CONTRIBUTIONS TO OUR KNOWLEDGE OF THE LIFE-HISTORY OF *PINUS LONGIFOLIA*

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CONTENTS.

Contributions to our Knowledge of the Life-history of Pinus longifolia.

I. Introduction.

The cytology of some foreign species of *Pinus* has been worked out but so far no Indian (Asiatic) species of the genus has been the subject of such study. It was therefore thought desirable to investigate the cytological life-history of an Indian species and the writer, acting on a suggestion of Prof. Kashyap, began this work in the winter of 1919–20.

There are four or five species of the genus *Pinus* which are found in India, *viz.* *Pinus excelsa*, *P. Gerardiana*, *P. Khasya*, *P. longifolia*. Of these four the last one is found at the lowest altitudes in the Himalayas (2,000–5,000 ft.) as compared with the other species. It is also very often cultivated in the Panjab plains, especially in gardens. As it is common in Lahore it was decided to investigate this species.

The present studies were carried out in the Botanical Laboratory of the Government College, Lahore, and the writer wishes to express here his best thanks to Prof. Kashyap for kind interest in the work and many helpful suggestions and criticisms.

II. Material and Methods.

The collection of material was begun in Jan. 1920 and was continued up to Jan. 1927 excepting the winter of 1923-24 when the writer was away from Lahore. Collections were made once a week or oftener. For following fertilization stages and the reduction divisions in the pollen-mother cells collections had to be made daily or twice a day. The material was taken from trees in the various gardens of Lahore, *viz.*, the Gol Bagh, the Botanical Garden of the Government College and the Lawrence Gardens. It was fixed immediately after removal from the tree. One collection of material was made near Chamba (Lat. 32° 29′ N., Long. 76° 10′ E., Alt. 3,027 ft. above sea-level) in the Himalayas in the month of July 1920 and it proved very fruitful. Another collection was made from a tree in the Royal Botanic Gardens, Calcutta, in February 1921, for which the writer is indebted to the Curator.

At first the following first two fixatives were used. Later on the third one, which had been used by Miss Ferguson (24) for *Pinus* species, was used :—

I.	Chromic Acid	...	...	3 grms.
	Glacial Acetic Acid	...	...	3 c c.
	Distilled water	...	...	100 c.c.
II.	Corrosive Sublimate	...	...	3 grms.
	Glacial Acetic Acid	...	...	3 c.c.
	Alcohol 70%	...	...	100 c.c.
III.	Chromic Acid	...	...	1·3 grms
	Osmic Acid	...	...	0·5 grm.
	Glacial Acetic Acid	...	...	8·3 c.c.
	Distilled water	...	...	160 c.c.

The following stains were tried at first :—Flemming's triple stain, Iron-alum Haematoxylin, Diamant Fuchsin and Light Green, and Gentian Violet and Orange G. Later on the Diamant Fuchsin and Light Green combination was dropped and only the others were used.

Since ovules in the second year of their growth in *P. longifolia* are pretty large their infiltration with paraffin offered great difficulty. They had to be kept in the paraffin-bath for three weeks continuously before they could be sectioned. Sections were cut 5 to 10 microns in thickness.

III. General Observations.

Pinus longifolia is not only the most easily accessible species of *Pinus* down in the plains of the Panjab because of its cultivation in the gardens but it is also the only species in the outer Himalayas at certain altitudes. The main upright trunk of the tree gives off several

tiers of branches. The habit is indicated in plate I fig. 1. The lower branches bear only the male cones and it seems that at first a young tree produces only male cones. Only the topmost branches and occasionally the lower branches give rise to shoots which bear the female cones. The branches are thus strictly male or female unlike the case well known in *P. silvestris*. On a male branch one can easily count thirteen years' growth externally. The remains of the whorls of scales which protect the young male cones at successive intervals indicate the growth of successive years. Similarly on a female branch one can easily see the cones of four successive generations attached to the branch (plate I fig. 2). It is rarely in the lower branches but not so rarely in the upper branches that male shoots are given off by the female ones.

In Lahore, the male cones are initiated in September. They grow slowly but continuously till pollen is shed in February. During the first week of February the male cones elongate considerably and in the second and third week of that month begin to discharge their pollen.

The female cones are initiated in January and become visible in February at the ends of the youngest female shoots. Generally two or three female cones are produced by a shoot but sometimes as many as five may be produced. The cones are reddish green in colour and open at first. The bract-scales are larger in size and more prominent than the ovuliferous scales at this time. After pollination, which takes place during the latter half of February, the ovuliferous scales grow more vigorously than the bract-scales and consequently the cones close up. Strasburger (67) stated in 1887 that 'after full pollination the fruit-scales close together again by their edges and are glued together by resin'. This statement was modified in 1900 (69) into: 'After full pollination the fruit-scales soon close together, and these are consolidated by the inter-growth of papillae'. The last statement stands as such in the 1924 edition (70) of Strasburger's book. In *Pinus longifolia* no papillae can be made out. The closure is simply due to the rapid growth of the ovuliferous scales. This growth is more rapid on the dorsal than on the ventral surface.

The safety of the pollen-grains within the pollen-chamber of the ovules is secured exactly in the same way as described by Ferguson (24) for *Pinus* species and by Ottley (54) for *Juniperus communis*.

The female cones even after pollination keep on growing till they become about an inch in length. After this their growth is stopped in May and they rest. In the resting condition the cones become brown externally.

The writer's observations fully support the statement of Ferguson (24): 'The evidence is conclusive that the ovules are not organized

in the species of Pinus studied by the writer until about three weeks or less before pollination'.

New foliage leaves come out in March and April in Lahore. The male branches produce the dwarf shoots just after producing the male cones and therefore above them. The female branches produce the dwarf shoots first and then the female cones. Therefore the dwarf shoots on the female branches are below the female cones produced in the same year.

Although a very large number of cones from a fairly large number of trees have been studied no bisporangiate cones were met with as reported by Steil (65) for *P. montana* and by Goebel (30) for *P. maritima*.

IV. Development of female prothallus, fertilization and embryo formation.

Female Cone and Megaspore-Mother-Cell.

As stated above, the female cones are initiated in Lahore in January. By the middle of February ovules show the integument and the nucellus. In the latter a deep-seated megaspore-mother-cell was seen on February 18, 20 and 22 in 1921. This megaspore-mother-cell is always more or less conical, the broader end being invariably towards the chalaza. The walls of this cell are very delicate. The cytoplasm is very finely granular and shows here and there a vacuole. The nucleus of the megaspore-mother-cell in the earliest stages is only slightly larger than the nuclei of the surrounding nucellar cells. The nucleus evidently grows and so does the cell as a whole. Ultimately the diameter of the nucleus becomes twice as large as that of the nuclei of the neighbouring nucellar cells, when the first heterotypic division takes place.

The nucleus of the resting megaspore-mother-cell does not stain so deeply as its neighbours (plate II fig. 3). The reticulum is very faint and the chromatin granules very small. It is only during the early prophase of the heterotypic division that there is an appreciable increase in the staining reaction of the nucleus.

The megaspore-mother-cell may continue to grow for three weeks. Fig. 5 represents it on February 18, while fig. 6 shows the stage of the nucleus which obtains on March 5. Both the figures have been equally magnified so that the increase in the size of the nucleus can be easily visualized.

This megaspore mother cell undergoes a reduction division. Plate II fig. 4 shows the nucleus of the megaspore-mother-cell during prophase when the chromatic material has organized itself into a spireme. Plate II fig. 5 represents the same undergoing the first

contraction. Plate II figs. 6 and 7 represent still later stages when the bivalent chromosomes are being formed. Other stages were not observed but it may be assumed that a linear tetrad is formed. A spongy tissue differentiates about the megaspore-mother-cell when the latter enters upon the first heterotypic division.

Only a few free nuclei are formed by the division of the megaspore nucleus before the resting period begins in May. The number of these free nuclei is never so large as 32 as Ferguson found in some cases but she states, 'the number may not be fixed in the same species, but it is certain that it is never very large' (24).

Growth in the female cones of the previous year is resumed every year in the month of February. The new parts of the ovuliferous scales formed after the resumption of this growth are green. Thus the apical brown region of each ovuliferous scale is carried up and represents the previous year's growth.

The endosperm develops vigorously, but till the last week of March only a peripheral layer of cytoplasm with free nuclei can be seen. Development seems more rapid in April, and by the middle of this month the endosperm is fully matured.

It is needless to mention the gradual transition from the free nuclear state of the endosperm to the fully formed female prothallus. No disparity is met with in *P. longifolia* in this regard when compared with the species worked out by Ferguson (24). Neither has the writer made an attempt to count the number of free nuclei which are formed before wall-formation sets in, this being particularly difficult in *P. longifolia* because of the very large size of the embryosac. The latter is about 8 mm. in length when wall-formation sets in.

External Morphology of Female Prothallus.

When mature the female prothallus is a colourless cylindrical body slightly tapering towards the two ends. It measures from 11 to 12 mm. in length. It is slightly compressed from the abaxial and the adaxial sides. The longer and shorter diameters are approximately 2·6 and 2·3 mm. respectively. At this time the glandular cells of the spongy tissue surrounding the prothallus which are so conspicuous in the early stages, were much reduced. Close to the micropylar end their crushed remains only could be seen, while at and close to the chalazal end the cells still persisted. The prothallus is externally bounded by the megaspore membrane which is quite conspicuous. This membrane is equally thick all round the prothallus except towards the micropylar end where it gradually becomes thin.

Thomson (74) who has made an extensive study of the megaspore membrane in five genera of the Abietineae states 'that in this group the coat is thick in the chalazal region, and thins out gradually towards

the micropylar portion of the prothallium being not more than one-third as thick at the apex as at the base of the macrospore.' Lawson (39) for *Pseudotsuga* and Miyake and Yasui (47) for *Pseudolarix* have made similar statements.

Internal Morphology of Female Prothallus.

The prothallus is made up of thin-walled polygonal parenchymatous cells which are compactly arranged. Two or three layers of cells towards the periphery are smaller than the cells towards the central region. The cells are uninucleate, contain very little protoplasm and very large vacuoles. No binucleate or multinucleate cells could be seen. The cells do not contain any starch granules at this stage. Later on the cells of the central region surrounding the growing embryo become very rich in starch.

Only the micropylar end of the prothallus bears the archegonia The number of archegonia ranges between 2 and 7; 3, 4 and 5 being the commonest numbers. The fertile end bearing the archegonia may be occasionally slightly constricted off from the rest of the prothallus. The archegonium is about one mm. in length. Its external shape and diameter depend upon the number of archegonia borne by the prothallus; if they are numerous they are narrow; if few they are broad. They are visible to the naked eye as opaque white oval bodies aggregated around the micropylar end. The longer axes of the archegonia are almost parallel to the longer axis of the prothallus or the necks of the archegonia may make acute angles with the longer axis of the prothallus. The archegonia have more or less deep archegonial chambers. Each archegonium has a neck usually consisting of eight cells in two tiers with four cells in each tier. In rare cases the archegonial neck consists of four cells only in a single tier at the mature stage. Ferguson (24) writing about the necks of the 5 species of pines which she studied states, 'In *P. Strobus* the typical neck of the archegonium consists of 4 cells, all lying in the same plane, while in *P. austriaca* and *P. rigida* it is made up of 8, disposed in two layers of 4 cells each, but there is a lack of uniformity both in the number and in the arrangement of these cells, not only in different but in the same species.'

All the archegonia have their necks opening towards the micropyle. Each archegonium is surrounded externally by its own layer of jacket cells characterized by large nuclei and specially granular cytoplasm. When the number of archegonia is small, the parenchymatous cells of the prothallus fill in the space between the jacket cells of the different archegonia, but when the number of archegonia is large, the jacket cells of adjacent archegonia are in contact with one another and there are no filling parenchymatous cells of the prothallus.

In some cases, though rarely, one finds two adjacent archegonia having a single layer of jacket cells in common between them for some distance. In describing the jacket layer of the archegonium in *Pseudolarix*, Miyake and Yasui (47) make a very similar statement. 'As in other *Abietineae* the archegonia are arranged quite separate from one another, and they may come in contact in the middle region where they are widest. Even there the egg-cells never come into direct contact as in the *Cupressineae* and some of the *Taxodineae* but they are always separated by one or two layers of jacket cells.' Doyle (19) describes an identical thing taking place in *Larix leptolepis*.

The Development of the Archegonium.

Generally the archegonial initials can be located when the prothallus shows a continuous tissue. This happens in the first week of April in Lahore. Near the micropylar end the cells of the prothallus are cylindrical with their longer axes parallel to the longer axis of the ovule. Later on the nuclei of some of these cells become larger than those of their neighbours. Such cells ultimately become the archegonial initials (plate II fig. 8).

The archegonial initial seems to grow rapidly in size. The nucleus at first occupies a central position but it soon moves towards the micropylar end of the cell (plate II fig. 9). After reaching this place the nucleus undergoes a mitotic division and the resultant two cells are the primary neck cell and the central cell (plate II fig. 10). The primary neck cell undergoes three repeated divisions and so forms 8 cells of the neck in 2 tiers of 4 cells each. The cells of the prothallus surrounding the developing archegonium near the apical region grow more vigorously than the former and so an archegonial chamber is formed. The central cell increases in size very rapidly and its nucleus lies just below the neck (plate V fig. 11 and plate III fig. 13). This sequence of development agrees closely with that described for other species of *Pinus* by Ferguson (23, 24).

The layer of jacket cells which invests each archegonium is not distinguishable till about the 14th of April *i.e.*, about 8 days after the initials are seen.

The Cutting off of the Ventral Canal Cell.

While lying just below the neck the nucleus of the central cell initiates the mitotic division which produces the egg-cell and the ventral canal cell (plate II fig. 12). This event took place on the 21st of April in Lahore in 1922. In the 1921 collection this division was observed on the 29th April. Some archegonia of this collection showed the maturation of the egg nucleus. Before division the diameter of the central nucleus measures about 54 microns. It contains one big nucleolus and two or three smaller nucleoli. The

bigger one occupies a central position while the smaller ones are peripheral. As the central nucleus prepares for division the cytoplasm of the egg becomes much more granular although the vacuoles in it are as numerous and prominent as before. The nucleoli disappear and the chromatin condenses at various places on the net-work. The spindle is formed wholly within the nucleus. It is bluntly pointed at the two poles but appears very broad if the sections do not pass through its median plane (plate II fig. 12). The chromosomes when they are orientated at the equator of the spindle are U or V shaped and not straight as Blackman (3) found in *P. silvestris*. This is not so important as chromosomes are known to undergo slight changes of shape at different periods in the same species. The equator of the spindle in the earlier stages of the division lies exactly between the two daughter nuclei and is straight. Later on it becomes concave on the side of the ventral canal nucleus (plate III fig. 14). A similar figure has been given by Chamberlain (7).

The Ventral Canal Cell.

The chromosomes which go to form the ventral canal cell do not round off after collecting at the pole but become aggregated in a more or less flat plate as seen in (plate III fig. 14). They do not undergo the typical telophasic changes which ordinarily result in a resting nucleus. The cell-plate is laid down and a fragile transverse wall is formed between the daughter cells. No nuclear membrane is formed around the ventral canal nucleus and it shows signs of disorganization from its very inception. The behaviour of the central nucleus during the cutting of the ventral canal cell agrees very closely with that described for *Pinus* species by Ferguson (24), for *P. silvestris* by Blackman (3), and for *Picea excelsa* by Miyake (46). Chamberlain (7), however, states that a nuclear membrane is formed around the ventral canal nucleus. The ventral canal cell is liable to split off from the egg cytoplasm in the region of the equator of the spindle and is generally to be seen as a mass of deeply staining substance with a little cytoplasm on the egg side of it. It persists sometimes till the fertilization time which occurs one week after the division of the central cell.

Although a fairly large number of ovules have been sectioned and a large number of ventral canal cells studied, in no case did the writer find it equal in size to the egg nucleus as reported by Chamberlain (7).

Maturation of the Egg Nucleus.

