

*Respiration Experiment.*Table XII.—*Phaseolus*.

Date.	Temperature, C.	CO per gm., dry weight per hour, c.c.	Remarks.
4.xi.19	11°-12°	1.40	Sown 29.x.19. Dry weight, 0.14 gm. Fresh weight, 0.74 gm.
5.xi.19	11°-12°	0.85	
6.xi.19	10°-11°	0.77	

Studies on Synapsis. I.—Oogenesis in the Hymenoptera.

By LANCELOT T. HOGBEN, M.A. (Cantab.), B.Sc. (Lecturer in Zoology,
Imperial College of Science, London).

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[PLATES 4-9.]

For establishing a correlation between genetic and cytological phenomena, the Hymenoptera furnish a series of forms which offer fertile possibilities; the production of males from eggs which segment without fertilisation makes it possible to state definitely the point in the germ cycle at which the determination of sex is effected. While, however, much work has been done from the cytological standpoint in connection with the group, our knowledge is still inadequate with respect to those species which produce females as well as males by parthenogenesis. What little is known of this subject has been derived exclusively from a study of the polar and cleavage mitoses; and in view of the importance of treating the germ cell cycle as a whole, and also of the unfavourable material which heavily-yolked ova necessarily afford, it seemed desirable to investigate oogenesis in typical and completely agamic Hymenoptera, more especially because data relating to the maturation prophase of the female germ cells in insects are much less numerous than in the case of spermatogenesis. The original purpose of the author in pursuing the present investigation arose out of previous work on nuclear phenomena in the late ovarian oocytes of *Neuroterus*, which yielded indications of post synaptic syndesis. Agar has described a second conjugation of chromosomes after a temporary dissociation of univalents in *Lepidosiren*, unconfirmed hitherto in any other animals; and since the

demonstration of such an occurrence in the oogenesis of insects might well provide a basis of reconciliation for the rival hypotheses of parasynapsis and metasynapsis (telosynapsis), the synaptic phase in the oocytes of the Hymenoptera was selected for the first of the present series of studies. In addition to the two-fold problem already stated, there have arisen several subsidiary questions in the course of the investigation, notably the peculiar character of the mitotic figure during ookinesis in certain families, and the significance of certain bodies characteristic of the Hymenopteran egg, namely, the so-called germ cell determinants and the remarkable secondary nuclei.

Wherever possible I have avoided the use of sublimate fixatives employed by previous workers. Bouin gave excellent results; but the best preparations were obtained by the use of Flemming modified to increase its penetrating power by means of a trace of urea.

1. *Cynips Kollari*.

Cynips kollari is the gall wasp which causes the exceedingly common marble gall to develop from leaf buds of the vegetative shoot on the oak. Its special interest for present purposes consists in the fact that unlike the other gall-makers which infest the oak, *Cynips* displays no alternation of sexual and parthenogenetic generations. A sexual brood is lacking in the life cycle; not only is the species exclusively agamic, but it is composed entirely of females. It has been chosen for investigation not with a view to elucidating directly the chromosome cycle of a form which produces females from unfertilised eggs, since the difficulty of inducing it to breed in captivity is too great; but it was hoped that it would assist in interpreting the phenomena which have already been investigated in another agamic Cynipid, *Rhodites*.

The somatic divisions were studied in the young pupæ in epithelia, nerve cells, developing wings and other centres of cell multiplication. The chromosomes in *Cynips* are unbent and filamentous; their number is 20, as in *Neuroterus*. The same number is found in dividing follicle cells, an anaphase of which is shown in Plate 4, fig. 2. I have not observed either asters or centrosomes in connection with the spindles of somatic figures, as illustrated in fig. 1 from the larval gut.

Early History of the Oocyte.

In the full grown larva the ovaries are small spherical bodies surrounded by a capsule of connective tissue and consisting of undifferentiated cells with granular nuclei (fig. 3). Mitoses are extremely rare; it seems that

the ovaries reach this stage of development at an early date in the life of the larva, remaining in this condition until pupation. All three types of cells in the ovariole of the mature ovary of *Cynips*—follicles, nurse cells, and oocytes—originate from germ cells. Certain of the primary oogonia by a series of divisions (probably synchronous) give rise to groups of cells, which correspond to the "rosettes" described in other insects. The rosettes consisting of 16-32 cells, when division has ceased, give rise both to the oocytes and nurse cells which are not distinguishable until the nuclei have been through the initial stages of synapsis.

Differentiation of oocytes and nurse cells takes place in the young pupa before the ovaries are clearly separated into strands; and no synapsis nuclei are found in the terminal portions of the egg tubes except in very young pupæ. The cells of a fully formed rosette at first possess nuclei the chromatin of which is present in masses of probably inconstant numbers (fig. 4).

The changes which ensue preparatory to differentiation are: (1) Leptonema, (2) Synizesis, (3) Break up of the post synaptic spireme.

(1) *The Leptotene stage* is characterised by the appearance of a dense tangle of pale and very much convoluted threads (fig. 5).

(2) *Synizesis* follows immediately upon the Leptotene stage (fig. 6 *a, b, c, d*).

(3) *Post Synaptic spireme*.—The chromatin emerges from the synaptic knot at first as an apparently continuous spireme. This, however, is soon seen to segment into separate filaments (fig. 8). From a careful examination of a large number of nuclei seen immediately after the break up of synizesis, it is almost certain that the haploid number, *i.e.*, about ten threads, make their appearance. Immediately after this, two sorts of nuclei can be distinguished: (*a*) those in which the chromatic elements lose their visible individuality (fig. 9*a*); (*b*) others in which the chromatin threads undergo fragmentation into innumerable minute granules (fig. 7*c*). The former develop into functional oocytes, the latter become nurse cells. A large number of cells were observed which indicate (fig. 7 *a, b*) the possible intercalation of a diplotene stage in the differentiation of the nurse cells; at the time when the latter can be first distinguished from oocytes by their nuclear behaviour, there are no recognisable differences in the size of the cell itself or the appearance of the cytoplasm.

The nucleus of the oocyte remains in the "diffuse" stage during the entire growth period, until the end of pupal life.

The Growth Period. General Characteristics of the Egg.

The young egg during the period of growth is oval; it is not completely surrounded by follicular cells at the extremities where the cytoplasm projects

into the adjacent nurse chamber entering into relation with the nurse cells. In the early growth period, before the deposition of yolk has begun, several spherical bodies of varying sizes staining with chromatin dyes are found lying in the cytoplasm; in one case two such bodies, one at the anterior end and one at the opposite pole were observed (fig. 9b). In other cases their number was more numerous; they appeared to increase in size in proportion as they were remotely situated from the nuclear membrane. Some sections showed minute granules lying within, upon and just outside the nuclear membrane; and the nucleus in such may show several karyosomes, so that possibly these bodies are the products of the oocyte nucleus. As in *Neuroterus* and *Andricus*, it is not possible to detect in the eggs of *Cynips* any region which can be said to correspond with the "germ-cell determinants" of *Copidosoma*. The full-grown egg, unlike the elongated type which is characteristic of the Hymenoptera, is fusiform, tapering to a fine stalk at one extremity (fig. 10).

Nuclear Phenomena in the late Ovarian Oocyte.

About the time when the egg reaches its maximum dimensions, *i.e.*, just before the imago bores its way out of the gall, the chromatin of the nucleus resolves itself into long filaments of which about 20 can be counted (fig. 11). These then become shorter and thicker, preparatory to conjugation in pairs, so that each member of a pair is in contact with its fellow by one extremity only. Since the number of chromosomes is reduced in the early oocytes of the young pupa, *it would appear that the chromosomes pair twice in the forms which are now under consideration.* Agar has described a reunion of univalents after a period of temporary separation preparatory to the formation of the maturation spindle in the spermatogenesis of *Lepidosiren*. Wilson has also stated that in the spermatogenesis of certain Hemiptera the X and Y chromosomes regularly conjugate at the poles of the spindle before the first maturation division is completed. Hegner believes that a pairing of chromosomes end to end immediately before the appearance of the maturation spindle occurs in *Copidosoma*. These bivalent elements, which are distributed at first without any definite arrangement (fig. 12), betray their double nature by a deep constriction in the middle. They soon begin to arrange themselves about a definite axis, and spindle fibres make their appearance, though no asters or centrosomes are visible. The number of doubles present at this stage is very easy to count, 10 being invariably present (fig. 13). They are now seen to array themselves in a parallel series upon the spindle with the point of contact of their univalent constituents in the equatorial plane. What follows corresponds exactly with what has been described in *Copidosoma*.

and *Neuroterus*. The chromosomes of the spindle come more closely into contact till they form a compact complex; the spindle disappears, the chromosome complex becomes more or less spherical, and the clear area occupied before by the nuclear sap is gradually invaded by yolk-laden cytoplasm, so that it is rendered inconspicuous (figs. 14-17). The condensation of spindles in the late ovarian oocyte has already been described in three Chalcid genera, *Ageneaspis* (Martin), *Copidosoma* (Hegner and Silvestri), and *Trichogramma* (Gatenby), in the Cynipids *Andricus* (Hegner) and *Neuroterus* (Hogben), as also in the Braconid *Apanteles* (Hegner).

The precise stage at which the nuclear membrane dissolves in the process here described is difficult to determine, owing to the sharply defined meniscus separating the clear space occupied by the nuclear sap and the surrounding heavily yolked cytoplasm. For this very reason it can be said, however, with some assurance that the spindle fibres originate in the area originally occupied by the resting nucleus. The existence of a maturation spindle in the ovarian oocyte of *Cynips* does not imply a reductive division in the formation of polar bodies. Reduction of the chromosomes in the oocytes of the agamic Spring females of *Neuroterus* occurs, but, while an abortive maturation spindle is found, no polar bodies are extruded in the female-producing ova. Possibly the same holds good for *Cynips*. Or alternatively, if polar bodies are produced, as is more usual in parthenogenetic ova, both divisions may be equational, and a subsequent disjunction of univalents may ensue. Whatever be the behaviour of the nucleus in *Cynips* after the egg is laid, it may be confidently asserted that the early history of the oocyte resembles that of other Hymenoptera parasitica, and the chromosomes, which are seen as the maturation spindle of the ovarian oocyte, are bivalent in character.

Rhodites rosæ.

Rhodites rosæ, which is responsible for the Bedeguar Rose gall on the wild rose, is too familiar to merit introduction. It has already been made the subject of cytological researches by two workers, but, since their conclusions are at variance, and do not seem to show an appreciation of the peculiar history of the nucleus in the later maturation prophase of the Cynipid egg, there is good reason for completing and criticising the accounts hitherto given.

Henking (1892) found that, in the full-grown oocytes, shortly before laying, nine chromosomes (which he concluded to be bivalent), are present. Two polar bodies are formed, and show 9-10 chromosomes, as does also the anaphase of the segmentation nucleus. According to this author, the segmentation nucleus, after a period of rest, during which its staining

capacity greatly decreases, shows 18–20 chromosomes on the equatorial plate of the first segmentation division. Segmentation mitoses showed the same number. Schleip reinvestigated the cytology of the egg in *Rhodites* in 1909. He gives the number of chromosomes in the young oocyte as 10–12, and denies that those of the female pronucleus are doubled before the first cleavage, finding that the segmentation mitoses show 12 chromosomes. He concludes that there is no reduction in *Rhodites*, though polar bodies are formed, *i.e.*, the maturation mitoses are equational. In heavily yolked eggs where the number of chromosomes is small, the segmentation divisions do not afford the most profitable basis for a study of the chromosome cycle, and, unhappily, neither of these investigators has added to his work data respecting the one point which would give most weight to the results obtained, namely, the number of chromosomes in the oogonial mitoses. In the present research, the somatic and oogonial mitoses of the larval and pupal stages have been thoroughly investigated, and the history of the oocyte, from its origin to the time of laying, has been worked out for the first time.

The Somatic Mitoses.

It has already been mentioned that neither Henking nor Schleip commenced their investigations on the chromosome cycle of *Rhodites* by a study of the mitoses in the larval or pupal stages, in which the oogonia are to be found dividing. As pupation is rapidly completed in *Rhodites*, the task of finding nuclei in division during the early stages is not a difficult one. In sections cut from material fixed in the inception of pupal life, mitoses are abundant in the developing wing epithelia, nervous system, and follicle cells. In all cases the number of chromosomes is at least 18; Henking gave 18–20 for the segmentation mitoses, and an element of uncertainty must be conceded in virtue of the extremely minute dimensions of the chromosomes in *Rhodites* as compared, *e.g.*, with *Neuroterus*, or, more particularly, with *Cynips*. The existence of 18 chromosomes in the dividing nuclei of the somatic tissues is not conclusive evidence that the same number occurs in the germ cells; in *Apis*, the somatic mitoses of the male show four times the number of chromosomes displayed by the segmenting spermatogonia, and in *Neuroterus* they display the number 20 (characteristic of the female) in both sexes, while spermatogonial counts give half this number. Nevertheless, the follicle cells in the ovaries present another case. Follicular cells have frequently been described as dividing amitotically in insects; in some cases, as in the paedogenetic Cecidomyid *Miastor*, oocytes alone are truly germinal in origin. In the Hymenoptera the follicle cells divide normally, and it has been well established by the work of Korschelt (1886), Pauleke (1907), and

Marshall (1907), that all three cell elements in the ovarioles of this group—nurse cells, oocytes, and epithelia—arise equally from the differentiation of germ cells. For two reasons, then, the mitotic figures in the follicular cells of *Rhodites* demand special consideration, as indicating the true diploid number of chromosomes: (i) in the allied Cynipid genus *Neuroterus*, according to Doncaster, dividing follicle cells give the same count as dividing oogonia, and the same is seen in my own preparations of *Cynips*, set forth above; (ii) in origin they are germinal. I have figured a particularly clear anaphase of a dividing follicle cell showing 18 chromosomes at one pole, and this avoids any suspicion that both sets of chromosomes have been counted in an equatorial plate; some stress is laid upon this, since Henking's count of the segmentation mitoses is almost twice that of Schleip (Plate 5, fig. 18).

The Synaptic Phase.

In the full-grown larvæ at the end of the first season the ovaries are inconspicuous ovoid masses of cells with granular nuclei. The ovary is surrounded by a capsule of connective tissue, and its component cells are undifferentiated. As in the case of *Cynips*, the fact that mitoses do not normally occur at this stage suggests that the ovaries are formed early and pass temporarily through a period of rest, during which there is no cell division. In the following spring (and sometimes in the autumn of the first season) changes begin to occur. Oogonia begin to divide into rosettes (fig. 20). The cells of a rosette divide synchronously, as in *Apis*.

Some 25 clear anaphase plates at this stage were observed, in which it was possible to count the chromosomes without difficulty, the number being the same—18—as that in the dividing follicle cells (fig. 19). This conclusively vindicates Henking's assertion regarding the somatic number in *Rhodites*.^{*} In early stages of ovaries at the inception of differentiation it is possible to distinguish three regions: (a) zone of rosettes; (b) zone of undifferentiated oogonia; (c) zone of flattened apparently vacuolated cells, which correspond to those which are found in the terminal filaments of the ovarioles of young pupæ. With the exception of the last, all cells rapidly

^{*} I find that several writers who have accepted Schleip's observations, including Miss Harvey and Cutler, states that he gives 10–12 as the number of chromosomes in the dividing oogonia. It is true that he says, in describing the segmentation divisions, "der normale Chromosomenzahl bei *Rhodites rose* ist also etwas 12; die gleiche Zahl müssen wir auch in den Oogonien annehmen"; but nowhere does he refer to preparations or give illustrations of oogonial mitoses—on the contrary he expressly asserts (erroneously), "die Zahl der Chromosomen in den Ureizellen ist nicht zu bestimmen."

become divided to form rosettes, and it is in the fully formed rosettes of the larva just before pupation that the synaptic stages are to be traced.

The stages of synapsis in *Rhodites* are here described for the first time, and I am able to supply fuller details than in the case of *Cynips*. As in the latter, the preleptotene nuclei display irregular masses of chromatin. Nurse cells cannot be distinguished from functional oocytes until after synizesis, and the nuclear history of the former may be dealt with first:—

1. *Leptonema*.—The chromatin of the nucleus forms a tangle of pale convoluted threads, which are too intricately crossed to permit the possibility of counting them (figs. 21, 22).

2. *Synizesis*.—The leptotene threads increase in staining capacity and contract away from the nuclear membrane in a compact knot (figs. 23–25).

3. *Post Synaptic spireme*.—As synizesis dissolves, a spireme emerges, which breaks up into pachytene filaments (figs. 26, 27), of which in over 20 counted about 9 are clearly present.

4. *Diplotene*.—Immediately after the foregoing, the pachytene threads are seen to be split lengthwise; these double threads shorten up into rectangular masses of ragged outline, which are still evidently double. These masses break up into minute granules which are dispersed throughout the nucleoplasm of the fully-formed nucleus of the nurse cell (figs. 28–30).

The foregoing account shows striking similarity to Dederer's recent work on the moth *Philosamia*, in which the history of the nurse cells is almost identical.

The young oocyte nucleus, after passing through the stages of leptonema, synizesis, and pachynema, begins to show a greatly diminished staining capacity; it becomes difficult to trace separate threads, though it seems likely from some preparations that the diploid number is present. A striking fact is that the diminution of the staining capacity of the nucleus coincides with the appearance of nuclear granules on the nuclear membrane within the nucleus and in the surrounding cytoplasm, thus confirming the opinion previously expressed that the chromatin-staining granules in the young egg of *Cynips* are of nuclear origin (fig. 30). During the greater part of the growth period the nucleus remains in the "diffuse" stage, *i.e.*, the visible identity of the chromatin elements can no longer be traced with certainty; but in the very late pupal ovary nuclei can be found in which long thin filamentous chromosomes are present, staining deeply; their number is about 18.

The oocyte is oval during the growth period. The cytoplasm at first projects into the adjacent nurse chamber; but by the time the imagines emerge the egg has reached the full size, the follicle is complete and reduced

to a thin lamella, while the nurse cells are no longer seen. The egg then is elongated, slightly pointed at each end, and tapering off into a stalk at the anterior extremity (fig. 31). At the posterior pole, in preparations that have been decolorised so as to remove the stain from the yolk, there is seen to be a region of deeply staining granules. The presence of this body was originally noticed by Weismann (1882), who applied to it the term "Furchungskern"; needless to say, it is not the cleavage nucleus, nor is there any reason to suppose that it is of nuclear origin. Tanquary, curiously, applied the same term to a similar collection of granules in the egg of the ant *Camponotus*. Hegner, who has not examined the egg of *Rhodites* himself, has suggested that these granules are of the nature of what he calls "germ-cell determinants." Their later history has not yet been followed, nor has that of the more definitive body described in the Cynipid *Diastrophus*; and in view of the conflicting accounts that have been given by Martin, Silvestri, Hegner, and others as regards both origin and fate of the "germ-cell determinants" in Hymenoptera, it might be advisable for the present to refrain from using this term until a more precise acquaintance with their genetic and developmental significance is forthcoming.

Nuclear Phenomena in the Late Ovarian Oocyte.

About the time that the gallfly emerges, the oocyte nucleus is large and conspicuous, occupying a position about midway from either pole. It has been said that, towards the end of the growth period, the chromosomes regain their visible identities, and appear in the diploid number (fig. 32). What follows has been described by Schleip, thus: "Zuerst sind sie Fädchen mit den bekannten Knotchen-förmigen Verdickungen und einer allerdings nicht sehr deutlichen Längspalte. Der relativ kleine Nucleolus bleibt erhalten bis etwas zu der Zeit wo die Kernmembran sich auflöst. Dann werden die Chromosomen dicker und erscheinen im Präparat schliesslich als unregelmässig geformte Klümpchen. Einige von ihnen sind deutlich ringförmig."

There are two points in this short account which compel comment. First, the chromosomes are not at this stage irregular. Originally in the form of filaments which are clearly double, like those of *Cynips* or *Copidosoma*, they shorten up into dumb-bell shaped bodies, that still preserve their bivalent character (figs. 33, 34). The only conclusion that can be drawn from Schleip's figures is either that they are very diagrammatic, or that they illustrate preparations imperfectly fixed. In the second place, none of the chromosomes are ring-shaped; at this

stage *ring-shaped* plasmosomes are frequently seen as in *Synergus* ;* and, as earlier described, the plasmosome of the preceding stage is seen to be vacuolated in preparation for its dissolution into ring-like bodies. This probably accounts for the fact that Schleip is able to count from 10 to 12 chromosomes (doubles), whereas I have never counted more than nine, thus agreeing with Henking, who also regards these chromosomes as bivalents. Those who have studied the late maturation prophases of the Cynipidæ would anticipate from these phenomena the subsequent formation of an abortive spindle such as has been demonstrated in *Andricus*, *Cynips*, and *Neuroterus*. Neither Henking nor Schleip have followed up the succeeding stages in detail. According to the former, who gives a much more thorough survey of the data, "lange bleiben die Chromosomen jedoch nicht von einander getrennt. In den etwas älteren Eiern bemerkt man nämlich dass deren mehrere zusammen zu treten beginnen, entweder zu Klumpen, oder auch Ketten. Je mehr das Ei reift, um so mehr schreitet dieser Process fort, so das schliesslich alle Chromosomen zu einem gemeinsamen Ballen vereinigt sind." Later he observes "der farblose Kernraum an Volumen eingebüsst hat." Henking does not mention the presence of spindle fibres, but, in reality, a very clear spindle is (figs. 35-37) formed, without either aster or centriole, and upon this spindle the bivalent chromosomes arrange themselves in a parallel series in the equatorial plane.

The mitotic figure now passes through the stages of condensation; the chromosome complex (figs. 37, 38), at first elongated with respect to the axis of the spindle, becomes spherical; the spindle itself disappears, and the so-called nucleus becomes lost in the surrounding yolk. One further point that may be mentioned is that the karyosome disintegrates into a number of granules that surround it.

