

pole to the chromosomes, and by their contractions pull the chromosome-halves apart towards the poles; this action is supplemented by an "antipodal cone" of astral rays opposite to the spindle-pole which draw the centers towards the periphery and thus cause them to move apart.<sup>1</sup>

Boveri's slightly later studies on the *Ascaris* egg ('88, 2) led to essentially similar conclusions. He demonstrated that during the fertilization of *Ascaris*, the astral rays become attached to the chromosomes of the pronuclei; that the longitudinal halves of each split chromosome become attached to rays connecting with the corresponding poles; that the chromosomes, at first irregularly scattered in the egg, are drawn into a position of equilibrium in the equator of the spindle by the shortening of these rays; and that *the rays thicken as they shorten.*

He concluded that the initial separation of the chromosome-halves in the early anaphases is not due to the action of these rays but to a *divergence of the centers*, caused by contractions of the antipodal rays. This was based on the fact that when

the daughter-chromosomes first separate in the earlier anaphases they do not come any nearer to the poles. The latter movement occurs only in

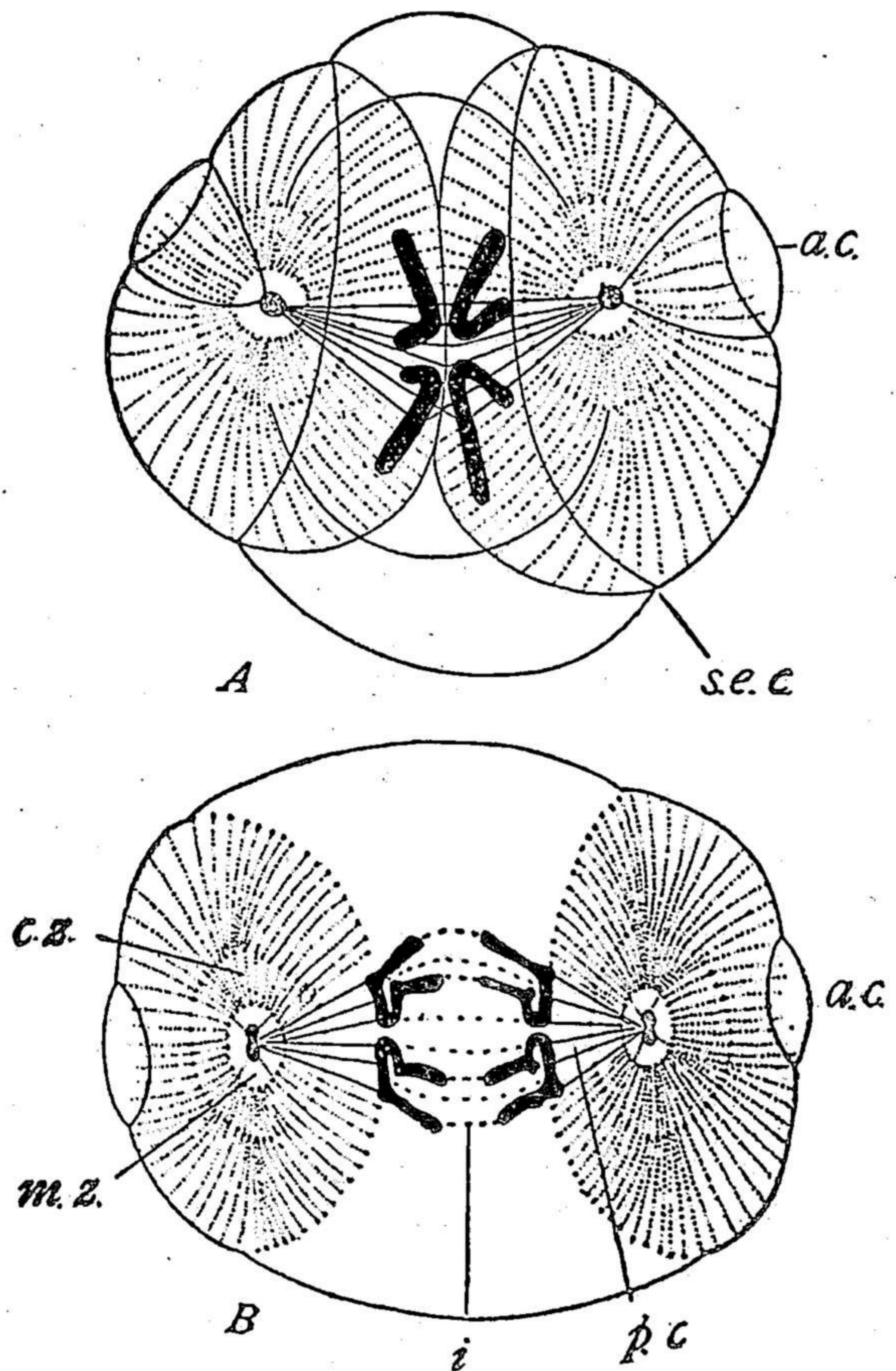


Fig. 76.—Slightly schematic figures of dividing eggs of *Ascaris*, illustrating Van Beneden's theory of mitosis (VAN BENEDEN and JULIN).

A, early anaphase; each chromosome has divided into two; B, later anaphase during divergence of the daughter-chromosomes; a. c., antipodal cone of astral rays; c. z., cortical zone of the "attraction-sphere"; i., interzonal fibers stretching between the daughter-chromosomes; m. z., medullary zone of the "attraction-sphere"; p. c., principal cone, forming one-half of the contractile spindle (the action of these fibers is reinforced by that of the antipodal cone); s. e. c., sub-equatorial circle, to which the astral rays are attached.

<sup>1</sup> Van Beneden describes a definite "polar circle" of microsomes marking the base of the antipodal cone, and also a "sub-equatorial circle" limiting the extension of the central rays towards the equator (Fig. 76); but this still lacks sufficient confirmation.

the later anaphases, a conclusion long afterwards substantiated by studies on monasters and on certain anomalies seen in merogonic fertilization (Fig. 71).

Boveri modified Van Beneden's conception by the assumption that the amphiaster is not a mere regrouping or image in a general reticulum but a new formation developed from a specific granular substance, *archiplasm* (originally written *archoplasm*) that collects about the central bodies and then differentiates into astral rays and spindle-fibers.<sup>1</sup> Rabl, on the other hand, extended Van Beneden's hypothesis by the assumption that the fibrillæ are persistent structures, permanently attached at one end to the original chromosome, at the other to the center. Division of the latter, therefore, leads to a corresponding division, first of the fibrilla and ultimately of the chromosome to which the latter is attached (Fig. 390). The hypothesis was further developed by Kostanecki in the hypothesis that the astral rays are likewise persistent structures that split lengthwise ("*omnis radius e radio*"); but this latter assumption was soon found to be untenable by numerous observations which proved that the old rays disappear after each mitosis to be replaced by new asters developed within the old (p. 680). On its physiological side the hypothesis was developed especially by Heidenhain ('94, '96), who devised ingenious models to simulate some of the phenomena of mitosis. In its simplest form the model consists of a ring to the periphery of which are attached at equal intervals a series of rubber bands (astral rays) the central ends of which are attached to a pair of small rings fastened together which play the part of central bodies. In the position of equilibrium, when the rays are stretched at equal tension, they form a symmetrical aster with the pair of rings at the center (Fig. 77). If the connection between the central rings be severed, they are immediately dragged apart to a new position of equilibrium with the rays grouped in two asters, as in the actual cell. If a round pasteboard box of suitable size (nucleus) be inserted between two of the rays, it assumes an eccentric position, the cell-axis being formed by a line passing through its center and that of the pair of small rings, and upon division of the aster it takes up a position between the two asters. In a second form of the model the peripheral ring is formed of two half-rings of flexible steel, joined by hinges; the divergence of the small rings is here accompanied by an elongation and partial constriction of the model in the equatorial plane; and if, finally, the hinge-connection be removed, each half of the ring closes to form a complete ring (Fig. 77). Heidenhain, like Rabl, assumed the astral rays to be permanent "organic radii," of equal length and tension, and permanently attached to the centers. In the resting cell they are commonly relaxed and

<sup>1</sup> For further account of this hypothesis, see p. 723.

lost to view, though sometimes permanently visible (leucocytes, pigment-cells). As mitosis begins, their tension increases by a tonic contraction, the astral rays then straightening and coming into view as such, while the equilibrium of the system is maintained by turgor of the cell. Upon separation of the two centers they are mechanically drawn apart while a spindle forms between them, and in the end the continued tension leads both to division of the cell-body and the continued divergence of the daughter-centers. A new condition of equilibrium is thus established in each daughter-cell until again disturbed by division of the center.<sup>1</sup>

The fibrillar interpretation of the aster received support through Schaudinn's ('96, 3) interesting discovery that the "central granule" of the *Heliozoa* (*Acanthocystis* and others, Figs. 85, 325), plays the part of a central body in mitosis. Further supposed support was found in the structure of

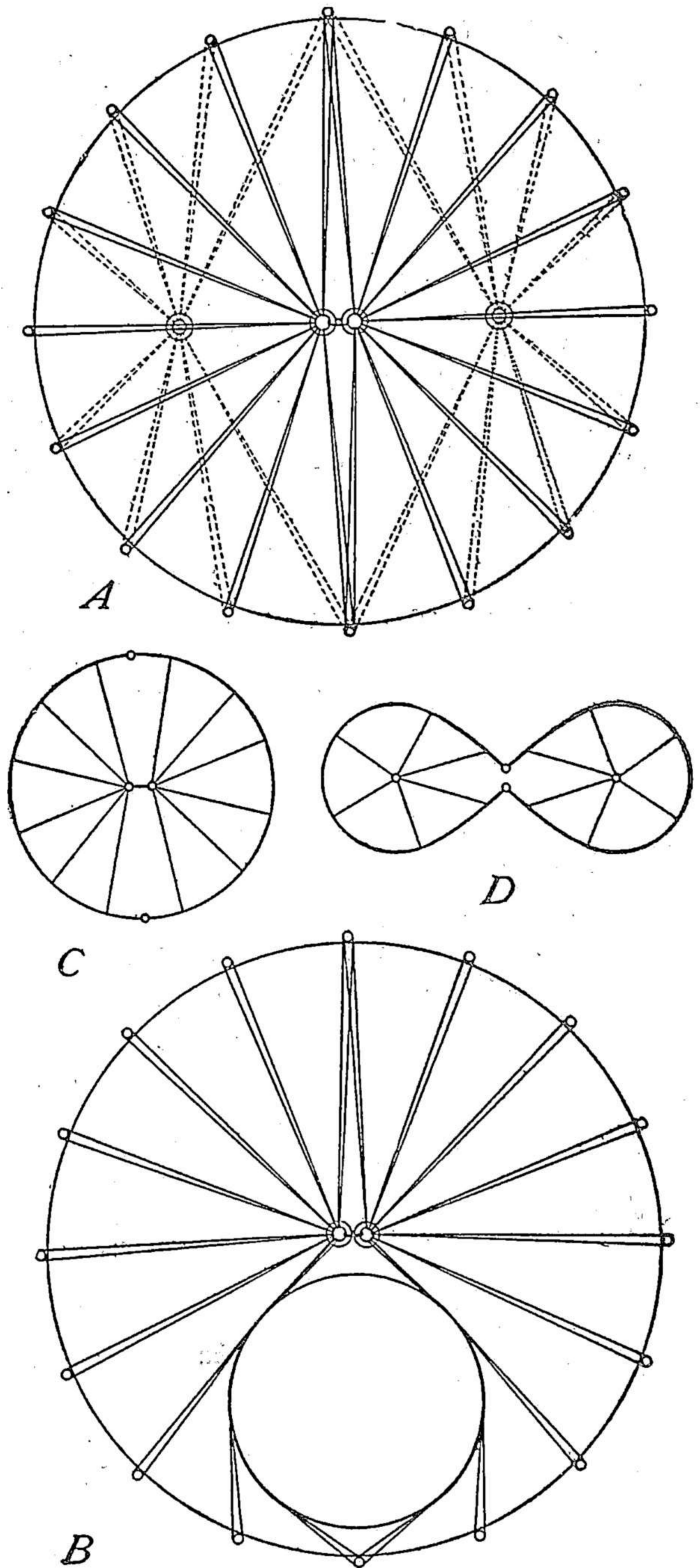


Fig. 77.—Heidenhain's model of mitosis (mainly from HEIDENHAIN).

<sup>1</sup>In a modification of the apparatus devised by Rhumbler ('97) the same effect is produced without the hinges.

A, dotted lines show position of the rays upon severing connection between the small rings; B, position upon insertion of "nucleus"; C D, models with flexible hinged hoops, showing division.

leucocytes and pigment-cells. In the former, of which Heidenhain ('93) made a close study, a large permanent aster is present, centering in a pair of centrioles and with rays extending far out through the cytosome (Fig. 10); and these were assumed by Heidenhain to be contractile elements by means of which the cell may change its form and creep about. A similar structure was found by Solger ('91) and Zimmermann ('93b) in the pigment-cells or chromatophores especially of fishes. Later researches have, however, much weakened the force of this comparison. The axial filaments or axopodia of the Heliozoa have been generally regarded as non-contractile supporting or skeletal structures<sup>1</sup> and the same view has been taken of the radiating structure of the chromatophore-cells (Franz, '08, etc.). Ballowitz, in a series of interesting works has advocated the view that this structure is a system of radiating, contractile intra-cellular canals within which the pigment-granules flow centrally or peripherally; but such a structure seems to offer little ground for comparison with the mitotic aster.<sup>2</sup>

No one who witnesses the operation of Heidenhain's models can fail to be impressed with its striking simulation of actual cell-division; and even if the whole fibrillar hypothesis be rejected, the analogy is of value as showing the effect of a dicentric system of radial strains in the cell. Nevertheless the whole hypothesis gradually lost ground and is now almost abandoned despite various attempts to modify and improve it.<sup>3</sup> So far as the asters are concerned the theory of fibrillar contractility breaks down in the case of anastral mitosis, where these structures are absent. It offers no explanation of the movements of the chromosomes during the later phases of mitosis, when the chromosomes proceed to the extreme end of the spindle and even beyond it, so as to enter bodily into the substance of the centrosome, while the "traction-fibers" have disappeared.<sup>4</sup> Its most serious weakness lies in the fact that it was based primarily on a conception of protoplasmic structure that proved to be untenable as a general theory. It steadily fell behind, therefore, as the proof accumulated that protoplasm has in general the properties of a colloidal system, and in the end was overshadowed by attempts to analyze the phenomena from the new standpoint thus given. It was proved, for instance, that the astral rays are paths of centripetal flow of hyaloplasm towards the astral centers; that they quickly

<sup>1</sup> See Doflein, '16a, '16b.

<sup>2</sup> See Ballowitz, '14a, '14b, etc. For general review see Schmidt, '18.

<sup>3</sup> Among these may be mentioned Hermann's hypothesis ('91) of the central spindle as a non-contractile supporting organ; that of Drüner ('95) that the central spindle is an actively elongating structure by which the centers are pushed apart (indicated by the contorted course of its fibrillæ during the anaphases); and the hypotheses of Watasé ('93) and of Meves ('97c) that all the fibrillæ of both spindle and asters operate as pushing organs, a view suggested by the inpushing of the nuclear membrane in the early stages of mitosis (p. 148). For a more recent careful study of the amphias-tral system and a critical review of the literature, see Yatsu ('09), Lams, ('10). Cf. p. 680.

<sup>4</sup> Wilson, '95, R. Hertwig, '98.

disappear under the action of such agents as cold, ether or chloroform, which diminish protoplasmic activity without destroying its structure, and redevelop upon restoration of the normal conditions; that the astral fibrillæ are not visible as such in the living object, but appear only as radiating tracts of hyaloplasm between the alveolar spheres; and that astral fibrillæ

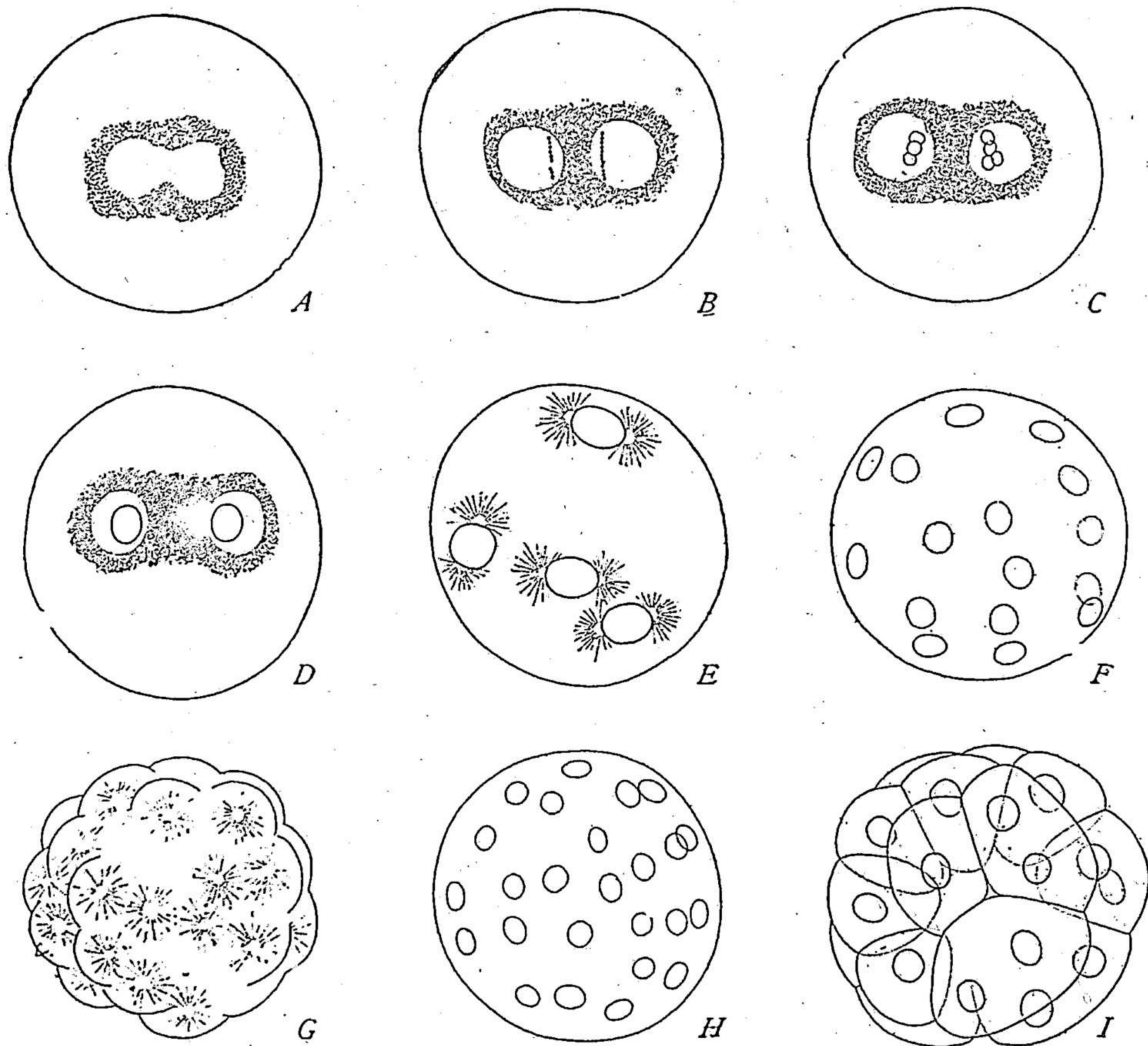


Fig. 78.—Cleavage in etherized sea-urchin eggs from life (*Toxopneustes*).

*A*, etherized in early anaphase, asters and spindle have disappeared; *B–D*, successive stages in a slightly etherized egg; *E*, 4-nucleate egg, after slight etherization; *F*, 16-nucleate egg; *G*, the same in the ensuing abortive division; *H*, 32 nuclei; *I*, blastula, from the division of a 16-nucleate egg when replaced in water.

may be produced as artificial coagulation-products along radial lines of strain in a homogeneous colloidal solution. All this helped to turn investigation of the subject into new channels, the result of which appears in the so-called "dynamical theories" of mitosis now to be considered. The theory of fibrillar contractility was in fact a first naïve attempt; nevertheless it gave a powerful stimulus to further inquiry, and served to place the whole problem before us in clearly defined form. In spite of all this,

in the writer's view it still seems far from certain that the "fibrillæ" seen in sections may not really preëxist approximately as such in the living cell—a possibility strongly suggested by the relations of the axial filaments of the Heliozoa (p. 680), by the active growth of the astral rays against the nuclear wall which they push before them (p. 148), by the definite and constant relations between the spindle-fibrillæ and the chromosomes (p. 130) and other facts. We should not, therefore, prematurely condemn a theory which may yet prove to be reconcilable with the so-called dynamical theories now to be considered and which, admittedly, have themselves thus far offered no more than a very inadequate explanation of mitosis.