While the ventral canal nucleus shows signs of disorganization very early, the egg nucleus begins to grow in size very soon. It begins to travel down towards the centre of the egg (plate V fig. 15). It may be seen travelling down while the fibres of the spindle of the

previous division have not as yet disappeared. The longer diameter of the nucleus before the commencement of this downward movement is about 18 microns but only a short distance below it measures 32 microns. Still lower it measures 90 microns and when it has reached the centre of the egg it attains the enormous longer diameter of 202·5 microns (plate V fig. 16). It is ovoid in shape at this stage. It shows a tangle of fine threads to which are anchored numerous small rounded vesicles. No vacuoles are to be seen in the egg cytoplasm at this time. The cytoplasm becomes much more granular than before and the granules are bigger in size. Proteid vacuoles make their appearance in the cytoplasm and they look like ordinary nuclei. Each one of these shows a fairly large deeply staining body lying in the centre and a peripheral lightly staining area bounded by a definite wall-like structure where it comes into contact with the cytoplasm. This can be made out in (plate V fig. 16). A similar structure of the proteid vacuole is described for *Picea excelsa* by Miyake (46). He states, 'The origin of the proteid vacuoles is not at all clear although there is no doubt about their being a kind of nutritive substance.' In regard to the migration of the whole nuclei from the jacket cells into the archegonium described first by Arnoldi (2), the present writer's observations are in accord with those of Ferguson (24), Murril (51), and Miyake (46) who all failed to see such migrations.

The writer opines that the proteid vacuoles are formed from ordinary vacuoles which are quite numerous in the egg. These vacuoles get filled up with a substance which is secreted by the egg cytoplasm and ultimately form what have been termed proteid vacuoles. Plate III fig. 17 shows vacuoles in the course of being filled up. It is believed that these proteid vacuoles serve a nutritive function. The writer had written out this part of the text when he saw a recent paper by Chamberlain (11) on the 'comparative morphology of cytoplasm and chromatin.' Chamberlain describes that ordinary vacuoles in the eggs of cycads get filled up with globules which stain deeply with safranin and iron-alum-haematoxylin. Obviously he is referring to the proteid vacuoles.

It may be noted in passing that all the archegonia in a prothallus at a certain time are at the same stage of development till the maturation of the egg. If in one the central cell is dividing to cut off the ventral canal cell, the others in the same prothallus would, it is highly probable, show a karyokinetic figure at the same stage.

The Receptive Spot.

When the egg nucleus has become mature, a large circular cavity appears in the egg cytoplasm just below the neck (plate III fig. 18).

Sometimes its outline indicates that it has been formed by the coalescence of two or more such cavities. The writer agrees with Ferguson (23, 24) in regarding this cavity as the 'receptive spot.' Other authors, for example Blackman (3), regard this cavity to have been formed by the sudden in-rush of the contents of the pollen-tube, but since it has been seen by the writer before the pollen-tube has discharged its contents into the archegonium, it is not formed as supposed by Blackman. It is very likely that this cavity or vacuole serves to keep a clear passage ready for the contents of the pollen-tube to enter the cytoplasm of the archegonium. This is further borne out by the fact that this vacuole becomes filled up with the cytoplasm discharged by the pollen-tube into the archegonium.

Contents of the Pollen-Tube.

The progress of the pollen-tube and the divisions of the nuclei in it have not been worked out by the writer in *P. longifolia*. When the pollen tube is just above the archegonium (*i.e.*, between the female prothallus and the nucellus) it contains the stalk cell, the tube nucleus and the two male nuclei enveloped in a common sheath of cytoplasm. Plate V fig. 19 shows the end of the pollen-tube just between the female prothallus and the nucellus containing the two male nuclei in the common sheath of cytoplasm. The male nuclei appear to be in advance of the other two at this stage. The stalk cell and the tube nucleus were seen in the other succeeding sections at about the same place. When the pollen-tube passes through the archegonial chamber of the egg and reaches the neck, the neck cells are in many cases crushed but in some cases they retain their identity. Then the pollen-tube opens by a definite pit and discharges all its contents into the egg. Some starch grains and some cytoplasm are also passed in. (The writer has not tried the iodine test on these particular grains but since the pollen-tube and the surrounding nucellar cells contain starch grains at a slightly earlier stage, it is highly probable that these grains which are passed in are also of starch). Generally the cytoplasm of the egg and that from the pollen-tube cannot be distinguished from each other but in some cases the cytoplasm from the pollen-tube is very large in quantity and takes a different shade of the stain from that which the cytoplasm of the archegonium takes. Plate III fig. 20 shows quite a large quantity of the cytoplasm which has been passed into the egg from the pollen-tube.

Fecundation

On entering the archegonium the two male nuclei free themselves from the enveloping sheath of cytoplasm. They differ slightly in size and it is the large one which is seen moving towards the female

nucleus just as Ferguson (24) found in the species of pines on which she worked (plate V fig. 21). Ferguson says that there is no bulging of the egg nucleus towards the male nucleus when the latter approaches the former, but in one of the writer's preparations (plate V fig. 22 and plate III fig. 23) the male nucleus is seen approaching the female nucleus and the latter has produced a distinct bulge towards the former. Corresponding to the bulge on one side of the female nucleus, there has been produced a concavity on the opposite side. But how far this is normal the writer is not prepared to vouchsafe. Blackman (3) in comparing the sizes of the larger male nucleus and the female nucleus states, 'The ratio of the diameter of the male nucleus (which has a nearly spherical form) to the long axis of the (ellipsoidal or egg-shaped) female nucleus at the time of the fusion is usually about one-third, but cases have been observed in which it is as much as one-half.' The present writer has not come across any cases in which this ratio approaches one-half. In all the cases which came under his observation the ratio was about one-third.

On reaching the female nucleus the male nucleus becomes lodged in a depression on the upper side of the former (plate V fig. 24). This is figured from a preparation fixed near Chamba on 1st July, 1920.

The next stage which the writer was able to secure corresponds exactly to Blackman's (3) fig. 23, plate 13. It shows the former limits of the female nucleus within which towards the upper side the first segmentation spindle is formed (plate III fig. 25). The spindle is wholly intra-nuclear and the paternal chromosomes cannot be distinguished from the maternal. A number of thin rod-like chromosomes can be seen. This stage was fixed at Lahore on 30th April, 1921. Now Chamba is 125 miles, as the crow flies, to the north-east of Lahore. The latitude of Lahore is 31° 30′ N ; the longitude is 74° 15′ E ; and the altitude 720 feet above sea-level. The latitude, longitude, and altitude of Chamba are 32° 29′ N, 76° 10′ E, and 3,027 feet above sea level respectively. For this distance and altitude the development near Chamba is late by two months.

The two nuclei formed as a result of the first division are seen in (plate V fig. 26). These nuclei divide simultaneously and very soon. Plate III fig. 27 represents one of them. The directions of these mitotic figures bear no definite relation to the direction of the preceding mitotic figure. The spindle is not surrounded by a clear space crossed by bands of protoplasm as figured by Blackman (3) for *P. silvestris*.

Fate of the other nuclei from the pollen-tube.

The second male nucleus, and the stalk cell are disorganized very quickly. In some cases by the time the fertilized nucleus has divided

into four segmentation nuclei they cannot be traced at all. The writer has seen one of these nuclei dividing mitotically just beneath the neck of the archegonium as described by Ferguson (23, 24), but it is extremely hazardous to state which it was. The tube nucleus which is fairly large (or which is the largest of all the nuclei in the pollen-tube) has been seen by the writer lying immediately below the neck-cells of the archegonium in many cases and so blocking the passage. It is often quite spherical and contains some dark staining granules. In other cases it is somewhat compressed and shows a distinct reticulum. The writer thinks that the former case shows only a disorganizing tube nucleus at a later stage than that at which it shows a distinct reticulum. When it is disorganized completely a very thick wall-like structure is to be seen below the neck which the writer takes to represent the last remains of the disorganized tube nucleus.

The Four Segmentation Nuclei.

The four segmentation nuclei when they lie about the centre of the archegonium are generally elongated in form. They are surrounded by fibres which lie very close to the nuclear membrane. These fibres become more pronounced during the descent of the nuclei towards the base of the archegonium (plate IV fig. 28). When the nuclei have reached the base of the archegonium they arrange themselves in one plane. No nucleoli are to be distinguished in these nuclei at any time. Plate IV fig. 29 shows the nuclei arranged at the base of the archegonium. They show an open reticulum at this stage and the staining material is rather small in comparison to the size of the nuclei. The cytoplasm about the nuclei shows a fine granular structure but the granules are slightly bigger than those of the cytoplasm in the upper part of the archegonium. Moreover this cytoplasm surrounding the four nuclei stains differently from the general cytoplasm of the archegonium. It takes the gentian violet readily. At this time the fibres surrounding the nuclei seem to have disappeared.

Pro-embryo and wall-formation.

The divisions of all the four nuclei take place simultaneously. The spindles show a range of variation, the poles being sometimes normal and sometimes truncated. A very obvious change has taken place in the cytoplasm surrounding the dividing nuclei. It shows very big coarse granules but there is an area of clear space with fine granules immediately surrounding each spindle. Only one of these spindles is seen in plate IV fig. 30, although as above remarked all the nuclei divide simultaneously.

The cross-walls in the pro-embryo are formed in connection with the first division of the four nuclei. Vertical wall-formation in the pro-embryo also begins at this stage. Kildahl (34) thinks that the vertical walls are formed in connection with secondary fibres which radiate from the spindle in the first division but since the present writer has not been able to see any radiating fibres, he thinks the fibres which go to form the vertical walls, arise independently in the cytoplasm. The formation of these vertical walls begins in the middle and proceeds both ways, towards the base and the top.

This wall-formation in *P. longifolia* does not fall in a line with the observations of Blackman (3) on *P. silvestris*. This writer reports wall-formation at the 4 nuclear stage. Ferguson, (24) however, disagrees with Blackman and states that 'in the five species of pines which I have studied cell-walls do not arise until after 8 nuclei have been formed.' This is exactly what obtains in *P. longifolia*.

Most of the coarse granules in the cytoplasm referred to above disappear when the pro-embryo shows four lower cells (completely walled) and four upper nuclei (because they are open above plate IV fig. 31).

The next division in the pro-embryo takes place in all the nuclei of the upper tier simultaneously (plate IV fig. 32 . Kildahl (34) states that it may take place in the lower tier also, but this has not been observed by the present writer. Miyake (46) found in *Picea excelsa* that the cells of the upper tier divide first as found in *P. longifolia*. Cross and vertical walls are formed as before. The pro-embryo thus reaches the three tiered stage (plate IV fig. 33) with four cells in each tier. The uppermost nucleus is not seen in the section sketched. The cells of the middle tier seem as if wedged in between the other two tiers.

The next and the last division in the pro-embryo takes place in all the cells of the lowermost tier simultaneously. This again is as it happens in *Picea excelsa* as reported by Miyake (46). Thus the pro-embryo reaches the four-tiered stage. The pro-embryo has now completed its development and consists of three tiers of four cells each, which are completely walled, and the uppermost tier of four cells which communicate with the cytoplasm of the egg on the upper side.

Formation of the Embryo and the Suspensor.

After the pro-embryo has completed its development the cells of the second tier (suspensor tier) from below or the third tier from above, begin to elongate (plate IV fig. 34). A very long suspensor is thereby produced which carries the lowermost tier of four cells (the embryo tier) down into the female prothallus. As the growth of the embryo proceeds the cells of the axial region of the female prothallus

NAME OF SPECIES.	Locality with Latitude and Longitude etc.	Initiation of Female Cone.	Megaspore-mother-cell	Reduction divisions in the Megaspore-mother-cell.	Second Winter Condition of female Proth.	Cutting off of the Ventral canal cell.	Syngamy	Pollination	Author
P. Laricio.	Chicago: Lat. 47° 50′ Long. 87° 37′	Late fall and winter Oct.?	May	...	Parietal layer of free nuclei	21st June	1st July	Mid. June	Chamberlain (7, 10, 14)
P. maritima.	Various Localities	Early March	...	....	32 or more nuclei	...	...	1st Oct.	Ferguson (24)
P. rigida.	Various Localities	Early March	15th May	5th June 1st division	32 or more nuclei	...	...	27th May	Ferguson (24)
P. Strobus.	Various Localities	Last week of April and May	20th June (about)	27th June	32 or more nuclei	...	21st June	Late May or early June	Ferguson (24)
P. austriaca.	Various Localities	...	...	6th June 1st division	32 or more nuclei	...	...	...	Ferguson (24)
P. silvestris.	...	Jan.-Feb.	...	...	...	...	...	End of May	Strasburger
P. longifolia.	Lahore: Lat. 31° 30′ Long. 74° 15′ Alt. 720 ft.	January	Mid.-Feb.	End of Feb. or early March	A few parietal nuclei	29th April, 1921 21st April, 1922	30th April	Mid. Feb. 2nd and 3rd week	M. L. Sethi
P. longifolia.	Chamba: Lat. 32° 29′ Long. 76° 10′ Alt. 3,027 ft.	...	...	...	...	...	1st July	...	M. L. Sethi

TABLE I.

assume an elongated form, parallel to the long axis of the prothallus. Thus they seem to offer less resistance to the downward pushing of the embryo. These prothallial cells and others at a fair distance from the embryo are rich in granular contents which have been tested for starch and give the characteristic blue reaction with iodine solution. This starch seems to be for the nourishment of the growing embryo.

Discussion.

It has been stated that the proximal parts of the basal whorls of scales protecting a cluster of male cones stick on to the branches for thirteen years in some cases. Such branches are almost equal in thickness near their apical and basal ends. Apparently, therefore, it seems that very little secondary growth takes place, if at all, during these years. It has been observed that however young an axis may be (even two or three years) if it happens to branch, secondary growth in the parent axis is very vigorous. As a result it grows in thickness very soon. If no branching takes place a shoot may remain without appreciable secondary growth for a long time.

In regard to the female cone and the megaspore-mother-cell the writer's observations are generally in a line with those of Ferguson (24) on *Pinus* species. The case of *Pinus Laricio* (14) in the vicinity of Chicago is rather interesting. In this species the female cone begins to develop at about the same time as in *P. longifolia* but development continues till October when the resting period begins. The resting period begins just in the beginning of winter. In *Pinus longifolia* the resting period begins in May which marks the beginning of summer in Lahore and continues through the succeeding winter. So while the cones in *P. Laricio* continue to grow through summer, those of *P. longifolia* stop their growth just in the beginning of summer. Table I has been compiled for the sake of comparison from the data available for different species of *Pinus*. It is obvious that the schedule for *P. Laricio* given by Coulter and Chamberlain (14) is applicable to *P. longifolia* in Lahore with slight modifications. Syngamy takes place in Chamba about the 1st July. This date is identical for *P. Laricio* in Chicago. It seems, although actual observations have not been made (except the one given above), that the schedule for *P. longifolia* in Chamba may be identical with *P. Laricio* in Chicago.

The megaspore-mother-cell in *P. longifolia* becomes evident very early in the life of the ovule. It is one of the deep-lying cells of the nucellus. The only reference which is not in accordance with this general rule is that for *Larix* (66). In this case Strasburger found an hypodermal archesporial cell, which divided periclinally into an outer primary wall cell(' tapetal cell ') and an inner primary sporogenous cell.

In having a single megaspore-mother-cell *P. longifolia* is similar to the other species of *Pinus* so far investigated and *Larix*. This number is constant in the Abietineae but is not so in Taxodineae and Cupressineae. Another feature which distinguishes the Abietineae from the Taxodineae is the presence of the spongy tissue about the megaspore-mother-cell in the former.

As in the other Abietineae this deep-seated megaspore-mother-cell undergoes meiosis. The female gametophyte develops in the same manner as in the other Coniferales and also in Cycadales and Ginkgoales. The archegonial initials can be located as soon as the gametophyte shows a continuous tissue. They follow the same course of development as those of other Pinaceæ. The venter of the archegonium shows a very vacuolate cytoplasm. When the archegonium nears maturity these vacuoles become filled with a certain substance and form proteid vacuoles. This is exactly similar to what has recently been described by Chamberlain (11) for Cycads. These proteid vacuoles were at one time supposed to be the nuclei of the surrounding jacket cells which had migrated into the archegonium. 'Even after Strasburger had shown that these deeply staining masses are not nuclear in nature, but the best organized are the so called proteid "vacuoles," Arnoldi (2) described the behaviour of migrating nuclei' (14). Coulter and Chamberlain (14) have argued that 'the bodily transfer of nuclei or any solid material is impossible.' Now Chamberlain for Cycads aad the present writer for *Pinus* have actually seen them arising within the egg cytoplasm, so that if final and definite proof against the migration of nuclei was needed, it has been given for two Gymnosperms.

