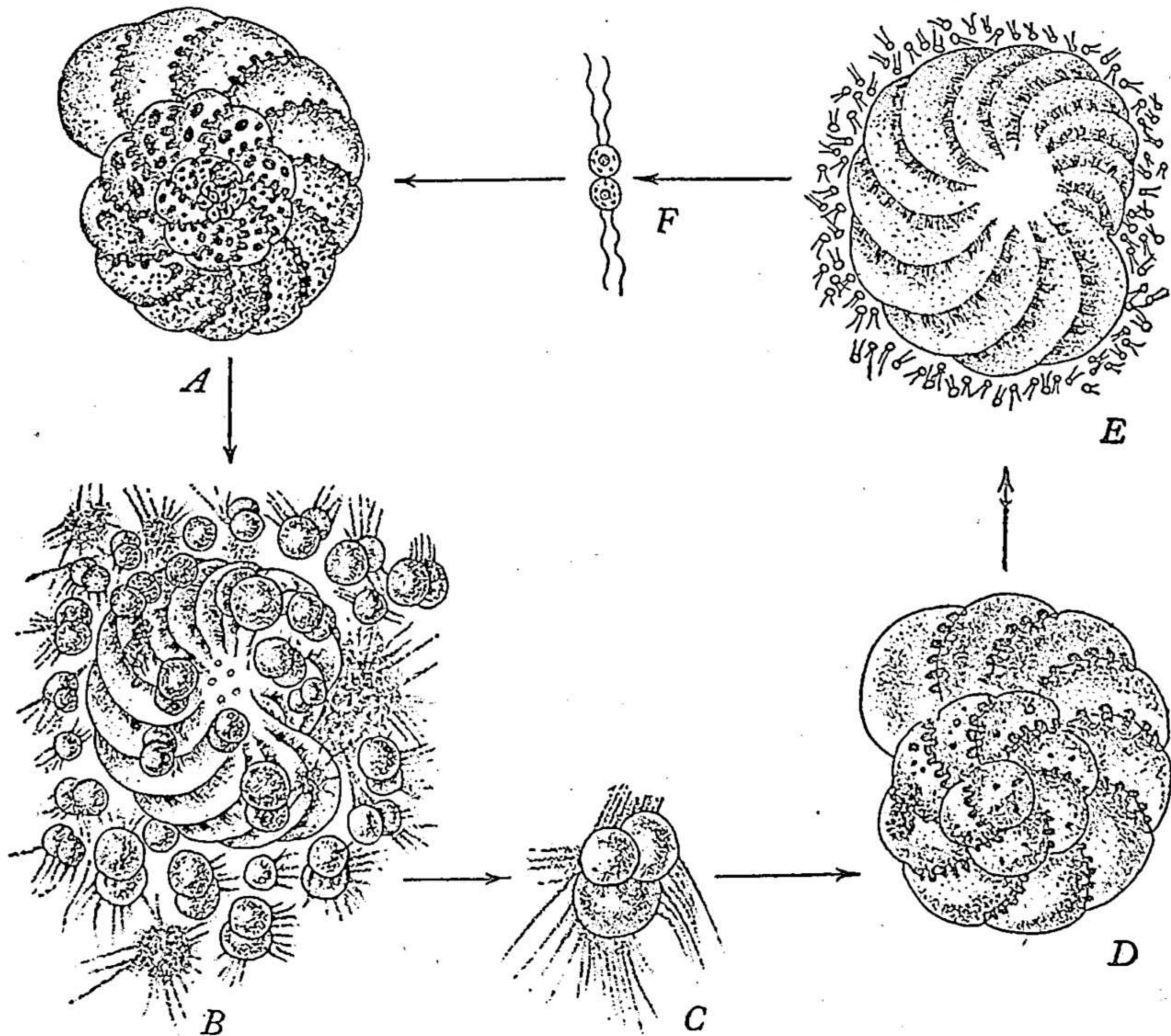

Fig. 284.—Life-cycle with alternation of generations in the rhizopod *Polystomella crispa* (from HARTMANN, after SCHAUDINN and LANG).

A, microspheric (asexual) generation or agamont; *B*, same, producing the asexual agametes, *C; D* macrospheric (sexual) generation or gamont, producing (*E*) biflagellate gametes; *F*, conjugation of the gametes.

which structurally, so far as we can see, are perfectly isogamous. This is seen in both exogamous and endogamous forms. An interesting example of the latter case is seen in the heliozoan rhizopod *Actinophrys*, in which the gametes are sister-cells. Bělař ('21, '23) has shown that although the two gametes are structurally alike one remains a passive rounded cell, while the other puts forth towards the passive gamete a group of pointed pseudopods which play the leading rôle in the subsequent fusion (Fig. 282).

In other isogamous forms exogamy seems to be strictly obligatory, *i. e.*, gametes from the same immediate source will not conjugate with one another, though they conjugate readily with gametes from a different source. For example, in the rhizopod *Polystomella*, a perfectly isogamous, merogamic form, Schaudinn states that only gametes of different parentage will conjugate with one another; and the same is true, according to Dodel and Klebs, of the isogamous flagellated gametes of the simple alga *Ulothrix* (Fig. 287).

Various interesting conditions exist among the green algæ and the fungi. In the group Zygnemaceæ exogamy is the rule, conjugation usually taking place between the cells of different filaments (Fig. 285, D); but there are im-- portant exceptions to this rule, and many gradations seem to exist in the physiological specification of the gametes. In some species of *Zygnema*, for example, they show no difference of structure or behavior, both gametes be- ing migratory and meeting halfway between the two conjugating filaments to form a zygote in the connecting tube (Fig. 285, A). In this case it is difficult to determine whether a physiological distinction of sex exists between the two filaments or the two gametes of each pair. That such a difference exists in other isogamous forms in this group is nevertheless certain. Many species of *Spirogyra* or *Zygnema* show definite physiological differences between the conjugating filaments, all the cells of the one being actively migratory or "male," those of the other passive, receptive, or "female" (Fig. 285). In these cases "male" filaments conjugate only with "female" and the fila- ments may appropriately be spoken of as diœcious. Most such species are morphologically isogamous, but in at least one case (*Spirogyra tenuissima*) a considerable degree of structural anisogamy is said to be recognizable, the male gamete being smaller than the female (Fig. 285, F). Some species that usually show a well-marked difference of sexual behavior now and then show "cross-conjugation," the direction of conjugation being reversed by certain pairs of gametes. In such cases (Fig. 285, E) the filament as a whole is physiologically to some extent bisexual and shows a certain analogy to the hermaphroditic condition of higher forms, though the cells may in- dividually be "male" or "female." Finally, at the opposite extreme from the exogamous forms are strictly endogamous species, such as *Spirogyra longata* (Fig. 285, C) in which the adjoining cells of the same filament unite by "lateral" conjugation, through a connecting tube formed between each pair, a condition which offers perhaps an analogy to the self-fertilizing types of hermaphrodites in certain higher plants and animals (*e. g.*, in some of the cestodes and nematodes).[1] In *Spirogyra inflata*, and a few others both

[1] Many hermaphrodites in higher forms are strictly exogamous. A remarkable example of this is offered by the ascidians, in which the eggs are fertilized in the sea-water and are infertile to sperms of the same individual though readily fertilizable by the sperms of any other individual (p. 421).

lateral and the ordinary or "scalariform" types of fertilization occur; and in this or a nearly related species Cunningham ('17) [1] found all three modes, the most common being the typical exogamous scalariform process, in which

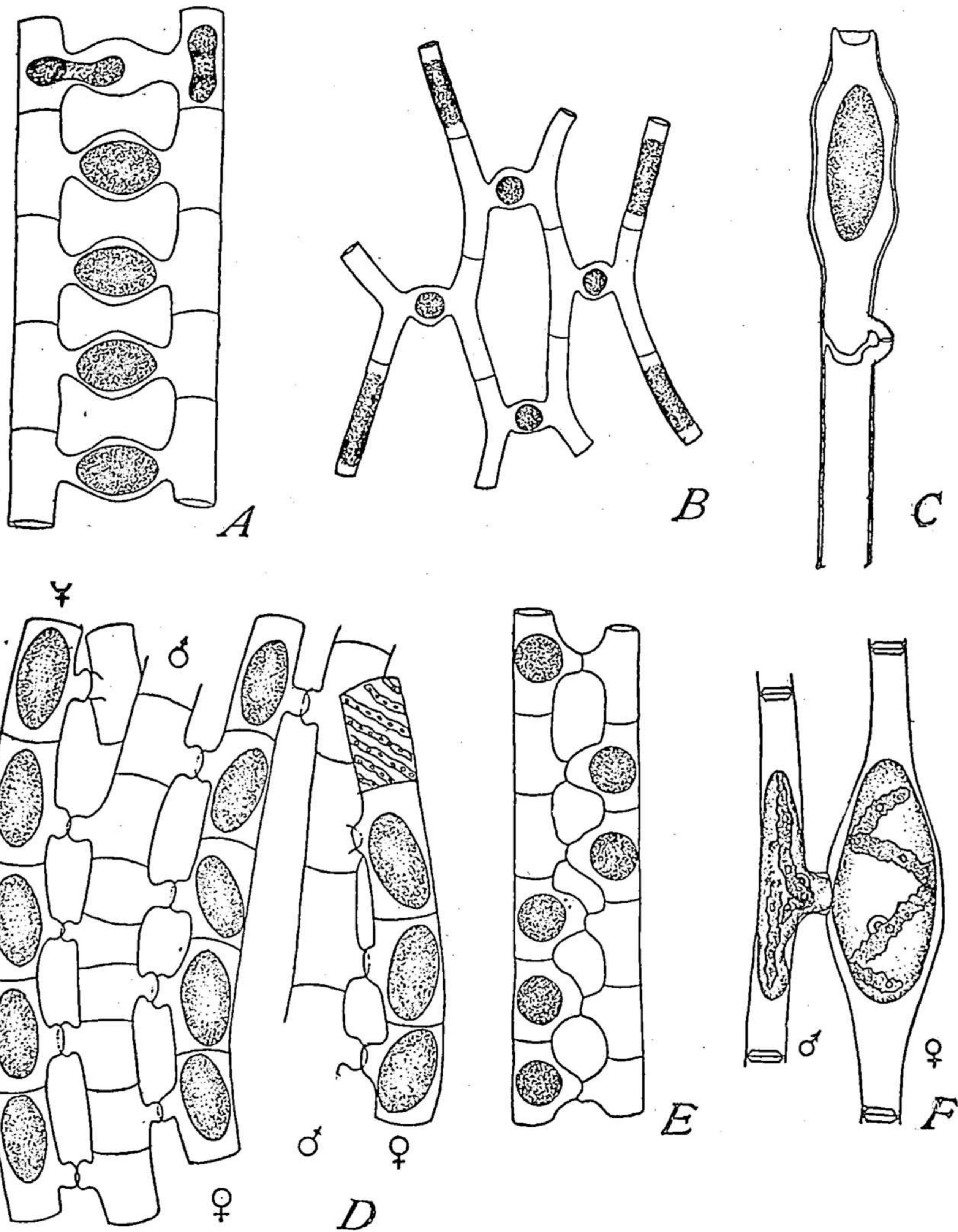

Fig. 285.—Sex-relations in the conjugate algæ. (A, B, C, and E from HASSALL, D, F, from WEST.)

A, *Zygnema stagnalis*, "scalariform" conjugation; B, *Debarya nummuloides* with several conjugating filaments; C, *Spirogyra quadratum*, with "lateral" conjugation; D, *Spirogyra nitida*, several conjugants showing sexuality of the filaments; E, *Zygnema insignis*, "cross-conjugation"; F, *Spirogyra tenuissima*, with slight anisogamy.

conjugation takes place in only one direction; but both lateral and cross-conjugation are occasionally found, sometimes in the same pair of filaments. These facts suggest that in this group of algæ as a whole the distinction

[1] This author has given a useful review and critique of the literature.

between the bisexual and unisexual condition, and possibly also that be-tween the sexes, has not yet become very firmly established.

Phenomena of analogous type have been made known by the illuminating researches of Blakeslee,[1] on the mucorine moulds (*Mucor, Rhizopus, Phycomyces*, etc.) which have recently been confirmed by a number of other observers both in the moulds and the smuts.[2] Most of these forms are structurally isogamous, but show physiologically quite definite sexual characteristics, some species being "heterothallic" (analogous to the dioe-cious or unisexual condition in higher forms) others "homothallic" or bisexual. In the homothallic forms the mycelia appear to be all sexually alike, and any two individuals, under suitable conditions, may conjugate together. In the heterothallic forms (*e. g., Mucor mucedo* or *Rhizopus nigricans*) the mycelia are of two kinds or strains analogous to male and fe-male. Blakeslee designates these as + and − forms, owing to the fact that in some species the + form has a somewhat more luxuriant growth than the −; though in others no visible difference between them appears. The two strains produce gametes structurally indistinguishable, but the + forms will conjugate only with − and *vice versa*, independently of the culture medium or other conditions of environment. If the two strains be kept separate and cultivated only by asexual sporangiospores they may be bred for an indefinite number of generations without conjugation and without modification of their sexual type; but when the two are finally brought together again they readily conjugate. The two strains must therefore be characterized by constant sexual differences, though often no morphological sign of them appears in the external aspect of the mycelia or the gametes which they produce.[3] We are here led to suspect that these differences may lie in the organization of the nuclei, such as are now known to exist be-tween the male-producing and female-producing gametes of many animals; and (in at least two cases) between the male-producing and female-pro-ducing spores of plants (p. 746). These differences are in some cases known to be quantitative (p. 812), which points to a similar type of sexual differ-ence in these lower plants. To a certain extent this is borne out by the experiments of Blakeslee and his collaborators on *Mucor* and *Cunning-hamella*[4] which indicate that the strength of the + and − sexual activity in different races varies markedly in degree. In *Cunninghamella* there appears to be a graded series in this respect, ranging from "sexually strong" races down to those that are "sexually weak," perhaps even

[1] '03, '04, '06, '15, '20, etc.
[2] See Burgeff ('14–'15) on *Phycomyces*, Kniep ('19) on *Ustilago*.
[3] *Cf.* Chapter X.
[4] Blakesley ('20), Blakeslee, Cartledge and Welch ('21).

to sexually neutral forms which thus far have shown no tendency to conjugate.[1]

4. Structure of the Gametes

Gametes of the hologamic type require no special description; they have in general the same structure—flagellated, amœboid, or non-motile—as the
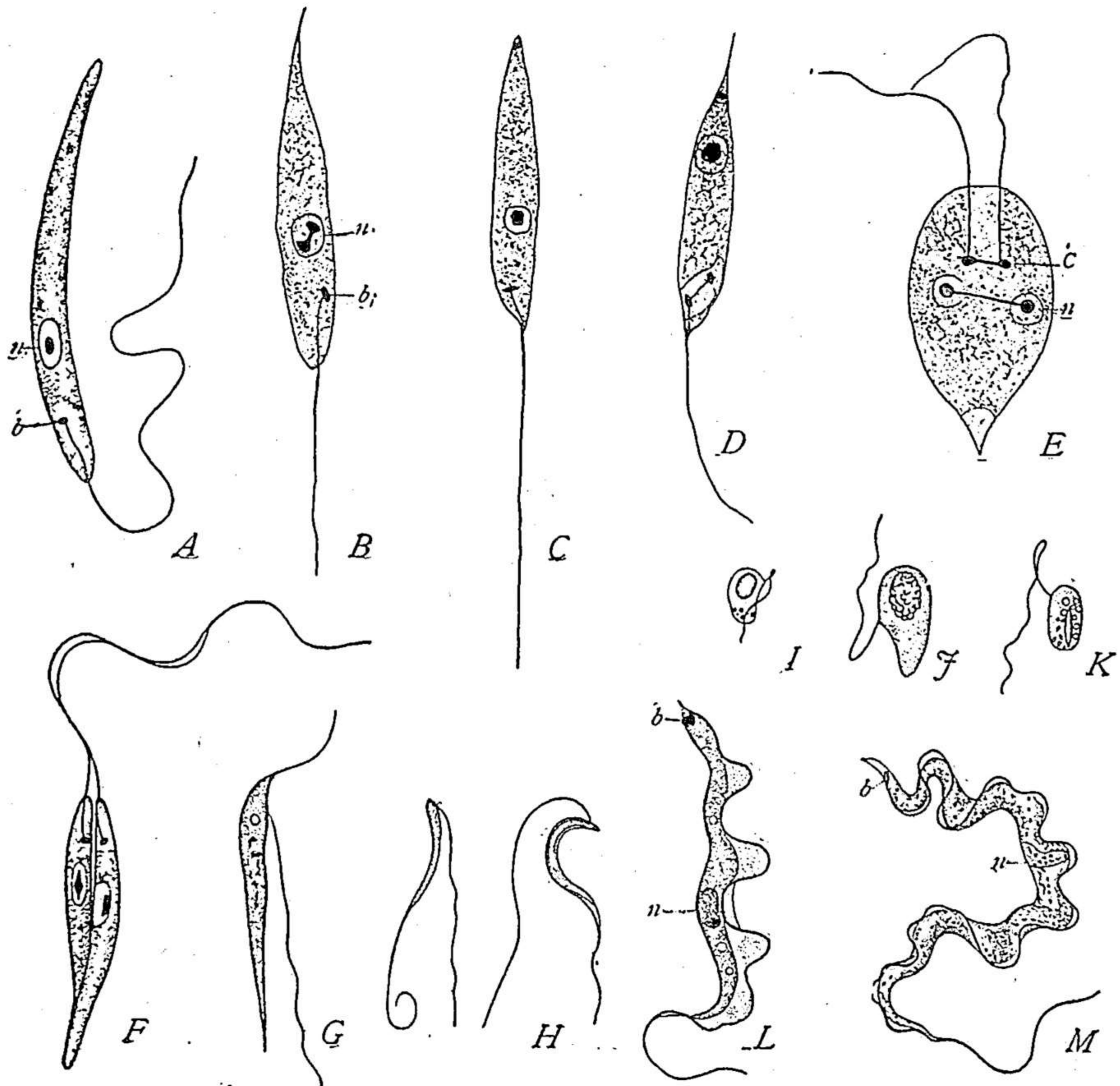

Fig. 286.—Some gametes and gamete-like flagellates.

A, Leptomonas jaculum (LÉGER); *B–E*, stages of longitudinal fission in *Leishmannia* (WENYON); *F*, final stage of fission in *Leptomonas* (LÉGER); *G, Bodo gracilis* (STEIN); *H*, microgametes of *Coccidium* (SCHAUDINN); *I*, microgametes of the radiolarian *Collozoum* (R. HERTWIG); *J*, macrogamete, and *K*, agamic swarmspore of same; *L, Trypanosoma tincæ* and *M, Trypanosoma granulosum* (MINCHIN).

vegetative cells of the species. The most interesting of such gametes occur in the flagellates; for the widespread occurrence of flagellated gametes in plants and animals generally leads to the conclusion that these probably

[1] Some observers have considered such gradations as sex-intergrades (*cf.* Burger, '19, etc.), but the work of Blakeslee and others indicates that they should not be so considered. There is, however, some evidence that true intergrades may exist in some of these fungi. (See Blakeslee, Cartledge and Welch, *op. cit.*)

represent the most primitive form; and many of the principal forms of flagellated sperms are paralleled with astonishing closeness among the adult forms of existing flagellates. The ordinary uniflagellated animal sperm is represented by various uniflagellated species, in particular by slender and elongated forms, such as *Leptomonas* or *Herpetomonas* (Fig. 286). Sperms that are provided with undulatory membranes, such as those of *Bombinator* or *Darwinia*, find their prototype in the trypanosomes (Fig. 286, L, M). Not less striking is the resemblance between the biflagellate sperms of thallophytes, bryophytes, certain platodes, the flagellated gametes of various rhizopods, and the biflagellated Protista. Gametes of this type are of two principal forms. In the more frequent both flagella are directed for-
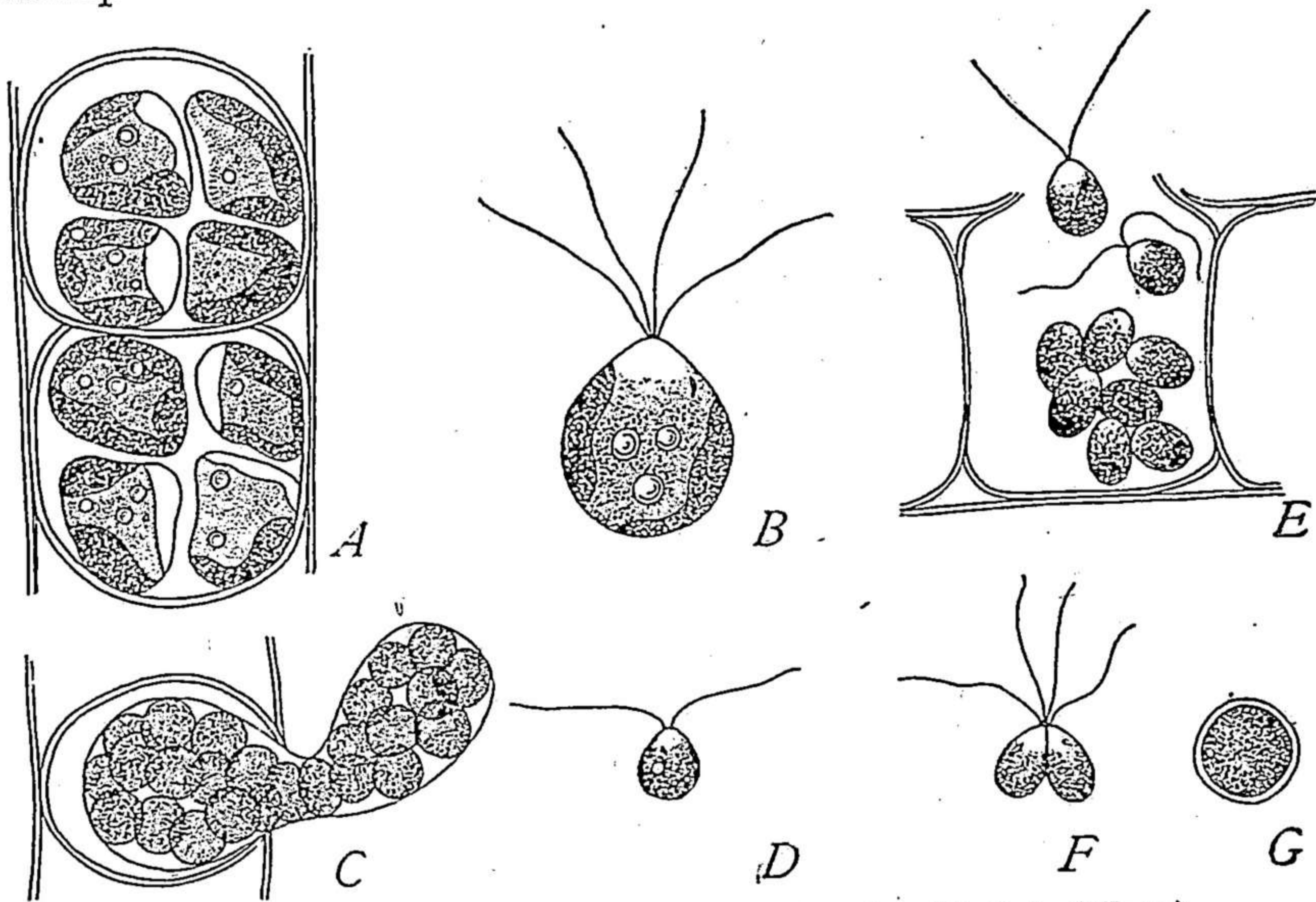

Fig. 287.—Gametes and zoöspores in the alga *Ulothrix* (WEST).

A, division of mother-cell to form the large quadriciliate zoöspores (macrogonidia) shown in *B*; C, division of mother-cell to form *D*, small, biciliate zoöspores (microgonidia); *E*, formation of the biciliate gametes; *F*, conjugation to form *G*, the zygote.

wards; this is seen in the rhizopods (*Polystomella, Trichosphærium, Paramœba*), in certain Sporozoa (*Aggregata*), and is widely prevalent in the green algæ (*Volvocaceæ, Ulothrix, Bryopsis*, etc.), where the gametes are closely similar to the sexual zoöspores or swarmers (Figs. 287, 290). In a second type, exemplified in various brown algæ (*Fucus, Cutleria*) one flagellum is directed forwards while the other trails behind (Fig. 311). Both these types also are paralleled by the adult or vegetative form of flagellates. The first type, with two forwards-directed flagella, is seen in such forms as *Chlamydomonas, Polytoma* or *Spongomonas* (four flagella are present in some forms, Figs. 290, 292). The second type, with one forward and one trailing flagellum is seen in *Bodo, Trypanoplasma,*

Trypanosoma, and their allies, and by a modification of this are formed such sperms as those of *Coccidium* (Fig. 286,H) with one anterior and one posterior flagellum. The resemblance is heightened by the fact that in flagellates generally the flagellum (or flagella) is attached at its base to a basal granule or *blepharoplast* from which it grows forth during its formation and which is, in some cases at least, identical with a central body and division-center (p. 690). The parallel between the flagellated gametes and the asexual swarmers or "zoöspores," which occur in certain rhizopods and Sporozoa and in many of the lower algæ, is even more striking; they possess, indeed, the same essential internal structure and may even be indistinguishable externally.[1] These resemblances point to the derivation of gametes from asexual zoöspores and more remotely from flagellated Protista. The most primitive forms, as has been indicated especially by Strasburger ('92, '00) are commonly isogamous, e. g., *Ulothrix* (Fig. 287), *Carteria*
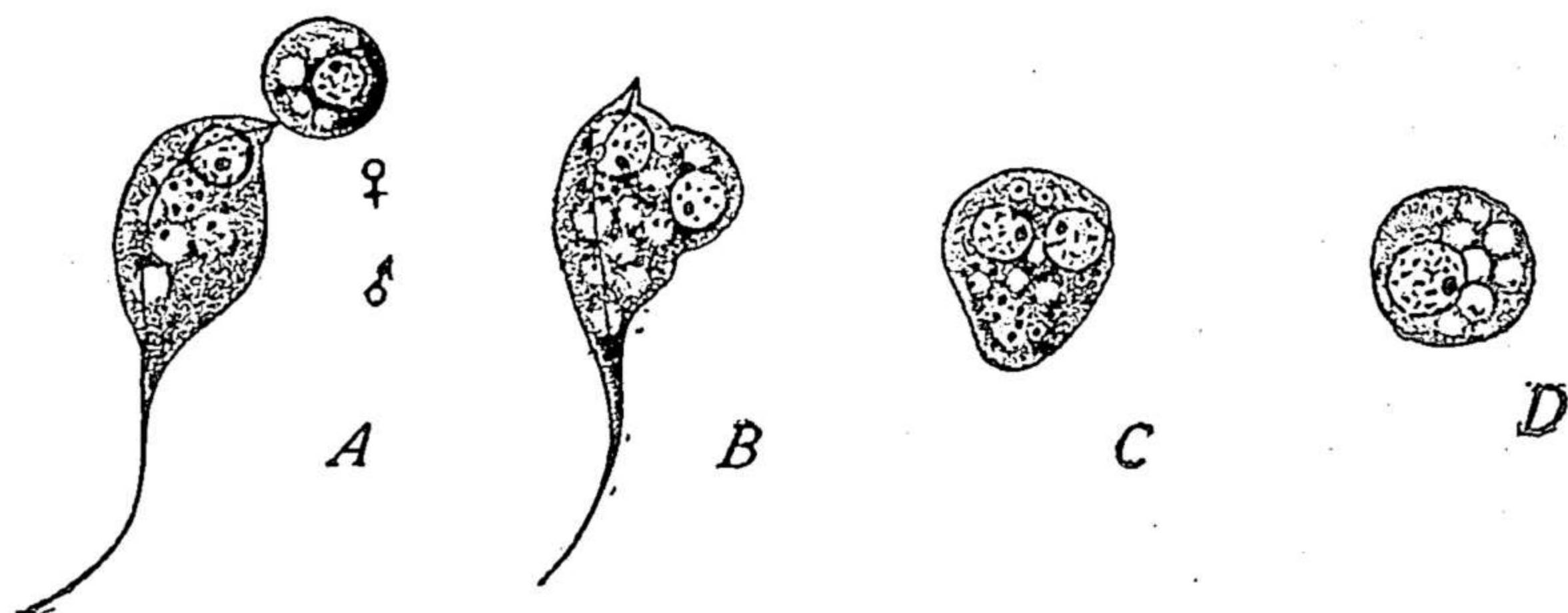

Fig. 288.—Fertilization in the gregarine *Stylorhynchus* (LÉGER).
A, pyriform sperm attached to the small spheroidal ovum; *B*, *C*, plastogamy; *D*, zygote.

(Fig. 290) or *Hydrodictyon*. These forms are more or less pear-shaped and pointed at one end at or near which are borne two flagella. Such gametes commonly contain a chromotophore and also a red "eye-spot" or "stigma" both of which are probably to be regarded as plastids or products of such bodies. The clear anterior region from which the flagella arise is considered by Strasburger to be largely composed of "kinoplasm"; and in the case of the closely analogous swarm-spores a localized blepharoplast may often be seen at the base of the flagella, (Strasburger, '92, '00, Timberlake, '02, etc.). In some cases the gametes contain pulsating vacuoles (*Hydrodictyon*, according to Klebs) as is also the case in certain zoöspores. A good example of microgametes of this type is offered by *Sphæroplea*, save that the flagella are attached at some distance from the anterior end. In Volvox (Fig. 291) the sperms are similar. This gives a transition to the type seen in *Cutleria* (Fig. 311) and other forms, where the two flagella are attached at one side

[1] Strasburger ('92, '00), Dangeard ('99), Timberlake ('02), Davis ('02, '04), West ('16), Oltmanns ('22, '23).

of the cell, or *Vaucheria*, where one is attached near the anterior end, the other backwards, reminding us of such flagellates as *Bodo* (Fig. 286, G). In the Charales appears a type of biflagellate sperm that is closely similar to that characteristic of the bryophytes generally (p. 307), and parallel to those of certain Turbellaria.

The transition from isogamy to heterogamy is shown by various intermediate stages in the simpler forms of both animals and plants, particularly in the merogamic Protozoa and in various groups of algæ. Among the merogamic rhizopods some forms are perfectly isogamous (*Polystomella*, *Trichosphærium*), others anisogamous in various degrees (*Arcella*, *Collozoum*) (Fig. 286, I, K). In Sporozoa nearly all gradations exist between complete isogamy (*Gregarina*) and a typical heterogamy such as appears in *Coccidium;* but many of these forms are non-flagellate. Here may be mentioned the case of *Basidiobilus* (one of the phycomycetes) as described by Fairchild ('97). In this form the two conjugants, formed as bud-like protuberances from neighboring cells of the mycelium, are at first alike (Fig. 289), but during fertilization the "female" gamete enlarges greatly, and after reception of the "male" nucleus gives rise to the zygote ("zygospore"). Here we see an original isogamy passing over into anisogamy during the act of fertilization.

The most complete series of transitional forms is seen among the algæ, exemplified by the series *Carteria*, *Monostroma*, *Pandorina*, *Bryopsis* (Fig. 290) and *Aphanochæte* (Fig. 292). In the last-named form the macrogamete, formed singly in the mother-cell (oögonium) is still flagellated but has lost the free-swimming habit, moving but feebly within its surrounding capsule and coming to rest as soon as fertilized. It is but a step from this condition to true heterogamy, where the egg has lost its flagella, is non-motile, and is fertilized without discharge from the oögonium. The latter condition, of widespread occurrence in lower plants, is exemplified by *Eudorina*, *Volvox*, *Vaucheria*, *Coleochæte*, *Fucus*, etc., and occurs in typical form in the coccidian Sporozoa. A curious exception to the usual rule in heterogamy is offered by the flagellate *Mastigella vitrea*, where, according to Goldschmidt ('07), the microgamete is non-motile, the macrogamete flagellated.

Attention has earlier been drawn to the widespread presence of plastids in the eggs of plants (p. 306). In the algæ plastids are commonly present in the gametes of both sexes, and also in the closely similar asexual swarmers or zoöspores. For example, in *Cutleria*, according to Yamanouchi ('12), the zoöspore contains upwards of 20 plastids, the macrogametes 30 or more, the microgametes usually but two (Fig. 311). In each the motile germ-cell contains a red stigma or "eye-spot." This structure appears in the motile zoöspores and gametes of many other algæ (*e. g.*, in *Ulothrix*, *Ectocar-*

pus and *Pandorina*) and also in the microgametes of various forms, such as *Eudorina, Volvox,* or *Fucus*.[1] From the bryophytes upwards plastids seem to be present only in the egg.

All these facts tell in favor of the view that the gametes of plants were originally flagellated, motile cells, closely similar to zoöspores and to the phytoflagellates. The existing types of hologamic non-flagellates, gametes such as appear in the diatoms, desmids and other Conjugatæ, have probably
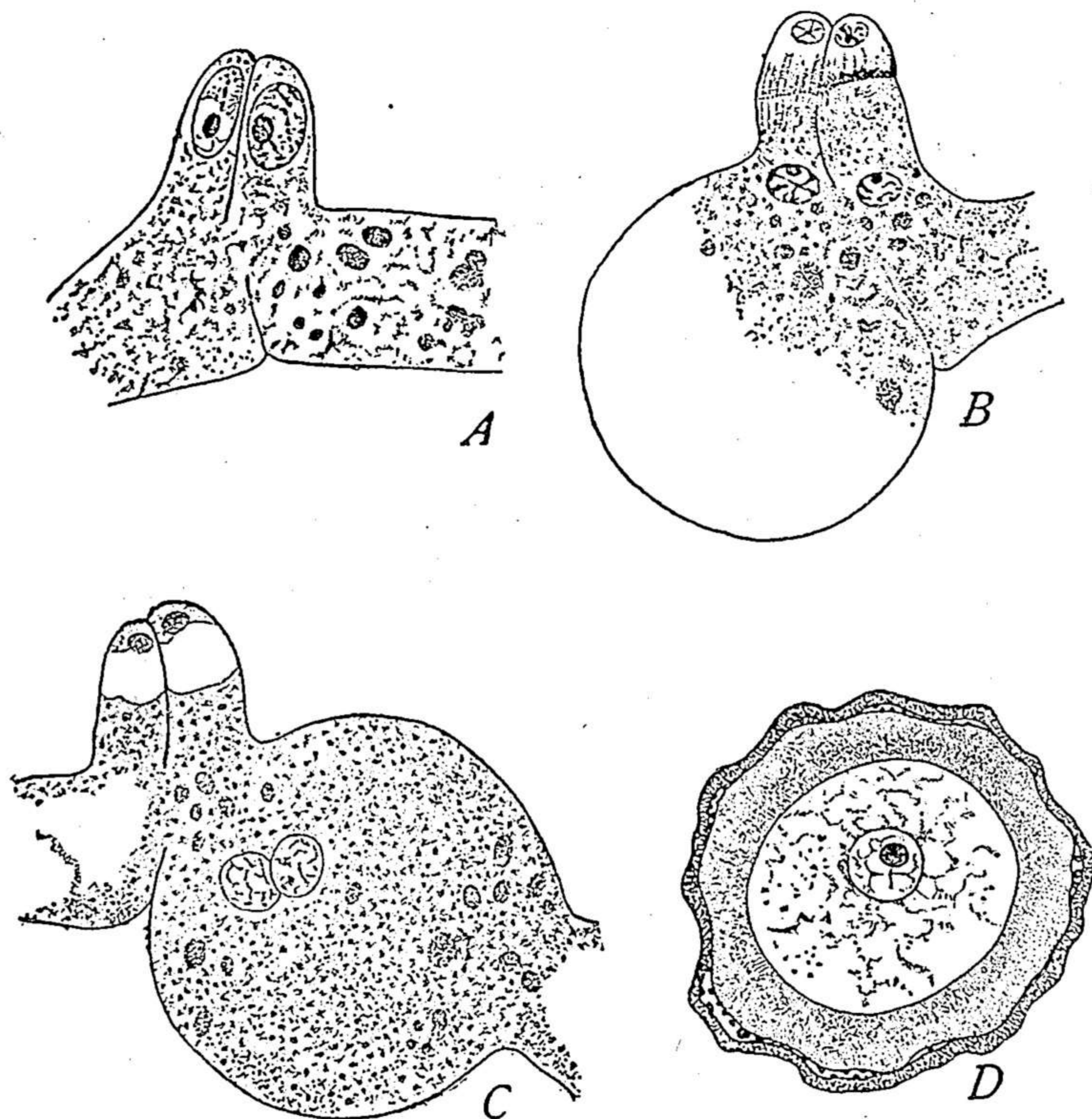

Fig. 289.—Fertilization in the fungus *Basidiobolus* (FAIRCHILD).

A, the two conjugants associated; *B*, two small sterile cells cut off, enlargement of one gamete; *C*, karyogamy; *D*, the zygote.

been derived secondarily from flagellated forms. A similar conclusion is indicated for animals, though the evidence is here less extensive.

In true heterogamous forms the macrogamete becomes a large, non-motile cell, essentially of the same type as in higher plants, but often highly colored through the presence of pigmented plastids or chromatophores. The microgametes or sperms are most commonly biflagellate and of varied type. In *Fucus* Retzius ('06) showed that the biflagellate sperm contains a "nebenkern-organ" apparently comparable to that seen in the middle-piece of certain types of animal sperms (p. 287); and the more recent work

[1] See especially Oltmanns ('22, '23).

of Meves ('18) establishes the correctness of this comparison by showing that it is composed of chondriosomes. Non-motile microgametes are found in the red algæ in the form of small, colorless, spheroidal, uni-nucleated cells constricted off from the vegetative cells and known as *spermatia;* and non-motile forms are all but universal among fungi. In many cases, however, the homology of the "gametes" of fungi is doubtful.