### 3. Dynamical Hypotheses Based on the Colloidal Nature of Protoplasm

In considering these hypotheses it must always be borne in mind that the amphiaser cannot be regarded as merely a temporary image or configuration of the colloidal cell-substance (alveolar cytoplasm, or the like), as it was regarded by Bütschli and even by some more recent writers. Undoubtedly it involves physical and perhaps also chemical changes in the material itself which gives to the amphiaser a considerable degree of coherence as a definite structure. This is demonstrated in several ways. The amphiaser may rotate or move bodily through the cytoplasm either in the normal condition of the cell (*e. g.*, in the polar mitoses, p. 493), or as a result of displacement by centrifuging or otherwise. This might indeed be due, as suggested by Lillie ('09), to a progressive regrouping of the cytoplasmic material as the centers move (as in a moving electrical or magnetic field in a suitable medium). Many observers have, however, observed distortions of the spindle or asters in smear-preparations, and especially in fixed eggs after centrifuging.<sup>1</sup> The spiral asters (p. 145) illustrate a similar phenomena; and Chambers ('17) has shown that the sperm-aster, or the amphiaser in the eggs of sea-urchins and nemertines may be pulled about, stretched or displaced with the micro-dissection needle, meanwhile undergoing a variety of distortions that are more or less persistent. These facts clearly indicate that, however the amphiaser may originally form, it finally becomes a coherent, and to a certain extent a persistent, structure.

*a. Hypothesis of the Polarized Field of Force.* Every close observer of the amphiaser must be impressed by its striking superficial resemblance to the polarized magnetic or electrostatic field of force. "The whole picture, which is of extreme clearness, vividly recalls the arrangement of iron filings about the two poles of a magnet."<sup>2</sup> This comparison was

<sup>1</sup> See Morgan, '08, '10; Lillie, '09; Spooner, '11; Conklin, '12, etc.

<sup>2</sup> Fol, '73. In the same work first appears also the idea that the astral foci are *centers of attraction*, a view afterwards advocated by Van Beneden, Boveri and other observers. See also Giard ('76).

developed by Errera ('80), Ziegler ('95) and Rhumbler ('03), who imitated some of the main phenomena by magnetic models, of which interesting photographs were reproduced. Hartog ('05) modeled the polarized magnetic field to a certain extent in three dimensions by suspending powdered

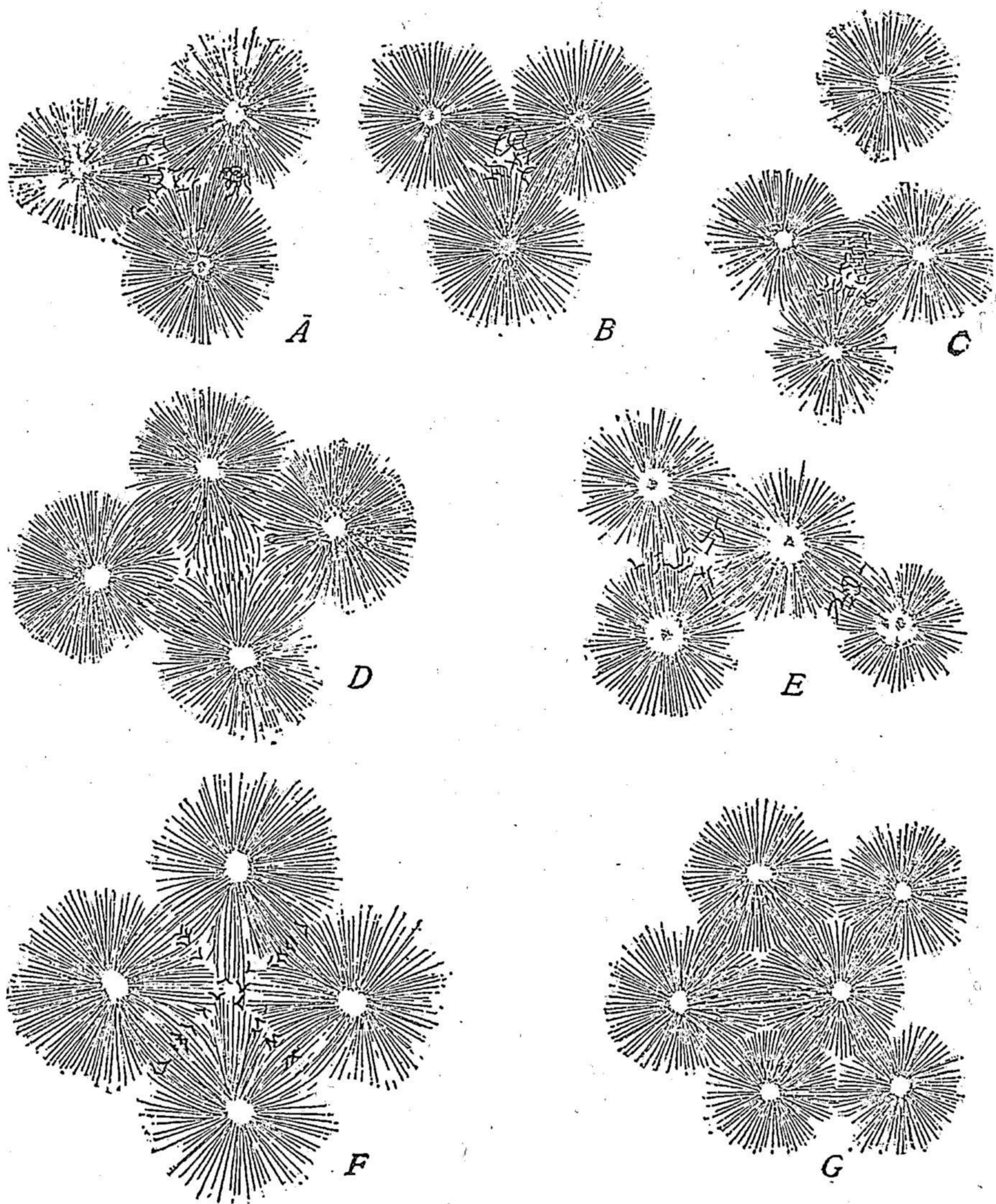


Fig. 79.—Multipolar mitoses in double-fertilized sea-urchin eggs (BALTZER).

*A, B*, triasters, the latter exactly symmetrical; *C-F*, various types of quadriaster figures; *D*, with one diagonal spindle, *F* with two; *G*, multipolar figure, with three acromatic spindles.

magnetite in glycerin in a magnetic field. Gallardo ('96), modifying an earlier experiment of Faraday's, produces a similar tridimensional model in the electrostatic field, by introducing two electrical poles into a glass trough containing finely powdered sulphate of quinine suspended in tur-

pentine. Gallardo at first advanced the view that the kinetic field is actually a bipolar electric field ('96) but greatly modified his interpretation ('06, '09, '12). Nevertheless, electrical hypotheses of mitosis continued to attract attention because of the fact, demonstrated by Hardy, R. Lillie and others, that the particles of colloidal solutions of various albuminous substances, and also cells or portions of cells, bear electric charges and react definitely to the lines of force in the electrostatic field (p. 189). The most recent advocate of the polarized field in mitosis is Hartog, who in a series of interesting papers (see especially '05, '09, '14) has contended that the amphiastrer is the expression of a polarized field of "mitokinetism" which he considers as a "new force" analogous to, but not identical with, electricity or magnetism.<sup>1</sup>

When critically examined, the striking superficial resemblance of the amphiastral field to a polarized field of force is found to be untenable, for many reasons. In the first place, numerous attempts to influence the mitotic field by causing cells to divide in the magnetic or electrical field, beginning with those of Errera ('90) and Roux ('91), have failed.<sup>2</sup> More specific difficulties arise from the following facts: (1) the common occurrence of tripolar (pathological) figures having an odd number of poles, and (2) of quadripolar figures in which occur diagonal spindles in addition to the four primary ones (Fig. 79); (3) the frequently observed crossing of astral rays from the two asters outside the spindle in the equatorial plane (Fig. 189 B); (4) the fact that separate asters (*e. g.*, cytasters) repel one another; (5) the fact that both astral rays and spindle-fibers anastomose, thus departing from the course of the trajectories in the polarized field. Of these difficulties the fifth and perhaps the third may be obviated. The remaining three are in the writer's opinion fatal to the hypothesis.

The fifth difficulty, as has been pointed out by Hartog ('05), is only apparent; for the amphiastral fibrillæ are either coagulation products or represent "chains of force," produced by the segregation of viscid protoplasmic substances, which, like the iron-filings in a magnetic field, can only approximately follow the lines of force. The third difficulty (Meves, '96, Wilson, '96), is more serious. Such a crossing of the rays is theoretically impossible in the bipolarized field; the lines of force are necessarily continuous from pole to pole.<sup>3</sup> Reinke ('00), it is true, proved that crossing of the rays may experimentally be produced in the magnetic field by intermittent and non-synchronous action of two opposite poles; but clearly the conditions of such an experiment are fundamentally different from those observed in the mitotic field.

<sup>1</sup> Literature in Hartog, '13.

<sup>2</sup> See Conklin '12.

<sup>3</sup> Gallardo's ('01, '02) attempt to show that the crossing of the rays is but an optical illusion due to foreshortening is obviously futile.



The most serious difficulty is offered by tripolar figures, or such as have any odd number of poles, a condition physically impossible in the polarized field since it is the essence of such fields that spindle-figures (in which the lines of force pass continuously from pole to pole) can only be formed between poles of different sign, *i. e.*, plus or minus. This is not alone true of the magnetic or electrostatic field but also of those in which the lines

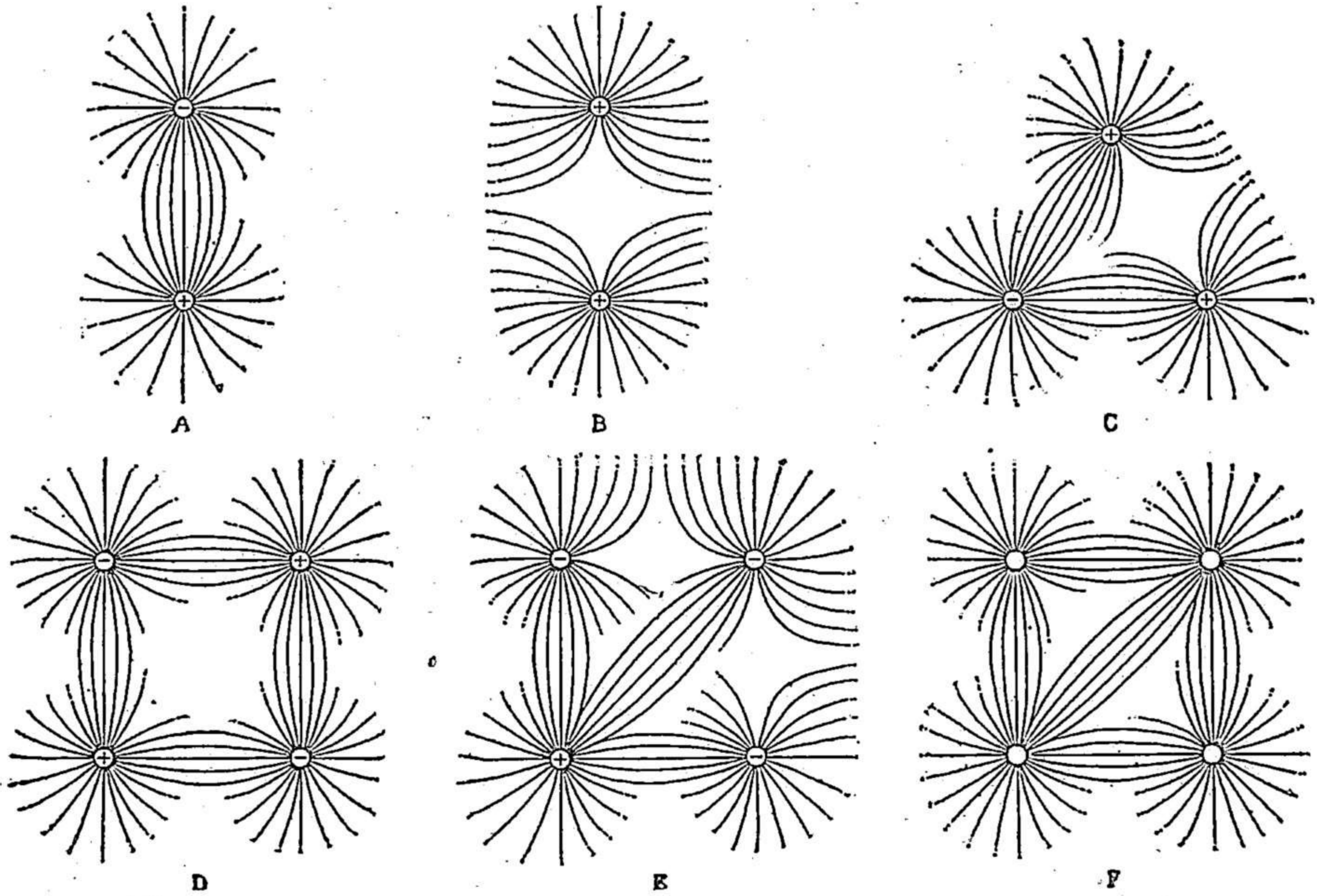


Fig. 80.—Diagrams of the polarized field (the curves only approximate).

*A*, bipolar field with unlike signs (spindle-figure); *B*, the same, like signs, anti-spindle figure); *C*, tripolar field; *D*, quadripolar field, with poles of alternating signs; *E*, quadripolar field arranged to give diagonal spindle (two anti-spindle figures); *F*, condition observed in the mitotic field.

of force or trajectories are represented by lines of liquid flow between a source and a sink; of heat-radiation between poles of higher and of lower temperatures (Maxwell, Kelvin), of oscillations in a liquid surrounding two bulbs that expand and contract in opposite rhythms (Bjerknes) or of diffusion-currents between centers of osmotic pressure and depression (Leduc, Damianowich).<sup>1</sup> Between two like poles, is formed an "anti-spindle" figure, in which the lines of force do not pass from pole to pole and have the opposite curvature to that of the spindle, *i. e.*, they are convex to the axis instead of concave (Fig. 80). In the mitotic field, however, multipolar figures (though commonly abnormal) are of common occurrence; and the evidence indicates that any two centers, if near enough together, may become connected by complete spindles. A pretty demonstration of this is offered in the *Ascaris* type of fertilization. When in such eggs the

<sup>1</sup> Cf. Hartog, '05, '14; Gallardo, '09, etc.; Leduc, '02, '04; Lamb ('08).

sperm-amphiaster approaches the second polar amphiaster (*é. g.*, in *Nereis*) the inner center of the latter may become connected by definite spindles with the sperm-centers (either or both) and thus produce striking triasters (Figs. 199, 470). Such figures afford crucial evidence against the whole hypothesis of polarized forces; for by the hypothesis two of the centers must be of the same sign.<sup>1</sup>

The difficulty presented by the diagonal spindles in quadripolar figures, as urged especially by Baltzer ('08) is of the same nature. A tetraster with consecutive spindles connecting the four poles is possible provided the centers be so placed that minus and plus poles alternate (Fig. 80). In such a figure diagonally opposite poles are of the same sign; nevertheless one or even two diagonal spindles connecting such poles are often seen (Fig. 79). This fact, as Baltzer shows, is readily demonstrated in dispermic eggs of sea-urchins, which (p. 917), typically develop four centers and divide at once into four. The double-fertilized egg sometimes gives rise to a triaster (owing to the failure of one sperm-aster to divide, and the same result may often be produced by shaking the eggs soon after fertilization.<sup>2</sup> In all these cases the facts clearly indicate that spindles readily form between any two adjacent asters, provided only that they are sufficiently near together during the prophase.

Lastly, separate asters do not attract but repel one another, an obvious indication that they are of like sign. M. Boveri ('03) proved that two separate asters (unconnected by a spindle) always move more widely apart than those which are connected by spindles; and Baltzer found a similar relation in case of the diagonal spindles of tetrasters. The spindle, therefore, seems to serve, at least in the earlier stages of mitosis, to hold the asters together in opposition to their reciprocal repulsion.

As a result of these difficulties Gallardo ('06) changed his original hypothesis, by the assumption that the centers are of like sign and the chromosomes of opposite sign; but again a fatal difficulty is offered by the existence of achromatinic spindles, wholly devoid of chromosomes (p. 176). Hartog's attempts to meet these difficulties ('05, '14) are ingenious, but hardly more convincing. The diagonal spindles of tetrasters may be imitated in the magnetic model, as he shows, by introducing a neutral pole of charcoal-iron into the center of the tetraphase figure, when chains of force detach themselves from the remainder of the system to anchor upon it, thus simulating the diagonal spindles. This seems, however, a very dubious analogy in view of the fact that the diagonal spindle is distinctly seen during

<sup>1</sup> See Rhumbler's excellent photographic figures ('03) illustrating these points. The difficulty offered by tripolar mitosis was first urged by Boveri ('88) and has been emphasized by many later writers. Cf. Ziegler ('95), Wilson ('00), Rhumbler ('03), Baltzer ('08), etc.

<sup>2</sup> Morgan, '95, Boveri, '02.

the anaphases of mitosis traversing the center of the field after the daughter-chromosomes have moved away from it (Fig. 430). Again, Hartog also shows that the triaster may be modeled in the bipolar magnetic field by introducing a neutral third pole (of soft iron), which then becomes connected by spindles with the two original poles. This effect is, of course, one of magnetic induction, the neutral pole becoming itself polarized. Since this pole is, however, necessarily weaker than the others, the two new spindles are correspondingly weaker, and such triasters are always asymmetrical, having the form of an obtuse-angled isosceles triangle. The triasters seen in cells may, however, be quite symmetrical;<sup>1</sup> and even were this not the case we should have to postulate the existence of three kinds of centers, plus, minus and neutral, practically a *reductio ad absurdum*.

For these various reasons the theory of the dual polarized field of force as applied to the mitotic figure seems to be untenable in any form thus far developed. We turn, therefore, to other attempts to arrive at a solution of the problem.

*b. Other Hypotheses concerning the Rôle of Electrical Phenomena.* A prominent place among attempts to advance our understanding of mitosis is occupied by the experiments of R. S. Lillie ('03-'11, etc.) which offer many important suggestions for further inquiry. Their point of departure was given by the demonstration (Lillie, '03) that a marked difference of electrical potential exists between the nuclear substance ("chromatin") and the cytoplasm, the former being on the whole electronegative, the latter electropositive. When suspended in a medium through which an electrical current is passed, free nuclei, or cells with a relatively large amount of nuclear substance (*e. g.*, sperm-heads, thymus-nuclei) move or tend to move towards the anode (positive pole) while cells with voluminous cytoplasm (large leucocytes, erythrocytes, smooth muscle-cells) show the reverse behavior. This result, extended to the chromosomes by the similar experiments of Pentimalli ('09) and McClendon ('10), is in harmony also with the fact (Hardy, '99, etc.) that the colloidal particles in a hydrosol are electrically charged, acid particles being negative and basic ones positive (p. 649). It was thus suggested that it may be such potential-differences which constitute the primary and determining conditions of mitosis (Lillie, '03, p. 275).

From this starting-point Lillie proceeded to an artificial imitation of many of the phenomena of mitosis by means of magnetic models.<sup>2</sup> The spireme-threads and chromosomes may be represented by magnetized needles, similarly oriented, each passed through a small cork float so as to stand vertically in the water and strung at short regular intervals along a silk thread to form a flexible filament (spireme-thread). Such a filament

<sup>1</sup> See Baltzer, '08, Figs. 3, 4.