The discovery of a precociously formed and interrupted maturation spindle in late ovarian oocytes of *Rhodites* is of some cytological interest, as throwing light on the character of the peculiar mitotic phenomena of oögenesis in certain Hymenoptera, and confirming the conclusions I have elsewhere expressed in regard to the figures originally described by Hegner, based on previous work on *Neuroterus*; for, according to Henking's account the preparation of the polar nuclei is very much like the corresponding process described by Doncaster in the eggs of *Neuroterus* and of a type differing widely from the ordinary form of mitosis. An atypical formation of polar bodies in *Trichogramma* and *Agéniaspis*, which also show abortive spindles in the ovarian egg, has been referred to earlier. Gatenby describes

* *Vide infra.*

the process loosely as "amitotic." Martin says that the mass formed by the condensation of the maturation spindle "divides in polar body formation without the presence of spindle fibres or asters." It may be confidently predicted that the formation of polar bodies by a similarly atypical procedure will be demonstrated by subsequent research in other forms, where the history of the egg nucleus shortly before deposition is comparable.

I have not investigated the maturation of the egg in *Rhodites*. The crucial difference between the rival conclusions of the two authors referred to lies in their statements regarding the segmentation mitoses, which, according to Henking display 18-20, according to Schleip 10-12 chromosomes. Concerning the latter view, I would here submit (1) that Schleip failed to appreciate the double nature of the chromosomes which he counted (together with karyosomes) in the oocyte nucleus; (2) that a study of the nuclear divisions in the segmenting egg does not provide such a satisfactory basis for counting the chromosomes, when small and numerous, as do the oogonia and somatic tissues of the pupa; (3) it is easier to underestimate the number of chromosomes than to count more than are actually present; (4) were the chromosome cycle in *Rhodites* such as set forth by Schleip, it would be remarkably unlike that of other Cynipids, notably, *Cynips kollari*. Furthermore, the last-named investigator seems to me to have approached the subject in an attitude suggestive rather of propaganda than of scientific impartiality, and appears over-anxious to show that Henking's conclusions are mistaken, because of their similarity to those of Petrunkevitch with respect to the drone bee. He criticises the alleged doubling (*i.e.*, disjunction) of chromosomes during interkinesis, on the ground that it does not fall in with accepted views regarding the persistent individuality of the chromosomes; but the facts of synapsis alone vitiate such an objection; and the fact that he finds only six chromosomes in the blastoderm stage confirms my belief that his preparations were not satisfactory. The events of the chromosome cycle in *Rhodites*, then, may be summarised as follows: (1) the somatic and oogonial mitoses show 18 chromosomes; (2) parasynapsis probably occurs in the young oocyte;* (3) at the conclusion of the growth period the diploid number reappears; (4) these filaments then conjugate, so that the full-grown oocytes show nine chromosomes, the origin of which is betrayed by their double character; (5) the haploid number of bivalents is arranged on an asterless spindle, which passes through the stages of condensation; (6) the segmentation nucleus, according to Henking, contains

* It is difficult to interpret the restoration of the diploid number by the longitudinal splitting of the pachytene threads without assuming that the latter arise by the side-by-side union of leptotene threads.

nine chromosomes, which number is doubled prior to the first segmentation division.

Significance of the Polar Divisions.

The character of the chromosome cycle in the production of drones in the Hymenoptera may now be regarded as settled. In those completely parthogenetic species which produce thelytokous ova without impregnation our knowledge is scanty, but it seems clear that the same uniformity does not prevail; the only really satisfactory account is that of Doncaster who investigated the agamic generation of *Neuroterus*, in which form there are no polar bodies extruded by those eggs which develop into females. This is not, however, typical of the Hymenoptera where in several cases agamic female-producing eggs are known to form two polar bodies, as in the case of *Rhodites*, *Pecilosoma* and *Cræsus*. These forms also contrast with the cytological behaviour of female-producing eggs among parthenogenetic organisms belonging to other groups, where as a rule females are produced from eggs which extrude only one polar body by an equational division.

The question now arises; what relation have the chromosomes that separate during the interkinesis preceding segmentation to one another? There seem to be two possible ways of interpreting Henking's "Verdoppelung": (1) the polar mitoses are both equational, and the doubling of the chromosomes in the female pronucleus is a disjunction of bivalents; or (2) the chromosomes which pair in synapsis are equivalent daughter halves of a single chromosome which has divided before segmentation begins.* Apart from the question of sex determination, either hypothesis is equally acceptable, an objection, however, to the first being the extraordinary similarity of the separation of polar nuclei in female-producing eggs of *Rhodites* and male-producing eggs of *Neuroterus*, which are probably reduced qualitatively and quantitatively. The second hypothesis in the case of *Rhodites* offers no explanation of the occasional appearance of males, and does not fall into line with conclusions regarding sex-determination based upon the study of other Hymenoptera. Nachtsheim, to account for the difference of sex in fertilised and unfertilised eggs of the bee, has put forward the theory that, of the 32 chromosomes in the oogonial mitoses of *Apis*, 30 are somatic and two are X-chromosomes; at maturation reduction occurs, so that the female pronucleus should have the constitution 15-X, thus a virgin egg would have a single sex chromosome, while a fertilised egg will have two. The sexual differentiation in the chromosome cycle of *Apis* is by this means made to agree with the case of those insects in which maleness is associated with a

* Nachtsheim's work on the segmentation of the drone eggs of the bee renders such a hypothesis not improbable *a priori*.

single X-chromosome and femaleness with a pair; as, however, there are no visible cytological peculiarities in these hypothetical X-chromosomes, and since moreover the question of sex-limited inheritance in the Hymenoptera is a subject which has not hitherto been investigated to my knowledge, it is only possible to accept Nachtsheim's suggestion with some reservation.

3. *Synergus Rheinhardii*.

In rearing *Cynips* a large number of individuals of *Synergus rheinhardii* and the smaller allied species *S. melanopus* emerged from the galls in which they pass their larval and pupal phases as inquilines. A study of the eggs of the inquiline *Synergus rheinhardii* revealed certain points and led to conclusions which seem to merit record.

The Oosoma.

In several respects the egg of *Synergus* resembles closely that of *Trichogramma* and *Copidosoma*. Like these it possesses when it has attained its full size a well defined body at the posterior pole staining deeply with chromatin dyes. This corresponds to what Silvestri originally called the "nucleolo," and Hegner the "Keimbahn" or "germ-cell determinants." Both of these authors originally believed that it arose from nuclear material. In view of its subsequent distribution in certain forms among the germ cells this view is a tempting one to those who advocate the doctrines of Weismann in their extreme form; but it has been shown by Gatenby in *Trichogramma*, Martin in *Ageniaspis*, and since reported by Hegner and Silvestri in *Copidosoma* that it is derived from the cytoplasm. In *Synergus* as in *Trichogramma*, the "germ-cell determinant" arises "as a cloud of granules which make their appearance spontaneously in the cytoplasm . . . become more and more heavily staining and denser until the determinant resembles a spherical ball at the end of the egg" (figs. 39-41). Further, in *Synergus* as in *Trichogramma*, the reaction of this body with respect to Gilson, Petrunkevitch, Flemming, and other acetic fixatives employed shows that it is neither wholly nor partially of mitochondrial matter as Martin believed. The fact that the "germ-cell determinant" is a cytoplasmic inclusion provides confirmation of the view of organ-forming areas in the cytoplasm of the egg. Gatenby has suggested that the function of the determinant is nutritive, preventing the germ-cell nuclei from being exposed to the uncertain conditions existing elsewhere in the segmenting egg and embryo. This might be correlated with its more frequent occurrence in parasitic forms. Such bodies do not occur in many gallmaking Cynipids, egg *Neuroterus*, *Cynips*, and *Andricus*: there is, however, a well defined structure at the posterior

pole of the egg in the blackberry knot gall-fly, *Diastrophus*. Since the fate of the "germ-cell determinant" has only been worked out in a few cases, it would appear advisable to adopt for the present Silvestri's newly proposed term "oosoma," or if the rule as to precedence is rigidly enforced, alternatively, let it be still called the "polar disc." This would obviate the drawback of being committed to any particular theory as to its physiological or genetic significance in the developmental process.

The Secondary Nuclei.

Gatenby (1918) has noticed in the egg of *Trichogramma* the presence of "large spherical granules" staining deeply with iron hæmatoxylin. They were also found in *Cynips* and smaller and more numerous bodies of similar origin in *Rhodites*, where they have been shown to be of nuclear origin, their appearance being correlated with the diminution of staining capacity exhibited by the nucleus during the growth period. Such granules are found at all stages after synapsis in *Synergus*. In the full grown egg little groups of granules are sometimes recognised in the peripheral region; these appear to be the result of fragmentation of the larger nucleoli. During the growth period the nucleus is seen to contain numerous granules of various sizes.

In addition, true secondary nuclei make their appearance in the peripheral cytoplasm towards the end of the growth period. Such nuclei are found in the eggs of aculeate Hymenoptera, but do not occur generally in other groups; they have, however, been described by Hegner (1914) in the oocytes of the Braconid, *Apanteles*. Those in *Synergus* agree remarkably closely with the description given for the latter genus. Each has a definite membrane and contains a substance staining like chromatin in the form of several small masses and a few strands of granules. That they arise *de novo*, that is to say, they do not originate by the division of pre-existing nuclei, is agreed by Hegner as regards *Apanteles* and shown in *Synergus* by: (1) the absolutely constant form of the germinal vesicle at the time when they appear precludes any possibility that they arise by budding from it; (2) the complete isolation of the oocyte by its follicle at the time when secondary nuclei are first seen excludes any possibility that they are derived from nuclei other than those of the follicle cells; (3) careful examination of the follicle cells and their nuclei at this stage makes it extremely improbable that there is any migration of nuclei from the epithelium into the peripheral cytoplasm of the egg. Beyond this, Hegner makes no suggestions as to their presence; but it seems to me clear that in *Synergus* they arise from the groups of granules in the periphery that have been referred to above. The

full story of their genesis would then be as follows: as the oocyte nucleus enters upon the diffuse stage its diminution of staining capacity is seen to synchronise with the ejection of chromatin-staining particles in the surrounding cytoplasm; these particles increase in volume and subsequently undergo fragmentation, becoming limited by an enclosing membrane, and so simulating the appearance of true nuclei. Hegner states that secondary nuclei in *Apanteles* are only found for a brief period in the egg: this is probably the case in *Synergus* also.

4. *Orthopelma luteolator*.

The later maturation prophases, as stated in the introduction to the present communication, have been described briefly by Hegner, Silvestri, Martin, and Gatenby in certain Chalcididae and in the Braconid, *Apanteles*, I have examined various species of both families, including *Torymus*, *Microgaster* and *Hecabolus*; but while confirming in general their conclusions, have insufficient new matter to merit record. To emphasise, however, the general occurrence of the phenomena that have been described in *Cynips* and *Rhodites* among the parasitic Hymenoptera, it will suffice to give a brief survey of the history of the oocyte in a representative of a family upon which no cytological work has been performed up to date. *Orthopelma luteolator* is one of the Cryptine Ichneumonidae, which parasitises the galls of *Rhodites* probably as a parasite on the gallmaker itself. A very large quantity of this species was bred in the course of my experiments with *Rhodites* itself; and I am indebted to Mr. Turner, of the British Museum, for assistance in the identification of this species.

In the larva, just before pupation begins, the ovaries consist of rosettes, the cells of which are undergoing segmentation by synchronous divisions. This is shown by the fact that mitoses are rare; but where they occur they are always seen in the same group of cells altogether (fig. 42). The number of chromosomes in dividing oogonia appears to be about 22. The eggs do not all develop at the same time, as in the Cynipidae, this insect having a longer life in the adult condition. In imagines that have just emerged, synapsis can still be studied in the terminal portion of the egg-tubes, which are of the usual monothalamous type. Unfortunately, however, the nuclei are minute, the whole egg in *Orthopelma* being in size about a fifth that of *Rhodites*. No differential divisions either of the nucleus or cytoplasm occur in the rosettes, the cells of which are equipotential. There is no visible distinction between nurse cells and oocyte until after synzesis (figs. 43, 44). The ovaries of the adult contain oocytes in all stages of growth situated at the posterior end. Nuclei in synzesis may be found in the ovaries of the

pupa, or of the early adults just after they make their way out of the gall. After the dissolution of the synaptic knot the differentiation of nurse cells and oocytes ensues immediately. The nurse cells in the region of differentiation possess nuclei with large plasmosomes and innumerable chromatic granules; but fragmentation is preceded by a stage in which the chromatin of the nuclei is distributed in the form of lumps of somewhat ragged outline (fig. 45). A large number of such nuclei were studied, and it seems that the number is constant and equal to half the number of chromosomes in the oogonia (10-12). I have been unable to satisfy myself that these chromatin masses show a division into two parts; but some sections suggest that this is the case, as in the young nurse cells of *Philosamia*.

The nucleus of the very young oocyte at the same stage shows what appears to be the haploid number of somewhat convoluted threads. At first I was of the opinion that there is no "diffuse" stage in the oocyte nucleus of *Orthopelma*; and Hegner omits any mention of one in the case of the Chalcid *Copidosoma*, or the more closely allied form *Apanteles*. This is comprehensible on account of the fact that owing to the rapid development of the oocyte, it is impossible to obtain a complete sequence of stages without the examination of a large number of preparations; and it is easy to attribute the disappearance of individually recognisable chromosomes to defective preservation. After comparing preparations of the ovaries from some fifty females, I am convinced that such a stage does occur, and that it is followed by the reappearance of the diploid number as in *Copidosoma* followed by an end-to-end pairing of univalent elements (figs. 47 and 48). I am convinced equally that if the case of *Copidosoma* or *Apanteles* were reinvestigated from this point of view a "diffuse" stage would be found to follow synapsis in these genera likewise. An abortive maturation spindle is formed by the arrangement of these pairs (eleven in number) on an asterless spindle (fig. 49). The bivalent chromosomes of the spindle are short stout rods as in *Rhodites*; in none of the forms examined either by Gatenby, Martin, or myself have the chromosomes been found to exhibit the filamentous form figured by Hegner, whose illustrations are evidently somewhat diagrammatic. As in all these cases the chromosomes condense, and the spindle fibres eventually disappear as the nucleoplasmic zone surrounding the chromatin complex is invaded by the yolk. In other forms I have examined the condensation figure is of homogeneous consistency; but in *Orthopelma* as in *Andricus* (Hegner) it exhibited a vacuolated structure (fig. 51).

Chromatin-staining granules occur in the young oocyte, but no secondary nuclei. In the full-grown egg there is a triangular area of minute granules

at the posterior pole which condense as in *Apanteles* to form a polar disc or oosoma of crescentic shape (fig. 52).

The foregoing account of the oogenesis of an Ichneumonid confirms the view that a remarkable similarity exists in the behaviour of the nucleus of the oocyte throughout the large families of the parasitic Hymenoptera (Chalcididae, Braconidae, Cynipidae, and Ichneumonidae). There can be little doubt that the phenomena described are general within the group and contrast strongly with the oogenesis of other Insecta and the remaining families of Hymenoptera. The most noteworthy features on which attention has been focussed are the peculiar interruption of the first polar mitosis and the existence of a second syndesis immediately before the formation of the maturation spindle.

5. *Lasius Flava*.

In order to contrast the peculiar phenomena which have been described in the oogenesis of the parasitic families of Hymenoptera, it is proposed to complete this account with a brief survey of the history of the egg nucleus in the yellow ant, *Lasius flava*, as exemplifying the Aculeata.

Though the maturation of the laid egg and the spermatogenesis of various Aculeata have been somewhat extensively studied, the synaptic phase has been overlooked by most authors, or at least treated very imperfectly. Paulcke (1900) made a study of nuclear differentiation in the ovarioles of *Apis*, and came to the conclusion that all the cell elements are germinal in origin, finding no visible distinction between oocytes and nurse cells until the zone of synapsis is passed. Nachtsheim's figures are similar.

Towards the end of pupal life in *Lasius* all stages of oogenesis can be found in the ovarioles, except the full-grown egg. The terminal filaments consist of flattened cells with granular nuclei, arranged in horizontal rows and extending across the entire width of the tube. At the point of origin of the "terminal chamber" mitoses are frequent. In a small number of very clear figures, 24 minute chromosomes were counted. The nuclei of the terminal chamber, as in *Campanotus*, are not limited by cell walls, but form a syncytium. In the chamber are found successively (a) rosettes; (b) a zone of synapsis; (c) a zone of differentiation, where nurse cells and oocytes become recognisably distinct. At first the nurse cells and oocytes are indiscriminately scattered. In the lower part of the ovariole they are arranged in the characteristic manner, the oocytes forming an axial series alternating with nurse chambers and surrounded by a definite follicular epithelium (fig. 57).

The large number and small size of the chromosomes in the Hymenoptera generally render the group most unfavourable for a crucial elucidation of the synaptic phase, but in studying the ovarioles of *Lasius*, the more satisfactory

seriation of stages permitted a better perspective with respect to those changes which occur immediately before the formation of the synaptic knot than forms previously discussed.

The preleptotene nuclei of *Lasius* exhibit a large plasmosome and the characteristic clumps of chromatin present in insect gametocytes at this stage, and it is possible to trace their transition into the paler and convoluted tangle of leptotene filaments in the following stage (fig. 53). In *Lasius* the leptotene threads definitely arrange themselves in such a manner as to converge towards one pole of the nucleus before passing into synizesis (fig. 53); further, it can be definitely stated that in some "bouquet" nuclei there are a large number of pale threads, and in others a much smaller number of thicker and more deeply staining filaments (fig. 53), indicating a possible condition of pachynema.

Synizesis is illustrated in fig. 54. Till this stage no distinction can be recognised in the cells of the terminal chamber. The haploid number of filaments appear after the break up of synizesis. Differentiation follows with remarkable alacrity. Already among the cells showing nuclei with separate pachytene filaments (after synizesis) two types can be distinguished. In the future oocytes the surrounding cytoplasm is already more bulky and granular. In the case of oocytes a diplotene stage is intercalated (fig. 56). What follows in each case is in the main similar to the course of events in the forms treated earlier in this paper. The chromatin of the nurse cell nuclei undergoes fragmentation into minute granules: at a very early stage the large and characteristic nucleolus can be seen. The oocyte nucleus shows no plasmosome; the filaments suffer a great diminution of staining capacity and lose their visible identity. There is contemporaneously a reduction of the chromatin-content of the nucleus. In the early stages of growth the nucleoli of the nurse cells constantly undergo fragmentation and eject particles, which can be seen to pass into the protoplasmic strand connecting the young oocyte with the nurse chamber.

The Late Ovarian Oocyte.

The nucleus remains in this condition until about the time the egg is laid, when various authors have described a typical spindle. None of the peculiar phases that are intercalated in the late ovarian oocyte of parasitic species like *Cynips* or *Copidosoma* occur. Since in both the ant and the bee the first polar spindle is already formed when the egg is laid the late maturation prophases must be studied immediately before the laying of the egg. With this end in view I have examined the oldest eggs in young queens of *Lasius* without success and preparations from *Formica rufa* of the ovarioles of queens

taken after the marriage flight from nests in which eggs were being laid, yielded equally fruitless results. There is, however, little reason to doubt that they are of the kind characteristic of insect oogenesis.

6. *Secondary Nuclei of Formica rufa.*

Secondary nuclei were first described in the oocyte of the Formicidæ by Blochmann who also described the presence of symbiotic bacteria in the eggs of some species. To the former Blochmann applied the term "Nebenkern." But as various cell structures, including the spindle apparatus, mitosome and the Golgi rods, have been by different authors described by the same word, it is entirely misleading to designate them thus. Their presence has been confirmed by several authors both in the ants, wasps and bees; but their origin and fate has proved fruitful of much controversy. Marshall and Blochmann both believed that they arise by budding from the germinal vesicle, but no actual stages in the budding were observed, and in view of the extreme improbability of amitotic division in germ cells, it is gratuitous to base such a conclusion on the such slender data as their first appearance in the neighbourhood of the oocyte nucleus and their irregular shape. Henneguy and Brunelli believe that they are derived from the follicular epithelium. Korschelt and Gross conclude the same in the case of *Bombus*, and Will (1884) finds that such bodies when they occur in the Hemiptera have a similar history.

The most thorough studies on the subject are those of Loyez who describes "pseudo-noyaux" in four species of *Bombus*, two of *Vespa* and in *Xylocopa*, and concludes that they originate from granules ejected from the nucleus of the oocyte, nurse cells and oocyte follicles, transformed into nucleiform bodies by the cytoplasm of the egg. This is what I believe to occur in *Synergus*, loss of nuclear matter in resting nuclei and its ejection as "chromidia" into the surrounding cytoplasm occurs in many insects, and clearly in the growth period form the germinal vesicle of *Formica rufa*.

Such secondary nuclei have been described in two species of *Campanotus* and in *Formica fusca* in the case of the ants. In the former they appear very early—before the egg is separately surrounded by a follicle, and as Hegner points out this is a potent objection to the theory that they arise by the migration of complete nuclei from the follicular epithelium. From my own preparations I cannot say that they appear at such an early date in *Formica rufa*, but the enormous number that are found in the full grown egg would inevitably be recognisable from the presence of follicular nuclei in the process of division were this hypothesis acceptable. Actually, however, follicular mitoses are not more numerous than would be expected, and no

evidence of indirect nuclear division or migration was observed. In the earliest stages on the contrary the secondary nuclei were always seen in the immediate neighbourhood of the oocyte nucleus. Further it may be added that no intermediate conditions were seen between the nuclei of the follicular cells and of the oocyte which are manifestly different. The nuclei of the follicles are of the characteristic granular type (fig. 60*a*), while those of the secondary nuclei (fig. 60*b*) were distinguished by chromatin-like masses often radially disposed and sometimes clumped together in a single mass (fig. 60*b*).

Regarding the subsequent fate of these structures there is insufficient ground for a final judgment in my own studies on *Formica rufa*, but on the whole I can confirm Loyez' view. The secondary nuclei in *Apanteles*, *Synergus* and *Rhodites ignota* appear to be temporary bodies which disappear without leaving any trace of their identity. According to Loyez those of *Bombus*, *Vespa* and *Xylocopa* are ultimately transformed into deutoplasmic spheres. Marshall believes that they assist the cytoplasm in the anabolic processes involved in the assimilation of material from the yolk discs. Hegner ('09), who has availed himself of every suggestion in favour of the nuclear origin of the "germ-cell determinants," has expressed the belief that they may migrate to the posterior pole, and take part in the formation of the latter; but no actual evidence either that they do migrate or have any connection with the "germ-cell determinants" is submitted. I do not believe that there is a "polar disc" or oosoma in *Formica rufa*, nor have I found any evidence of the migration of secondary nuclei to the posterior pole of the egg, so that the last hypothesis may be rejected. That of Marshall is suggestive and not improbable; but it seems to exceed the legitimate limits of inference on the data available. Loyez claims that all stages between the typical secondary nuclei described above and older nuclei simulating the condition of synizesis and homogeneous globules can be observed.