5. Gametes and Gametocytes. Cœnogametes

In certain Protista, and even in some of the multicellular plants, a preliminary conjugation takes place between cells that are not yet gametes
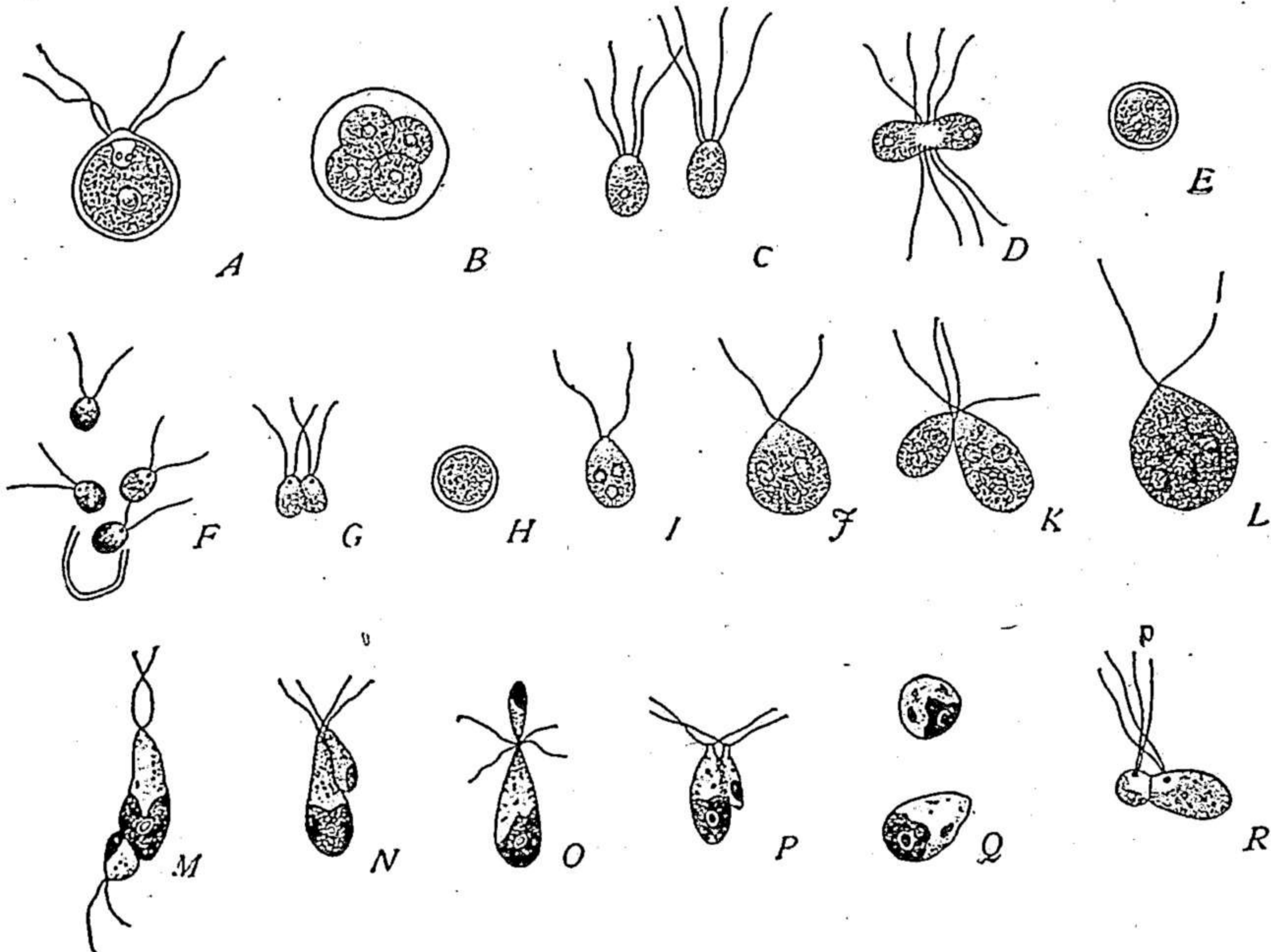

Fig. 290.—Isogamy and anisogamy in algæ.

A–E, isogamy in *Carteria* (WEST); F–H, anisogamy in *Monostroma;* I–L, anisogamy in *Phyllobium;* M–Q, anisogamy in *Bryopsis* (OLTMANS); R, in *Pandorina* (KLEBS).
A, vegetative cell; B, its division to form C the gametes; D, syngamy; E, zygote; H, zygote; I, microgamete; J, macrogamete; K, syngamy; L, zoöspore (zygote); M–P, various forms of syngamy; Q, zygotes.

in the strict sense but *gametocytes* which later give rise by division to the true gametes or gamete-nuclei. This is mostly clearly shown, perhaps, by the phenomenon in the gregarines, where conjugation is typically of this form, the primary union taking place between two "sporonts" or "gametes" (gametocytes) which become closely associated and inclosed in a common cyst but without fusion ("pseudo-conjugation"). The nucleus of each gametocyte now divides successively to form a number (often very large) of gamete-nuclei which pass to the periphery and here are budded off, together with a small quantity of protoplasm, to form minute true gametes.

The latter then unite two by two, one member of each pair being derived from each of the original conjugants, as is made evident in the anisogamous forms. The distinction between the gametocytes and the true gametes is here obvious; and it is also evident that the original "pseudo-conjugation" of the gametocytes is no more than a prelimary to the true syngamy of the gametes.[1]

Closely analogous to this are the phenomena in some of the fungi, where the primary conjugants are large cells which are multinuclear like the mycelium from which they arise, and constitute the so-called *coenogametes*. In *Mucor* they are nearly or quite isogamous and fuse completely. In *Pyronema* and *Albugo* they are unequal, the male conjugant ("antherid-
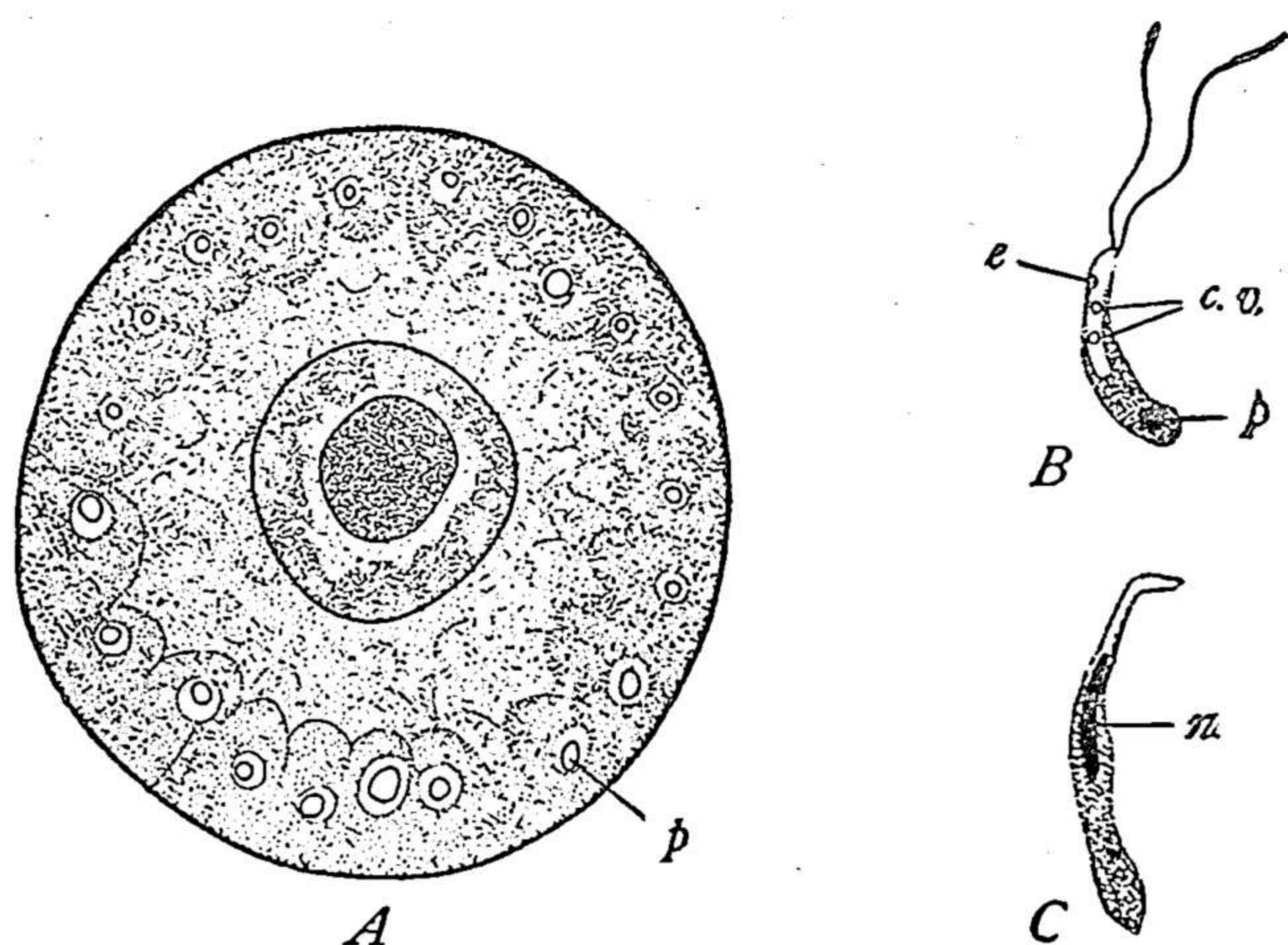

Fig. 291.—Germ-cells of *Volvox* (OVERTON).

A, Ovum (oösphere) containing a large central nucleus and a peripheral layer of chromatophores (plastids); *p*, pyrenoid; *B*, spermatozoid; *c*, *v*, contractile vacuoles; *e*, "eye-spot" (chromoplastid); *p*. pyrenoid, *C*, spermatozoid stained to show the nucleus (*n*).

ium") being much smaller than the female ("oögonium"), and sending into the latter an in-growing "antheridial" tube, the contents of which enter the oögonium. In *Pyronema* the numerous nuclei which it contains conjugate two by two with those of the oögonium (Fig. 293), and the same is true in some species of *Albugo*. In *A. candida*, on the other hand, only a single antheridial nucleus enters and conjugates with one nucleus of the oögonium, and the remaining nuclei ultimately disappear (Fig. 294).[2] Analogous to this are the phenomena in the ciliates, where each conjugant produces at least five nuclei, and sometimes a larger number, of which only two take part in conjugation (p. 609). In all these cases, evidently, the

---

[1] All doubt concerning this interpretation seems to be removed by Nusbaum's ('03) observations on *Schaudinella*, where pseudo-conjugation of the gametocytes does not take place, though the gametes are formed as usual.

[2] See Harper ('00), Stevens ('99, '01), review in Davis ('04).

original conjugants can be called gametes only by courtesy; and even in case of the animal ovum the sperm often enters before the polar divisions have been formed while the egg is still technically a gametocyte or oöcyte (p. 398).

6. The Chromidial Formation of Gametes. Chromidiogamy

A remarkable phenomenon said to occur in certain Protista is the formation of gamete-nuclei from chromidia extruded from the nuclei, or even, it is said in certain cases, a union of chromidia from the two conjugant-nuclei without preliminary formation of gamete-nuclei. The first of these condi-
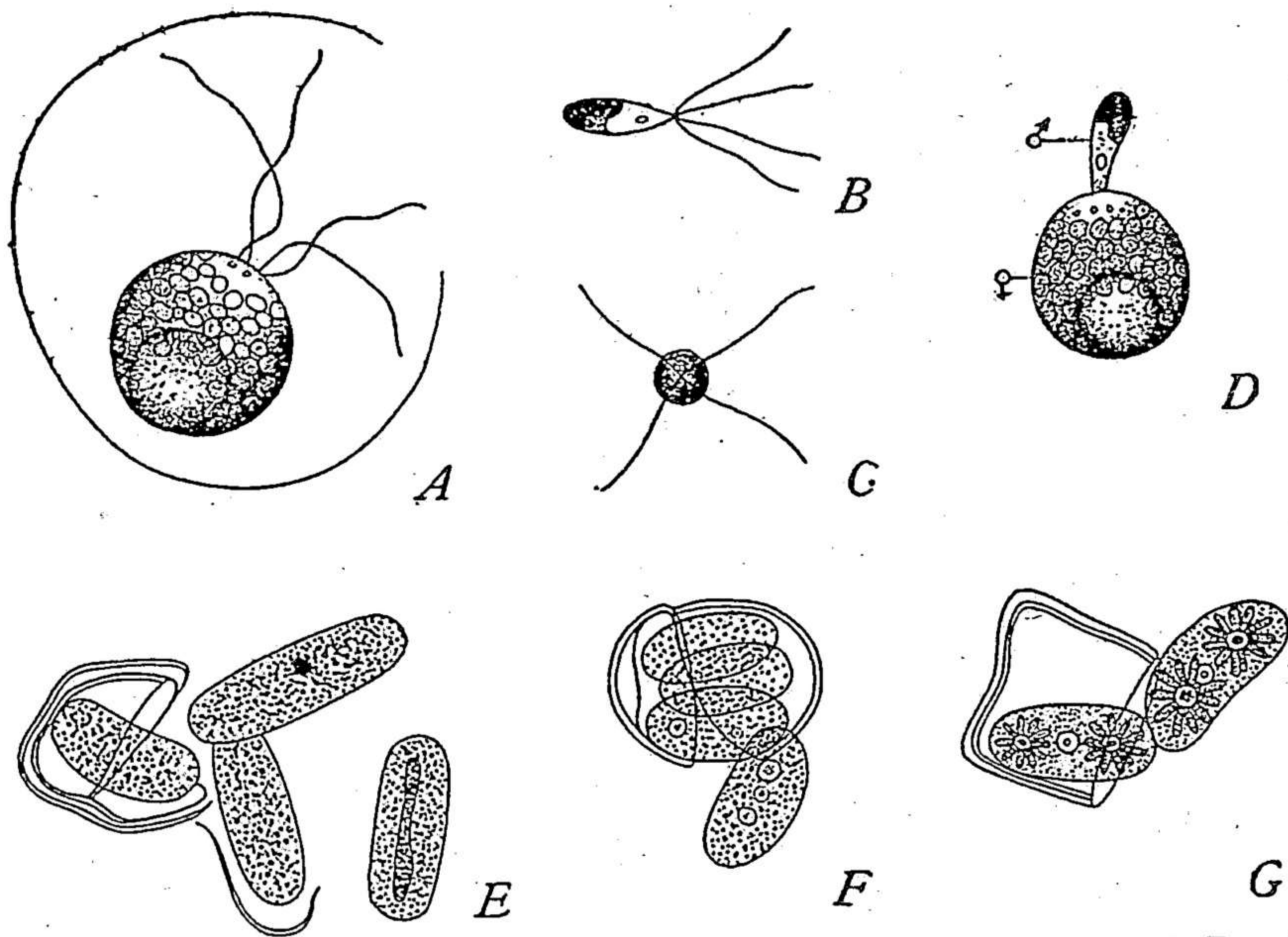

Fig. 292.—Reproduction in green algæ (A–D, from HUBER; E–G, from DeBARY).

A, macrogamete of *Aphanochæte* ready for conjugation; B, C, different views of microgamete; D, conjugation; E, germination of the zygote in the desmid *Mesotænia*, producing four functional products (zygotic meiosis); F, similar process in *Mesotænium*; G, the same in *Cylindrocystis*, two functional products.

tions was described by Schaudinn in the Foraminifera *Polystomella*, *Centropyxis* and *Chlamydophrys*, later by several observers especially in rhizopods and gregarines.[1] In these rhizopods the scattered chromidia are said to enlarge to form the nuclei of minute flagellated or (in *Arcella*) amoeboid gametes that conjugate in pairs. In the gregarines an interesting series of conditions leading up to such a condition has been observed. In most of the gregarines the gamete-nuclei are formed by repeated division of the original nucleus of the gamont (gametocyte) to produce minute gamete-nuclei. In certain

[1] R. Hertwig ('99); Schaudinn ('99, '00, '04); Elpatiewsky ('07) (*Arcella*, flagellates, gregarines); Kuschekewitsch ('07); Swarzewsky ('10, '12) (gregarines), etc. See Fig. 342.

cases,[1] the primary nucleus is said to break down into, or to give off, chromidia from which is re-formed a single nucleus, which divides progressively to form the gamete-nuclei. In *Gregarina cuneata* and *Lankesteria sp.* the primary nucleus breaks down into a mass of chromidia from which are said to arise many small nuclei (as in the Foraminifera) each of which divides twice to form four gamete-nuclei.[2] The climax is offered by *Difflugia areolata* (a multinucleate species) in which, according to Sülzer ('04) after fusion of the two conjugants (gametocytes) the nuclei give off most of their chromatin in the form of chromidial granules which become closely intermingled and supposedly fuse (chromidiogamy); and from the products are said to arise new nuclei for the new generation.

None of these accounts, especially the last one, seems to the writer to be sufficiently demonstrated; and they have in fact been viewed with considerable scepticism by specialists in this field.[3] In any case all call for the most careful reëxamination.

II. ILLUSTRATIVE EXAMPLES

1. Hologamy and Isogamy with Gametic Meiosis

A striking example of this is offered by the heliozoan rhizopod *Actinophrys sol*, in which the cytological phenomena have been made known in greater detail than in any other recorded case. An extended account of these phenomena, illustrated by numerous excellent figures, is given by Bělař ('21, '23).[4]

As above stated (p. 582) the conjugants are in this case (Fig. 282) sister-cells, arising by a "progamic" mitosis, the two cells thus produced becoming surrounded by a common cyst. In this mitosis, as in the vegetative mitoses, appear 44 chromosomes (the diploid number) which arise from elongate spireme-threads, split lengthwise, and show marked size-differences. In each conjugant the nucleus now undergoes two meiotic divisions, producing successively two small reduction-nuclei or "polar bodies," which approach the cell-periphery and quickly degenerate. *In the course of this division the number of chromosomes is reduced from 44 to 22.* The remaining nucleus in each gamete now enlarges, becomes reticular; and this is followed by complete fusion of the gametes. Fusion of the cytosomes (plastogamy) is followed by that of the two gamete-nuclei, thus completing the formation of the zygote which, after a period of rest within the cyst,

[1] Léger et Dubosc, '09.

[2] This suggests a process of gametic meiosis; and such a process had actually been described in *Monocystis* by Mulsow ('11). On the other hand, in *Aggregata* and *Diplocystis* Dobell has found meiosis to be zygotic (p. 491).

[3] See Doflein, '19, Kofoid, '21.

[4] See also Schaudinn, '96, Keysselitz, '08, Distaso, '08, Prowazek, '13.

escapes and assumes the ordinary vegetative form. It is here evident that the original conjugants are not gametes but gametocytes (p. 582). According to the account of Bělař, the history of the chromosomes, during meiosis shows a very exact parallel, which extends to many of the finer details, to that seen in higher animals. In the early prophase of the first meiotic division the nucleus of each conjugant develops a leptotene spireme which quickly becomes polarized, and passes through a typical amphitene stage in the course of which the threads conjugate side-by-side. Thus arises a polarized pachytene which later loses its polarization, becomes longitudinally double (diplonema) and twists to form a strepsinema. A typical diakinesis now ensues, in which may be seen some of the tetrad forms occurring in higher animals (transverse rod-tetrads, double crosses) and showing some
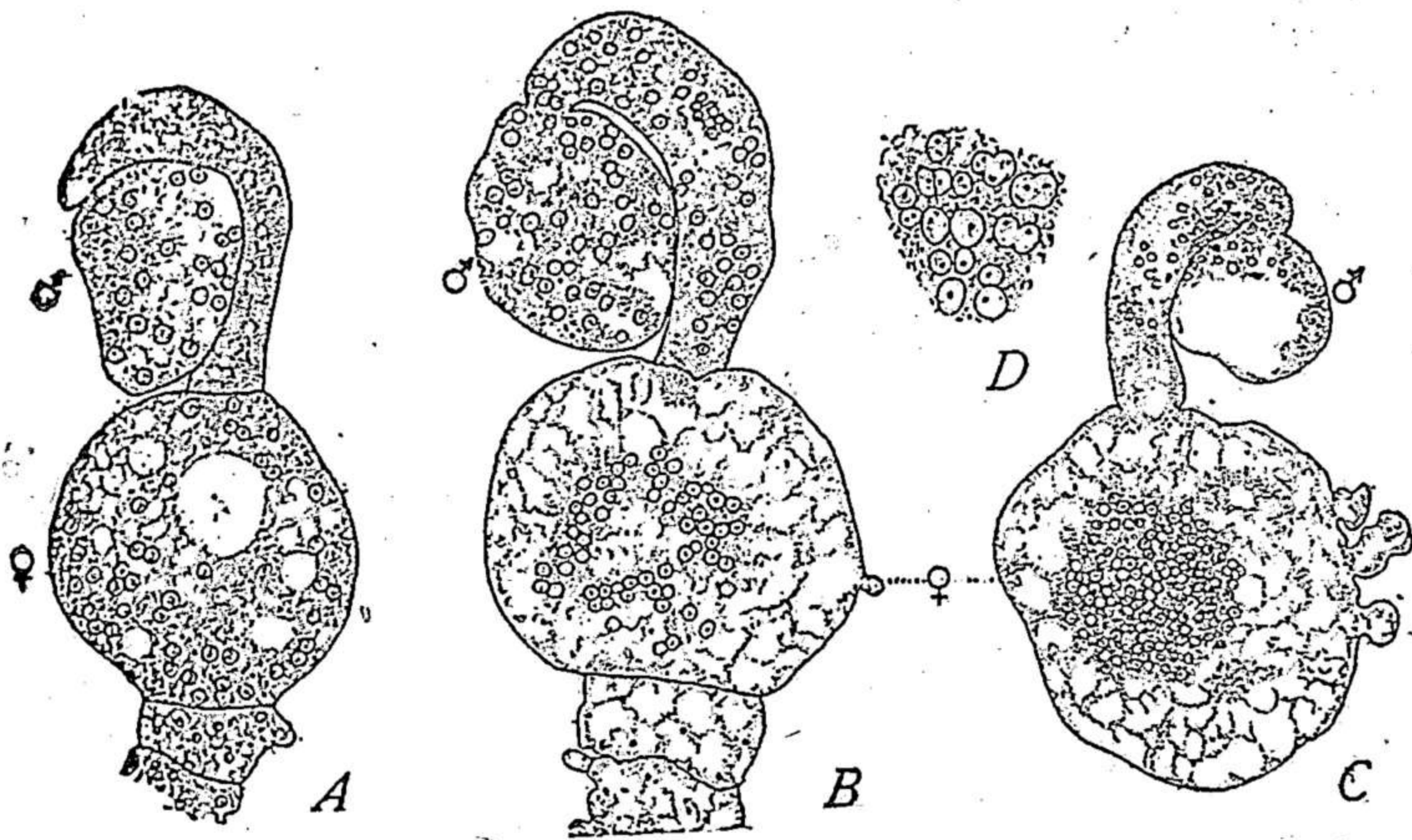

Fig. 293.—Multinucleate "gametes" (gametocytes) and fertilization in the fungus *Pyronema* (HARPER).
A, the multinucleate "oögonium" (♀), to which is attached the male gametocyte (♂); B, entrance of sperm-nuclei into the trichogyne; C, intermingling of sperm-nuclei and egg-nuclei; D, conjugation of nuclei in pairs, in various stages.

of the same variations in mode of spindle-attachment. The resemblance extends even to the interkinesis, which clearly shows the single crosses, so often seen in higher forms. In this rhizopod, accordingly, it would seem that the *entire mechanism of meiosis exists substantially in the same form as in a fish, a salamander or a mammal.*

No other case is so completely known as this; but many less complete observations indicate that an essentially similar process may take place in other Protista. An example of this in a flagellate is described by Dobell ('08) in *Copromonas subtilis.* The conjugants are here typical flagellates, indistinguishable in size or appearance from the vegetative cells or from each other. Union of the conjugants is followed by complete fusion, during which the flagellum first of one gamete and then of the other is lost. Before

union of the nuclei each divides twice by a simple type of heteropolar promitosis, thus giving three nuclei in each gamete, of which two quickly degenerate as "reduction-nuclei" while the third persists as the gamete-nucleus or pro-nucleus. Karyogamy now follows the two nuclei completely fusing into one, after which the zygote may at once resume its ordinary free-swimming type, or may first undergo a period of rest while encysted.
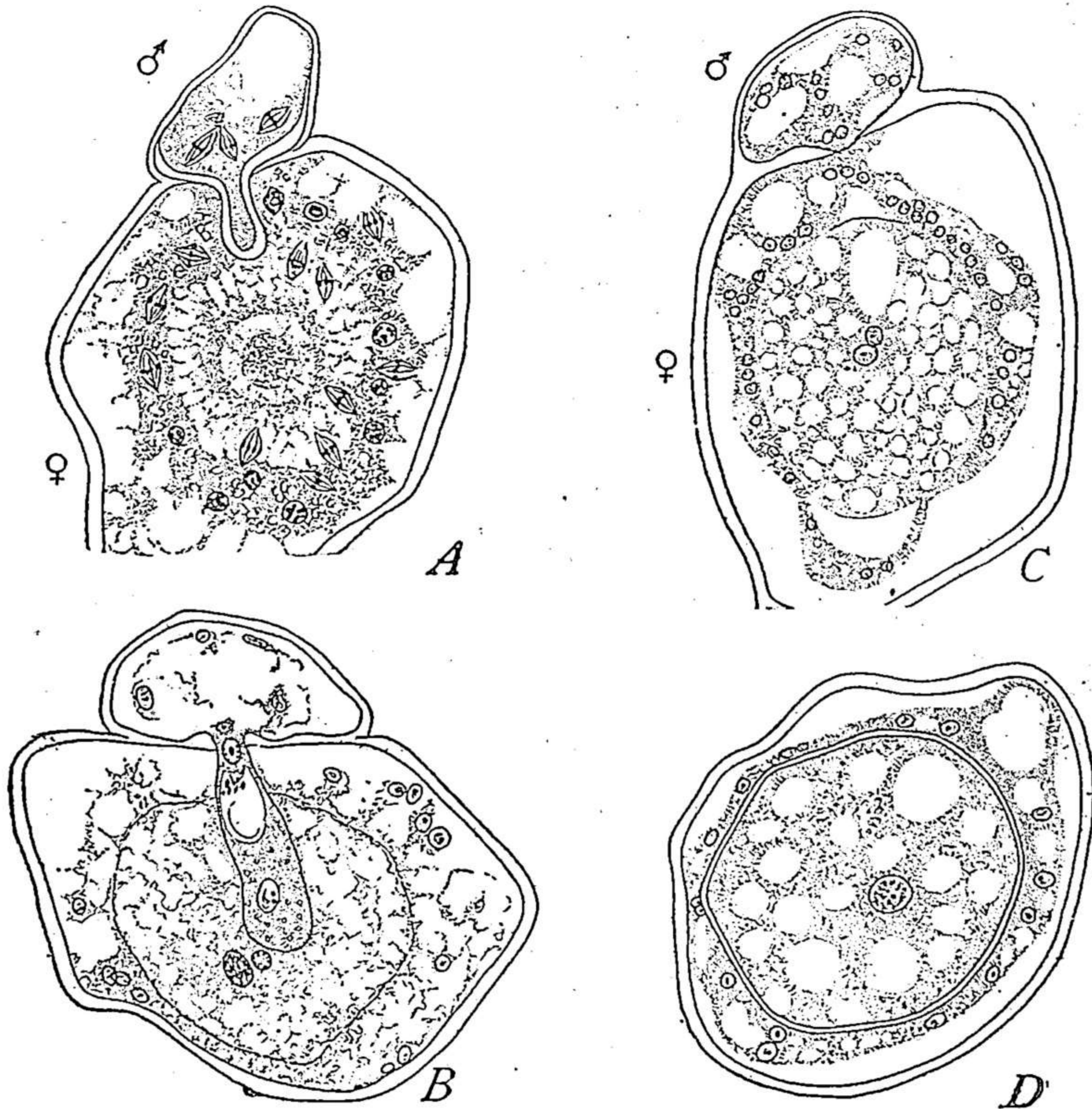

Fig. 294.—Fertilization in the fungus *Albugo* (*Cystopus*) *candidus*. (*A, B*, from Davis, *C, D* from Wager.)

A, antheridial tube from male gametocyte (♂) penetrating the oögonium or female gametocyte (♀); *B*, later stage, ovum formed, with single nucleus (near it the cœnocentrum); one sperm-nucleus in antheridial tube; *C*, union of gamete-nuclei; *D*, zygote.

The history of the nuclear elements is not known; but these observations suggest that the nuclei are originally of diploid organization, and that the two divisions preceding karyogamy are meiotic in character and effect a reduction of the nuclei to the haploid condition.[1]

A third example of this type, with certain interesting modifications, is seen in the diatoms, among which three different conditions have been described. In *Surirella* (Karsten, '00) the phenomena are essentially as

[1] Some doubts have been expressed as to whether the cycle described by Dobell may not represent stages of fission rather than of conjugation. Berliner's results on *C. major* ('09) are, however, in harmony with Dobell's, though in some respects less complete.

in the foregoing cases; the nucleus of each conjugant divides twice, producing four nuclei, of which one becomes the gamete-nucleus while three degenerate as reduction-nuclei. More complicated conditions are found in a number of other diatoms [1] where the phenomena are of particular interest since *two of the products of the maturation-divisions are functional instead of one.* In *Rhopalodia*, as described by Klebahn (Fig. 295) the process begins as in
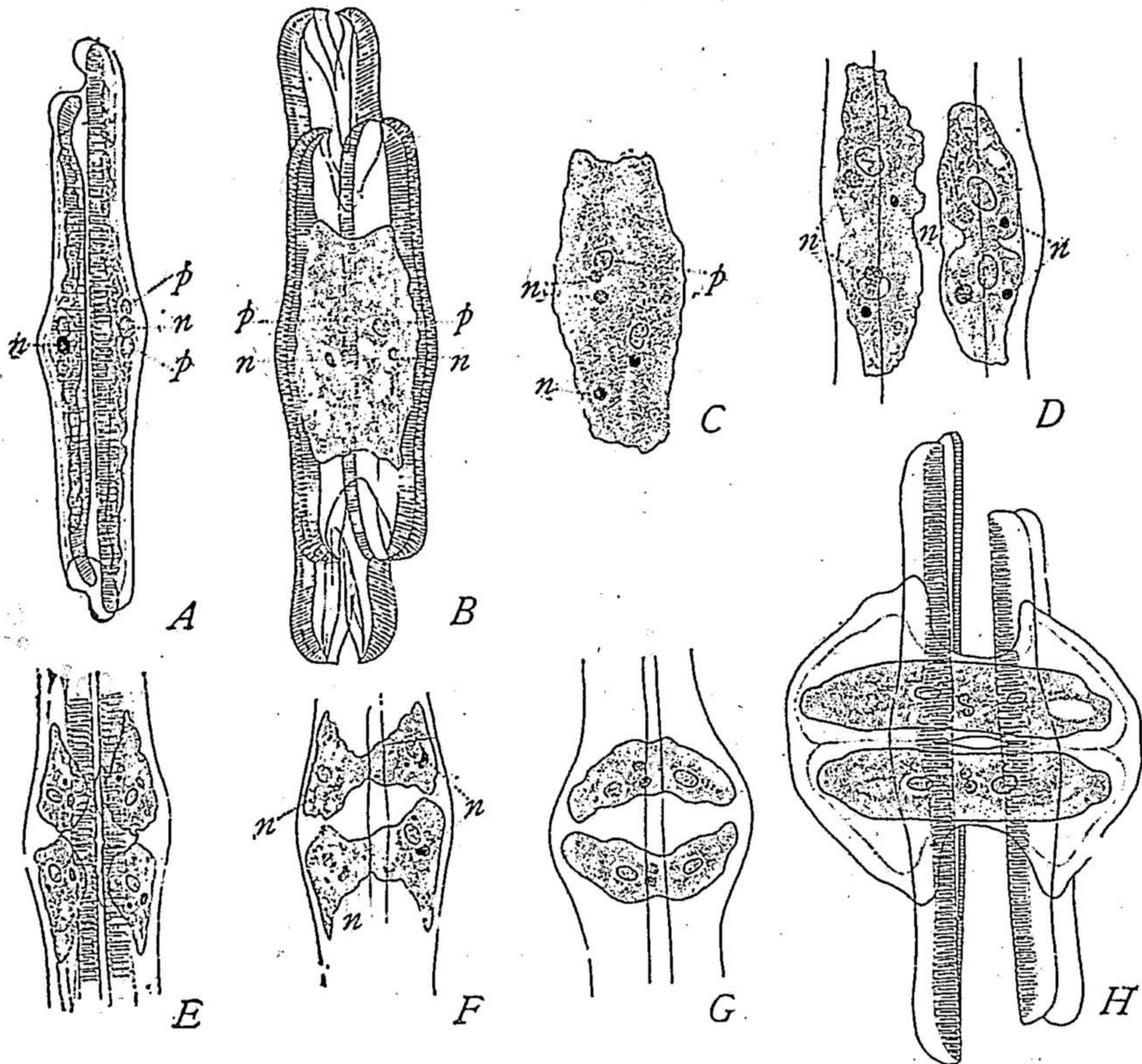

Fig. 295.—Conjugation and gametic meiosis in the diatom *Rhopalodia* (KLEBAHN).

A, union of the conjugants in side-view, each having one nucleus (*n*) and two pyrenoids (*p*); B, later stage, from above, showing two nuclei (result of first division) in one conjugant; C, protoplast of one conjugant after second division, four nuclei, two pyrenoids; D, later stage of the two conjugants, side-view, each with four nuclei, two large and two small ("polar bodies" or reduction-nuclei); E, division of each conjugant, to form two gametes, each with one functional nucleus and one reduction-nucleus; F, G, fusion of the gametes two by two, disappearance of the reduction-nuclei; H, the two resulting zygotes, gamete-nuclei not yet fused.

Surirella, each nucleus dividing twice to form four nuclei, but is later complicated by the fact that in each conjugant two of the four nuclei enlarge and become functional while two degenerate as reduction-nuclei; and the protoplast now divides into two gametes each of which receives one of the functional nuclei. Thus arise four gametes which now fuse two by two, the two gametes of each pair being derived respectively from the two original conjugant con-

[1] Karsten, *op. cit.*, Klebahn, '96, '02, Oltmanns, '22.

jugants, so as to effect an exogamous union. The final result is to produce two zygotes instead of one (Fig. 295, H).

The process is essentially similar in *Epithemia, Amphora, Navicula* and some other forms. It is here perfectly clear that the original conjugants are not gametes but gametocytes (p. 582), and the same conclusion follows, strictly speaking, for all cases of gametic meiosis in which the meiosis first takes place after union of the conjugants, even when only a single gamete-nucleus results from the process.[1]

A remarkably interesting case is offered by *Amœba diploidea* as described by Hartmann and Nägler ('09).[2] In this rhizopod the ordinary vegetative individuals are visibly diploid (though the chromosome-number is not known), being regularly binucleate, precisely as in the sporophytic generation of the *Uredinales* and some other fungi (p. 624), the two nuclei being closely appressed and dividing side by side by "conjugate division" (Fig. 296). This condition arises from the fact that in conjugation the two gamete-nuclei become closely associated during this process but do not actually fuse until near the end of the vegetative cycle—an ideal example, as Hartmann has said, of gonomery (p. 433). In conjugation (Fig. 296) these *Amœbæ* first become associated two by two, and without immediate fusion become inclosed in a common cyst. Subsequently the two nuclei in each conjugant at last fuse to form a single diploid nucleus. This is followed by complete plastogamy, the two diploid nuclei still remaining separate (Fig. 296, F). Now, as in *Actinophrys* or *Copromonas*, each of these nuclei is said to undergo two successive heteropolar divisions of simple promitotic type, three of the products in each case being abortive reduction-nuclei while the fourth is the gamete-nucleus. The two gamete-nuclei thus produced finally conjugate with each other without fusion to form the double zygote-nucleus for a new vegetative cycle (Fig. 296, H). If this account is correct it seems clear that the case is one of gametic meiosis quite comparable to that seen in other rhizopods (*Actinophrys, Actinosphærium*). Owing, however, to the complete autonomy of the two gamete-nuclei throughout the vegetative cycle, the phenomena offer a superficial resemblance to the zygotic type, since the meiotic divisions take place just after final fusion of the original gamete-nuclei. In reality the two cases are radically different; for in zygotic meiosis the cells of the vegetative cycle are haploid, in *Amœba diploidea* diploid. The final fusion of the nuclei

---

[1] *Cf.* p. 597. Among the pelagic diatoms a number of forms have been described in which the phenomena are widely different, conjugation being of the merogamic type, and *followed* by meiosis. In *Corethron*, for example (Karsten, '04), the protoplast divides to form numerous small gametes which fuse in pairs with those from other cells to form zygotes. Two nuclear divisions now follow, the zygote dividing into two cells each containing two nuclei of which one degenerates. This process is closely parallel to that seen in the desmids.

[2] See also Nägler ('09) and Erdmann ('10, '13).

just preceding maturation in the latter case is in a broad sense comparable to synapsis, and like the latter may be regarded as "the final step in the conjugation of the germ-cells" (Montgomery, '01, p. 223).