A 'notable feature of the Abietineae is the formation of a true ventral canal cell.' Among the Taxodineae the ventral canal nucleus is cut off but no cell-plate is laid down. This nucleus is sometimes very ephemeral. In the Cupressineae the ventral nucleus is cut off but it is very evanescent, perhaps more so than in the Taxodineae. Among the Araucarineae in *Agathis* a ventral canal nucleus is cut off (Ghose 29) but it is not mentioned whether it is as ephemeral as in the Taxodineae and Cupressineae. In *Araucaria* Burlingame (5) states 'the central cell of the archegonium divides very late or perhaps not at all, except in cases of delayed fertilization.' So *Araucaria* most nearly approaches, in this respect, *Torreya* (15) in which there is no ventral canal cell, the nucleus of the central cell becoming the egg nucleus.' From these observations the general tendency among the Coniferales towards the elimination of the ventral canal cell is quite obvious.

In regard to the period that elapses between pollination and syngamy recorded cases according to Coulter and Chamberlain (14

fall into two categories. One of them includes cases in which pollination occurs in one season and syngamy the next. The other in which pollination and syngamy occur during the same season. *Pinus longifolia* belongs to the first category. Table I gives the relative dates for different species of *Pinus* in different localities.

The female nucleus does not get surrounded by a cytoplasmic mantle as is known for *Torreya taxifolia* (15), *Juniperus* (52, 53) *Taxodium* (13), *Sequoia* (37),,*Thuja* and *Ephedra* (36). From what has been stated above about syngamy, it is clear that *P. longifolia* agrees with other species of *Pinus*. A close series of stages being not available to the writer, he is not in a position to say whether cross-segmentation of haploid number of chromosomes after pairing does take place in *P. longifolia* or not as has been described in *Abies* by Hutchinson (33).

In *P. longifolia* and in all the species of *Pinus* on which Ferguson (24) worked, she found that cell-walls do not arise in the pro-embryo till after eight nuclei have been formed. In *P. silvestris* it is stated by Blackman (3) that wall-formation takes place at the four nuclear stage. So within the limits of a single genus in which the various species show so close a resemblance in other respects, the wall formation in the proembryo may not be similar. In the genus *Picea* (46) wall-formation takes place exactly as in the majority of *Pinus* species. Then in regard to the mode of origin of these walls there seems to be some disagreement. Kildahl (34) states that the vertical walls are formed in connection with secondary fibres which radiate from the spindle in the first division. Ferguson (24) does not say anything on this point. The present writer thinks that these walls arise quite independently of the spindle fibres because he was unable to see any radiating fibres. This mode of wall-formation in the pro-embryo of *Pinus* finds an analogy, though very remote, to the condition met with in *Stangeria* (9). From the figures which Chamberlain (9) gives for this genus, it seems that walls in the pro-embryo are not laid down in connection with any spindle fibres but arise independently in the cytoplasm.

V. Microsporogenesis.

Introductory.

In the years 1920, 1924 and 1925 male cones of *Pinus longifolia* were sectioned by the writer by the paraffin method. Flemming's weak and strong, Herman's, Bensley's and corrosive sublimate (aqueous) solutions were tried as fixatives. It was found that excepting the earlier stages of development the paraffin method after any of the above fixatives was not successful. By this method good preparations were secured up till the formation of the microspore-mother-cells. But as soon as the mother cells entered upon the first

heterotypic division the fixatives failed to fix properly. This seems to be due to the inability of the fixatives employed to enter the cells. And the cause of this impermeability of the cells, the writer opines, is the large quantity of a resinous substance in the sporophylls and the axis of the male cone.

Lawson (37) in his paper on *Sequoia sempervirens* makes the following statement:—"There are few groups of plants that offer more difficulties in the way of cytological research than the Coniferales. The structures that are of greatest cytological interest are usually buried deep in the other tissues thus requiring very careful dissection before being placed in the killing fluids. Then, again, if resin is present, as is usually the case, a rapid penetration of the fluid is impossible." Burlingame (4) working on *Araucaria brasiliensis* mentions some similar difficulties and because of these he could not follow the reduction divisions closely. He figures only a few stages.

Chamberlain (10) recommends a method for following spermatogensis in *Pinus*. That method was tried but could not be followed because the divisions in the microspore-mother-cells of all the sporangia in a cluster of male cones are almost simultaneous. It was always found that the same stage of development obtained in the apical male cones of a cluster as the one found in the basal male cones. So that material had to be fixed in the field day after day.

In the winter of 1925–26 Flemming's weak fluid was again tried. The material after being fixed and washed was dehydrated very carefully beginning with 5% alcohol and brought up through 10%, 20% etc. It was found that even with all these precautions the mother-cells were plasmolysed. In January 1926 Taylor's (72) smear method was tried for the reduction divisions. Taylor in describing his method recommends slides cleaned by a long stay in battery fluid to ensure fixation of the mother-cells. The present writer used ordinary new slides and felt no difficulty whatever. Before making a smear stamens from a cone were always crushed in water and examined in the field to see if they were at the right stage. After this preliminary survey, a cone from the vicinity of the one which showed any desirable stage was taken and cut longitudinally into two halves. One of these two halves was always discarded and the other rubbed smartly with the cut surface downwards on a clean slide to obtain a smear. Examination of the slides after staining showed that by far the best preparations were those in which the cut cone was dragged but once on the slide. Obviously this gave a thin smear and took less time than otherwise. Moreover a second rub would mechanically tend to remove the material which might adhere to a slide after the first rub. The smear being made the slide was then placed with the smeared face downwards on a glass rod in a petri-dish containing

the fixative according to Taylor (72). In doing this great care had to be exercised to bring the entire smear into contact with the fixative all at once. The slide was allowed to remain in this condition for two or three minutes and then turned right side up. The fixative was allowed to act for 15 minutes in all. The slide was then transferred to a tube containing water. A number of slides prepared in this way after one or two changes of water were carried to the laboratory while still in water. Washing in water was done usually for about an hour or so. After bleaching with hydrogen peroxide solution the slides were stained as usual. Heidenhain's iron-alum-haematoxylin was used for staining throughout. No difficulty was met with in obtaining brilliantly stained preparations.

By the above method very good slides were secured and the reduction divisions followed in a very short time indeed. The smear method proved successful where the ordinary paraffin method had failed.

Taylor (72) has employed this method mainly for Monocotyledonous plants. The experience of the writer shows that it can be used for Gymnosperms as well.

The fixative used in 1926 did excellently for the nucleus. When living material was again examined in 1927 it was found that the fixation of cytoplasm was not so perfect. Therefore to fix the cytoplasm more satisfactorily Gatenby's (26) suggestion of leaving out acetic acid from the fixative was followed. Flemming's strong without acetic, Taylor's without acetic, vapours of osmic followed by Regaud's were used simultaneously with Taylor's to compare and contrast the effects on cytoplasm. The fixation of cytoplasm was no better with the first two fixatives than heretofore while Regaud's proved a failure.

Sequence of Development in the Male Cones.

The clusters of male cones are initiated in the month of September in the vicinity of Lahore, Lat. 31° 30′ N., Long. 74° 15′ E. and Altitude 720 feet above sea-level. Material fixed on the 26th September, 1920 showed the individual male cones as conical axillary structures consisting exclusively of meristematic cells. During early October the individual microsporophylls become distinguishable. On the 18th October, 1920 the fertile proximal part of the microsporophyll was observed marked off from the sterile distal part. In cones fixed on the 26th October, 1920 the two microsporangia were seen delimited from each other in as much as there were two or three layers of vegetative cells between two patches of meristematic cells on the two sides. It should not be understood that all the cones in a cluster are at the same stage of development. While those near the base

approximately show the stages for which the dates have been given above, those near the apex are usually much younger at this time.

Gradually by the 11th November the 3 or 4 wall layers of the sporangium get defined. By the 15th December the cells of the outermost layer of the wall get filled up with resin. Such cells in sections appear filled up with a homogeneous substance. Ferguson (24) states that such cells lose their nuclei. The writer has observed nuclei in these cells in cones fixed as late as January 24th. By this time most of the cells of the sporophyll and the cells of the wall except the two innermost layers get this resiniferous deposit (plate VI fig. 35). It is this deposit, the writer thinks, which hinders the penetration of the fixatives by the ordinary paraffin method.

Demarcation of Dehiscence.

The cells of a longitudinal plate of 3 or 4 cells towards the mid-ventral line of the sporangium do not get filled with the resiniferous substance (plate VI fig. 35). This is about the third week of January before the reduction divisions. It is in the middle line of this plate of cells that dehiscence takes place later on when the spores are ripe. So far as the writer is aware no one has made a mention of this demarcation of dehiscence or figured this plate of cells in *Pinus*. In the microsporangium of *Ceratozamia*, however, Chamberlain (8) has described and figured a similar structure.

The tapetum as a distinct layer was made out in cones fixed on the 15th December, 1924. It appears to be the modified outermost sporogenous layer because some cells more like the sporogenous cells lie in between the tapetal cells. Ferguson (24) states that in the species of *Pinus* which she studied except *P. Strobus*, the tapetum cannot be distinguished during the early stages of development from the other tissues. It is first clearly differentiated in spring when the mother-cells are in the early prophase of the heterotypic division. The present writer's observations go to show that the tapetum as a distinct layer is differentiated in *P. longifolia* much earlier in the life-history when compared with *P. rigida*, *maritima*, etc.

The growth and divisions of the sporogenous cells since the earliest stages, are continuous and uninterrupted till the pollen-mother-cells become recognizable in January. In *Pinus longifolia* there is no winter rest either before or after the formation of the mother-cells or pollen grains. The mother-cell stage in *P. longifolia* is reached in January after continued growth and division. Before the reduction divisions the scales in the axils of which the male cones arise are closely appressed to the cones themselves. When the mother-cells have been fully formed and are ready to enter upon the heterotypic division, the scales open out. This is apparently due to

the growth of the male cones which takes place vigorously at this time. So the position of the scales can serve as an index for the reduction divisions. In the year 1925 the reduction divisions in the pollen-mother-cells were observed from the 23rd January to the 1st of February. In 1926 these divisions were observed from the 24th of January to the 1st of February. This overlapping of dates was a very fortunate coincidence. It should not be supposed that reduction divisions take place during the same days every year. There may be a slight shifting backwards or forwards of this time in different years. This shifting has been attributed to climatic factors by Chamberlain (10).

During the first week of February the male cones elongate rapidly, the sporangia open along the middle line of the plate of cells already indicated and the pollen grains are shed.

Tha Reduction Divisions in the Pollen-Mother-Cells.

The mother-cells before entering upon the heterotypic division.— The reduction divisions as stated above take place in *P. longifolia* during the last week of January. The mother-cells immediately before this are very closely packed within the sporangium. Only the tapetal cells are loose and seem to be undergoing disorganization. The mother-cells themselves are polygonal (plate VI fig. 36). The cell walls are thin. The nucleus is very large when compared with the size of the cell and the quantity of cytoplasm contained. The nucleus seems to fill the entire cell cavity, the cytoplasm being confined to the corners only (plate VI fig. 37). In form the nucleus is approximately spherical. It shows a close linin network with small chromatin granules uniformly distributed upon it. The chromatin granules are not confined to the net-knots of the linin threads only. They are found along the threads too, although the size of those granules found at the net-knots may be appreciably larger than those of the rest. The number of nucleoli has been counted in sections and may be 3 or 4 in any one of them. It is very difficult to count their actual number in a nucleus. Their staining reaction is very faint and some of them are vacuolate.

The structure of the nucleus at this time in *P. longifolia* is similar to that described by Lewis (42) for *Pinus Strobus*. The writer can fully support the view expressed by Lewis for *P. Strobus* and Woolery (76) for *Smilacina* that the number of chromatic bodies in the resting nucleus bears no definite relation to the number of somatic chromosomes as has been shown for a number of plants, *viz.* in *Thalictrum purpurascens*, *Helleborus foetidus*, and *Campanula grandis* by Overton (55) and in *Carex* by Stout (71).

The cytoplasm of the mother-cell is vacuolate. There are mitochondria or chrondriosomes both of the filamentous and the spherular type. These latter structures the writer interprets as such because Mottier (50) working by a similar method found chondriosomes in *P. banksiana*. The spherular type of mitochondria were visible in the material in the living condition when examined in the field in water. At that time they looked like blue-green homogenous bodies.

Prophase.—During early prophase the staining reaction of the chromatin granules increases remarkably (plate VI fig. 38). The granules increase in size also till they look like droplets. Their size and number is so large that the original linin threads get obscured. This proceeds on gradually till the reticulum retracts from the nuclear membrane (plate VI fig. 39), and forms a close knot (the synizesis stage) along one side and the major part of the nuclear cavity remains empty (plate VI fig. 40). In many mother-cells it was found that the synizetic knot remains connected with the nuclear membrane by strands as shown by Mottier (49), and Woolery (76). No membrane, however, could be indentified around this synizetic knot as reported by Latter (41) for *Lathyrus*.

Near the close of synizesis stage a continuous thin spireme can be made out within the synizetic knot (plate VI fig. 41). This continuous chromatic thread shows alternating heavier and lighter parts along its length.

These prophasic changes may not be simultaneous in all the mother-cells. While some may be showing early synizesis others may represent close of synizesis. In the cytoplasm the spherular type of mitochondria become more prominent.

The writer could not make out in the pollen mother-cells of *P. longifolia* anything like the fibres which have been figured by Allen (1) in the pollen mother-cells of *Larix* during prophase. Neither has Lewis (42) seen anything similar to them in *Pinus* and *Thuja*.

The chromatic thread seen during the close of the synizesis soon uncoils itself and becomes uniformly distributed within the nuclear membrane but more or less peripherally (plate VI fig. 42). Only one or two nucleoli can be made out within the nucleus at this time, the others seem to have disappeared. The staining reaction of the nucleus as a whole seems to decrease. This may simply be due to the spreading out of the spireme and thus may be only apparent and not real. The spireme is so long that after complete recovery there are so many turns and twists in it that one cannot trace its course within the nucleus. This is the open spireme stage.

It is during this recovery after the first contraction that the mother-cells round off and glide on one another and so become loose.

Lewis (42) reports the loosening of the microspore-mother-cells in *P. Strobus* at exactly the same stage.

After the cells have become loose the spireme shows longitudinal splitting at certain places (plate VII fig. 43).

The spireme gradually shortens and its thickness gradually increases and the split disappears. This is the pachynema stage. Soon after this a second contraction takes place (plate VII fig. 44). As this figure shows this contraction does not result in the formation of such a compact knot as the first contraction. In some cases a more well pronounced second contraction was seen (plate VII fig. 45) but the writer is not sure whether it is well marked in all cases. Fig. 45 shows some very fine achromatic strands radiating from the nuclear knot at this time and reaching the nuclear membrane. By means of these the nuclear knot seems to remain anchored within the latter. Lewis (42) and Ferguson (24) make no mention of this second contraction. When these writers published their accounts the first contraction even was held in doubt. It was believed to be an artifact. Cardiff (6) wrote in 1906 as follows:—'In fact, the knot seems to be as often, if not more often, on the side of the cell where there is least cytoplasm. It was generally found, however, that in any one sporangium or group of sporangia all the knots occupy the same relative position in the nuclei. I offer as a tentative explanation of this, that the chromatin mass is of greater density than the nuclear sap and the position of the nucleolus and knot is due to gravity.' Keeping this in mind the second contraction which is not so pronounced might have been overlooked by these writers, but since that time the first contraction has been recognized as a normal phase in the heterotypic division.

When the chromatic thread recovers from this second contraction it is very much thicker than during any of the earlier stages and shows a number of loops (plate VII fig. 46). No nucleoli could be made out in the nucleus at this time. These loops have been reported for *Lathyrus* by Latter (41) and by Gates and Rees (28) for *Lactuca*. But in these plants the number of loops corresponds to the number of haploid chromosomes. The writer could not decide this point for certain in *P. longifolia*. Plate VII fig. 46 represents a cell where recovery has progressed to a certain stage, while plate VII figs. 47 and 48, where it is complete. The chromatic thread shows a number of large chromomeres. This thread undergoes segmentation and each loop represents a bivalent pair. It can be deduced from the behaviour of the chromatic thread that the univalents came together first of all end to end and then curved over. This shows that the chromosomes in *P. longifolia* conjugate telosynaptically as reported by Lewis (42) for *Pinus* and *Thuja*. Thus the sequence of events corresponds to

Scheme B of Sharp (63). In figures 47, 48, young bivalent chromosomes are more or less clear. At this time their outline is not quite smooth. Each pair shows a number of very large chromomeres. Later on in several bivalents the univalents get twisted about each other. Plate VII fig. 49 represents one such bivalent. So this is the strepsinema stage. The bivalent chromosomes go on condensing and thickening till the diakinesis stage is reached (plate VII fig. 50 and plate X fig. 51). At this stage the bivalents exhibit fairly smooth outlines. They are found very much scattered within the nucleus.