In favour of this view, *Formica rufa* supplies the following data: (i) towards the end of the growth period and immediately before the first appearance of yolk spheres, the secondary nuclei cease to form a compact group round the germinal vesicle and are distributed throughout the egg, particularly in the periphery; (ii) yolk deposition proceeds from the periphery inwards; and the yolk discs are large bodies of dimensions comparable to those of the "pseudo-noyaux; (iii) in my own preparations, the later secondary nuclei are seen in a condition simulating synizesis. On the whole, therefore, there is some degree of probability in favour of the view that the secondary nuclei of the aculeate Hymenoptera are destined to become transformed into deutoplasmic spheres; that they are connected in any way with the "polar disc" seems both improbable and unacceptable.

Synopsis.

(1) The chromosome cycle has been investigated in relation to the production of females from virgin eggs in the Hymenoptera; the process of synapsis and the maturation of prophases of certain parasitic Hymenoptera have been studied and contrasted with similar stages in the Aculeata; the origin and fate of secondary nuclei has also been considered.

(2) With respect to three genera of parasitic Hymenoptera, the origin, differentiation, and nuclear history of the oocytes have been investigated, viz., *Cynips* and *Rhodites* (Cynipidæ) and *Orthopelma* (Ichneumonidæ). The results confirm the existence of a somewhat unique type of oogenesis, as suggested by available particulars relating to Braconid, Chalcid, and Cynipid genera described by Hegner and other authors. The more salient points are:—

(a) *Origin of the Oocytes*.—All three types of cells (oocytes, follicle, and nurse cells) in the ovary originate from germ cells. The divisions of the oogonia are apparently equipotential, and no distinction exists between nurse cells and oocytes until after synapsis.

(b) *Synapsis*.—After synizesis the haploid number of pachytene threads appear, and in *Rhodites* split lengthwise.

(c) *The "Diffuse" Stage*.—During the growth period there is a diffuse or confused stage.

(d) *The Double Conjugation of the Chromosomes*.—At the termination of the growth period the diploid number of chromosomes reappears, and they pair end to end, as already described by Hegner in the Chalcid *Copidosoma*, so that, as in *Lepidosiren*, a temporary separation of the diplotene threads is intercalated between synapsis and the first polar mitosis. *This is the first confirmation of Agar's account of a double syndesis yet published. The restoration of the diploid number by the lengthwise splitting of the haploid pachytene threads permits the inference that the first conjugation is of the type described as "parasyndesis."*

(e) *The Abortive Maturation Spindle*.—Immediately after this the maturation spindle appears precociously in the late ovarian oocyte; it shows no asters or centrosomes. The chromosomes on the equatorial plate condense to form a solid "chromatin nucleus" without proceeding to the poles of the spindle, which eventually disappear. Such behaviour is without a parallel in the oögenesis of other forms previously investigated. In all genera where such abortive spindles have been shown to occur, the separation of the first group of polar chromosomes is atypical. There can be little doubt that we have here an interrupted mitotic process.

(3) *Agamic Female Production*.—With regard to the production of females from parthenogenetic eggs, the case of *Rhodites* and *Cynips* has been considered, and Henking's account of the former has been vindicated. *It appears that there is a reduction of the number of chromosomes in the young oocyte of both these forms, and it may be assumed that Henking rightly described the double-equational formation of polar bodies with subsequent disjunction of univalent halves in the female pronucleus of the former.* This is not in conflict with the case of *Neuroterus*, where, although no polar bodies are formed in the female-producing ova of the agamic generation, all the oocytes appear to show the reduced number of chromosomes at the corresponding stage.

(4) A study of the early oogenesis of *Lasius* demonstrated that synizesis is followed by the appearance of the haploid number of chromatin threads, and the differentiation of nurse cells from functional oocytes; and the oocyte nucleus remains in the "confused" stage till immediately before it is laid.

(5) *The Secondary Nuclei*.—Secondary nuclei have been described in *Synergus* (Cynipid) and *Formica rufa*, and their genesis has been examined; my conclusions with regard to their origin and fate are, that in both the case of *Synergus* and *Formica*, they arise from nuclear particles, the ejection of which synchronises with a diminution of staining capacity in the germinal vesicle. These migrate to the cytoplasm, fragment, and acquire an enclosing membrane; they are transitory structures. Similar conclusions have been recorded by Hegner in regard to *Apanteles* and *Rhodites ignota*.

(6) *The Oosoma*.—The Oosoma, or so-called germ-cell determinant, is not of nuclear origin, as believed formerly by Hegner and Silvestri, but arises, as Gatenby affirms, from cytoplasmic granules which are not mitochondrial.

The present research was commenced at Birkbeck College. The greater part has been conducted at the Imperial College. I must express my thanks to Prof. MacBride, who has kindly read through the MS., and assisted me with helpful criticism. To Prof. Doncaster, of Liverpool—whose kindness in sending material of *Neuroterus* for a previous paper first directed my attention to the cytological interest of the Hymenoptera—I owe special gratitude, for continuous help with regard to bibliography, and suggestive advice throughout.

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EXPLANATION OF PLATES.

Illustrations were outlined with camera lucida and studied with Leitz 2 mm. and No. 12 apochrom. Single cells of individual plates are drawn at the same magnification. Figs. 9*b*, 10, 30, 31, 39, 40, 41, 46, 52, are not drawn at the same magnification.

PLATE 4.—*Cynips Kollari*.

Fig. 1, *a*, *b*, *c*.—*Cynips Kollari* side views of mitoses in somatic tissues.

d, *e*.—Anaphase plates from gut of larva: 20 chromosomes visible.

Fig. 2.—Follicular mitoses with 20 chromosomes.

Fig. 3.—Cell from ovary of larva during resting period.

Fig. 4.—Rosette cell, showing irregular clumps of chromatin in the nucleus.

Fig. 5.—Leptonema. Dense tangle of pale threads in the nucleus.

Fig. 6, *a*, *b*, *c*.—Stages in synizesis.

Fig. 7, *b*, *c*.—Young nurse cells.

Fig. 8.—Young oocyte showing pachytene threads haploid number.

PLATE 5.—*Cynips Kollari* (continued).

Fig. 9, *a*.—Young oocyte, "diffuse stage." Chromosomes with reduced staining capacity, identity no longer traceable.

b.—Somewhat later oocyte less magnified, showing follicle nurse cells and protoplasmic strand of egg protruding into the nurse chamber.

Fig. 10.—Diagrammatic representation of the full-grown egg.

Fig. 11.—Nucleus of oocyte at end of the growth period. Diploid number of filaments visible.

Fig. 12.—End to end pairing of the same.

Figs. 13, 14.—Maturation spindle of the late ovarian oocyte with 10 doubles.

Figs. 15–17.—Stages in condensation of the abortive maturation spindle: disappearance of spindle fibres and reduction of the nucleoplasmic zone.

PLATE 6.—*Rhodites Rosæ*.

Fig. 18.—Follicular mitoses.

Fig. 19.—Oogonial mitoses showing 18 chromosomes.

Fig. 20.—Rosette from ovary of late larva with chromatin of nucleus in form of irregular masses.

Figs. 21, 22.—Leptotene nuclei.

Figs. 23–25.—Synizesis figures.

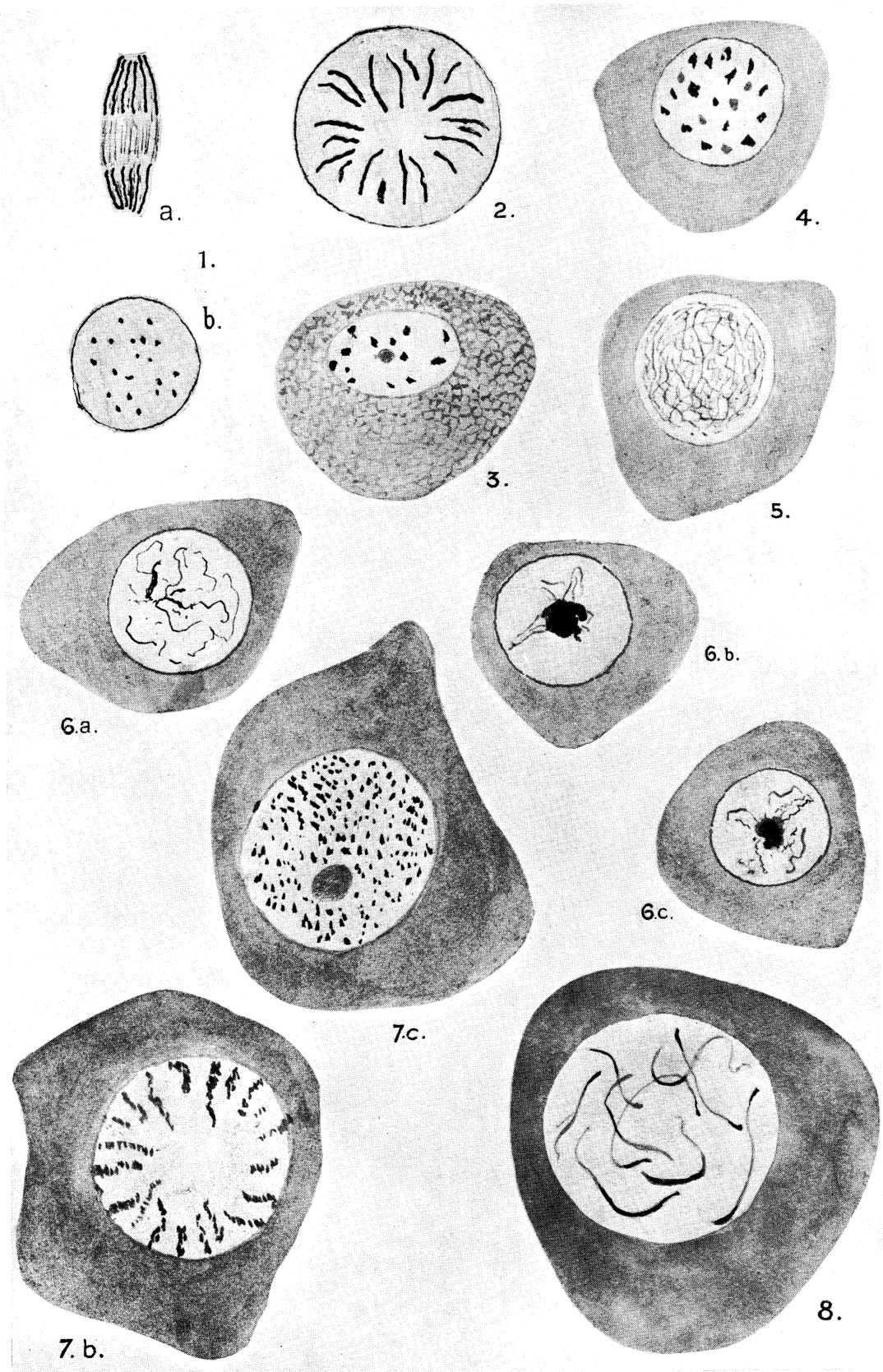
Figs. 26, 27.—Post-synaptic spireme segmenting into 9 pachytene threads.

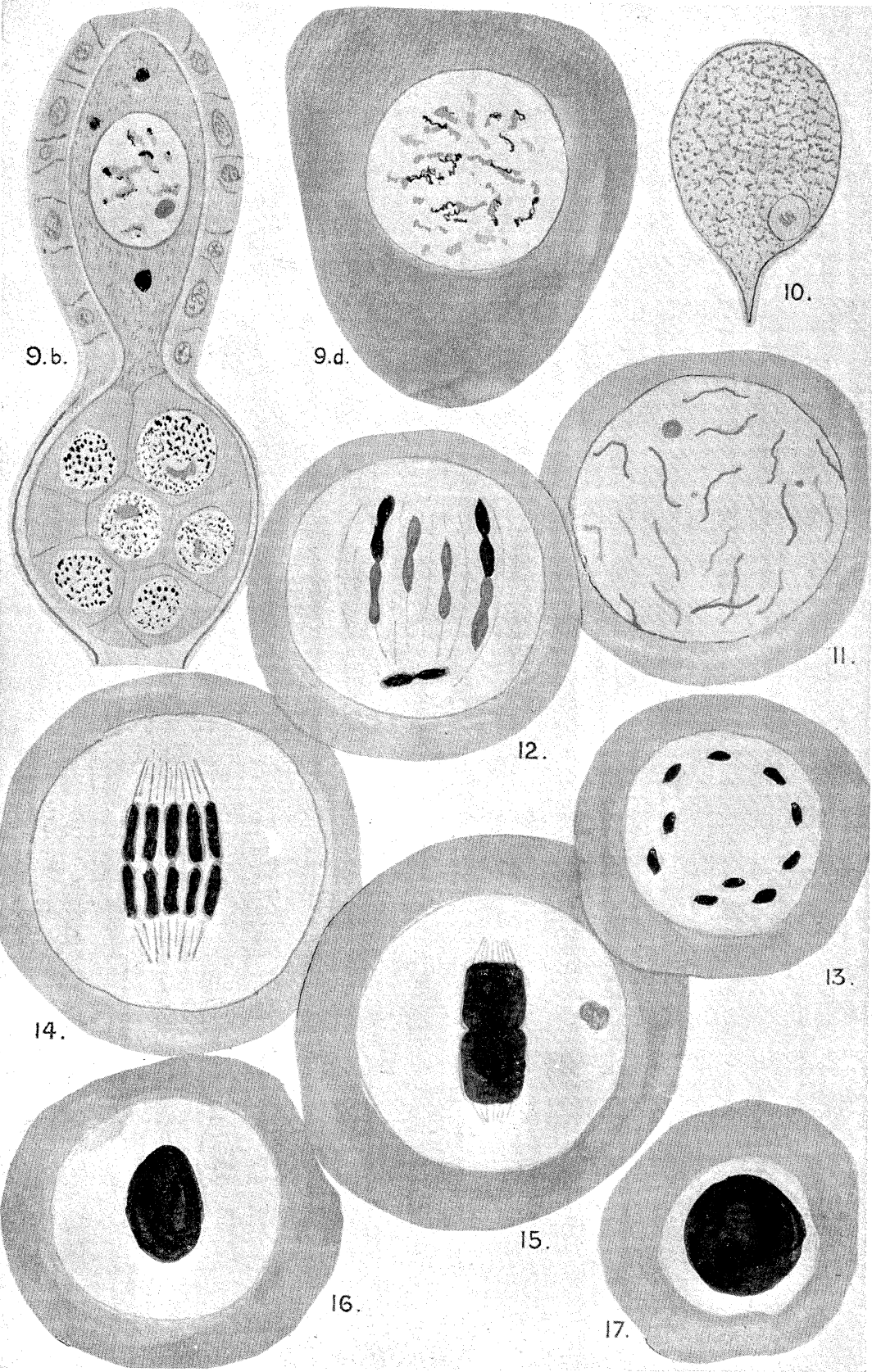
Fig. 28.—Diplotene nucleus.

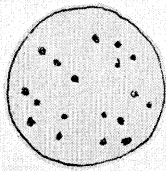
Fig. 29.—Late diplotene in a young nurse cell comparable to that described by Dederer in *Philosamia*.

PLATE 7.

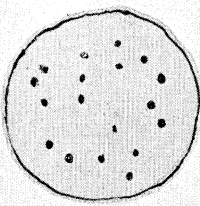
Fig. 30.—Very young oocyte and adjacent nurse chamber. The oocyte nucleus is in the diffuse stage, with chromatin granules in the surrounding cytoplasm. The nuclei of the nurse cells are granular. (Magnification reduced.)



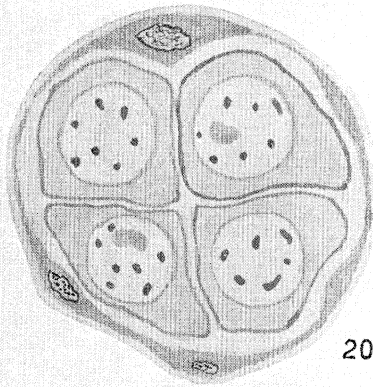




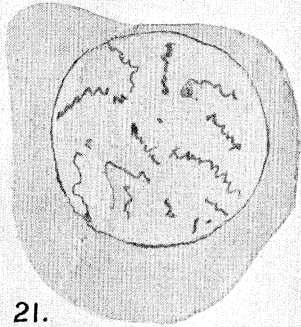
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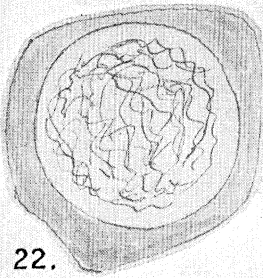
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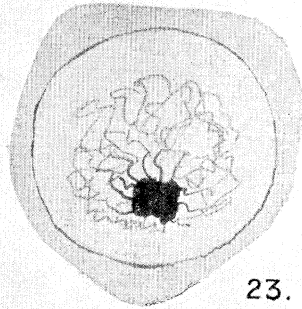
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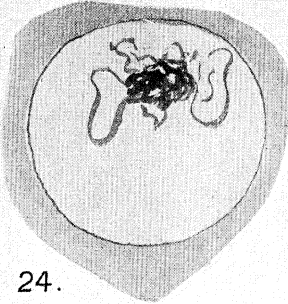
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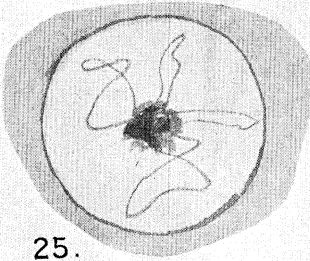
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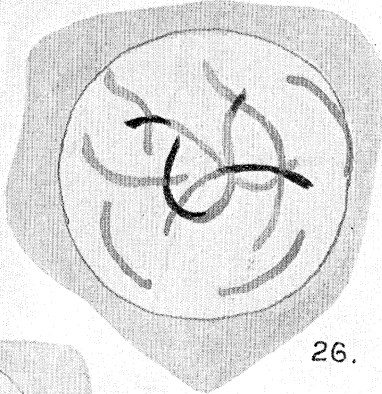
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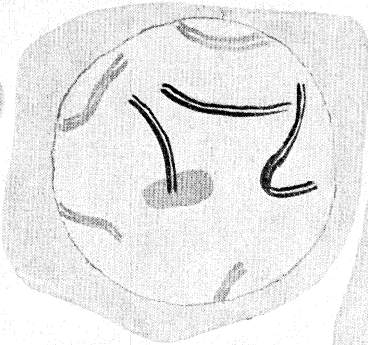
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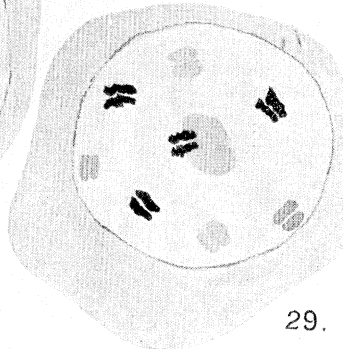
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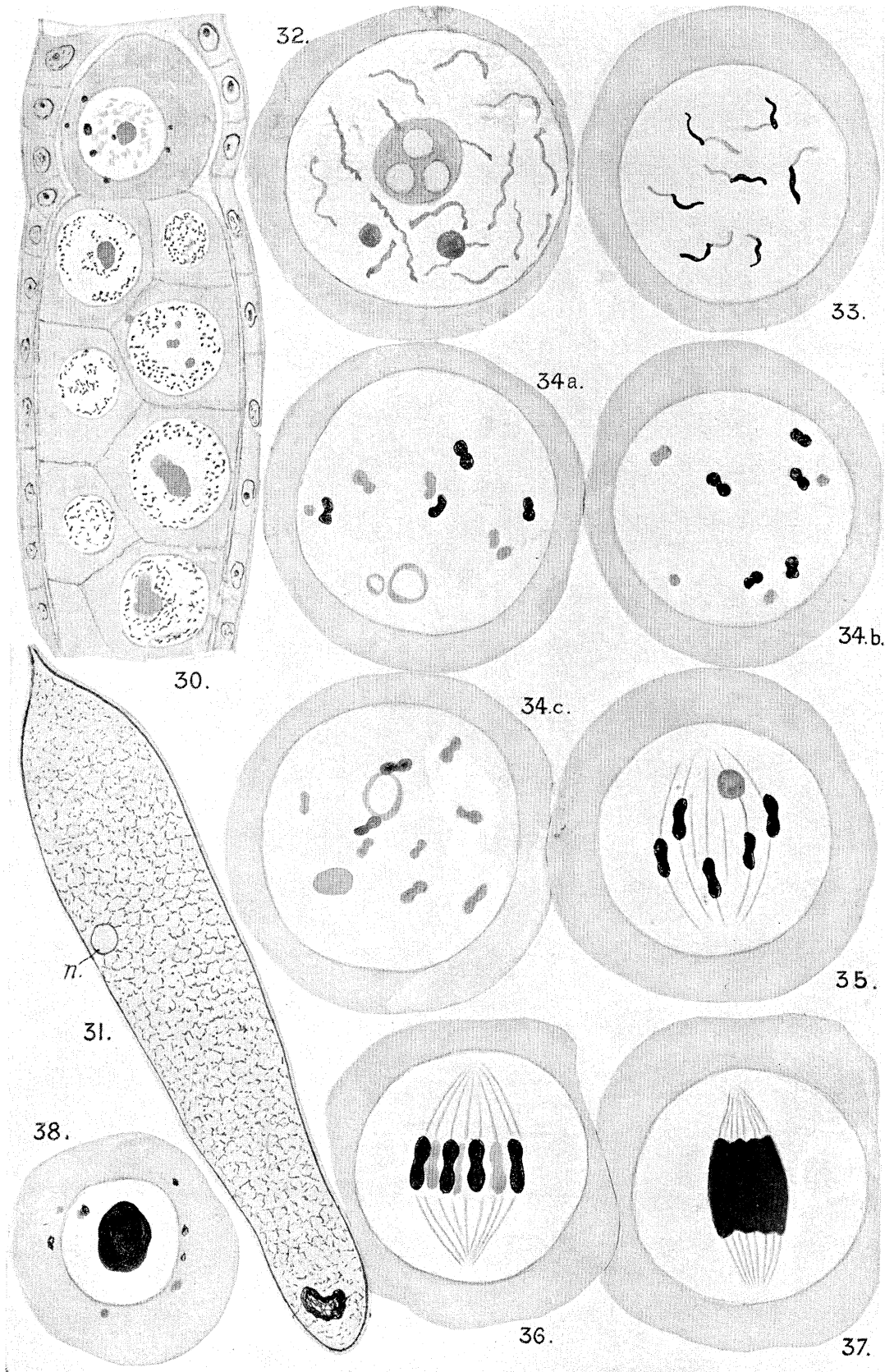
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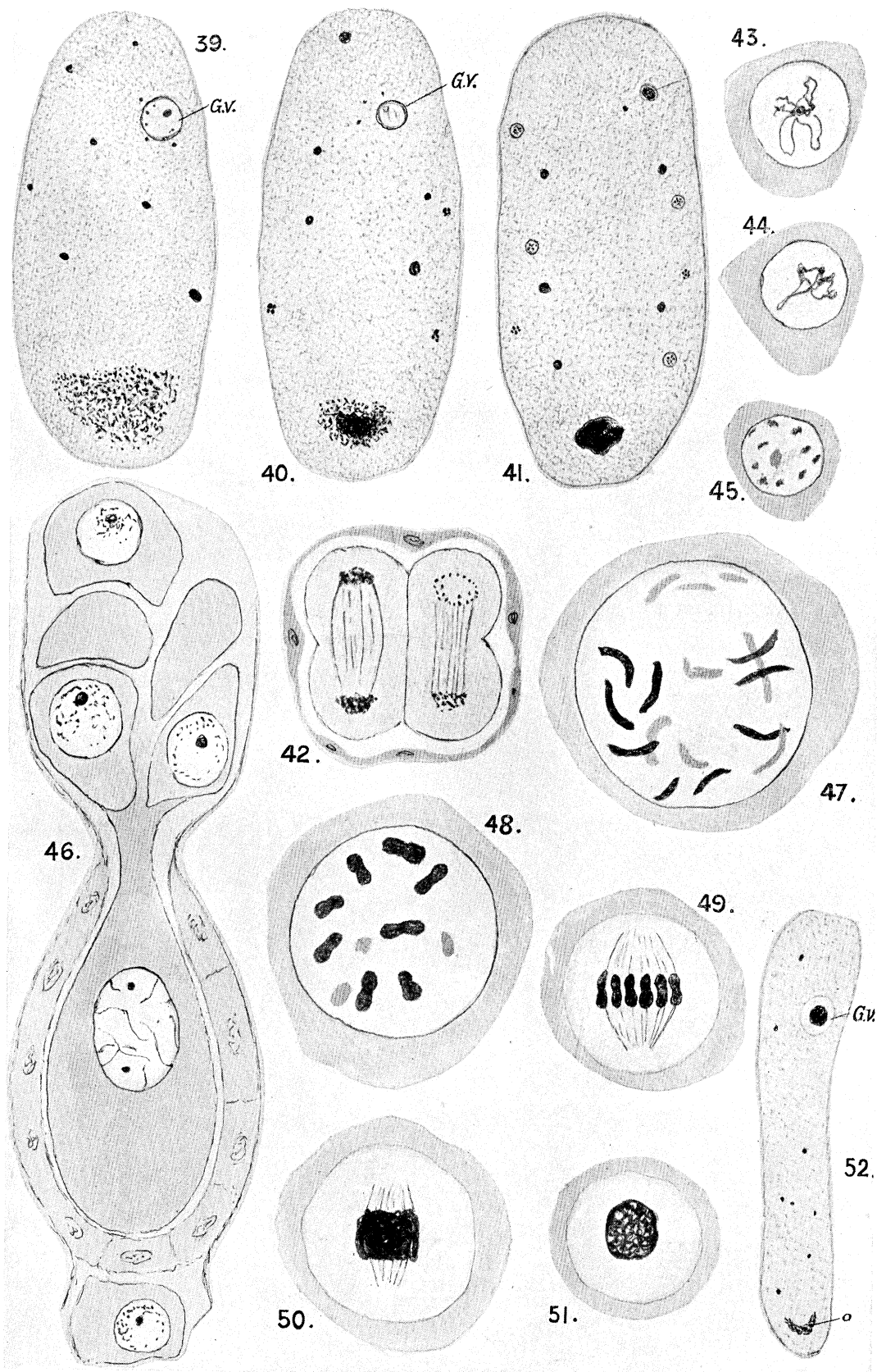