In none of the foregoing cases have the chromosome-numbers been determined; but it seems probable that all belong to the gametic or terminal type of meiosis. It is a fact of much theoretic interest that in *Rhopalodia*
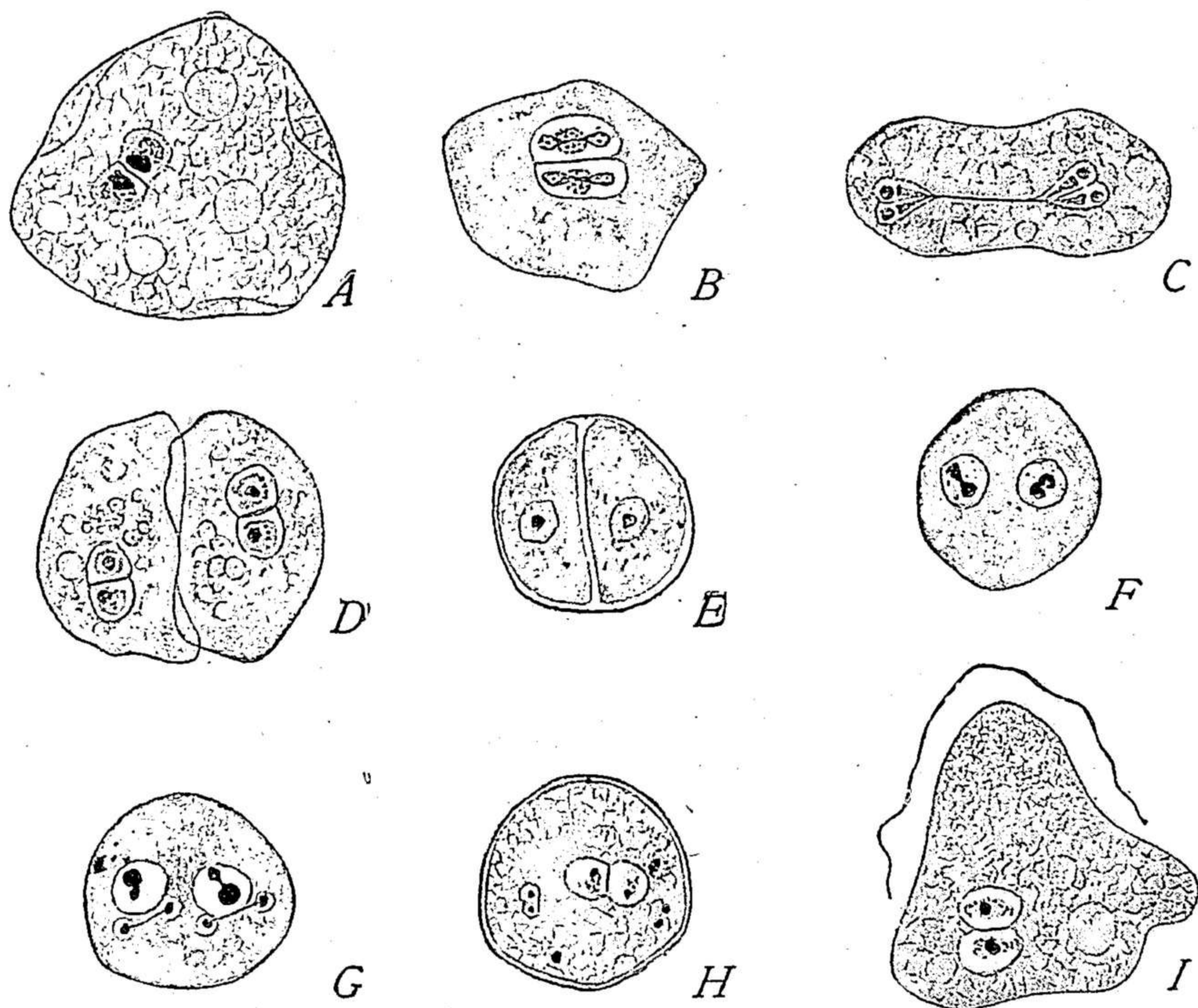
Fig. 296.—Nuclear cycle in *Amœba diploidœ* (HARTMANN and NAEGLER).

A, binucleate individual of ordinary vegetative type; *B, C,* ordinary mode of division, by conjugate promitosis; *D,* conjugation; *E,* fusion of nuclei (= synapsis) in each conjugant; *F, G,* supposed meiotic divisions of simple type; *H,* union of the gamete-nuclei without fusion; *I,* escape of zygote from cyst.

two of the products of meiosis are functional gamete-nuclei, instead of only one (as in the animal egg) or all four (as in the animal sperm).

2. Hologamy and Isogamy with Zygotic Meiosis

In the cases now to be considered meiosis is initial or zygotic, immediately following syngamy and karyogamy instead of preceding them. Among animals this type is known only in certain gregarines (p. 605), but it is more frequent in lower plants, and is well illustrated in the conjugate algæ (Conjugatæ). An interesting series of forms here exists.

In the Zygnemaceæ (*Spirogyra, Zygnema*) conjugation is typically holo-gamic, any or all of the cells of the linear cell-aggregates being able to con-jugate with little visible alteration of structure beyond a contraction of the protoplast into a rounded form. Complete plastogamy or fusion of the gametes is at once followed by complete fusion of the nuclei in karyogamy; and only after the completion of this process do the meiotic divisions take place. In both cases there are, as usual, two of these divisions,[1] producing four nuclei, of which one enlarges to form the initial functional nucleus while three remain of small size, forming reduction-nuclei that finally degenerate
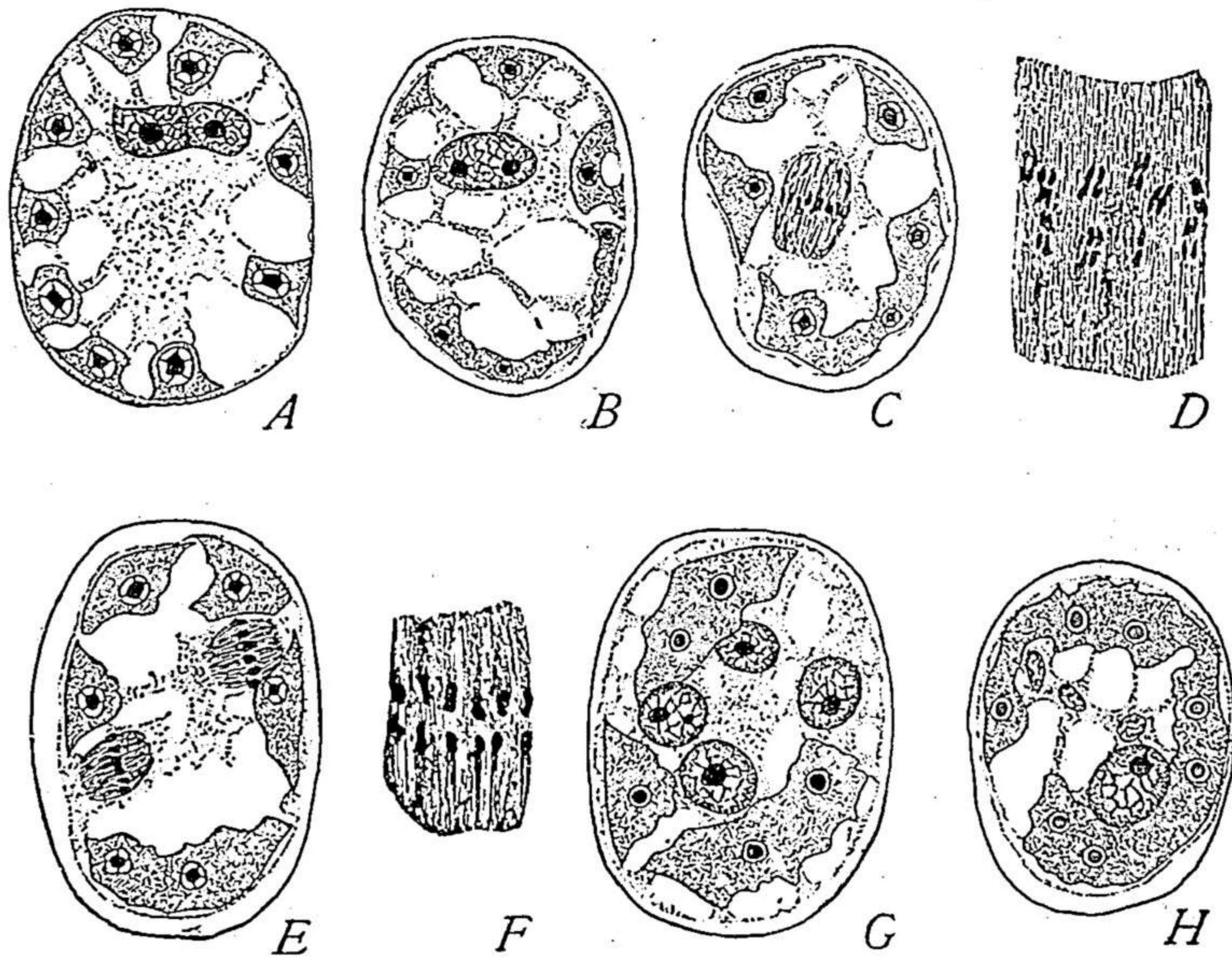

Fig. 297.—Zygotic meiosis in the alga *Spirogyra elongata* (TRÖNDLE).

A, zygote, gamete-nuclei uniting above (the peripheral dark bodies are pyrenoids); *B*, fusion of gamete-nuclei; *C*, first (heterotypic) resulting division; *D* (from *S. neglecta*) tetrads in this division; *E*, *F*, second division; *G*, resulting 4-nucleate stage; *H*, later stage, one functional and three de-generating nuclei (polar bodies?).

(Fig. 297). This condition, evidently, is comparable with that seen in animal ova generally, excepting that meiosis occurs at the beginning of the vegetative cycle instead of at the end. This interpretation seems to be placed beyond doubt in the case of *Spirogyra* by the chromosome-number, which is said to be in the second division but half that present in the first, the numbers found by Karsten in *S. jugalis* being 28–14, while Tröndle found in *S. neglecta* 24 and 12, in *S. longata* 20–22 and 10–11, and in *S. colo-spora* 18–9. The primary functional nucleus is therefore haploid, as are also the nuclei of the filament developed from the zygote. *The ordinary vegetative or "adult" plant is therefore a haploid organism or haplont*, like the

[1] See for *Spirogyra*, Karsten ('00) and Tröndle ('11) and, for *Zygnema*, Kursanow ('11).

haploid or gametophyte generation of higher plants, and the diploid number exists for only a brief period in the unicellular zygote. This condition is unknown in higher plants and animals.

In the Zygnemaceæ but one of the four nuclei resulting from the meiotic divisions is functional, and but one embryo is produced from the zygote (zygospore) upon its germination. The same condition is said to exist in certain of the desmids (Gonatozygæ, according to De Bary);[1] but more usually two or even four embryos are produced from a single zygote. In *Closterium* and *Cosmarium*, as shown by Klebahn ('90), meiosis follows upon complete fusion of the gamete-nuclei as in *Spirogyra* or *Zygnema;* and is accomplished by two divisions; but the first of these divides the zygote into two complete and equal cells, while the second is merely a division of the nucleus in each of these forms to form one functional nucleus and one small reduction-nucleus (Fig. 298). Each of the two cells thus produced forms the starting point for a new vegetative cycle of division; and although the chromosome numbers are not known, there is every reason to believe that the ordinary vegetative individuals are haploid as in the Zygnemaceæ. In certain of the Mesotæniaceæ, finally, the zygote typically produces four functional embryos, though sometimes two (species of *Mesotænia, Spirotænia,* and *Cylindrocystis,* Fig. 292). Here again the cytological detail is not yet known, but it seems probable that in all these cases alike meiosis is zygotic, and is accomplished by two divisions.[2] This is supported by the more recent observations of Pascher ('16), on the phytoflagellate *Chlamydomonas* in which total conjugation is said to be followed by a process of zygotic meiosis during which the number of chromosomes is reduced from 20 to 10. The interesting fact here is that even in this simple unicellular form meiosis is effected by two divisions, all four of the products being functional, flagellated swarm-spores, each of which initiates a haploid cycle of successive cell-divisions that ultimately ends with conjugation.[3] This leads to the suspicion that zygotic meiosis may occur in various other cases among algæ in which the zygote produces four swarm-spores (e. g., in *Œdogonium* or *Bulbochæte,* Fig. 306), and that this type of meiosis may be of widespread occurrence in the lower plants.

We may conveniently consider here the only known cases of this type among animals, though both these belong to the merogametic forms, and one of them is heterogamous. These cases are offered by the coccidian

[1] See West, '16, p. 375.
[2] See Hartmann ('14), Kaufmann ('14), Oltmanns ('22).
[3] That this is the correct interpretation is indicated by the fact that different species of *Chlamydomonas* may be crossed and the offspring reared until the next enduring conjugation. According to Pascher the offspring of the zygote show varying combinations of the gamete-characters which point to a segregation during the spore-producing (meiotic) divisions of the zygote like that seen in higher forms (p. 930).
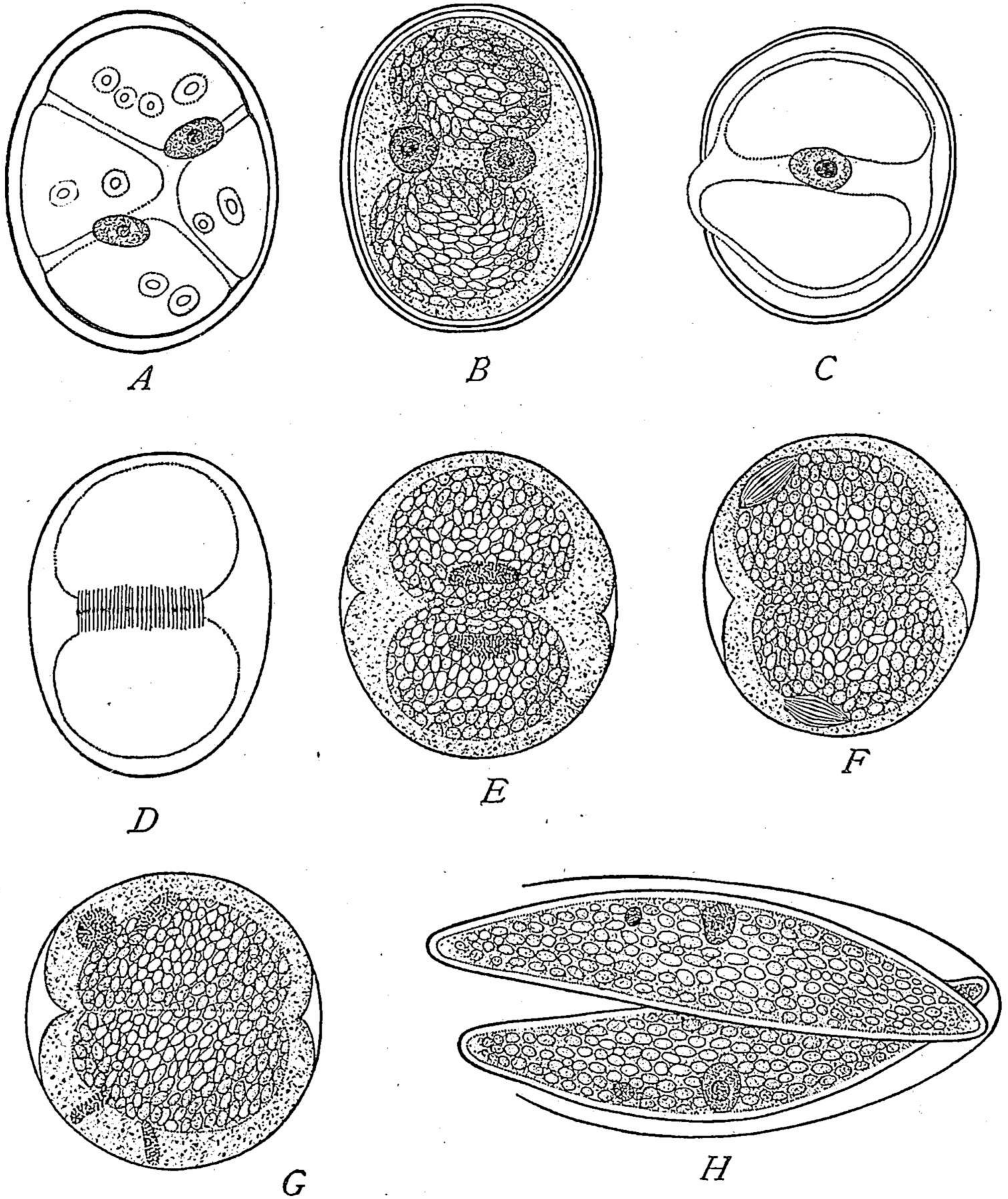
Fig. 298.—Conjugation and zygotic meiosis in *Closterium* (KLEBAHN).
A, soon after union, four chromatophores; B, chromatophores reduced to two, nuclei distinct; C, fusion of the nuclei; D, first cleavage of the zygote; E, resulting 2-cell stage; F, second mitosis; G, resulting stage, each cell bi-nucleate; H, separation of the cells; one of the nuclei in each enlarging to form the permanent nucleus, the other degenerating.

sporozoan *Aggregata eberthi* and the gregarine *Diplocystis schneideri*, as described by Dobell and Jameson ('15) [1] in both of which the chromosomes can readily be counted. The diploid and haploid numbers are respectively

[1] These results are contrary to those of several earlier observers, who described more or less definitely a process of gametic reduction. The most important of these is that of Mulsow ('11) who described such a process in the gregarine *Monocystis rostrata*.

in *Aggregata*, 12 and 6, and in *Diplocystis*, 6 and 3. Reduction occurs in the first division of the zygote, and the haploid number (6) thereafter appears in each succeeding division, producing the sporoblasts and sporozoites, and also in the divisions of the resulting schizonts. The haploid number thus occurs during both the asexual and the sexual cycle, *i. e.*, throughout the entire life-history with the single exception of the zygote-nucleus—a condition in all essentials identical with that seen in *Spirogyra*. The two cases are noteworthy in that meiosis *seems* to be effected in both by one division, in *Aggregata* the first, in *Spirogyra* the second—but this evidently calls for further study.

3. Merogamic Syngamy and Meiosis

A typical example is given by *Polystomella*.[1] In this form there is a true alternation of generations (in the zoölogical sense), the zygote developing into an asexual form (microspheric type) in which numerous nuclei are produced by progressive division. These nuclei (which in the meantime are said to break up into a chromidial form) ultimately become centers of small amœboid asexual germ-cells or *agametes*, formed by a fragmentation of the protoplasm; and these develop, without conjugation, into the sexual, or macrospheric form (Fig. 284, E), which likewise contain numerous nuclei progressively formed. When mature the macrospheric form in its turn fragments, producing a multitude of minute, uninucleate biflagellated gametes which have no resemblance whatever to the amœboid vegetative type.[2]

Theses gametes are perfectly isogamous, but Schaudinn states that gametes from the same macrospheric individual will not unite with one another. Conjugation is therefore exogamous, and one may infer that some sort of physiological difference exists between "male" and "female" individuals as in the moulds (p. 588).

The chromosome-numbers are unknown; but it seems clear that the zygote in this case must be of diploid organization and the gametes of haploid. The point at which meiosis takes place is not certain. Schaudinn, Lister and Winter state that the last two divisions, leading to the gamete-formation, differ in type from those which precede them and may perhaps be meiotic; but this question still remains undecided. The same gap exists in our knowledge of the rhizopod *Trichosphærium*, which has a life-history closely parallel to that of *Polystomella* (Schaudinn, '03) save for the fact that the small gametes are not flagellated and are somewhat unequal in size. Similar

---

[1] Schaudinn ('03), Lister ('05), Winter ('07).

[2] Lister has shown, however, that the macrospheric individuals may produce amœboid agametes instead of gametes. The alternation is therefore not strictly obligatory.

doubts hang over many other cases of merogamic conjugation in plants (e. g., among the green algæ) as well as in animals.

4. Partial or Temporary Conjugation

There is now a general agreement among protozoölogists that conjugation of this form is a secondary process that has been derived from a more primitive total type. It possesses, nevertheless, a high interest, especially
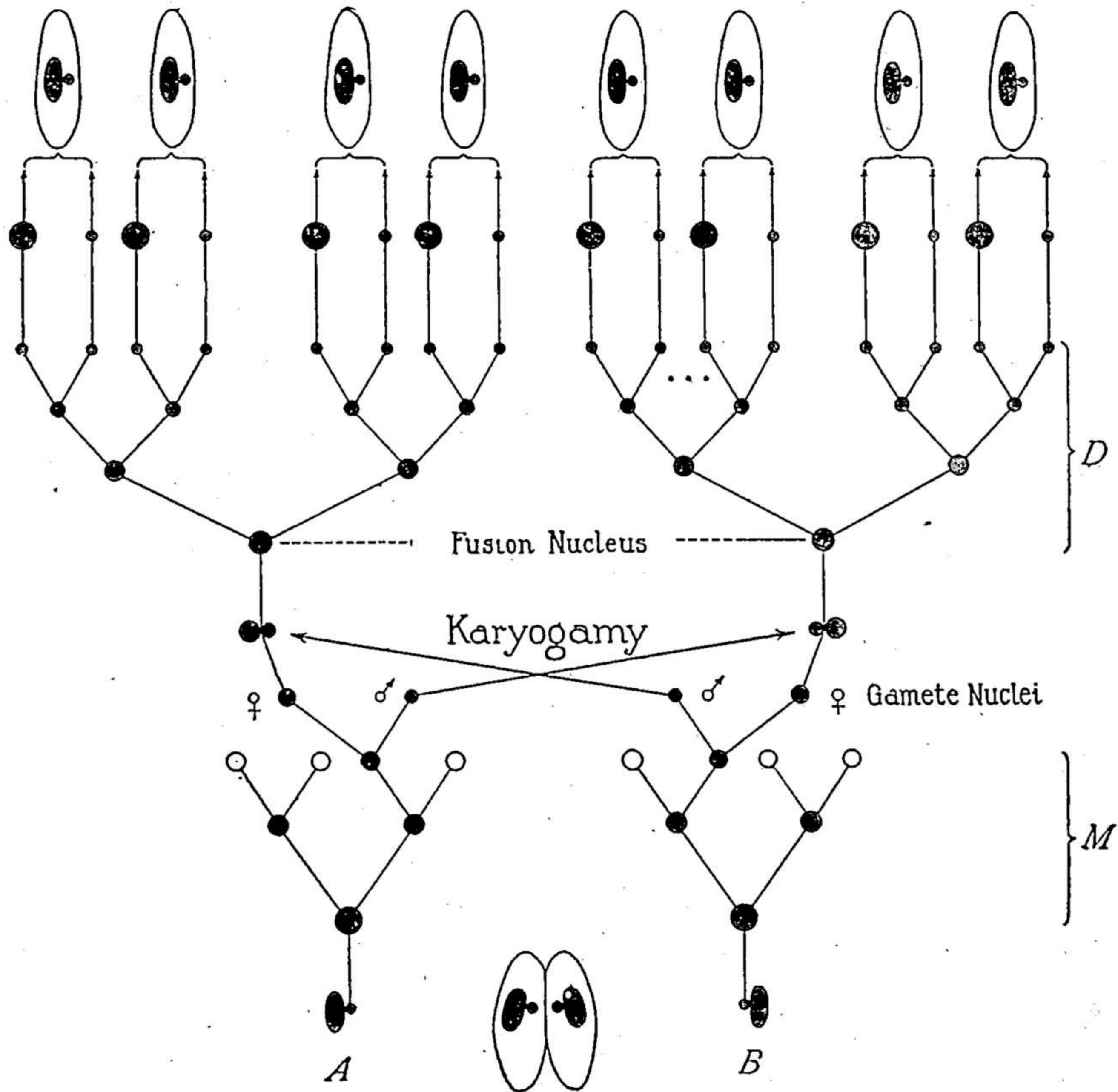
Fig. 299.—Diagram showing history of the nuclei in the conjugation of *Paramœcium caudatum.* (Following MAUPAS and CALKINS.)

A and B represent the nuclei of the two conjugants, M the supposed meiotic divisions of the micronucleus in each, and D the division of the zygote-nucleus in each conjugant (*cf.* Fig. 300, *I–M*); the final result of the three divisions gives four reconstructed ex-conjugants as show above.

because of its historical association with the experimental and cytological researches of Bütschli, Engelmann, Maupas, R. Hertwig, and their successors, on the general problem of senescence and rejuvenescence in unicellular organisms.

Partial conjugation is known only in the ciliates, is always hologamic, and usually isogamous. The general course of the process is largely con-

ditioned by the fact that the ciliates typically possess two kinds of nuclei, a large macronucleus and much smaller micronucleus, the former generally regarded as a vegetative or somatic nucleus, the latter as a reserve or generative one. Both nuclei divide at each mitosis; but in conjugation the micronucleus alone is concerned.[1]

We select as a type the classical object *Paramœcium caudatum*, in which a single macronucleus and micronucleus are present. In all cases the macronucleus degenerates and disappears during the process, and in species
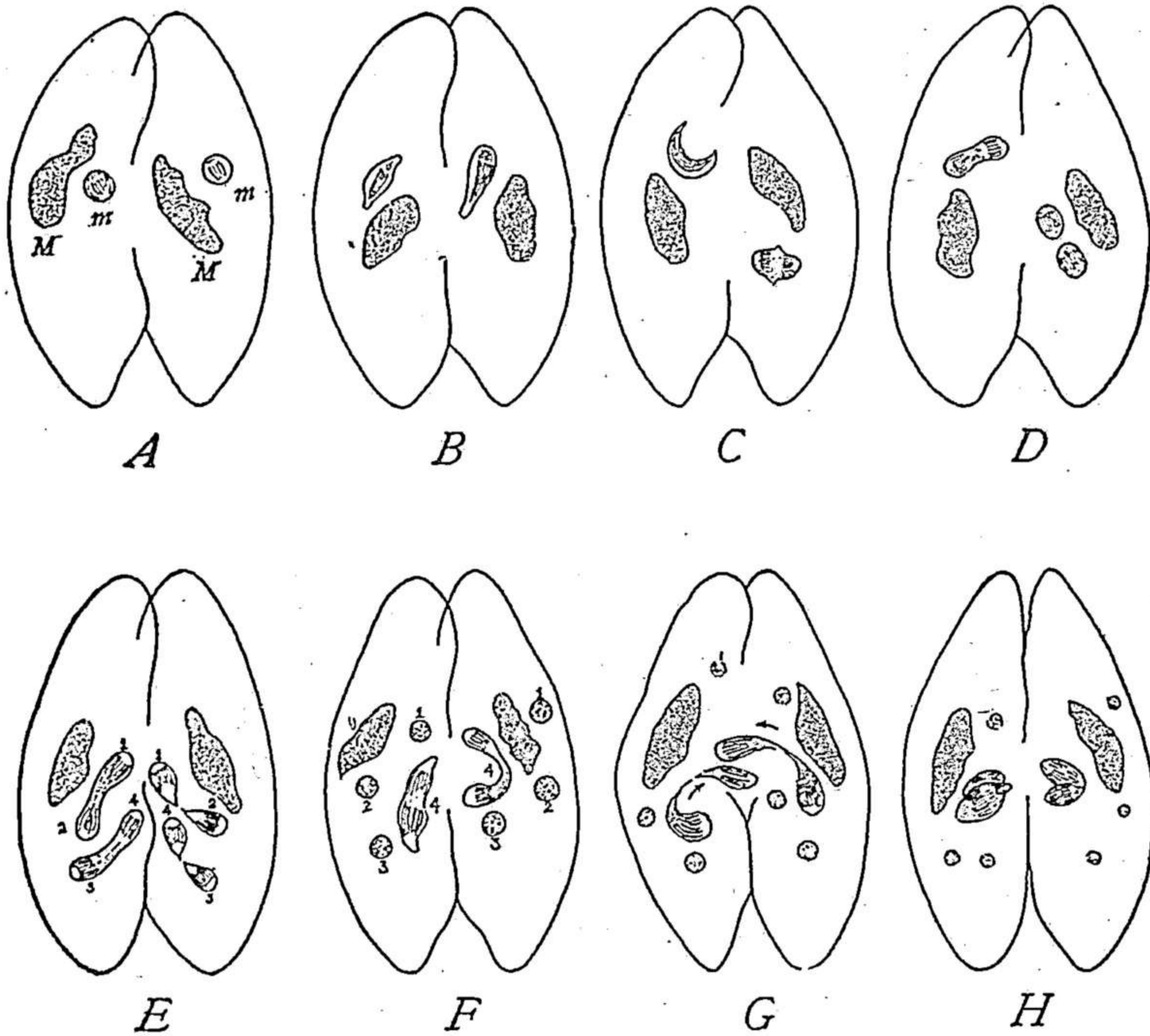
Fig. 300.—Conjugation and gametic meiosis in the ciliate *Paramœcium caudatum* (CALKINS).
A, the two conjugants (gametocytes) united; M macronucleus; m, mincronucleus; B–E, division of each micronucleus into four (meiosis); F, third division of one micronucleus in each conjugant 4 and 4) to produce the pronuclei, leaving three reduction-nuclei in each conjugant.

possessing more than one micronucleus all of them save one likewise degenerate. Although conjugation is here of the hologamic type the conjugants are measurably smaller than in ordinary vegetative forms, a fact noted by many observers and demonstrated by Pearl ('07) by extended statistical studies. Two such individuals become attached by their oral surfaces and partially fuse over a rather small area. This is followed by two successive divisions of the micronucleus, a little later by a fragmentation and final disappearance of the macronucleus, which becomes

[1] In many forms more than one micronucleus is present, and not infrequently more than one macronucleus.

irregular in shape (Figs. 299–301) and finally breaks up into large spheroidal granules, which finally disappear leaving no trace. Meanwhile three of the four products of the micronucleus in each conjugant degenerate as "reduction-nuclei" (or "polar bodies"), while the fourth remains as the only persistent derivative of the old nuclear apparatus.

A third division now ensues to form two spindle-shaped gamete-nuclei in each conjugant. One of these, formerly called the "female pronucleus"
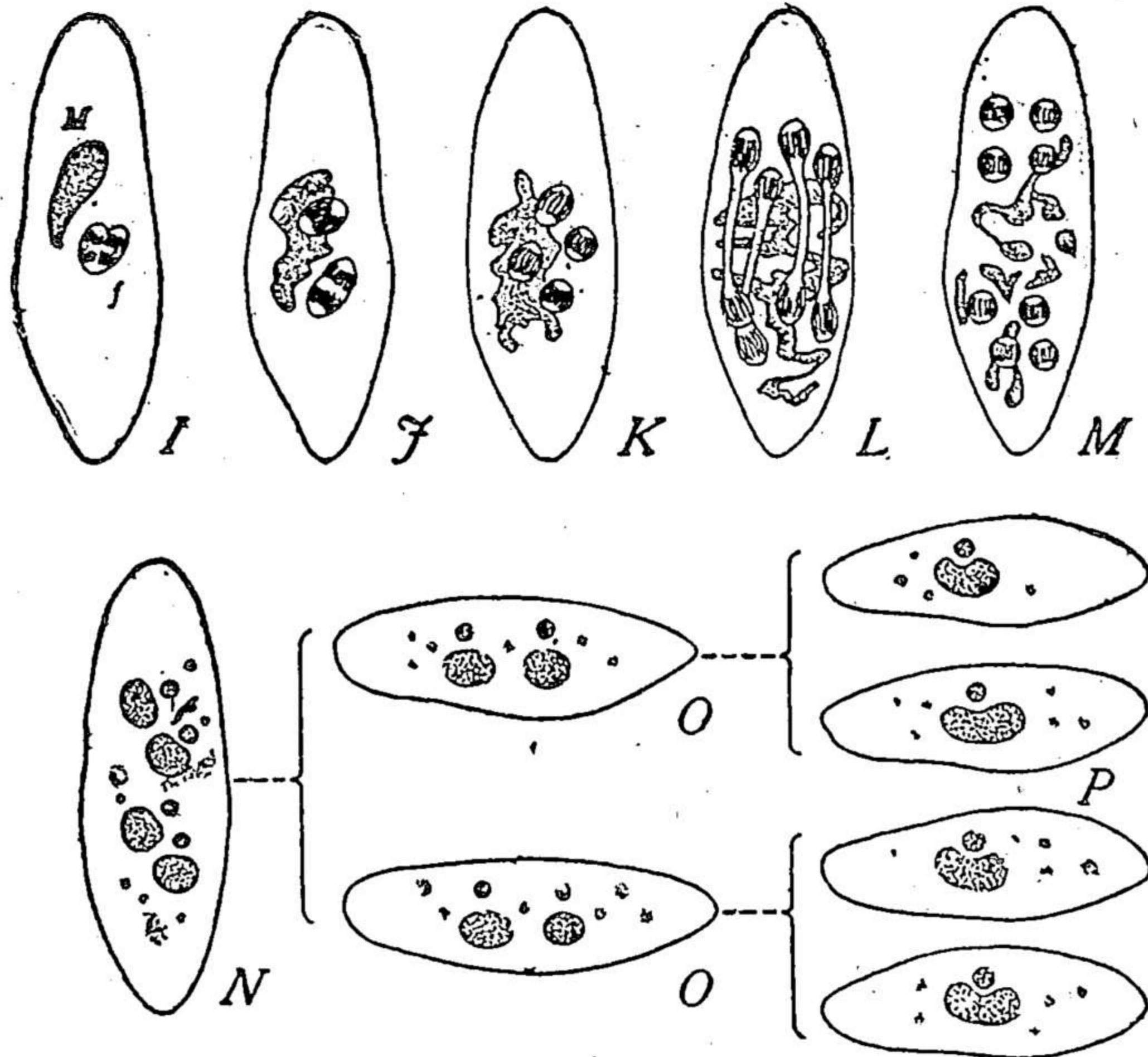
Fig. 301.—Later stages of conjugation in *Paramœcium caudatum* (CALKINS).

I, ex-conjugant (zygote), with fusion-nucleus (*f*) and macronucleus (*M*); *J-M*, division of fusion-nucleus to form eight small nuclei; breaking up of macronucleus; *N*, differentiation of four new macronuclei and four micronuclei; *O, P*, distribution of the products, by two fissions, to four resulting individuals of ordinary vegetative type.

and now usually designated as the *stationary nucleus* remains in the conjugant; the other or *migratory nucleus* ("male pronucleus") passes over into the opposite conjugant and there fuses with the stationary nucleus of the latter, after which the two conjugants separate. By this process the two conjugants have reciprocally fertilized each other, each now containing a single fusion-nucleus or zygote-nucleus, equally derived from the original micronucleus of the two. From this nucleus a new nuclear apparatus is rebuilt as follows: Separation of the conjugants is followed by three successive divisions of the fusion-nucleus in quick succession, producing in each ex-conjugant eight nuclei, of which four enlarge to form new macronuclei, while four remain small as micronuclei (Fig. 301). By two

ensuing fissions of the entire cell the four new macronuclei are distributed, one to each of the resulting individuals. In some species the four micronuclei seem to be distributed in the same way, but in *Paramœcium caudatum* (Maupas) [1] three of the four degenerate, to be replaced by derivatives of the fourth. In either case the final result is a group of eight individuals, each containing a micronucleus and macronucleus derived from the fusion-nucleus, and hence equally from the micronuclei of the original conjugants. The original macronucleus has meanwhile disappeared, and the cells have now entered upon a new cycle of vegetative activity during which one fission follows another in the usual manner. Essentially similar facts have
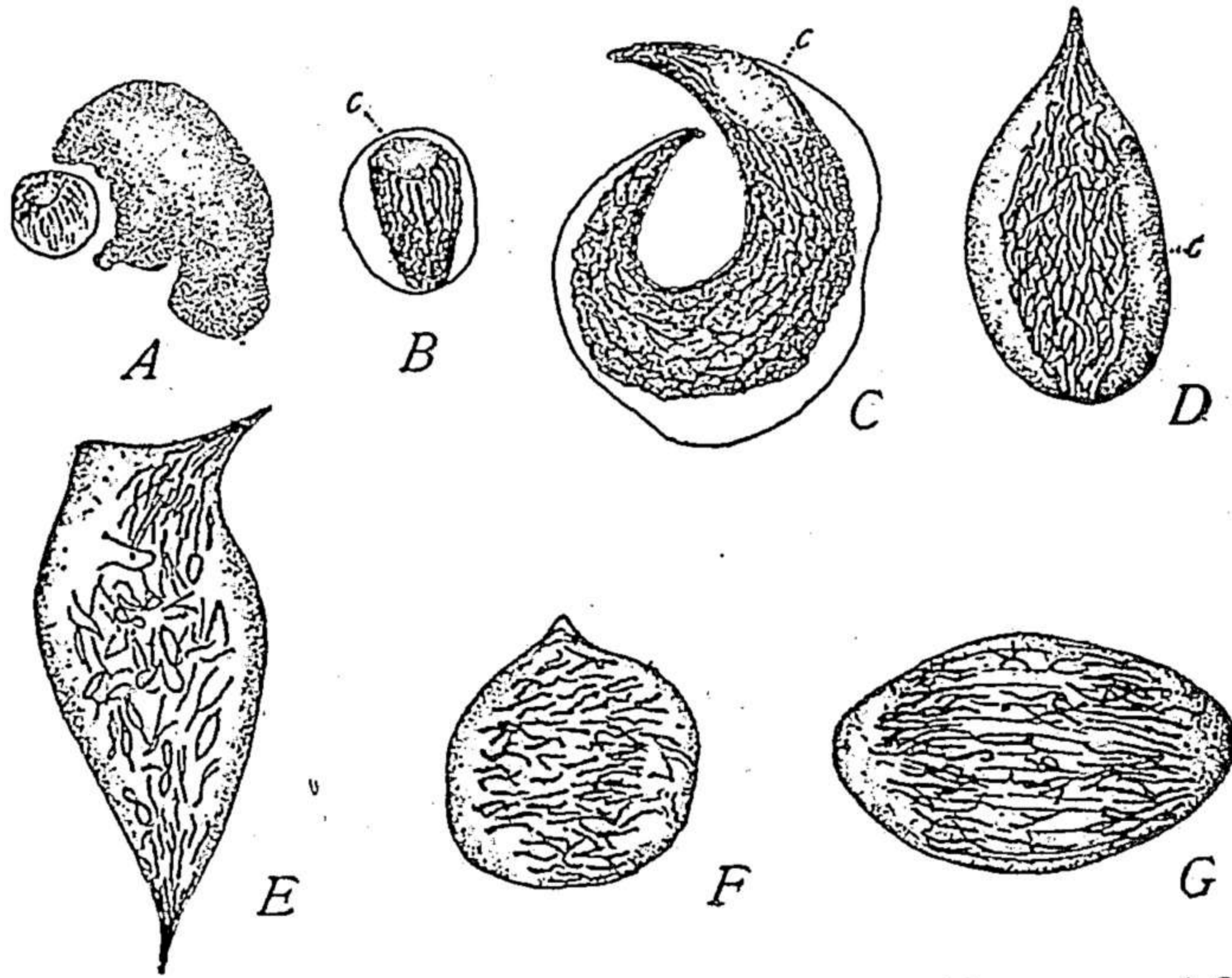
Fig. 302.—First division in *Paramœcium caudatum* (CALKINS and CULL).