At the diakinesis stage, (plate VII fig. 52 and plate X fig. 53), each univalent chromosome of a bivalent pair, in some cases, shows a longitudinal split. The writer is not sure whether this is the reappearance of the same split which is seen during earlier prophase. This results in the formation of chromosome tetrads. In many cases the chromosomes remain adherent at one end but lie widely apart at the other end. Some of these bivalent chromosomes may remain in telosynaptic union till this stage. Such telosynaptic bivalents have been described during late diakinesis for *Oenothera* (Gates 27) and *Carex* (Stout 71).

No worker, so far as the writer is aware, has reported chromosome tetrads in any species of *Pinus*. They have been reported however in a large number of animals and some plants.

Metaphase.—At the close of the diakinesis the nuclear membrane disappears and the spindle fibres make their appearance. The spindle fibres converge to very fine points at the spindle poles. During metaphase the chromosomes are very thick, stumpy and deep staining. In these metaphasic chromosomes the longitudinal splits which had resulted in chromosome tetrads during prophase become invisible. These splits become visible again during the close of metaphase (plate VIII fig. 54). The chromosomes in this figure are just going to part company. At this point the writer finds evidence to support Lewis (42) rather than Ferguson (24). The former states that the bivalents separate into constituent univalents, while the latter supposes that the chromosomes separate along the longitudinal splits and so half of each somatic chromosome passes to each daughter nucleus and thus this mitosis according to Ferguson (24) effects an equational division.

In figure 54 the split univalent chromosomes are like Vs, the angle of the V being directed towards the pole. The univalent members of a bivalent pair are very much alike in size and shape, *i.e.*, they would coincide exactly if placed one over the other. Bundles of spindle fibres can be seen attached to the apices of Vs.

Anaphase.—As soon as the anaphase begins the distance between the arms of Vs decreases and chromosomes look like Us. Plate VIII fig. 55 represents a cell in which anaphase has progressed pretty far. The chromosomes are pretty long (*i.e.* they are longer than during metaphase or prophase). It may be concluded that they get elongated during anaphase due to a pulling strain being exerted on them by the spindle fibres. However, all are not of the same length. In the two groups of chromosomes, the longer and shorter all show their former mates of corresponding size and shape. From plate VIII fig. 55 one can infer that the separation of the univalents in all the bivalents took place almost simultaneously. The split halves of chromosomes have a tendency to approximate still more closely and may ultimately come to lie almost parallel to each other. These observations are almost identical with those made in *Pinus Strobus* and *Thuja* by Lewis (42).

In each of the two batches of chromosomes four very long chromosomes can always be detected. They show constrictions near their ends which are towards the equator of the achromatic figure.

Telophase.—The chromosomes having migrated to the poles the spindle fibres become very prominent in the region of the equator (plate VIII fig. 56), and can be fairly well made out in living material in the field. There is considerable overlapping of the fibres in the equatorial region. After reaching the poles the outlines of chromosomes remain distinct for a time and the splits are also visible in many of them.

The chromosomes of each daughter nucleus contract into a very compact mass which shows very few light areas. The outlines of individual chromosomes are obviously lost (plate VIII fig. 57). The chromosomes again separate and organize themselves into a network by branching. The branches may be attenuated. The branches fuse and ultimately typical resting nuclei are developed (plate VIII figure 58). This behaviour of telophasic chromosomes corresponds to mode 1 of Litardière (43). There is no thickening of spindle fibres or deposition of granules in the equatorial region of the spindle but in a few cases a very fragile cell-plate has been seen.

No nucleoli appear in the daughter nuclei during interkinesis. Ferguson (24) makes a similar statement.

Cytokinesis.—Karyokinesis is not followed by cytokinesis as is generally the rule. The cell-plate is not to be seen during late telophase. It is concluded that it disappears very soon (plate X fig. 59.)

A constriction furrow develops between the two daughter cells during interkinesis. It is fairly well marked but does not make much progress till after the second homotypic division. Harper (31) found in

Larix that no cell-plate followed the first division of the pollen-mother-nucleus and that the spindle fibres of the primary mitosis were untilized in the formation of the spindle fibres for the second division.

Ferguson (24) on this point states, "Contrary to the observations of Hofmeister (32) no cell wall is laid down and in only a very few instances has a slight thickening of the spindle fibres in the region of the cell-plate been observed." She found no spindle fibres of the first division persisting till the second division as Harper did. The writer does not agree with Ferguson. And neither does she say anything nor does she figure the invagination.

Second homotypic division.—The second homotypic divisions are initiated simultaneously in the two daughter cells formed after the first heterotypic division. The relative position of the two mitotic figures is very variable.

Prophase.—The earlist prophase is denoted by the reticulum of the nucleus becoming more open (plate VIII fig. 60). This is followed by an increase in size of the chromatin granules and their reaction to stains. Some of them coalesce into one another. This proceeds in such a way that a more or less continuous spireme is formed. This spireme in the beginning is not of uniform thickness but looks jagged. Gradually the chromatic material becomes evenly distributed along the length of this spireme. It undergoes a certain amount of shortening accompanied by consequent thickening. Ultimately it segments into a number of chromosomes (plate VIII fig. 61). They are fairly and uniformly thick. The chromosomes lie entangled among themselves. The entire mass of chromosomes fills about 2/3 of each cell (plate IX fig. 62).

Here again, in the behaviour of the spireme the writer finds evidence to support Lewis (42) rather than Ferguson (24). The latter states that the spireme forms loops across the spindle and as cross-segmentation proceeds the loops undergo longitudinal fission. According to Ferguson the spireme takes its position on the equator of the spindle, while according to Lewis and the writer the spireme segments much earlier into the chromosomes. The nuclear membrane disappears and the chromosomes occupy the equator of the achromatic figure (plate IX fig. 63).

Metaphase.—The chromosomes are pretty long structures at this time but not so very thick (plate IX fig. 63). When lying at the equator of the achromatic figure their longer axes are parallel to the longer axis of the spindle. They show longitudinal splits very clearly. The equatorial plate of chromosomes is fairly broad and almost fills the whole of the cell breadth-wise. This mitotic figure presents a very sharp contrast to the same stage of the previous heterotypic division.

The spindle fibres are very weakly developed at this time or they are not brought out very clearly by the smear method. Ferguson (24) also does not figure them very prominently, so that one can infer that they are really weakly developed and that the smear method is not to blame.

The metaphase when compared with the prophase lasts for a shorter time. This, the writer deduces from the fact that in preparations where many cells show prophasic changes in progress cells showing metaphase are much fewer in number.

Anaphase.—When moving towards the poles the chromosomes become bent and so assume the form of Us or Vs (plate IX fig. 64). They lie fairly apart from one another and their angles point towards the poles. During this time the cleavage furrow which appears during the preceding interkinesis makes some progress but it is not sufficient to pinch off the two daughter cells.

Telophase.—The chromosomes after reaching the poles remain grouped together for some time with their ends pointing towards the equator (plate IX fig. 65). The outlines of individual chromosomes can be made out more or less clearly at this time. The spindle fibres which are not conspicuous either at metaphase or during anaphase become very conspicuous (plate IX fig. 65). Soon after the chromosomes contract into a spherical nucleus which develops a nuclear membrane. Within this membrane the chromosomes become united and form a skein (plate IX fig. 66). The skein seems to be peripherally placed and spirally coiled. This is quite in conformity with Ferguson's (24) observations. The skein branches. The branches run into each other. Gradually certain spots on the skein fail to take up the stain. Ultimately the resting nucleus is formed.

Since no vacuolation in the chromosomes could be made out the telophasic changes fall into group 2 of Litardière (43).

While the second homotypic division is in progress the cleavage furrow laid down during the preceding interkinesis makes some progress but does not separate the two daughter cells quickly (plate X fig. 67).

An ephemeral cell-plate can be made out during the second homotypic division. Ferguson (24) does not say anything at all in regard to this cell-plate. During the close of the first division the cytoplasm of the mother-cell separates from the wall. The furrow previously laid down proceeds afar and another invagination appears between the grand daughter nuclei (plate X fig. 67). In this way the cytoplasm of the mother-cell cleaves into four parts. While this is going on a thickening appears on the cell-wall opposite to the first furrow. This grows centripetally. Another thickening but not so large as the former

appears on the cell-wall opposite the second furrow. By the centripetal growth of these thickenings, the mother-cell which has already cleaved into four parts, becomes quadri-partitioned. The tetrads (plate IX fig. 63) may be tetrahedral or bilateral. The young spores develop their wings within the partitioned mother-cell. They ultimately come out through definite openings appearing in the outer walls of the mother-cell.

These observations on cytokinesis were confirmed in January 1927. It is advisable to remark here again that the slight amount of shrinkage which is seen in plate VIII figs. 54, 55 is not at all due to the action of the fixative because the cytoplasm of the mother-cell has been seen detached from the surrounding wall in living material examined in water. So the shrinkage is natural and cannot be attributed to the fixative or any subsequent operation.

So far as the writer is aware, among Gymnosperms, Saxton (61) only has definitely described cleaving of the microspore-mother-cells in *Tetraclinis*. He states 'the cytoplasm cleaves into four equal parts.' It is not stated whether the mother-cell becomes partitioned subsequently or not. In regard to *Juniperus* the evidence is conflicting. Norens (53) states that four spores are formed in an unpartitioned mother-cell. Nichols (52) working on the same genus maintains that the mother-cell becomes partitioned. But more of this hereafter in the discussion.

The Number of Chromosomes.—The diakinesis stage is specially well suited for counting the number of chromosomes. Accordingly at this stage several counts were made. In all clear cases 12 bivalent chromosomes were found. Among these 12, four very long ones could invariably be detected (plate VII fig. 50 and plate X fig. 51). All the rest were almost of the same length. Approximately the longer ones were to the shorter ones as 3 is to 2. So the haploid and the diploid numbers are twelve and twenty-four respectively. For counting the number of chromosomes the smear method is an advantage over the paraffin method, in as much as here one counts them in entire cells and the possibility of a mistake which is always present in counting them from sections is avoided. This is a very real advantage when one has to deal with sporogenous tissue or any other tissues in which the cells are large in size and divide simultaneously, it being very difficult to spot the same cell in two successive sections.

Starch grains in the mother-cells.—Starch grains make their appearance in the cytoplasm of the mother-cells during early prophase. They are to be seen in the cells throughout meiosis and are present in the pollen grains also. They are visible in the living material n the field and stain deeply with iodine. They cannot be seen in

preparations stained with haematoxylin after any of the above fixatives. This is the reason why they have not been shown in the figures. Their structure is homogeneous and a hilum like granule as figured by Saxton (61) in the starch grains of *Tetraclinis* cannot be seen.

The Male Gametophyte.

Two prothallial cells are cut off while the pollen grain is still within the sporangium. These cells are more evanescent perhaps than in the other species of *Pinus* because they disorganize very soon and the pollen at the time of shedding stage shows no indications of them.

Discussion.

The sequence of development of the microsporangia in *P. longifolia* is not in accord with the statements of Chamberlain (10), and Coulter and Chamberlain (14) who state that 'in most of the recorded cases the microsporangium passes the winter approximately in the mother-cell stage.'

In *P. longifolia* the writer has observed the sporogenous cells in an active state of division in December. December, it should be remembered, means mid-winter in the Panjab. The writer's experience is more in accord with Ferguson's (24) who seems not to agree with Coulter and Chamberlain in regard to *P. Laricio*. She states, 'Had Coulter and Chamberlain examined the microsporangia in the latter part of March they would doubtless have found typic divisions taking place in the archesporial tissue.'

In this connection it will be of interest to mention Lawson's (38) work on *Cryptomeria japonica*. In this plant male cones appear in October. Reduction divisions take place in the latter part of October or November, pollen grains are separated before the first of December and pollination takes place in the following March. Obviously in *Cryptomeria* there is no winter rest preceding the reduction divisions. In *P. longifolia* though it takes almost the same time for ripening the pollen, as has been shown above, the development proceeds in a leisurely fashion *i.e.* the first few stages take more time while the last few take less time when compared with *Cryptomeria*.

Robertson (58) reports, the male cones of *Torreya californica* pass the winter in the mother-cell condition'. The present writer has carefully gone through Robertson's paper and finds that it is difficult to reconcile this observation with what she states further on in the paper. The first collection which Robertson studied was made in November and the male cone showed 'pollen-mother-cells already formed and the cell division taking place. The nuclei are large and fill the cells. Further, 'in cones of this

date (March 4th) a certain number of sporogenous cells are in a state of division though these are fewer than in the November material.' So if divisions in the sporogenous tissue take place in March the mother-cells are organised in spring.

Dupler (20) reports that in *Taxus canadensis* the winter at Huntingdon, Pennsylvania, is passed in the microspore stage.

So as against five plants in which there is, according to Coulter and Chamberlain, a winter rest, the writer can cite the following plants: *P. longifolia*, *P. patula*, *P. rigida*, *P. resinosa*, *P. austriaca*, *P. Strobus*, *P. maritima*, *Cryptomeria japonica*, *Juniperus virginiana*, *Taxus canadensis*. This makes the number of genera 4 and species 10 in which there is no winter rest and if Robertson's *Torreya californica* in included the number of genera would come up to 5 and the number of species to 11. Over and above these facts, the doubts which Fergusou (24) entertains are well worth a consideration.

So winter rest is not essential for all Coniferales.

Meiosis.

From the description which has been given above it can be easily inferred that the heterotypic division follows the scheme indicated by Farmer and Moore (21) and designated as scheme B by Sharp (63, 64). Ferguson's (24) conclusions are not in a line with those of Farmer and Moore (21), Lewis (42), and other writers of that school.

It is not proposed here to discuss the differences in the views held by the telosynaptists and parasynaptists, but merely to put forward the evidence afforded by *P. longifolia* supporting telosynaptic interpretation.

Writing on Synapsis (Synizesis) in 1911 Lawson (40) interpreted the phenomenon as 'a growth period of the nucleus.' According to him synizesis consists in an 'extension of the nuclear cavity.' 'The chromatin mass is left behind and its characteristic position at one side of the nuclear membrane is a perfectly natural one.'

On this Saxton (61) writes, 'But even his (Lawson's) own figures, as mentioned in a criticism by Professor Farmer, do not support his contention.' The present writer believes that expansion of the nuclear cavity and contraction of the nuclear reticulum both proceed simultaneously, otherwise the tight synizetic knots figured for various plants and animals cannot be explained: simply by saying that the chromatic material is left behind by the growing nuclear membrane.

Sharp (64) gives a list of some plants in which chromosome tetrads have been described so far. Sakamura (59) and Chambers and Sands (12) interpret reported plant and animal tetrads as pairs of constricted chromosomes. Refering to Taylor's (73) work on *Gasteria* Sharp (64) states, 'On the other hand the newer evidence

brought forward by W. R. Taylor in his studies on the chromosomes of *Gasteria* affords strong confirmation of the suspicion that the gemini of plants really have a quardruple constitution.'

In *Pinus longifolia* the explanation of Sakamura (59) and Chambers and Sands (12) does not seem adequate. In the case of the tetrad marked A (plate VII fig. 52 and plate X fig. 53) and some others the question of constriction does not arise at all. The formation of this tetrad is clearly due to longitudinal splits appearing in the two conjugating mates. The tetrads observed in *Pinus longifolia* conform to the description of the Rod-tetrads of Wilson (75). Some are of Anaschistic type while others are of Diaschistic type.

In regard to the separation of chromosomes in the anaphase of the heterotypic division, Ferguson (24) holds that half of each somatic chromosome passes to each daughter nucleus. The writer has carefully compared Ferguson's figures with his own, and believes that there is only a difference of interpretation. If what Ferguson considers halves of two somatic chromosomes be taken for the split halves of a single univalent chromosome, as Lewis did, her observations can be brought into conformity with those of Lewis and the present writer.

Cytokinesis.