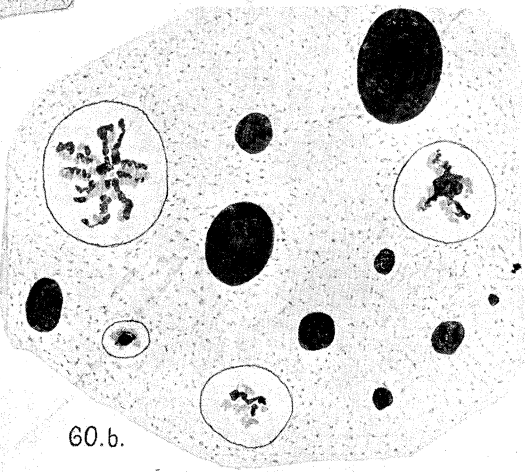
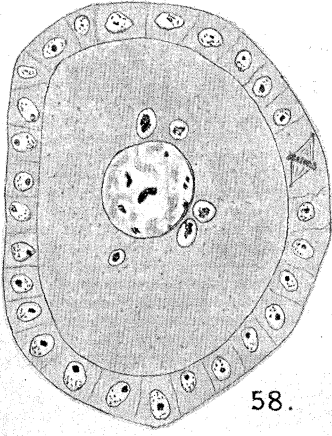
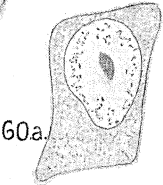
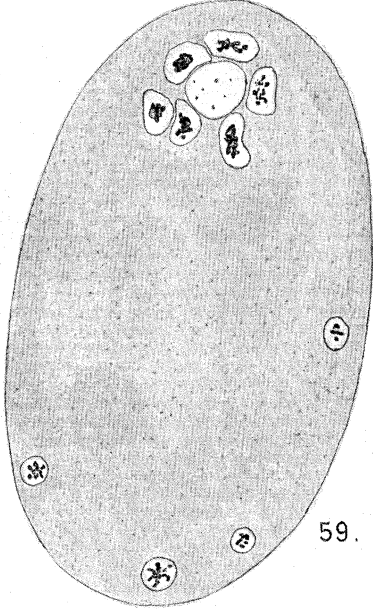
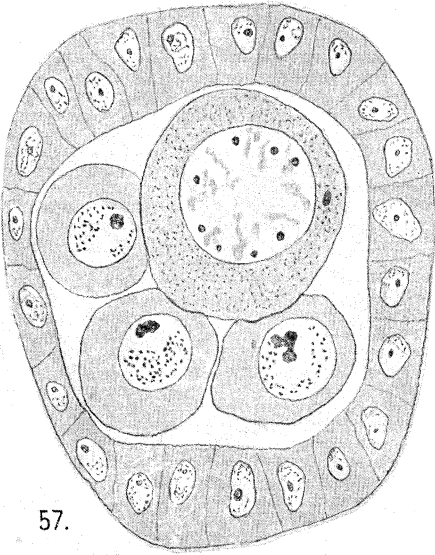
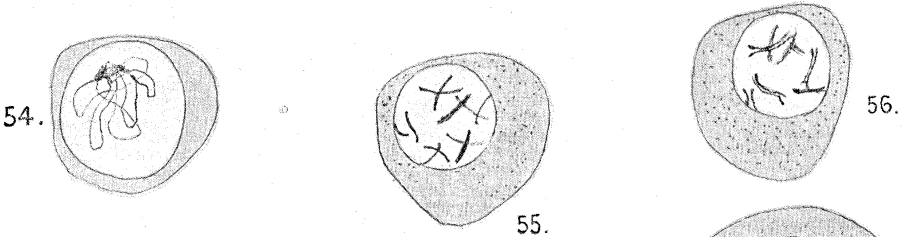
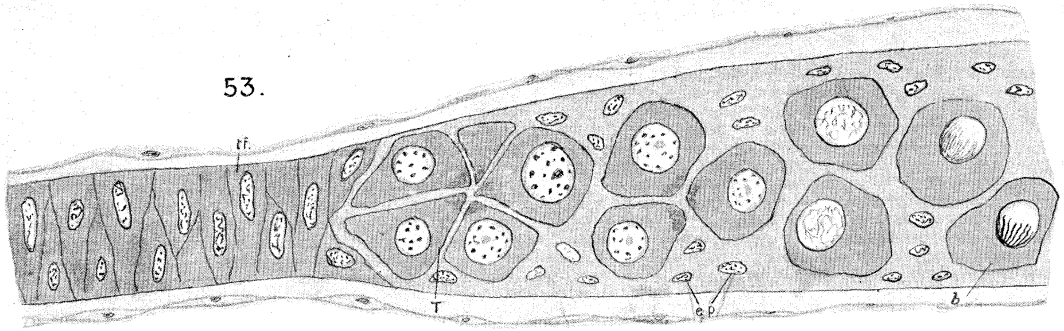
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- Fig. 31.—Full-grown egg (diagrammatic), showing deeply staining body (polar disc) at posterior pole and nucleus at the periphery at one side.
- Fig. 32.—Nucleus after the "diffuse" stage with diploid chromosomes: vacuolated plasmosome and two karyosomes.
- Fig. 33.—End to end pairing of filamentous chromosomes.
- Fig. 34, *a*, *b*, *c*.—Showing haploid number of bivalents with condition indicating their double nature; ring-like karyosomes and plasmosomes.
- Fig. 35.—Formation of maturation spindle.
- Fig. 36.—Abortive maturation spindle.
- Figs. 37, 38.—Condensation of same.

PLATE 8.—*Synergus* and *Orthopelma*.

- Figs. 39–41.—*Synergus Rheinhardi*. Formation of Oosoma and secondary nuclei.
- Fig. 39.—Cloud of granules at posterior end: spherical chromatin granules disposed in the cytoplasm.
- Fig. 40.—Granules at posterior pole condensing. Large chromatin granules fragmenting in the periphery of the egg.
- Fig. 41.—Secondary nuclei in periphery of egg; *g.v.* = germinal vesicle.
- Figs. 42–52.—*Orthopelma luteolator*.
- Fig. 42.—Rosette showing synchronous division of cells, and 22 chromosomes at one end of spindle, -- larva shortly before pupation.
- Figs. 43, 44.—Synizesis nuclei.
- Fig. 45.—Young nurse cell: haploid number.
- Fig. 46.—Young oocyte and nurse chamber. Nucleus shortly before "diffuse" stage shows haploid number of bivalents. (Magnification reduced.)
- Fig. 47.—Later stage: diploid number of chromosomes.
- Fig. 48.—Second syndesis.
- Fig. 49.—Abortive maturation spindle: no asters or centrosomes.
- Fig. 50.—Condensation of same.
- Fig. 51.—"Chromatin nucleus," showing vacuolated structure: just before laying.
- Fig. 52.—Full-grown egg, "chromatin nucleus" in nucleoplasmic zone, oosoma, etc. (Diagrammatic.)

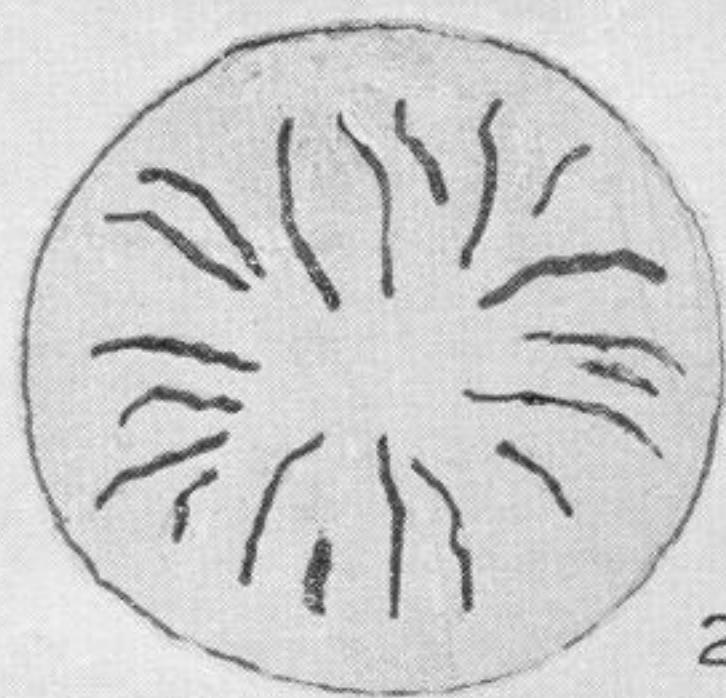
PLATE 9.—*Lasius* and *Formica*.

- Figs. 53–57.—*Lasius flava*.
- Fig. 53.—Terminal chamber (*t.c.*) and filament (*t.f.*), rosettes (*r.*), epithelial nuclei (*ep.n.*), "bouquet" nuclei (*b.*)
- Fig. 54.—Synizesis.
- Fig. 55.—Haploid number of pachytene threads from post-synaptic spireme.
- Fig. 56.—Diplotene nuclei.
- Fig. 57.—T.S. first zone of growth; nurse cells, follicular epithelia and oocyte nucleus in "diffuse" stage.
- Figs. 58–60.—*Formica rufa*.
- Fig. 58.—Young oocyte with follicle complete: secondary nuclei around germinal vesicle.
- Fig. 59.—Later: migration of secondary nuclei to periphery.
- Fig. 60*a*.—Follicle cell and nucleus.
- Fig. 60*b*.—Yolk and secondary nuclei of full-grown egg.