A, macronucleus and micronucleus, initial stage; *B*, micronucleus elongating, with "division center"; *C*, crescent stage of same; *D*, *E*, later stages, formation of (bivalent?) chromosomes; *F*, *G*, anaphases.

been observed by many observers in a large number of forms. In cases of permanent conjugation, as in *Vorticella*, where a smaller microgamete unites with a larger macrogamete, the process is essentially the same, though the details are more complex.

Maupas and Hertwig concluded that the first two divisions of the original micronucleus (preceding karyogamy) are comparable to the maturation-divisions; and this is to a considerable extent borne out by more recent observations. Calkins and Cull ('07) offer a careful study of these divisions in *Paramœcium caudatum*, showing that in the early prophase the micronucleus first assumes the form of a spindle near the end of which appears a large, nucleolus-like body or "division-center," and then bends into a

[1] Maupas's conclusions on this point are confirmed by Klitze ('14) and (in *P. pritinum*) by Doflein ('07), but have been questioned by some observers. See Woodruff and Erdmann ('14).

characteristic crescent or sickle (as already described by Maupas and Hert-wig), regarded by Calkins and Cull as the synaptic stage (Fig. 302). Still later the crescent is converted into a mitotic anastral spindle by elongation in an axis at right angles to that of the crescent, while the "division-center" disappears from view, and the chromatin network of the nucleus gives rise to numerous elongate chromosomes, which show many of the characteristic forms of bivalents, such as rings, 8-figures, and Y's, and divide to form two groups. These observations seem clearly to show that the first division is heterotypic in form.

The third division is of especial interest because of the fact, first clearly made out by Prandtl in *Didinium* and confirmed by Calkins and Cull in
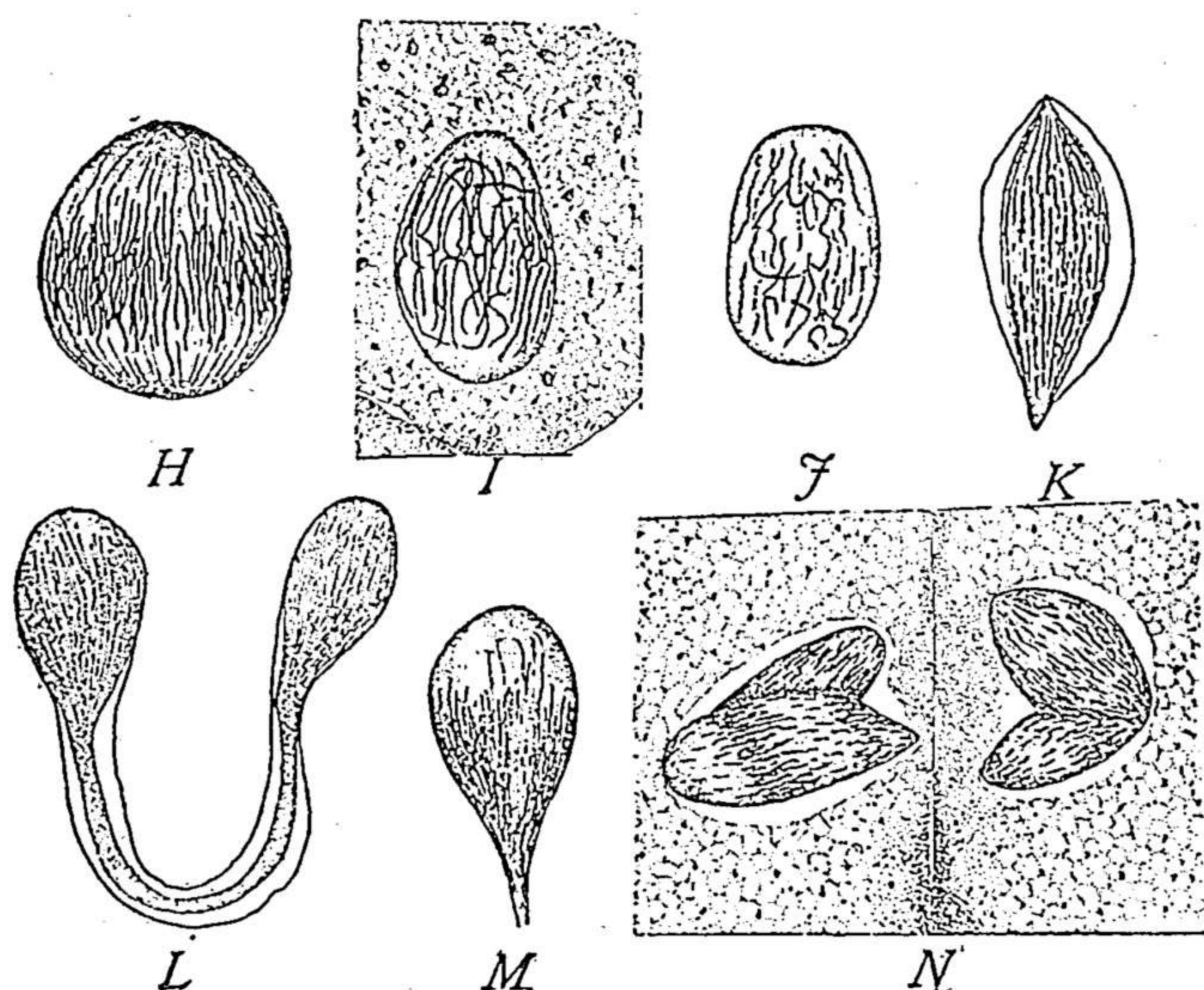
Fig. 303.—Later stages of maturation and syngamy in *Paramœcium caudatum* (CALKINS and CULL).

H, I, stages in the second division; *J, K,* the third division; *L,* telophase of third division, showing heteropolar condition; *M,* the larger pronucleus after the third division; *N,* karyogamy, showing size-difference of the gamete-nuclei.

Paramœcium, that it is in some cases heteropolar (Fig. 303), producing a larger and a smaller nucleus, of which the former is the stationary or "female" gamete-nucleus, the latter the migratory or "male." [1] Thus, although the conjugants appear to be isogamous the gamete-nuclei are distinctly anisogamous, a fact which sustains the view that the conjugating ciliate is a gametocyte (p. 582) and emphasizes the analogy between the conditions seen in ciliates and the hermaphroditism of higher forms. The third division, here obviously correlated with the reciprocal fertilization

[1] Dogiel ('23) has recently found, in the parasitic ciliate *Cycloposthium,* that the migratory nucleus in each conjugant is accompanied by a long cytoplasmic structure (derived from the spindle) so as closely to stimulate a flagellate sperm.

of each other by the conjugants, is unknown in higher animals and has no precise parallel in the total conjugation of most Protozoa (the Vorticellids are an exception). As will later appear (p. 616) it raises the question of an antithetic alternation of generations in the Protozoa. The facts also obviously suggest that the infusorian conjugant should be regarded as a gametocyte, derived from a form having merogamic conjugation and producing a large number of small gametes.[1] This view, now widely held,
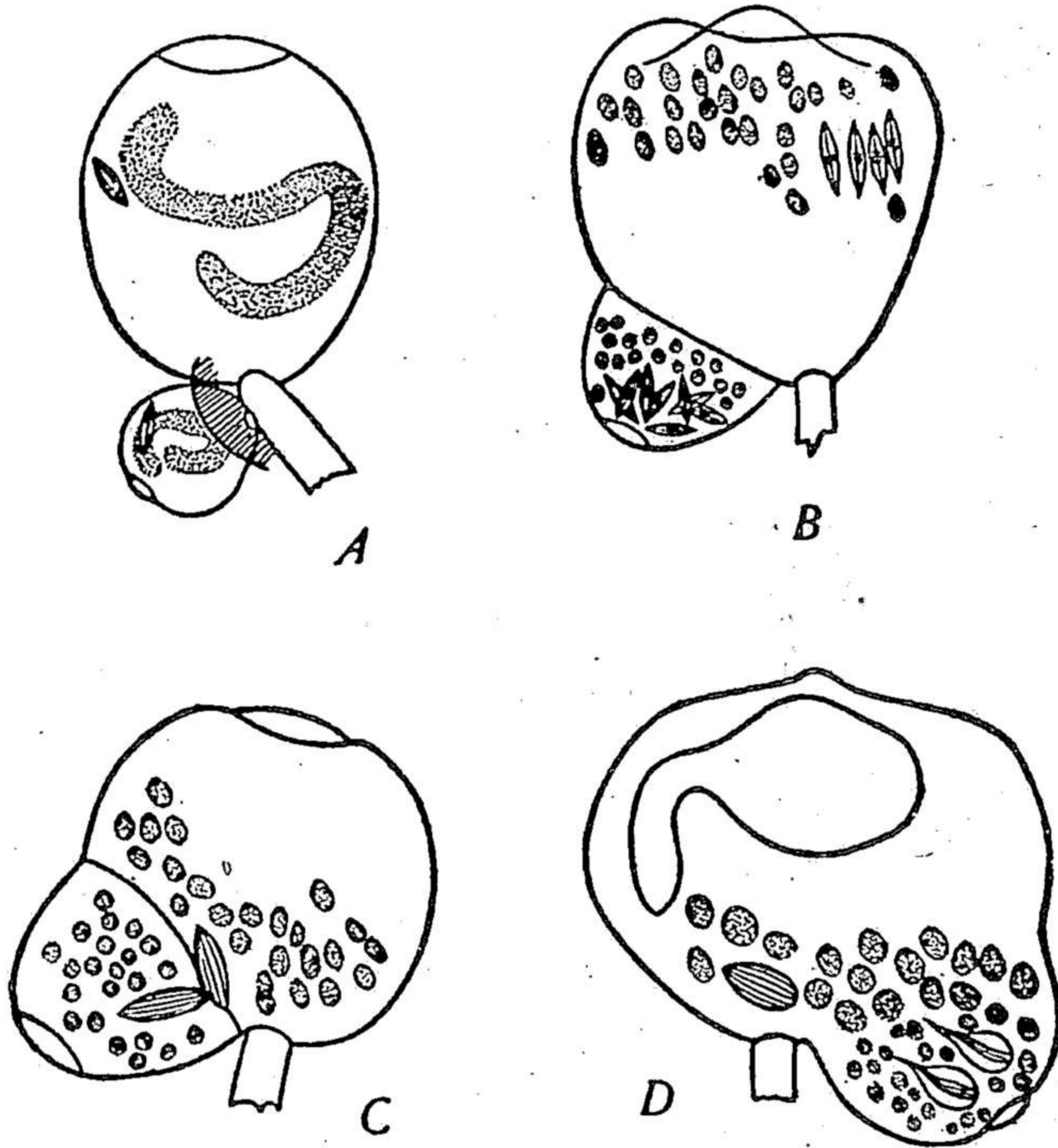
Fig. 304.—Conjugation of Vorticellids (MAUPAS).

A, attachment of the small free-swimming microgamete to the large fixed macrogamete; micronucleus dividing in each (Carchesium); B, microgamete containing eight micronuclei; macrogamete four (Vorticella); C, all but one of the micronuclei have degenerated as polar bodies or "corpuscules de rebut;" D, each of the micronuclei of the last stage has divided into two to form the pronuclei; two of these, one from each gamete, have conjugated to form the zygote-nucleus seen at the left; the other two at the right, are degenerating.

is supported by the fact that in the micro-conjugant of Vorticella (Maupas) and Carchesium (Popoff) the three divisions produce eight (instead of five) nuclei, while a fourth division of one of these gives rise to the gamete-nucleus (Fig. 304). In the multinucleate form Opalina, conjugation is actually merogamic in type, the vegetative cell dividing up into many small uninucleate or binucleate gametes (Metcalf, Neresheimer); but the significance of this is doubtful in view of the parasitic habit of Opalina and its wide divergence from the typical forms of ciliates.

[1] Lühe ('02), Popoff ('08), Hartmann ('09, '14).

To sum up: the most essential result of the process is to bring about in each conjugant a radical reorganization of the nuclear apparatus of both conjugants as follows:

(1) In each ex-conjugant the old macronucleus is wholly lost, to be replaced by one equally descended from both of the gamete-nuclei.

(2) The new micronucleus in each is likewise equally derived from both of the gamete-nuclei.

(3) The gamete-nuclei, though derived from the original micronuclei, are not identical with them, each of the latter having undergone the maturation divisions, during which, if we may accept an analogy with higher forms, a re-grouping of nuclear elements has probably taken place. This conclusion must, however, be accepted with some reserve.

5. Endomixis and Parthenogenesis

As earlier indicated (p. 243), nuclear reorganization may be accomplished by a process of *endomixis* in a single individual without any process of conjugation or amphimixis. This process is analogous to the asexual process of parthenogenesis in higher organisms, and bears a similar relation to the sexual process of conjugation. The cytological changes in *Paramœcium aurelia* (which possesses two micronuclei) are in broad outline as follows (Fig. 305).[1]

As in conjugation, the macronucleus disintegrates and finally disappears. Meanwhile the two micronuclei migrate away from their normal position beside the macronucleus, and towards the end of the macronuclear disintegration undergo two successive divisions, thus producing eight micronuclei in all. This, again, is precisely as in conjugation, but is not followed by the third division which in the latter case gives rise to the gamete-nuclei (p. 609).

The reconstruction is now initiated by the disappearance of all but one or two of the eight micronuclei. Most commonly each of these divides twice to form four products, of which one enlarges to form a new macronucleus, the other persists as a micronucleus. These are then distributed by one or two cell-divisions in such a manner that each resulting cell receives one macronucleus and one micronucleus, the latter finally dividing to form two which thereafter persist. This is closely analogous to the reconstruction following conjugation, as may be seen from the diagram (Fig. 305), which shows, at the right, one of the four variants of endomixis, recognized by Woodruff and Erdmann, for comparison with the conjugation-process, as shown at the left.

From a cytological standpoint the most noteworthy features of endo-

[1] Woodruff and Erdmann ('14).

mixis are: (1) the occurrence of two "maturation divisions" of the micronucleus which seem to be quite comparable with those that take place in conjugation; and (2) the lack of the third division which in the conjugating cell gives rise to the gamete-nuclei. At first sight this seems analogous to the diploid type of parthenogenesis in such animals as the aphids, or daphnids (p. 467); but further consideration reveals difficulties that can only be cleared up by further research. If we adopt the current assumption that reduction takes place during the first two micronuclear divisions we
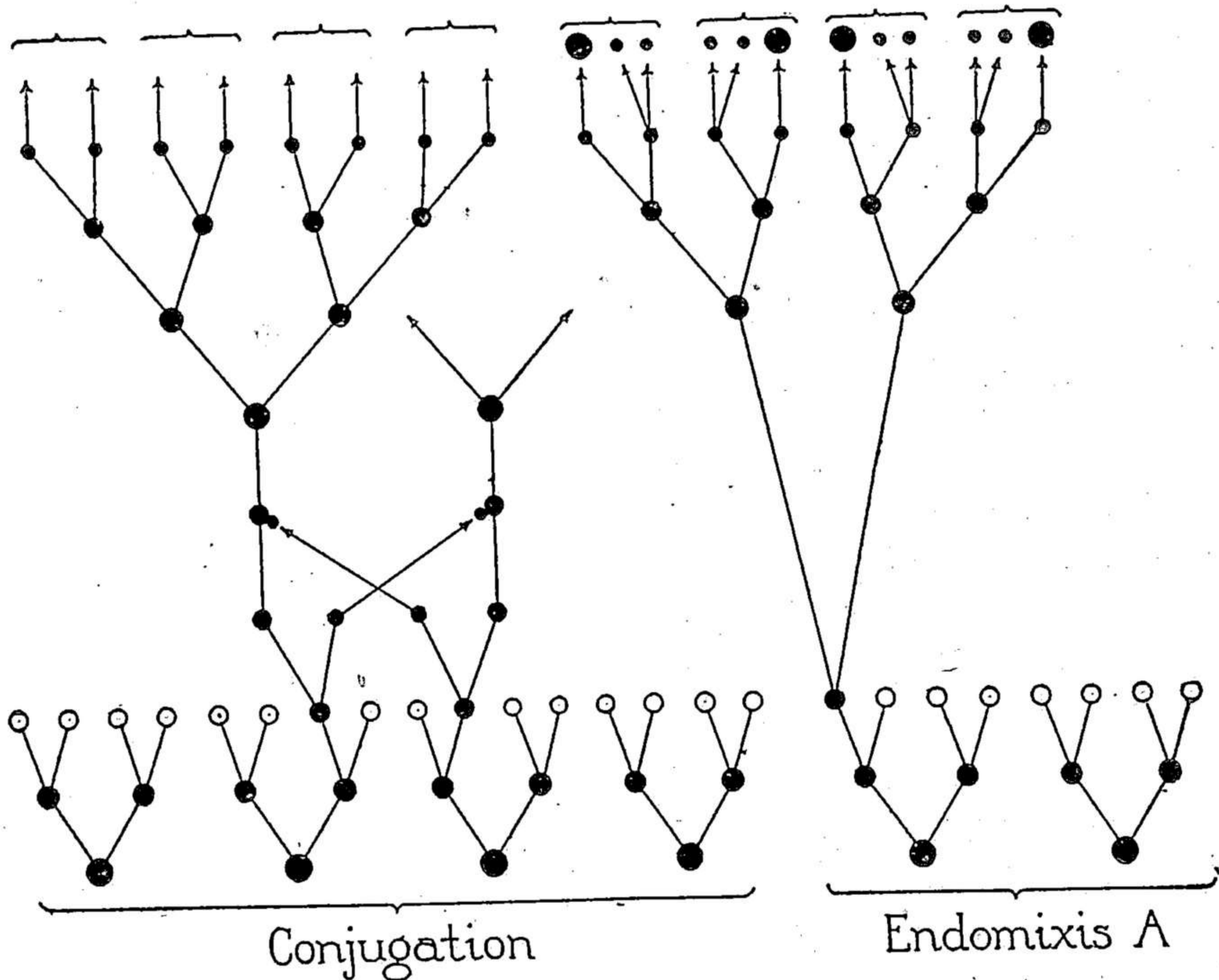
Fig. 305.—Diagram, Comparison of Conjugation and Endomixis showing history of the micronuclei (after WOODRUFF and ERDMANN).

fall into the following dilemma. If the two divisions in endomixis likewise effect reduction, then the reorganized *Paramœcium* and all of its descendants should be of haploid nuclear constitution, which contradicts the primary assumption concerning the maturation divisions. The alternative to this is to suppose either that a process of autogamous syngamy has been overlooked in endomixis, or that a compensatory doubling is effected. All difficulties would disappear under the conclusion that it is the third division by which reduction is effected; but this is in contradiction with the results of all previous cytological investigation of the problem (p. 610). This whole question must therefore be left open for the present.

As a supplement to the foregoing brief outline, attention may be called

to the possibility that endomictic reorganization may be widespread among Protozoa in connection with the process of encystment. The latter process, common in Protista, offers a means of protection against or recovery from the effects of unfavorable conditions, and is often accompanied by changes in the consistency of the protoplasm and nucleus and the loss and subsequent redifferentiation of various specialized cell-organs, such as cilia. Encystment may or may not be preceded by a process of conjugation, and in either case it may be followed by a marked renewal of vitality as shown by the division-rate; in *Didinium nasutum*, for example, Calkins ('16) found that encystment (without conjugation) is followed by a division-rate from five to seven times that of the same rate prior to encystment. A number of the earlier observers described marked changes in the nuclear apparatus at this time; and more recently it has been shown that these changes involve a nuclear reorganization of the same general type as that effected by endomixis or by conjugation. In *Stylonychia* (Fermor, '13) and in *Didinium* (Calkins, *op. cit.*) it was found that the old macronucleus breaks up and degenerates while a new nuclear apparatus arises by the division of the micronucleus and subsequent differentiation of its products.[1] Many interesting possibilities for further researches on encystment are here raised; though this process and endomixis are of course not necessarily connected.

III. GENEALOGICAL CONSIDERATIONS

The phenomena of reproduction in lower organisms bring to our attention three closely connected historical problems, namely, the origin of syngamy, the primitive relation between syngamy and meiosis, and the origin of antithetic alternation of generations. None of these has yet been completely solved, but certain interesting possibilities may here briefly be considered.

1. The Origin of Syngamy

This question still remains in the stage of more or less plausible guesswork. It has been suggested that syngamy first arose as a process of reciprocal cannibalism or "autophagy," two unicellular organisms devouring each other to the refreshment and advantage of both. This notion, suggested by Van Rees ('87), is developed in a suggestive manner by Dangeard ('99). In his view the gametes are "hungry cells" which lack sufficient energy to continue their development, but are rescued by a union of their resources. Such a speculation is more picturesque than convincing; never-

---

[1] According to Fermor the reorganization in *Stylonychia* is preceded by a conjugation of the two micronuclei. If this is correct endomixis here would seem to approach autogamous conjugation.

theless, as an historical explanation of syngamy it makes a certain appeal to the imagination. More plausible, perhaps, is the assumption of some kind of quantitative difference between the primitive gametes, the prototype of which appears in the suggestion of Bütschli [1] that syngamy and the primitive gametic differentiation resulted from imperfections in the mechanism of cell-division and consequent inequalities of distribution of the nuclear components. Thus arose some cells having an excess of chromatin, others a deficiency; and by the union of such contrasting individuals (now gametes) the normal balance was restored.

By later writers this hypothesis was given a different direction in accordance with certain views concerning the dualism of the cell-substance. Strasburger ('92, '98) assumed that the primitive gametic differentiation arose through variation in the relative amounts of trophoplasm and kinoplasm (p. 723), the female gamete containing an excess of the former, the male gamete of the latter. Syngamy, the result of a chemotactic interaction between the two, restored the normal balance and thus became a condition of survival. This conception emphasized the cytoplasmic differences of the gametes rather than the nuclear. On the other hand, the hypothesis of Schaudinn ('05) and his followers, assumed the primitive gametes to have differed in respect to "kinetic" and "trophic" chromatins, the former relatively in excess in the male, the latter in the female (p. 726).[2] More recent cytological studies on sex have in fact given some reason to suspect that the primitive sex-differentiation may have taken its origin in a quantitative difference of chromatin—perhaps a particular kind of chromatin—a difference at first irregular and fluctuating, later regular and constant, as is now the case in many higher animals (p. 816). "The sexual differentiation may therefore be rooted in a simple principle of plus and minus that holds true of all sexual organisms and may be an expression of a fundamental principle of metabolism." [3] The weak point in this hypothesis is its failure thus far to give us a clear view of the origin of hermaphroditism and its relation to diœcism.[4] To spin theories concerning this inaccessible field of inquiry is, however, no part of our purpose.

2. The Origin of Meiosis and Its Primitive Relation to Syngamy

This question is closely bound up with that of the origin of antithetic alternation, from which it is here separated only as a matter of convenience. By Weismann and other earlier writers meiosis was treated teleologically as an adaptive process, though which the gametes are "prepared" for their

[1] ('87–'89.)
[2] See Prowazek ('07), Hartmann ('09); also the critical reviews in Doflein ('11), and Minchin ('15)
[3] Wilson, '10 '11), p. 266.
[4] Cf. Doflein ('11), Minchin ('12, '15).

subsequent union in syngamy and the summation of the germ-plasms in successive generations prevented. This view involved, tacitly at least, the assumption that the diploid number was historically the original and fundamental one, and the haploid a derivative from it. Great difficulties stand in the way of such a view; for it provides us with no explanation of the double character of the diploid groups or the mechanism of meiosis. The problem suddenly took on a new form with Strasburger's ('94) [1] illuminating suggestion that the reverse conclusion is more likely to be, the correct one, namely that the haploid condition was the original one, inherited from an early prototype in which conjugation or fertilization had not yet arisen. Thus regarded, the periodic doubling of chromosomes appears as a secondary or derived phenomenon, a consequence of syngamy. "The morphological cause of reduction and the equality of chromosome-number in the germ-cells of each species is, according to my view, a phylogenetic one. I consider the process as a return to the original generation from which, through the development of the sexual process, first arose forms possessing the double chromosome-number . . . . The reduction of this number to one-half represents the restoration of the original number present in the nuclei of those organisms in which the sexual process first took its origin" (*op. cit.*, p. 523). This conclusion was suggested by the fact that the gametophyte and sporophyte generations in plants are characterized respectively by the haploid and diploid numbers of chromosomes respectively (p. 492). Its plausibility was increased by the later discovery of haploid parthenogenesis even in bees and other animals of relatively high grade of organization (p. 794). There is, therefore, nothing unreasonable in the hypothesis that the primitive organisms were asexual (as appears still to be the case in the Bacteria and Cyanophyceæ), that they were of haploid constitution, and that the diploid condition was a later acquisition.

It is of course unknown at what period in the life-history the process of reduction primitively took place. Theoretically, we might perhaps expect meiosis to have been originally of the zygotic type, *i. e.*, to have followed soon after syngamy as a kind of regulative process by which the original haploid condition was quickly restored; and it is interesting to note that the only cases of this type occur in very simple forms such as the algæ and the Protozoa.

3. Antithetic Alternation of Generations

Before the announcement of Strasburger's conclusions botanists had begun to turn towards the early view of A. Braun (1877) that the gametophyte or sexual generation of higher plants represents the older and preëxistent

[1] This remarkable work abounds in fruitful suggestions bearing on the whole series of problems here involved.

generation, and that the sporophyte is a neomorph, secondarily interpolated into the life-history. This view, developed in detail and with much ingenuity by Bower ('90, '94, '19) and later writers, has been widely accepted by botanists. The origin of the sporophyte was sought in a division of the zygote to produce spores, such as still occurs in certain of the algæ.

"The gradual development of this generation (the diploid) from the sexual product of the first generation can be actually traced step by step phylogenetically. The first indication of this development is apparently to be found in the Algæ; at least the life-history of Œdogonium, Coleo-
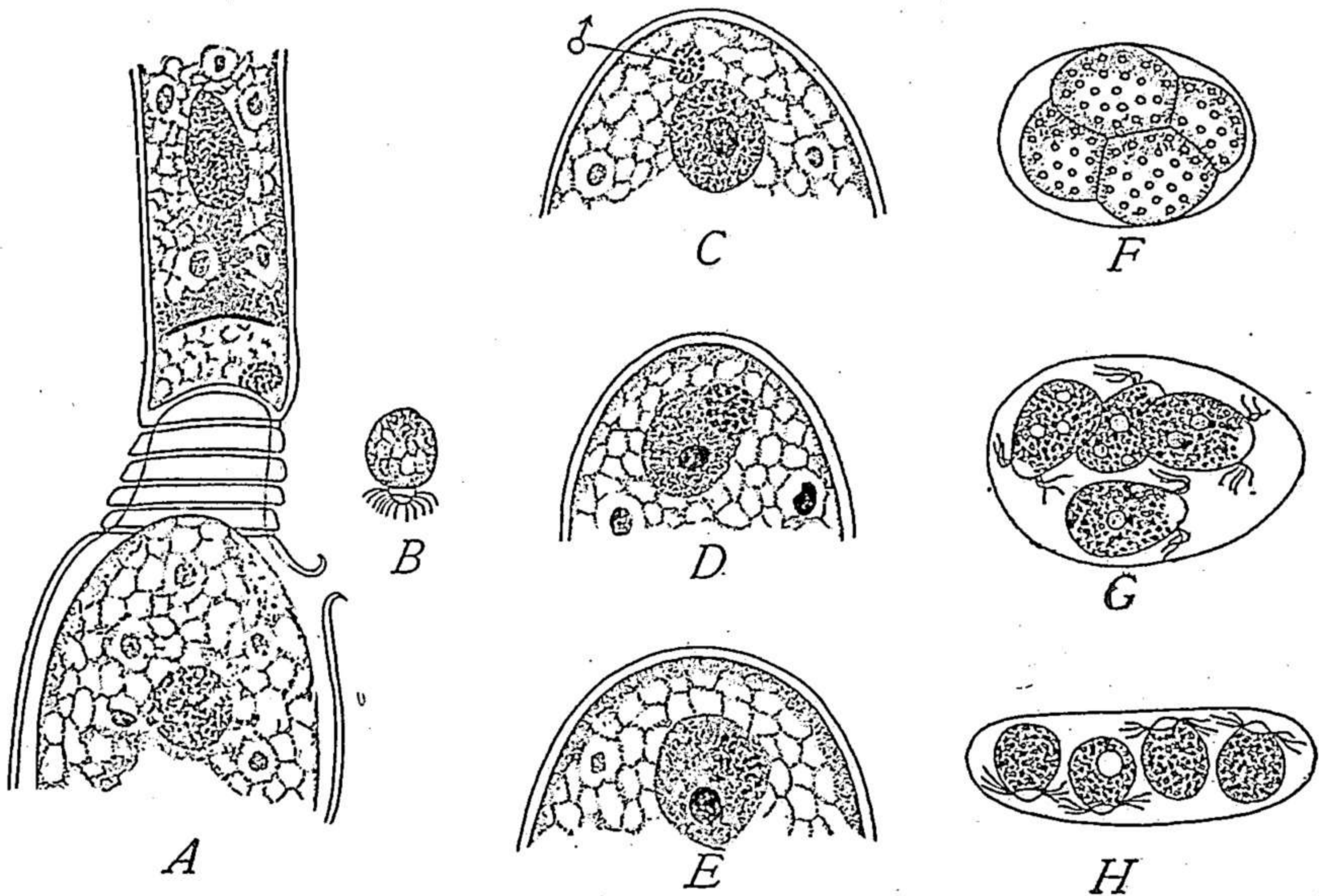
Fig. 306.—Fertilization in the alga Œdogonium (A–E, from KLEBAHN; F, G, from JURANYI; H, from PRINGSHEIM).
A, oögonium (below), just after opening, containing single ovum; B, sperm-cell; C–E, union of gamete-nuclei in the egg; F, G, division of zygote (possibly zygotic meosis) to form four zoöspores; H, corresponding quartet in Bulbochæta.

chæte, and the Floridea, may be interpreted in this sense. In Œdogonium, four swarm-spores are formed from the fertilized ovum (see Fig. 306); whilst in Coleochæte a small multicellular body is developed from the cells of which swarm-spores are formed; in both cases the swarm-spore gives rise to the first generation. In the Floridea the cystocarp is developed from the fertilized ovum, and the spores of the cystocarp give rise to individuals of the first generation. The Muscineæ and the Pteridophyta can readily be traced to the Chlorophyceæ: in the Muscineæ the fertilized ovum gradually developed into a sporogonium, and, in the Pterodophyta, into a sporangium-bearing cormophytic plant."[1]

[1] Strasburger ('94, p. 285). Cf. the discussion of Klebs ('98), Campbell ('05), Lotsy ('05), Cook and Swingle ('05).

Following strictly the logic of his theory, Strasburger adopted a similar interpretation of animal evolution, suggesting that in animals the haploid or gamete-producing generation has all but disappeared, its last vestiges *being represented by the three polar bodies or polocytes formed during the maturation of the egg.*[1] *This hypothesis assumed more definite shape in* the development given it by Chamberlain ('05, '08) and his followers, who
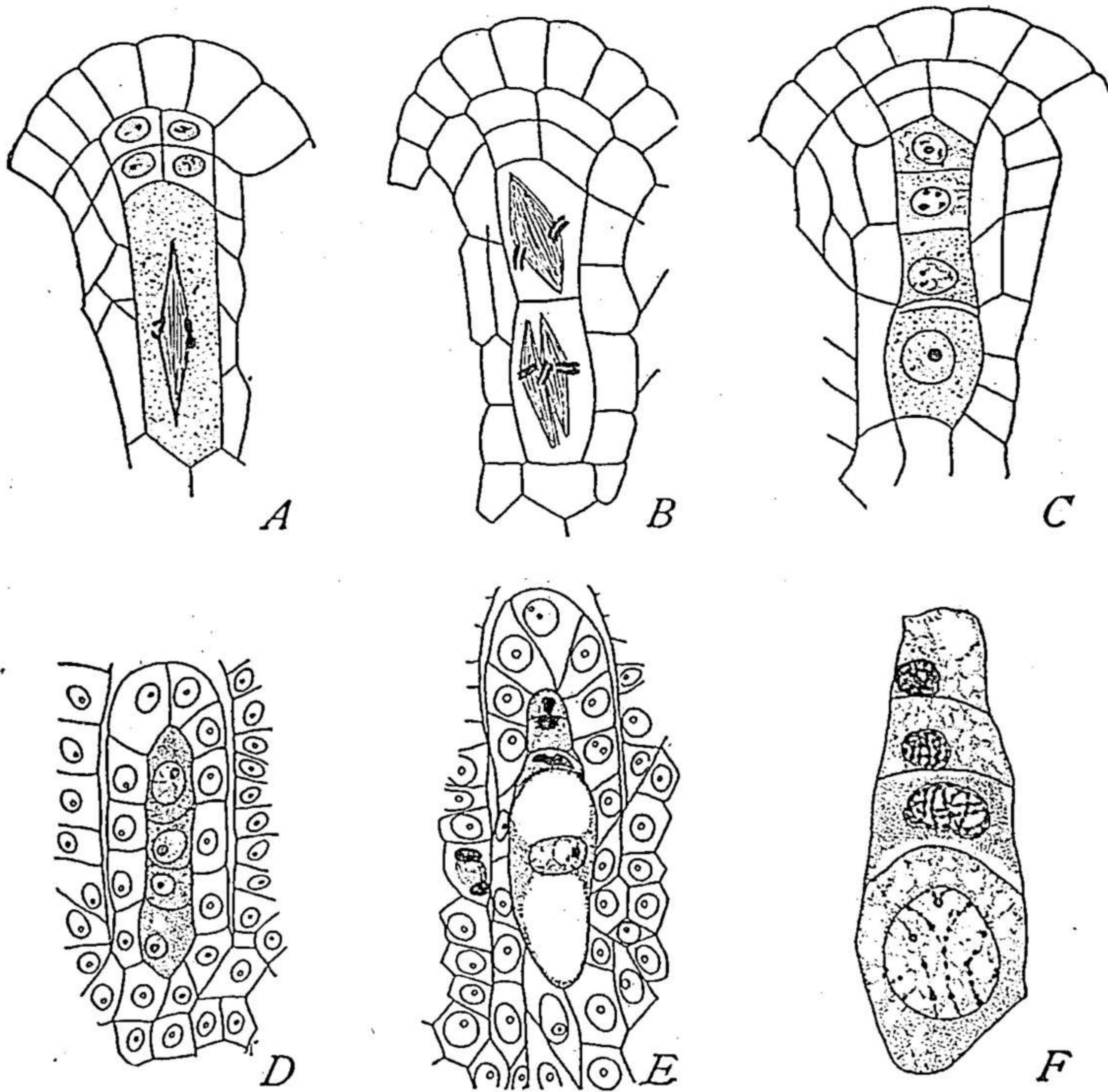
Fig. 307.—The megaspore-quartet in seed-plants.

(*A–C*, from SCHNIEWIND-THIES; *D*, *E*, from MERRILL; *F*, from FERGUSON.)

A-C, division of megaspore mother-cell (primary sporocyte) of *Galtonia* to form the four megaspores; *D*, early megaspore-quartet in *Silphium*; *E*, later stage of same, enlargement of inner cell to form functional megaspore; *F*, early stage of megaspore-quartet in *Pinus*.

have pointed out the close analogy between the animal egg with its three polocytes and the megaspore-quartet and its products in the higher plants. In both cases, only one of the four cells is functional (in the animal, this cell is the egg, in the plant the embryo-sac), while the other three are reduced in size and sterile (Fig. 307). In both cases the four cells of the

---

[1] Conclusions more or less akin to this have been suggested by a number of other writers. Whitman ('78) early suggested that the polar bodies might be the remnant of a former sexual generation, though the chromosome relations were at that time unknown. Beard ('95a), following out the suggestions of Strasburger and the earlier ones of Bower ('87) on apospory, endeavored to show that metazoan development actually involves an antithetic alternation. Lotsy ('05) considers that the animal body is an asexual phase or diploid generation, while the sexual phase or haploid generatior is confined to the germ-cell. These views are well criticised by B. M. Davis ('05).

quartet result from the two meiotic divisions, in the course of which the number of chromosomes is reduced from diploid to haploid. The analogy between the three vestigial megaspores and the polocytes is obvious.