<sup>2</sup> '05, 1, '05, 2.

straightens out by reciprocal repulsion of its similarly charged units; but if a large magnetic pole, of opposite sign to the upper needle-poles be brought vertically above the filament its attraction causes the filament to draw together into a convoluted spireme-like form. Several such filaments in association offer a close semblance to a segmented spireme, the filaments crowding together but without touching one another (owing to mutual

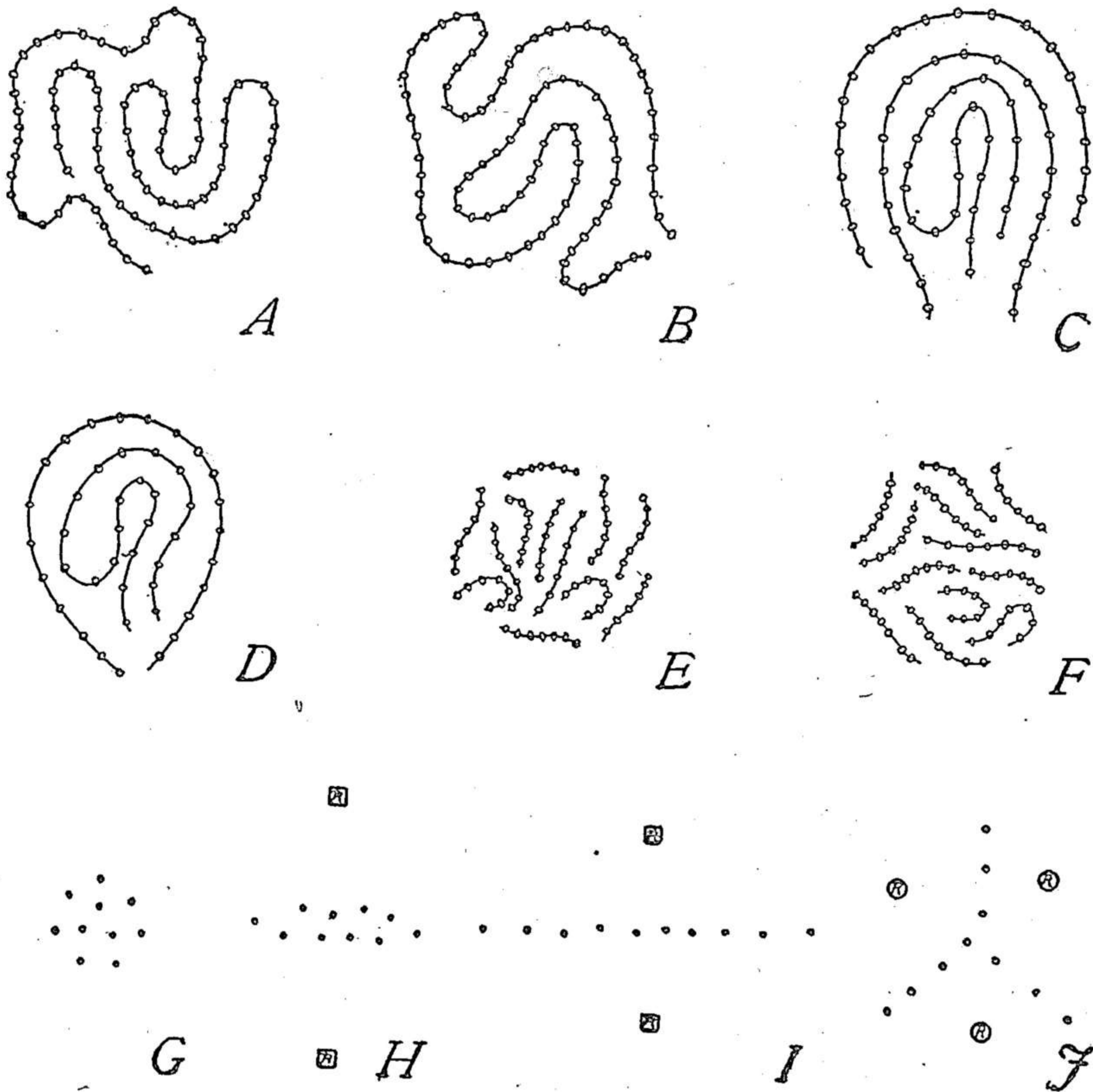


Fig. 81.—Magnetic models of mitosis (R. S. LILLIE).

*A, B*, spireme-configuration of floating magnetized needles strung on single silk-thread, drawn together by larger attractive pole; *C, D*, similar figures given by several separate threads (segmented spireme); *E, F*, figures with short flexible threads; *G*, group of single floating magnetized needles, drawn together by larger attractive pole; *H*, similar group between two repellent poles, *R, R*; *I*, similar group with repellent poles nearer equatorial plate; *J*, tripolar figure, with three repellent poles.

repulsion). If the needles be strung upon flexible wires bent into U-shapes, etc., to imitate chromosomes, the centripetal attraction of the large magnet causes them to assume groupings closely similar to those seen in the actual equatorial plate (Fig. 81). If a group of separate needles thus centripetally aggregated by a magnetic pole vertically above them be approached by two additional poles of like sign (and opposite to the overhead pole) the repul-

sion of the latter causes them to aggregate in a linear series transverse to the axis connecting the repellent poles.

All this, evidently, suggests that the spireme-threads tend to become straight, but are thrown into convolutions because of their confinement within the nuclear membrane. The elongate chromatin-threads of the spireme repel one another because of their similar (negative) electric charge, but are held together (action of the vertical magnet) by the nuclear membrane; hence the close convolution of the threads *without coming into contact*, as actually observed. When set free by dissolution of the nuclear membrane the chromosomes approach the equator of the spindle and spread out in a flat equatorial plane because this is the position of equilibrium resulting from: (1) their repulsion by the two negatively charged poles; (2) their attraction by the positive mid-region of the spindle; (3) their reciprocal repulsion by one another. The initial separation of the daughter-chromosomes may likewise be due to such a repulsion; but this leaves unexplained the later movement to the poles, which indeed seems to contradict the assumption of a negative charge at the poles.

In spite of the latter difficulty it is possible that thus far the analogy drawn by Lillie may have a substantial basis. His attempts to explain the achromatic figure, are less convincing. Both spindle-fibers and astral rays are assumed to arise by the polarization of the colloidal particles lying between regions of relatively positive and negative charge, and the consequent serial alignment of these particles. The centers or spindle-poles are assumed to be electronegative, the equatorial region electropositive; hence the formation of continuous linear cytoplasmic tracts (spindle-fibers) between the former in spite of their similar charge. The astral rays are assumed to arise in like manner between the electronegative centers and the electropositive cell-periphery, the peripheral positive charge being ascribed to a depolarization of the plasma-membrane caused by changes of permeability, which operate as a stimulus to cell-division, and to which the whole series of phenomena may be traceable.

Unfortunately this part of the hypothesis encounters many difficulties and becomes quite unmanageable when the attempt is made to apply it, for instance, to the formation of great numbers of cytasters scattered through the cytosome (p. 482); but it nevertheless offers many suggestions to the cytologist. One of the most interesting of these is the possibility that the serially aligned polarized colloidal particles may fuse to form fibrils ('11, p. 728) by a process analogous to coagulation and once formed may long persist after the originating conditions have disappeared. This helps us

to understand how fibrillar formations may come and go in a structure that primarily is alveolar and has the general properties of a colloid. We see further how spindle-fibers or astral rays, once formed, may undergo twisting (as in the spiral asters) or other deformations, such as have been described by many observers; how they may differ in physical consistency and staining-reaction from the general protoplasmic meshwork; and how asters and spindles, once formed, may move through the protoplasmic substance. It also may help us to understand how changes of protoplasmic viscosity may play an important part in mitosis as demonstrated by the recent work of Chambers and Heilbrun (p. 197).

On the whole, however, Lillie's analysis leaves us far short of any adequate understanding of mitosis; and as he himself points out, the problem is no doubt far too complex to be solved by so simple a series of assumptions. This will be more evident after considering another series of facts and assumptions as follows:

*c. Diffusion-streams, Protoplasmic Currents and Surface-tension in Mitosis.* That the astral rays may represent lines of diffusion-currents or of protoplasmic flow was suggested by some of the earliest observers of mitosis.<sup>1</sup> Auerbach regarded the rays as an "expression of the paths along which fine currents of nuclear sap pass outward into the protoplasm." Bütschli ('76, '03) considered the aster as an expression of radial diffusion-currents; and with various modifications this has been accepted by many later observers. Hertwig, Fol and Strasburger regarded the currents as a *centripetal* flow of the hyaloplasm towards the astral centers, where a progressive accumulation of this substance as the aster grows may readily be observed in the living egg. This observation has been confirmed by many later observations<sup>2</sup> and the fact may now be regarded as well established.

This flow may readily be observed in the living eggs of *Toxopneustes* (Wilson, '01, 2), where, as the aster enlarges, the hyaloplasm accumulates at the center while the alveolar spheres move away from it. At the height of its development the center of the aster is occupied by a large *hyaloplasm-sphere*, which shows no trace of alveolar structure, while around it the alveolar spheres are disposed in radiating rows between which lie the astral rays. In the living object the latter appear simply as radiating tracts of hyaloplasm between the alveoli and cannot be seen as fibrillæ; and Chambers ('17) has more recently shown that small protoplasmic granules, artificially introduced into the astral ray in the living object by means of the microdissection needle, are carried by the stream inward towards the center.

<sup>1</sup> Auerbach ('74), O. Hertwig ('75), Bütschli ('76), Fol ('79) and Strasburger ('80).

<sup>2</sup> Ziegler ('95), Rhumbler ('86, '99), Wilson ('01b), Giardina ('02), Teichmann ('03), Vejdovský and Mrazek ('03), Chambers ('17).

Bütschli ('76) suggested that the movements of the chromosomes towards the poles might be due to protoplasmic currents in the spindle; also that the ultimate effect of diffusion-currents, set up in the astral rays as a result of specific chemical changes in the centers, must be a *change of surface-tension*; and this, he argued, will be greatest when the peripheral actions of the two asters coincide, *i. e.*, in the equatorial region. Assuming the change of surface-tension to be an increase, the effect will accordingly be greatest at the equator, least at the poles; and from this should result the karyokinetic elongation in the axis of the spindle (p. 157), and ultimately the equatorial constriction and cleavage. In an important later modification and development of this view ('92, 1900) he assumes an absorption of liquid and its chemical fixation in the central region so as to cause here a diminution of volume. The result of this must be a tractive force upon the surrounding region to which the aster owes its origin, and which may also play a direct part in cell-division. This was supported by the bubble-experiments (p. 66) which prove that both asters and spindle (*i. e.*, an amphiaster) may be closely simulated by the coagulation of hot melted gelatin-solution about two adjoining air-bubbles, the tractive force being here exerted by the contraction of the bubbles, as they cool.<sup>1</sup>

These conclusions are developed at length by Rhumbler ('96 '99) who combines Hertwig's and Fol's conclusion as to a centripetal flow of hyaloplasm with Bütschli's conception of an absorption of liquid at the centers. The latter process, evidently, must give rise to a progressive increase of viscosity along the astral rays from the center towards the periphery; and should create adhesion-stresses or tractive forces in the astral rays, having the same general configuration as the tensions postulated by Heidenhain, Van Beneden and Boveri. Rhumbler thus reaches a conception essentially in harmony with that of fibrillar contractility or tension, though under a widely different view of the structure of the amphiaster.<sup>2</sup> On the other hand, Giardina ('02) and Teichmann ('03) reject the assumption of traction in the astral rays; each in his own way explains cleavage as due to a change of surface-tension directly caused by the central movement of hyaloplasm.<sup>3</sup>

Rhumbler, like Bütschli, emphasizes the effect of surface action at the equator, but imagined this as a process of increased growth which finally produces an infolding of the superficial layer at this point; and the way for this is prepared by the formation of a modified protoplasmic region or "diastem" through the equatorial plane, likewise determined by the traction of the astral rays. Of similar type is the assumption of Ziegler ('98,

<sup>1</sup> See the remarkable photographs in Bütschli's work of 1898.

<sup>2</sup> See also Wilson, '01, 2.

<sup>3</sup> See also Gurwitsch, '04 and Bonnevie, '06.

'03) that the infolding is directly caused by the hyaline or ectoplasmic layer described beyond (pp. 261, 413), thus explaining the remarkable cleavage of the ctenophore egg in which the thickening or "cleavage-head" cuts downward from one pole through the whole egg (Fig. 83). This assumption seems, however, to break down in view of Herbst's discovery (p. 1046), that in calcium-free sea-water the ectoplasmic layer disintegrates completely or disappears, yet constriction and division proceeds as before though the reuniting cells do not hold together.

*d. Surface-Tension and Vortical Currents in Mitosis.* The assumption that changes of surface-tension may constitute the immediate agent of

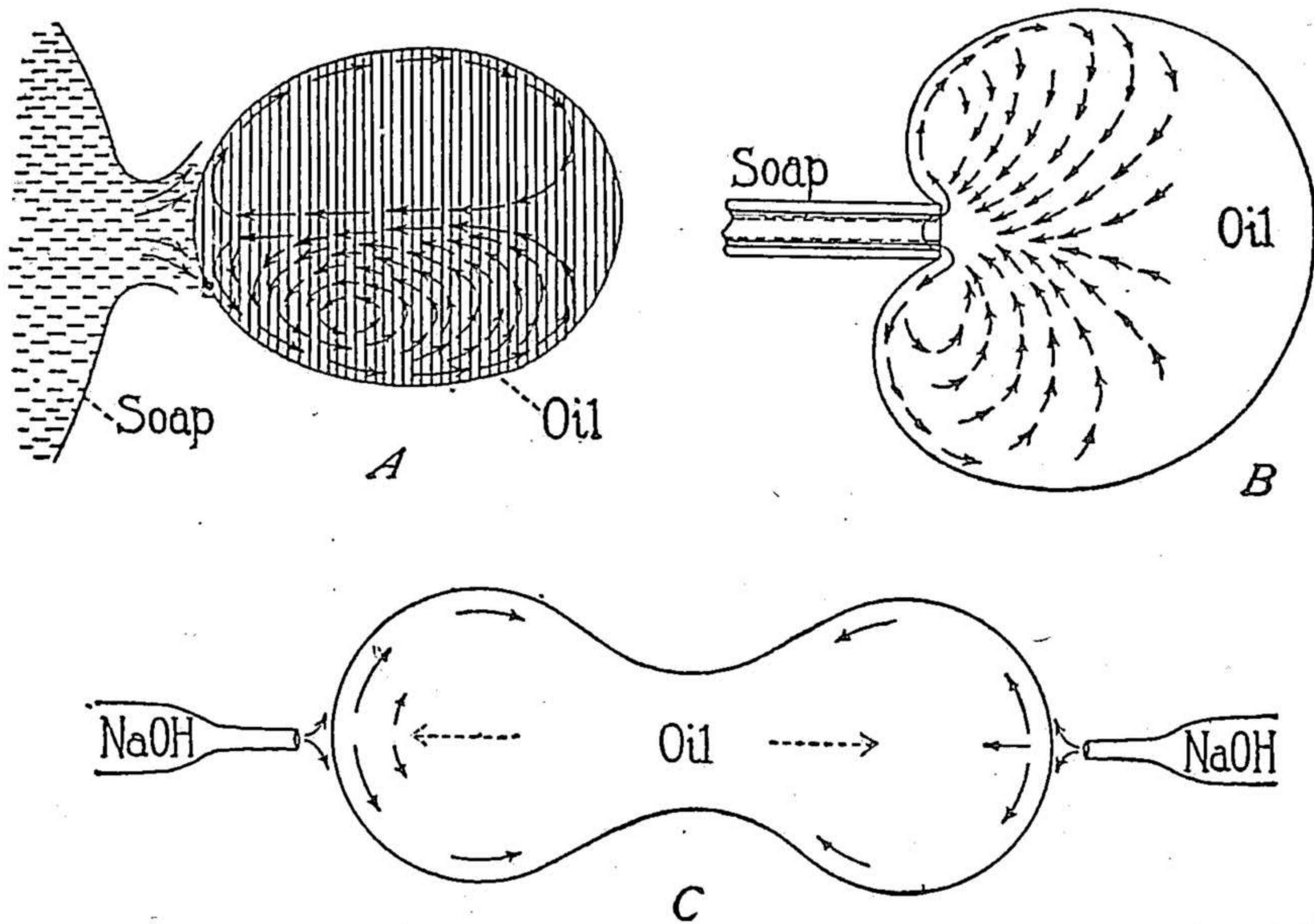


Fig. 82.—Surface-tension, cortical currents and division in oil-drops (*A*, *B*, from BÜTSCHLI; *C*, constructed from two figures by McCLENDON).

*A*, oil-drop suspended in water, in contact with soap-solution at left pole; *B*, similar drop, soap applied by means of pipette; *C*, drop of oil and chloroform suspended in water with simultaneous application of NaOH solution at opposite poles.

cleavage is made plausible by experiments on oil-drops which clearly bring out also the relation of such changes to currents in the cortical cytoplasm as recorded by many observers, especially in the later stages of mitosis. Bütschli's important studies on oil-drops ('92, '00) were based on investigations, especially by Quincke ('88, '89)<sup>1</sup> which proved that whenever the surface-tension of a drop of liquid (such as oil) be locally diminished (*e. g.*, by the local application of soap-solution) streaming movements are produced in the drop which flow away from the area of diminished tension, and thus give rise to vortical movements. This is shown in Fig. 82 in which the currents, indicated by the arrows, were made visible by mixing lamp-

<sup>1</sup> See also Berthold, '86, Lehmann, '88.



black with the oil. As shown in A, the superficial currents pass inwards as they approach the pole farthest from the soap to form an axial current within the drop passing forwards to the pole next the soap. Corresponding currents are likewise produced in the soap-solution; but these may here be left out of account. The same result may be produced by applying the soap-solution by means of a capillary pipette in which case the action is more gradual and the currents may continue for hours.

If we imagine two such areas of lowered surface-tension at opposite poles of a liquid drop, or (what amounts to the same thing) an increase of tension in the equatorial zone midway between the poles, two sets of vortical currents should be set up, having the disposition shown in C, superficial currents flowing from both poles towards the equator and thence into the interior. *Vortical currents of the same type occur in the dividing cell*, as was early observed by Bütschli's pupil, Erlanger ('97). This observer was able to see, in the living eggs of certain nematodes during division, definite streaming movements of the protoplasm which pass superficially from the regions opposite the spindle-poles towards the equator and thence along the cleavage-furrow into the interior of the egg (Fig. 83). In the mean time several observers had observed movements of pigment-granules towards the cleavage-furrow during or just previous to cleavage,<sup>1</sup> and Loeb was also led to the conclusion that vortical movements of the protoplasm may play an important part in protoplasmic cleavage. Conklin's studies ('99, '02) on the eggs of the gasteropod *Crepidula* showed that as the spindle elongates in the later stages of mitosis the yolk-spherules at the periphery of the cell move towards the equator, and thence in toward the middle of the cell in the plane of the future cell-wall.

More recently these conclusions have received a striking confirmation in the work of Spek ('18), who describes vortical currents in divisions of the living eggs of various forms, including nematodes, leeches, copepods and gasteropods. In the simplest of these cases division is symmetrical, the cleavage-furrow cutting in equally around the whole equator of the egg. In such cases, as is occasionally seen in the first cleavage of *Rhabditis dolichura*, the superficial currents conform exactly to the general theoretic scheme (Fig. 83), the flow being symmetrically from the poles to the equator. The movements are more complicated in the frequent case when the furrow is one-sided, first cutting into the egg from one pole and only later from the opposite pole. In such cases, a reversal of movement occurs, the currents at first flowing towards the first-formed furrow, but later

<sup>1</sup> In the embryonic cells of Amphibia by Nussbaum ('93) and Van Bambeke ('96), in segmenting eggs of acoelous Turbellaria by Gardiner ('95), and in the segmenting eggs of the fish *Ctenolabrus* by Loeb ('95).

in the opposite direction towards the delayed furrow. In all such cases the direction of flow is always towards that furrow which is actively cutting into the egg, while that on the opposite side has come to a standstill. We may therefore expect, as Spek points out, to find in the ctenophore egg (where, as above indicated, p. 194, the furrow is formed only on the upper side of the egg) that the vortical currents flow only towards one side; but

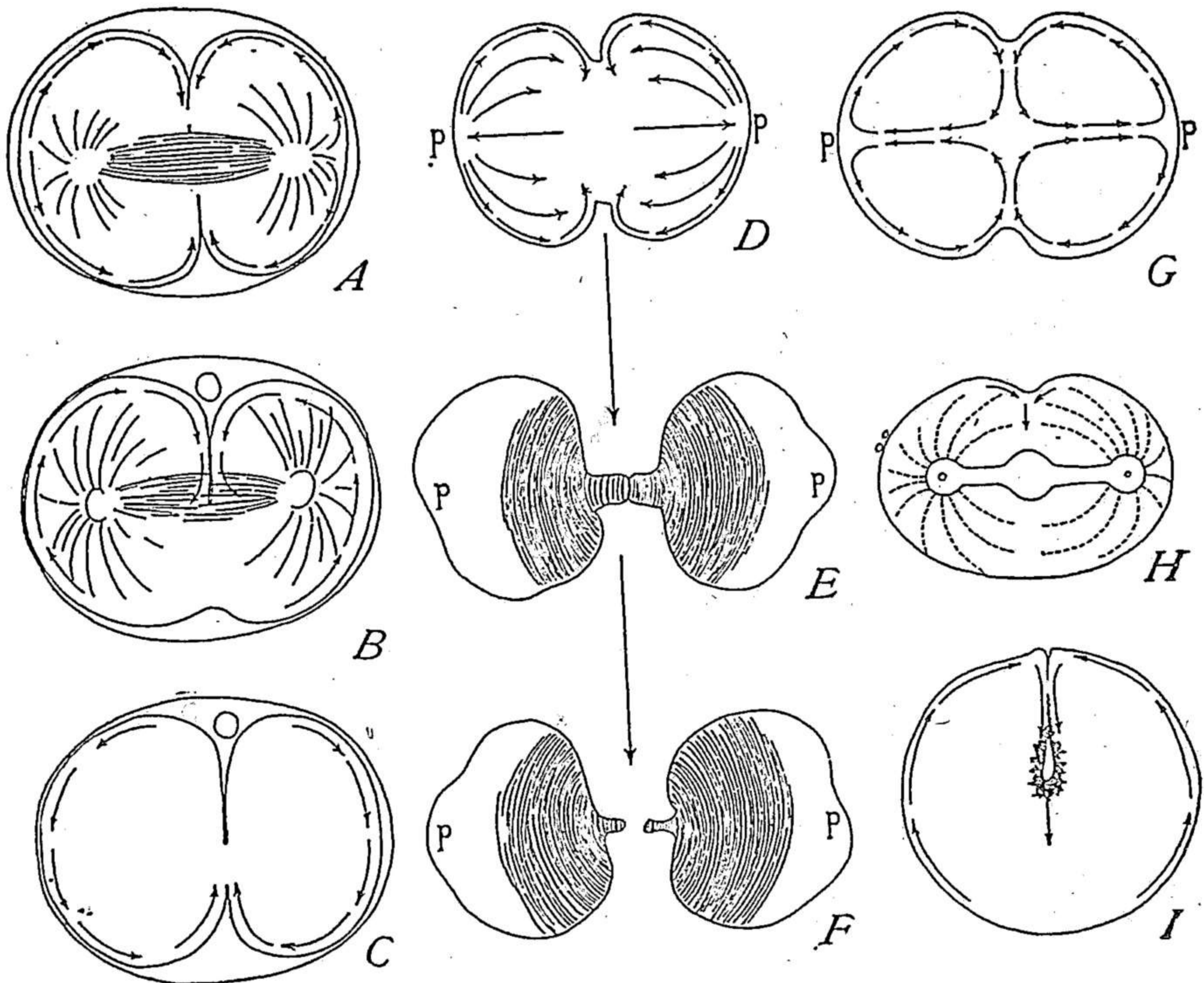


Fig. 83.—Surface-tension and vortical currents in mitosis (SPEK).