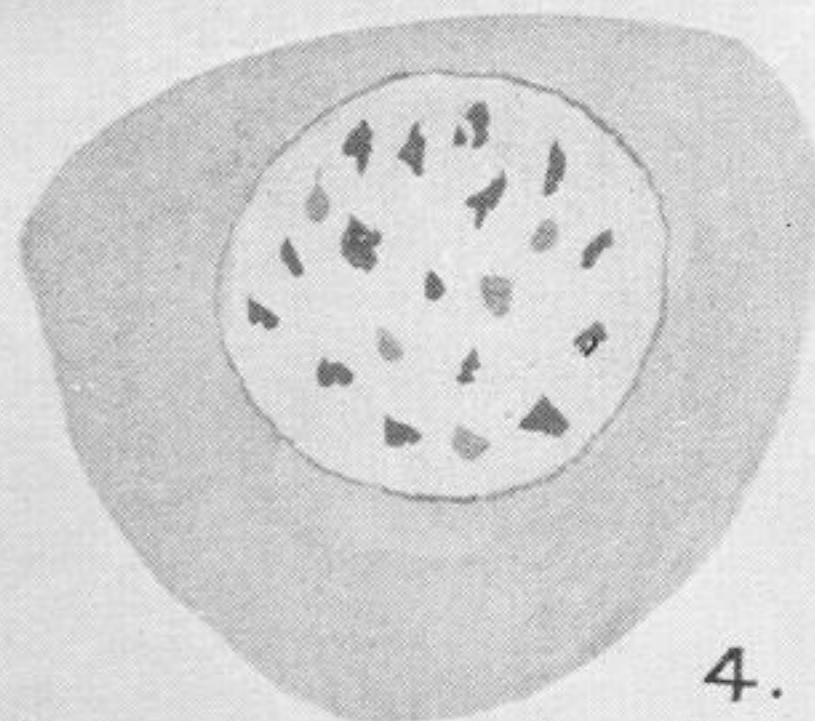


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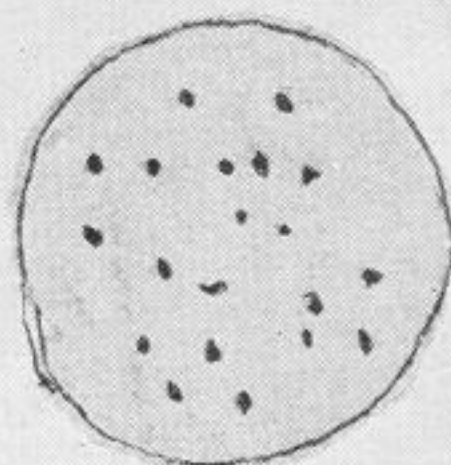
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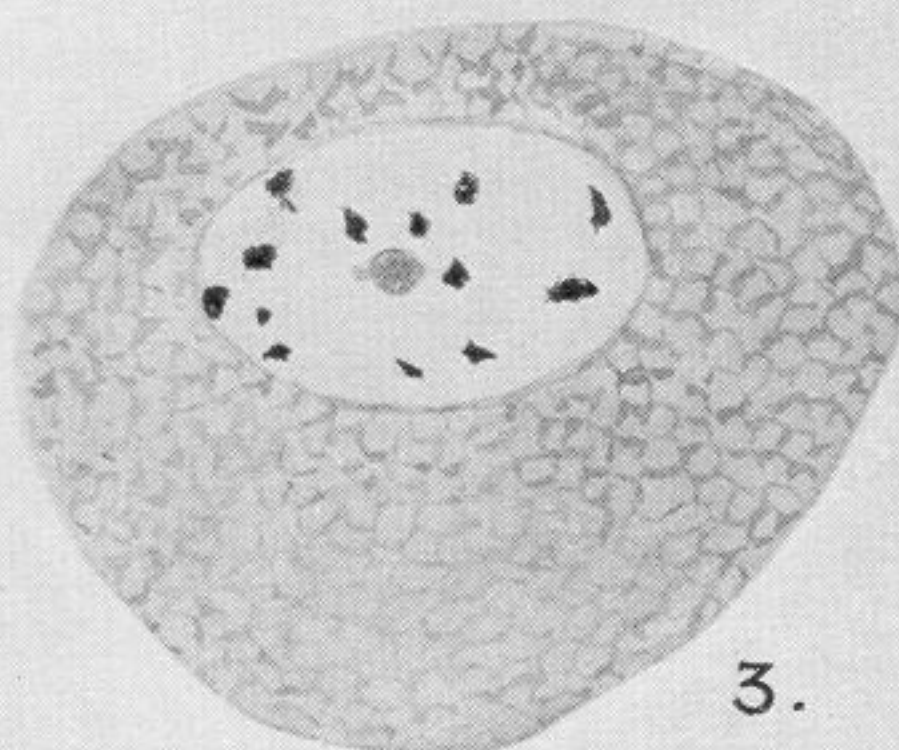
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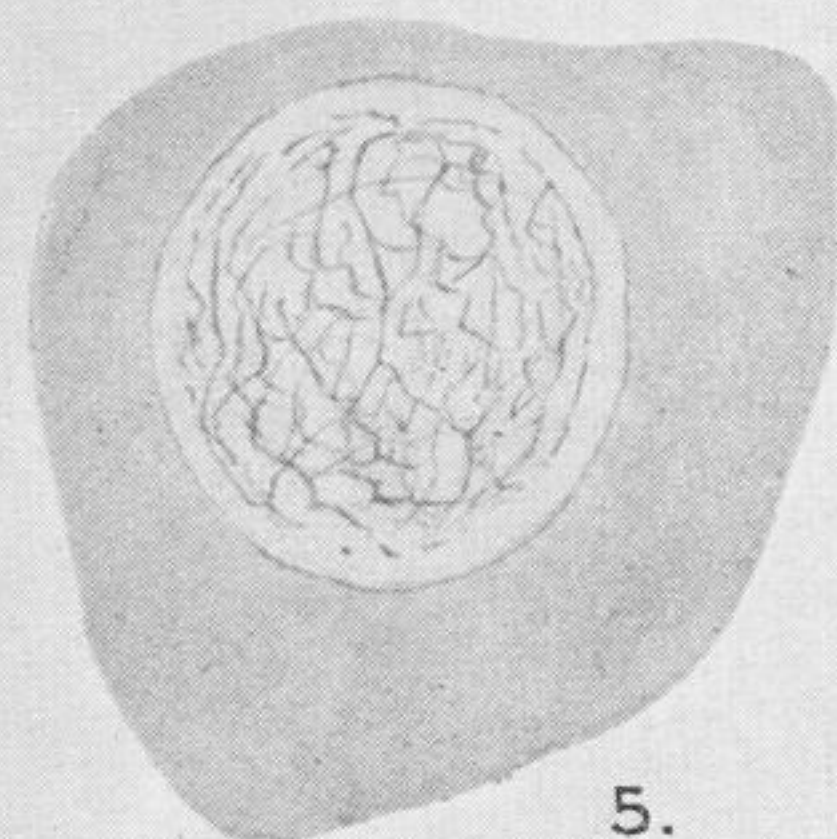
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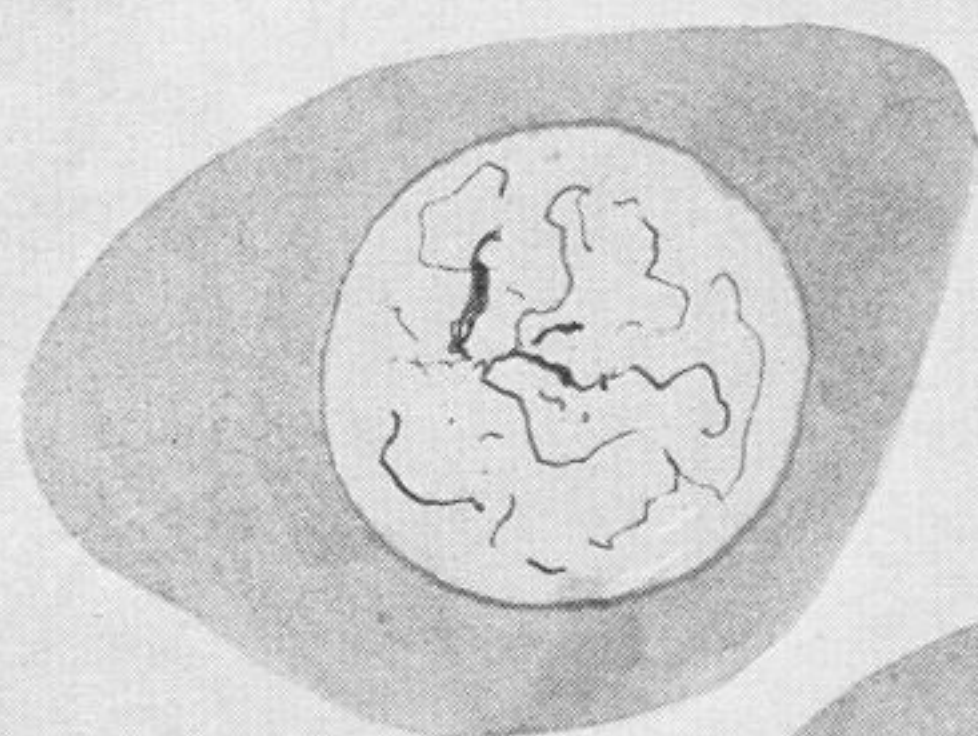
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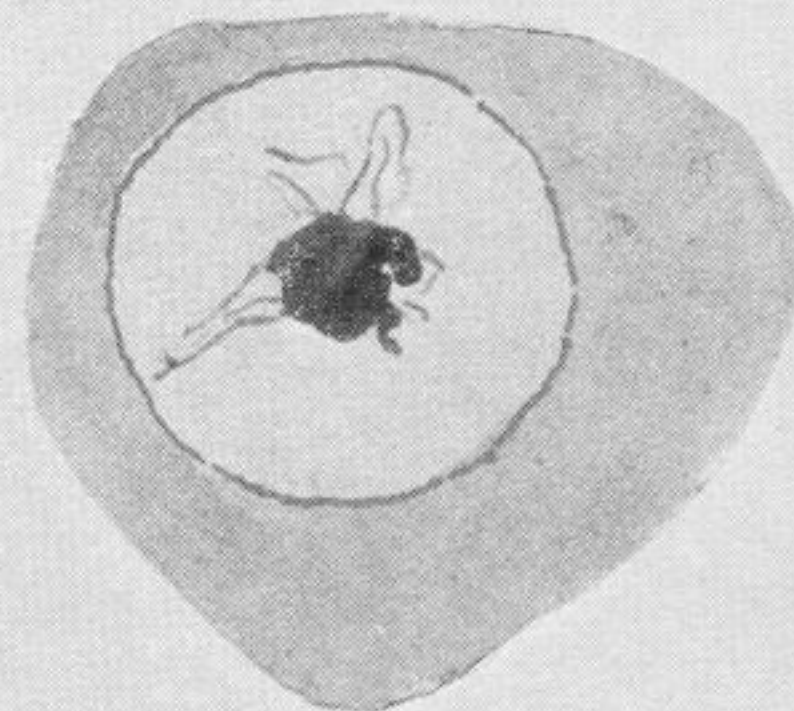
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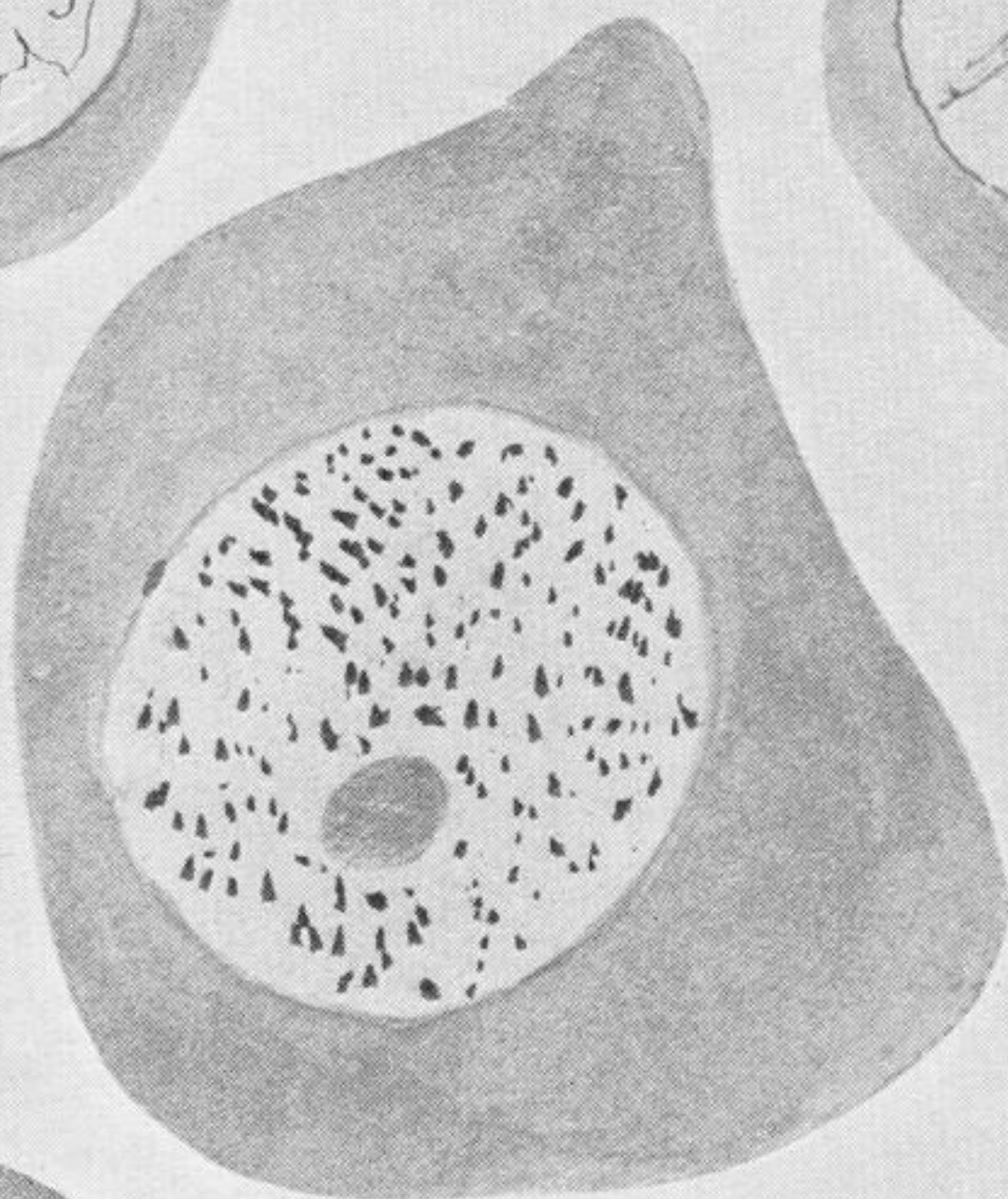
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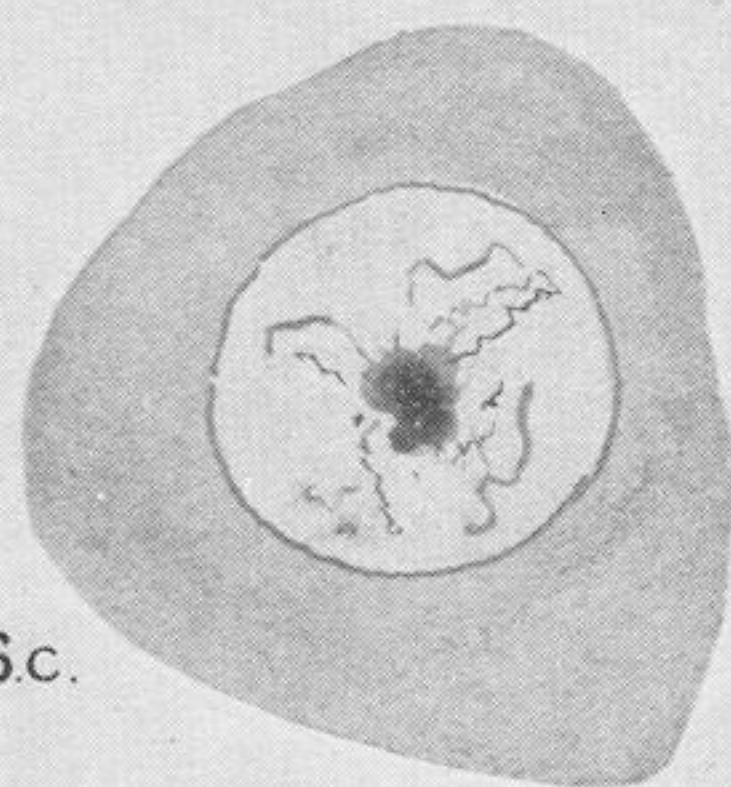
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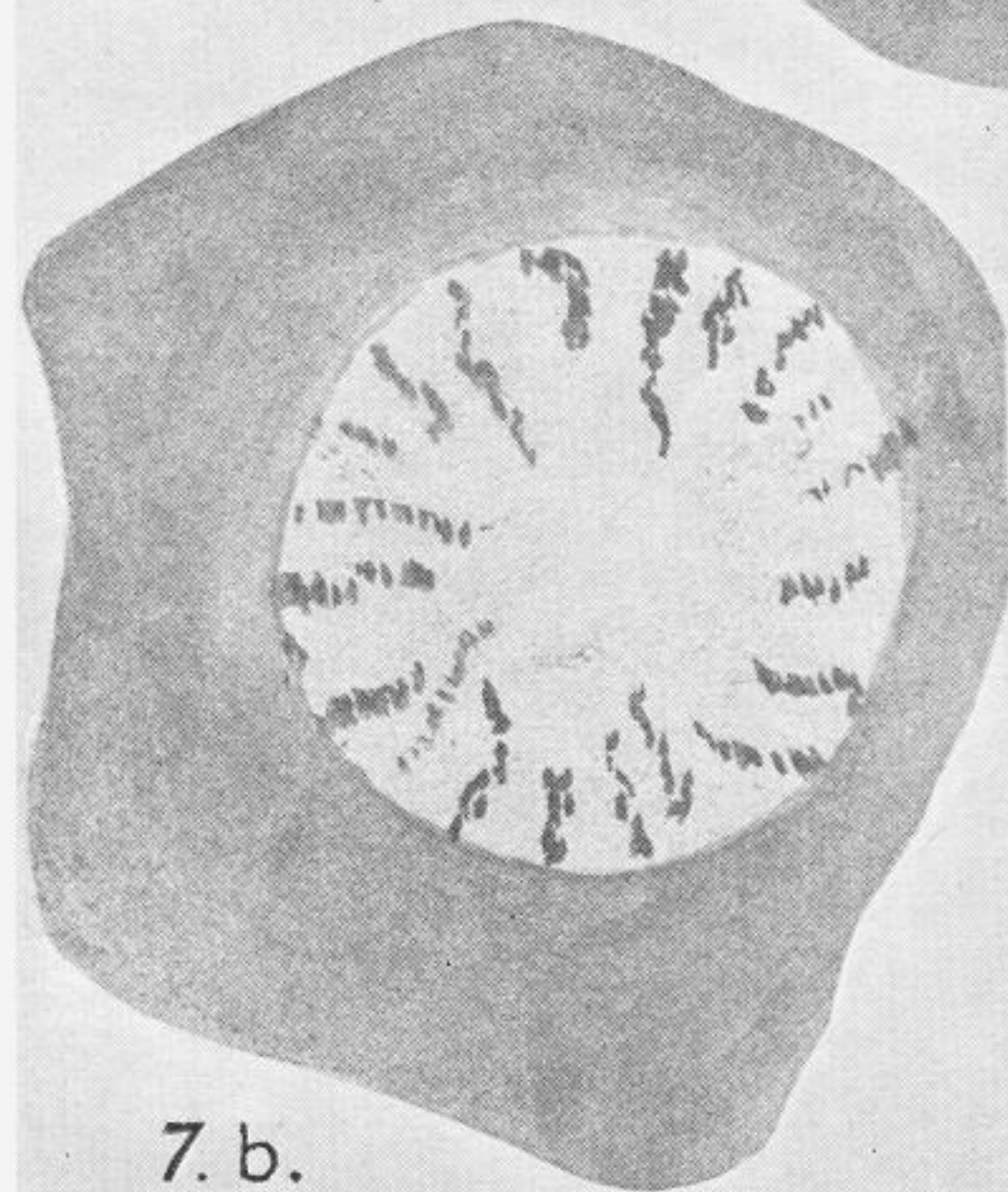
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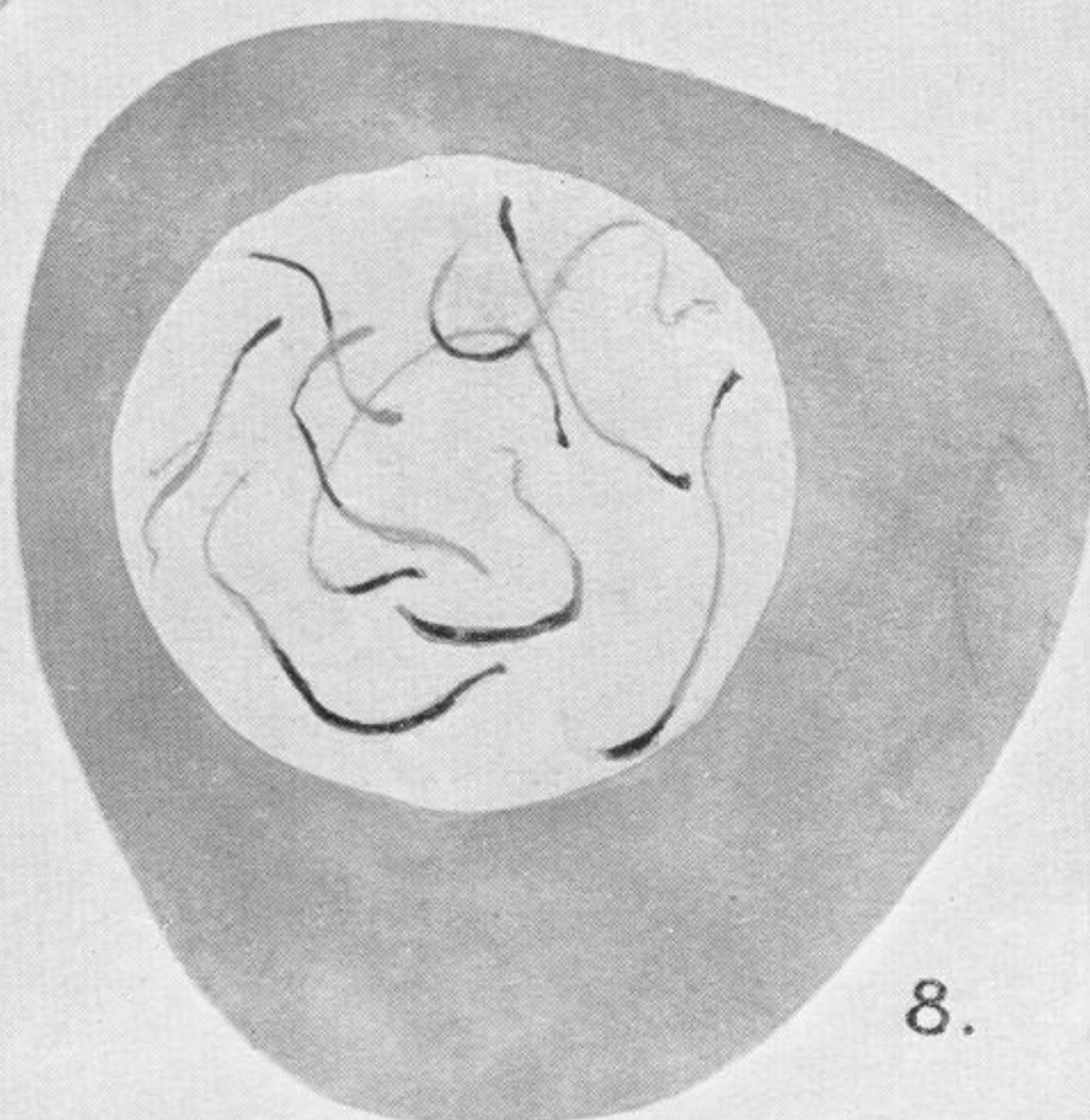
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6.c.



7. b.



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9.b.

9.d.

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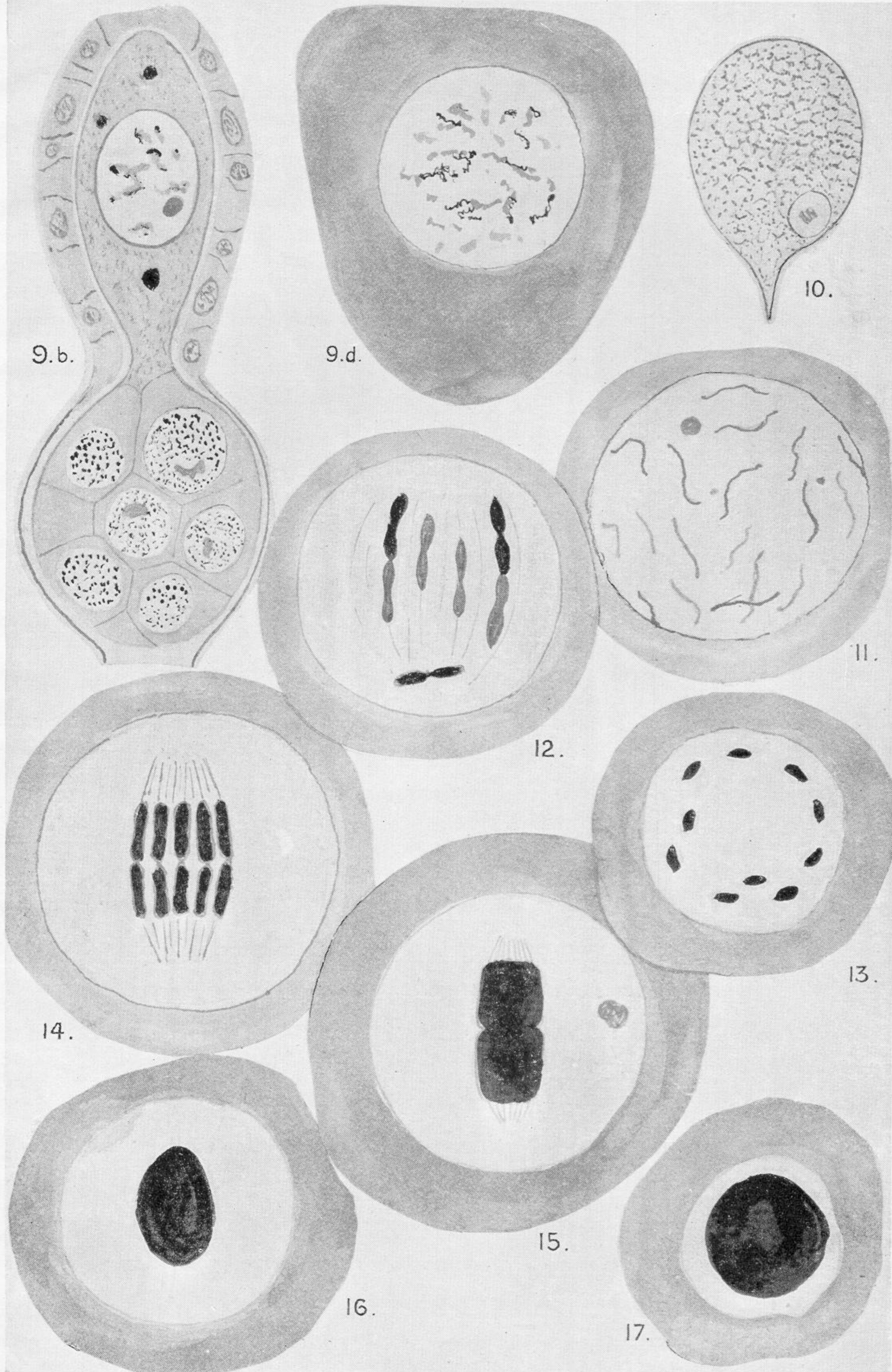
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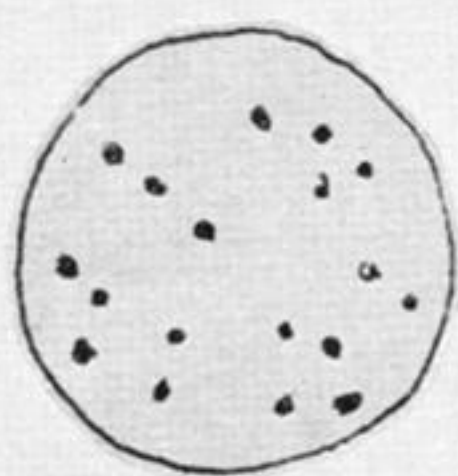
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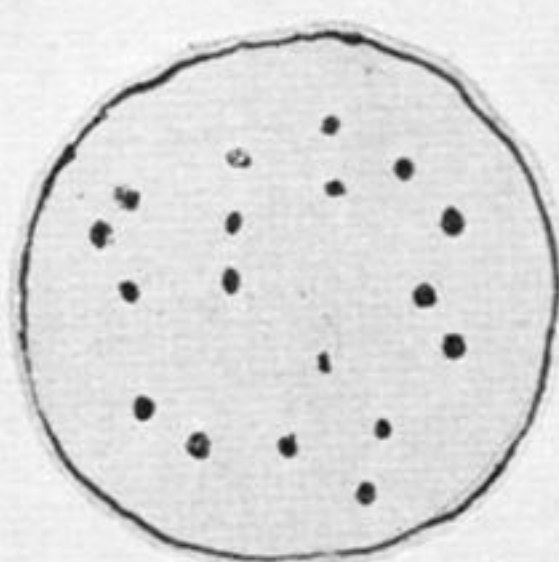
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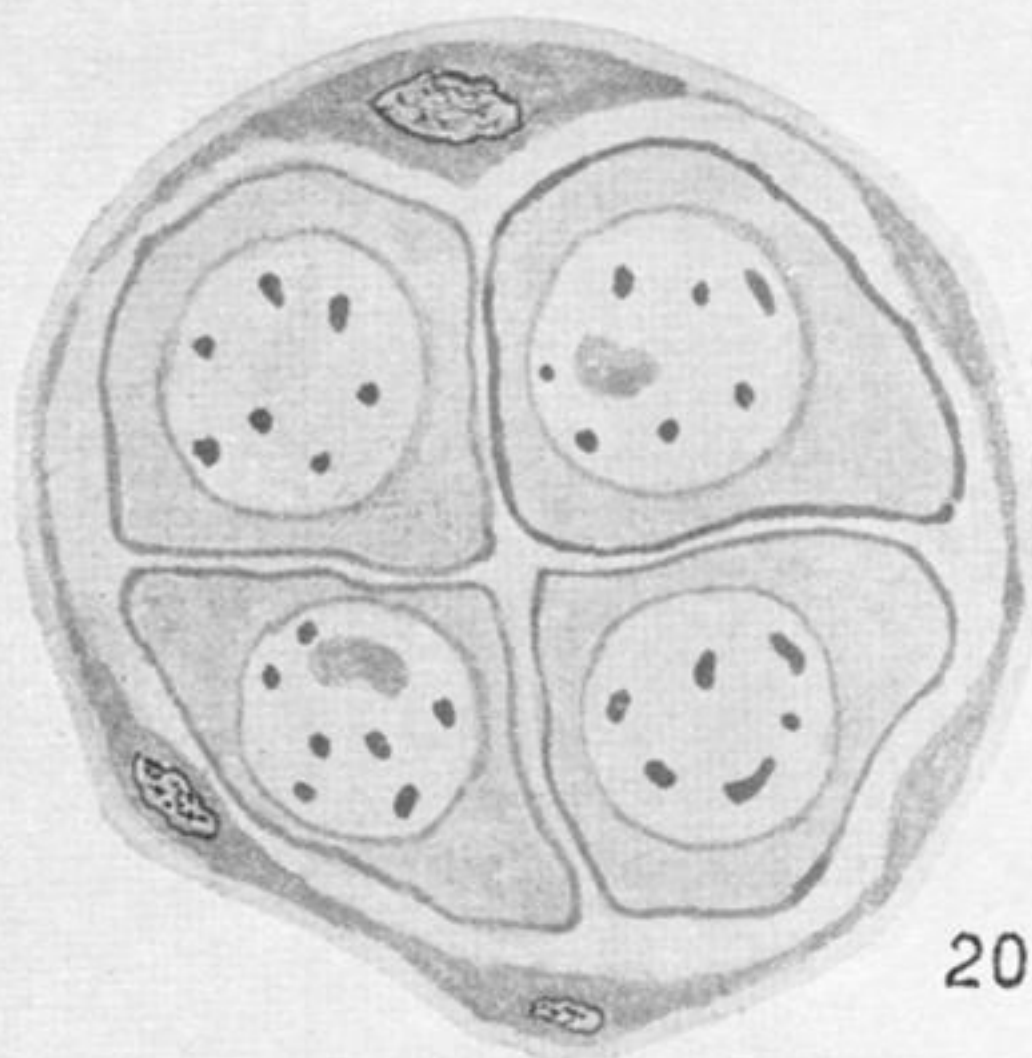