How the condition seen in animals might have been reached is clearly shown by an almost continuous series of intermediate conditions still exist-
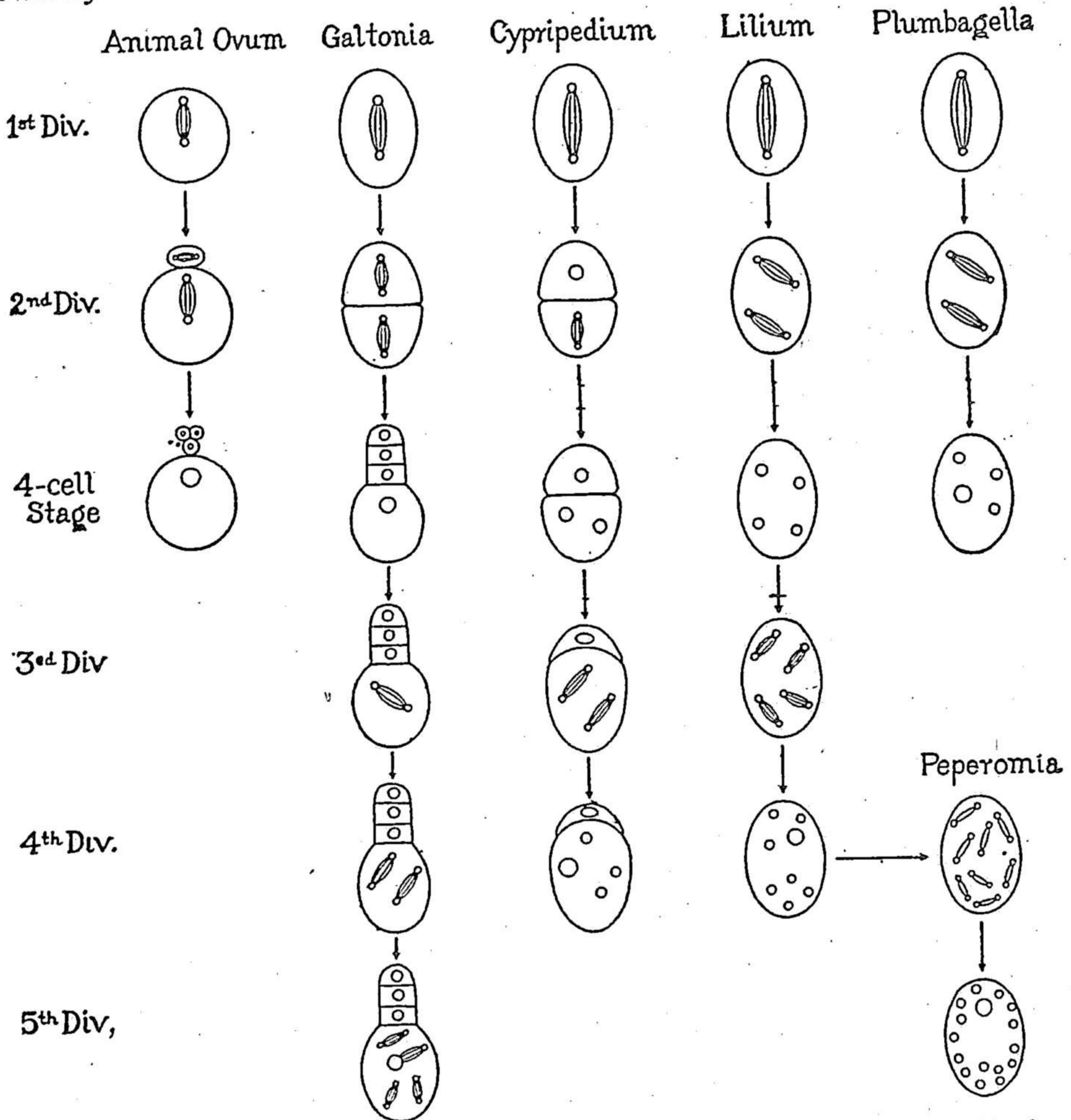
Fig. 308.—Diagram illustrating the parallelism between meiosis in the animal egg and in the megaspore (or embryo-sac) in angiosperms. Based on diagrams by CHAMBERLAIN, PACE and others. Galtonia shows the typical condition in angiosperms, with three abortive megaspores and one functional (embryo-sac) the latter producing eight nuclei (one egg, seven prothallial cells). In Cypripedium but two actual megaspores are formed; in Lilium, Peperomia and Plumbagella the primary mother-cell is directly converted into the embryo-sac. The total number of divisions varies, as shown.

ing in the seed-plants. In all cases reduction is effected by the first two divisions of the spore-mother-cell (typically producing the megaspore-quartet), and in nearly all these are followed by a certain number of additional divisions in which the haploid chromosome-number persists, thus

giving origin to a more or less reduced haploid generation or female gametophyte. The number of these divisions in different species varies (Fig. 308), and with it the degree of the reduction of the female gametophyte. In many gymnosperms, e. g., in *Pinus* (following Chamberlain's discussion) the embryo-sac produces thousands of nuclei, in *Gnetum* they are numbered by hundreds, while in the angiosperms the number is usually reduced to eight. A slight further reduction would produce a condition closely analogous to that seen in animals, and such a case has actually been reported recently by Dahlgren ('15) in *Plumbagella*, a form in which (as in *Lilium*) both megaspore-producing cleavages have been suppressed and the primary sporocyte is directly converted into the embryo-sac. The nucleus of this cell divides but twice altogether (meanwhile undergoing reduction), one of the four resulting nuclei becoming the egg-nucleus, one an antipodal cell, while the remaining two fuse to form the endosperm-nucleus (*cf.* p. 454). Here the whole haploid generation (other than the egg) is reduced to three cells, just as Strasburger conceived to be the case in animals. The relation of this case to the more typical ones in the angiosperms is indicated by the diagram (Fig. 308), as explained in the subjoined footnote.[1]

From a merely logical standpoint, clearly, the general comparison, as above outlined, is quite admissible; but it rests upon a mere analogy, and one that probably indicates no real genetic relationship between the two cases. Among the Metazoa there is no trace of such a mode of evolution.

[1] This relation can only be fully grasped when we take into account the total number of divisions involved, including those by which the megaspores are formed. This number is typically five, of which the first two are always the meiotic divisions (heterotypic and homeotypic respectively) however the external aspect of the process may vary otherwise. Typically (as in *Galtonia*), these two divisions produce four actual megaspores (one functional and three vestigial), while the remaining three take place within the functional megaspore (now the embryo-sac) to produce eight nuclei; one of the latter is that of the egg while the other seven constitute the vestigial gametophyte (p. 496). This process may be modified and abbreviated in two ways, namely, (1) by the suppression of one or both megaspore *cleavages* (the nuclear divisions taking place as usual), and (2) by reduction of the total number of divisions from five to four, three or finally to two. When both megaspore protoplasmic cleavages are omitted (as in *Lilium*) the primary sporocyte or megaspore mother-cell is directly converted into the embryo-sac and the meiotic divisions take place as the first two nuclear divisions within it. One or both megaspore-producing divisions may thus, as it were, be telescoped into the embryo-sac; and by the continuation of this process, together with a reduction in the total number of divisions, have arisen many curious modifications of the typical process, some of which are shown in a schematic form in the diagram (Fig. 308). The typical process is here illustrated by *Galtonia*, where the meiotic divisions produce one functional and three vestigial megaspores, followed by three other divisions in the embryo-sac. In *Cypripedium* (Pace, '07) the number of divisions is reduced to three, the first meiotic division producing one vestigial macrospore and one functional (the embryo-sac), while the second and third take place in the embryo-sac. The latter, accordingly, contains but four nuclei, one of which is the egg-nucleus. In *Lilium* and some other cases the number of divisions is likewise reduced to three; but the result is different, since the spore-mother cell is directly converted into the embryo-sac; and the three divisions take place within the latter, thus producing eight nuclei. In *Plumbagella*, the process is the same as in *Lilium*, but only two divisions take place, giving a result like that in *Cypripedium*, but again by a different process. In *Peperomia*, finally, the process begins as in *Lilium*, but is followed by a fourth division, producing 16 nuclei.

The lowest Metazoa (sponges, cœlenterates) show precisely the same conditions as the highest; and nowhere in the great series of forms between these extremes do we find an antithetic alternation between haploid and diploid generations, unless we choose so to regard the case of the Hymenoptera and Rotifera, where the males are haploid, the females diploid. With these very special exceptions alternation of generations in Metazoa, in particular in the Cœlenterata, is of the homologous type, i. e., both sexual and asexual generations are diploid; and this is even true, as Hartman has shown, of the Dicyemids, which are among the simplest of the Metazoa, though probably degenerate forms. By the zoölogist, accordingly, the view that the condition existing in animals generally has resulted from the gradual loss of a haploid generation, or gametozoön, can hardly be taken seriously. At best such an hypothesis can only have application in the earliest stages of sexual evolution, and in Protista.

Strasburger's view clearly implies that meiosis was originally of the zygotic or initial type; and this has met with some support in the demonstration that this type of meiosis has been more or less clearly demonstrated, or made probable, in a considerable number of forms that are unicellular or represent simple types of cell-aggregates. This type of reduction seems also to occur in various other simple algæ, including *Coleochæte*, desmids, certain diatoms, the Zygnemaceæ, in some of the red algæ (*Scinaia*, *Nemalion*, *Batrachospermum*) and possibly in some other cases. That the zygotic type may be still more widely prevalent is suggested by the formation of four swarm-spores from the zygote in *Œdogonium*, *Bulbochæte* (Fig. 306) *Hydrodictyon* and other cases, and also by the frequent formation of four spores in the Basidiomycetes, or of four (and then of eight) spore nuclei in the Ascomycetes, in each case preceded by a process of nuclear fusion.[1] It must, however, be borne in mind that the sexual phenomena in fungi are believed to be in many cases of highly modified or quite secondary nature.

The foregoing cases may well be cited in favor of the view that zygotic reduction is a very primitive and perhaps original type. On the other hand, some studies in this field lead us to consider the possibility that antithetic alternation may likewise have been a very early type. It seems admissible to assume that the primordial process of syngamy may from the first have been followed by a considerable series of diploid divisions before reduction took place, thus producing immediately an antithetic succession of haploid and diploid cycles.[2] Such a primitive succession, we may surmise, is likely to have been at first irregular and facultative, only later becoming estab-

---

[1] See Blackmann ('04), Harper ('10), Guilliermond ('13), Bower ('19), etc..
[2] The view here suggested is essentially in harmony with that advocated by W. H. Lang ('09) in his suggestive discussion on the origin of alternation in the archegoniate plants.

lished as a regular alternation. Such a succession may well have formed the starting point from which arose all the existing types of meiosis, the sporic by persistence of both haploid and diploid cycles, the zygotic by the reduction and final disappearance of the diploid cycle until only the zygote remained, the gametic by a similar disappearance of the haploid cycle, until nothing remained but the two meiotic divisions.

Some such hypothesis as this, I venture to think, claims our attention because of the fact, now apparently well established, that antithetic al-
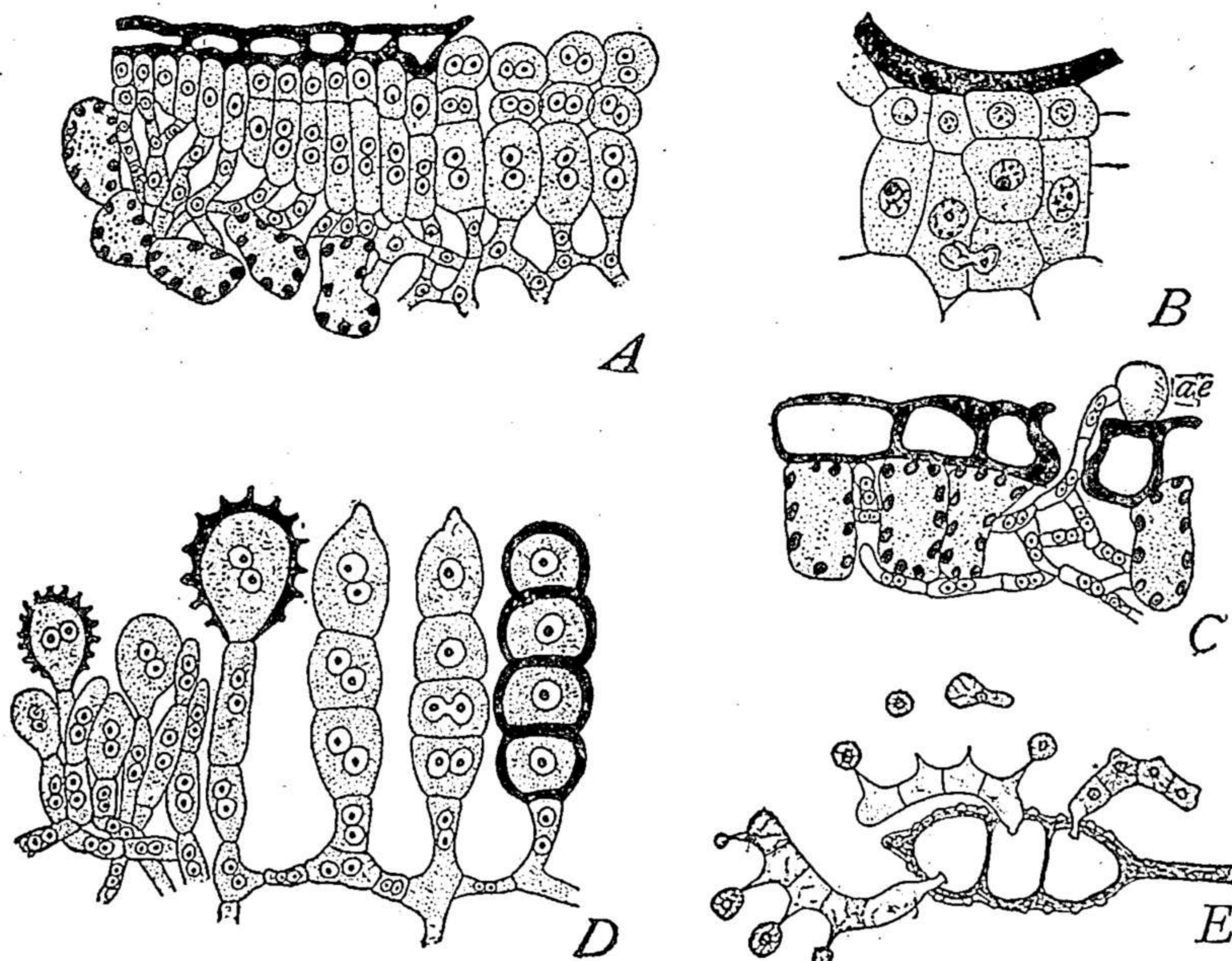
Fig. 309.—Alternation of generations in the rusts (Uredinales) slightly schematized (*A–D*, GUILLERMOND; *E*, SAPIN-TROUFFY).
(All the figures from *Phragmatobium violaceum*.)
A, portion of the æcidium formed by the uninucleate mycelium with binucleate æcidiospores above (products of the diploid zygote); *B*, supposed fertilization in cells of the uninucleate mycelium (gametophyte) by migration of nucleus; *C*, germination of the binucleate æcidiospore (*æ*), producing binucleate mycelium (sporophyte), here entering stoma of wheat; *D*, formation of binucleate teleutospores in which fusion of the two nuclei (? synapsis) finally takes place; *E*, three teleutospores germinating to produce promycelium and four basidiospores by two divisions, believed to be of meiotic type; from these spores arises the original uninucleate mycelium (*A*) or gametophyte.

ternation between independent and equally well developed generations already exists in certain of the algæ and fungi (p. 626).[1] Though the fungi are perhaps too far removed from the direct line of evolution of higher plants to bear very directly upon our general problem they display a highly

[1] No attempt will here be made to consider the problems of sexuality in the fungi generally, for the homologies of the gametes, and the relations of the phenomena to those seen in algæ are too doubtful to be discussed profitably by a botanical layman. See Harper ('10). An interesting review of this subject is given by Guilliermond (Les Progrès de la Cytologie des Champignons) in *Progressus Rei Botanicæ*, IV, 3, 4, 1913.

suggestive series of nuclear phenomena certain of which may here be illustrated by the life-history of the rusts (*Uredinales*), which has been the object of numerous cytological studies. These fungi show a regular antithetic alternation between haploid and diploid mycelia, similar in general morphological type but readily distinguishable by their nuclei, which are single in the haploid and double in the diploid. In this respect the cells of the diplont are quite analogous to those of *Amœba diploidea* (p. 601).[1] The general life-history is briefly as follows (Fig. 309). Fertilization takes place in the *æcidium* (a structure produced by the uninucleate mycelium)[2] but is not followed by fusion of the nuclei, the zygote remaining binucleate (quite as in *Amœba diploidea* and giving rise by "conjugate division" to cells that are binucleate like itself (Fig. 309, *A*). These cells give rise to a series of binucleate "æcidiospores" which are set free, and upon germination give rise by continued conjugate division to a binucleate mycelium. From the latter ultimately are produced binucleate "teleutospores" in each of which the two nuclei finally fuse into one.

By the earlier observers this fusion was considered as the act of fertilization. The work of Maire and Blackman makes it probable, however, that this fusion is the preparatory act of meiosis, and is compared by Blackman to synapsis, again as in *Amœba diploidea* (p. 602). After fusion of the nuclei the teleutospore (now uninucleate) undergoes two divisions producing a "promycelium" which quickly forms four uninucleate spores ("sporidia"); and by germination of the latter arises the uninucleate mycelium which ultimately produces the *æcidium*, the starting-point. The number of chromosomes cannot be certainly determined, but there is now a fairly general agreement that the two divisions of the teleutospore are identical with the meiotic divisions. If this be correct the case is clearly one of sporic meiosis, the teleutospore being analogous to the primary sporocyte, the sporidia to the spores, the uninucleate mycelium to the haploid gametophyte, and the binucleate mycelium to the diploid sporophyte.

Among the algæ we find many forms that are primitive in respect to both gametes and the fertilization process; and they are of especial interest because many botanists have held that the nearest approach to the ancestors of the cormophytic plants are probably to be sought among the green algæ or Chlorophyceæ. Unluckily, the relations of the chromosomes in these particular algæ are unknown, save in a few cases (Zygnemaceæ, Desmidia-

---

[1] See especially Sapin-Trouffy ('96), Maire ('98, '00, '03, '10), Blackman ('04), etc.

[2] The details of fertilization seem to vary considerably in different species, being effected in some cases by a migration of the nucleus of one cell into an adjoining cell (*Phragmidium violaceum, Uromyces Poæ*), in other cases by a lateral fusion of cells (*Ph. speciosum, Uromyces Caladii*, etc.), but in every case the result is a binucleate zygote. The exact homologies of the conjugants is uncertain, and it is doubtful whether they are morphologically comparable to the "true" gametes of algæ.

ceæ), and these give no clue to the origin of antithetic alternation. On the other hand, among the brown algæ (Phæophyceæ) and red algæ (Rhodophyceæ) are conditions which prominently raise the question of an origin of antithetic alternation differing materially from the one adopted by Bower, Strasburger and their adherents.

It seems to be clearly established that in some of these cases no antithetic alternation exists; further, that in some of them meiosis is of the gametic type (as in animals generally) in others of the zygotic (as in *Spirogyra*). An example of gametic meiosis is offered by the brown alga *Fucus* (Phæophyceæ) where reduction takes place in the course of the first two divisions
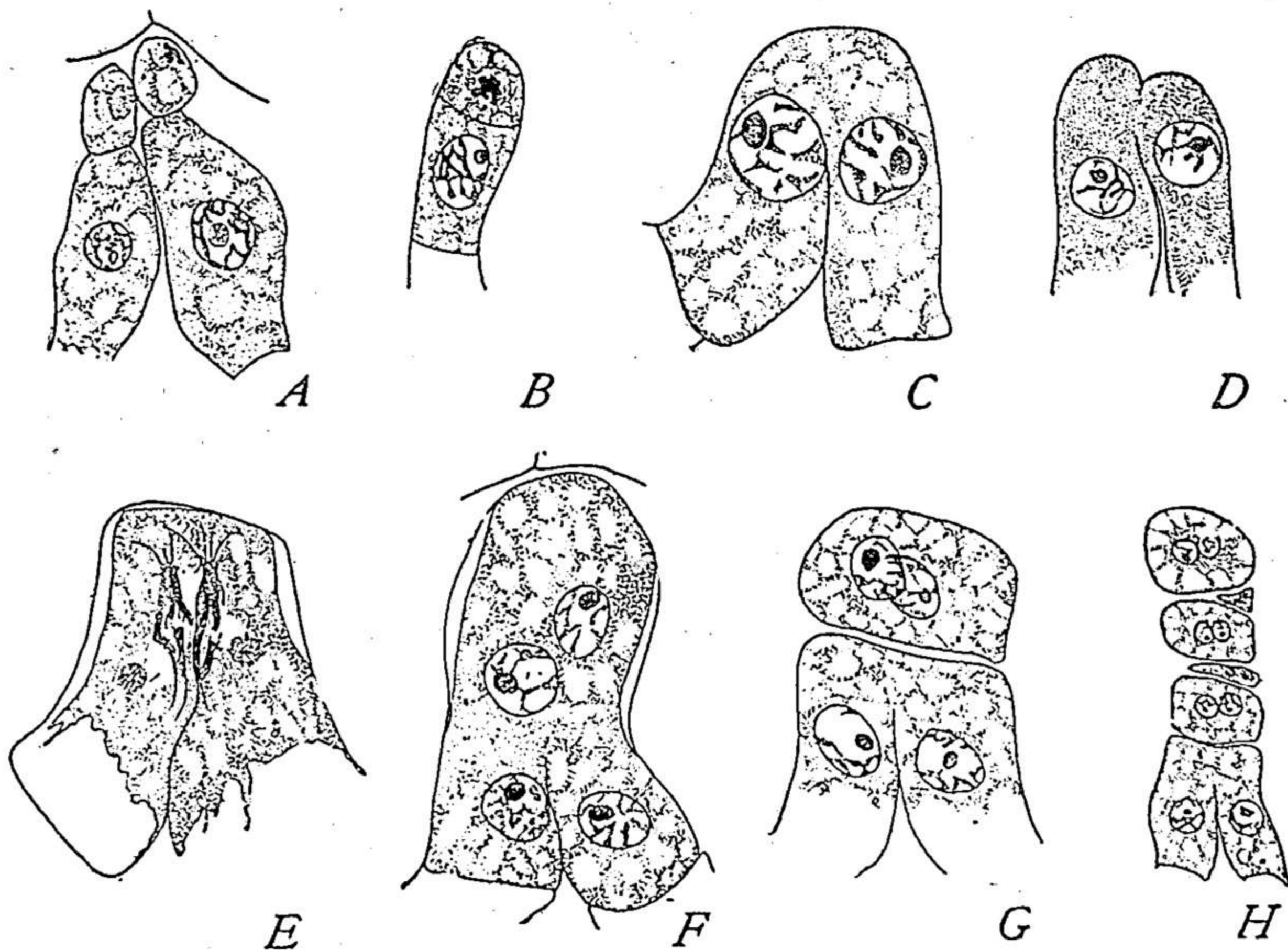
Fig. 310.—Sexual process in the rusts (CHRISTMAN). (All the figures from *Phragmidium speciosum*, except *D* from *Uromyces*.)

A, gametes ready to fuse after cutting off two sterile cells; *B*, single gamete and sterile cell; *C*, *D*, fusion of gametes without karyogamy; *E*, conjugate division following fusion; *F*, *G*, resulting binucleate æcidiospores; *H*, series of æcidiospores and sterile intercalary cells.

of the primary oöcyte (oögonium), quite as in animals (Yamanouchi, '09), though a complication arises from the fact that these two divisions are followed by at least an additional one, producing (usually) eight mature ova. The egg, after fertilization, develops with the diploid number of chromosomes (64 in *F. vesiculosus*) and the same number persists in the resulting thallus. In this case, accordingly, there is but one generation, and this is diploid, as in animals. At the opposite extreme are the Zygnemaceæ (p. 603) in which meiosis is zygotic, following immediately upon syngamy; so that the only existing generation is haploid, like the gametophyte of higher forms. It is a remarkable fact that in certain groups both types co-

exist; for instance in the diatoms, the ordinary forms (*Surirella, Navicula*) display meiosis of the gametic type, the pelagic forms the zygotic (p. 599). Here, accordingly, the ordinary vegetative individuals appear to be in some species of diploid constitution, in others of haploid.

In a third class of cases appears a typical antithetic alternation, highly interesting for the general problem because of the fact that the haploid and
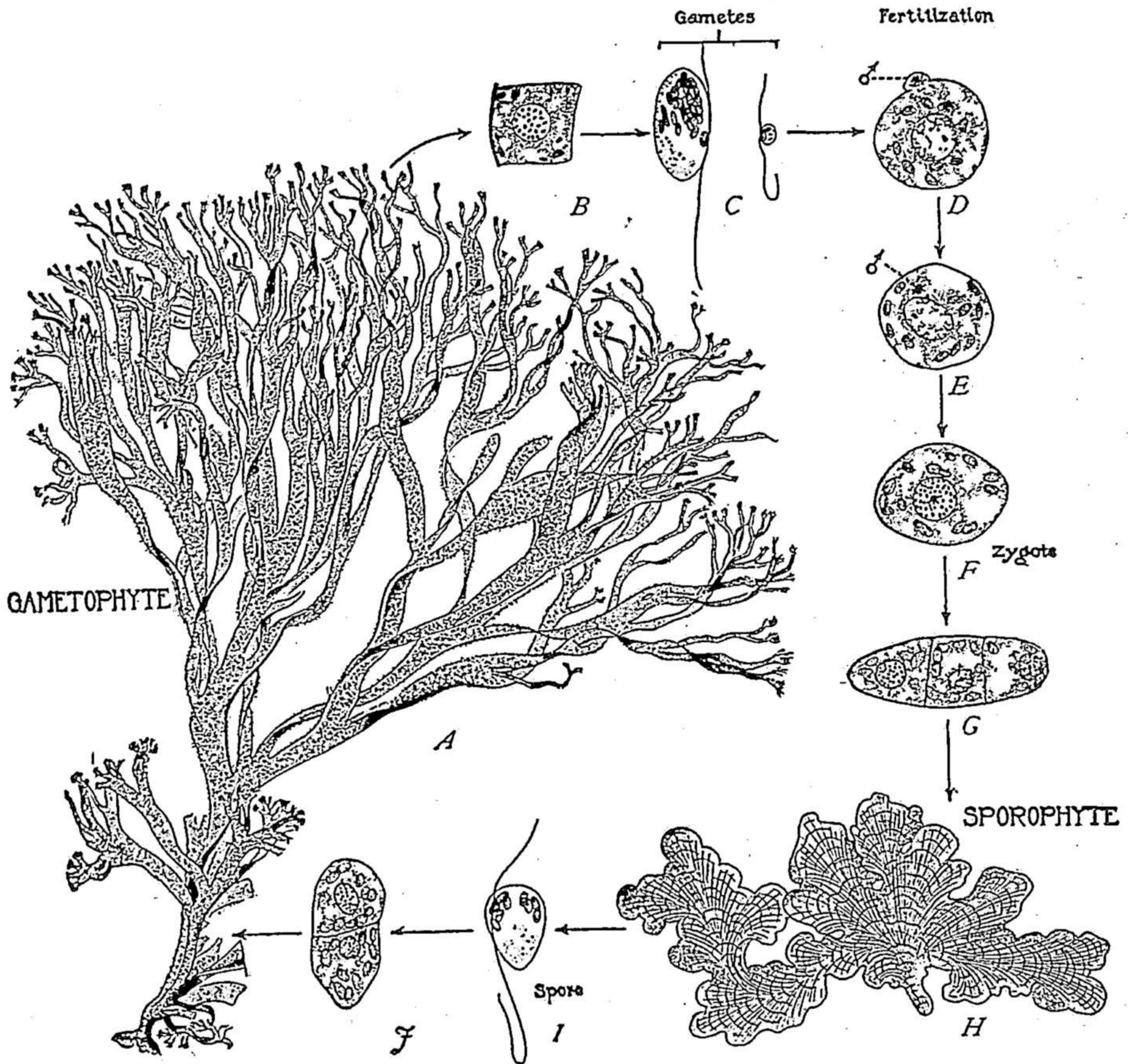
Fig. 311.—Alternation of generations in the alga *Cutleria-Aglaozonia* (*A*, from THURET and JANCEWSKI; *H*, from FALKENBURG; the remaining figures from YAMANOUCHI).
A, mature *Cutleria*, the haploid gametophyte; B, cell of female gametangium, 24 chromosomes; C, gametes; D, E, fertilization; F, zygote, 48 chromosomes; G, embryo; H, mature *Aglaozonia*, the diploid sporophyte; I, motile spore (zoöspore); J, embryo, 24 chromosomes.

diploid generations are of similar, and in some cases of identical, morphological type save in respect to the reproductive organs. In most of these cases the diplont or sporophyte produces its spores (either motile or non-motile) in groups of fours, hence known as *tetraspores*, by two meotic divisions, as in higher forms).

An example of these cases is offered by *Cutleria-Aglaozonia* (Phæophyceæ), a form in which the two generations are separate plants both of thal-

loid type but differing so considerably in external appearance that they were originally described under different generic names. The main features of this life-history are shown in Fig. 311. An interesting feature of this case is the fact that all the germ-cells, whether gametes or asexual spores, are of primitive biflagellate type, and all contain plastids and a red pigment-spot associated with one of the plastids. The haploid gametophyte or sexual generation (*Cutleria*) is a more or less branched thallus (Fig. A), while the diploid sporophyte, or asexual generation (*Aglaozonia*) is an irregularly lobed encrusting form (H). The following facts are from the important paper of Yamanouchi ('12).

Cutleria multifida (usually diœcious), shows 24 chromosomes in the division of all its cells and the same-number is delivered to the gametes (B, C). The latter are typical, biflagellate, free-swimming forms, very unequal in size, but of exactly similar type, except in regard to the number of plastids, of which the macrogamete contains 30 or more, while the microgamete has but two (Fig. 311). By their union (D E,) is formed a zygote (F), which contains 48 chromosomes, and germinates to give rise to the diploid *Aglaozonia* (H), the cells of which likewise divide with 48 chromosomes. At maturity the *Aglaozonia* produces numerous zoöspores (1) closely similar in type to the gametes, but varying in size with the number of divisions of the mother-cell by which they are produced (as is often the case in other algæ). The spores usually contain 20 or more plastids. Without conjugation the zoöspore germinates to produce the haploid *Cutleria*, thus completing the cycle. The reduction of the chromosome-number is effected, in the first two divisions within the zoöspore mother-cell in the zoösporangium and is accompanied according to Yamanouchi by the occurrence of a polarized synaptic stage and the formation of 24 heterotypic chromosomes. Reduction is therefore complete after completion of the second division; but from one to three additional divisions take place, thus producing from 8 to 32 zoöspores, from each of which (Fig. 311) a haploid *Cutleria* plant may develop.[1]

Another step brings us to forms in which the alternation appears in still more generalized form, the diploid and haploid generations being entirely similar in general type. Among the brown algæ this condition is known only in *Dictyota*, where the generations differ only in the fact that the diploid one produces *tetraspores*, by the germination of which arise the haploid sexual individuals which produce the gametes. In these algæ both tetraspores and ova are large, non-motile cells, while the sperms are minute

[1] This is analogous to the multiplication of the primary oöcyte in *Fucus* (above described), though the starting-point is in the one case a zygote, in the other case the terminal product (oöcyte) of a zygote.

and flagellated. The work of Mottier ('oo) and of Williams ('o4) proved that meiosis here takes place in the two tetraspore-forming divisions, the diploid or sporophytic number being 32, the haploid number (found in the tetraspore and the gametophyte to which it gives rise) 16.

The essential facts (disregarding certain complications of detail) are essentially similar in certain of the red algæ, as shown especially in *Polysiphonia* by Yamanouchi ('o7) whose results have been confirmed in other forms particularly by Svedelius.   Yamanouchi proved, in the case of *Polysiphonia vidolacea* that the two tetraspore-forming divisions are of meiotic type and effect a reduction of the chromosome-number from 40 to 20; and this has been confirmed in several other red algæ by other observers.[1]   The life-history of *Polysiphonia* is therefore as follows: The tetraspore-producing plant, of diploid constitution (40 chromosomes), corresponds to the *Aglaozonia* generation (sporophyte) of *Cutleria*.   By this plant are produced tetraspores, each containing 20 chromosomes; and from such a spore arises a haploid sexual plant quite similar in general structure and appearance to the diploid generation.   From these plants are formed the gametes (*carpogonium* = ovum, and *spermatium* = sperm), each of which receives 20 chromosomes, and by their union is produced the zygote, containing 40 chromosomes, from which ultimately arises the diploid sporophyte.   All the essential features of this account, even the same chromosome-numbers, are confirmed in the related form *Delesseria* by Svedelius; and important confirmatory evidence has also been contributed by the studies of Lewis on *Griffithsia* and of Kylin on both this form and *Rhodomela*.

It is remarkable that in another series of red algæ (*Scinaia, Batrachospermum, Nemalion*) no tetraspore-bearing plants appear, the tetraspores being replaced by monospores, which are formed singly on the sexual plants and develop directly into new sexual plants.   This case formerly offered a cytological puzzle which was solved by Svedelius ('15) in his studies on *Scinaia* by the surprising discovery that meiosis is here of zygotic type, and no diploid generation exists.   Svedelius showed that the monospores are of haploid constitution and, like the gametes, are produced by ordinary somatic (non-meiotic) mitosis from the haploid sexual plant.   Fertilization is immediately followed by meiosis in the zygote (as in *Spirogyra*).[2]   The formation of monospores thus seems analogous, broadly speaking, to the vegetative apogamy of higher forms.

We have here a remarkable example of the readiness with which the type of meiosis may change, even as between rather closely related forms.   The extreme plasticity of these phenomena shown in the red algæ and the dia-

[1] Lewis ('o9, '12), (Svedelius ('11, '12, '14), Kylin ('14, '16).
[2] For the nearly similar case of *Nemalion* see Davis ('10) and Kylin ('16).

toms (p. 599) is emphazised by the classical experiments of Klebs ('96, '99, etc.) which show how readily the sexual processes of the green algæ may be modified by change of external conditions such as light, temperature, chemical medium and the like. In case of the red algæ, Svedelius considers the *Scinaia* type, with zygotic meiosis, to be the more primitive and suggests that the *Polysiphonia* type, with antithetic alternation, may have arisen suddenly by a postponement of the meiotic divisions, the diploid number of chromosomes having been carried forward into the carpospores which accordingly give rise to a diploid generation or true sporophyte. It seems, however, at least equally plausible to assume with Lang (p. 622) the correctness of the reverse view, *i. e.*, that the *Polysiphonia* type, with alternation of equally developed haploid and diploid generation, was the primitive one.

The origin of the internal phenomena of meiosis is still wholly unknown. What led to the conjugation of the chromosomes and their subsequent disjunction in orderly system, and what part this process may have played in the physiological activities of the cell can only be conjectured. It is easy to offer hypothetical teleological answers to such questions. We may say that only through these processes could the normal organization and activities of the species be maintained, the "summation of ancestral germ-plasms" prevented, the species held true to its type, or (conversely) provided with a fruitful source of variation and natural selection; but such "explanations" leave us no wiser than before. If the cytological problems here involved remain unsolved they are none the less real and offer an interesting field for further inquiry.

LITERATURE VII

(See also III, V, VI. For abbreviations see General Literature List.)

Bělař, K., '23. Untersuchungen an *Actinophrys sol.* I: *A. Zf.*, XVI.

Bensaude, M., '18. Recherches sur la cycle evolutif et la sexualité des Basidiomycetes: (See *B. G.*, LXVIII.)

Blackman, V. H., '04. On the Fertilization, Alternation of Generations and General Cytology of the Uredineæ: *A. Bot.*, XVIII.

Blakeslee, A. F., '04. Sexual Reproduction in the Mucorineæ: *P. A. A. A. S.*, XL, 4.
    '06. Differentiation of Sex in Thallus, Gametophyte and Sporophyte: *B. G.*, XLII.
    '15. Sexual Relations between Hermaphroditic and Diœcious Mosses: *B. B.*, XXIX, 2.
    '20. Sexuality in the Mucors: *Sci.*, LI.

Bonnet, J., '14. Reproduction sexuée et alternance des génération chez les algues: *P. R. B.*, V.

Bower, F. O., '89-'91. On Antithetic as distinguished from Homologous Alternation of Generations in Plants: *A. Bot.*, IV.
    '19. The Botany of the Living Plant: *Macmillan, London*.

Burgeff, '14–'15. Üntersuchungen über Variabilität, Sexualität und Erblichkeit bei *Phycomyces: Flora*, CVII, CVIII.

Calkins, G. N., 'o1. The Protozoa: *New York.*

'o9. Protozoölogy: *Philadelphia.*

Campbell, D. H., 'o5. The Structure and Development of Mosses and Ferns: *The Macmillan Co., New York.*

Christman, A. H., 'o7. The Alternation of Generations, etc., in the Rusts: *B. G.,* • XXXIX.

Coulter, J. M., '14. Evolution of Sex in Plants: *Chicago.*

Cunningham, B., '17. Sexuality of the Filament of *Spirogyra: B. G.,* LXIII, 6.

Davis, B. M., 'o1. The Origin of Sex in Plants: *Pop. Sci. Monthly,* LX.

'o3. The Evolution of Sex in Plants: *Ibid.,* LXII.

'10. Nuclear Phenomena of Sexual Reproduction in Algæ: *A. N.,* 44.

Dobell, C., 'o9, '14. (III, IX).

Doflein, F., '16. Lehrbuch der Protozœnkunde: 4 Auflage, *Jena.*

Enriques, P., 'o7. La coniugazione, etc., negli Infusori: I, *A. P.,* IX.

'o9. La sexualité chez les Protozoaires: *Rev. di Scienza.*

'16. *Mem. R. Accad. Sci. Bologna,* VII, 3.

Farmer and Williams, '98. Contributions to our Knowledge of the Fucaceæ: *P. T., B.,* 190.

Fortpflanzung der Gewächse, in *Handwört. der Naturw. Jena,* 1913. *Algæ,* F. Oltmanns; *Fungi,* E. Fischer; *Archegoniata,* F. O. Bower; *Mosses,* G. Tischler; *Ferns,* F. O. Bower; Intermediate Forms, between Ferns and Seed-plants, N. Arber; Seed-plants, A. Ernst; Physiology, G. Klebs.

Guilliermond, A. '10. La sexualité chez les champignons: *B. S. F. B.,* 44.

Harper, R. A., 'oo. Sexual Reproduction in *Pyronema confluens,* etc.: *A. Bot.,* XIV.

'10. Nuclear Phenomena of Sexual Reproduction in Fungi: *A. N.,* XLIV.

Hartmann, M., 'o9. Autogamy bei Protisten, etc.: *A. P.,* XIV.

'13. Flagellata: *H. Nw.,* III, *Fischer, Jena.*

'14. Der Generationswechsel der Protisten, etc.: *V. D. Z. G.,* XXIV.

'18. Ergebnisse und Probleme der Befruchtungslehre im Lichte der Protistenforschung: *Die Naturwissenschaften,* VI.

'21 (III).

Hartmann and Schilling, '17. Die Pathogenen Protozoa: *Springer, Leipzig.*

Hertwig, R. '89 (III).

'o2. Die Protozoen und die Zelltheorie: *A. P.,* I, 1.

Jennings, H. S. (III).

Jollos, V., '21. Untersuchingen über Varietät und Verebung bei Infusorien: *A. P.,* XLIII.