A, superficial vortical currents (indicated by arrows), in the living egg of the nematode *Rhabditis dolichura*, with symmetrical furrows; B, C, the same, with asymmetrical furrows; D, E, F, vortical currents, leading to division in an oil-drop to which a crystal of soda is applied at each pole (*p*); G, theoretic course of the currents in a drop with diminished surface-tension at the poles; H, Erlanger's figure of the currents in *Rhabditis*; I, cleavage of the ctenophore-egg, showing expectation concerning the currents.

this has not yet been observed. All these observations, obviously, bring support to Bütschli's hypothesis.

Evidence which seems to be conclusive is given by further ingenious experiments on oil-drops by McClendon and Spek, by which the actual division of the cytosome, may be imitated in the artificial model. McClendon ('10, '12, '13) employed drops of olive oil floating on the surface of water, or drops made heavier than water by the addition of chloroform to the oil. To opposite poles of these oil-drops is applied a solution of NaHO by means of two fine pipettes (Fig. 82), thus diminishing the surface-tension

at these points, so that a relative increase is produced in the equatorial zone. The result is a constriction and then a complete division of the drop in the equatorial zone, midway between the pipettes; and McClendon also showed, by adding fine particles of lampblack to the oil, that vortical currents are produced in the drop which flow from the poles to the equator and thence into the interior, precisely in accordance with the theoretical postulates of Quincke and Bütschli and with the vortical currents actually observed in living cells, as described above. These results are fully confirmed by Spek, who improved the method by employing in place of the pipettes solid crystals of "soda." Drops thus treated show the vortical currents, as described by McClendon, followed by an equatorial constriction and often by complete division (Fig. 83). So striking is the analogy between these drops and the actual division of cells that the correctness of the conclusions of Bütschli and his followers can hardly be doubted. If they are finally substantiated important progress will have been made towards a comprehension of cleavage, at least in its more general aspects.<sup>1</sup>

*e. Changes of Protoplasmic Viscosity in Relation to Mitosis.* Changes in the physical consistency of the protoplasm during the mitotic activities were indicated by early observations on alterations of the refractive index of cells during division (Flemming, '82) and were later noted by many other observers, such as Driesch, Morgan, Albrecht and Yatsu, who were engaged in experiments on shaking or cutting eggs to pieces. More recently this phenomenon has been examined by Heilbrunn and Chambers<sup>2</sup> by more effective methods, the former observer by studying the rate and degree of displacement of the protoplasmic granules when the egg is centrifuged at different periods during artificial parthenogenesis and fertilization, while Chambers relied upon direct observation by means of the micro-dissection needle (p. 55). Both observers observed a marked increase of viscosity in the prophase, and Chambers found (in the eggs of sea-urchins and nemertines) that this continues throughout mitosis, reaching a maximum just before actual cleavage begins and later diminishing. Heilbrunn found (in the eggs of echinoderms, mollusks and annelids) two maxima, one in the prophase and a second just before cleavage, with a decided decrease in the intermediate period.<sup>3</sup> Chambers' observations proved that the increase in

<sup>1</sup> Roberston ('09, '11, '13) reached a contrary conclusion by applying to the oil-drops threads soaked in the NaHO solution, whereupon division took place in the plane of the thread; he therefore concluded in opposition to all others, that division takes place along a plane of *diminished* surface-tension. The explanation of this result is not yet entirely clear. Both McClendon and Spek have criticised it in detail. The weak point in Robertson's conclusions seems to be his failure to take into account the vortical movements, which afford so strong a confirmation of McClendon's conclusions.

<sup>2</sup> See especially Heilbrunn, '15, '17, '19, '21; Chambers, '17a, b, '19; Seifriz, '18, '20.

<sup>3</sup> Seifriz on the other hand, using the same method as Chambers, found a decided decrease in viscosity of the central region from the middle prophase up to the late anaphase. This result hardly seems tenable in view of the essential agreement between the other observers.

viscosity affects especially the asters, spreading out in all directions as they grow, and diminishing from the central region outwards; the firmness of the protoplasm is thus greatest near the central hyaloplasm-sphere and least towards the periphery. The aster as a whole thus becomes a "sphere of solidification," which extends itself as the aster grows until only a relatively small peripheral zone remains liquid. Since two such growing spheres are present the egg elongates (karyokinetic elongation) as a result of their reciprocal pressure and finally divides along the more liquid equatorial zone (Chambers, '19).

This work further shows that the solidification of the astral region affects only the alveolar protoplasm lying *between* the true astral rays. Like his predecessors Chambers found the rays themselves to be composed of liquid hyaloplasm flowing centripetally into the central hyaloplasm-sphere. On the other hand, F. Lillie ('09) and Conklin ('17) believe the rays to be of firm or even elastic consistency, though Conklin also accepts the fact of a centripetal flow along them. Chambers found further, that the return of the semi-solidified protoplasm to the more liquid state first begins in the equator of the egg, spreading thence towards the poles as the hyaloplasm collects at the astral centers and the cell divides.

These various observations constitute an important advance in our knowledge of the mechanism of mitosis, though they have not yet been brought into very definite relation with the changes of surface tension meanwhile in progress. An important point established by Heilbrunn's work is that the solidification-process is favored by abstraction of water from the egg; and he would thus interpret the effect of hypertonic solutions on dividing eggs or in artificial parthenogenesis (p. 473). Since Chambers has proved that the viscosity of the aster decreases from the center towards the periphery we can thus in a measure bring all these observations into harmony with the view of Bütschli and of Rhumbler (p. 193) that the aster as a whole is a radial system of tractive forces. An important modification of this conception is necessitated by Chambers' demonstration that it is not the astral rays themselves but the alveolar inter-radii in which the change takes place; but so far as cytokinesis is concerned the effect would be the same.

#### 4. Division of the Chromosomes <sup>8</sup>

Since division of the chromosomes is not dependent upon that of the central bodies or the formation of a spindle (see monocentric mitosis, p. 168) the conclusion is clearly indicated that the primary act of division is due to an autonomous activity on the part of the chromosomes, essentially like that seen in the division of the central body or a plastid in the cytosome. It is an interesting fact that the mitotic transformation of the nucleus very

rarely if ever takes place without the appearance of at least one central body and aster. An exception seems to be offered, according to Kautzsch ('12) by abnormally large second polocytes occasionally found in *Ascaris*, in which the nuclear changes are said to take place in the entire absence of centers, asters, or spindle. The chromosomes here form and split lengthwise as usual but the daughter-chromosomes fail to separate (as in monocentric mitosis).<sup>1</sup>

The nature of the initial act of doubling in the spireme-thread (or earlier) is still unsettled.<sup>2</sup> Some recent writers have questioned the traditional conception that the doubling of the thread, or of smaller components from which it is built up, is fundamentally an act of fission. It has been conjectured<sup>3</sup> that each ultimate component of the threads (or of the nucleus from which they arise) may attract from the surrounding medium (cytoplasm?) its component materials and by a process of "autocatalysis" mould them into its own counterpart, in immediate juxtaposition to itself. Such a process would involve an accurate meristic duplication of the nucleus without involving any actual division. At present, however, this remains a pure speculation, and there is nothing in the facts thus far known to contradict the more usual view. The question here raised belongs, however, to the fundamental problem of growth and self-perpetuation in living things generally and has not yet found an adequate biophysical or biochemical answer.

## V. MITOSIS IN LOWER ORGANISMS

### 1. In Lower Metazoa and Metaphyta

As earlier indicated (p. 152) the anastral mitoses of higher plants, and of the animal ovum in certain cases (p. 508), are undoubtedly of secondary origin and derived historically from the amphiastral type. In animals the latter type is found in all Metazoa including the coelenterates, sponges and even such simple forms as the dicyemids.<sup>4</sup> Among multicellular plants amphiastral mitoses with both asters and central bodies are of common occurrence in the thallophytes, including both algæ and fungi. Typical

<sup>1</sup> A remarkable feature of the case is the fact that in spite of the absence of an achromatic figure these cells may divide by a constriction occurring after reconstruction of the nucleus and often dividing the latter irregularly. These facts are interpreted to mean that the central bodies are not primary or even necessary organs of division but are rather regulative or directive agents which bring about a more precise division than could otherwise take place. See also Boveri ('97) and Hogue ('10). It seems possible, however, that the process observed by Kautzsch is no more than a kind of fragmentation without special significance.

<sup>2</sup> Bonnevie ('08), has described the chromosome as traversed by a central axis which splits lengthwise as division begins, but this has not yet been confirmed.

<sup>3</sup> See Troland ('17), Muller ('22), Bridges ('22), Sands ('22). The prototype of these speculations is found in Haeckel's hypothesis of the plastidules and their "perigenesis" (1874).

<sup>4</sup> It was in these animals that Van Beneden first discovered the central bodies (1876).

examples of this are seen in the diatoms (Lauterborn, '96, Karsten, '00) in the algæ *Sphacellaria* and *Stypocaulon* (Swingle, '97) *Dictyota* (Mottier, '00), or in the fungus *Phyllactinia* (Harper, '05). In all these the central body is an extra-nuclear centriole lying in the protoplasm as in *Ascaris*, and the

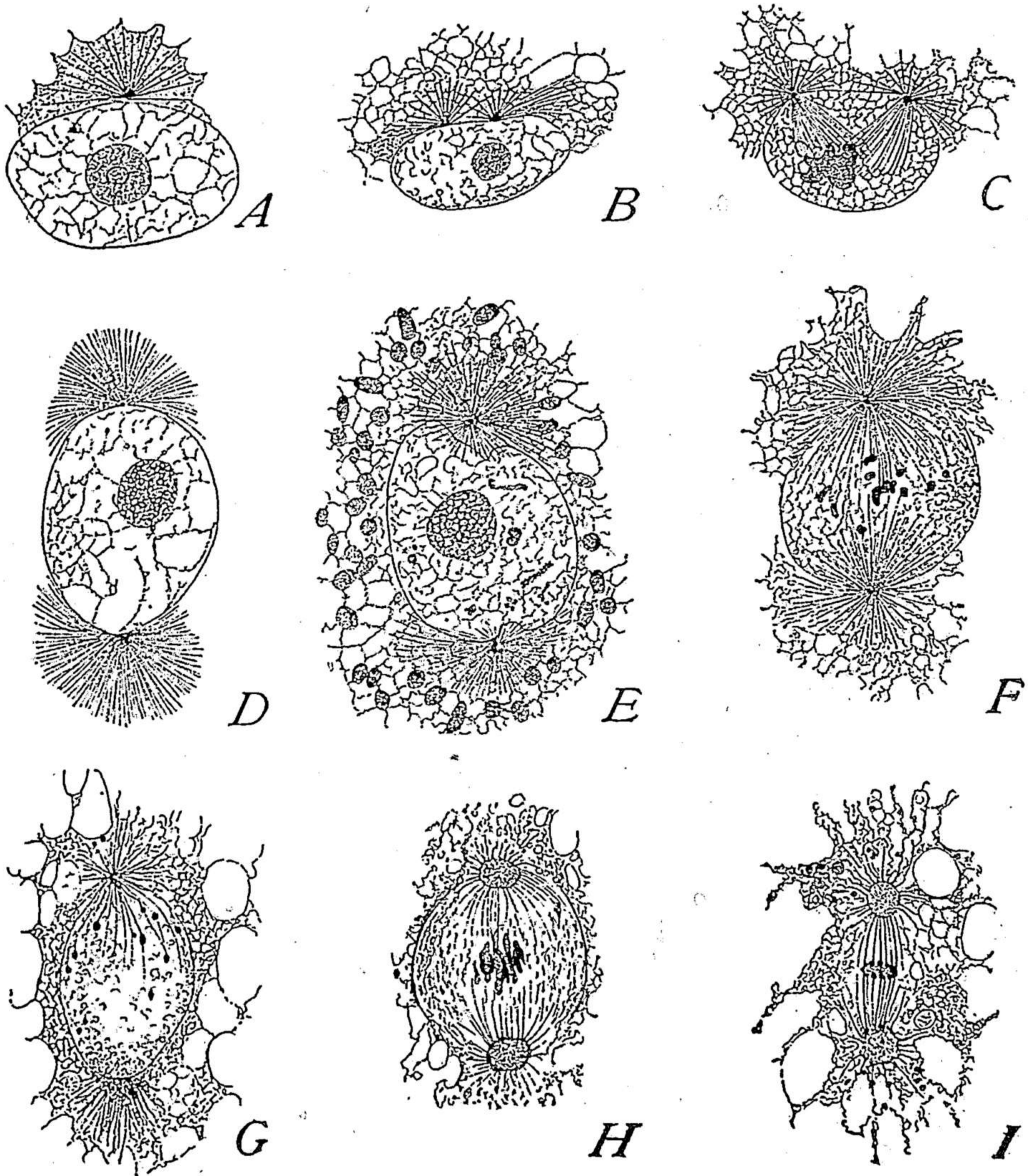


Fig. 84.—Central bodies in algæ (A–G, *Dictyota* from MOTTIER; H, I, *Corallina* from DAVIS). A, “resting”-nucleus of epidermal cell; B, C, prophases of second division of tetraspore mother-cell; D, prophase of first division of same; E, F, G, later stages of same; H, I, prophase and metaphase in *Corallina*, showing “centrospheres.”

aster or its center appears to be a persistent structure that arises by division, quite as in *Ascaris* or in *Acanthocystis*. The mode of spindle-formation in these forms (Fig. 84), commonly approaches the second type as seen in animals. In some of these cases the nuclear membrane seems wholly to

disappear during mitosis (e. g., in *Dictyota*, or *Stypocaulon*) in others to persist throughout the whole process (*Erisiphe*, *Synchytrium*).

In some of the thallophytes the centers of the asters are said to be formed by "centrospheres" (i. e., centrosomes) in which no centrioles have yet to be distinguished, and which seem not to arise by division but by forming separately and *de novo*.<sup>1</sup> Such conditions have been supposed to represent an early condition from which by disappearance of the rays might have been derived the polar caps of the cormophytic plants and ultimately the multipolar spindle-formation of the spore-forming divisions. Possible intermediate conditions are found in certain of the bryophytes and pteridophytes. In the moss *Polytrichum* Allen ('12) found each pole of the spindle occupied by a flat polar plate which behaves as a division-center though not surrounded by an aster. This body persists in the resting cell, divides in the prophase to form two polar plates from which protoplasmic fibrillæ grow towards the nucleus and finally form a spindle. In the later divisions the polar plate is represented by a group of granules or "kinetosomes" which divides into two similar groups during the prophase, as before. Comparable with this, possibly, is the division in *Isoëtes* as described by Marquette ('07). Here a large rounded "starch-body" containing starch-granules, lies at each pole of the spindle, persists as a single body in the resting cell and divides to form two similar bodies, which become flattened in the prophase while a spindle develops between them, very much as in *Polytrichum*. The resemblance of this body to a plastid lends some color to the attempt of Sapèhin ('13) to identify Allen's polar plates, and central bodies generally, with plastids.

That this series represents any approach to the actual phylogenetic series is, however, very doubtful. It seems probable that more thorough studies will reveal the presence of centrioles within the "centrospheres" of the algæ. The true nature of the pole-plates of mosses and the starch-bodies of *Isoëtes* is still uncertain, while the work of Devisé, as earlier indicated, (p. 154) raises still further doubts concerning the nature of the polar caps and the multipolar spindles of the seed-plants. This part of the subject, therefore, does not yet seem ripe for critical discussion.

## 2. Mitosis in Protista

In turning to the mitosis of Protista we find ourselves on somewhat more secure ground, though here too we are confronted by many difficulties. The phenomena in these simplest plants and animals might be expected to throw light upon those seen in higher forms; and in fact these organisms

<sup>1</sup> E. g., in *Fucus* (Farmer and Williams, '06, '98), *Corallina* (Davis, '98), shown in Fig. 84, *Erisiphe* (Harper, '97, '99), and *Pellia* (Farmer and Reeves, '94, etc.)

exhibit many interesting modifications and simplifications of the process.<sup>1</sup>

From the multitude of varied phenomena that have here been recorded two salient facts stand out. First, a few of the Protozoa, including representatives of both the rhizopods and the flagellates, exhibit a process of mitosis that appears to be in all essential respects of the same type as in higher animals. Secondly, this process appears to be connected by successively simpler types with modes of division hardly, if at all, distinguishable from direct or amitotic division. This series is most evident in case of the "achromatic" structures, although it is now certain that definite chromosomes are present in some Protista, and there is reason to suspect that an evolution of the chromosomes, as well as of the achromatic elements, may yet be traced among these organisms. Taken as a whole the phenomena unmistakably point to the general conclusion that the entire mitotic apparatus was originally of nuclear origin. Concerning the evolution of the more complicated types of mitosis in detail no general agreement has yet been reached. It is difficult, therefore, to give a brief connected account of the matter or to offer a simple grouping of the phenomena, as may be judged from the fact that while Chatton ('10) distinguishes three distinct types of nuclear division in the Protozoa, Alexeieff ('13) increases this number to five, or if the sub-types be reckoned in, to twelve, without counting various forms of multipolar division.

*a. The "Achromatic" Structures.* It must be borne in mind that the various modifications of the chromatic and the achromatic structures in Protista by no means run closely parallel.

(1) In a first group may be placed the comparatively rare cases in which both nucleus and cytoplasm contribute to the formation of the mitotic figure ("metamitosis" of Chatton) in a process that agrees in its essential features with that seen in the Metazoa generally. Examples of this are offered by certain of the flagellates, Sporozoa, diatoms, and by the heliozoan rhizopods *Acanthocystis* (Schaudinn, '96), *Wagnerella* (Zülzer, '09) and *Oxnerella* (Dobell, '17). All these are characterized by the presence of a persistent central body or division center, lying in the cytoplasm outside the nucleus, which elongates and finally divides to form the basis of a central spindle.

The best examples are offered by the Heliozoa above mentioned (Figs. 85, 325). The division-center is here a rather small, well-defined spheroidal body or *centroplast*, long known under the name of the "central granule." This body occupies in the vegetative cell the center of a very large per-

<sup>1</sup> The term Protista will here be employed to designate all unicellular organisms, whether plants or animals.



manent aster, the rays of which are definite fibrillæ extending out into the radiating pseudopodia, of which they constitute the axes (axopodia). So far as the achromatic figure is concerned the general history of the mitosis is here almost identical with that seen in higher forms, the centroplast dividing into two halves which pass to opposite poles of the nucleus and play the part of division-centers during the ensuing mitosis.<sup>1</sup> The centroplast is said to contain a sharply defined central granule or centriole (Key-

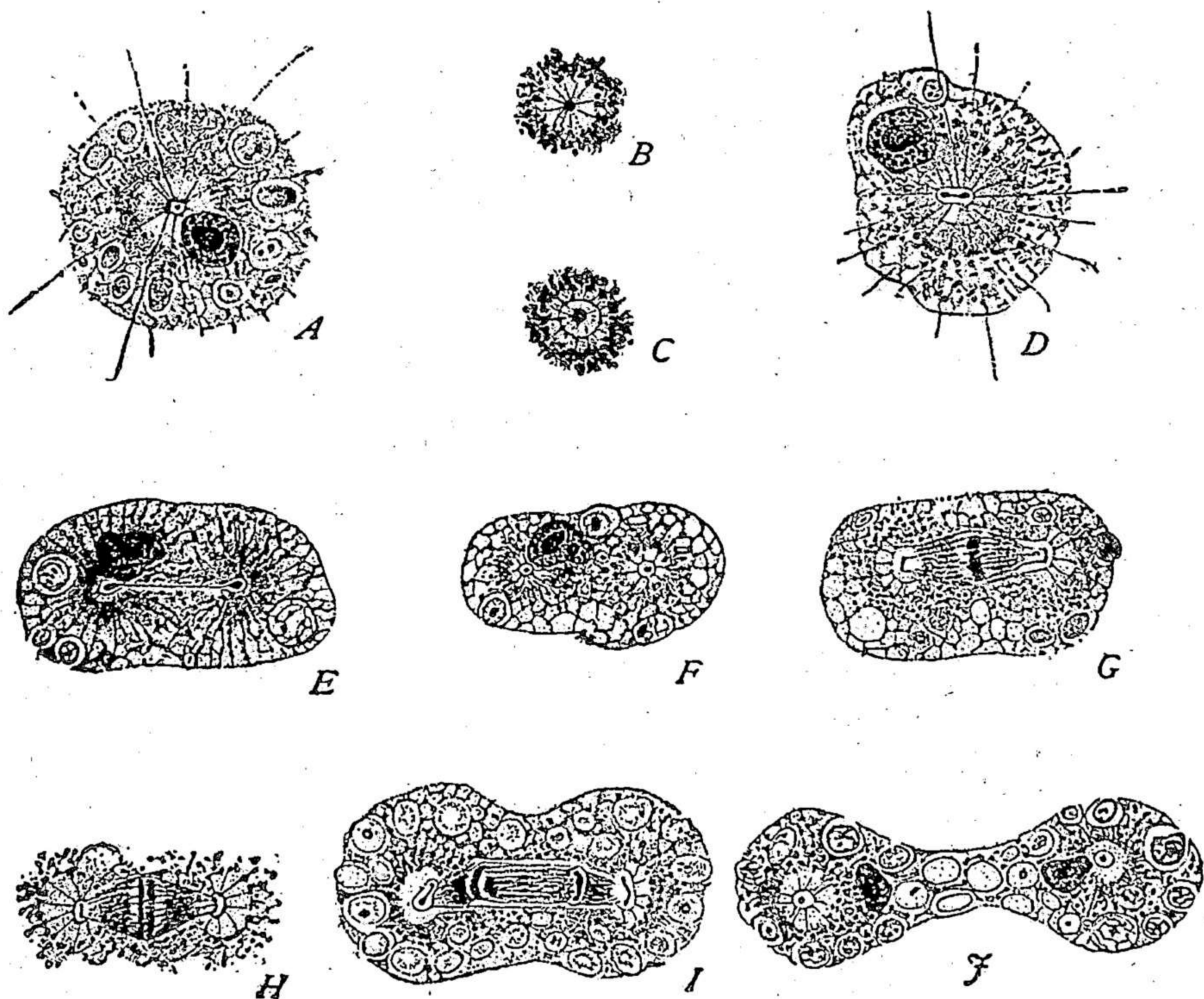


Fig. 85.—Mitosis in the heliozoön *Oxnerella* (DOBELL).