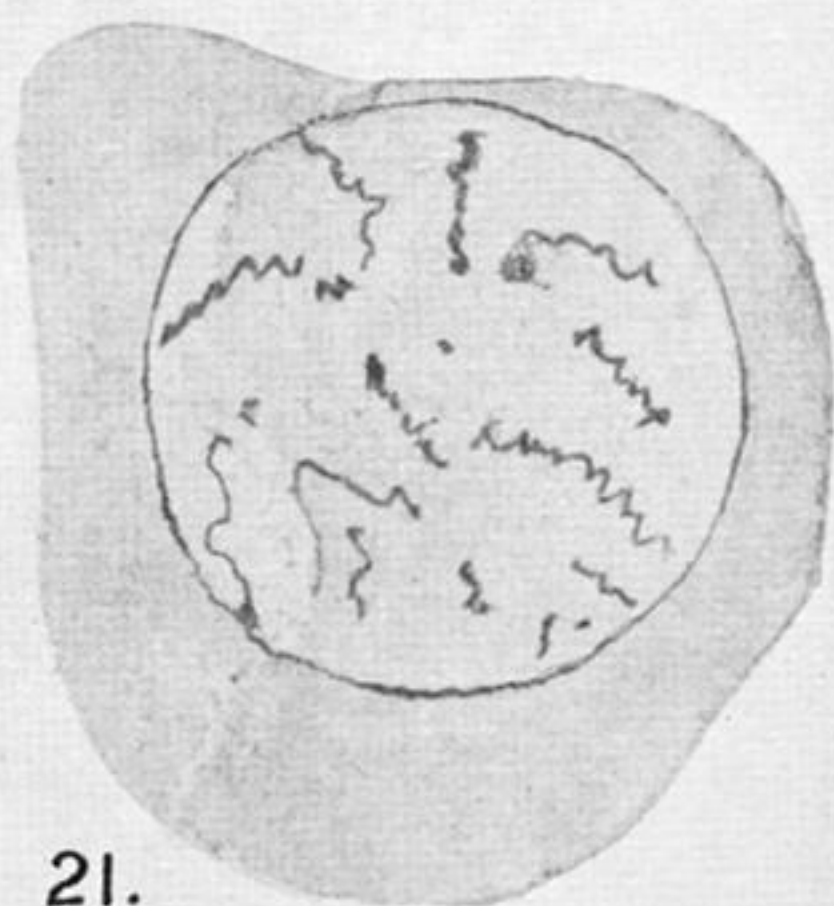
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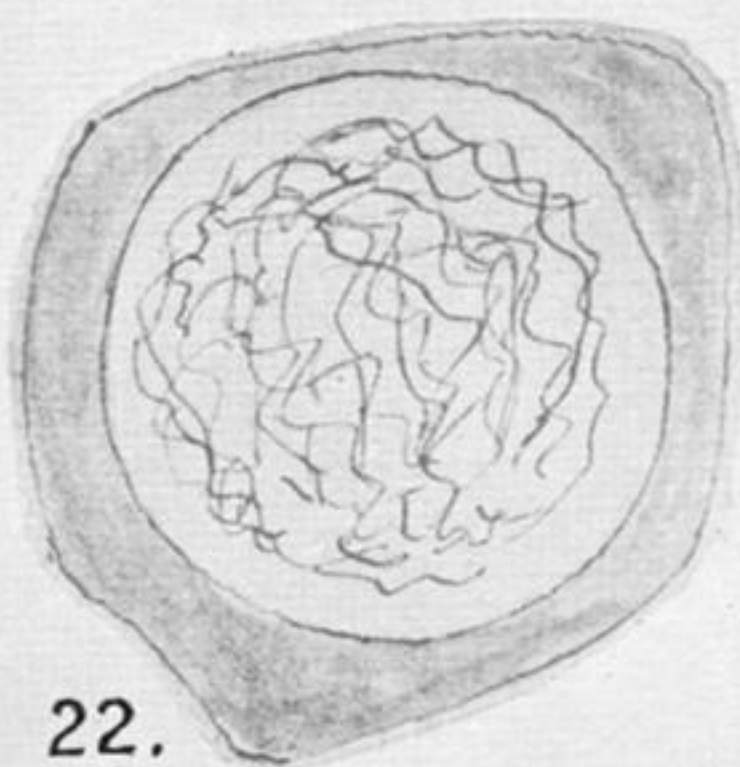
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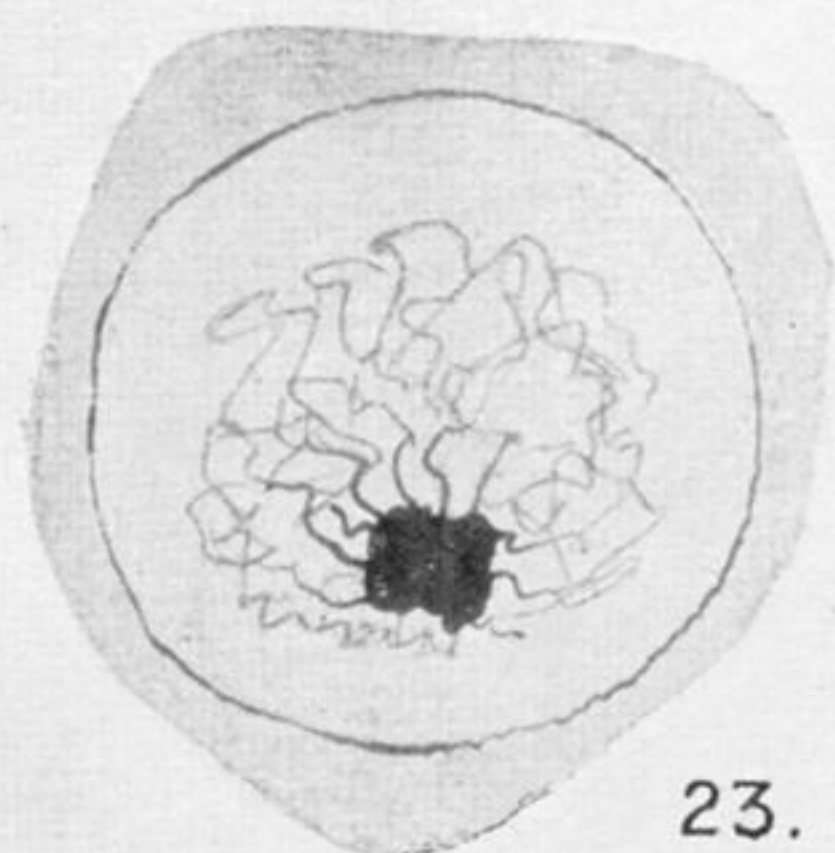
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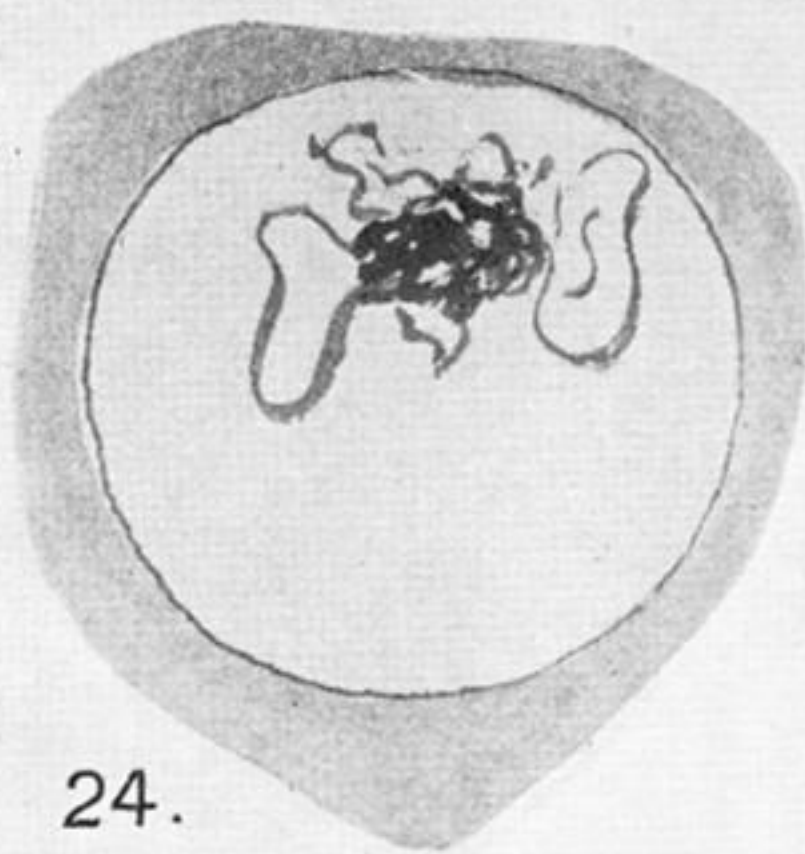
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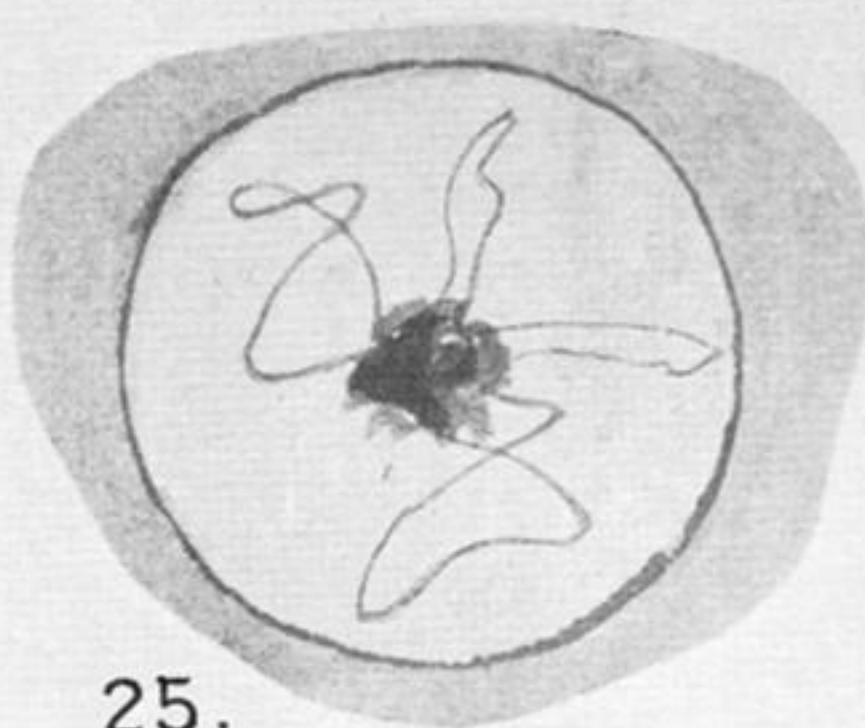
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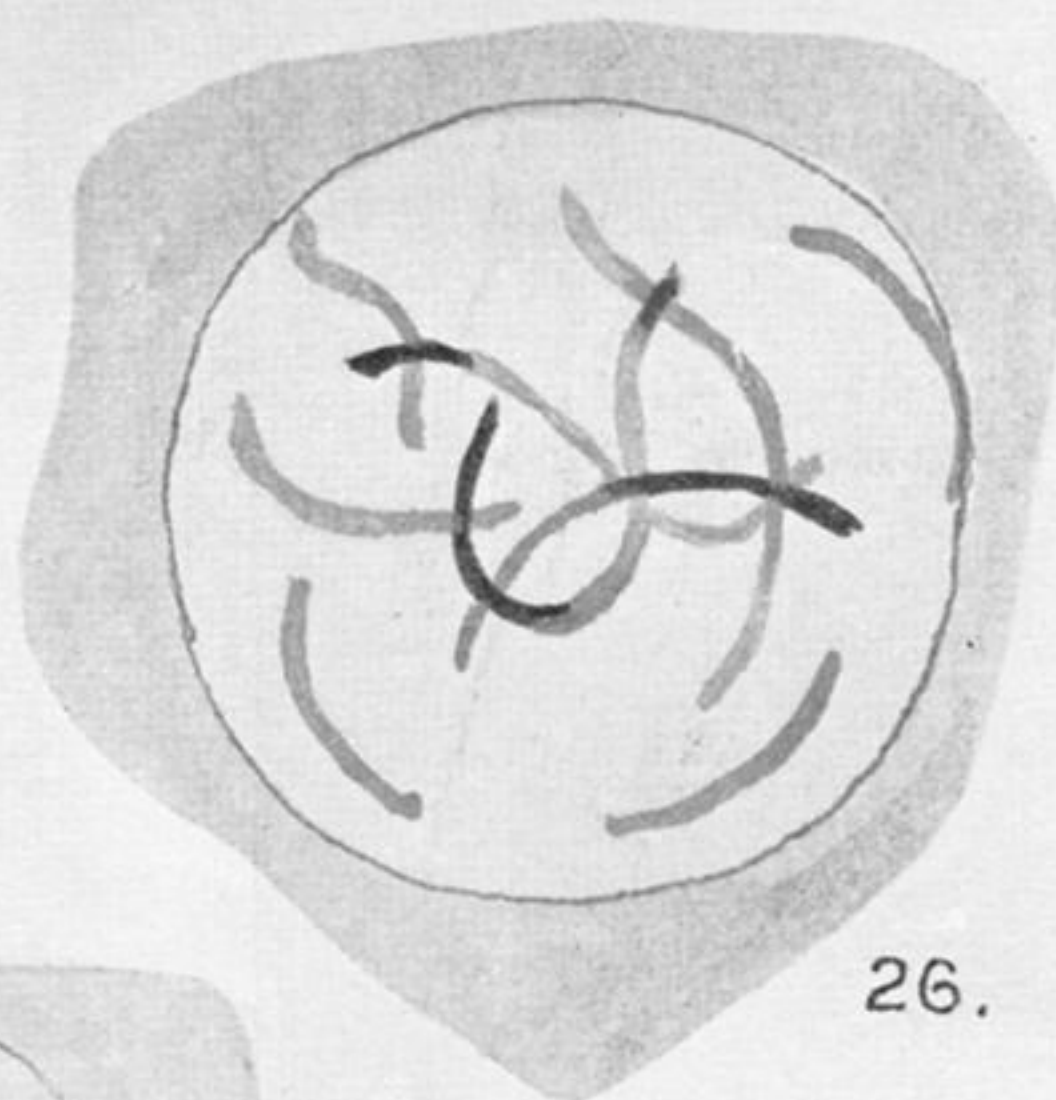
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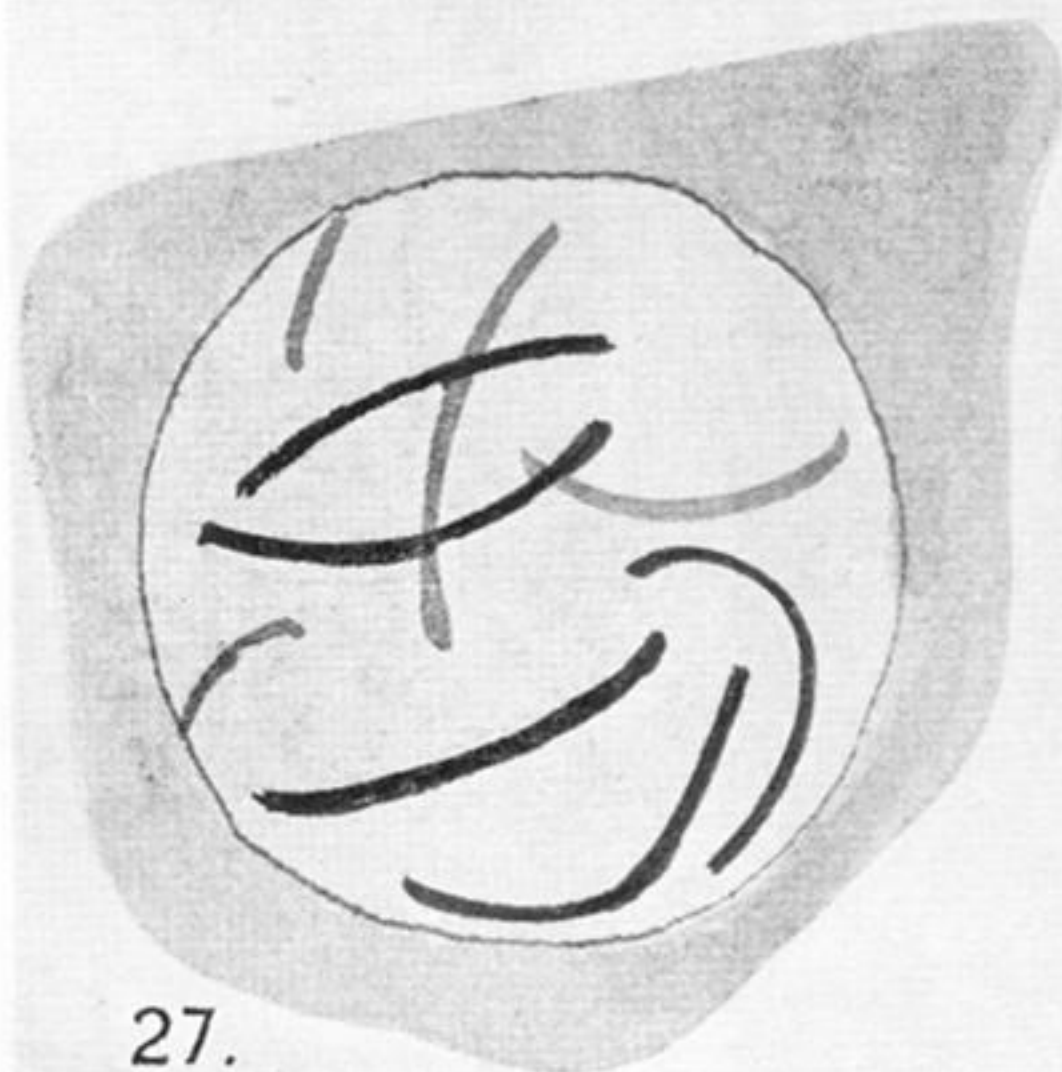
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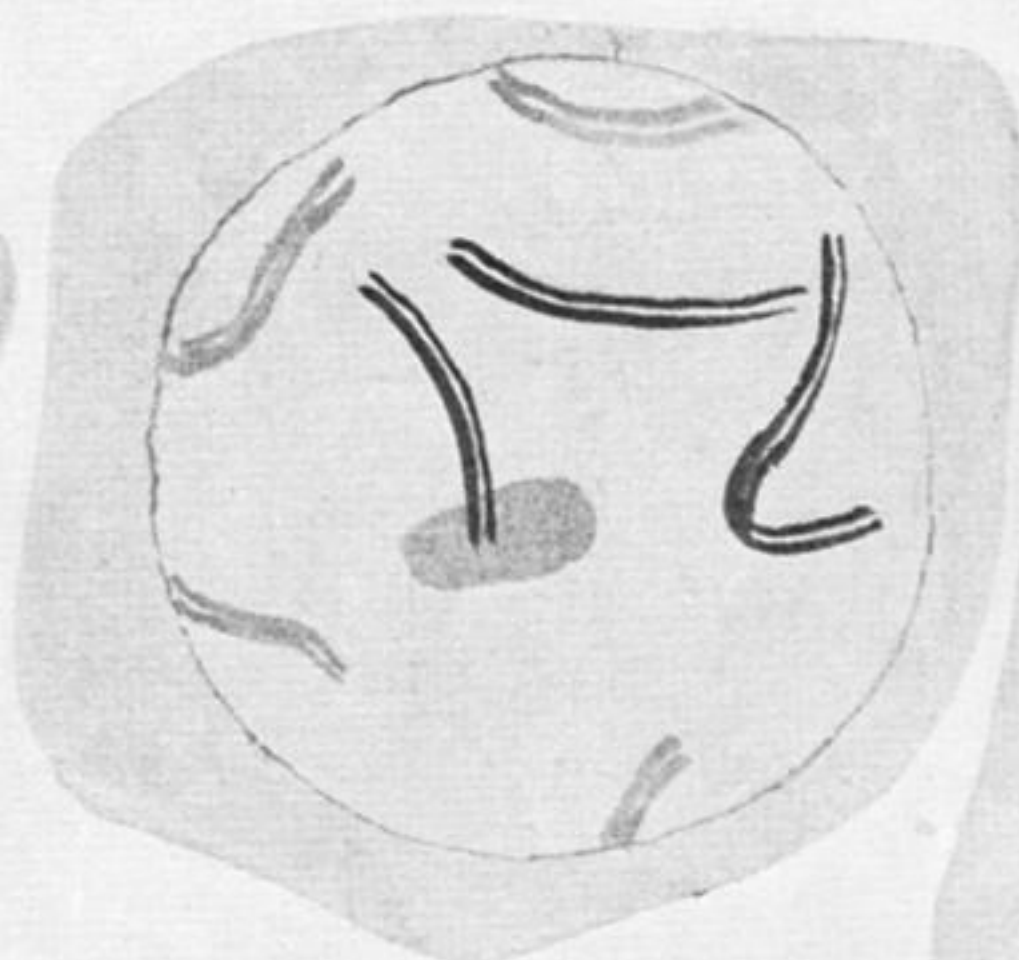
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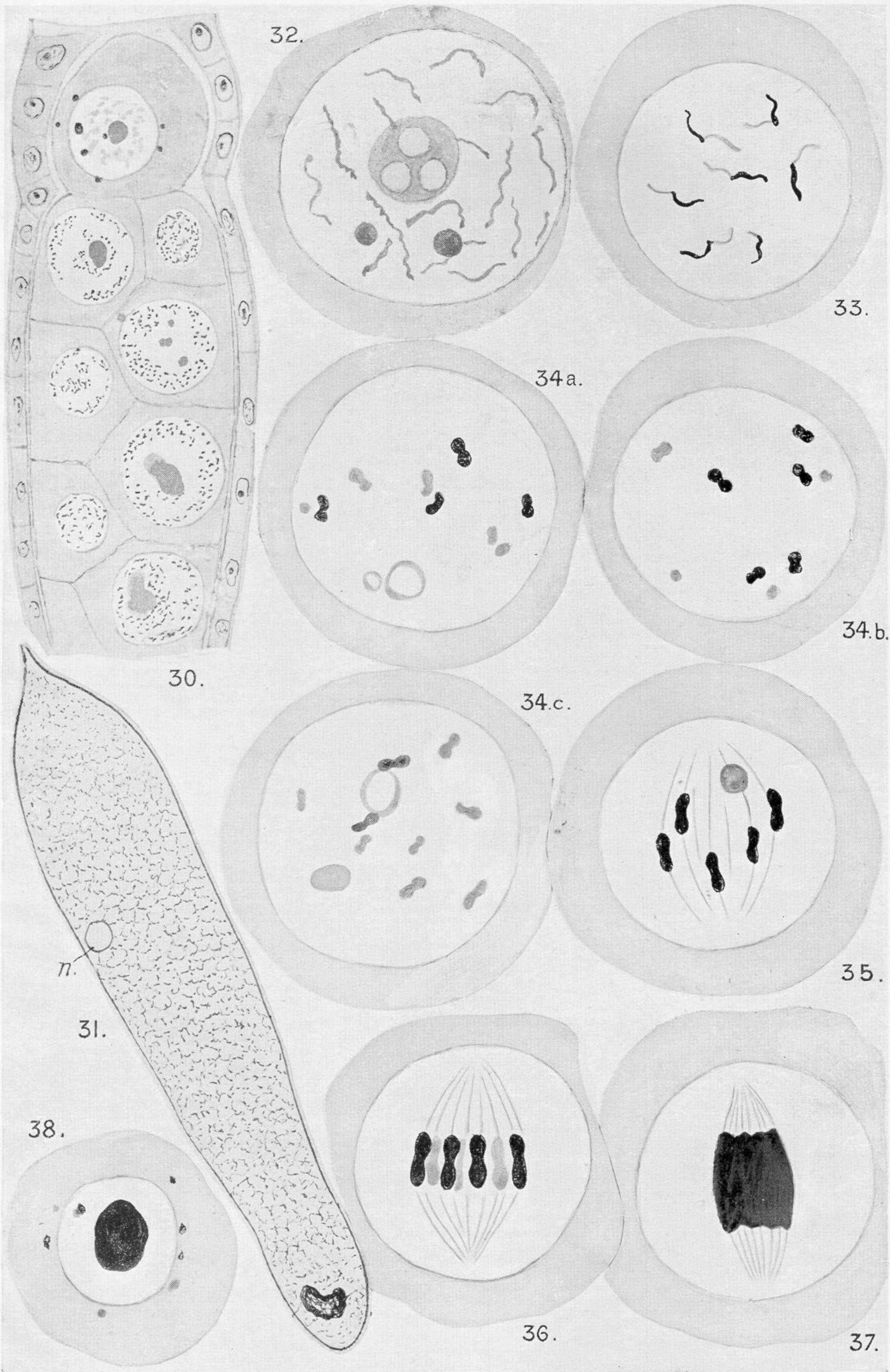
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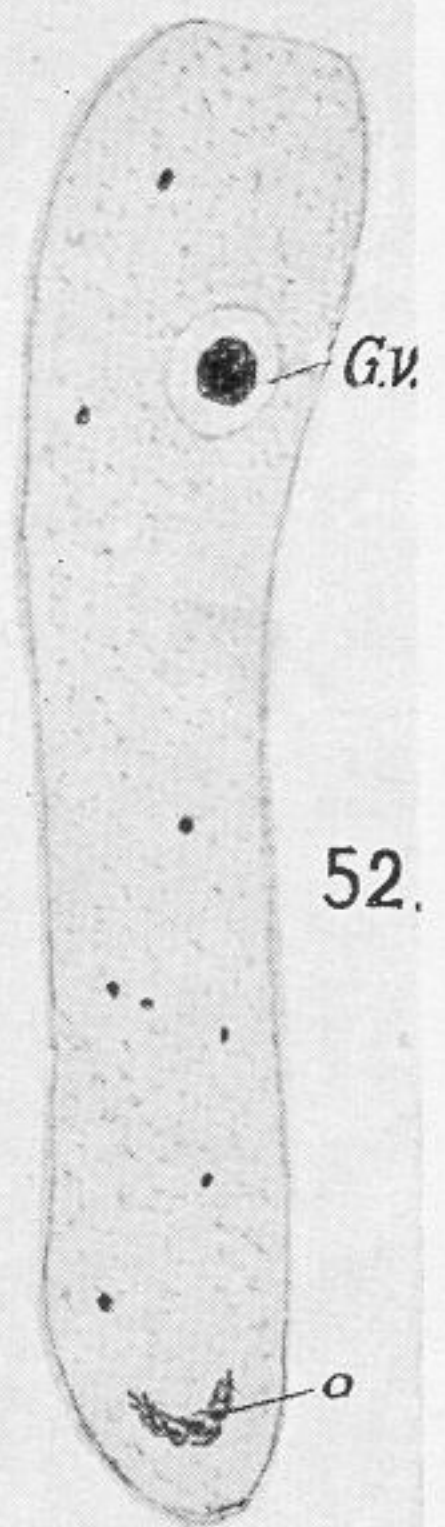
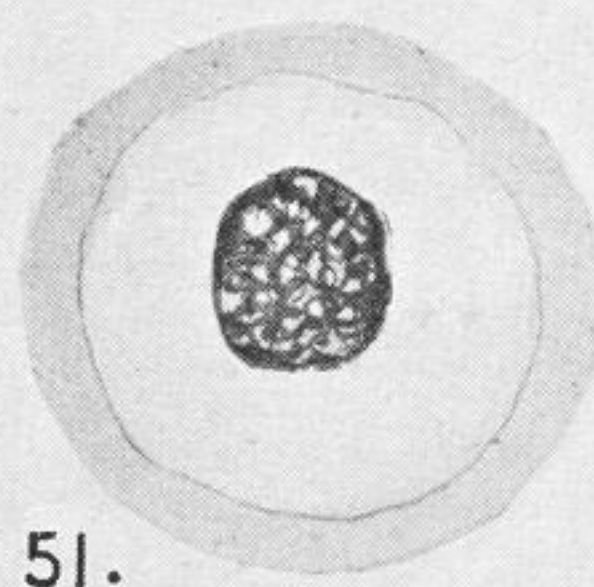
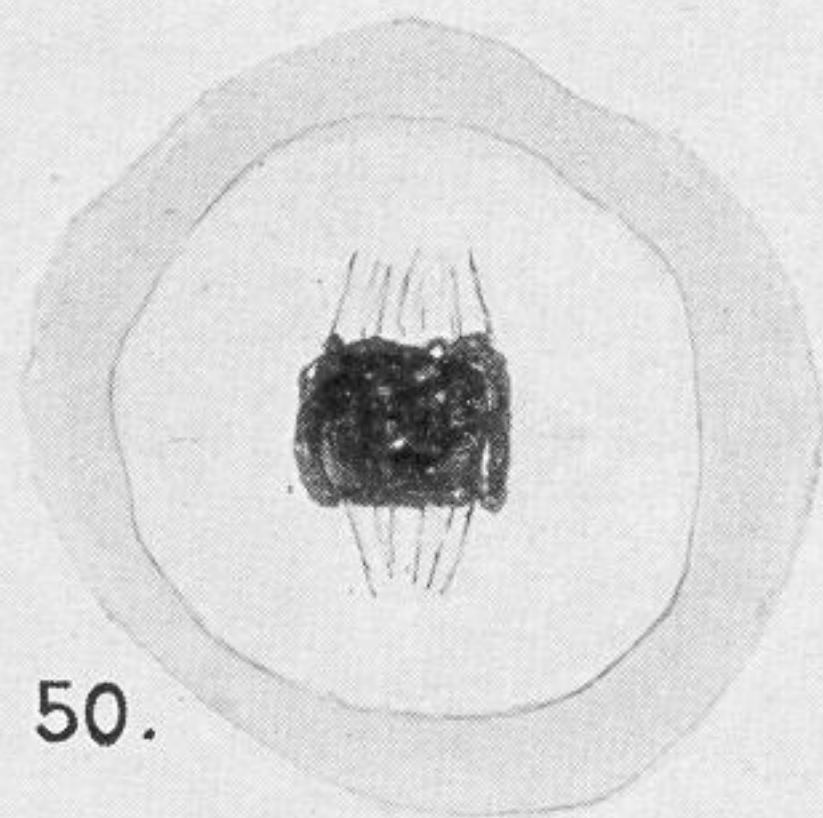
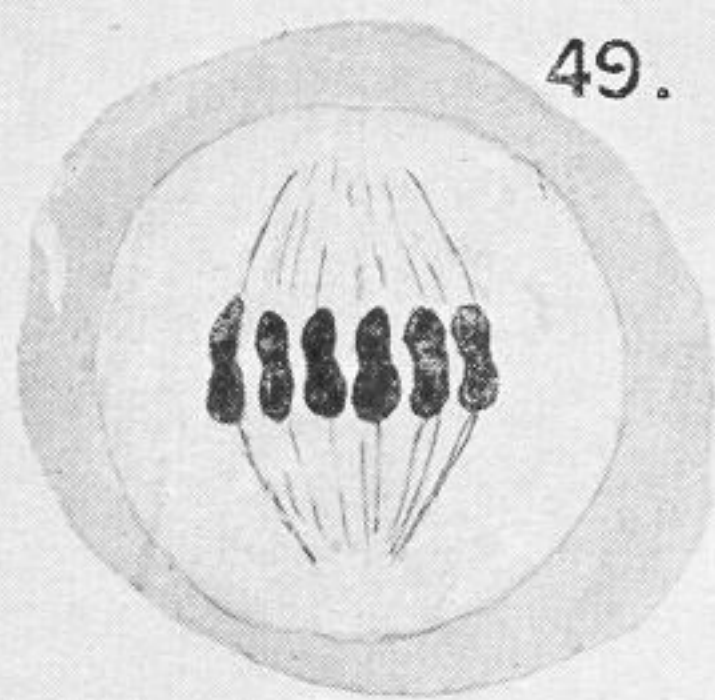
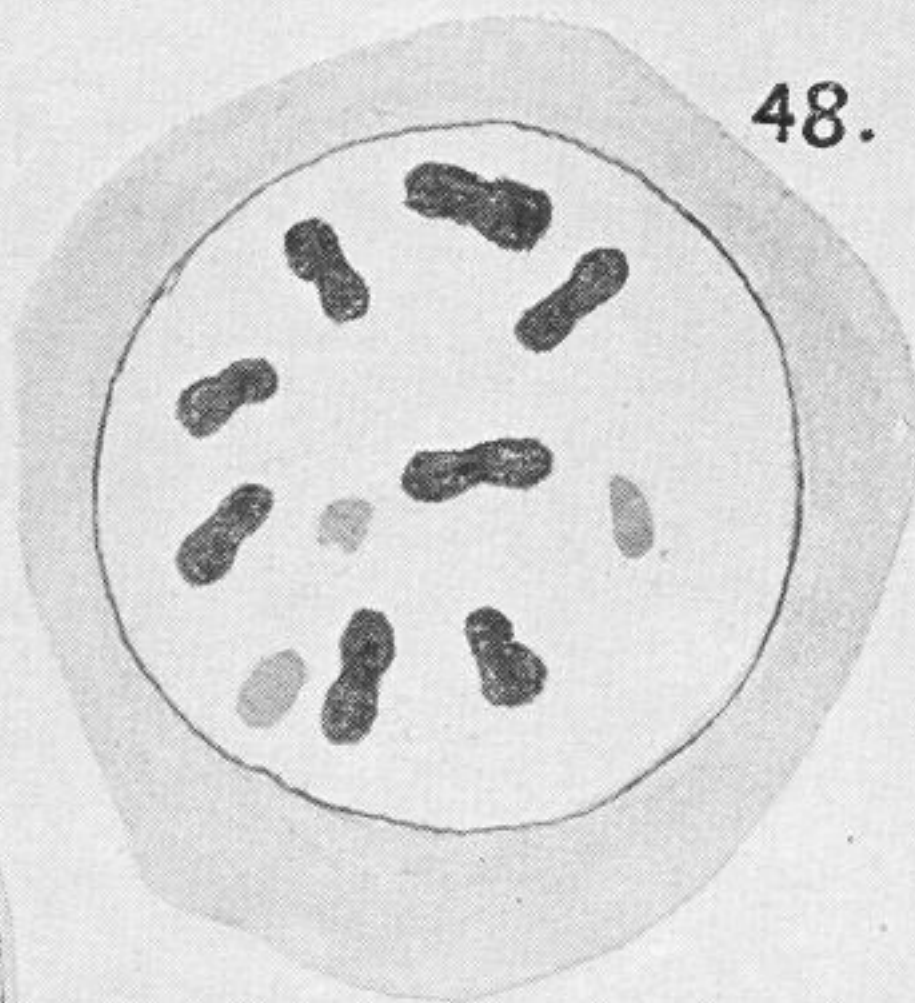
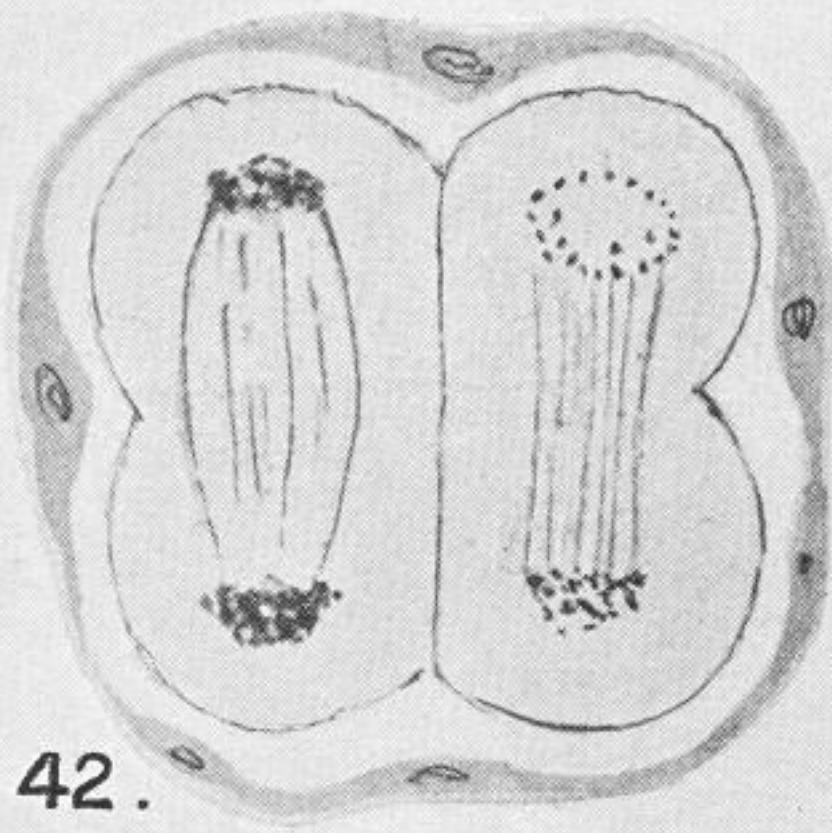
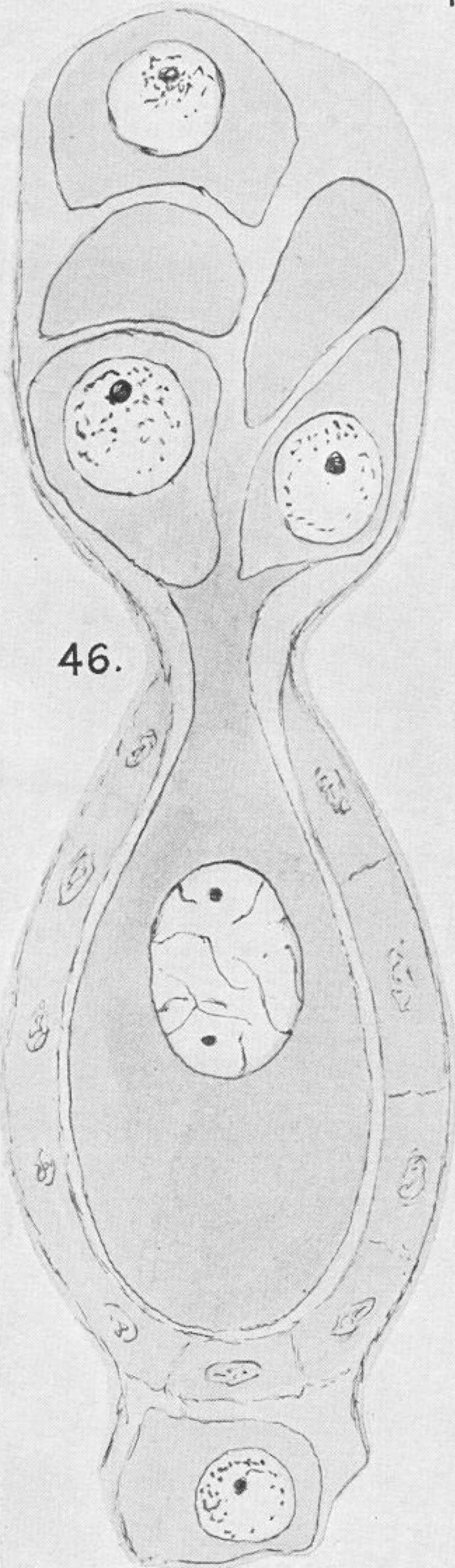
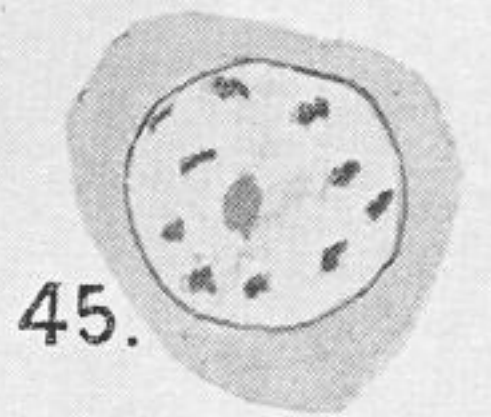
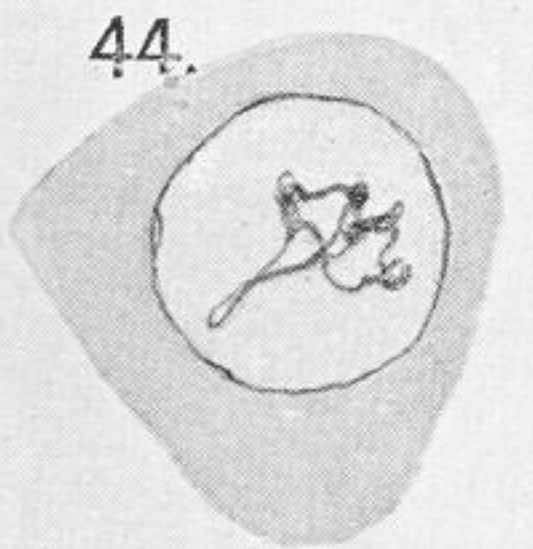
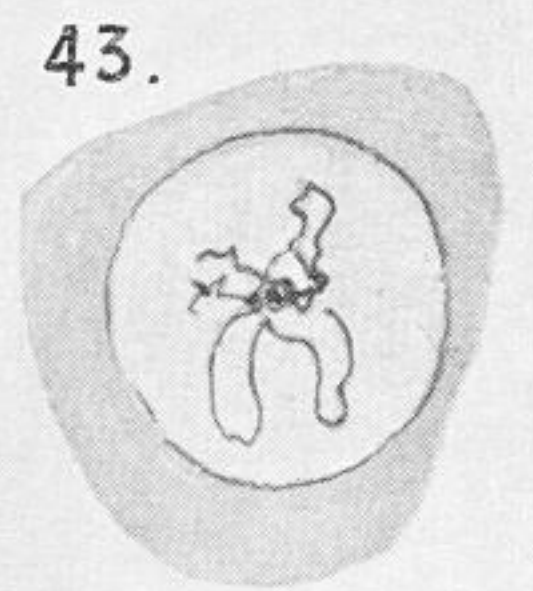
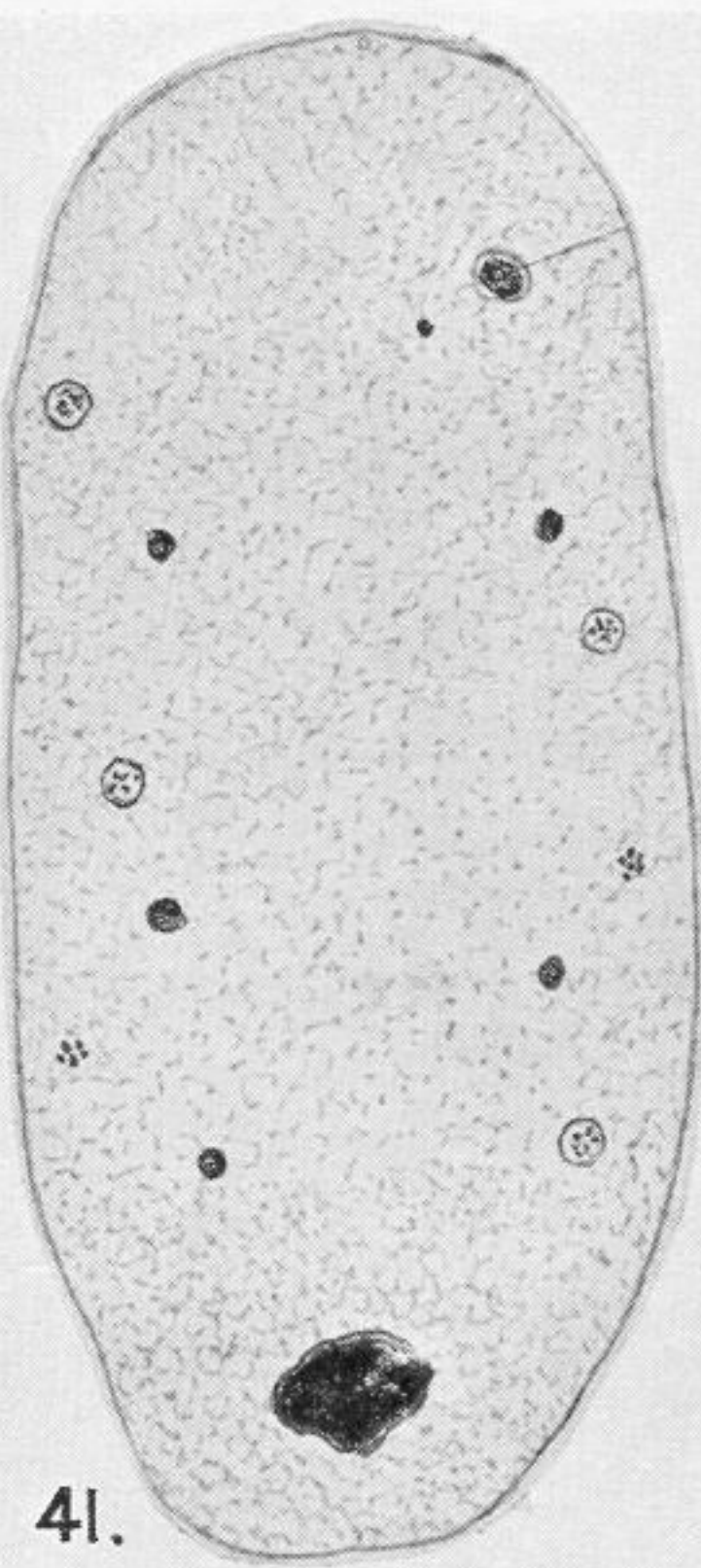
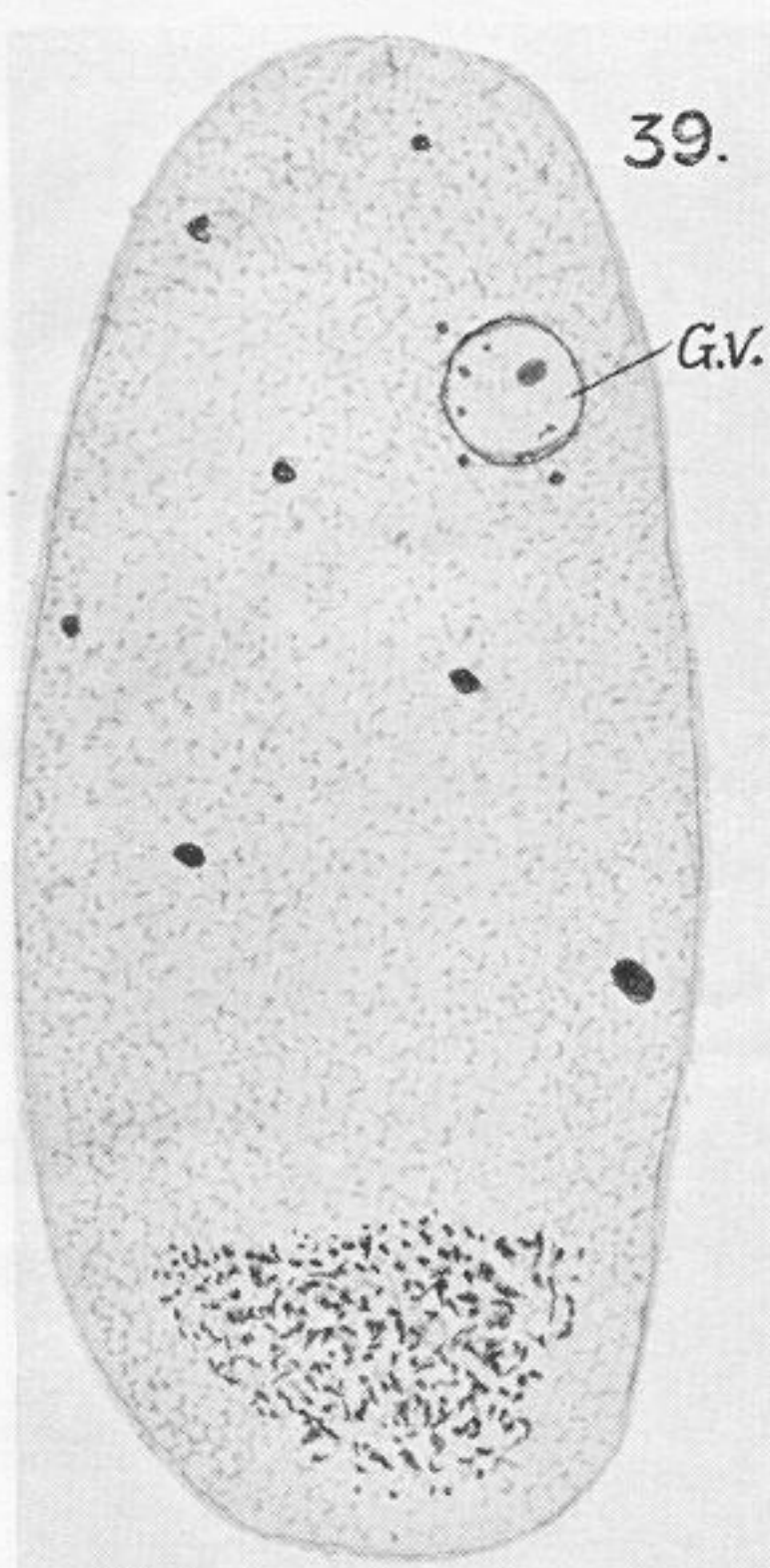