Klebs, G., '96, '12, (III).

'99. Ueber den Generationswechsel der Thallophyten: *B. C.,* XIX.

Kofoid, C. A., '23 (III).

Marchal, Élie et Émil, 'o6, 'o7. Aposporie et sexualité chez les mousses: *B. A. B.,* 'o9, *Ibid.* (see also XI).

Maupas, E., '88, '89 (III).

Minchin, E. A., '12. An Introduction to the Study of the Protozoa: *London.*

Oltmanns, F., '22, '23. Morphologie und Biologie der Algen, I, II, III. 2nd Ed.: *Fischer, Jena.*

Prowazek and Nöller, '20. Handbuch der Pathenogenen Protozoa: *Barth., Leipzig.*

Svedelius, N., '11. Ueber den Generationswechsel bei *Delesseria sanguinea: Svensk. Bot. Tidskrift,* V.

'15. Zytologisch-entwicklungsgeschichte Studien über *Scinaia furcellata,* etc.; *Nova Acta Reg. Soc. Sc. Upsaliensis,* Ser. 4, IV.

West, G. S., '16. Algæ, I: *Cambridge Univ. Press, Cambridge.*

Woodruff and **Erdmann,** '14 (III).

Yamanouchi, S., '06. The Life-History of *Polysiphonia: B. G.,* XLI, XLII. Also Univ. Press, *Chicago,* '07.

'12. The Life-History of *Cutleria: B. G.,* LIV, 6.

CHAPTER VIII

SOME ASPECTS OF CELL–CHEMISTRY AND CELL–PHYSIOLOGY

"The synthetic act by which the organism maintains itself is at the bottom of the same nature as that by which it repairs itself after undergoing a mutilation, or by which it multiplies and reproduces itself. Organic synthesis, generation, regeneration, reintegration, the healing of a wound, are but different aspects of a single phenomenon."

CLAUDE BERNARD. [1]

"The morphologist, on the one hand, strives to elucidate the structure of protoplasm down to its finest details; the biochemist, on the other, with his apparently ruder yet still more searching methods, seeks to determine the chemical functions of the same protoplasm; broadly speaking, they are only dealing with two different sides of the same thing."

HOFMEISTER. [2]

No attempt will here be made to consider the general chemistry and physiology of the cell; such a task, manifestly would lie far beyond both the scope of this work, and the competence of its author. In practice, however, the modern study of cytology, cellular embryology and genetics, especially on its experimental side, passes insensibly into that of cell-physiology and cell chemistry. We cannot for instance, intelligently employ the modern technique of cytological staining without some knowledge of its chemical and physical basis. Such topics as the structure of protoplasm, the mechanics of cell-division, or the phenomena of fertilization cannot adequately be discussed without keeping in view at least some elementary notions concerning the chemical basis of protoplasm and the properties of colloidal systems. Again, we can only skirt the margin of the problems of development and heredity so long as we deal only with their morphological aspects. As the study of embryology, cytology and genetics goes forward we see more and more clearly that it can only approach its final objectives through a close alliance with biophysics and biochemistry. Even a work that deals primarily with cytology must therefore attempt in some degree to orient itself with respect to these other sciences. As such an attempt, and no more, the following brief discussion is offered.

---

[1] *Leçons sur les phénomènes de la vie*, p. 517, 1878.
[2] *Die chemische Organization der Zelle*, p. 29, 1901.

I. GENERAL VIEW

1. The Cell a Colloidal System

Physico-chemically regarded, as has been indicated (pp. 60, 73) the cell appears as a complex system largely composed of colloidal material. That it may profitably be treated from this point of view is proved by many of the most important physiological advances of our time; life as we know it is indeed inseparably bound up with matter in the colloidal state.[1] This conception seems likely to prove as fruitful in cytology as it has been in physiology. Under its influence many of the leading problems of cytology took on a widely different aspect when once the subject had thrown off the dominance of those earlier conceptions of the cell-substance that found expression, for instance, in reticular theories of protoplasmic structure and the nearly allied hypothesis of fibrillar contractility in mitosis. More modern attempts to consider the structure and transformations of protoplasm, the mechanism of mitosis, the nature of fertilization, the nature and function of the cell-membrane and the physiological relation between nucleus and cytoplasm, have for the most part taken the colloidal nature of protoplasm as a common starting point; and numerous recent observations on both living and fixed cells tend to demonstrate that we are here on the right track. On the physiological side of the subject this is even more obvious, and many striking cell-phenomena have in a measure been imitated in artificial colloidal systems: for instance, the properties of surface-films or membranes, the antagonistic effects of inorganic salts on the nature and stability of the system, or the changes of viscosity in the living cell-substance.

Obviously, however, the cell-substance is not to be thought of as a simple diphasic system. The cell is a highly heterogeneous or polyphasic system, in which the disperse phases are represented by many different kinds of suspended particles of all degrees of magnitude, differing among themselves more or less widely in degree of aggregation, physical consistency, chemical nature, and physiological activity, and varying endlessly from species to species. The system also contains various inorganic salts, either in combination or in solution, which play a most important part in controlling the equilibrium of the system. Protoplasm considered as a colloidal system thus offers a problem of extreme intricacy, alike from the morphological, physiological and the physico-chemical point of view.

To the cytologist one of the most interesting parts of this problem concerns the nature of the ultra-microscopical dispersed particles, both in the hyaloplasm and in artificial colloidal solutions of protein and related sub-

---

[1] *Cf.* Loeb ('06, '22), Bechold ('12), etc.

stances; but the questions here raised are still too largely a matter of controversy among students of colloidal chemistry to be profitably discussed at length by a non-specialist. Until recently it has been the prevalent opinion that in colloidal solutions (e. g., of proteins) the dispersed particles are not ions or molecules but larger molecular aggregates, to which have conveniently been applied the term *micellæ*, adapted from Nägeli's earlier use of the word (p. 718).[1]   Important recent researches, however, point to the conclusion that the degree of dispersion may often be molecular, and that even protein solutions differ from true solutions only in the greater size of the dispersed molecules and of the ions into which they may break up.[2]   That the dispersed particles, though still below the horizon of microscopical vision, are relatively of very large size is shown by the extremely slow rate of diffusion of protein solutions, by their viscosity and often by their opalescence (Tyndall effect); and by the fact that by the use of the "ultra-filter" [3] the particles of egg-albumin, gelatin, various albumoses, and even of dextrin, may be separated from their watery solutions (Bechold). The opalescence of certain protein solutions shows that the suspended particles are large enough to disperse and reflect part of the transmitted light; and it has been shown that the smallest particles capable of thus scattering light are from 50 to 100 times smaller than the mean wave length of light (0.5μ). They must, therefore, have a diameter of at least $5–10\mu\mu$ ($0.005–0.010\mu$) and this is believed to be about the size of the smallest particles revealed by the ultra-microscope (p. 33). According to the calculations of Robertson ('18), the diameter of a molecule of casein, which has a molecular weight of nearly 16,000 (based on Nernst's estimate of the diameter of the CO_2 molecule) is less than half this ($\pm 2.4\mu\mu$). It can not be concluded from this alone that the dispersed particles are necessarily molecular aggregates; on the contrary Robertson has urged the view that not only the Tyndall effect and non-filterability of the particles in protein solutions but also their viscosity may be due to an association of the protein ions with a large amount of water to form complexes of great size.[4]   Apart from all this, however, it is evident that the hyaloplasm of living protoplasm, though apparently structureless, is not merely a solution of colloidal material. Many facts indicate that in addition to proteins, carbohydrates, salts, and other substances in true solution, it may contain great numbers of larger molecular aggregates[5] which form the original source of many of the still larger

[1] For a presentation of this view see Zsigmondy ('18).
[2] See especially Robertson ('18), Loeb ('22).
[3] Bechold, 1906.
[4] *Op. cit.*, p. 344, etc.
[5] This is admitted by Robertson, who also holds that many proteins enter into true solution, becoming molecularly dispersed in accordance with Avogadro's law ('18, p. 341). See also Bayliss ('11, '21, '23), Bechold ('12), Höber ('14), Mathews ('15), Maclendon ('17), Zsigmondy ('18), etc.

bodies that emerge into microscopical view as the smallest visible formed components of protoplasm. At this point we only emphasize from the cytological point of view the conclusion of Bayliss that "no hard and fast line is to be drawn between matter in pieces visible to the naked eye, down through ultra-microscopic particles to molecules."[1] The field open to the cytologist forms the middle portion of this series, ranging from the largest formed elements of the cell down to those just perceptible by the highest powers of the microscope (p. 720); but we may regard it as certain that the diminishing series does not end at the purely artificial boundaries determined by the working limit of our instruments.[2]

2. The Cell a Chemical Machine

Modern physiology unhesitatingly accepts the fundamental conclusion that all vital energies are traceable to the chemical energy of food-stuffs that have been incorporated into the cell-substance and are there set free by oxidations and other destructive chemical processes of metabolism. Physiologically, therefore, the cell may be regarded as an apparatus for the transformation and application of chemical energy. In the phrase of Loeb, it is a chemical machine.

Within certain well-defined limits the activities of every cell are of specific type—the muscle-cell, the nerve-cell or the gland-cell displays its own characteristic performance just as the egg of each species has its own mode of development, or each species of protozoön its own specific forms of behavior. We assume, as our fundamental working hypothesis, that the specificity of each kind of cell depends essentially upon what we call its *organization, i. e.,* *upon the construction of the cell-machine,* in some sense or other—morphological, physical or chemical. Even the most superficial acquaintance with the cell-activities shows us that this conclusion cannot be taken in any crude mechanical sense—the difference between the cell and even the most intricate artificial machine still remains too vast by far to be bridged by present knowledge. Nevertheless we accept the hypothesis that the difference is one of degree rather than of kind because it has proved itself fruitful in discovery and has kept us moving in the right direction (p. 1116).

Fundamentally the cell-system is a *reaction-system,* which responds to a multitude of *modifiers* (Bechold) by activities that differ with the nature both of the system and of the modifier. Some of these agents are *activators,* which incite or accelerate action; others *inhibitors,* which suppress or retard it; still others *transformers,* which change the character of activities in progress. Some modifiers are external to the cell, and include those agents which effect the coördination of action between different tissues and cells and the

[1] Bayliss, '11, p. 20.   [2] Wilson, '99, '23.

maintenance of the general organic equilibrium. Some of these (such as the nerves) belong to the obvious mechanical structure of the body as a whole. Others are soluble chemical substances, such as the enzymes and hormones, that are independent of fixed structural relations and hence capable of being transported from one part to another in the blood or lymph or by diffusion from one cell to another. This subject is too large to be entered upon here; but we may emphasize the interest for the student of the development of the hormones or related soluble substances that call forth or maintain character-. istic structural relations, sometimes of a very complicated type. It must here suffice merely to mention such well-known hormones as thyroidin, or the pituitary hormone, both of which powerfully affect growth and metabolism. The sex-hormones, produced by certain cells of the reproductive organs, bring the subject conspicuously into relation with our conceptions of development and heredity, especially in the production of the secondary sexual characters. The remarkable morphological, physiological, and even psychical reactions of the organism to castration have long been familiar; and equally striking effects are often produced by the transplantation of the gonads of one sex into the body of the other sex. These effects are now known to be due to specific hormones produced by the interstitial cells or other non-germinal cells of the gonads; the growth and functional activity of the mammary gland in the female, for example, is incited by a hormone derived from the foetal cells. An interesting demonstration of the action of the sex-hormones is made by F. R. Lillie ('16) in his solution of the long-standing puzzle of the "free-martin" twin. In this case, the female characters of a female twin are more or less completely suppressed, and certain male organs may develop, as a result of anastomosis of the placental blood vessels with those of the associated male twin. This apparently can only be due to the influence of soluble substances derived from the male, and transferred to the female by the blood.

Loeb ('16) points out the manifest analogy between such phenomena and the effects of food in inciting the production of the sexual forms in the polymorphic social insects,[1] or in the rotifers, as demonstrated by Whitney and Shull (p. 229). These effects too must be due to a reaction of the organism to substances in the blood, either introduced as such in the food or produced by its transformation, and having effects analogous to those of hormones, even though not technically so called. Loeb has suggested that in the same category with the hormones should be placed the "formative stuffs" of Sachs, suggested by so many facts of regeneration and development (p. 1065); and this view is supported by a number of recent investigations.[2]

[1] See Caullery, '13.

[2] See Bayliss, '18.

This conclusion may open the way to a more adequate conception of localization than has hitherto been possible (p. 1062).

A similar view may be taken of the individual cell, if only for the reason that the organism as a whole is a single cell at an early period of its existence (*cf*. p. 1). The intracellular coördinations, like the intercellular, may be effected by structural relations, in some cases of high complexity, as seen, for instance, in the neuro-motor apparatus of the flagellates and ciliates (p. 695) or in the relations between the central bodies, the astral formations, and the movements of the chromosomes. Other internal modifiers undoubtedly are soluble enzymes or hormones. A probable example of this is offered by the fact (p. 405) that so long as the germinal vesicle of the egg is intact, the cytoplasm is in general incapable of aster-production under the influence of either the sperm or of parthenogenetic agents. As soon as the germinal vesicle breaks down and nuclear substance mingles with the cytoplasm, the latter becomes capable of fertilization, and is able, upon appropriate stimulus, to produce an astral system, and to proceed with its development (p. 405). The effect of the sex-chromosomes and of other chromosomes on development may be thought of in the same way (p. 815). We may emphasize, lastly, the fact that since the reaction of the cell to its modifiers is of specific type it must somehow depend upon its own specific type of organization. In the words of Bechold, "The order maintained among the activities of the vast number of modifiers in the body must itself be referred to an organization of the body as a whole, and ultimately to that of the germ-cell from which it has been derived" ('12, p. 31).

The cell, like the whole organism, thus appears to us as a delicately balanced moving equilibrium, offering a picture that is continually changing yet always remains within the boundaries of a specific type. Nothing is more remarkable than that a thing so delicate and plastic should run so true to form through countless generations. How this is possible we can hardly imagine. We can but record the observed fact that it is effected by an inherent power of adjustment or self-regulation that holds the cell fast to its own type; and by processes of assimilation, growth, and cell-division that ensure an unbroken protoplasmic continuity between successive generations.

II. CHEMICAL RELATIONS

1. General

As has often been remarked, the chemical conditions existing in living cells are of necessity imperfectly known, because every attempt to examine them by precise methods kills the cell. In the main, therefore, our statements concerning these conditions are limited to inferences based on the

chemical behavior of dead cells or their components. Even as thus circumscribed, investigation is beset with formidable difficulties. Neither protoplasm nor nucleus consists of a single substance of fixed composition. Both are made up of a great number of different chemical components, themselves often highly complex, and in a continual flux of chemical transformation. Only in very restricted degree is it possible to isolate these components for accurate chemical analysis; and our so-called "micro-chemistry"—i. e., observation by the microscope of the effect of treatment by dyes, and other reagents—is still in too rudimentary a state of development to give us more than a few rough qualitative indications. There is good reason to conclude, further, that many of the most significant of the chemical properties of the cell-components (for instance, the nucleo-proteins) are too subtle to be recognized by our present methods of analysis; we know nothing, for example, of the chemical differences between different chromosomes of the same group; yet the experimental evidence makes it certain that such differences exist (p. 916). In these considerations we find sufficient explanation of the fragmentary and unsatisfactory existing state of our acquaintance with the chemical physiology of the *cell* as such, and the great distance by which our knowledge of this subject is still separated from that of the chemistry of organic substances.

Regarded from a purely chemical point of view the cell-substance is a complex mixture of substances, of which a large percentage (60 to 90%, or more) consists of water, while 1%, more or less, is formed by various inorganic salts. Among the latter the compounds (mainly chlorides and phosphates) of sodium, potassium, calcium and magnesium preponderate; but small quantities of iron are probably also always present and sometimes manganese, silicon, copper and other elements. It is an interesting fact, that the chief inorganic constituents of the living body (as indicated by the salt-composition of the blood) are the same as those of sea-water; and this has ingeniously been conjectured to indicate that living matter first appeared in the sea and has thus from the first maintained an adjustment or colloidal equilibrium with its characteristic salts or their ions (Macallum, '04). This is supported by the familiar fact that in marine invertebrates both the osmotic pressure of the blood and the nature and proportions of the inorganic salts are approximately the same as those of the sea-water.[1] In higher forms the osmotic pressure of the blood is much lowered and the Mg-content largely decreased. M. R. Lewis ('16) has shown that the embryonic tissues of vertebrates (birds) may be successfully grown in sea-water diluted until isotonic with the normal blood-plasma of the

[1] See Höber ('14),

animal; it is well known also, that some of the most useful of the "normal fluids" employed for culture-fluids (such as the solutions of Locke or of Ringer) approximate in salt-proportions to sea-water, though of different concentration. The notion, therefore, that the similarity of salt-content between the cell-substance and sea-water has a real historical significance is hard to escape, even though it be (as Loeb has said) no more than a "poetical dream."

However this may be, the work of many observers, prominent among them Herbst, Loeb, Osterhout and R. S. Lillie, has shown that the salts of the sea-water and those of the blood or lymph alike form "physiologically balanced" solutions, *i. e.*, they are present in such proportions as to maintain a normal equilibrium with the cells of the organism. Some of these salts are actually poisonous when acting alone, but their injurious effect is counteracted or antagonized by the presence of other salts; sodium chloride, for instance, is thus antagonized by calcium chloride, and a similar balance exists between magnesium and calcium. A wide vista here is opened of the fundamentally important rôle played by the inorganic salts in protoplasmic action, and even in animal behavior,[1] but this subject lies outside the scope of this work.[2]

The organic constituents of the cell [3] are for the most part compounds of carbon with oxygen, nitrogen and other elements, and some of these are the most complicated of known chemical compounds. The time-honored classification of these substances is into (1) *carbohydrates* (2) *fats*, and (3) *proteins*, the first two being non-nitrogenous compounds of carbon, hydrogen and oxygen, while the third contain also nitrogen, and commonly small quantities of sulphur and sometimes of phosphorus. Many recent writers, however, include the true fats and oils in a larger group of *lipins* to which are assigned various other fat-like bodies such as lecithin or cephaline which contain nitrogen and phosphorus in addition to carbon, hydrogen and oxygen. All the substances of this group agree in their solubility in chloroform and other fat-solvents, and their insolubility in water.

---

[1] *Cf.* Loeb ('06, '16).

[2] According to Macallum ('08), the nucleus contains no potassium, no chlorides and no phosphates, and probably no sodium or magnesium; but iron and calcium appear to be generally present. Macallum believes that inorganic salts are in general absent from the nucleus and that when such substances as calcium, or iron are present, they are always in organic combination. This fact is ascribed by him to the impermeability of the nuclear membrane to inorganic salts; and he makes the interesting suggestion that it may thus serve to protect the intra-nuclear nucleins, or nucleo-proteins from the action of inorganic salts contained in the cytoplasm and thus to insure their stability. The osmotic properties of the nuclear membrane may thus be a factor in heredity (*op. cit.*, p. 650). *Cf.* Mathews, p. 176.

[3] *Cf.* Mathews, *Physiological Chemistry*, 1915, and Halliburton, *Essentials of Chemical Physiology*, 9th Ed., 1916.

2. The Proteins and their Derivatives

The proteins are of especial interest since they are the most complex and varied of the cell-components, and there is also reason to believe that they form the main chemical basis of the apparatus of heredity. Recent research, it is true, has emphasized anew the importance of both the carbohydrates and the lipins in the fundamental operations of the cell; nevertheless it seems certain that in most cases the proteins (together with water) constitute the main bulk of the active cell-substance and of its more constant structural components, including the hyaloplasm and many of the formed elements such as plastids, many forms of granules, fibrillæ, chromosomes and nucleoli, and the material of the astral rays and spindle-fibers. A great number of proteins are known. A large and increasing body of evidence shows that some of them differ characteristically from species to species[1] and even indicate that they may constitute the fundamental chemical basis of heredity.

  a. General Nature. The proteins habitually appear in the colloidal state, and some of them have the largest and most complex of known molecules, as is shown by their high molecular weight, low diffusibility, and the great number and complexity of their cleavage-products. Chemical formulas for the proteins have little meaning apart from the structure of the protein molecule as revealed on the one hand by its breaking up by means of hydrolysis into simpler components (the building-stones or "Bausteine" of Kossel), on the other by the progressive artificial recombination or synthesis of these components to form more complex substances. Some of the compounds thus artificially produced, such as the polypeptids, are in the view of Emil Fischer actually proteins, or at least would have been classed as such had they first been met with in nature (Robertson, '18).

  By these methods it has been established that the protein molecule is in general built up from a series of the *amino-acids,* familiar examples of which are *glycin* ($C_2H_5NO_2$), *leucin* ($C_8H_{13}NO_2$), and *tyrosin* ($C_9H_{11}NO_3$). The smallest number of component amino-acids occurs in the protamines, which therefore may be regarded as the simplest of proteins; and it was especially their study which led Kossel to his general hypothesis concerning the constitution of the protein molecule in more complex forms. Proteins vary with the nature, number and proportions of these component units and also with their mode of linkage; hence the immense number of possible combina-

---

[1] This is shown, for instance, by the fact that the hæmoglobins of different species differ characteristically in form of crystallization (Reichert and Brown, '09); by the remarkable specific precipitin-reactions of blood-sera from different animals (Nuttall, '04); by the phenomena of anaphylaxis; and by the differences shown by the protamines from the sperm-nuclei of different fishes. (See tables in Mathews, '15, pp. 128, 129). An interesting discussion of the general subject is given in Loeb's *The Organism as a Whole,* Chap. III, 1916.

tions and the corresponding diversity which distinguishes the proteins above all other known substances. To employ a figure of Kossel's, just as the letters of the alphabet may be variously grouped to form an immense multitude of words, so a comparatively small number of amino-acids (about a score of them are known) may by different combinations and groupings build up a practically unlimited number of distinct proteins. Kossel even went so far as to maintain that every peculiarity of the species and every occurrence affecting the individual may be indicated by special combinations of these "building stones" or molecular components; and physiologists have shown a growing readiness to accept the conclusion that the specificity of organisms generally may have its root in that of their component proteins.[1]

In the "simple proteins" the molecule on being treated by enzymes or by acids breaks up wholly into the amino-acids or their derivatives; such proteins are the protamines, histones, albumins or globulins. These proteins readily combine with other substances to form salt-like compounds, thus giving rise to still more complex "compound" or "conjugated" proteins. The substances with which such unions may take place are of varied nature; they include many acids, bases and salts, both organic and inorganic, various lipoids, and perhaps such substances as toxins, anti-toxins, hormones and enzymes.[2] Proteins readily combine with other proteins, and many of the proteins of the tissues and tissue-fluids are believed to be thus united, forming complexes which, as a number of biochemists have suggested, may be characteristic of these several tissues and thus offer a key to their physiological individuality. Many proteins or their components will unite with *dye-stuffs* to form colored compounds—a fact of fundamental importance for the theory of cytological staining, though the precise nature of the combination is still under discussion (p. 646). It is also of cytological interest that some of the natural pigments (*e. g.*, hæmoglobin or the phycoerythrin of plants) are conjugated proteins.

A remarkable peculiarity of the proteins, especially interesting for the theory of cytological staining, is their *amphoteric* character, *i. e.*, the capacity of one and the same protein to form a salt-like compound with either an acid or a base. This property is due to varying electrical charges, the protein-radical being in the one case electro-positive, in the other electro-negative. Some proteins, such as protamine, are predominantly basic, others predominantly acid, such as casein, or the "nucleins." It

---

[1] "There can be no doubt that on the basis of our present knowledge proteins are in most or practically all cases the bearers of this specificity" (Loeb, '16, p. 61). "The specific character of every animal or plant may be determined ultimately by the specific characters of their structure-forming proteins" (R. S. Lillie, '18, p. 77).

[2] Robertson, '18, p. 156.

was demonstrated experimentally by Hardy ('oo) that the charge of the suspended particles in a simple protein solution is determined by the reaction of the medium; in an acid medium (*i. e.*, in the presence of an excess of H-ions) they become electro-positive and behave as bases, while in the presence of alkali (with an excess of OH-ions) they become electro-negative and behave as acids.[1] At an intermediate point, which is neutral or isoelectric, they are chemically indifferent and unable for the time being to enter into combination. The extreme importance of these facts for the practical use of staining agents will later be indicated (p. 649).
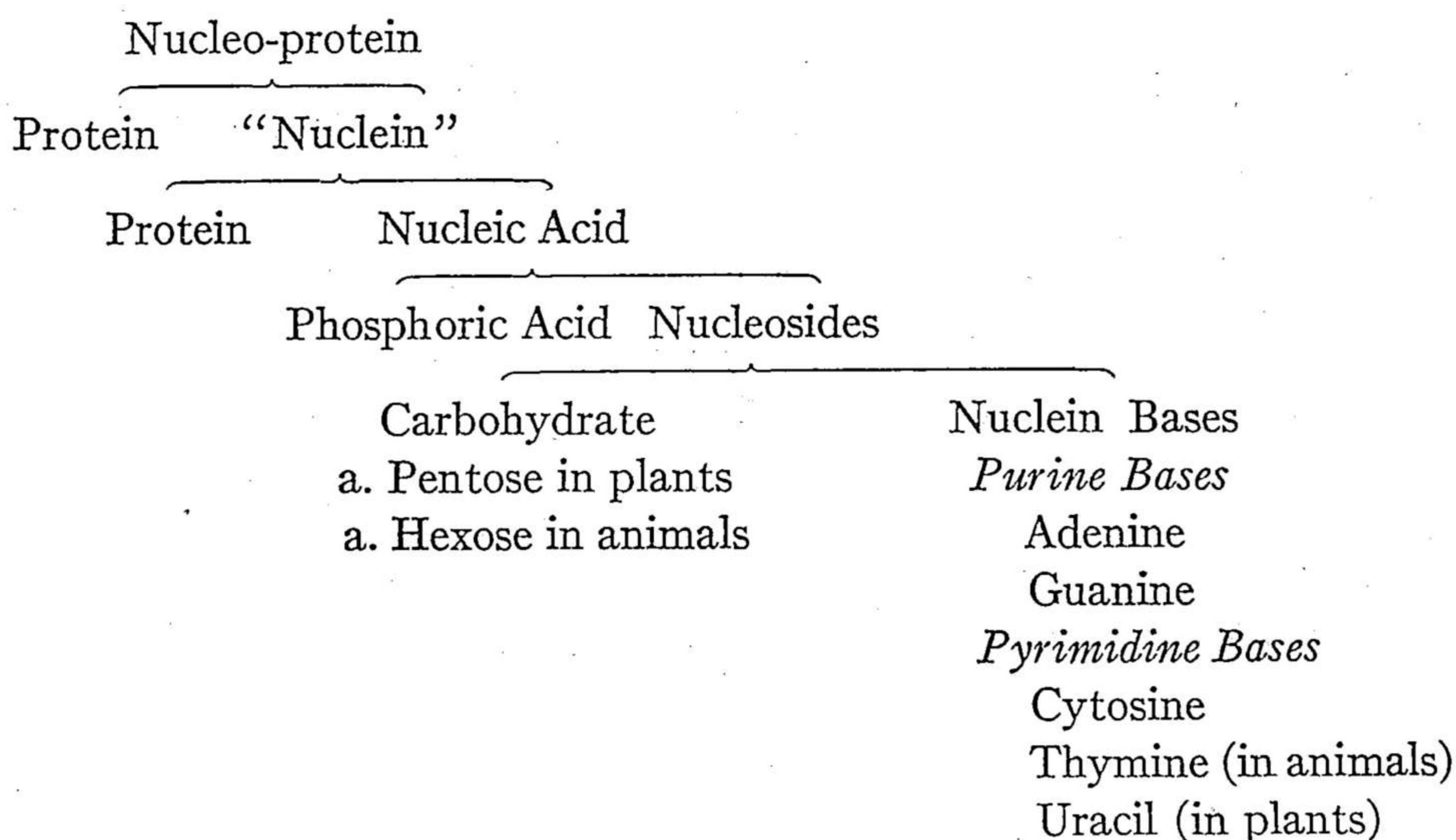
b. The Nucleo-proteins. Among the compound proteins a special interest is offered by the nucleo-proteins since they form the principal chemical basis of the nuclear substance and by earlier writers were identified with the "chromatin" of cytologists. Substances related to nucleo-proteins occur, however, also in the cytoplasm, and it should also be borne in mind that the word "chromatin" is no longer employed to denote a single or definite chemical compound (p. 88). It was shown by Miescher in 1871, that when cells can be obtained of practically unmixed type and in large quantity (*e. g.*, pus-cells, fish-sperm, or yeast-cells, etc.) and are treated with artificial gastric juice (hydrochloric-pepsin) the cytoplasm is digested, leaving only the nuclei. Nuclear substance ("chromatin") thus obtained in large quantity and practically pure for quantitative analysis was found to consist largely of a complex protein, rich in phosphorus, which Miescher called "nuclein," giving to it the formula $C_{29}H_{9}N_{9}P_{3}O_{22}$. By Altmann, it was later shown (1889) that Miescher's "nuclein" may be split into a protein base, rich in nitrogen (protamine or histone in case of the sperm-nucleus) and a complex organic acid containing phosphorus to which he gave the name of nucleic acid: further, that "nuclein" may be synthetically re-formed by recombination of these two substances. It thus became clear that in some cases "nuclein" or nucleo-protein is a salt-like compound in which a simpler protein plays the part of a base (*i. e.*, is electro-positive),[2] and this was confirmed by many later workers.

The simplest and best-known of the nucleo-proteins have been obtained from the sperm-nuclei in fishes which the work of Kossel and his followers showed to be a protamine in salt-like union with nucleic acid; and Kossel likewise demonstrated that the nucleo-proteins of the thymus gland and of the erythrocytes of birds is a salt-like compound between histone and

[1] For the explanation of this see p. 649. *Cf.* Robertson ('09), Loeb ('04, '22), Mathews ('15,) Bayliss ('18.)

[2] Since the amount of phosphorus in nucleic acid is constant, the total amount of this substance in nucleo-proteins may be taken as a measure of the relative proportions of nucleic acid in the nucleo-protein compound. It was at first assumed that "chromatin" might in some cases even be pure nucleic acid (Kossel, Halliburton), but this has not been substantiated.

nucleic acid. In sea-urchin sperm Mathews found the basic component to be *arbacin*, a substance related to both protamine and histone. In general, however, the nucleo-proteins are of more complex composition, containing a larger proportion of protein, and a greater number of protein radicals; and much evidence exists to show that a large number of such protein bases and their combinations may exist, among which may possibly be organic enzymes and hormones, though this is conjectural.[1] The components of the nucleo-proteins in general, and the order of their disassociation upon hydrolysis are shown in the following scheme.[2]

Nucleo-protein

Protein   "Nuclein"

Protein   Nucleic Acid

Phosphoric Acid   Nucleosides

Carbohydrate   Nuclein Bases
a. Pentose in plants   *Purine Bases*
a. Hexose in animals   Adenine
                            Guanine
                           Pyrimidine Bases
                            Cytosine
                            Thymine (in animals)
                            Uracil (in plants)

As is here shown, the nucleic acid itself splits into phosphoric acid and nucleosides, which are compounds of various bases with carbohydrate. The nucleic acids are, however, of two types, characteristic respectively of animals and of plants. In percentage composition the two are not dissimilar, as may be seen from the formulas $C_{43}H_{57}N_{15}O_{30}P_4$ for animal thymus nucleic acid (Steudel), and $C_{38}H_{55}N_{15}O_{32}P_4$ for that from yeast (Levene), and both alike yield as cleavage products the purine derivatives guanine and adenine,[3] and also the pyrimidine derivative cytosine. On the other hand, plant nucleic acid yields uracil in place of thymine, and a carbohydrate pentose group in place of a hexose.[4] Miescher showed that nucleo-proteins are more or less resistant to pepsin-hydrochloric digestion, a fact

[1] Mathews, p. 180.

[2] From Sherman ('18), based on the works of Wells and of Jones, and the earlier results of Kossel.

[3] In this respect, according to Kossel, the true nucleic acids of the nucleus differ from the nucleo-proteids or "pseudo-nucleins" of the cytoplasm (prepared from egg-yolk or milk). Some biochemists have concluded, accordingly, that nucleic acid and true nucleo-proteins are found only in the nucleus (Mathews, '15, p. 174).

[4] These statements are given on the authority of Jones ('14).

of importance when taken in connection with the staining-reactions as an indicator of nucleo-proteins ("chromatin") in the cell (p. 652). It has been commonly assumed that the variations shown in respect to this resistance are due to varying proportions between the nucleic acid and the protein component; but Kossel's later work has shown that another factor probably lies in varying degrees of the firmness of the union between them.[1] The peptic digestion test of "chromatin," like the staining tests (p. 650), is therefore by no means a trustworthy one. To some extent, both these tests are supplemented by the nuclease test, introduced into cytological technique by Oes in 1908–10 and more recently extended by Van Herwerden.[2] Nucleic acid is readily split up by the nucleases (enzymes found in the pancreas and many other kinds of cells) and most nuclei thus treated lose more or less completely their basophily. This test, too, is defective; the sperm-nuclei of mammals, for example, resist nuclease-digestion as they do peptic (Van Herwerden, '16). It must be admitted, therefore, that we still have no certain means of identifying "chromatin" in the cell apart from its morphological history. Opinion among biochemists as to whether true nucleo-proteins (i. e., such as contain true nucleic acid) occur in the cytoplasm seems to be somewhat conflicting. Since nuclear substance is discharged into the cytosome at every mitosis (p. 126) we should expect to find nucleo-proteins there; and there is some direct evidence that such is the case—for instance, Masing's ('10) studies on the amount of nucleic acid in sea-urchin eggs at the end of cleavage as compared with that in the unsegmented egg. Indications in the same direction are offered by the observation of Van Herwerden ('13) that the basophilic microsomes of these eggs are dissolved by nuclease, as is also basichromatin.

It is an interesting fact, which has been emphasized by biochemists, that apart from the characteristic differences between animals and plants, referred to above, the nucleic acids of the nucleus are on the whole remarkably uniform, showing with present methods of analysis no differences in any degree commensurate with those from the various species of cells from which they are derived. In this respect they show a remarkable contrast to the proteins, which, whether simple or compound, seem to be of inexhaustible variety. It has been suggested, accordingly, that the differences between different "chromatins" depend upon their basic or protein components and not upon their nucleic acids.[3] Interesting questions are here raised

---

[1] For instance, the sperm-nuclei of various fishes and echinoderms examined by Kossel are readily attacked (so as to lose their basophily) by 1% hydrochloric acid, while those of mammals are but slightly affected. See Burian, '06; Kossel, '13, '14; Van Herwerden, '16.

[2] See Oes ('10); Van Herwerden ('13, '14, '16).

[3] See Mathews ('15), Levene ('17).

concerning the qualitative differences of the chromosomes and of their changes of staining-reaction at different periods of the nuclear cycle (p. 652).

3. Staining-Reactions of the Cell-Substance [1]

Staining agents are indispensable adjuncts to our microscopical analysis of the cell; but experience has shown that they may form a prolific source of error when employed as tests for the chemical nature of the cell-components or even for their morphological identification. Certain elementary sources of error must first be excluded.

First, color in itself is devoid of all significance. For example, by the use of methyl-green and eosin the nucleus is typically stained green and the cytoplasm red; but the same cell gives the reverse color effect if stained by safranin and light green, the nucleus now being red and the cytoplasm green.

Secondly, the most diverse structures may often be stained alike by the same dye. In cartilage, for example, both the basichromatin of the nuclei and the inter-cellular matrix (a non-protoplasmic product of the cell) are alike stained by methyl green, though unrelated chemically. Again, many kinds of granules and other formed cytoplasmic elements resemble the nuclear basichromatin in staining-reaction, and hence have actually been regarded as "cytoplasmic chromatin"; but we cannot for this reason alone conclude that they are of nuclear origin or chemically similar to the nuclear "chromatin." The naïve conclusion, without further evidence, that similarity in staining-reaction necessarily indicates similarity of chemical or morphological nature has indeed been one of the most frequent sources of error in cytological work.

Thirdly, the character of staining-reactions, as every cytologist knows, is profoundly affected by the reaction of the medium and by the processes of fixation, mordaunting, and the like. This applies especially to treatment by metallic salts, which may enter into combination with the organic components of the cell so that they cannot be removed by simple washing. This process, which is really a kind of mordaunting, may be favorable to certain stains, and unfavorable to others. The effects and nature of staining are therefore best examined after fixation by non-electrolytes, such as alcohol or heat, which produce a minimum of chemical change in the organic cell-constituents; but in other respects, unluckily, such agents are usually far inferior.

Even when all such sources of error have been eliminated the nature of

---

[1] A useful discussion of this subject is given by Mann ('02). Among other important works may be mentioned those of Mathews ('98), Heidenhain ('02, '07, '14) and earlier works, Michælis ('09, '10, '20), Bechold ('12), and Bayliss ('21, '23).

the process of staining offers a complicated problem that has been but incompletely solved; for it is still uncertain how far dyeing is a chemical process and how far a physical one, *i. e.*, in what measure it involves a chemical union between the dye-stuff and the stainable material, and in what measure a merely physical process or adsorption of the dye by surface-action. Nevertheless, it now seems certain that the effect is often to a considerable extent conditioned by the chemical relation between the dye-stuff and the dyed material. Cytological staining methods may, therefore, have a certain value as *indicators;* but since the physical phenomena of adsorption also may play an important part in the process, we must admit that for the present *the main use of cytological staining processes is to make the cell-components more clearly distinguishable by the microscope.*[1]

The foundation of the chemical theory of staining was laid by Ehrlich ('77, '91, etc.). Cytologists long since classified coloring agents as "nuclear" and "plasmatic" (or "plasma") dyes, the former (such as carmine or hæmatoxylin) displaying a marked tendency to stain the nucleus, the latter (such as eosin) staining more especially the cytoplasm.[2] Ehrlich first pointed out, in case of the coal-tar or "anilin" dyes, that this difference is correlated with a definite difference of chemical composition. These dyes are in general organic compounds in which (to state the matter in the most general way), a color-determining radical (chromatophore) is united with another radical to form a salt-like compound. In the various dyes thus formed, the color radical plays the part sometimes of the anion (acid), sometimes of the kation (base)—*i. e.*, in some cases is electro-negative, in others electro-positive; hence Ehrlich's designation of these dyes as "acid" and "basic" respectively. These terms do not refer to the reaction of the dye itself, but only to the nature of the color-determining component; an "acid" dye may indeed actually have a neutral or alkaline reaction, and *vice-versa.*[3] It seems preferable, therefore, to designate the "acid" dyes as *acidic.*

A typical example of an acidic dye is eosin, which is a sodium salt of the color-acid tetrabrom-fluorescein; and of a basic dye, methylen-blue, which is the chloride of a color-base, tetramethyldiphenthiazin.[4] As already indicated, this distinction bears no relation to actual color. Basic dyes may be blue (methylen-blue, gentian violet), red (safranin, basic fuchsin),

[1] *Cf.* Bayliss ('21).