A, vegetative cell (pseudopodia mostly retracted by fixation), showing nucleus, central body (centroplast) and axopodia; B, C, enlarged views of centroplast in different phases; D, E, F, division and separation of centroplast; G, metaphase; H, I, anaphase; J, telophase.

sellitz, '08, Zülzer, '09, Dobell, '17) the division of which initiates the whole process of mitosis. It seems clear, therefore, that in these animals the centroplast and central granule play the parts respectively of centrosome and centriole; and the observations of Zülzer and Dobell indicate that here too the centriole is the only permanent central body while the centroplast is a temporary structure (p. 681).

Cases of this type also occur among the gregarines. An example is *Monocystis magna* (Doflein, '11), in which the extra-nuclear centers are

<sup>1</sup> Sasaki ('94) in *Gymnosphæra*, Schaudinn ('96) in *Acanthocystis* and several other forms, Zülzer ('00) in *Wagnerella*, Dobell ('17) in *Oxnerella*.

likewise surrounded by conspicuous asters by the rays of which the nuclear wall is pushed in very much as in the first maturation-mitosis of many animal eggs. In *Noctiluca* (Ishikawa, '94, Calkins, '98) the division-center is a large cytoplasmic "sphere" which by its division gives rise to daughter-spheres connected by a conspicuous fibrillated spindle; in the daughter-spheres, distinct centrioles are found at least at certain stages, but true astral rays seem to be wanting. In the diatoms (Lauterborn) the division-center ("centrosome") is much smaller and is surrounded by conspicuous astral rays in the resting-cell, but the latter disappear as the centrosome elongates to form the spindle. No centrioles are here seen. In both these cases the nuclear membrane for the most part persists and the spindle becomes more or less completely surrounded by the nucleus, and in *Noctiluca* the membrane fades away along the surface of contact where very distinct "traction-fibers" grow into the nuclear cavity and become attached to the long, thread-like chromosomes. The latter split lengthwise as in higher forms.

It is an interesting fact that in some of the flagellates belonging to this type the division-center or central body is identical with, or is very closely associated with, the basal body or "blepharoplast" that lies at the base of the flagellum; and the same is true of the flagellated swarm-spores of certain Myxomycetes (Jahn, '04). Examples of this are offered by various monads, in some of which the flagella persist during the whole mitosis. A well determined case of this kind is shown in *Trichomonas* (Fig. 86).<sup>1</sup> The blepharoplast here divides into two parts which migrate to opposite poles of the nucleus, within which a spindle is meanwhile developed. Asters appear to be wanting unless they be represented by the flagella.

(2) In many other Protozoa the entire mitotic apparatus is developed from the nucleus alone and the division-center, when such is present, lies inside the nucleus. A transition from the conditions seen in the first group occurs in *Acanthocystis*, where the center is said to have an extra-nuclear position only during the process of binary fission. In the budding process this center takes no part, a new center arising in the buds inside the nucleus (Fig. 325) from which it is finally extruded, taking up a cytoplasmic position that is retained through all subsequent processes of fission until budding again occurs. In *Centropyxis* the division-center is said to be permanently intra-nuclear (Fig. 90).

In many cases the intra-nuclear division-center is a large, nucleolus-like body, usually staining deeply with nuclear dyes, and variously known as the "endosome," "karyosome," or "nucleocentrosome"; and within this a number of observers have described a minute central body, or centriole.

<sup>1</sup> See Kuczynski ('14), Kofoid and Swezy ('15), Wenrich ('21).

By Nägler ('09) the mode of division thus characterized has been termed *promitotic* (promitosis); and this term will here be employed, though its significance has been considerably modified by later writers.<sup>1</sup> In nuclei of this type the chromatin may be wholly confined to the karyosome or may also be present as "peripheral chromatin" in the space surrounding the karyosome; and in mitosis the chromosomes or chromatic elements may apparently be derived from either or from both sources. An intra-nuclear

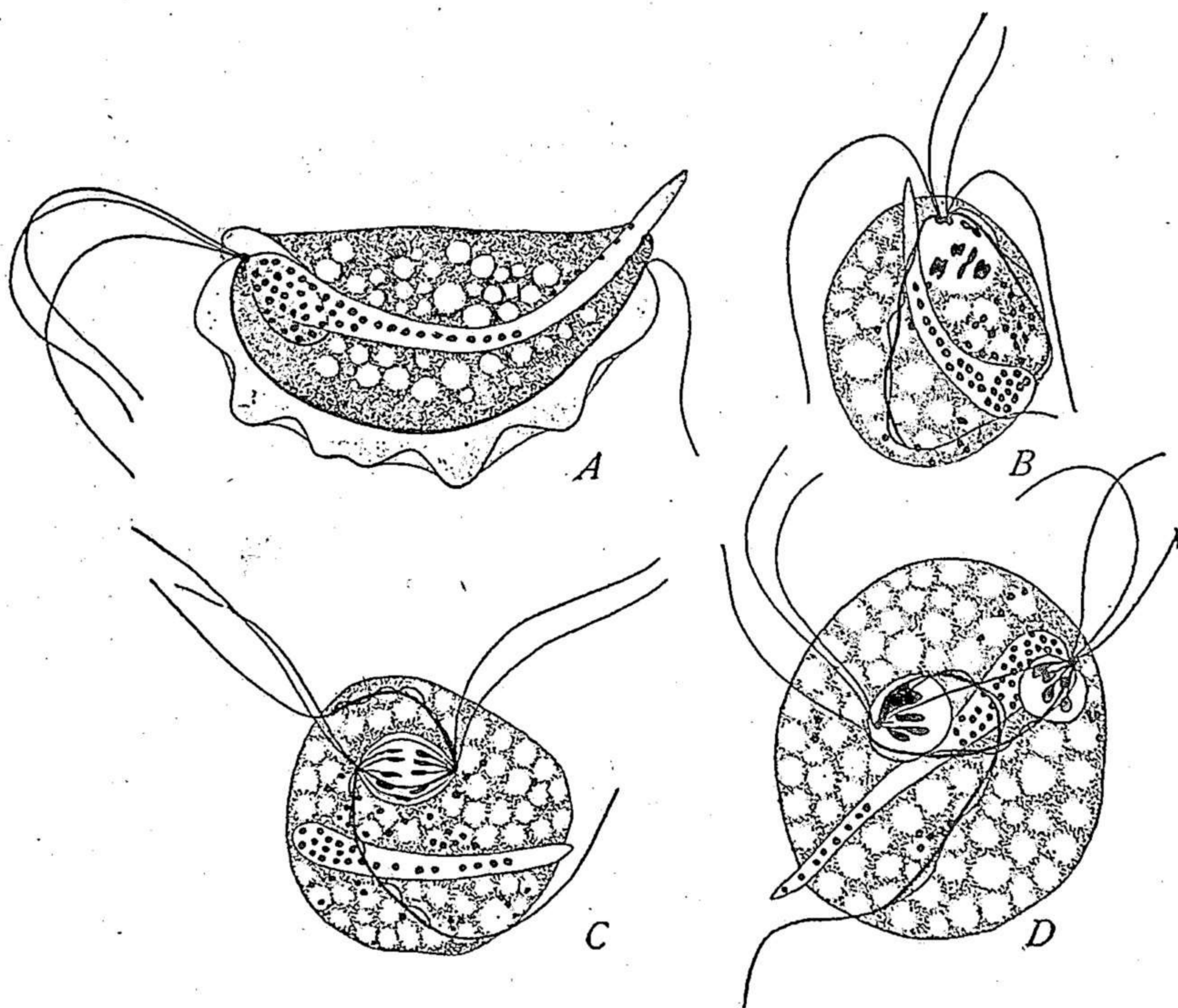


Fig. 86.—Mitosis in the flagellate *Trichomonas* (KOFROID and SWEZY).

A, vegetative individual (trophozoite); B, same, blepharoplast (b) divided; C, early anaphase; D, telophase.

spindle is found by elongation and ultimate division of the karyosome, a process in which the way is led by division of the centriole when this is present. The poles of the spindle, in typical cases, are occupied by deeply staining "polar masses" or "polar caps" derived directly or indirectly by division of the karyosome. The spindle itself appears to be formed in some cases entirely from the karyosome (e. g., in *Arcella*, Swarczewsky, '08), in other cases in part at least from the linin-substance of the peripheral nuclear zone surrounding the karyosomes (Fig. 87).<sup>2</sup> As a rule, neither karyosome nor centriole is surrounded by astral rays; nevertheless it is evident that they correspond in a general way to the centrosome and cen-

<sup>1</sup> Cf. Alexeieff, '13.

<sup>2</sup> *Amæba tachypodia*, Gläser, '12.

triole of metazoan mitoses,<sup>1</sup> whence Keuten's term "nucleo-centrosome" ('95).

Interesting questions grow out of the various relations of the karyosome to the chromatic elements; for, as stated above, this structure not only acts as a division-center but may also give rise to chromosomes. Three cases are here to be distinguished. In *Euglena* and certain other euglenoid flagellates the karyosome, though intensely basophylic, seems to take no

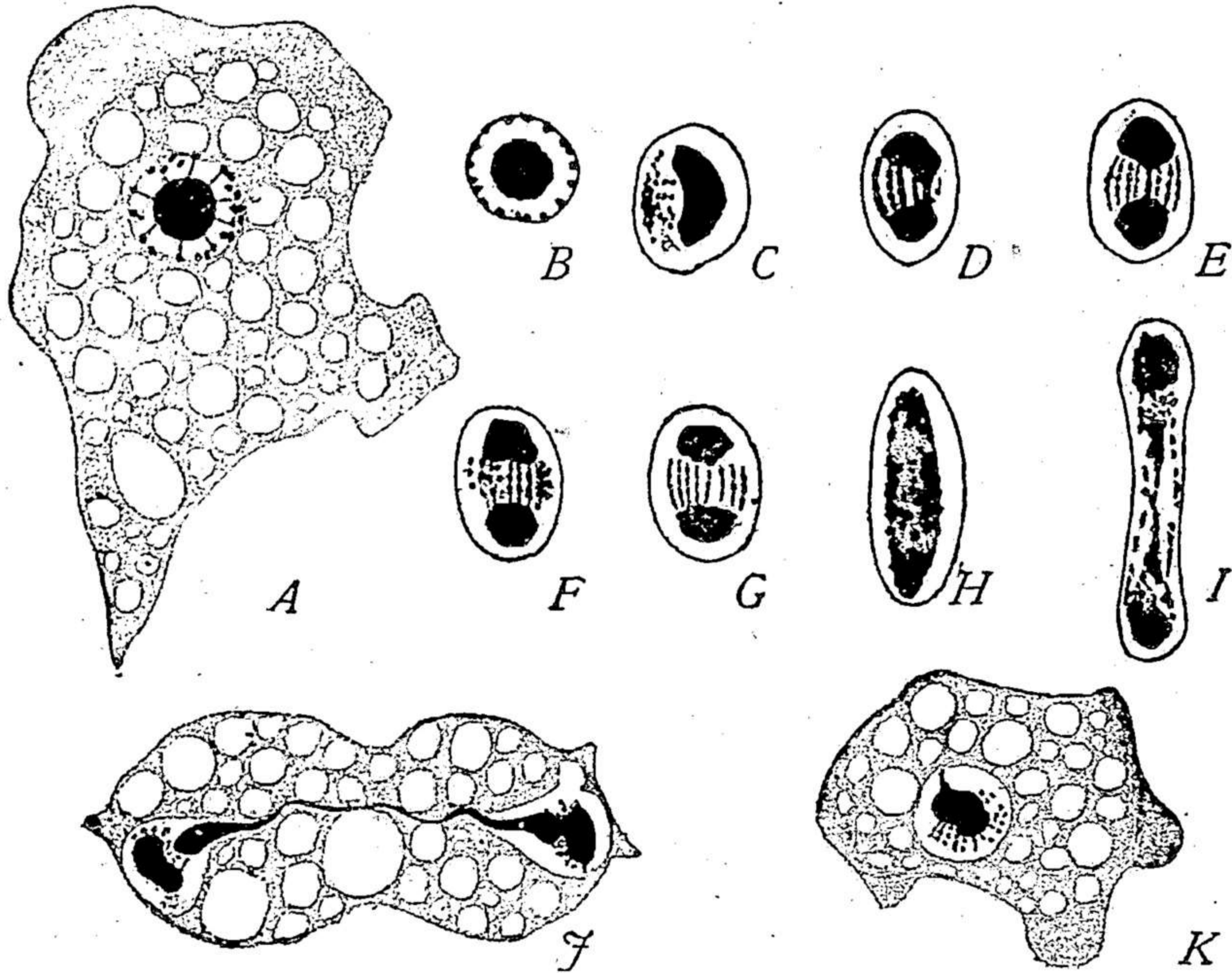


Fig. 87.—Promitosis in *Amœba tachypodia*, from fixed preparations (GLÄSER)

A, vegetative individual with "resting" nucleus showing large karyosome and peripheral chromatin; B, nucleus more extracted, to show structure of karyosome; C-F, elongation and division of karyosome to form "polar bodies"; G, a more extracted form like F; H, breaking up of polar bodies; I, J, telophases; K, daughter-cell, just after division.

part in the formation of the chromatic elements, these being derived wholly from the peripheral chromatin. In case of *Euglena* it is doubtful whether a centriole is present in the karyosome, though its presence has been maintained by some observers (Haase, '10). In the related form *Peranema* a distinct centriole is described by Hartmann and Chagas ('10), while in *Astasia* Bělář ('16) demonstrates its presence and division by means of photographs<sup>2</sup> (Fig. 88). Examples of the same type of karyosome in

<sup>1</sup> Intra-nuclear asters seem to be found in *Mastigella* (Goldschmidt), and *Centropyxis* (Schaudinn), while Gläser ('12) describes distinct cytoplasmic asters in *Amœba verrucosa*. Definite centrioles appear to be absent in the last case.

<sup>2</sup> The relations between karyosome, centrosome and centriole constitute an intricate question of which some discussion is given at another place (p. 690). Some protistologists (e. g., Hartmann, Nägler, Chatton, Minchin, Bělář) regard the centriole as of wide if not universal occurrence; others (e. g., Dangeard, Dobell, Gläser, Alexeieff) believe it to be exceptional.

the rhizopods are given by *Chlamydomphrys* (Doflein, '09) and *Amæba crystalligera*. In both these, according to Schaudinn, the chromatic elements are formed entirely from the peripheral chromatin, while the karyosome produces only the spindle (as in *Euglena*); and the same is described by Keysselitz in *Oxyrrhis* (Fig. 89). In the former species well-defined chromosomes appear, in the latter they seem to be absent.

In a second series of forms, illustrated by certain Amœbas of the *limax* type, a part of the chromatic elements are derived from the peripheral

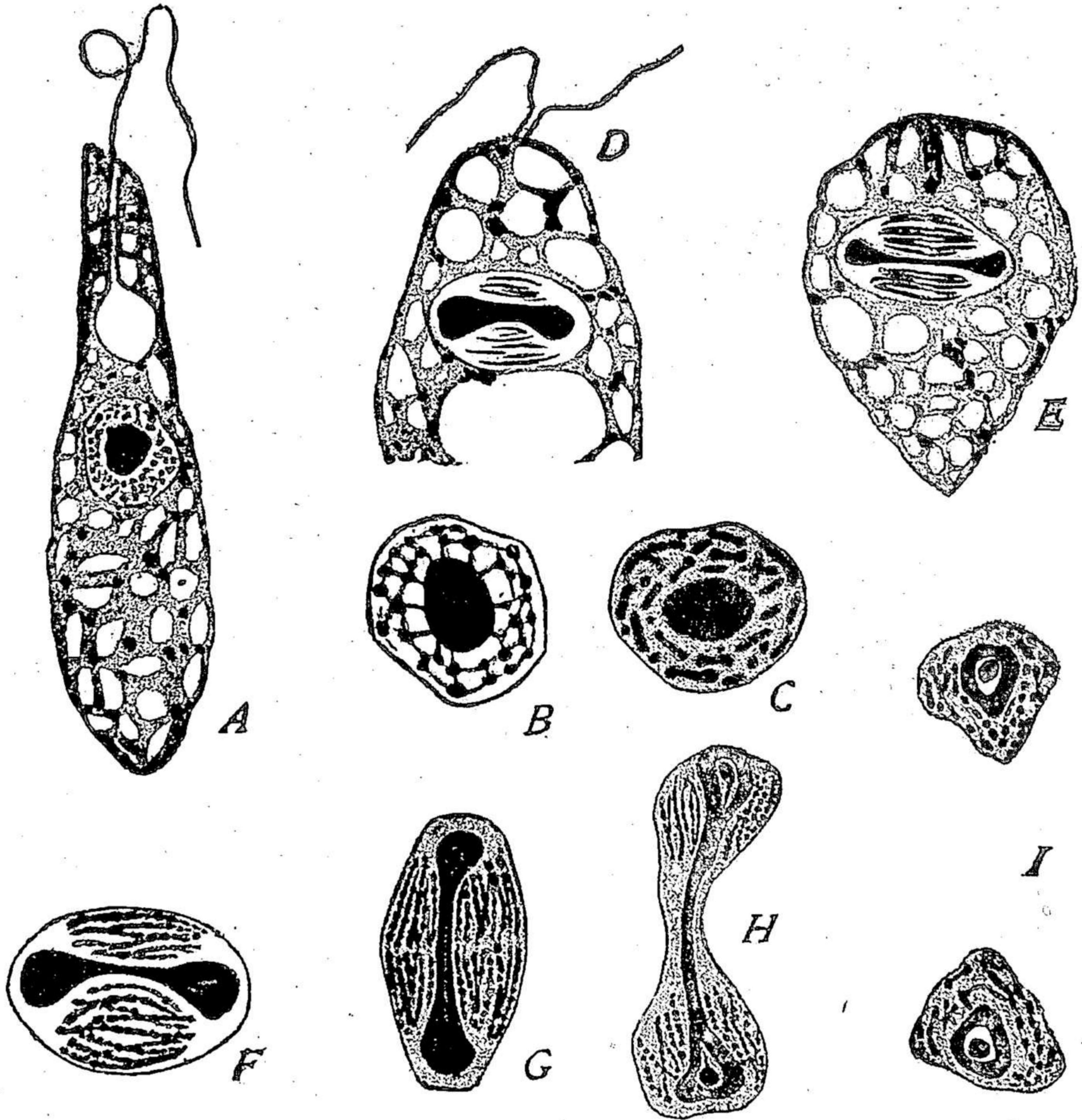


Fig. 88.—Mitosis in the euglenoid *Astasia* (BĚLAŘ).

*A*, vegetative individual; *B*, nucleus more enlarged; *C*, later stage, more extracted, showing dividing centriole and chromosome-formation, from peripheral chromatin; *D-F*, metaphase figures; *G-I*, later stages, apparently transverse division of chromosomes.

zone, a part from the karyosome. An example of this is given by *A. diplomitotica*, in which, according to Aragao ('09) definite rod-shaped chromosomes are formed, both from the peripheral chromatin and the karyosphere, the latter then dividing to form the polar masses while the chromo-

somes remain in the equatorial region. In this species two modes of mitosis are described. In one a definite equatorial plate occurs, dividing into two daughter-plates that pass as such to the poles; in the other no definite equatorial plate forms but the chromosomes scatter irregularly along the spindle, and pass some to one pole, some to the other, without visible process of division. Gläser ('12) has more recently given a careful description of *A. tachypodia*, producing clear evidence of a similar double origin of the chromosomes, and confirming Aragao's observation that the karyosome may by proper staining be differentiated so as to show a polar basis of "plastin" in which very definite chromatin-granules are imbedded (Fig. 87).