Evanescent cell-plates are laid down during the telophase of both divisions. A constriction furrow appears after the first division which makes, at any rate, a slow progress. After the second homotypic division another constriction furrow develops. These furrows cleave the mother cell into four spores. Later on due to centripetal growth of thickenings the mother-cell becomes partitioned. So the quadripartition of the mother-cell is brought about primarily by furrows and secondarily by the centripetal growth of cell-walls.

These conclusions are at variance with those of Ferguson (24) who definitely denies the presence of any cell-plate in the first heterotypic division, and makes no mention of the cell-plate in regard to the second division. She, however, did not bestow her special attention on cytokinesis.

Saxton in his paper on *Tetraclinis* (61) reviews the situation in other gymnosperms on this point. 'In Cycads investigated and *Ginkgo*, the mother-cell is chambered, the partition walls being thick and persistent.' In *Pinus* Hofmeister (32) described a cell-wall after the first heterotypic division but contrary to his observations Ferguson (24) found no cell-plate in connection with the first division. In regard to the cell-plate after the second homotypic division Ferguson does not say anything. The present writer has found evanescent cell-plates and cleavage furrows in connection with both these divisions.

After these furrows have cleaved the cell into four parts, centripetal walls divide it into four chambers. In *Larix* Devise (17) reports that the cell-plate formed after the first division disappears and the division of the mother-cell into tetrahedral spores is brought about by cell-plates formed after the second mitosis. Burlingame (4) on this point in regard to *Araucaria* states 'no walls are formed, apparently, until after the spore nuclei have passed into the resting condition, when a system of fibres is present between the nuclei, on which the plasmatic membranes separating the young spores arise.' In *Thuja* Land's figures imply an unpartitioned mother-cell, as is also claimed for *Juniperus* by Norens (53) with whose results Nichols (52) is not in agreement. In *Torreya* (58) Robertson's figures imply an absence of chambering. In *Actinostrobus* and *Cupressus* according to Saxton (61) the spores are formed free in the mother-cell. In *Tetraclinis* only (61) has cleaving been definitely described by Saxton.' In Gnetales *Ephedra* and *Welwitschia* (56) are not chambered, while *Gnetum africanum* and *G. scandens* (57) show thick partition walls.

Farr (22), and Gates and Rees (28) and some Swedish investigators have described a number of Angiosperms in which quadripartition by furrows takes place in the pollen mother-cells. In a recent paper McPhee (45) describes the same thing for *Cannabis*. Farr (22) makes a mention of some plants *viz.*, *Magnolia*, *Nelumbo*, *Carex* and others in which transitory cell-plates are formed. The case of *Pinus longifolia* is very similar to *Carex* in which they are formed after both the divisions.

Although according to Saxton (61), and truly enough, this character is one which cannot on any scheme of Gymnosperm classification be of any phylogenetic importance, it still serves to bridge that gulf which at one time stretched between cytokinesis in animals and plants.

Chromosome Numbers.

Ferguson (24) has reported 12 and 24 chromosomes for five species of *Pinus* viz., *Strobus*, *rigida*, *austriaca*, *resinosa*, *montana* var *uncinata*. Chamberlain (7) reports the same numbers for *P. Laricio*. Blackman (3) and Dixon (18) for *P. silvestris* report the same numbers. Strasburger (68) has also reported 12 and 24, but his paper being not available to the writer, he cannot say on which species Strasburger worked. Thus the same haploid and diploid numbers of chromosomes have been reported for eight species of *Pinus*, including the writer's *P. longifolia* and excluding Strasburger's species.

Not only in *Pinus* but in several other Coniferales the same numbers have been reported *viz* :—*Larix*, *Thuja*, *Taxodium*, *Araucaria*, *Callitris* (14) and *Tetraclinis* (61).

In this connection the following extract from 'General Cytology' (16) would be of interest:—'The most striking case of this marked uniformity of the chromosome complex is shown in extensive studies upon the short-horned grass-hoppers by McClung and his students. This family in North America consists of about 100 genera, including 800 species, and most of these have now been studied. It thus appears that throughout the group the somatic cells of the male contain twenty-three chromosomes while those of the female have twenty-four. Correspondingly, in the germ-cells the diploid numbers are twenty-three and twenty-four and the haploid eleven or twelve in the male and always twelve in the female. The significance of these figures cannot be over-estimated, for nothing but the most extreme precision of organization could preserve this common series of chromosomes through the millions of years in which this group of animals has existed, and in the innumerable multitudes of cells composing them. There is absolutely nothing to suggest the intrusion of chance into this incomprehensible picture of organic constancy.'

Sharp (63) discussing the bearing of the number of chromosomes on the origin of species and varieties states that 'the number shown by the species of a given genus or even of an entire family form a series of multiples.' 'From this it is to be inferred as suggested by McClung (44) that there is a relationship of some sort between the constitution of the chromosome complement and the externally visible taxonomic characters.' In support several cases are cited. For example, *Chrysanthemum* (species with 9, 18, 27, 36 and 45 pairs of chromosomes); *Triticum* and *Avena* (with 7, 14 and 28 pairs of chromosomes). Recently Ferguson (25) has reported seven and fourteen chromosomes for four genera of Aloinae, in each of which several species have been studied.

Now, how to explain, according to the chromosome theory of heredity, the origin of species of *Pinus* in which 8 species have been recorded to have the same chromosome numbers? There are no triploid and tetraploid or any such species. There are two ways to explain the problem. Firstly, by assuming that there is really only one species, but this would go against taxonomic conclusions which seem quite sound. The other alternative solution of the problem is that although the numbers are the same, the chromosomes are constitutionally different from one another, *i.e.*, they differ in their factors or genes. These species might have arisen from the prepetuation of mutations which arose in the chromosomes, the number of the latter remaining constant. Change of type taking place through mutations of genes has already been emphasized by Sharp (64), Morgan (48) and others.

VI. Notes on Abnormal Female Prothalli.

In this note the writer proposes to give an account of those abnormal female prothallia he came across during his work on *P. longifolia*.

Prothallus No. 1.

This prothallus was found in an ovule collected at Lahore from a tree in the Gol Bagh on the 10th May 1920. Its abnormal external characters struck the writer as the material was being passed through the grades of alcohol. It was forthwith singled out and a detailed study of the internal structure was carried out in serial transverse sections which revealed some more points of interest in addition to its external morphology. (See plate X fig. 71).

External characters.—The prothallus was of the normal length *i.e.*, about 11 mm. It was much more compressed than a normal prothallus from the abaxial and the adaxial sides, the shorter and the longer diameters being 1.2 and 2.6 mm. respectively. When these measurements are compared with those of a normal prothallus which are 2.3 and 2.6 mm. the contrast comes out clearly. This prothallus was further peculiar in having all the archegonia aggregated towards one flat side (plate X fig. 71), the other flat side being quite devoid of archegonia. The archegonia were not situated at the extreme tip of the prothallus but some distance below. In a normal prothallus the archegonia are disposed radially around the micropylar end. The writer is not prepared to hazard a guess as to the position of the side bearing the archegonia *i.e.*, whether it was adaxial or abaxial because it was not noted at the time of the dissection of the prothallus preparatory to fixation.

Internal structure.—There were 11 archegonia according to one interpretation, or 12 according to another, borne by the prothallus. The largest number in this species, in normal prothallia, is 7. So far as the writer is aware the highest number of archegonia, which has been recorded for any species of *Pinus* is 9 (Ferguson 24, *P. montana var. uncinata*). In other species on which Ferguson worked she found that the highest number was 5 (24). In the writer's specimen as many as 9 could be counted in a single section. Owing to the large number of archegonia and the limited volume of the prothallus the archegonia were not oval as usual, but were compressed out of their shape by mutual pressure. The necks of all the archegonia opened on the side on which they were aggregated and not towards the apex as is generally the case. The longer axes of the archegonia did not coincide with the longer axis of the prothallus but they were either at right angles to or oblique to the longer axis of the prothallus.

Each archegonium possesses a distinct jacket all round. When the number of archegonia is small in a prothallus the space between different archegonia is filled with parenchymatous cells of the prothallus, but when the number is large the jacket cells of adjacent archegonia come into contact with each other but yet the identity of the two jacket layers is maintained. In this prothallus it was noted that in many cases there was no parenchyma between the jackets of adjacent archegonia which thus came into contact. Two archegonia were exceptional in having only one layer of jacket cells between them (plate X fig. 69).

In spite of such a large number of archegonia borne by the prothallus all of them were normal except one. Most of them showed different stages before, at and after fertilization. In fig. 69 the small male nucleus is seen in the egg cytoplasm in the left hand archegonium.

One of these archegonia was peculiar in having a bulge on one side (plate X fig. 69). This bulge appears in sections as a small archegonium with a jacket of its own. This jacket is distinct all round the so-called archegonium except at the place where it comes into contact with its larger neighbour, where there is only a thin membrane separating the two (plate X fig. 70). In sections taken at a lower level this membrane begins to disappear. Finally the membrane disappears altogether and the cytoplasm of the so-called archegonia is seen fusing (plate X fig. 69). At still lower levels the smaller bulge like structure disappears but its larger neighbour continues. It is at these lower levels that the persisting archegonium shows four nuclei of the pro-embryo. The bulge-like structure which does not extend throughout the whole length of the larger archegonium shows no nucleus and the writer was not able to see a definite neck attached to it.

Now there are only two ways in which this peculiar structure can be explained. Either it is in reality a bulge given off by the larger archegonium or it is itself an archegonium, which has got fused with its neighbour. The first alternative seems rather unlikely because there is such a large number of archegonia borne by the prothallus, that the possibility of a protrusion being given off by one of them is excluded on physical grounds. The other alternative appears to be more sound; though there are the following two serious objections to it:—Firstly, the smaller archegonium possesses no neck and secondly it shows no nuclei at all. The large number of archegonia and the resulting mutual pressure go in favour of the second alternative (*i.e.*, two adjacent archegonia have fused). It is also a fact, as noted above, that there are two archegonia, which have

got a common layer of the jacket cells between them. In this case the mutal pressure and the limited space available have resulted in the elimination of both the jacket layers between the two archegonia under consideration and the consequent fusion of the two archegonia. As to how the neck and the nucleus of the smaller archegonium disappeared remains unexplained. No traces of a disorganizing nucleus could be made out.

This prothallus agrees with the one described by Saxton (60) in *P. maritima*. In that prothallus the longer axes of the archegonia were at right angles to the longer axis of the prothallus and they opened laterally. The prothallus No. 1 described by the present writer stands alone by itself in the two fusing archegonia or the one bulging archegonium.

After the writer had found the female prothallus described above he kept a look-out for any other abnormalities. Both of the abnormal prothallia described below were collected on the 15th May, 1920.

Prothallus No. 2.

This prothallus bore only three archegonia which were laterally placed. Their longer axes were oblique to the longer axis of the prothallus. Their venters instead of their necks made acute angles with the longer axis of the prothallus. Their necks opened out towards the side and not towards the apex (plate X fig. 72).

Prothallus No. 3.

This prothallus was in reality a transitional stage between No. 2 and a normal prothallus. In this there were three archegonia ; one was situated at the apex and opened apically, while the other two opened laterally. Plate X fig. 73 shows the prothallus as seen externally.

Conclusions.

The lateral position of the archegonia resembles that met with in *Araucaria* and *Agathis*, but their aggregation towards one side only resembles the condition met with in *Callitris*.

Seward and Ford (62) regard this feature in *Araucaria* and *Agathis* as a primitive feature, and Saxton (60) holds the same view in regard to *Callitris*. If the view of these writers is correct the occurrence of lateral archegonia in *Pinus* would be a sort of reversion to a primitive character and the prothallia Nos. 2 and 3 would represent the intermediate stages towards the realisation of the apical position of the archegonia. The view advanced by Saxton (60) in his note on *P. maritima*, that the lateral position may be of the nature of a mutation cannot be held in view of the transitional stages described by the present writer.

The disappearance of one layer of jacket cells between two archegonia where they touch points towards the origin of the archegonial complex in the *Cupressineae*. Coulter and Chamberlain (14) state that 'all the evidence of morphology, vascular anatomy and history favours the belief that the *Taxodineae* and the *Cupressineae* have been derived from the much more primitive stock of the *Abietineae*.' This view coincides well with the explanation offered above for the disappearance of one jacket layer between two archegonia, or the disappearance might have no phylogenetic significance, having resulted from purely mechanical causes.

VII. Abnormalities in the male cone-bearing shoots.

I.

Occasionally in the cluster of male cones, the writer found a few of the cones replaced by dwarf-shoots. From their position on the long shoots, the dwarf-shoots and the male cones are held to be homologous organs. The evidence from abnormalities further supports this conclusion.

II.

In one case the writer found that a branch after producing a number of male cones had divided. A number of male cones were borne by each of the two branches. A little higher up the branches had again fused and a number of male cones were borne on a single axis. Higher up still there was a single apical bud of young dwarf-shoots.

VIII. Summary.

In *P. longifolia* most of the lower branches bear clusters of male cones only. The female cones are found on branches high up in the tree. These branches bear only the female cones, and very rarely give rise to a branch which bears a cluster of male cones.

The male cones are initiated in September in Lahore. They grow continuously till pollen is shed in February. The female cones are initiated in January. After pollination which takes place in February the female cones close up but continue to grow till about May when they become about an inch in length and growth is stopped. A deep-seated megaspore-mother-cell is formed about the 18th or 20th of February. This undergoes a reduction division.

Only a few free nuclei are formed by the division of the functional megaspore before the resting period ensues in May. Growth is resumed in February next. Till the last week of March only a peripheral layer of cytoplasm with free nuclei is seen. The compact tissue of the female prothallus arises in the same way as in other Coniferales.

The fully formed female prothallus is a cylindrical body tapering towards the two ends (11-12 mm.). It is slightly compressed from the adaxial and the abaxial sides. Only the micropylar end of the prothallus bears the archegonia. The number of these may be between 2-7. The archegonium is about 1 mm. in length. Its neck shows 2 tiers of four cells each. The archegonia never come into direct contact.

The development of the archegonium agrees closely with those of the other Abietineae. The archegonial initials become visible in the first week of April in Lahore. The ventral canal cell is cut off in the usual way in the last week of April. The ventral canal nucleus shows signs of disorganization very early. The egg nucleus travels down to the centre of the archegonium and grows very rapidly in size. When fully grown its longer diameter measures 202·5 microns. Ordinary vacuoles in the egg cytoplasm get filled up and form what are called proteid vacuoles.

The fully formed archegonium before syngamy shows a receptive spot.

The pollen-tube discharges all of its contents into the archegonium through a pit. A large quantity of cytoplasm and some starch grains are also passed in.

On entering the archegonium the male nuclei slip out of the enveloping sheath of cytoplasm. The larger one moves towards the female nucleus and becomes lodged in the latter in a cavity towards its upper side. Syngamy in Lahore takes place about the 30th of April. In Chamba it takes place about the 1st of July. The first segmentation spindle is intranuclear. The two segmentation nuclei divide simultaneously. The four segmentation nuclei travel to the base of the archegonium, and become arranged in a single plane. All of these divide simultaneously. Vertical and cross-walls in the pro-embryo are formed immediately after this mitosis. The next division takes place in all the nuclei of the upper tier simultaneously. The last division takes place in all the cells of the lowermost tier simultaneously and the pro-embryo completes its development. The cells of the suspensor tier (second from below or third from above) begin to elongate and carry the embryo tier (lowermost) down into the female prothallus.

Some abnormal female prothalli are described.

Reduction divisions in the pollen mother cells have been followed by the smear method. The male cones are initiated in Lahore in September. By the middle of November the three or four wall-layers of the sporangium get defined. By the third week of January most of the cells of the sporophyll and the cells of the wall except the two

innermost layers get a deposit of a resiniferous substance. Demarcation of dehiscence in the sporangium occurs soon. The cells of a longitudinal plate of 3 or 4 cells towards the under side do not get this resiniferous substance and in the middle line of this plate of cells dehiscence takes place when the sporangium becomes ripe. A tapetum as a distinct layer is marked off rather very early when compared with the other species of *Pinus*.

Growth and division of the sporogenous cells are continuous and uninterrupted till the formation and the dispersal of the pollen grains. Pollen mother cells become recognizable in January. Reduction divisions take place during the last week of January and pollen grains are shed in the middle of February. There is no winter rest.