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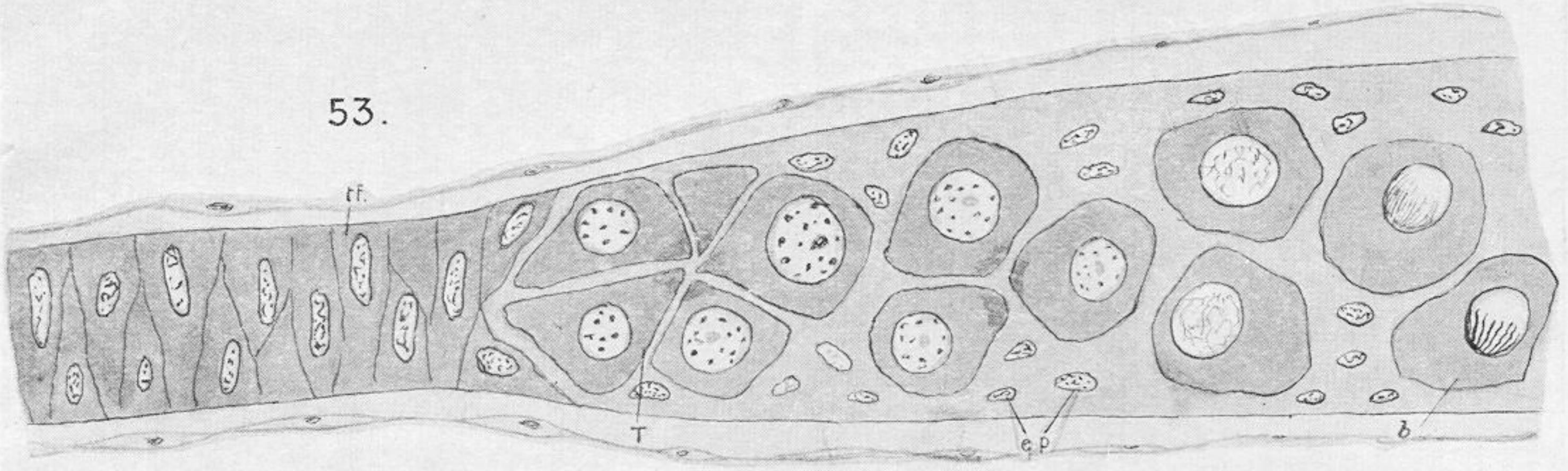