In a third type all the chromosomes arise from the karyosome. Examples are given by *Amæba lamellipodia* and *A. verrucosa* (Gläser, '12), *A. glebæ* and *A. fluvialis* (Dobell, '14), and *A. diplogena* (Bělař, '15). This author ascribes a distinct centriole which by its division leads the way in division of the karyosome; on the other hand, careful study (*e. g.*, by Gläser, Dobell and others) has failed to reveal the presence of such a structure in several other *Amæbæ*.

In the typical form of promitosis, as already mentioned, the karyosome divides bodily to form the deeply staining polar caps; and in such cases the spindle during the late anaphases and telophases often becomes compact and deeply staining, so that the chromosomes can for a time no longer be distinguished. A very characteristic appearance is thus given, for instance, in *Amæba tachypodia* (Fig. 87). There are, however, many modifications of this process. In *Arcella*, for example (Swarcewsky, '08) the polar caps are said not to be formed by direct division of the karyosphere, but by the breaking up of the latter into fine granules which then accumulate at the two poles, while the chromosomes appear in the equatorial region. In other cases (which Alexeieff, '12, places in an entirely distinct class) the karyosome breaks up but no polar caps are found, nor are centrioles present at the spindle-poles (*e. g.*, *Amæba glebæ*, *A. fluvialis*). Such cases are analogous to the anastral polar spindles of certain metazoan eggs later described (p. 508), while the phenomena in *Arcella* may give the key to the mode of division seen in Infusoria which are placed below in a fourth group.

(3) In a third group may be placed forms in which division is characterized by the presence of distinct centrioles, separate from the karyosome, which play the part of division-centers, and occupy the spindle-poles during mitosis. The karyosome no longer divides to form polar caps, but either gives rise to the chromosomes (like the karyosphere of metazoan nuclei) to a nucleolar-like body of reduced size, or even disappears altogether.

This is the *mesomitosis* of Chatton ('10), who has shown that it is connected with the preceding type by many intermediate steps. In these forms pointed spindles are found with centrioles at their apices, much as in Metazoa, but the whole spindle is still intra-nuclear. In certain cases (*Centropyxis*) towards the close of division the nuclear membrane disappears at the poles, and the result is the appearance of *cytoplasmic* asters about them (Fig. 90). In other cases the karyosome seems to disappear altogether, leaving only the centriole, or becomes much reduced. According to Chatton *Euglypha* is probably of this type, though the division of the center has not actually been seen (Fig. 91).

(4) In a fourth group may be placed certain forms, in which individualized division centers have not yet very definitely been recognized. Prominent among these are various ciliates, in which the whole nucleus transforms itself bodily into a spindle, at the poles of which appear rather definite polar plates; but it is doubtful whether the latter arise by the division of a single division center or by a process similar to that mentioned above in the case of *Arcella*. In *Paramœcium* Calkins and Cull ('07) conclude that the division of the "division-center" "is in reality only a flow of substance in opposite directions" to the poles of the spindle. Such a process would seem to approach in some respects the mitosis of higher plants in which individualized division-centers seem to be wholly absent (p. 152).

(5) We may lastly place in a distinct group the division of the chromidial nuclei by a process of constriction, without the formation of chromosomes or the participation of a division-center; and such a process, evidently, is strictly amitotic. Such a mode of division was long since described by Bütschli, Schewiakoff, and others in bacteria and related organisms, more recently by Schaudinn ('02), Dobell ('11) and others. Nothing like a "division-figure" is here found, the scattered granules merely separating into two groups as the cell divides; but in *Achromatium* Schewiakoff asserts that the individual chromatin-granules multiply by fission

(Fig. 32). Such a mode of division graduates by intermediate stages, on the

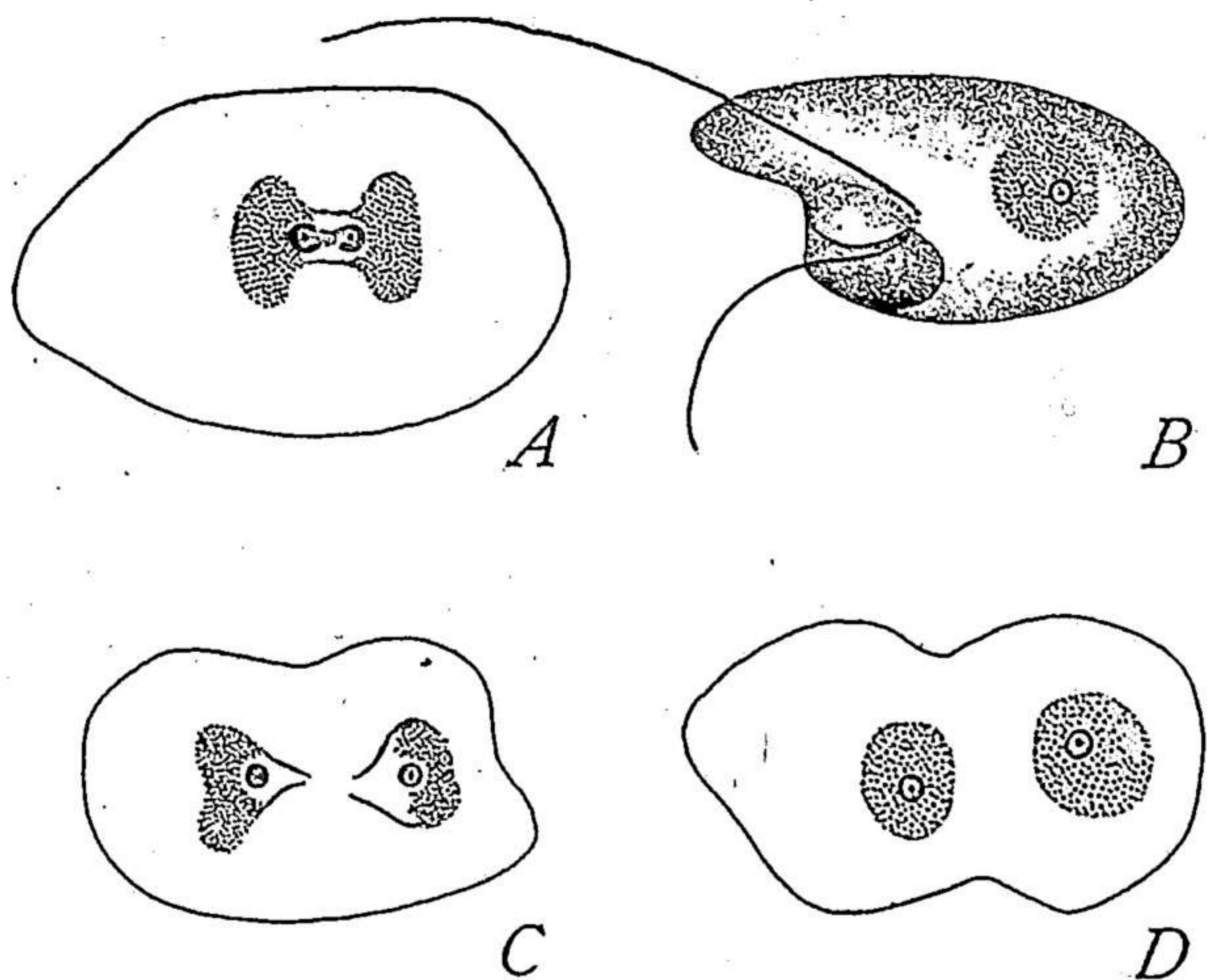


Fig. 89.—Mitosis in the flagellate *Oxyrrhis* (KEYSSELITZ).  
B, vegetative individual; A, C, D, successive stages of division.

one hand, into the direct division of a more compact type of nucleus, as Dobell in particular has shown in the case of bacteria (Fig. 33). On the other hand, the chromidial mode of division seems to show possible transitions to the mitotic processes of higher forms. In the much debated case of the Cyanophyceæ, for instance, where the nuclei approach the chromidial type, different observers have been unable to agree as to whether division is mitotic or not even in case of the same species.<sup>1</sup>

A surer basis of comparison is given by certain of the flagellates. In *Tetramitus* as described by Calkins the nucleus during the vegetative phase is in the form of scattered chromidia, but a small, spheroidal "division center" is also present in the cell. At the time of division the chromidia are said to aggregate into a single mass about the center, the latter divides into two halves which separate, the chromidial mass likewise dividing into two and afterwards breaking up into granules scattered through the daughter-cells (Fig. 32). Such a mode of division leads into that seen in other flagellates, such as *Oxyrrhis* where the nucleus consists of a localized mass of granules (chromidia) not surrounded by a membrane and inclosing a karyosome which acts as a division-center (Fig. 89). Similar types of nuclei are seen in *Trachelomonas*, *Lagenella* and *Chilomonas*, and also in the euglenoid flagellates, though in the latter case the chromidial granules seem in some cases to be inclosed by a definite nuclear membrane. From such a mode of division it is easy to pass to the more complicated form of mitosis.

*b. The Chromatin.* Some features in the history of the chromatic elements have been indicated above, but as already indicated it is not yet possible to give an adequate account of this part of the subject in Protozoa. It seems beyond doubt that in some cases definite thread-like chromosomes are found, often preceded by a spireme-stage, and splitting lengthwise quite as in higher forms. Chromosomes of this type are seen in *Noctiluca* (Calkins, '98), in *Paramecium* (Calkins and Cull, '08), *Actinophrys* (p. 597), and are said to occur also in *Euglypha* (Schewiakoff, '00), *Acanthocystis* (Schaudinn) and some other forms. In other cases, such as *Trichomonas* (Kofoid and Swezy, '15, Wenrich, '21) the chromosomes are much shorter and thicker, but still appear to split lengthwise in the prophase when in the form of a thick contorted spireme. In many cases, however, the chromosomes are much smaller and more numerous and the question of their division becomes correspondingly difficult.

There is considerable ground for the conclusion that among the Protozoa longer thread-like chromosomes, which split lengthwise (as in the forms just mentioned) arise by the linear aggregation of much smaller elements or

<sup>1</sup> This subject is reviewed in Sharp ('21). See also Acton ('14).



chromioles that are comparable to the chromidia of the scattered or chromidial nuclei, and may be capable of independent growth and division; but the data are as yet too uncertain to justify any very definite conclusions on this point. Of special interest is this connection is the type of mitosis called *haplomitosis* (Dangeard, '01), which is characteristic of the euglenoid flagellates and appears in some other Protozoa. In this type it was at first supposed that true chromosomes are not formed; and such is possibly the correct view, though it has recently been disputed. In *Oxyrrhis*,<sup>1</sup> for example (Fig. '89), nothing resembling chromosomes is seen, the nucleus consisting of a mass of fine granules and dividing as such bodily into two. But

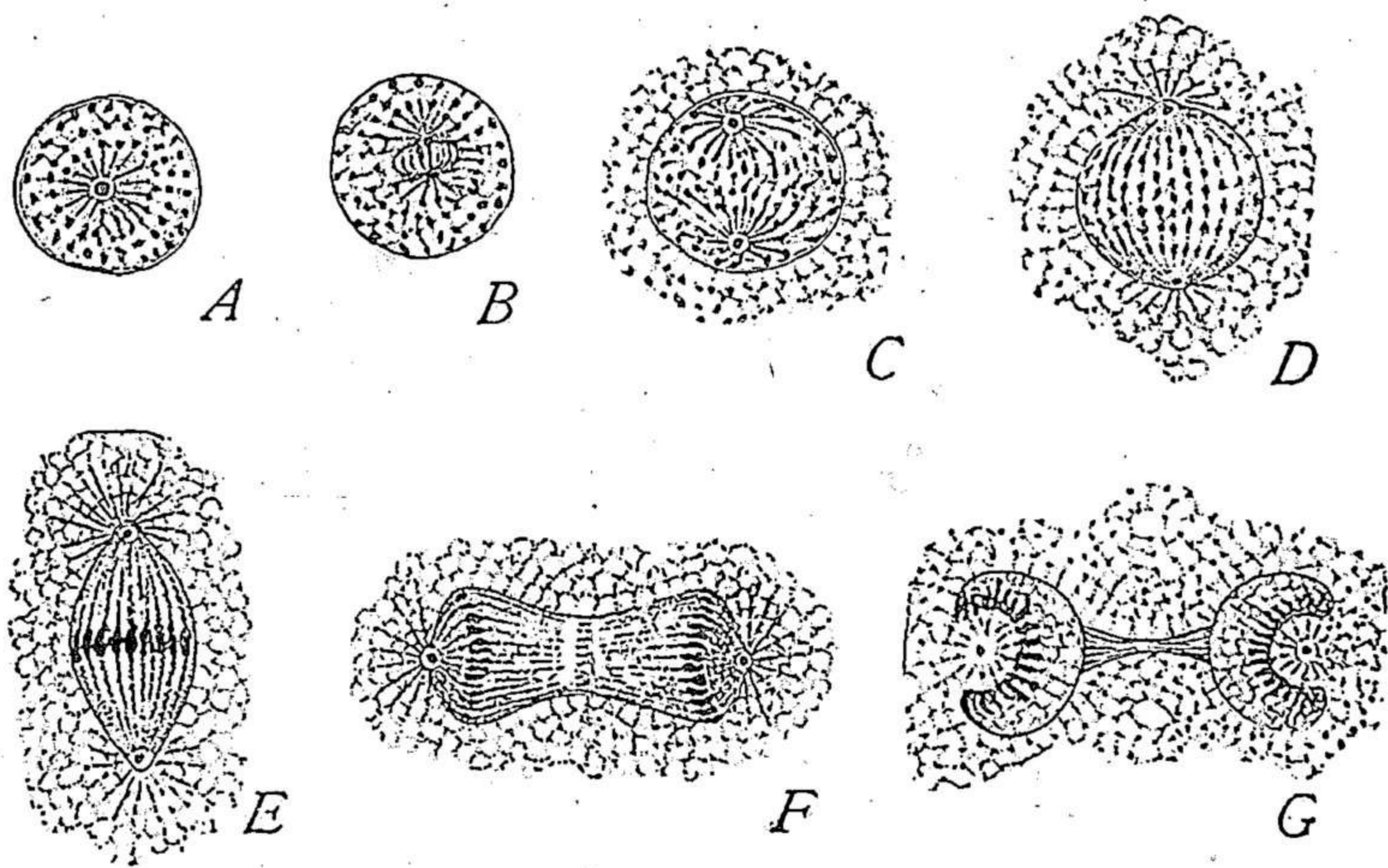


Fig. 90.—Intra-nuclear division-centers in the rhizopod *Centropyxis* (SCHAUDINN).

for the presence of the division-center this would doubtless be regarded as a process of amitosis. More or less similar to this is the process, according to Alexeieff ('11) in *Scytomonas* and some other flagellates, where the chromatin-granules, without forming spireme-threads, draw apart into two masses which pass to opposite poles of the elongating division-center to form "pseudo polar plates" (*crypto-haplomitosis* of Alexeieff). In *Euglena* and other forms, on the other hand, the nuclear granules give rise to spireme-like, moniliform threads or "chromospines" (Dangeard), but these seem not to split lengthwise, either drawing apart into two groups without division (hence Dangeard's term *haplomitosis*), or placing themselves parallel to the spindle and dividing transversely at the middle point. An example of the latter process is seen in the euglenoid *Astasia* (Fig. 88), as recently described by Bělař ('15); and a similar transverse division of "chromospines" or chromosomes has also been asserted to take place in various other Protozoa, of which conspicuous examples are offered by *Aulacantha* and *Ceratium*, as described by Borgert ('09, '10).

<sup>1</sup> See to the contrary, Hall, 1925. *Univ. Calif. Pub. Zoöl.*, 16.

At first sight this seems quite in opposition to what is known in higher forms; but a possible explanation is offered by the recent work of Tschenzoff ('16) on *Euglena*. This observer produces evidence that a longitudinal division takes place during the *anaphases* as the sister-chromosomes separate. The double chromosomes thus produced build up the daughter-nuclei, and the duality is assumed to be retained during the vegetative stage, the daughter-halves coming together again in the succeeding prophase, to be finally separated in the anaphases. The appearance of transverse division in the

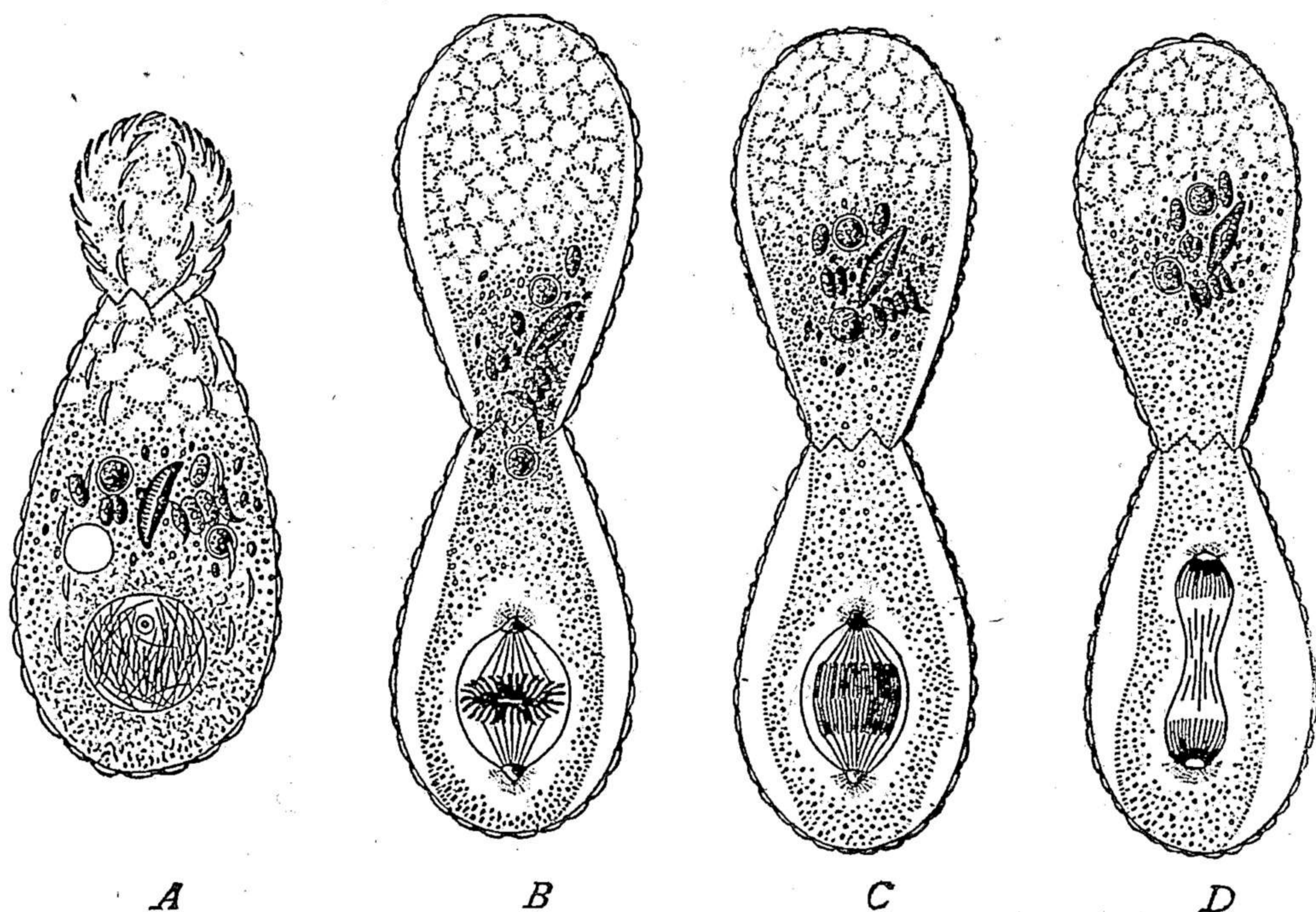


Fig. 91.—Mitosis in the rhizopod, *Euglypha* (SCHEWIAKOFF).

In this form the body is surrounded by a firm shell which prevents direct constriction of the cell-body. The latter therefore divides by a process of budding from the opening of the shell (the initial phase shown at A); the nucleus meanwhile divides, and one of the daughter-nuclei afterwards wanders out into the bud.

metaphase is considered by Tschenzoff to result from a terminal attachment of the chromosomes, quite as in higher forms. The general interpretation thus offered is in accord with that of various observers who have accepted the anaphasic splitting in higher forms generally (p. 138). In view, however, of the doubts hanging over this point the case of *Euglena* and similar ones call for further critical study.