The pollen mother-cells are polygonal and show very large nuclei. The cytoplasm contains at first mitochondria but starch later on. During the prophase the nucleus undergoes the first contraction. Small starch grains make their appearance in the cytoplasm at this time and remain there throughout. Near the close of the first contraction a continuous thread can be made out in the synizetic knot. This thread uncoils itself and becomes peripherally distributed in the nucleus. The mother-cells become loose at this time. In the spireme a longitudinal split appears. The spireme shortens and thickens. The split becomes invisible. This is followed by the second contraction. On recovery from the second contraction the spireme shows a number of loops. The spireme gets segmented. Each segment corresponds to a pair of somatic chromosomes. It is believed that conjugation is telosynaptic. At the diakinesis stage chromosome tetrads become visible. During metaphase the spindle fibres are not so well developed. The bivalents separate into constituent univalents which move towards the spindle poles. They become somewhat longer at this time. They show longitudinal splits. In each of the two batches of chromosomes four very long chromosomes can always be detected. During early telophase the spindle fibres become very prominent. The chromosomes of the two groups gradually organize themselves into the typical resting nuclei. They form a continuous beaded thread. Branching in the chromosomes is brought about by vacuolation. No nucleoli appear in the daughter nuclei and an evanescent cell-plate is laid down between them. A constriction furrow appears between the two daughter cells during inter-kinesis but it does not separate the two daughter cells rapidly.

The second homotypic division is simultaneous in the two daughter cells. In the prophase a more or less continuous spireme is formed which segments into the reduced number of chromosomes. Thesechromosomes when they lie on the equator of the spindle are

pretty long and therefore this metaphase presents a sharp contrast to the preceding metaphase of the heterotypic division. During the anaphase the chromosomes move to the poles of the spindle and become bent. The two batches of chromosomes form the two daughter nuclei. The chromosomes are thin and the telophasic changes are brought about by branching. An ephemeral cell-plate is formed after telophasic changes. The cleavage furrow laid down between the daughter nuclei of the previous heterotypic division develops still further. A second furrow appears between the grand-daughter nuclei and so the mother-cell cleaves into four parts. Opposite the furrows thickenings are laid down in the wall of the mother-cell. These grow centripetally in between the furrows and the mother-cell becomes quadripartitioned.

Twelve bivalents have been counted at the diakinesis stage, so the haploid and the diploid numbers of chromosomes are 12 and 24 respectively.

The male gametophyte consists of two lenticular cells which disorganize very soon. They cannot be made out in pollen grains at the time of shedding.

The male cones and the dwarf shoots in *Pinus* are believed to be homologous organs. This conclusion is supported by the evidence afforded by abnormalities met with in the clusters of male cones, where some of them may be replaced by dwarf shoots.

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X. Explanation of Plates.

All the microscopic figures with the exception of photomicrographs have been drawn with a camera lucida.

Plate I.

Fig. 1. Photograph of a tree.
Fig. 2. A branch showing female cones of 4 generations.

Plate II.

Fig. 3. Fully formed resting megaspore-mother-cell. × 3325.
Fig. 4. Nucleus of the megaspore-mother-cell showing the spireme during the prophase of the first heterotypic division. × 400.
Fig. 5. Nucleus of the megaspore-mother-cell during the synizesis stage. × 2355.
Figs. 6. 7. Formation of the bivalent chromosomes in the nucleus of the megaspore-mother-cell (heterotypic division). × 2355, 3325.
Fig. 8. Archegonial initial. × 800.
Fig. 9. Archegonial initial. Older stage than Fig. 8. × 800.
Fig. 10. The archegonial initial has divided into the primary neck cell and the central cell. × 800.
Fig. 12. Central cell dividing to produce the ventral canal cell and the egg. × 640.

Plate III.

Fig. 13. Nucleus of the central cell before division lying just below the neck. × 800.
Fig. 14. Later stage in the division than that shown in Fig. 12. × 800.
Fig. 17. Proteid vacuoles being filled up. × 650.
Fig. 18. Egg showing receptive spot. × 120.
Fig. 20. Egg cytoplasm with a large quantity of cytoplasm passed from the pollen tube. × 220.
Fig. 23. A reconstruction of Fig. 22 showing all the nuclei. × 120.
Fig. 25. First segmentation spindle. × 800.
Fig. 27. One of the two segmentation nuclei dividing. × 1690.

Plate IV.

Fig. 28. Four segmentation nuclei. (Only three are visible).
Fig. 29. Four segmentation nuclei arranged at the base of the archegonium. × 650.

Fig. 30. First division in the nuclei of the pro-embryo (only one is seen). × 650.
Fig. 31. Pro-embryo showing four upper nuclei and four lower cells. × 650.
Fig. 32. Pro-embryo showing division in the nuclei of the upper tier. × 650.
Fig. 33. Pro-embryo showing three tiers. × 650.
Fig. 34. Pro-embryo showing early suspensor formation. × 650.

PLATE V.

Fig. 11. Archegonium before the cutting of the ventral canal cell.
Fig. 15. Egg nuclei are seen travelling down towards the centre of the archegonia. The ventral canal cells are also visible.
Fig. 16. A mature egg nucleus.
Fig. 19. The end of the pollen tube is seen between the female prothallus and the nucellus. The two male nuclei are embedded in a common sheath of protoplasm.
Fig. 21. Two male nuclei are seen in the egg. The female nucleus is below.
Fig. 22. The male nucleus is seen approaching the female and the latter has produced a projection towards the former.
Fig. 24. The male nucleus is lodged in a concavity of the female nucleus.
Fig. 26. Two segmentation nuclei.

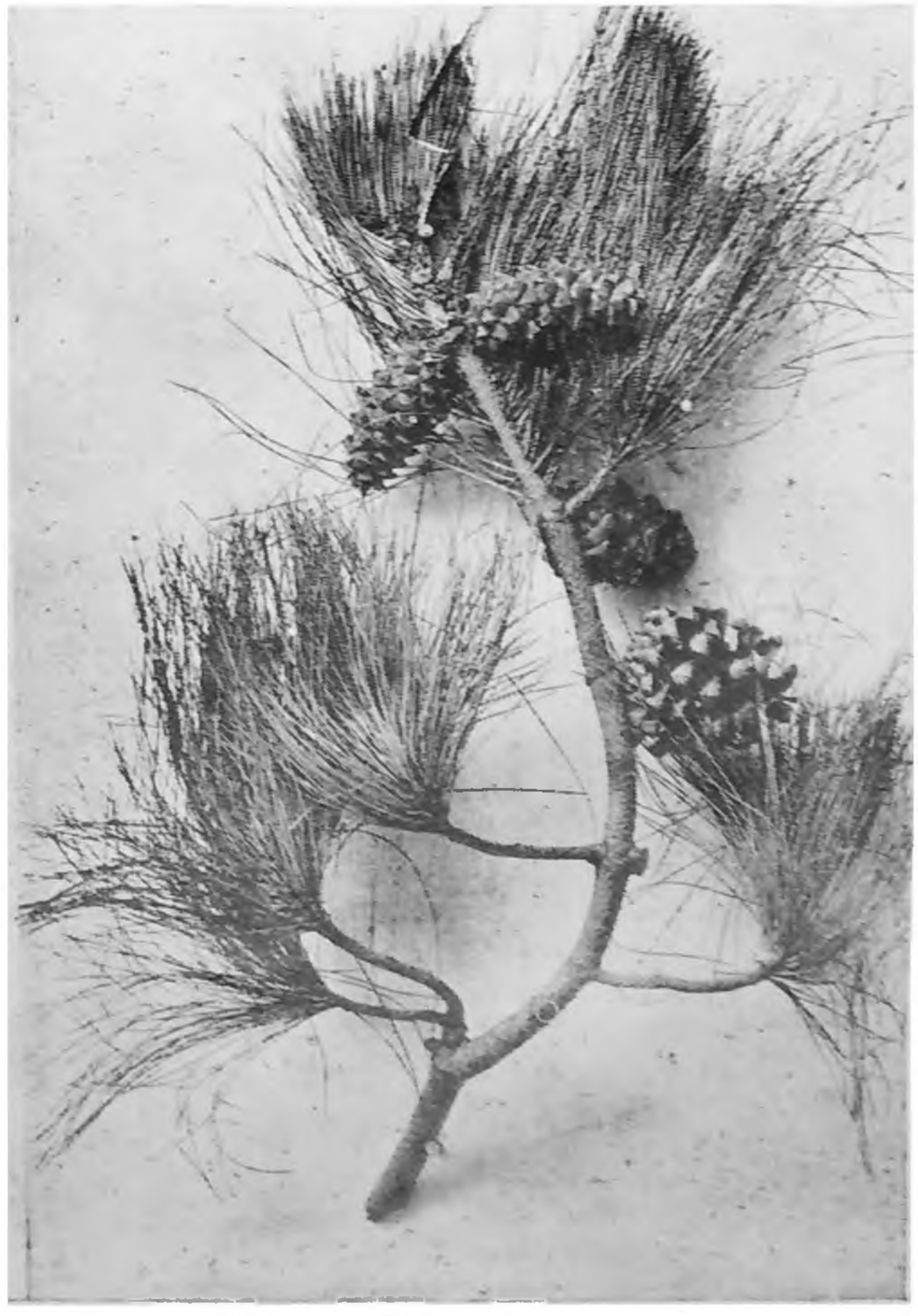
PLATE VI.

Fig. 35. Cross section of microsporangium. All the cells of the wall are filled with resin except those between which dehiscence takes place.
Fig. 36, 37. Pollen mother-cells. × 2350, 3325.
Fig. 38. Early prophase. × 3325.
Fig. 39. Later than Fig. 38. × 2350.
Fig. 40. Synizesis. × 3325.
Fig. 41. Close of synizesis. A continuous chromatic thread can be seen within the synizesis knot. × 3325.
Fig. 42. Chromatic thread uniformly distributed within the nucleus. × 3325.

PLATE VII.

Fig 43. Spireme split at certain places. × 1690.
Fig. 44. Second contraction. × 1690.
Fig. 45. Ditto. × 1690.

M. L. S. Phot. 1.



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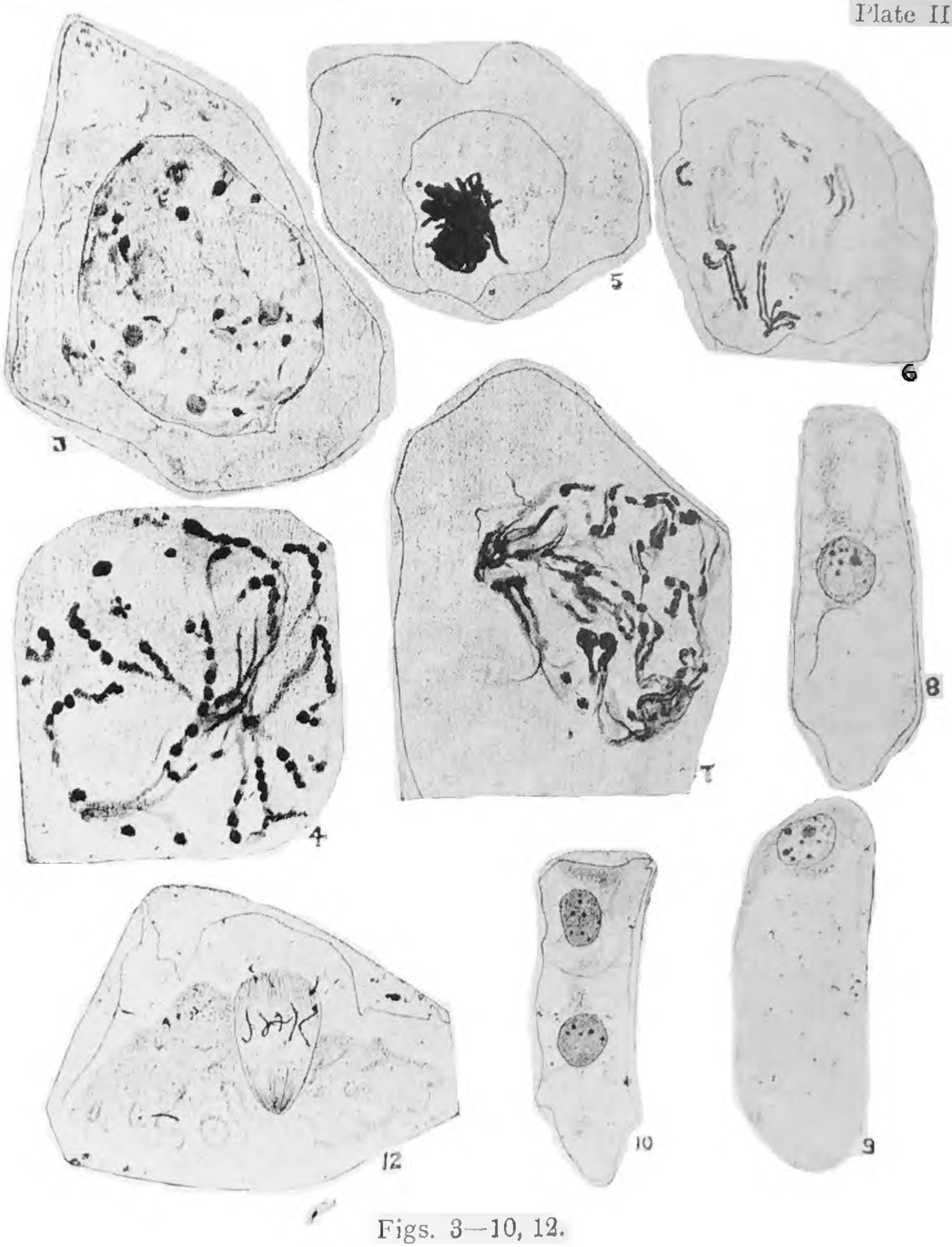
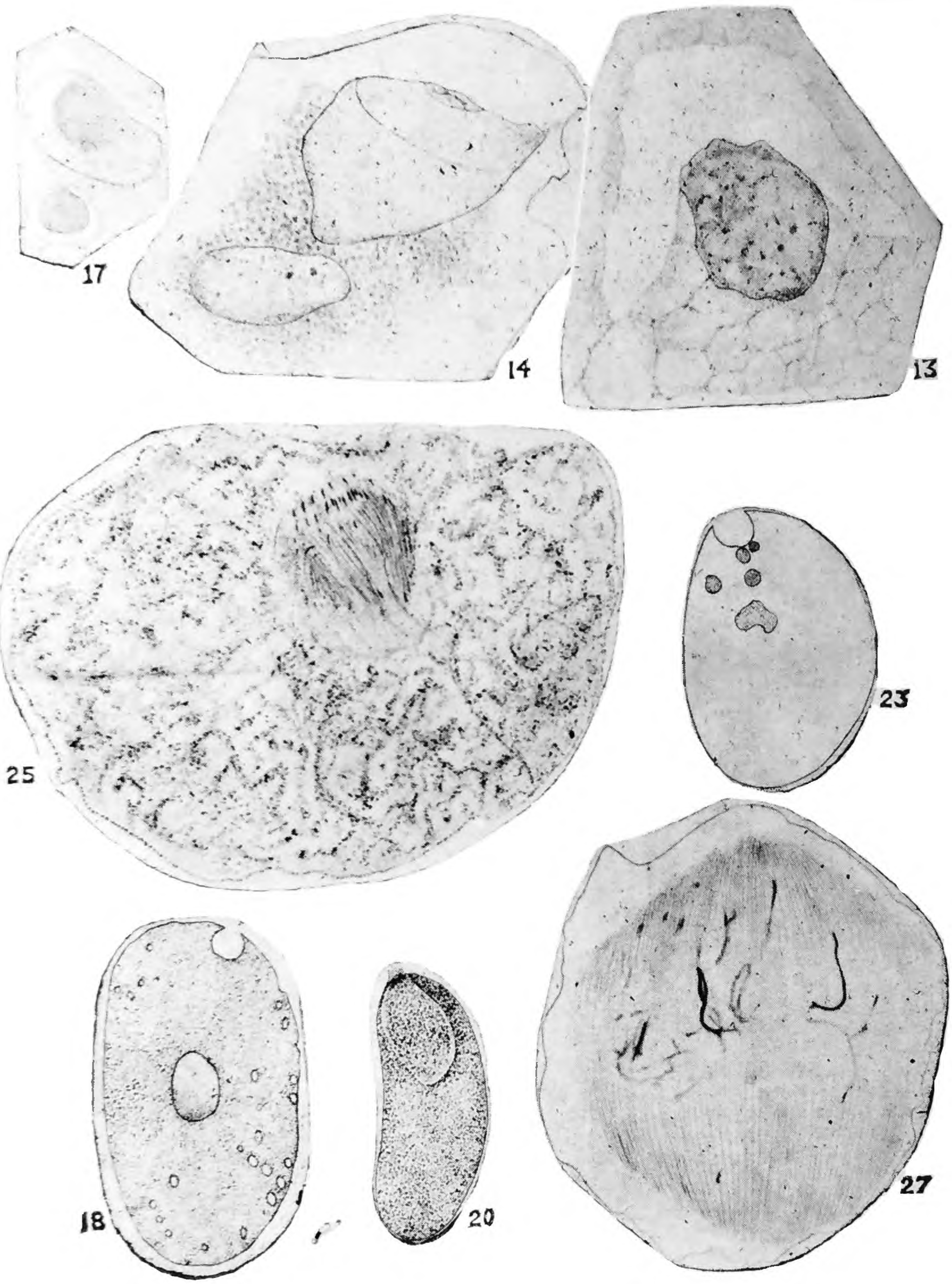
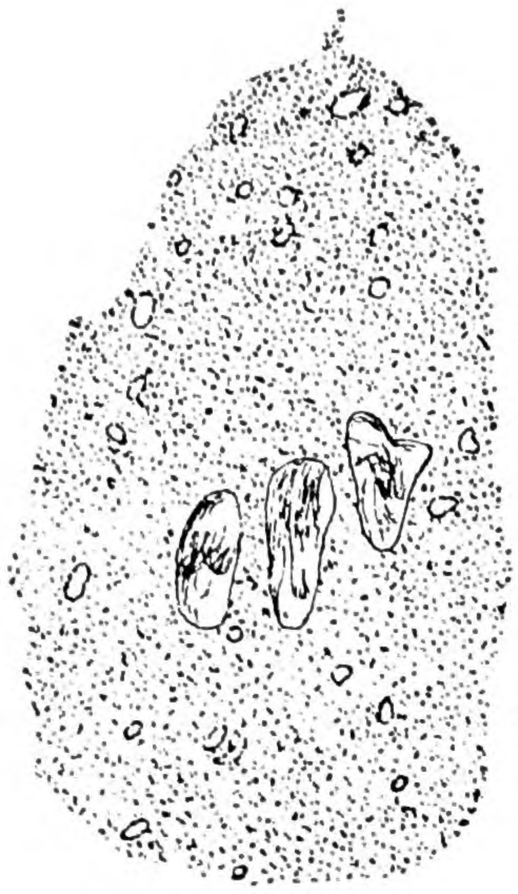