[2] The history of the subject from 1850 to 1880, is considered in Mann's work, above cited, and also in the interesting articles by Gierke in early volumes of the *Zeitschrift für wissenschaftliche Mikroskopie.* Here we only refer to the introduction of the use of carmine by Hartig and Gerlach (1854–58), of double staining by Schwarz and Ranvier (1867–68), of hæmatoxylin by Waldeyer (1863) and Böhmer (1865) and of the coal tar-colors by Beneke, Frey and Waldeyer (1862–63).

[3] Ehrlich also distinguished as "neutral" dyes those in which a color-base and a color-acid are united in the same compound; but these need not here be considered.

[4] From Mann.

green (methyl green), or yellow (Bismarck brown). Similar differences are shown by the acidic stains. Examples are methyl-blue, eosin, Congo red, light green and orange. In some cases the same color-group may play the part of either a base or an acid, an example of which is fuchsin, a red dye of which the color-determining component is rosaniline. By appropriate chemical treatment this may appear in either the "basic" or "acidic" form, being in the former case a hydrochloride of the base rosaniline, in the latter a sodium salt of sulphonic acid (a derivative of rosaniline).

The most convincing demonstration of these distinctions is offered by the simultaneous use of basic and acidic dyes in a mixture,[1] the one most commonly employed containing basic methyl-green and acidic red (acid fuchsin), which acts electively on the cell in such a manner as to stain the nucleus (basichromatin) green and the cytosome red. As Ehrlich pointed out, however, both nucleus and cytoplasm usually contain elements of opposite staining-capacity to that of their principal components. Thus, the cytoplasm, while predominantly *oxyphilic* (*i. e.*, showing an affinity for acidic stains) nevertheless often contains granules or other structures that are intensely *basophilic*. Again, in the case of the nucleus only certain constituents are basophilic ("chromatin" in the older sense, karyosomes) while others are strongly oxyphilic (plasmosomes, "linin" in the older sense); hence Heidenhain's distinction between "basi-chromatin" and "oxychromatin" (p. 88).

These various facts led Ehrlich to conclude that staining results from a chemical union between dyes and the tissue-elements; and this conclusion has been supported by many later students of the subject. On the other hand, it has been demonstrated also that many dyes are energetically taken up from their solutions by powdered charcoal, filter paper, silk, and other inert substances in a state of fine subdivision. Doubts, therefore, were early raised concerning the chemical theory of staining by Gierke ('99), Rawitz ('97) and others, above all by A. Fischer ('99), who brought forward a large body of evidence demonstrating the important effect upon cytological staining of the physical processes of adsorption and the like.

Fischer showed that in combinations of different stains the effect is largely influenced by such factors as the relative size of the granules or other stainable elements, the order in which the dyes are used, their relative concentrations, and their differences in rate of diffusion. He showed that the double staining of granules of coagulated proteins by the simultaneous use of an acidic and a basic dye, or even of two acidic or two basic dyes,

---

[1] Similar results can of course be produced by their separate and successive action, but the interpretation is here always more doubtful because of the artificial factor introduced by the manipulation of the two dyes.

may be thus wholly determined. If these granules be stained with a suitable mixture of picric acid and acid fuchsin, or of eosin and light green (all acidic dyes) the large granules are in the first case yellow and the small red, while in the second case, the large ones are red and the small ones green. The double staining is here due to different rates of diffusion, the more rapid dye taking possession of the granule before the slower has had time to act; but in the end all the granules ultimately stain alike, since the slower dye finally displaces or covers the faster. More recently, Bechold ('12) has shown by accurate studies that the density of the stainable object has an important effect upon the rate of diffusion of the dye, and hence on the staining-capacity.

Such facts clearly show that physical factors play an important part in cytological staining-reactions; nevertheless the evidence now seems to indicate that with certain reservations the chemical theory of staining is still tenable. It has been clearly demonstrated by Heidenhain, Michælis, Bechold, and others that the adsorption of dyes by insoluble substances in fine suspension (or otherwise finely divided) is conditioned by the chemical nature of those substances. Substances that are electro-negative (*e. g.*, kaolin, siliceous marl, silicic acid) adsorb in general only basic dyes; those that are electro-positive (clay, iron hydroxide) adsorb only the acidic; while the amphoteric substances are stainable by both kinds of dyes, and the indifferent faintly by both.[1] All this indicates that the staining-process is here not one of adsorption alone but involves a chemical reaction; and this seems to be proved in certain cases by the fact that products of the chemical exchange are later found in the liquid (Michælis, '20).

To return to the chemical theory of staining, it was shown by the early work of Miescher (1874) that isolated nucleic acid will form insoluble intensely colored precipitates with basic tar colors. Malfatti ('91) and Lilienfeld ('93, '94) observed that in a mixture of basic methyl-green and red acid fuchsin free nucleic acid stains intensely green while albumen is stained red. The same observers demonstrated that compounds of metaphosphoric acid and albumin in various proportions stain bluish red graduating into pure red as the percentage of phosphorus diminishes. Observations of this type indicate that the differences of staining capacity displayed by different forms of nuclei, or by different conditions of the same nucleus, may be interpreted as due to varying proportions between the nucleic acid and the basic protein components, the former being at a maximum during mitosis when the chromosomes are so markedly basophilic. In harmony with this are the later experiments of R. Lillie, Pentimalli, and others on the behavior of nuclei and chromosomes in the electrostatic

[1] See table in Bechold ('12, p. 29).

field, which demonstrate their electro-negative or acidic character (p. 189). It has been supported by numerous more direct experiments on staining reactions, and especially those of Mathews ('98), Mann ('02), Heidenhain ('02, '03, '07, etc.), and Loeb ('18).

Mathews found that acidic dyes (used in the form of neutral salts) have no effect upon solutions of albumin or albumose until a small amount of free acid is added when an intensely colored, insoluble precipitate (a combination of the color-acid and protein) is at once thrown down. With basic dyes the opposite result is obtained. If added to neutral or acid solutions of albumose they produce no effect; but if the solution be made slightly *alkaline* a deeply colored insoluble compound is precipitated. In like manner, egg-albumin coagulated by heat or alcohol fails to stain in neutral solutions of either acid or basic dyes [1] but is instantly and intensely colored by acidic dyes if the latter be acidified, in basic dyes if these be rendered slightly alkaline. Conversely, no effect, or but a slight one, is produced by acidified basic dyes or by acidic ones rendered slightly alkaline.

It is the same with the staining of cells (from the liver, pancreas, and muscles) fixed in neutral or acid alcohol. When such preparations are treated with neutral solutions of basic dyes (such as methyl green, safranin or thionin) a pure "chromatin stain" results, but if the section be first treated, even for a very short time, in a weak alkaline solution (of sodium carbonate), or if the staining fluid itself be made slightly alkaline, not only the chromatin but also the *cytoplasm* is intensely and permanently stained. In this way the cytoplasm of these cells may be given a powerful "plasma-stain" by the use of various typical "nuclear" (basic) dyes. With acidic dyes, on the other hand, the cytoplasm is stained only in acid solutions.

These results, confirmed fully by the work especially of Heidenhain, are explained under the chemical theory of staining by the amphoteric nature of the proteins. After treatment by acids (which are ingredients of most cytological fixing agents), or upon staining in acidified solutions, the cytoplasmic proteins are in general oxyphilic, *i. e.*, they behave as bases and hence combine with acidic dyes. In alkaline solutions they show the reverse behavior, becoming acid and hence combining with basic dyes. The nuclear proteins, on the other hand, are for the most part electro-negative or acid, because of the large proportion of nucleic acid which they contain; and in general, therefore, are basophilic. Quite similar are the results of Loeb ('18) on gelatine. Stained in neutral red (a basic dye) it gives off its color in acid solutions; stained in acid fuchsin the color is given off in alkaline solutions. For in the first case gelatine becomes electro-positive or basic, in

---

[1] A temporary physical imbibition of the dye may take place, but the color thus produced may readily be washed out in water.

the second electro-negative or acid and fixes (*i. e.*, combines with) or gives off the dye accordingly.

The simplest interpretation of these results, evidently, is that staining involves an actual chemical combination to form a salt-like combination; and this explanation has been adopted by Heidenhain as a result of his above-cited extended experiments and by Bechold, Michælis, Loeb and many others. Bayliss ('21, etc.) has made an interesting attempt to interpret results of the foregoing type in terms of surface-action and electrical charges; but at the same time he emphasizes the fact "that the physical properties of a surface ultimately depend on its chemical nature" ('23, p. 27). Bechold, Michælis and others have recognized the probability that the part played by the physical process of adsorption is a preliminary one, the dissolved or finely suspended dye-stuffs being first taken up by a process of surface-action on the part of the protoplasmic particles and then more intimately combined with them by a process which to say the least approaches a true chemical union.

All this makes it highly probable that the oxyphilic or basophilic staining-reactions of the cell-components are in greater or less degree determined by chemical conditions; and the possibility is here undoubtedly offered of achieving ultimately a rational micro-chemistry of the cell, such as Ehrlich always had in mind. It must, however, be admitted that we are still very far indeed from its realization. It may again be emphasized that mere similarity of staining-reactions is always an unsafe guide either to morphological or chemical nature. Basic dyes, for example, are not in any sense specific tests either for "chromatin" (basichromatin) or for nucleo-proteins. For, as Mathews' experiments showed, even the basic or neutral cytoplasmic proteins readily become basophilic in alkaline solution; and there are also a number of strongly basophilic compound proteins known to be of cytoplasmic origin and to contain no nucleic acid. Examples of these are offered by mucin or the inter-cellular substance of cartilage, both of which are conjugated proteins containing a complex organic acid (chondriotic) other than nucleic. The basic dyes are therefore merely indicators for proteins in the acid state, or for basic proteins linked with acids.

With these results in mind it will be instructive to glance at certain problems concerning the staining-reactions of the cell-components which first took on definite form with the classical studies of Heidenhain on leucocytes ('93, '94) by the use of simultaneous staining with mixtures of acidic and basic dyes. Heidenhain emphasized the conclusion that the periodic changes of staining-reaction on the part of the nuclear structures may largely be due to changing properties between the basic (protein) and the acid (nucleic acid) components of the "chromatin." Basichroma-

tin and oxychromatin ("chromatin' and "linin" in the older sense), as was earlier emphasized by Van Beneden, are by no means to be regarded as permanent or fixed bodies, but may change their color-reactions by combining with or giving off phosphorus (nucleic acid), according to varying physiological conditions of the nucleus or of the cell. Many examples of such changes of chromatophily have been given in the foregoing pages, for example: the intense increase of basophily on the part of the chromo-

Fig. 312.—Chromosomes of the germinal vesicle in the shark *Pristiurus*, at different periods, drawn to the same scale (RÜCKERT).

A, At the period of maximal size and minimal staining-capacity (egg 3 mm. in diameter); B, later period (egg 13 mm. in diameter); C, at the close of ovarian life, of minimal size and maximal staining-power.

somes during the prophases of mitosis, the corresponding decrease following its final phases in ordinary somatic cells, and the still more marked decrease so often seen in the auxocytes, particularly in the germinal vesicle of the animal ovum (p. 351). The decrease of basophily is accomplished by marked, and often extremely striking processes of growth on the part of these structures (Fig. 312). Not less striking is the fact that their later

resumption of basophily is accompanied by a marked decrease in size, very often also by a giving off or leaving behind of a quantity of residual oxyphilic or but slightly basophilic material (p. 355).

All this falls in line with the assumption that during the vegetative activities of the cell the protein bases of the nucleus increase in quantity, the acidic character of the chromatin (and hence its basophily) correspondingly diminishing as the affinities of the nucleic acid radical are more completely satisfied. Whether this means an actual increase in the protein components, or a diminution of the nucleic acid, can only be surmised. Perhaps both may be true; for the enormous growth of the chromosomes, accompanied in some cases by a complete reversal of their staining-reactions seems to indicate a progressive accumulation of protein-components and a giving up, or even a complete loss, of the acid component (p. 650). One is tempted to suspect that the basophily of the nucleoli so often observed at this time may mean that they are storehouses of nucleic acid that has been given off from the chromosomes; further, that as the nucleus prepares for mitosis the accumulated protein-components are in considerable measure set free while the remainder are left in combination (or recombine) with nucleic acid to form the spireme-threads and chromosomes. The chromatin is thus restored to its original basophilic condition in preparation for division, only the essential components being carried over into the next cell-generation. From the point of view thus suggested it is interesting that the cast-off material (which may contain both oxyphilic and basophilic components) is often thrown out into the cytoplasm as "residual substance" when the nucleus breaks down in preparation for the ensuing mitosis. In part, perhaps, this process may be a mere discarding of material that has played its part during the growth and differentiation of the cell,[1] but it is probable that in some cases the residual substance plays an active and important part in the constructive processes of the cytoplasm.

An important, little explored field of inquiry is here opened. At this point we may mention the hypothesis that the various nucleo-proteins (or chromatins) are determined by their protein rather than by their nucleic-acid components (p. 644). Mathews suggested that in a chemical sense the nucleic-acid component may be regarded as "a colloidal, gelatinous substratum in the nature of an organic skeleton to which the specifically active, more labile albuminous constituents, possibly of a catalytic nature, may be attached.[2]" Goldschmidt emphasized the biochemical evidence that certain enzymes (oxydases, hydrolytic ferments) are often linked with nucleo-proteins. "The thought is thus suggested that it is the function of

[1] Cf. the "histogenetic plasm" of Weisman, p. 498.
[2] Mathews. '15, p. 176.

chromatin to adsorb the heredity-enzymes and to serve as their skeleton." [1] This suggestion is of much interest, but to the cytologist it seems to reverse the conditions as actually observed. For, as has been indicated (p. 351), in some cases the chromosomes may become nearly or quite oxyphilic without loss of their identity; and in others, the nuclear network to which they may give rise undergoes a similar change; hence the conclusion of certain observers that the "chromatin" may wholly disappear from the nucleus. So far as the staining-reactions show, therefore, it is not the basophilic component (nucleic acid) that persists, but the so-called "achromatic" or oxyphilic substance. The nucleic acid component comes and goes in different phases of cell-activity, and it is the oxyphilic component that seems to form the essential structural basis of the nuclear organization. This would accord perfectly with the "achromatin hypotheses" of chromosome continuity (p. 895) and also with the accumulation of basophilic material ("chromatin") in the nucleoli while the chromosomes lose their basophily, and often also, in appearance, their identity in the general nuclear framework. Mathews seems to have overlooked these facts in his criticism of theories of heredity "based on the behavior of the nucleic acid of the nucleus, *that is, the behavior and number of the chromosomes*" (italics by the writer).

III. GENERAL PHYSIOLOGICAL RELATIONS BETWEEN NUCLEUS AND CYTOSOME

1. General

In his celebrated work, *Leçons sur les phénomènes de la vie* (1878), Claude Bernard grouped the constructive processes of the cell in the two categories of *chemical synthesis* by which specific organic substances are formed, and *morphological synthesis* by which these substances are built into a specifically organized fabric. Bernard was one of the first clearly to see that the two categories represent fundamentally only different phases or degrees of the same general phenomenon. The primary agent in both he believed to be the nucleus, considering the cytoplasm to be characterized by the predominance of the destructive operations by which energy is set free. This highly fruitful generalization now rests on a solid basis of known fact; but it was orginnally stated in too simple a form. All physiologists, probably, will agree that the principal arena for the liberation of energy is the cytoplasm, and that this process may for a time go on in the absence of a nucleus (p. 659). It is equally certain that the nucleus plays an important part in organic synthesis. It is impossible, however, to consider the nu-

---

[1] Goldschmidt, '17a, p. 94. See also '17b, p. 608.

cleus as the sole agent in synthesis, as is made obvious by the fact, among many others, that the photosynthesis of carbohydrates from H_2O and CO_2 (the most fundamental of all organic syntheses) takes place only in the chloroplasts, which are strictly cytoplasmic structures; and it has further been shown that photosynthesis continues in chlorophyll-containing masses of protoplasm from which the nucleus has been removed (Klebs, '79, '87), and according to Molisch ('04) even in isolated chloroplasts.[1] There is now indeed little or no evidence to show that the nucleus is the actual formative center of the cell.

The influence of the nucleus upon organic synthesis nevertheless remains a fundamentally important fact. How this influence is exerted still remains almost unknown; but it is probably connected with exchanges of material between nucleus and cytosome which continually or intermittently take place. This fact is most obvious in the cyclical reproductive processes of the cell. At the time of mitosis, when the nuclear membrane breaks down, a considerable and sometimes very large amount of nuclear material is directly set free into the cytoplasm. Conversely, at the close of mitosis the nucleus undergoes extensive growth, the material for which process, obviously, must be taken into the nucleus from the surrounding cytoplasm. But there is other ground for the conclusion that exchanges of material between nucleus and cytoplasm likewise go on during the vegetative state of the cell, whether by processes of ösmosis or, as some observers maintain, by an actual extrusion of formed elements through the nuclear membrane. The opinion has steadily gained ground that the nucleus may be a storehouse of enzymes, or of substances that activate the cytoplasmic enzymes, and that these susbtances may be concerned with synthesis as well as with destructive processes.[2] To cite Hopkins "We have arrived, indeed, at a stage when, with a huge array of examples before us it is logical to conclude that all metabolic tissue reactions are catalyzed by enzymes, and, knowing the properties of these, we have every right to conclude that all reactions may be so catalyzed in the synthetic as well as in the opposite sense." Too little is known as yet concerning this subject to justify its extended discussion here; but it may be pointed out that the suggestions which it offers have already opened interesting possibilities concerning the problems both of genetics and cytology.

2. Nuclear Size and Cytoplasmic Growth

We may here again refer to observations and experiments which prove that artificial increase in the size of the nucleus leads to a corresponding increase

[1] Jost ('07), p. 107.
[2] See, for example, Bayliss ('13, '18). An interesting discussion of the enzymes is given by Loeb ('18). See also Loeb and Chamberlain ('15) and Goldschmidt ('16, '17, '20).

in the size of the cytosome by a compensatory growth of the cytoplasm (pp. 100, 729). Especially convincing is the demonstration of this offered by Gerassimoff's experiments on *Spirogyra* (Fig. 313).[1] Since these experiments involve no disturbance of the cytoplasm other than that which results from the nuclear change, they seem to afford decisive proof that the
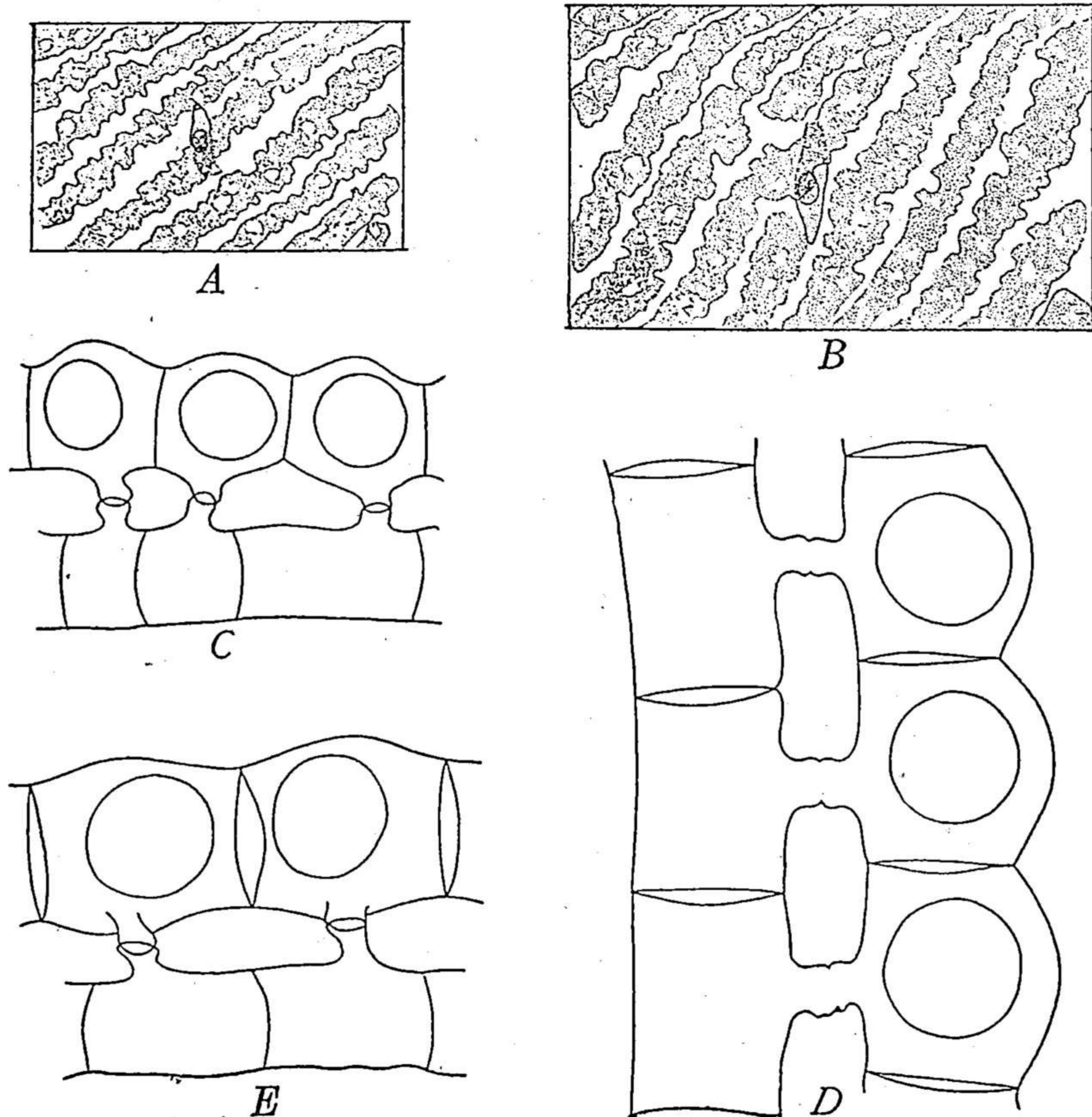

Fig. 313.—Normal and giant forms of *Spirogyra bellis* (GERASSIMOFF).

A, cell from a normal (haploid) plant; *B*, cell from an artificially produced giant (diploid) plant of the same species; *C*, part of two conjugating normal filaments; *D*, corresponding view of conjugating diploid filaments, forming tetraploid zygospores; *E*, conjugation of normal (haploid) and giant (diploid) filaments, to form triploid zygotes. *C*, *D*, and *E* drawn to the same scale.

nucleus is the *primary* agent in the constructive processes of cytoplasmic growth. The experiments of Boveri, the Marchals, and others (p. 729) though in some respects less direct, have placed this conclusion on a broader basis by the demonstration that within a given cell-type the cytoplasmic volume varies with the number of chromosomes that enter the nucleus (p. 729). In the mosses the relation seems to be essentially the same as

[1] See p. 25.

Spirogyra; for the Marchals found (p. 730) that in diploid or tetraploid gametophytes the cells, both somatic and germinal, are approximately two or four times that of the normal (haploid) forms. This applies to nucleus and cytosome alike (as in *Spirogyra*), the normal karyoplasmic ratio being thus retained. In sea-urchin eggs, the proof is less evident because the cell-size is here affected by compensatory changes in the rate of cell-division. The segmenting eggs may here be either haploid (parthenogenetic), diploid (normal, fertilized eggs) or tetraploid (fertilized monaster-eggs, p. 729) and the size of the resulting cells differs correspondingly. In each of these cases the ratio of cytoplasmic volume to nuclear is the same as in the normal egg (as is the case in the tetraploid cells of *Spirogyra* or the mosses). Here, however, the size-differences do not result from different rates of growth (as in *Spirogyra*), but from different rates of cleavage, the haploid cells dividing most rapidly, and the tetraploid most slowly (p. 728).[1] The result is the same, though here seen from a different angle.

Winkler ('16) shows that in tetraploid *gigas* mutants of the tomato (*Solanum*), not only are the cells (including the pollen-grains, Fig. 350 A-H) larger than those of the normal diploid form but *also the chloroplasts* (I, J). This seems clearly to show that the increase of nuclear content affects the growth not merely of the cytosome as a whole, but also that of its formed components (*Cf.* p. 732).[2]

The relation between chromosome-number and nuclear size is well shown in larvæ (usually abnormal) from dispermic eggs, in which cleavage is typically multipolar and usually leads to an irregular distribution of the chromosomes to the cells of different embryonic regions. The size of these cells, and their nuclei, is likewise larger or smaller according to the varying number of chromosomes that they receive (Fig. 432) though the numerical relations cannot here be so exactly determined. In dispermic or trispermic eggs of frogs, as shown by Brachet and by Herlant, the case is still clearer; for here but one sperm-nucleus unites with the egg-nucleus, while the other one (or two) divides separately (p. 418). Both the diploid and the haploid nuclei thus produced continue to divide, finally producing larvæ in which the cells of certain regions (sometimes in one-half of the body) contain diploid nuclei, while in the remaining regions they are haploid. Both nuclei and cytosomes, as might be expected, are much smaller in the haploid regions than in the diploid (Fig. 314).

In the foregoing cases we are dealing with only quantitative effects.

---

[1] This difference is due to the fact that the cleavage-cells do not grow noticeably, cytoplasmic growth for these stages having already been accomplished by the egg before cleavage begins.

[2] It should, however, be recalled that the polymegalous spermatocytes of Hemiptera, though all alike diploid, likewise show corresponding variations in the size of the cytoplasmic components (p. 304). Perhaps, therefore, another factor may be concerned, as in certain diploid giants (p. 101).

In later chapters we shall become acquainted also with qualitative effects in the cytoplasm due to the influence of the nucleus; but attention may here briefly be drawn to them. Boveri's celebrated experiments on multipolar mitosis ('02, '07), demonstrated that normal development and differentiation are dependent on the presence of the normal combination of chromosomes in the nucleus. When this combination is impaired or modified without disturbance of the cytoplasmic substance development is correspondingly impaired or modified, leading to the production of asymmetrical, incomplete, pathological or monstrous forms. This can only mean that constructive morphological processes of the cytosome are conditioned by the nucleus in a qualitative as well as a quantitative sense (p. 920).
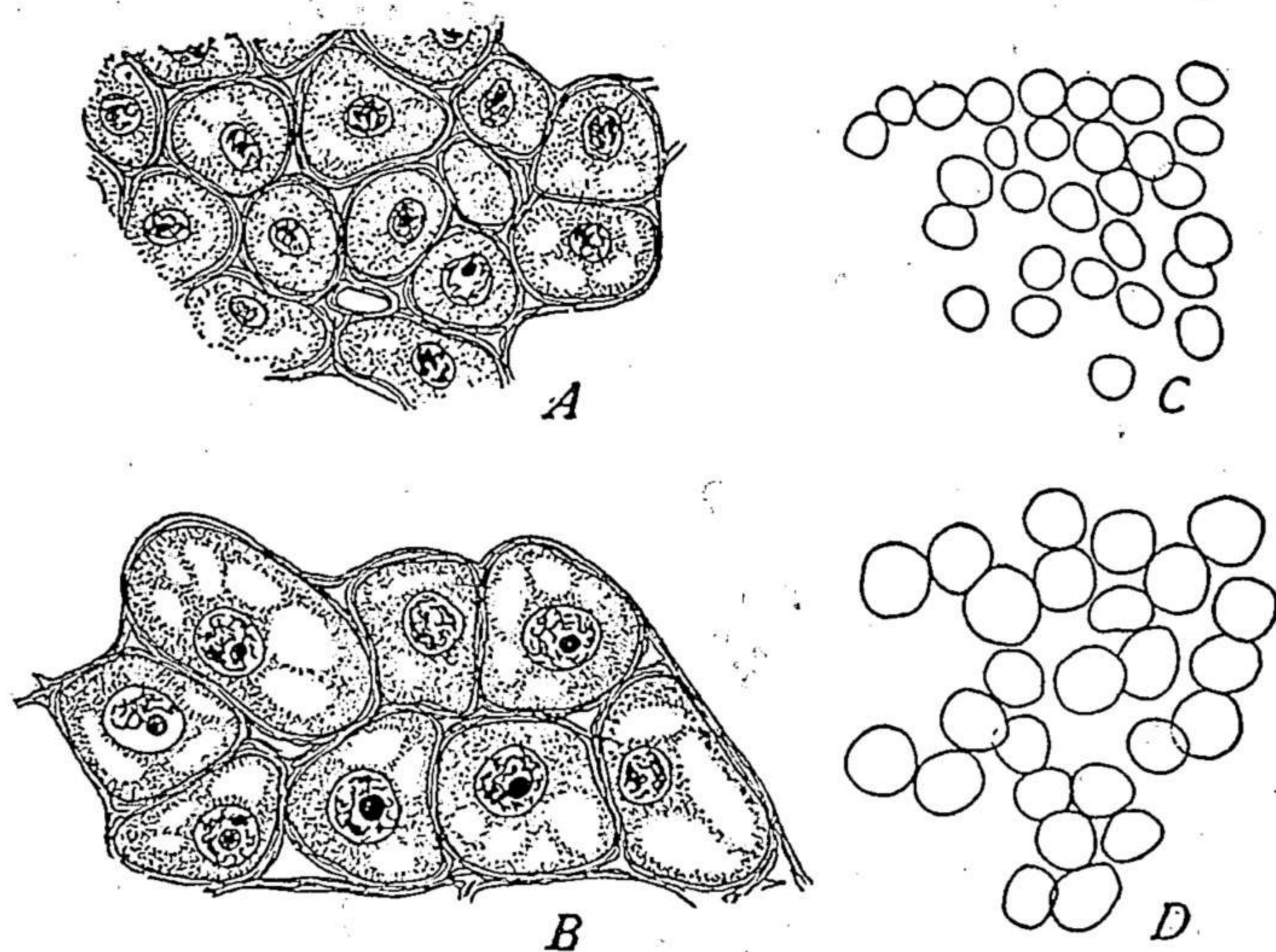

Fig. 314.—Cells and nuclei from trispermic frog-larvæ (HERLANT).

A, cartilage-cells from right side, B, from left side, larva of 10 days; C, nuclei from central nervous system, right side, D, from left side, larva of 54 days.

The same conclusion follows from the relations of the chromosomes to sex, as will be shown in the tenth chapter; for it is now known that the production of the male or female characters in many animals, and in a few plants, is dependent upon the nature of the chromosome-combination (pp. 816, 817, etc.).

3. Nucleated and Non-nucleated Cell-fragments

Equally convincing, if less direct, is evidence drawn from the remarkable contrast between nucleated and non-nucleated cell-fragments in respect to synthetic processes. The earliest observations on this subject were the classical ones of Waller (1852) on the regeneration of nerve-fibers, which proved that this process only takes place when the axis-cylinders remain in

connection with the nucleated cytosomes of the nerve-cells. When a nerve-fiber is severed the distal portion degenerates, while the proximal portion (still connected with the nerve-cell and its nucleus) may readily grow forth until the missing portion is restored. This observation, repeatedly confirmed by later observers, was not wholly decisive, but nevertheless gave the first clear indication of the necessity of the nucleus for growth, regeneration and differentiation. Conclusive evidence was later obtained by experimental studies on the cells both of Protista and of higher forms.
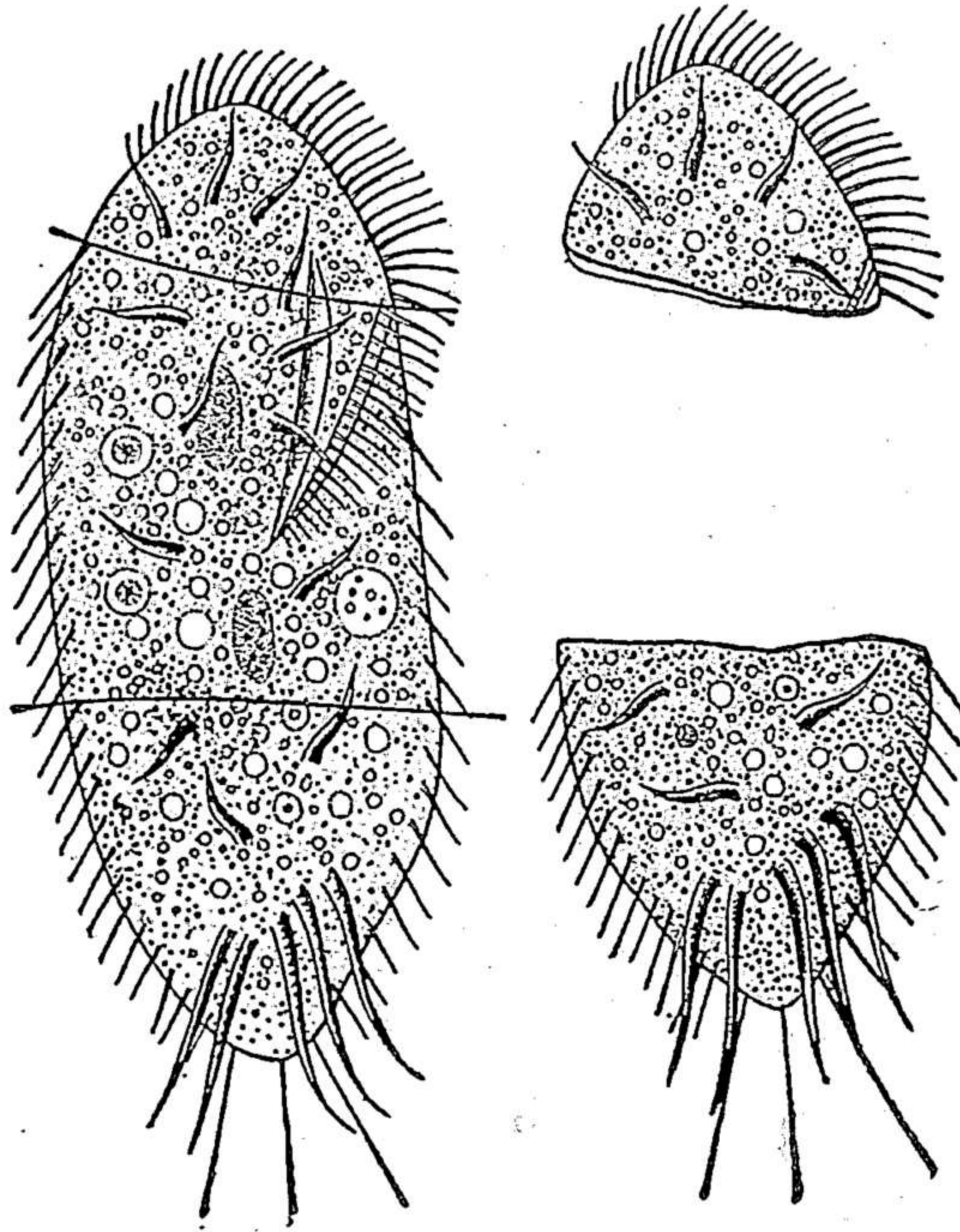

Fig. 315.—Regeneration in *Stylonychia* (VERWORN).

At the left an entire animal, showing planes of section. The middle piece, containing two nuclei regenerates a perfect animal. The enucleated pieces, shown at the right, swim about for a time, but finally perish.

Brandt ('77) long since observed that enucleated fragments of *Actinosphærium* soon die, while nucleated fragments heal their wounds, and continue to live. The first decisive comparison between nucleated and non-nucleated masses of protoplasm was, however, made by Moritz Nussbaum in 1884 in the case of a ciliate, *Oxytricha*. If one of these animals be cut into two pieces, the subsequent behavior of the two fragments depends on the presence or absence of the nucleus or a nuclear fragment. The nucleated fragments quickly heal the wound, regenerate the missing portions, and thus produce a perfect animal, while enucleated fragments, consisting of cytoplasm only, quickly perish. Gruber ('85) obtained a similar result in the case of *Stentor*, another ciliate (Fig. 316). Fragments possessing a large fragment of the

nucleus completely regenerated within twenty-four hours. If the nuclear fragment were smaller, the regeneration proceeded more slowly. If no nuclear substance were present, no regeneration took place, though the wound closed and the fragment lived for a considerable time. The only exception was the case of individuals in which the process of normal fission had begun; in these a non-nucleated fragment in which the formation of a new peristome had already been initiated healed the wound, and completed the formation of the peristome. Lillie ('96) later found that *Stentor polymorphus* may be shaken into fragments of all sizes, and that nucleated fragments as small as 1/27 the volume of the entire animal are still capable of complete regeneration. In all the foregoing cases all non-nucleated fragments perish.[1] In *S. cœruleus* Morgan ('01) showed that nucleated fragments not more than 1/64 the normal size might still produce a complete individual.