Among the rhizopods the facts seem somewhat clearer. In some species of *Amæba* no equatorial plate is formed and the "chromosomes" or chromatic granules wander irregularly towards the poles (*Amæba lacertæ*, Dobell,

'15) the whole karyosome meanwhile drawing out into a spindle-shape and finally dividing. It is doubtful whether we can here speak of "chromosomes" or even of "mitosis"; but such a type of division, evidently, might well form the point of departure for the evolution of a true mitotic process. On the other hand, in *Amæba glebæ* numerous minute and granule-like "chromosomes" are formed from the karyosome, and become aligned in a single series to form a spireme which finally assumes the shape of a ring. This lies at the equator of a spindle developed by elongation of the karyosome, and splits lengthwise—a process which seems to be brought about by division of each constituent granule or "chromosome."<sup>1</sup>

*Conclusion.* The foregoing brief survey indicates five main conclusions:

(1) It seems beyond question that in some unicellular forms a true process of mitosis has been evolved, and one that in all essential features is of the same type as in the higher animals.

(2) From such a condition, most clearly evident in the heliozoan rhizopods (*Acanthocystis*, *Oxnerella*, etc.), progressively simpler conditions can be traced in almost unbroken series to anastral types of mitosis (ciliates, *Amæba*, etc.) and finally to conditions hardly to be distinguished from amitosis.

(3) This series leads to the conclusion that the most primitive forms of mitosis were of the anastral type; further, that the division-centers and spindle were primitively intra-nuclear (as in the existing forms of pro-mitosis); while the asters were a later acquisition, probably developed in the cytosome. The loss of the asters in various higher forms might thus in a sense be regarded as a return to a more primitive type; and the same view might be taken of the intra-nuclear origin of the anastral spindles in many cases (p. 156).

(4) We may therefore entertain the hypothesis that mitosis has arisen from a condition superficially similar to amitosis but in which the basic phenomenon is the growth and multiplication of small bodies, such as chromidia or chromioles, by the aggregation and alignment of which have arisen the spireme-threads and chromosomes of higher forms.

(5) The central bodies or division-centers have probably arisen by the localization of a substance ("plastin" or the like?) which once formed the general basis of the nucleus in which the chromioles were suspended. By the gradual separation from the latter of this substance arose a distinct division-center, which ultimately assumed an independent existence in the cytosome and became the center of the astral system.<sup>2</sup>

<sup>1</sup> This whole process is remarkably like that described by Stout ('12) in the sedge *Carex* and is also comparable to the pro-somatic divisions of *Ascaris megalocephala* (p. 323).

<sup>2</sup> Cf. p. 69<sup>6</sup>

## VI. DIRECT DIVISION. FRAGMENTATION. AMITOSIS

## 1. General Outline

Amitosis differs from mitosis essentially in that neither spireme nor chromosomes are formed, the nucleus dividing bodily into two by a simple mass-division. A classical example is given by the leucocytes, where the process was long ago continuously followed in the living object by Ranvier, Arnold and other observers. Here the nucleus elongates, assumes an hour-glass shape and finally draws apart into two, sooner or later followed by a similar division of the cell-body (Fig. 92). This type of division has been described more recently by Nowikoff ('09, '10) in the cells of various connective tissues (Fig. 93). In a second type, of common occurrence, the

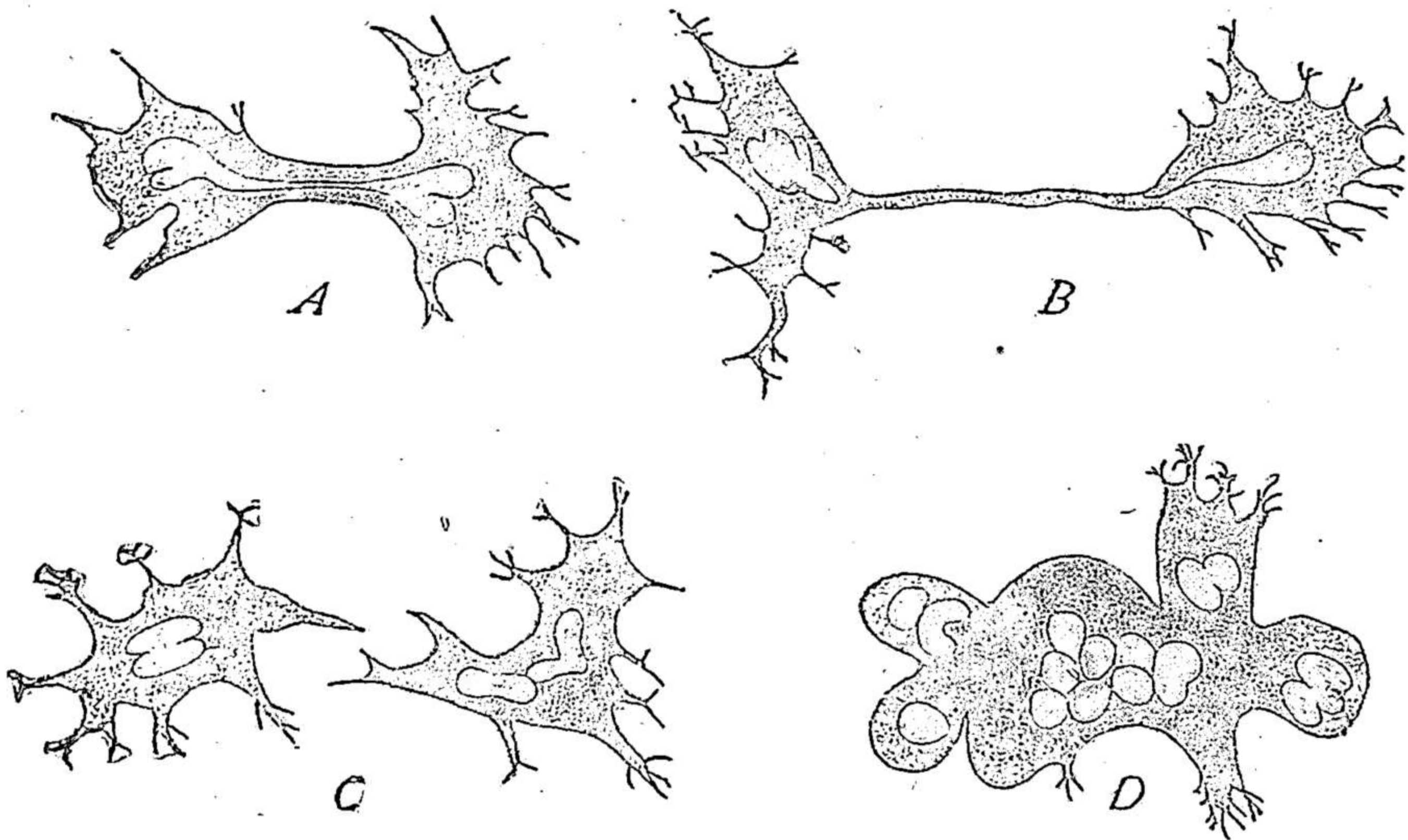


Fig. 92.—Amitosis in wandering cells (leucocytes) of the frog, from life (ARNOLD).

A-C, successive stages of division in the same individual, A at 11.00, B, 11.30, C, 11.50; D, multi-nuclear leucocyte, presumably arising by amitotic division of the nucleus.

nucleus divides by the formation of a transverse partition or "nuclear plate" by which the nucleus is cut in two.<sup>1</sup> The two types may occur side by side in the same tissue (*cf.* Gross, '10), and are connected by various intermediate forms. In either case division of the nucleus may be preceded by that of the nucleolus, as in Remak's original scheme (Fig. 44); but the nucleolus often seems to remain undivided, passing into one of the daughter-nuclei.

There are some cases in which a single central body or "sphere" is present and seems to play some rôle in the nuclear division. For example, in the testis-cells of urodeles Meves ('91) found that the sphere lies in a nuclear constriction, and gives rise to a ring-like band encircling the nucleus which

<sup>1</sup> Wassielewski ('02) proposed to designate these respectively as *diaspasis* (in Latin form *distrac-tion*); and *diatmesis* (*dissection*); but these terms are not in common use.

seems by contraction to cut the latter in two. Again, in leucocytes, according to Heidenhain, the astral sphere always lies in a bay on the concave side of the nucleus and may be concerned in its division (Fig. 10). Such cases are, however, exceptional.

If the chromosomes possess a persistent individuality or are genetically connected from one cell-generation to another (p. 828) amitotic division should produce a halving or irregular reduction in the number of chromosomes. It is therefore a fundamental question whether amitosis accompanied by complete cell-division may intervene in the succession of mitotic division as part of the normal process of development. Apart from this question, however, an interesting problem is offered by the physiological significance of amitosis.

## 2. Physiological and Theoretical Aspects of Amitosis

*a. Early Views.* With the discovery of mitosis and the demonstration of its widespread occurrence, two views arose concerning its relation to amitosis. Already in 1876, in his classical work on the dicyemids, Van Beneden drew a sharp distinction between mitosis ("nuclear division") and amitosis ("fragmentation"), regarding the latter as a rare and special phenomenon of secondary origin. For a time it was supposed by some writers that amitosis represents a primitive type of division from which mitosis has been derived, and is perhaps a direct survivor of a simpler type occurring in the Protista.<sup>1</sup> This view soon gave way, however, to the reverse conclusion, developed especially by Ziegler, Flemming and vom Rath, that amitosis is a secondary and simplified type that is an accompaniment of extreme specialization, and is commonly a symptom of approaching degeneration and death; and this is the conclusion which on the whole still seems most probable, though it requires some qualification.

With the progress of the inquiry the following main facts gradually became evident. If we leave aside the Protista and certain other low forms, mitotic division is highly characteristic of vigorous and actively proliferating cells, as seen for example in the cleavage of the ovum; in the division of embryonic cells generally, both in normal development and in regenerative processes, and in the maturation of the germ-cells—in short, in cells of un-specialized character, unimpaired vitality and high reproductive capacity. Amitotic division, on the other hand, is of frequent occurrence in cells of the opposite type, *i. e.*, such as are of weakened vitality, highly specialized, or on the road to degeneration. Some of the most striking examples of this are as follows:

Amitosis is especially frequent in cells of transitory nature, such as those

<sup>1</sup> Strassburger, '82, Waldeyer, '88.

of the vertebrate decidua, the embryonic envelopes, the periblast of meroblastic ova, the accessory nutritive cells connected with the developing germ-cells, and the like. In many cases such cells may be seen side by side with those of embryonic or progressive type, the former dividing amitotically, the latter mitotically. In the fish-egg, for example, all the earlier divisions are of strictly mitotic type; but the resulting cells later become differentiated into two groups, a central one belonging to the complete cells

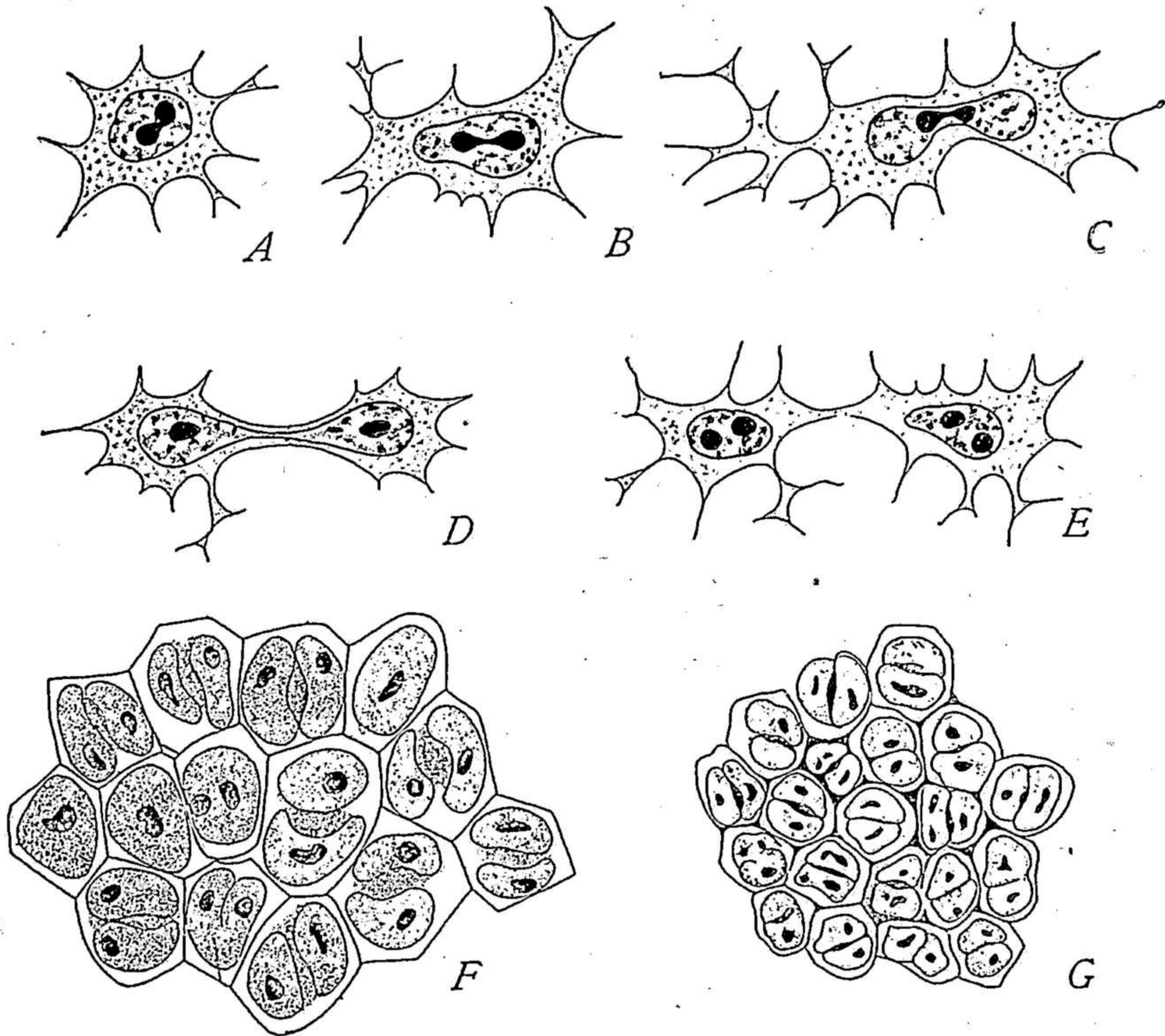


Fig. 93.—Amitotic division (A–E, from NOVIKOFF, F, from PREUSSE, G, from GROSS).

A–E, stages selected from fixed and stained preparations of sinew-cells in the mouse, arranged to show a series of apparent amitotic divisions; F, follicle-cells from the ovary of the bug *Nepa*; G, similar view from *Syromastes*, showing various stages of supposed amitosis.

of the embryo proper, and a peripheral group which lie free in the extra-embryonic region, are not surrounded by cell-boundaries, and constitute a merely transitory structure, the periblast, concerned in the absorption of the yolk. As shown by Ziegler ('87, '91, '96) and many later observers, the cells of these two groups show a remarkable contrast in respect to their later division. For a time both groups of nuclei divide by typical mitosis, but this only continues in case of the embryonic nuclei of the central group, while those of the periblast soon begin to show simplified forms of mitosis and finally numerous amitoses. In many other cases it was found that amitotic division of the nucleus is often not followed by cleavage of the

cytosome, so that binucleate or multinucleate cells are formed (Fig. 93). A striking example is offered by the observations of Dogiel ('90) on the stratified epithelium from the bladder of the mouse. Here the nuclei of the more superficial layers regularly divide amitotically giving rise to large binucleate or multinucleate cells which finally degenerate and are cast off, while the new cells that take their place are always formed by mitotic division of cells of the deeper layers

These conclusions have been supported by many other observations on various animals, though some of the facts are still to some extent in dispute. In the case of insects and other arthropods many of the earlier observers (Mayer, Carnoy, Will, Brandt, Korschelt, Preusse) emphasized the frequency of amitosis in the embryonic envelopes, and in the nutritive cells and follicle-cells of the ovary. Vom Rath's studies on the testis of vertebrates, mollusks and arthropods ('93) led him to conclude that amitosis never occurs in the sperm-producing cells (spermatogonia, etc.), but only in the supporting cells (Randzellen, Stützzellen). The question here raised has been carefully studied in the ovaries of insects with results which on the whole sustain the conclusions of Ziegler, Flemming and vom Rath. The careful studies of de Bruyne ('99) and of Gross ('01) on the testes of Hemiptera and other insects, with which the observations of the writer are in agreement, show that the ovary grows largely by the multiplication of an apical group of cells near its tip, which always divide mitotically. From the products of these cells arise (a) the oögonia and ova, (b) in Hemiptera the nutritive cells of the end-chamber, by which the growing ova are nourished, and (c) the follicle-cells surrounding the ova, which likewise probably contribute for a time to the growth of the egg, and ultimately secrete the thick chorion or secondary egg-envelope. According to nearly all observers mitosis is confined to the actively dividing apical group, to the oögonia, and to the young follicle-cells, while amitosis is characteristic of the nutritive cells and of the older follicle-cells. In all these cases we are struck by the correlation between the mode of division and the functional character of the cells, and also by the failure of nuclear amitosis to be followed by cleavage of the cytosome. The same is true of various gland-cells, such as those of the mucous skin-glands of the Amphibia (Klein) or the salivary glands of isopods (*Anilocra*, vom Rath, '95). In all these, amitotic nuclear division seems clearly not to be a step in cell-division but, as held by Korschelt, Chun and Flemming, is rather a means of increasing the nuclear surface. In all, again, amitosis is characteristic of cells that are highly specialized or are entering upon a period of degeneration, soon to be terminated by death. Another striking example appears in the ciliates in which both *macro-nucleus* and *micro-nu-*

*cleus* divide at each cell-division (Fig. 301), the former by an amitotic process, the latter by one which though of simple type, is clearly mitotic in character. This difference is obviously correlated with profound functional differences between the two nuclei, the macro-nucleus being an active metabolic nucleus, especially concerned with trophic functions and periodically undergoing complete disorganization and death, the micro-nucleus a generative nucleus from which, or its products, the macro-nucleus is periodically rebuilt (p. 608).

On the basis of such facts Flemming ('91) concluded that so far as the higher plants and animals are concerned amitosis is "a process which does not lead to a new production and multiplication of cells, but wherever it occurs represents either a degeneration or an aberration, or perhaps in many cases (as in the formation of multi-nucleated cells by fragmentation) is tributary to metabolism through the increase of nuclear surface." In this direction Flemming sought an explanation of the fact that leucocytes may divide either mitotically or amitotically (Peremeschko, Löwit, Arnold, Flemming). In the normal lymph-glands, where new leucocytes are continually regenerated, mitosis is the prevalent mode. Elsewhere (wandering cells) both processes occur. "Like the cells of other tissues the leucocytes find their normal physiological origin in mitosis. Only those so produced have the power to live on and reproduce their kind through the same process. Those that divide amitotically are on the road to ruin." Amitosis in the higher forms was thus conceived as a purely secondary process, not a survival of a primitive process of direct division from the Protozoa, as earlier supposed.<sup>1</sup>

*b. Later Views.* The most recent and thorough studies in this field have left little doubt that the conclusions of Flemming and Ziegler were correct in principle, though they require some qualification. Those conclusions met, however, with energetic opposition; and the enunciation of the so-called "mitosis dogma"<sup>2</sup> was followed by a marked, though temporary, reaction of opinion. It was clearly proved by a number of observers that the occurrence of amitotic division of the nucleus by no means precludes a subsequent resumption of mitosis. Meves ('94) showed that in the salamander the nuclei of the spermatogonia may divide amitotically at certain seasons of the year, subsequently resuming the mitotic mode of division and (presumably) giving rise to normal sperms; and these results were subsequently confirmed in *Amphiuma* by McGregor ('99). A considerable number of later observers have reached similar conclusions, both in adult

<sup>1</sup> "When once a cell has undergone amitotic division it has received its death-warrant; it may indeed continue for a time to divide amitotically, but inevitably perishes in the end" (vom Rath, '91, p. 331).

<sup>2</sup> Wassielewsky, '02.



and in embryonic tissues. Examples of this are given by the work of Bardeen ('02) on regenerating planarians, and especially of Child ('04, '07) on various invertebrates and vertebrates, in particular in the spermatogonia of cestodes, in the developing hydranths of hydroids, in regenerating plattodes, in the larvæ of *Amphioxus*, and in the cells of chick embryos. Patterson ('08) reports similar results from various stages of development in the pigeon's egg; Maximow ('08) in the embryonic tissues of animals; Nowikoff ('09, '10) in cells of the connective tissues; Wiemann ('10) for the early germ-cells of the beetle *Leptinotarsa*; Cilleuls ('14) in the regenerating uterine epithelium of mammals, and in other cases. C. W. Hargitt ('04-'06) even went so far as to assert that in hydroids the cleavage of the ovum, perhaps even the maturation-divisions, were accomplished by amitotic division. The farthest point in the reaction was reached by Child. Especially in the cestodes (*Moniezia*) this observer asserted the occurrence of numerous amitoses not only in various tissue-cells but also in the embryonic cells of the most rapidly growing regions, in the oögonia and spermatogonia, and even in the early cleavage of the ovum, mitosis being rarely seen after the first cleavage, while amitosis is of frequent occurrence. Even in higher forms he found in actively growing embryonic tissues very few mitoses, while amitoses frequently occur. From all this Child concluded that amitosis is to be regarded as "an important factor in growth in many organisms, and in some cases at least, either form of division may be changed into the other by altering the conditions" ('07, II, p. 211). The implications of this statement were towards the conclusion, that the occurrence of amitosis does not *per se* constitute any evidence of degenerative or senescent character of the cell; that such division may be an important means of progressive cell-multiplication; that it may alternate with mitosis; and that under certain conditions it may play an equally important rôle in development.