Figs. 3—10, 12.

Plate III.



Figs. 13, 14, 17, 18, 20, 23, 25, 27.



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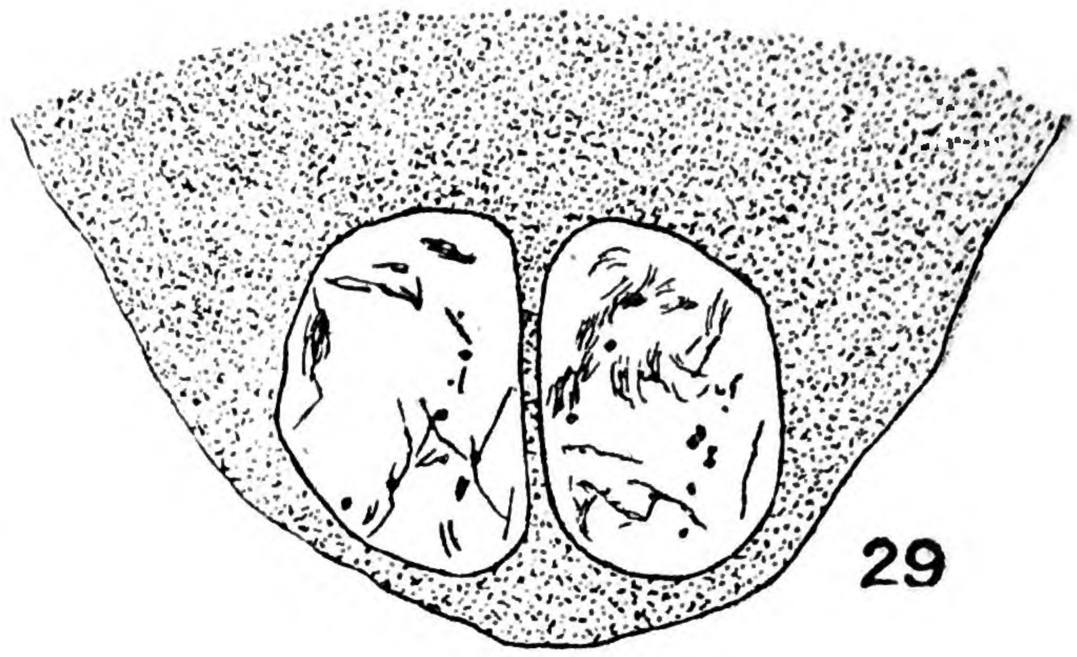

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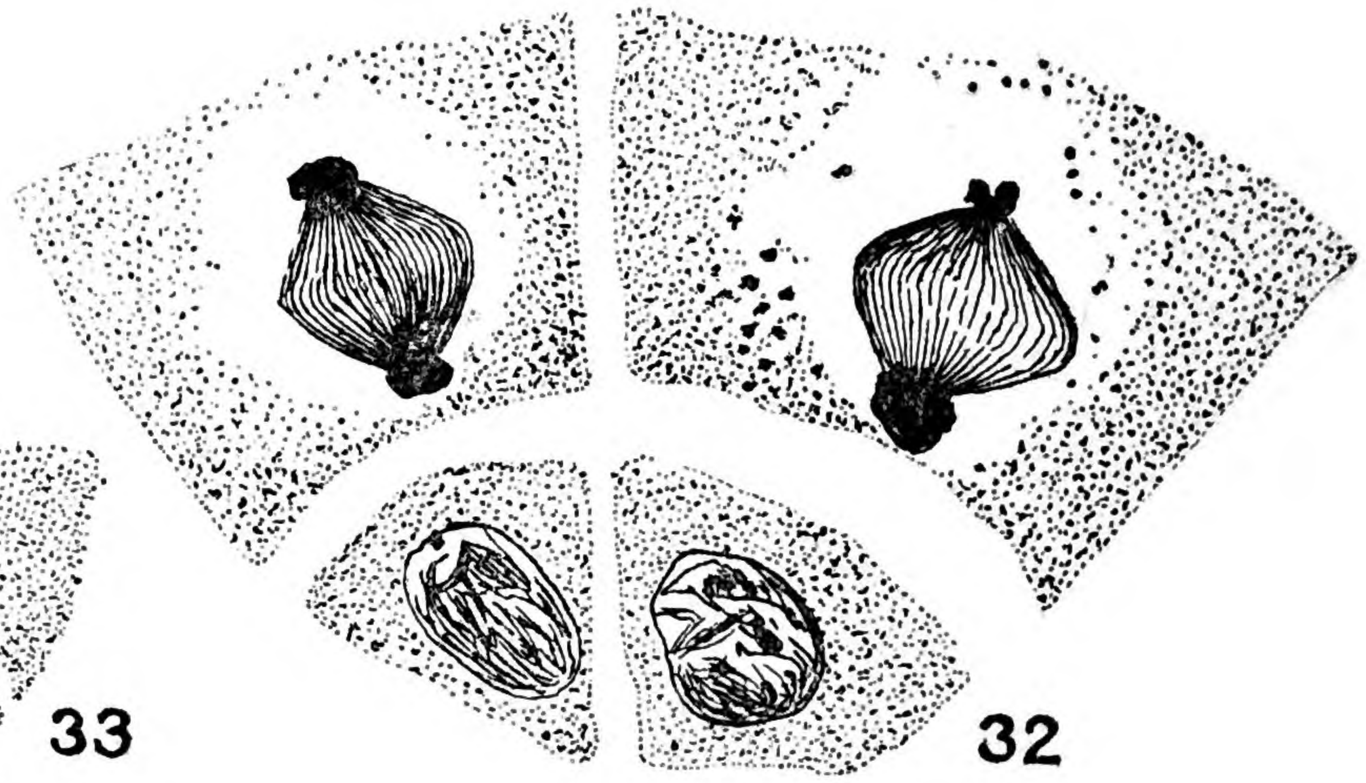

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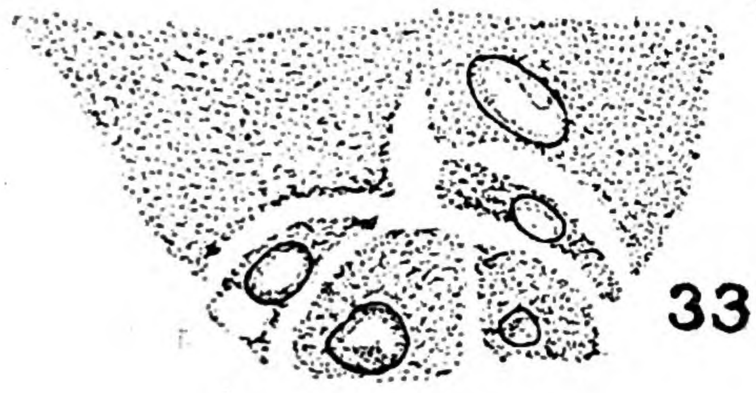

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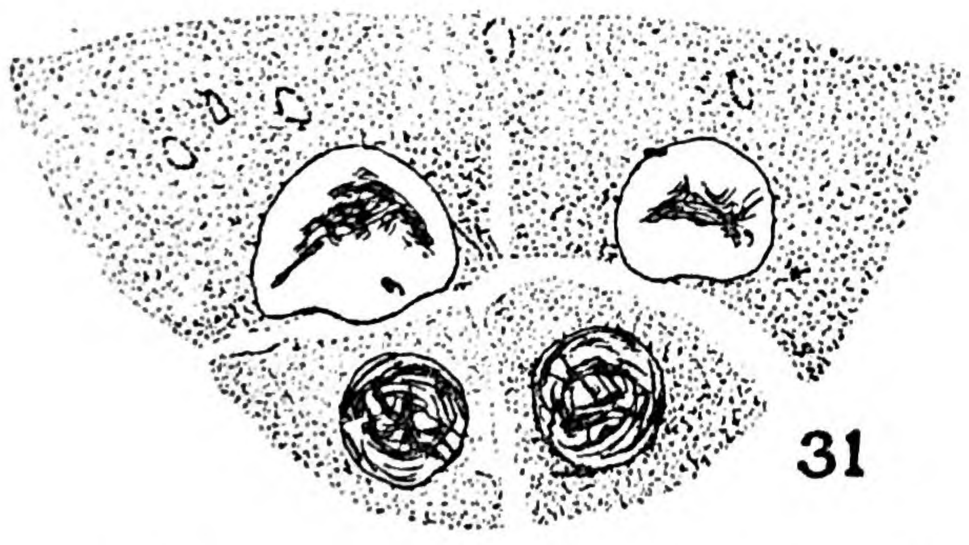

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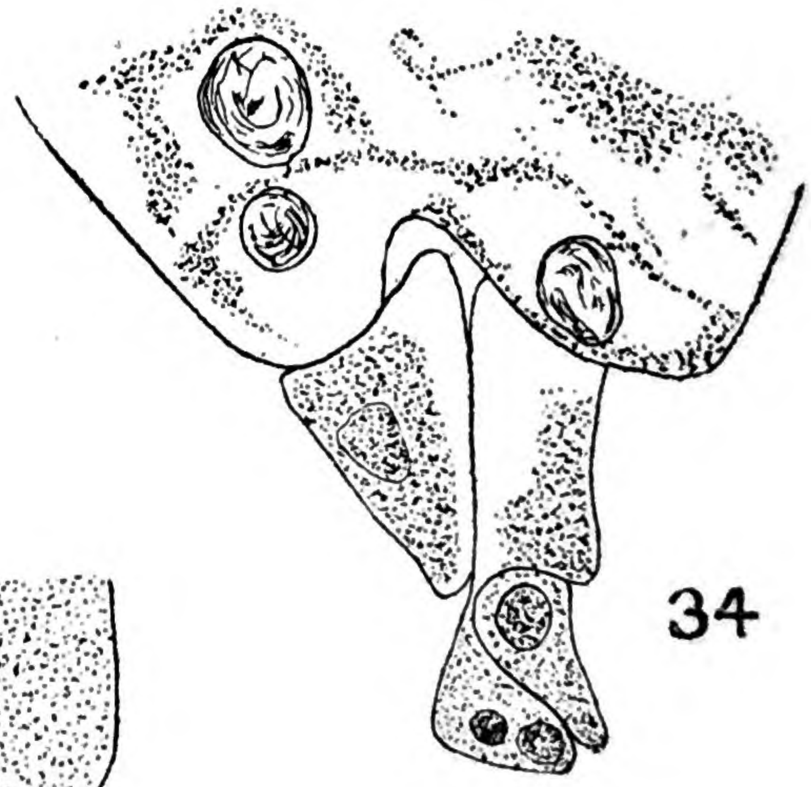

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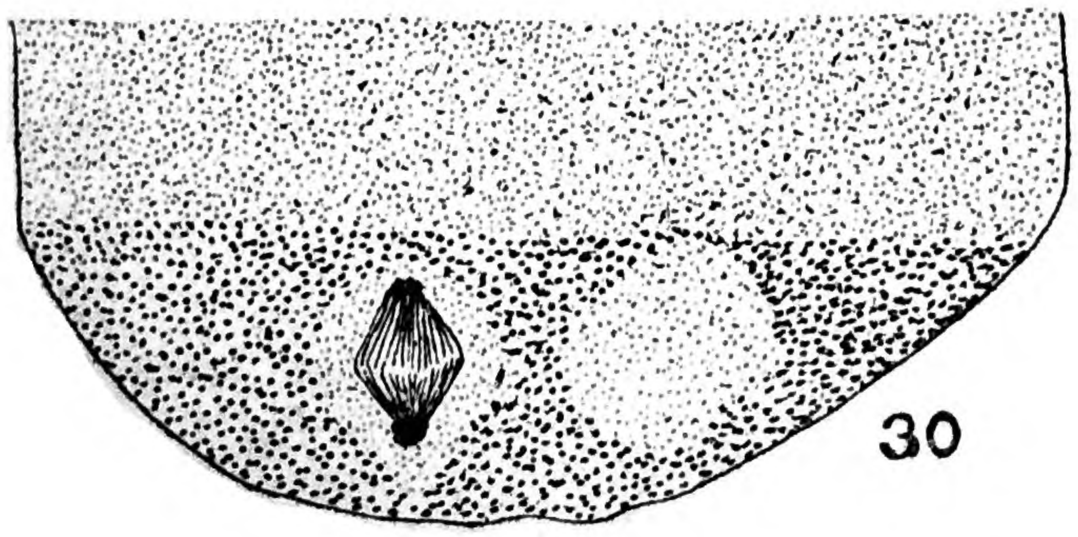

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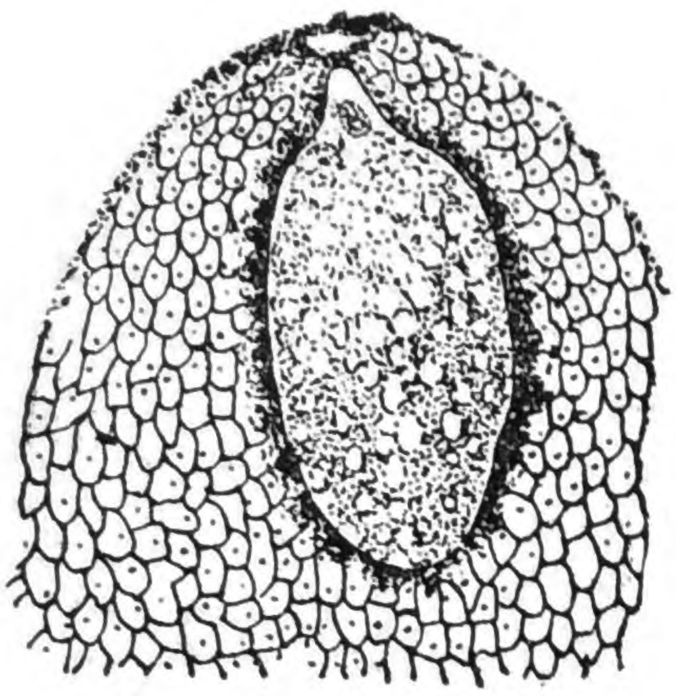
Figs. 28—34.