These studies were extended to the rhizopods by other observers. Verworn ('88) proved that in *Polystomella*, one of the Foraminifera, nucleated fragments were able to repair the shell, while non-nucleated fragments lack this power. Balbiani ('89) found that although non-nucleated fragments of ciliates had no power of regeneration, they might nevertheless continue to live and swim actively about for many days after the operation, the contractile vacuole pulsating as usual. Hofer ('89) found in *Amœba*, that non-nucleated fragments might live as long as fourteen days after the operation (Fig. 317). Their movements continued, but were somewhat modified, and little by little ceased, but the pulsations of the contractile vacuole were but slightly affected; they lost more or less completely the capacity to digest food, and the power of adhering to the substratum. Verworn's later experiments ('89) on various Protozoa confirmed the accounts of his predecessors, and added many important results. Non-nucleated fragments both of ciliates (*e. g., Lachrymaria*) and rhizopods (*Polystomella, Thalassicolla*) may live for a considerable period (Fig. 315), performing perfectly normal and characteristic movements, showing the same susceptibility to stimulus, and having the same power of ingulfing food, as the nucleated fragments. *They lack, however, the power of digestion and secretion.* Ingested food-matters may be slightly altered, but are never completely digested. The non-nucleated fragments are unable to secrete the material for a new shell (*Polystomella*) or the slime by which the animals adhere to the substratum (*Amœba, Difflugia, Polystomella.*)

These results have been confirmed in their main features by the work of many later observers.[2] With certain variations of detail they establish

---

[1] As Calkins has pointed out, the phenomena in ciliates are less demonstrative than in rhizopods, owing to the difficulty of making sure of the presence or absence of micronuclei in the fragments.

[2] Lists and reviews of the literature will be found in K. Gruber's work on *Amœba* ('13) and especially in that of Sokoloff ('24), received too late for further mention here.

the fact that destructive processes and the liberation of energy, as mani-
fested by coördinated forms of protoplasmic movement, may go on for some
time undisturbed in a mass of cytoplasm deprived of a nucleus. On the
other hand, the building up of new chemical or morphological products by
the cytoplasm is only initiated in the presence of a nucleus, and soon ceases in
its absence. The nucleus must, therefore, play an essential part both in the
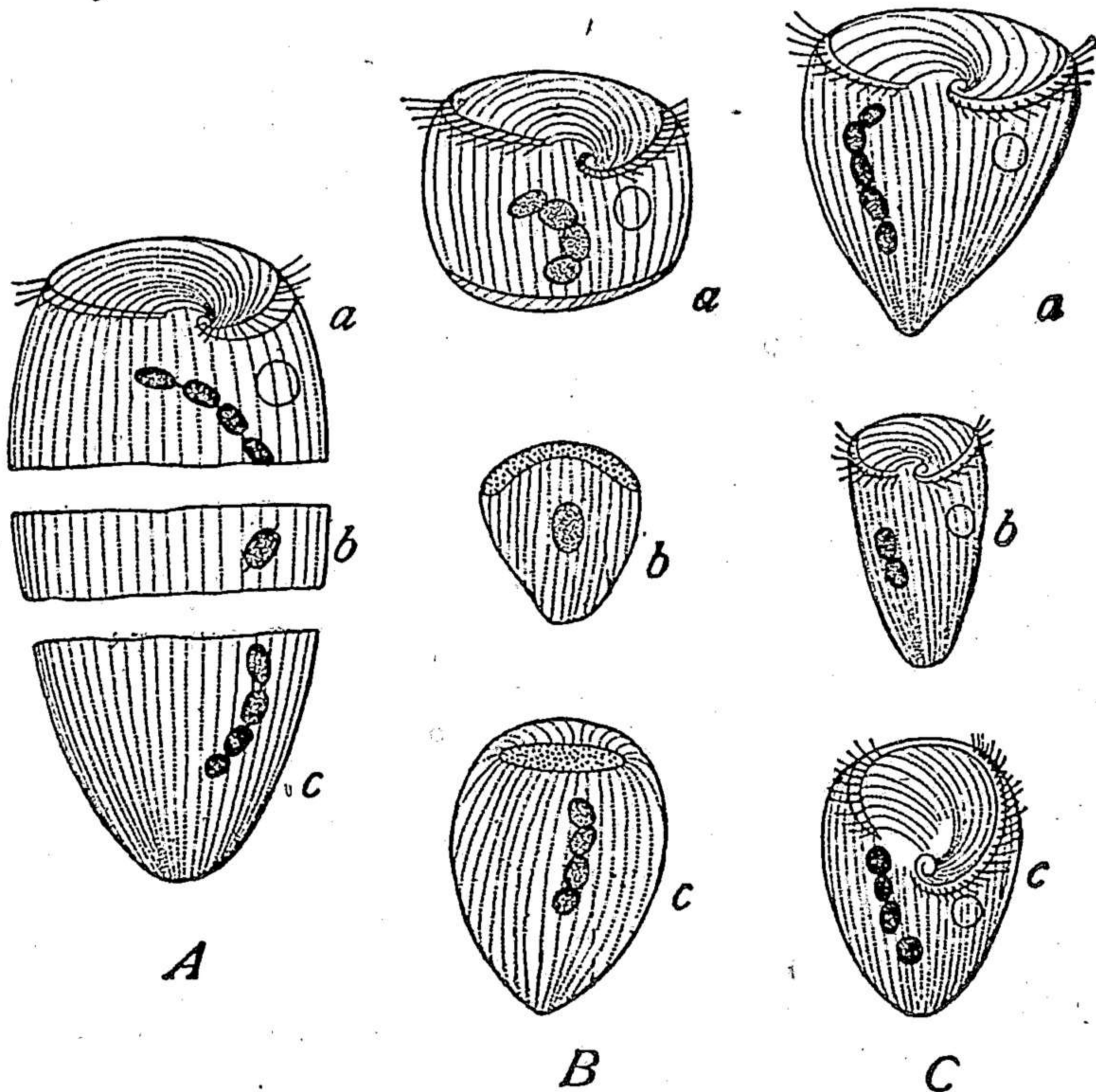operations of synthetic metabolism or chemical synthesis, and in the *morpho-*

Fig. 316.—Regeneration in the unicellular animal *Stentor* (from GRUBER after BALBIANI).
A, animal divided into three pieces, each containing a fragment of the nucleus; *B*, the three
fragments shortly afterwards; *C*, the three fragments after twenty-four hours, each regenerated to a
perfect animal.

logical determination of these operations (*i. e.*, the morphological synthesis of
Bernard)—a point of capital importance for the theory of inheritance.

Convincing experiments of the same character and leading to the same
result have been made on the cells of plants. Francis Darwin ('77) long
since observed that movements actively continued in protoplasmic filaments,
extruded from the leaf-hairs of *Dipsacus*, that were completely severed
from the body of the cell. Conversely, Klebs ('79) showed that naked
protoplasmic fragments of *Vaucheria* and other algæ were incapable of
forming a new cellulose membrane if devoid of a nucleus; and he afterward
showed ('87) that the same is true of *Zygnema* and *Œdogonium*. By plas-

molysis the cells of these forms may be broken up into fragments, both nucleated and non-nucleated. The former surround themselves with a new wall, grow, and develop into complete plants; the latter, while able to form starch by means of the chlorophyll they contain, are incapable of utilizing it, and are devoid of the power of forming a new membrane, and
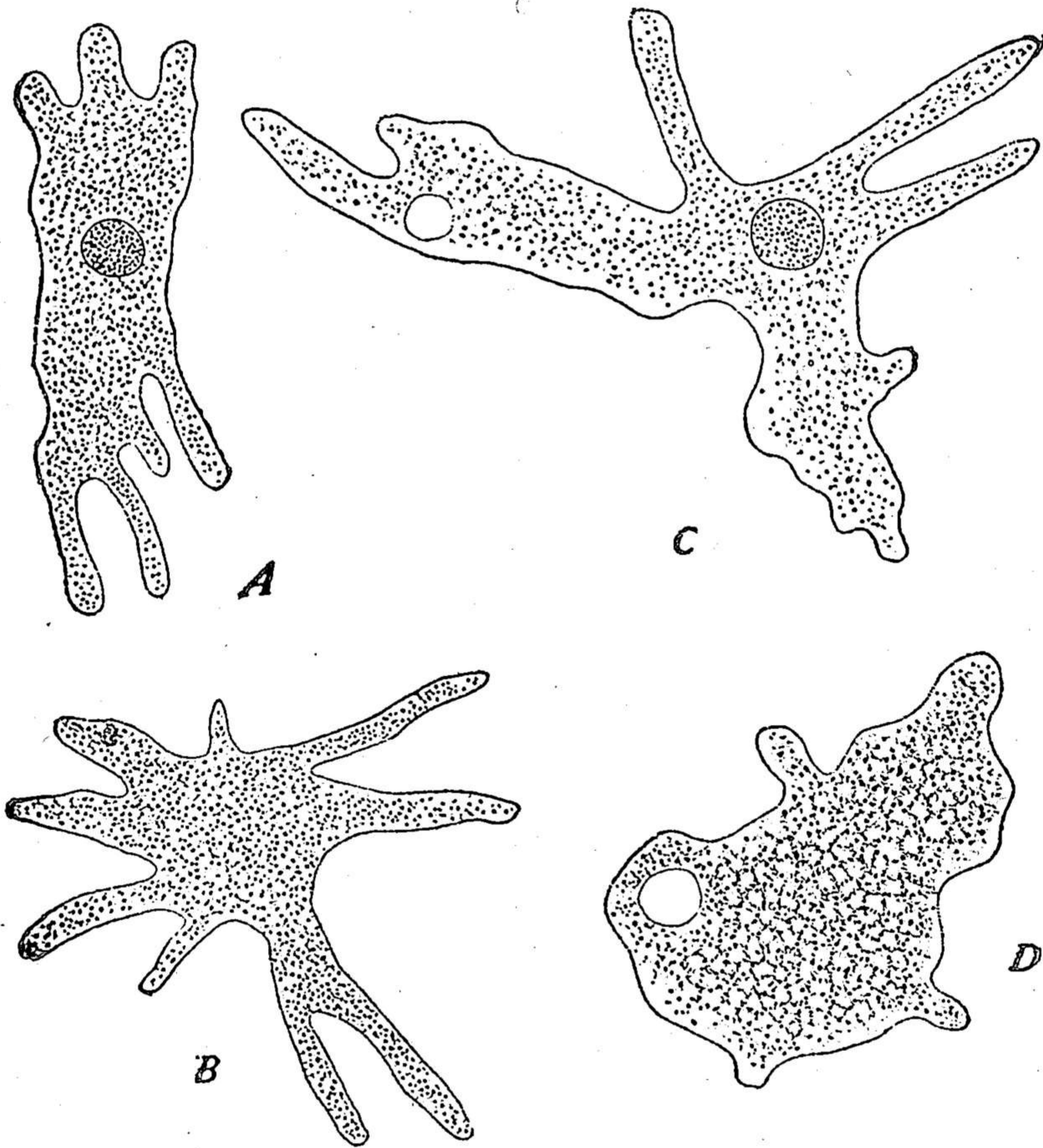

Fig. 317.—Nucleated and non-nucleated fragments of *Amœba* (HOFER).

A, B, an *Amœba* divided into nucleated and non-nucleated halves, five minutes after the operation; *C, D,* the two halves after eight days, each containing a contractile vacuole.

of growth and regeneration. A striking confirmation of this was given by Townsend ('97), who found in the case of root-hairs and pollen-tubes, that when the protoplasm is thus broken up, a membrane may be formed by both nucleated and non-nucleated fragments, by the latter, however, *only when they remain connected with the nucleated masses* by protoplasmic strands, however fine. If these strands be broken, the membrane-forming power is lost. Of great interest is the further observation (made on leaf-hairs in *Cucurbita*) that the influence of the nucleus may thus extend from cell to cell, an enucleated fragment of one cell having the power to form a

membrane if connected by intercellular bridges with a nucleated fragment of an adjoining cell (Fig. 318).

4. Form, Position, and Movements of the Nucleus

Many observers have approached the same problem from a different direction by considering the position, movements, and changes of form in the nucleus with regard to the formative activities in the cytoplasm. To
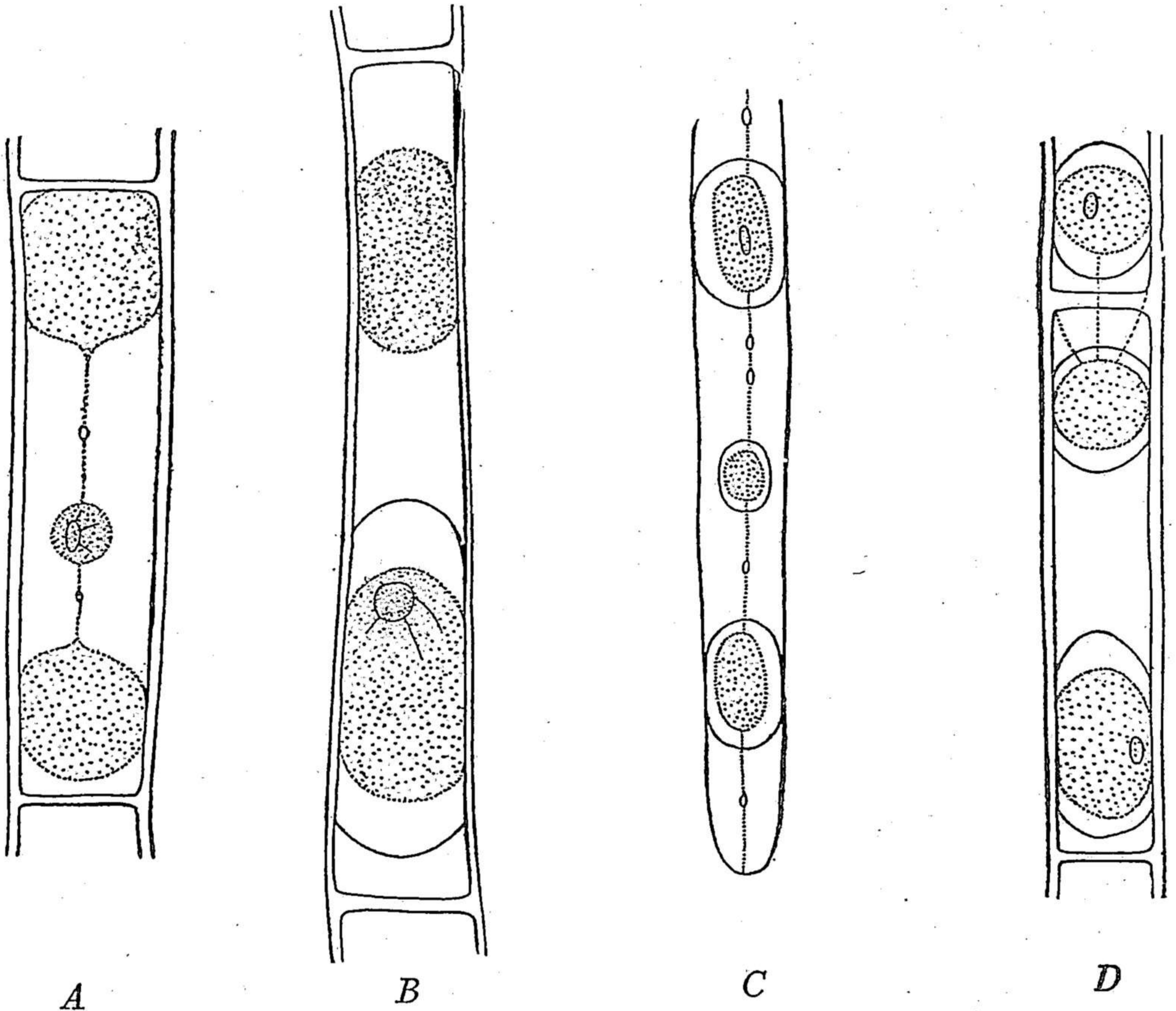

A   B   C   D

Fig. 318.—Formation of membranes by protoplasmic fragments of plasmolyzed cells (TOWNSEND).

A, plasmolyzed cell, leaf-hair of *Cucurbita*, showing protoplasmic balls connected by strands; B, calyx-hair of *Gaillardia;* nucleated fragment with membrane, non-nucleated one naked; C, root-hair of *Marchantia;* all the fragments, connected by protoplasmic strands, have formed membranes; D, leaf-hair of *Cucurbita;* non-nucleated fragment, with membrane, connected with nucleated fragment of adjoining cell.

review these researches in full would be impossible, and we shall limit our consideration to the well known researches of Haberlandt ('77) and Korschelt ('89). Haberlandt's studies related to the position of the nucleus in plant-cells with especial regard to the growth of the cellulose membrane.

He determined the very significant fact that local growth of the cell-wall is always preceded by a movement of the nucleus to the point of growth. Thus, in the formation of epidermal cells, the nucleus lies at first near the center, but as the outer wall thickens, the nucleus moves toward it, and remains closely applied to it throughout its growth, after which the nucleus
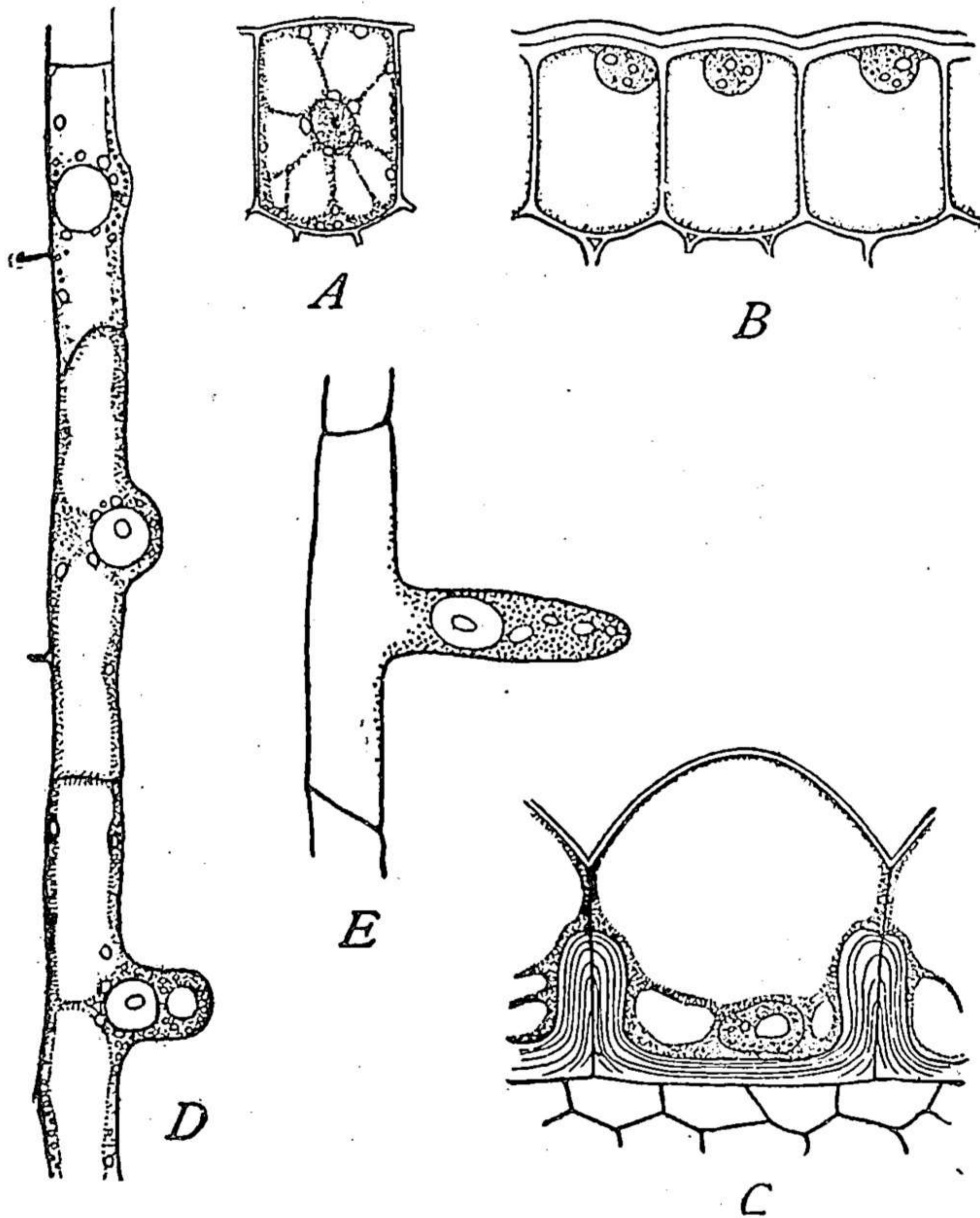

Fig. 319.—Position of the nuclei in growing plant-cells (HABERLANDT).

A, young epidermal cell of *Luzula* with central nucleus, before thickening of the membrane; *B*, three epidermal cells of *Monstera*, during the thickening of the outer wall; *C*, cell from the seed-coat of *Scopulina* during the thickening of the inner wall; *D*, *E*, position of the nuclei during the formation of branches in the root-hairs of the pea.

often moves into another part of the cell (Fig. 319, A, B). That this is not due simply to a movement of the nucleus toward the air and light is demonstrated in the coats of certain seeds, where the nucleus moves, not to the outer, but to the inner wall of the cell, and here the thickening takes place (Fig. 319, C). The same position of the nucleus is shown in the thickening of the walls of the guard-cells of stomata, in the formation of the peristome of mosses, and in many other cases. In the formation of root-hairs in the pea, the primary outgrowth always takes place from the immediate neighborhood of the nucleus, which is carried outward and remains near the tip

of the growing hair (Fig. 319). The same is true of the rhizoids of fern-prothallia and liverworts. In the hairs of aërial plants this rule is reversed, the nucleus lying near the base of the hair; but this apparent exception proves the rule, for in this case growth of the hair is not apical, but proceeds from the base! Very interesting is Haberlandt's observation that in the regeneration of fragments of *Vaucheria* the growing region, where a new membrane is formed, contains no chlorophyll but numerous nuclei. Again it was shown by Tangl, Nestler, Nemec, and others, that after injury to the epidermis of plants the processes of healing and regeneration are initiated by a movement of nuclei and protoplasm towards the wounded surface. The general result, based on the study of a large number of cases, is, in Haberlandt's words, that "the nucleus is in most cases placed in the neighborhood, more or less immediate, of the points at which growth is most active and continues longest." This fact points to the conclusion that "its function is especially connected with the developmental processes of the cell" and that "in the growth of the cell, more especially in the growth of the cell-wall, the nucleus plays a definite part."

Korschelt's work dealt especially with the correlation between form and position of the nucleus and the nutrition of the cell, and bore more directly on chemical than on morphological synthesis. The results showed that there is a definite correlation, on the one hand, between the position of the nucleus and the source of food-supply; on the other hand, between the size of the nucleus and the extent of its surface and the elaboration of material by the cell. In support of the latter conclusion many cases are brought forward of secreting cells in which the nucleus is of enormous size and has a complex branching form. Such nuclei occur, for example, in the silk-glands of various insect larvæ (Meckel, Zaddach, etc.), which are characterized by an intense secretory activity concentrated into a very short period. Here the nucleus forms a labyrinthine network (Fig. 31), by which its surface is brought to a maximum, pointing to an active exchange of material between nucleus and cytoplasm. The same type of nucleus occurs in the Malphigian tubules of insects (Leydig, R. Hertwig), in the spinning-glands of amphipods (Mayer), and especially in the nutritive cells of the insect ovary (p. 665). Here the developing ovum is accompanied and surrounded by cells which there is good reason to believe are concerned with the elaboration of food for the egg-cell. In the earwig *Forficula* each egg is accompanied by a single large nutritive cell (Fig. 320), which has a very large nucleus rich in chromatin. This cell increases in size as the ovum grows, and its nucleus assumes the complex branching form shown in the figure. In the butterfly *Vanessa* there is a group of such cells at one pole of the egg, from which the latter is believed to draw its nutriment (Fig.

153). A very interesting case is that of the annelid *Ophryotrocha* (p. 333). Here, as described by Korschelt, the egg floats in the perivisceral fluid, accompanied by a nurse-cell having a very large chromatic nucleus, while that of the egg is smaller and poorer in chromatin. As the egg completes its growth the nurse-cell dwindles away and finally perishes (Fig. 151).
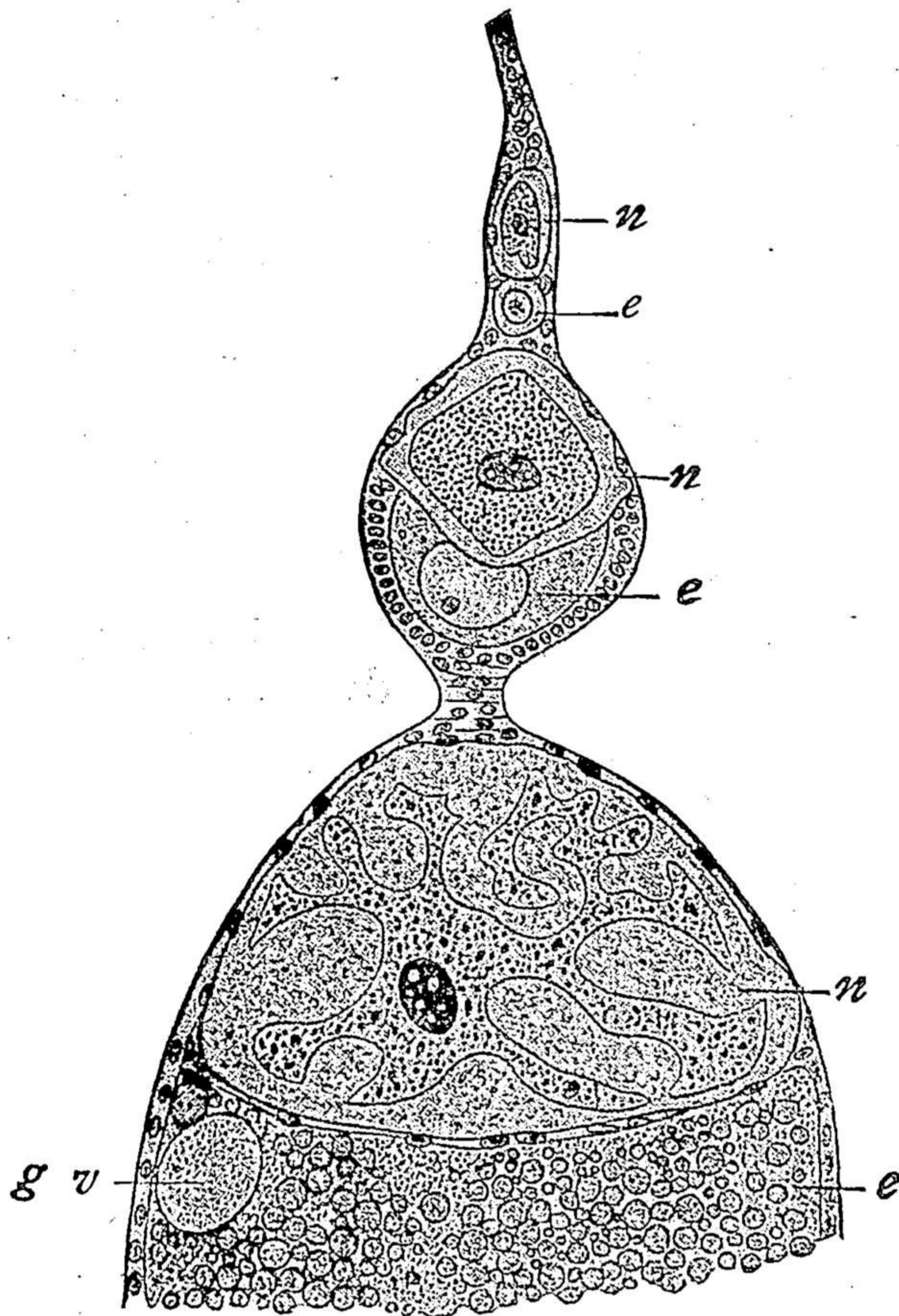

Fig. 320.—Upper portion of the ovary in the earwig *Forficula*, showing eggs and nurse-cells (Korschelt).

Below, a portion of the nearly ripe egg (*e*), showing deutoplasm-spheres and germinal vesicle (*g v*). Above it lies the nurse-cell (*n*) with its enormous branching nucleus. Two successively younger stages of egg and nurse are shown above.

In all these cases it is scarcely possible to doubt that the egg is in a measure relieved of the task of elaborating cytoplasmic products by the nurse-cell, and that the great development of the nucleus in the latter is correlated with this function.

Regarding the position and movements of the nucleus, Korschelt reviews many facts pointing toward the same conclusion. Perhaps the most

suggestive of these relate to the nucleus of the egg during its ovarian history. In many of the insects, as in both the cases referred to above, the egg-nucleus at first occupies a central position, but as the egg begins to grow, it moves to the periphery on the side turned toward the nutritive cells. The same is true in the ovarian eggs of some other animals, good examples of which are afforded by various cœlenterates, e. g., in medusæ (Claus, Hertwig) and actinians (Korschelt, Hertwig), where *the germinal vesicle is always near the point of attachment of the egg.* Most suggestive of all is the case of the water-beetle *Dytiscus*, in which Korschelt was able to observe the movements and changes of form in the living object. The eggs here lie in a single series alternating with chambers of nutritive cells. The latter contain granules which are believed by Korschelt to pass into the egg, perhaps bodily, perhaps by dissolving and entering in liquid form. At all events, the egg contains accumulations of similar granules, which extend inward in dense masses from the nutritive cells to the germinal vesicle, which they may more or less completely surround. The latter meanwhile becomes amœboid, sending out long pseudopodia, which are always directed toward the principal mass of granules (Fig. 153). More recently Wiemann ('10) has shown with great clearness, by the use of differential stains, that in the beetle *Leptinotarsa* the nutritive cells of the end-chamber of the ovary elaborate basophilic granules that flow downward into the eggs through the protoplasmic pedicles by which the latter are connected with the end-chamber (p. 335). These granules flow into the main body of the egg, and towards the germinal vesicle, around which they form a characteristic broad zone (*cf.* Fig. 155). Their precise fate is uncertain, but undoubtedly they provide material at the expense of which the egg grows. The similar observation of Nussbaum-Hilarowicz are elsewhere reviewed (p. 336).

IV. THE NUCLEUS AND THE PROBLEM OF HEREDITY

The close relation between the nuclear activities and the synthetic processes of the cell points unmistakably towards its important relation to heredity, which, as Darwin long ago remarked, may be looked upon as a form of growth (p. 1035). The theory that hereditary characters are transmitted by the nucleus, as independently advanced by O. Hertwig and by Strasburger (1884), was based originally on the history of the gamete-nuclei in fertilization. The conclusions of both were reached through direct observation, but were considerably influenced by more theoretical considerations on heredity developed by Nägeli in his notable work on the idioplasm, which appeared earlier in the same year (p. 722). Nägeli himself did not

attempt definitely to localize the idioplasm or germ-plasm in the cell, but emphasized the equal parts played by the gametes in heredity, despite their enormous difference in size. Hertwig and Strasburger transferred this argument to the nucleus, contrasting the exact equivalence of the gamete-nuclei in fertilization (as demonstrated especially by Van Beneden) with the extreme disproportion between the gametes in respect to the cytoplasm. In respect to the chromosomes, which embody the primary essential and fundamental nuclear substance, the parent germ-cells make equivalent contributions to the offspring (with the special exception of the sex-chromosomes), while with respect to the cytoplasm a very great discrepancy exists—a significant difference when we consider that so far as can be determined by experiment the two parents contribute equally, on the whole, to the hereditary endowment of the offspring. Additional weight was brought to this consideration by the wide contrast between nucleus and cytosome during mitotic division. The nucleus undergoes an exactly equal and a meristic division, effected by the spinning out of its substance into fine threads, and the longitudinal splitting of the thread; hence the conception of Roux ('83) that the nucleus consists of numerous smaller elements or "qualities," each of which must be transmitted unimpaired to the daughter-nuclei (p. 908). The cytosome, on the other hand, offers the general aspect of a mass-division, and is often unequal, sometimes in an extreme degree. There is, it is true, increasing evidence that the cytoplasm may contain numerous independently dividing bodies, such as plastids, central bodies, and possibly chondriosomes and other formed components; but in none of these cases is there evidence of exactly ordered distribution of the division-products, such as is so strikingly displayed in the division of the nucleus.

General considerations of this type long since caused the widespread acceptance of the nuclear theory of heredity; but its complete demonstration only came through the close coöperation of genetic experiment and cytological studies on the chromosomes which followed the rediscovery of Mendelian heredity in 1900.[1] This must not be taken to mean that the cytoplasm is without significance for these phenomena, as some opponents of the nuclear theory have pretended; it is indeed probable that in a large sense the whole cell-system is involved in the production of every hereditary trait. Most adherents of the nuclear theory have gone no further than to treat the nucleus as an essential, but not the exclusive, factor in heredity.[2] The point of view thus indicated will later be made clear (pp. 637, 916).

[1] See Chapters X, XII.
[2] See for instance Godlewsky ('06), Wilson ('12, '14).

LITERATURE VIII

(For abbreviations see General Literature List.)

Aberhalden, E., '09. Abbau der Proteine: *Handbuch der Biochemie*, I.

'14. Defensive Ferments of the Animal Organism: 3d Ed., Eng. Trans., *London.*

Adami, J. G., '08. Principles of Pathology: *Lee and Fibiger, Philadelphia and New York.*

Bayliss, W. M., '11. The Nature of Enzyme Action: *Longmans, Green & Co.*

'18. Principles of General Physiology, 2d Ed.: *Longmans, Green & Co.*

'21. Staining. Chap. XI, in *Lee's Microtomist's Vade-mecum; 8th Ed., London.*

'23. The Colloidal State: *London.*

Bayliss and Starling, '06. Die chemische Koordination der Funktionen des Körpers: *E. P.*, I, 2.

Bechold, H., '12. Die Kolloide in der Biologie und Medizin: *Dresden.*

Bernard, Claude, '78. Leçons sur les phénomènes de la vie; 1st Ed.: '78; 2d Ed., '85. *Paris.*

Burian, R., '06. Chemie der Spermatozoen: *E. P.*, V, 1.

Clowes, G. H. A., '16. Protoplasmic Equilibrium: *Journ. Phys. Chem.*, XX, 5.

Cohnheim, O., '11. Chemie der Eiweisskörper: 3 Aufl. *Braunschweig.*

Czapek, F., '11. Chemical Phenomena of Life: *N. Y.*

Dakin, H. D., '12. Oxidations and Reductions in the Animal Body: *Longmans, Green and Co.*

Furth, O. v., '12. Probleme der Physiologischen und Pathologischen Chemie: *Leipzig, Vogel.*

Haberlandt, G., '87. Ueber die Beziehungen zwischen Funktion und Lage des Zellkerns: *Fischer, Jena.*

Halliburton, W. D., '16. The Essentials of Chemical Physiology: 9th Ed., *Longmans, Green & Co.*,

Hammarsten, O., '09. A Textbook of Physical Chemistry. 5th Ed., *N. Y.*

Hardy, W. B., '13 (I).

Hatschek, E., '22. An Introduction to the Physics and Chemistry of Colloids: 4th Ed. *Philadelphia.*

Heidenhain, M., '02. Ueber chemische Umsetzungen zwischen Eisweiskörpern und Anilinfarben: *A. Ph.*, XC, III, 4.

'10. Färbungen: *Handbuch d. Mikr. Tech.*, 2te Aufl. I.

Höber, R., '14. Physikalische Chemie der Zelle und der Gewebe. 4te Aufl.: *Engelmann, Leipzig and Berlin.*

Hopkins, F. G., '13. The Dynamic Side of Biochemistry: *Nature*, XCII.

Jones, Walter, '14. Nucleic Acids: *Longmans, Green & Co.*

Kanitz, A., '09. Biochemie der Zelle: *Handb. der Biochemie des Menschen und der Tiere*, I.

'10. Das Protoplasma als chemisches System: *Ibid.*, II, 1.

Korschelt, E., '89. Beiträge zur Morphologie und Physiologie des Zellkerns: *Z. J.*, IV.

Kossel, A., '12. The Proteins, Herter Lecture: *Johns Hopkins Hospital Bulletin, Baltimore.*

Lillie, R. S., '18. Heredity from the Physico-Chemical Point of View: *B. B.*, XXXIV.

Loeb, J., '18. Amphoteric Colloids: I, *J. G. P.*, I, 1; II, *Ibid.*, II, 2.

Macdougal, D. T., and Spoehr, H. A., '20. The Components and Colloidal Behavior of Plant Protoplasm: *Proc. Am. Phil. Soc.*, LIX.

Mann, G., '02, '06. Physiological Histology: *Clarendon Press, Oxford*.

Mathews, A. P., '98. A Contribution to the Chemistry of cytological Staining: *A. J. P.*, I, 4.

'15. Physiological Chemistry: *Wood & Co., New York*.

Molisch, H., '13. Mikrochemie der Pflanze: *Jena*.

Nuttall, '04. Blood Immunity and Blood Relationships: *Cambridge Univ. Press*.

Ostwald, W., '09. Die wichtigsten Eigenschaften des Kolloiden Zustandes der Stoffe: *Handbuch der Biochemie*, I.

Pratje, A., '20. Die Chemie des Zellkerns: *B. G.*, XL.

Reichert and Brown, '09. Differentiation and Specificity of Corresponding Proteins and other vital Substance in Relation to Biological Classification and Organic Evolution: *Carnegie Inst.*   ,

Schittenhelm, A., '11. Der Nukleinstoffwechsel: *Handbuch der Biochemie*, IV, 1.

Sokoloff, B., '24. Das Regenerationsproblem bei Protozoen: *A. P.*, XLVII.

Spaeth, R. A., '16. The Vital Equilibrium: *Sci.*, XLIII.

Spiro, K., '09. Physikalische Chemie der Zelle: *Handbuch der Biochemie*, I, 2.

Verworn, '91. Die physiologische Bedeutung des Zellkernes: *A. Ph.*, LI.

'03. Die Biogenhypothese: *Jena*.

'09. Allgemeine Physiologie, 5te Aufl: *Fischer, Jena*. (Eng. Tr. by Lee, from 2nd German Ed., London, Macmillan, '99.)

'15. Zellphysiologie: *H. Nw.*, X, *Fischer, Jena*.

Warburg, '13. Ueber die Wirksamkeit der Strukturen auf die chemischen Vorgänge in Zellen: *Jena*.

Zacharias, E., '10. Die chemische Beschaffenheit von Protoplasma und Zellkern: *P. R. B.*

Zsigmondy, R. '20. Kolloidchemie, 3te Auflage.