The foregoing conclusions, based on the observation of cell-division under normal conditions, seemed at first to be borne out by experimental studies. Pfeffer ('99) and Nathansohn ('00) in a widely cited series of experiments, found that if the cells of *Spirogyra* be slightly etherized the nuclei seem to divide by amitosis, but upon restoring the normal conditions mitotic division is resumed, and normal growth proceeds; hence the conclusion (probably erroneous) that, in this case, at least, an organism which normally divides by mitotic division is capable under appropriate conditions of continued amitotic division, of producing cells that contain all of the embryonic potentialities unimpaired, and of resuming the normal mode of mitotic division. In like manner Wassielewski ('02, '04) found that under the action of solutions of chloral hydrate the nuclei of root-tips in higher plants showed many

amitotic divisions which were succeeded by mitotic division upon restoration of the normal conditions.

More critical examination showed that these observations are open to a quite different and far more probable interpretation. Haecker ('00) showed that slight etherization of the segmenting eggs of copepods (*Cyclops*) caused the nuclei to divide by a process that gives a deceptive appearance of amitosis (Fig. 94), but actually is only a modified type of mitosis in which

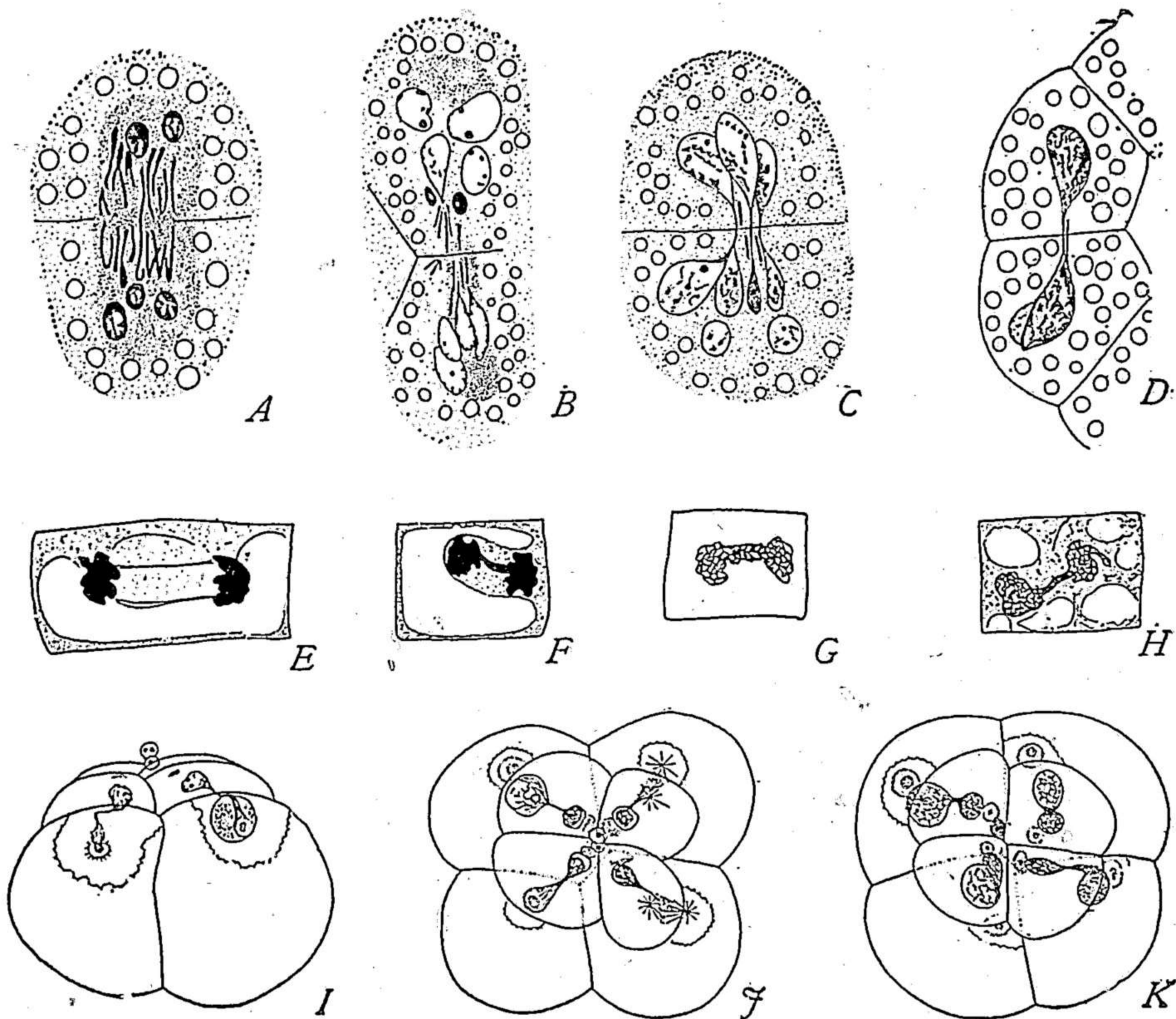


Fig. 94.—Artificially induced figures simulating amitosis.

*A-D*, early cleavage-figures of *Cyclops* slightly etherized (HAECKER); *E-H*, from root-tips of *Vicia* after slight chloralization (NEMEC); *I-K*, from early cleavage of *Crepidula* in dilute sea-water (CONKLIN). The final stages in all (*D, G, H, J, K*) simulate amitosis owing to the telophasic transformation of lagging chromosomes.

the chromosomes are formed and divide but fail to separate in normal fashion; and this was confirmed by Schiller ('09) and by the studies of Nemeec ('04) on chloralized root-tips. These observers showed that the effect of the narcotic is to cause a degeneration or suppression of the spindle-fibers and often an incomplete separation of the daughter-chromosomes. Thus in the telophase may readily be formed two nuclei in close apposition or connected by a narrow bridge (Fig. 94); and many evidences were also found that two separate nuclei thus formed may subsequently

fuse together.<sup>1</sup> It is obvious that such conditions of incomplete separation or of fusion subsequent to separation might readily be mistaken for amitosis. Results essentially similar to these were also reached by Conklin ('12, '17) in an important study of gasteropod eggs (*Crepidula*) after treatment by hypotonic solutions (Fig. 94).

In all these cases the constriction between two nuclei is shown to offer "no evidence of amitosis, but rather of the scattering of chromosomes along the spindle, at the previous division of these cells" (Conklin, 552). It is clear, therefore, that evidence of amitosis, unless based on direct study of the living cell, must be received with the greatest caution; and it is extremely probable that the results of Sachs and Nathansohn are susceptible of an interpretation similar to the foregoing one.

As was urged by Boveri, most of the observations on amitosis have failed to demonstrate (1) that the two nuclei of binucleate cells actually result

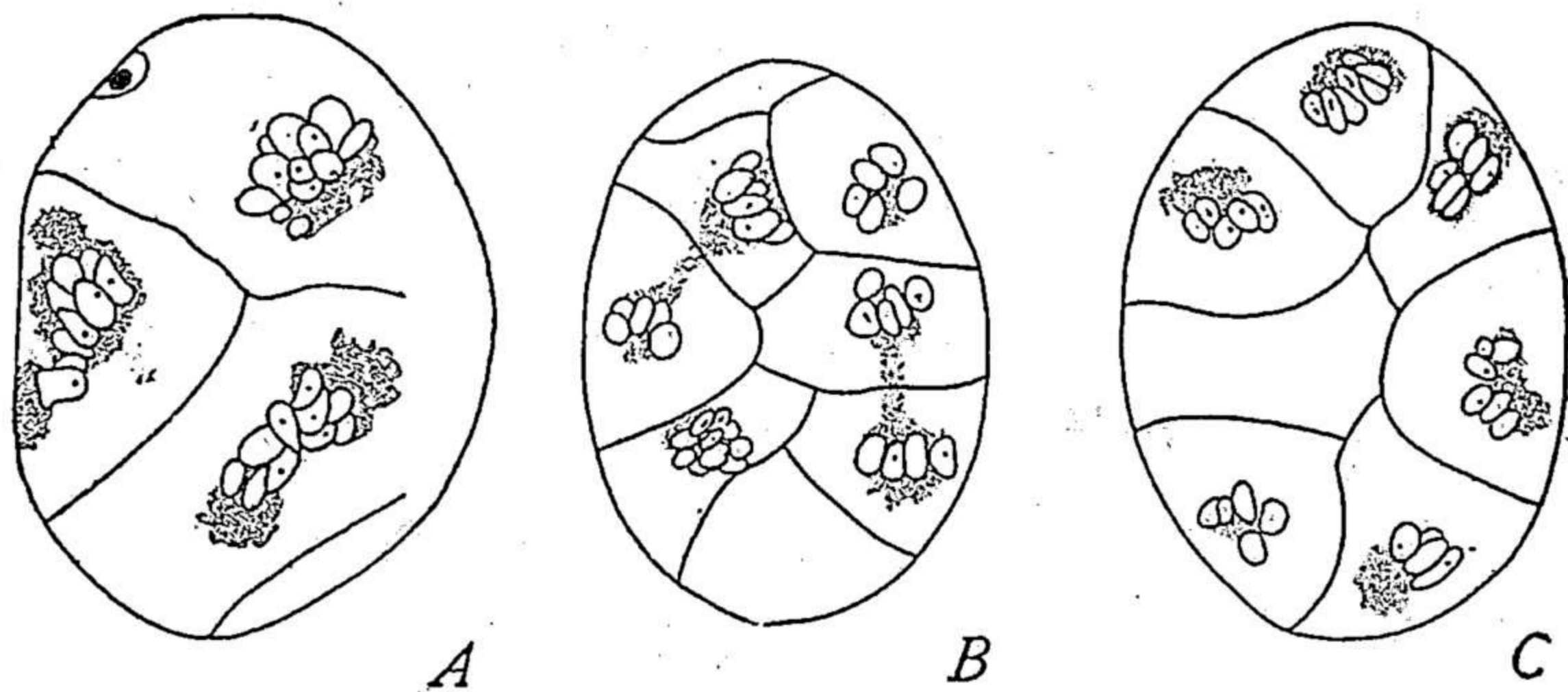


Fig. 95.—Polymorphic nuclei resulting from failure of the telophase-karyomeres to fuse. From the cleavage of the tardigrade *Macrobiotus* (WENCK).

A, from section of 7-cell stage; B, C, from 16-cell stage.

from division; (2) that division of the nucleus is actually followed by that of the cell-body; (3) that the cells thus produced subsequently divide mitotically with the normal number of chromosomes. In point of fact the so-called "amitotic cleavage" of the egg in hydroids was subsequently proved by Beckwith ('09) and G. T. Hargitt ('09) to be illusory, arising merely from the fact that the karyomerites or chromosomal vesicles formed in the telophase of each division do not completely fuse to form a single nucleus (Fig. 95). Again, although cytoplasmic cleavage following upon amitotic nuclear division has often been described and figured, nearly all such cases are based on the study of fixed material where decisive proof cannot be obtained. That cytoplasmic cleavage *may* follow upon amitotic nuclear division seems it is true to be clearly established by the observations of

<sup>1</sup> All this is in harmony with the phenomena earlier observed by the Hertwigs ('87) and the writer ('01, a, b) in etherized sea-urchin eggs (*cf.* p. 183).

Ranvier and Arnold on living lymphocytes (Fig. 92); but the existing evidence indicates that this is a rare and secondary occurrence.

In the case of cestodes the careful studies of Richards ('09, '11) have shown that mitosis is more frequent and amitosis less frequent than Child believed; that no evidence could be found of cytoplasmic cleavage following nuclear division; that the cleavage of the ovum is almost certainly mitotic throughout; and that the pre-oögonial and oögonial divisions are also probably mitotic. Similar contradictions exist in case of the insects. In Hemiptera Foot and Strobell ('11) concluded, in opposition to the earlier conclusions of Gross and others, that the oöcytes of *Protenor* are derived by amitotic division from cells of the nutritive end-chamber (p. 332). This, however, is contradicted by Payne ('12) who found in *Gelastocoris* that the oöcytes are derived directly from the apical group, passing thence downwards through the nutritive end-chamber but always distinct from them. Again, E. B. Harvey ('13) concluded that the binucleate cells so often observed in the follicle-cells are not due to amitosis but to mitotic division of the nucleus without cleavage of the cytosome. It is nearly certain that this is to a certain extent correct, because of the frequent occurrence of multiple or polyploid chromosome-groups in the follicular epithelium (p. 870).

It must be admitted that the questions here involved are in a somewhat unsettled state; nevertheless it has become evident that nearly all of the evidence supposed to demonstrate the occurrence of complete cell-division by amitosis as a normal process in the genetic continuity of cells is untrustworthy. The facts now indicate rather the correctness of Flemming's conclusion that amitosis means merely a fragmentation of the nucleus, often of temporary nature, which is only rarely followed by cleavage and probably plays some special part in the cell-metabolism by increase of the nuclear surface. The frequent occurrence of amitosis in embryonic cells, therefore, by no means proves that the multiplication of *cells* is thus effected. An important contribution to this question is made by the studies of Macklin ('16) carried out by means of continuous observations on living cultures of embryonic cells of various tissues from the chick. Binucleate cells are frequently observed in such cultures, and this condition certainly results in some cases from typical amitotic division of the nucleus, as was determined by continuous observation of the living cells (Fig. 96). *In no case, however, was amitotic division found to be followed by division of the cytosome.* The cytoplasmic cleavage of a binucleate cell seems only to take place after a subsequent mitotic nuclear division; and Macklin was able to observe in the prophases of such a mitosis in the living cell that *the two original nuclei fuse together and give rise to a single group of chromosomes associated with a single spindle.* The amitotic nuclear division here

forms no part of cell-division but is only a temporary fragmentation of the nucleus into two karyomerites. Such a process, evidently, is in no way incompatible with the occurrence of perfectly normal mitosis following

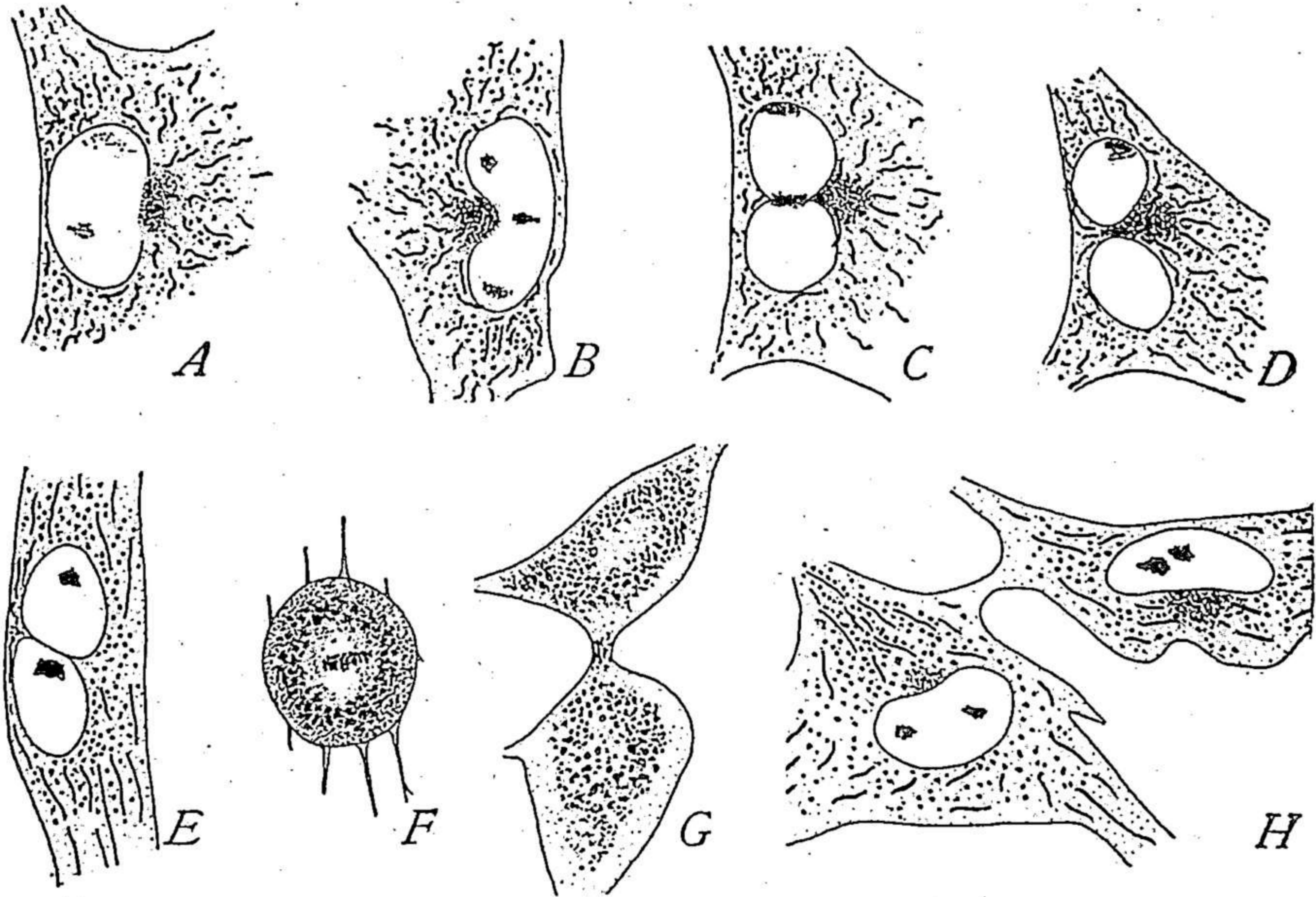


Fig. 96.—Nuclear amitosis in living cells (MACKLIN).

*A-D*, successive drawings of a living connective-tissue cell (cytosome only partly shown) in hanging-drop culture, from an embryo chick of five days, showing complete division of nucleus without division of cytosome; the dark bodies are chondriosomes, in some degree massed about the "centrosphere" at one side of the nucleus; *E-H*, successive stages of mitosis of a binucleate cell from life; the bipolar mitotic figure *F* was followed by complete division.

upon amitosis, and does not contradict the individuality or genetic continuity of the chromosomes.

In the light of the foregoing observations it may fairly be concluded that the general conclusions of Flemming and of Ziegler still remain unshaken and that they may probably be accepted as valid in principle. No doubt the subject will repay further critical study; and it would be rash to deny that amitosis may play a larger rôle in cell-division, particularly in cells of highly differentiated type, than now seems probable. That some sort of amitosis may play an important part in the multiplication of some unicellular organisms seems very probable; but it is also probable that in these cases the nucleus may be of far simpler composition than in higher forms and that its division may involve a correspondingly simpler mode of distribution. In higher forms, on the other hand, the whole force of the accumulating evidence, direct and indirect, tends to emphasize the fundamental importance of mitosis as a primary factor in every form of reproduction.

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## CHAPTER III

### REPRODUCTION AND THE LIFE CYCLE. INTRODUCTORY

“In both the rejuvenated infusorian and the fertilized egg-cell we see the onset of an energetic multiplication by cell-division which leads in the one case to the formation of a multicellular organism and in the other to a series of cell-generations. Morphologically, therefore, we must compare the sum of all the individuals of these generations to the multicellular higher organism that arises from the fertilized egg and itself in turn gives rise to eggs.”

BÜTSCHLI.<sup>1</sup>

Growth and cell-division constitute the central phenomena in every process of reproduction; upon them, therefore, depend the genetic continuity of living organisms and the phenomena of heredity. The cyclical character so conspicuously shown in the life-histories of plants and animals generally prominently raises the question whether growth and cell-division are fundamentally rhythmic. We shall here briefly examine this subject from a general and elementary point of view in order to prepare the way for the cytological problems of reproduction to be considered in the four succeeding chapters.

It is obvious that the conditions on which the life-cycle depends are in part internal, in part external to the organism, but the relation between these has not yet become entirely clear.<sup>2</sup> We must also distinguish between the life-cycle of the individual and that of the species as shown in the succession of generations. In higher animals generally the individual life runs its allotted course in a certain sense automatically, in the absence of any corresponding cycle of external conditions. Given the normal conditions, and barring accident, every individual displays a succession of youth, maturity, old age and death as progressive phases of a process that goes forward without pause from the moment that the egg begins its development. In youth the constructive activities are in the ascendent, in age the destructive, while maturity is a period of relative physiological balance. To a certain extent the course of this process can be diverted by experimental modifications of external conditions; but sooner or later the end is inevitable.

In lower organisms many cases are known in which the life-cycle of the *species*, as shown by the alternation or succession of generations, is dependent upon changes of the external environment (for instance, in the

<sup>1</sup> *Studien über die ersten Entwicklungsvorgänge der Eizelle, die Zelltheilung und die Conjugation der Infusorien: Abh. d. Senckenberg. Naturforsch. Ges. X, 3, 4, Frankfurt, 1876, p. 420.*

<sup>2</sup> For an interesting discussion of these relations see Jost, '07, '13.