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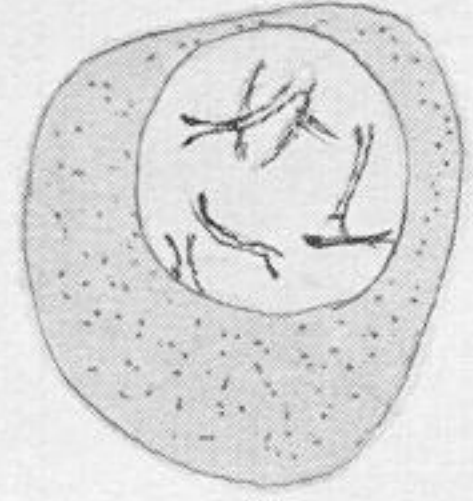
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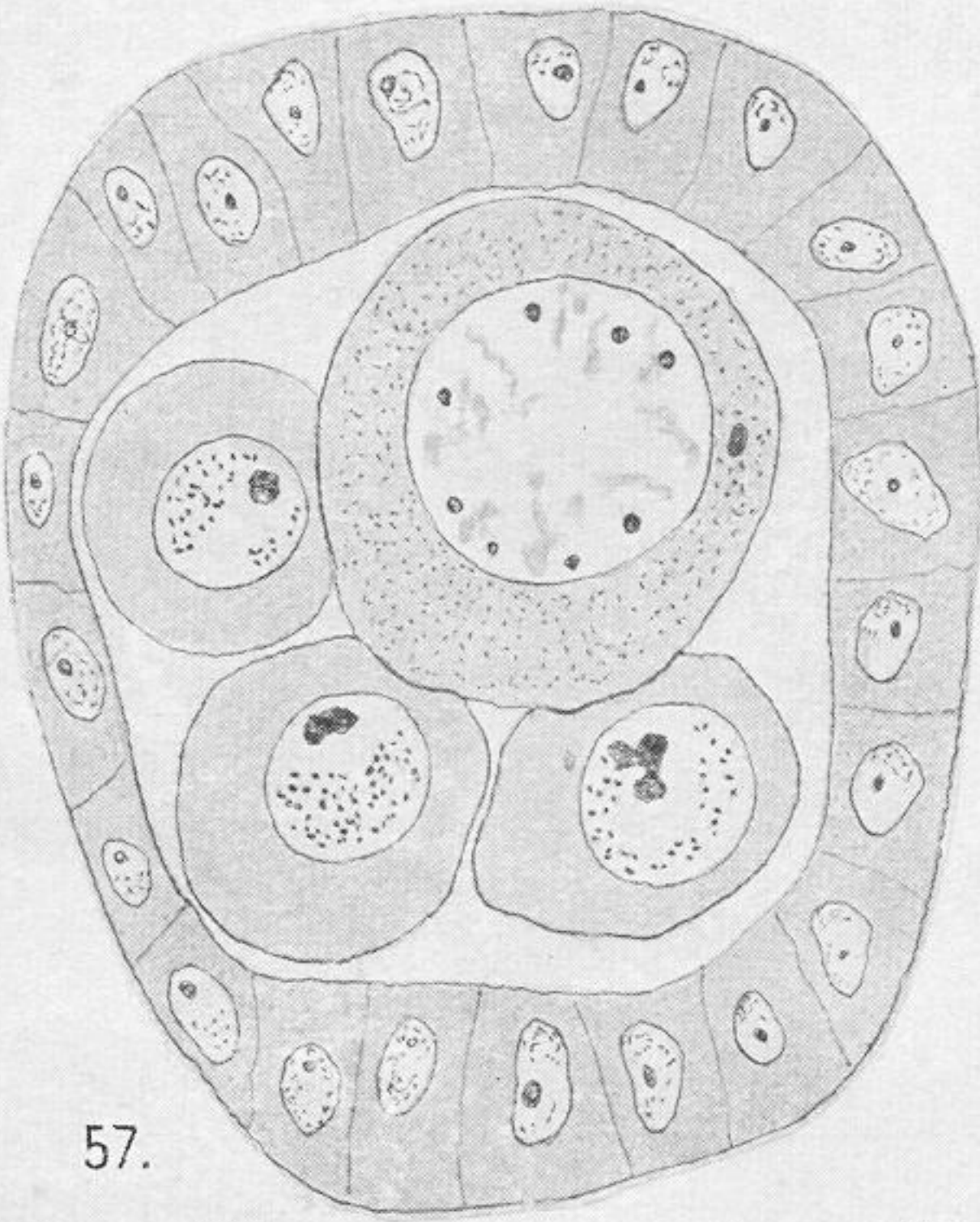
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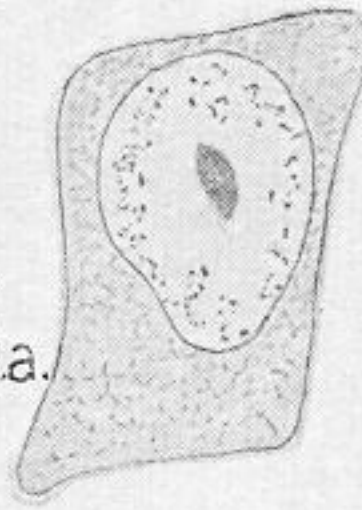
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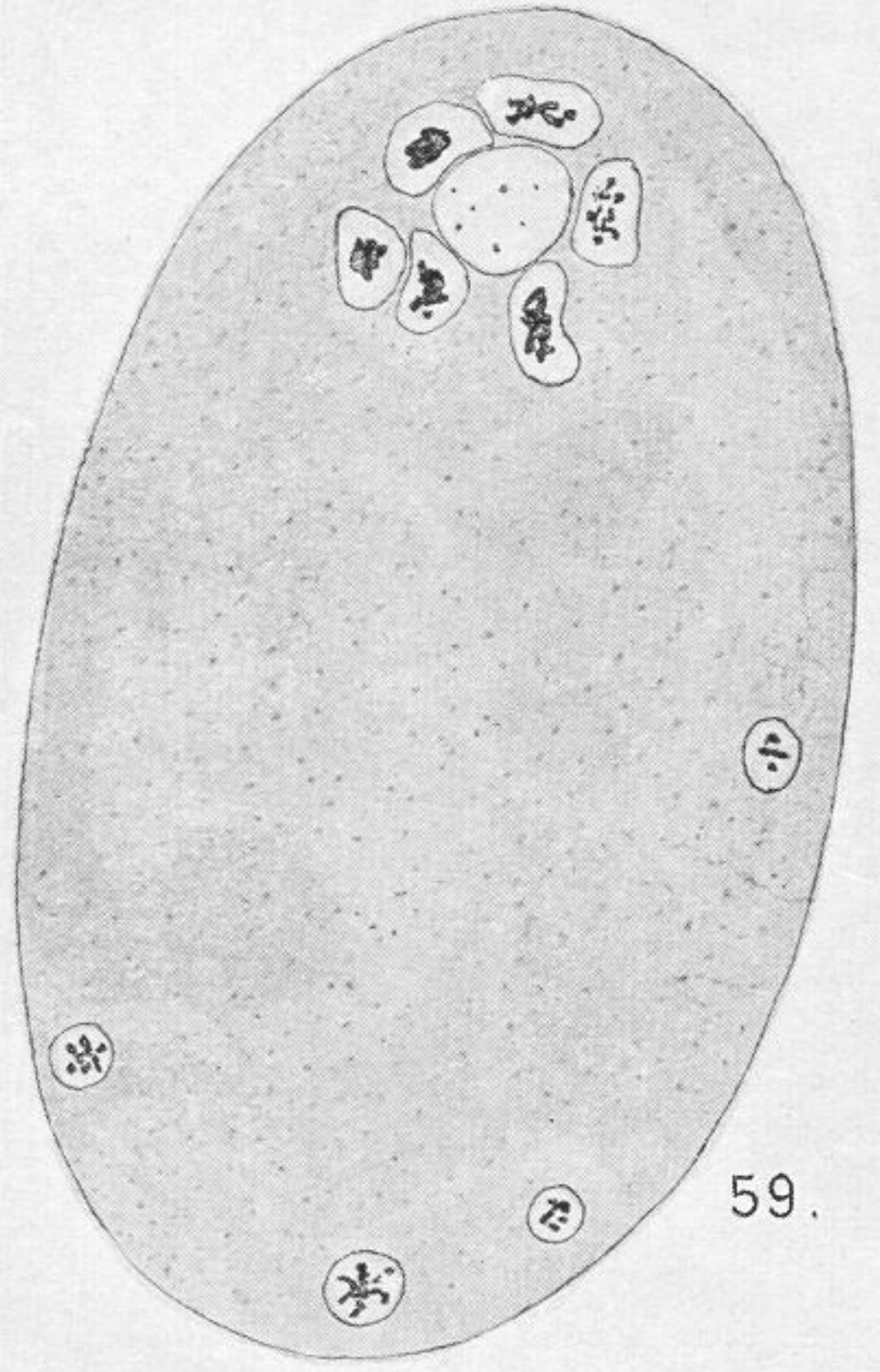
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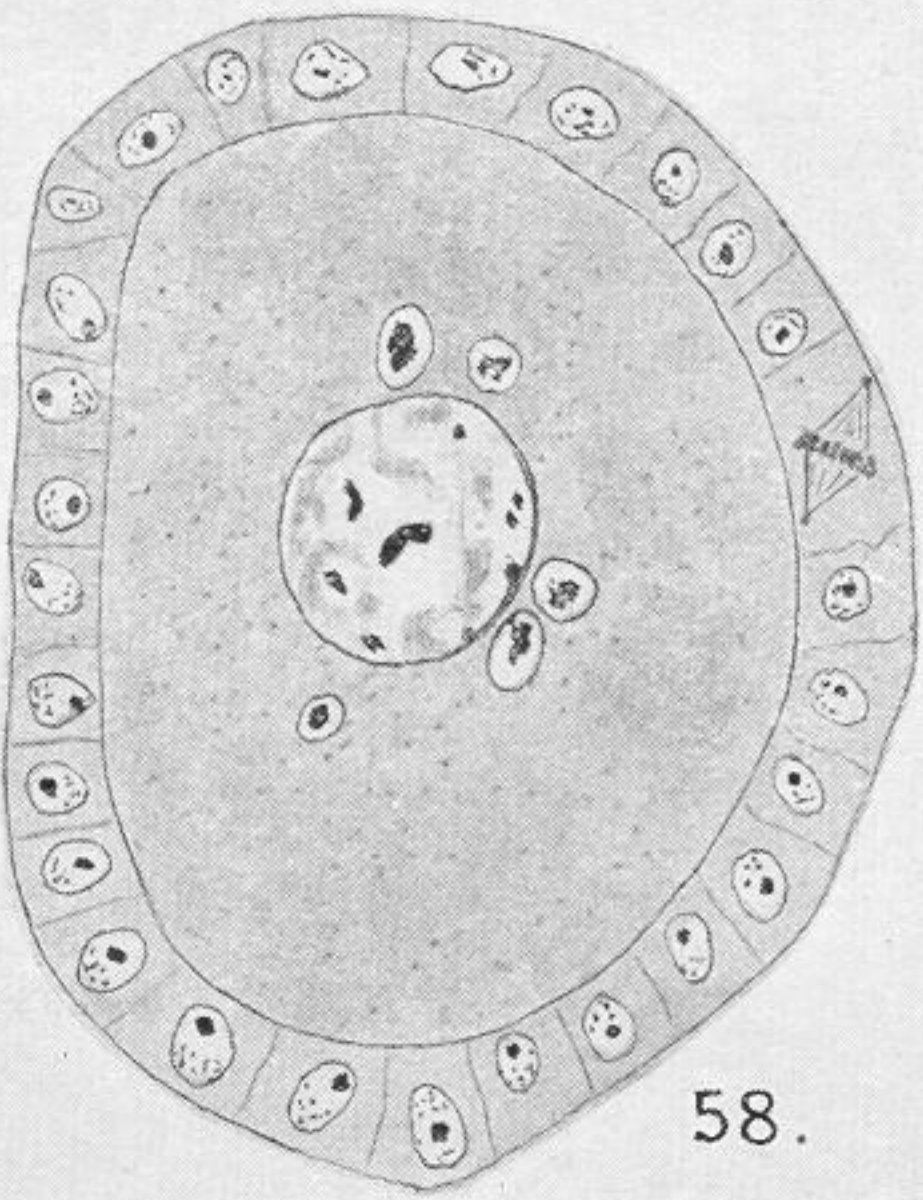
60a.



59.



58.



60.b.