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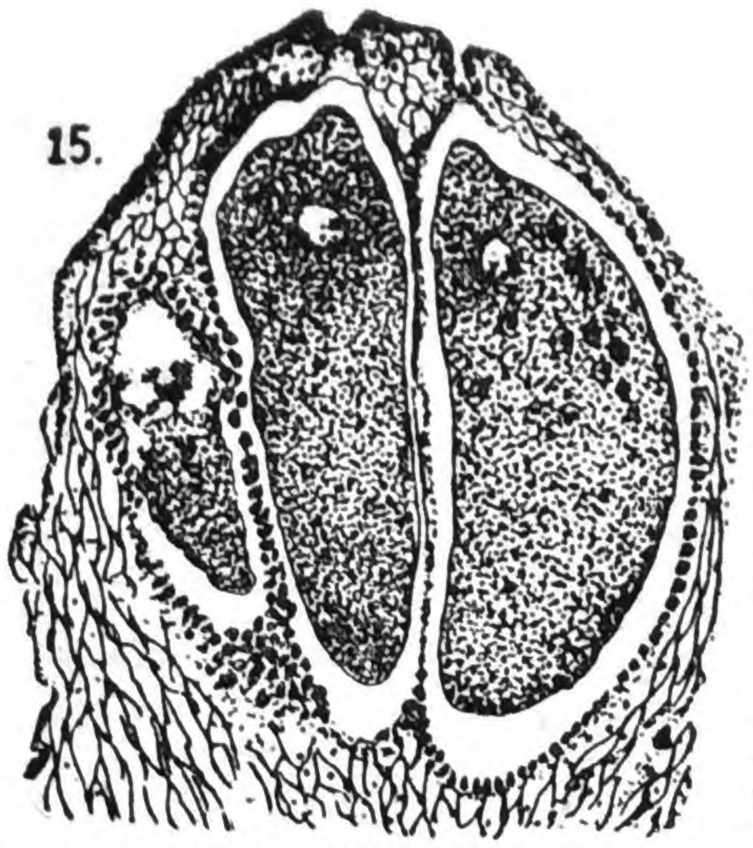

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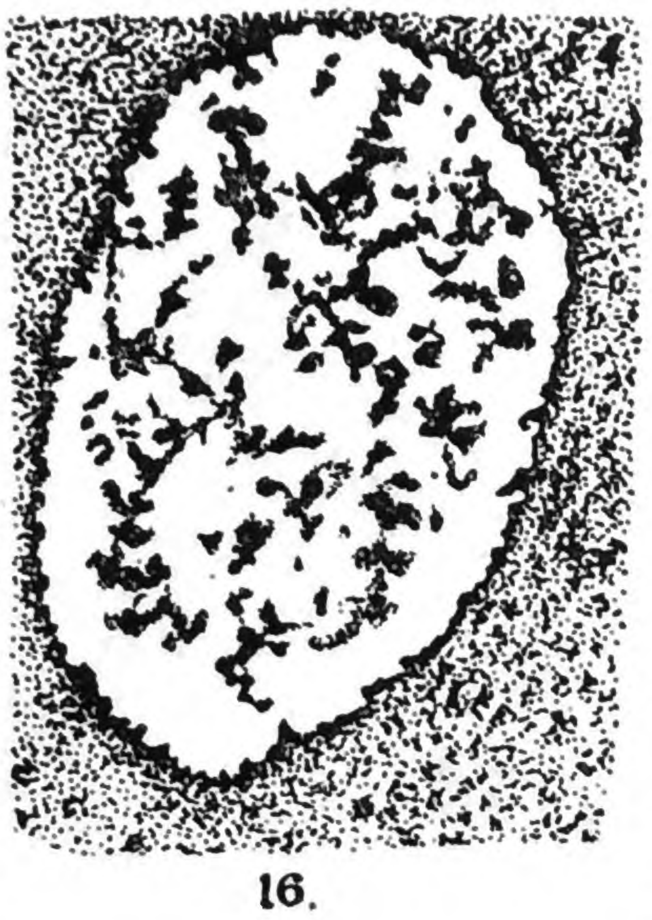

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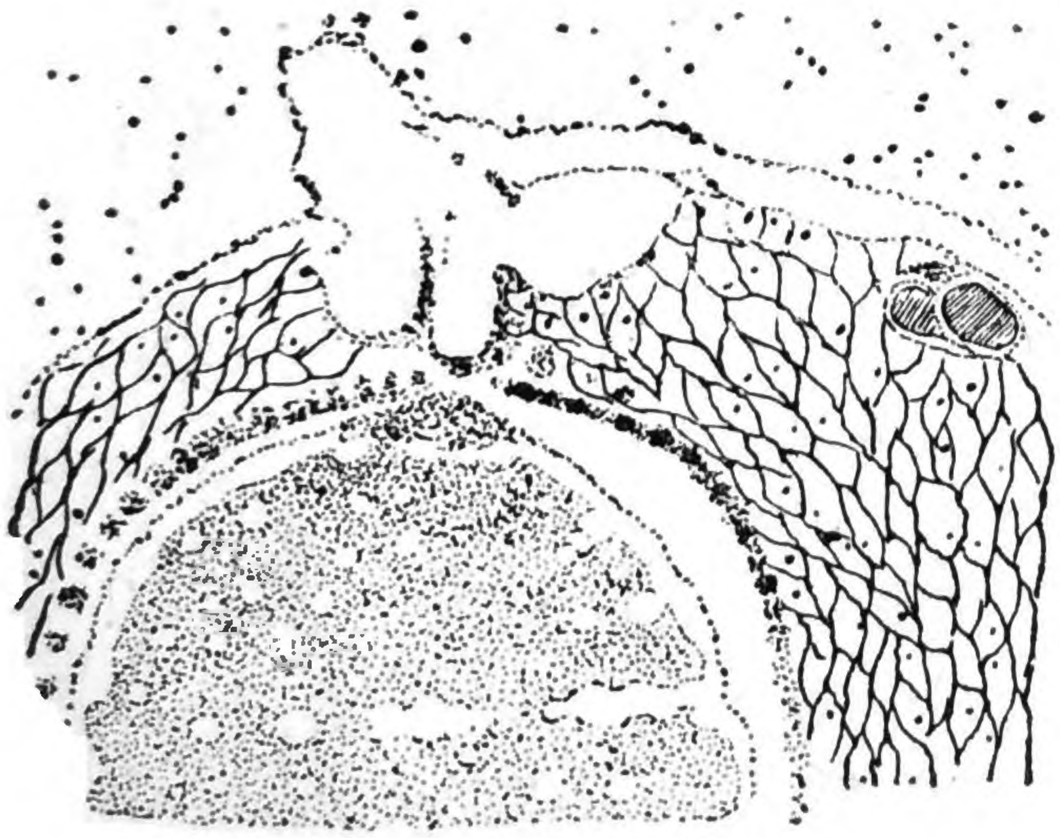

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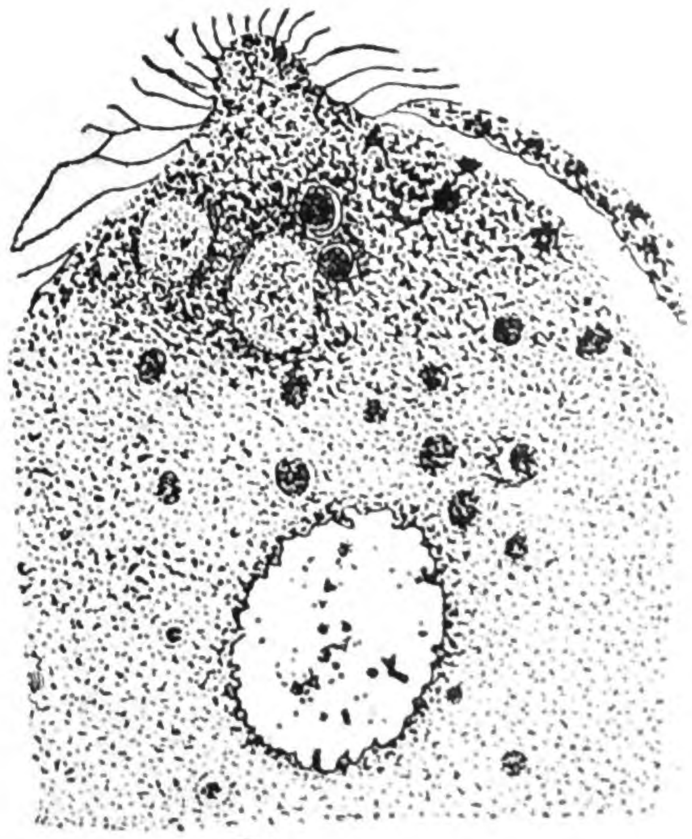

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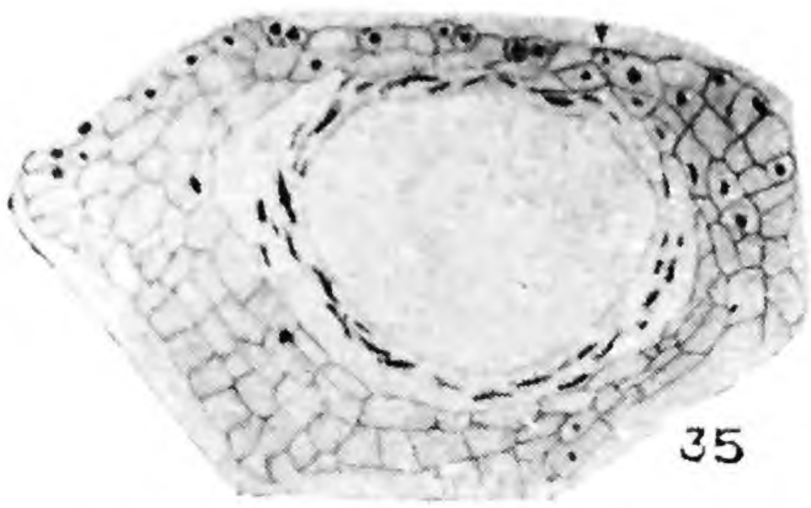
Figs. 11, 15, 16, 19, 21, 22, 24, 26.

Plate VI.



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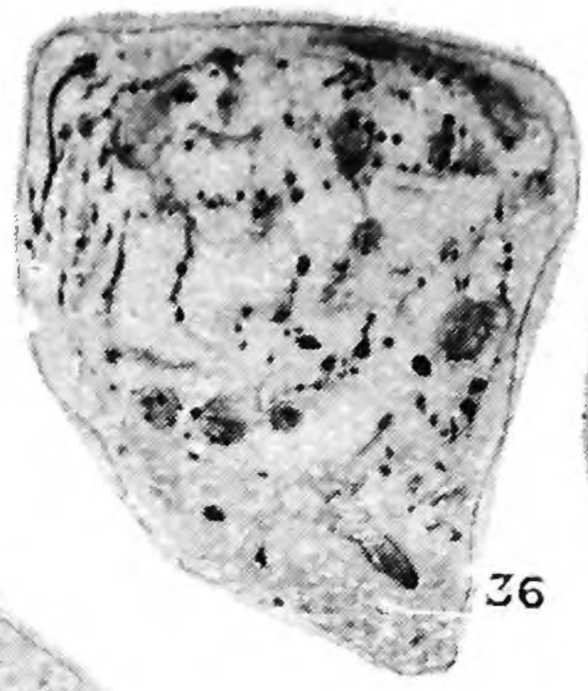

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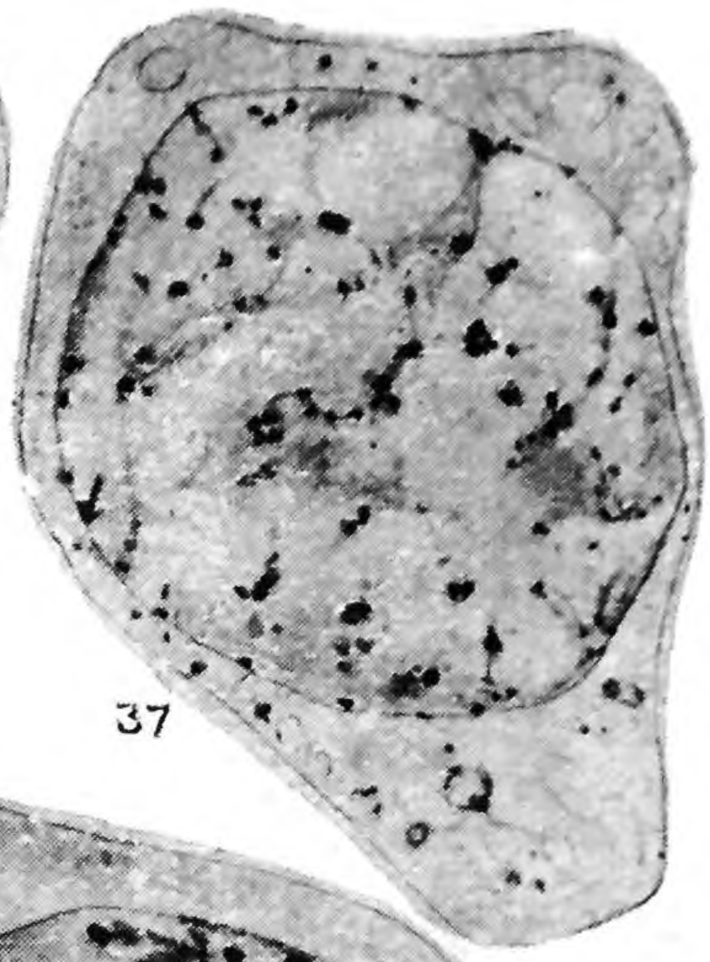

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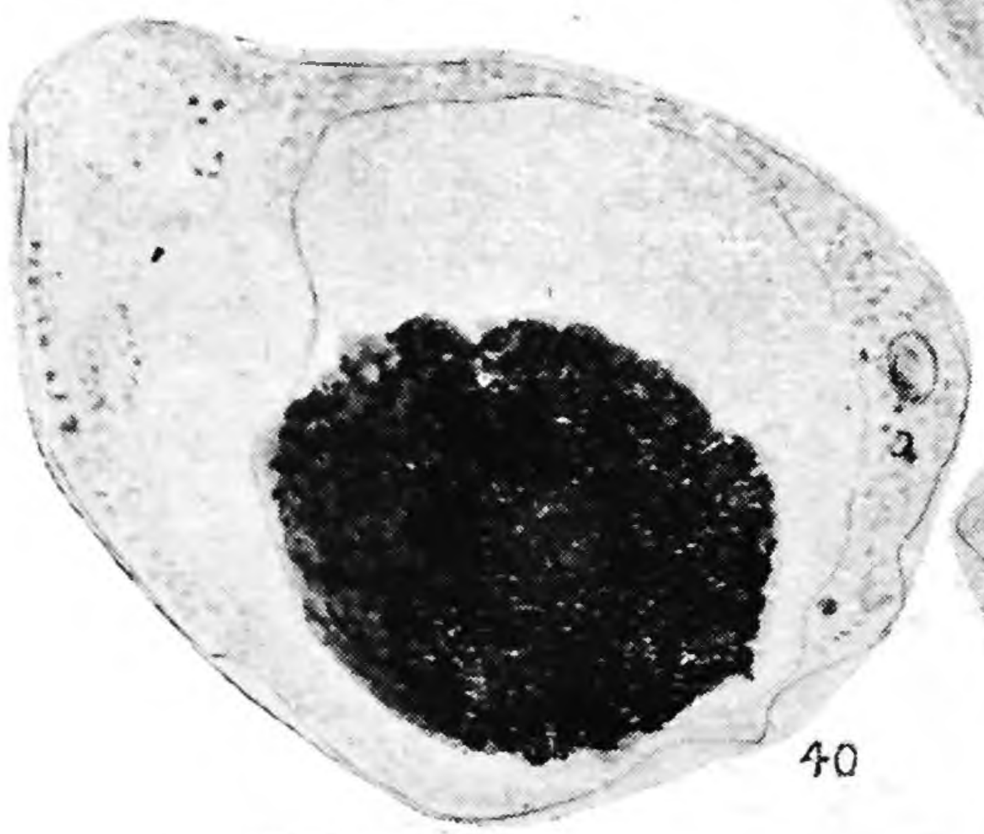

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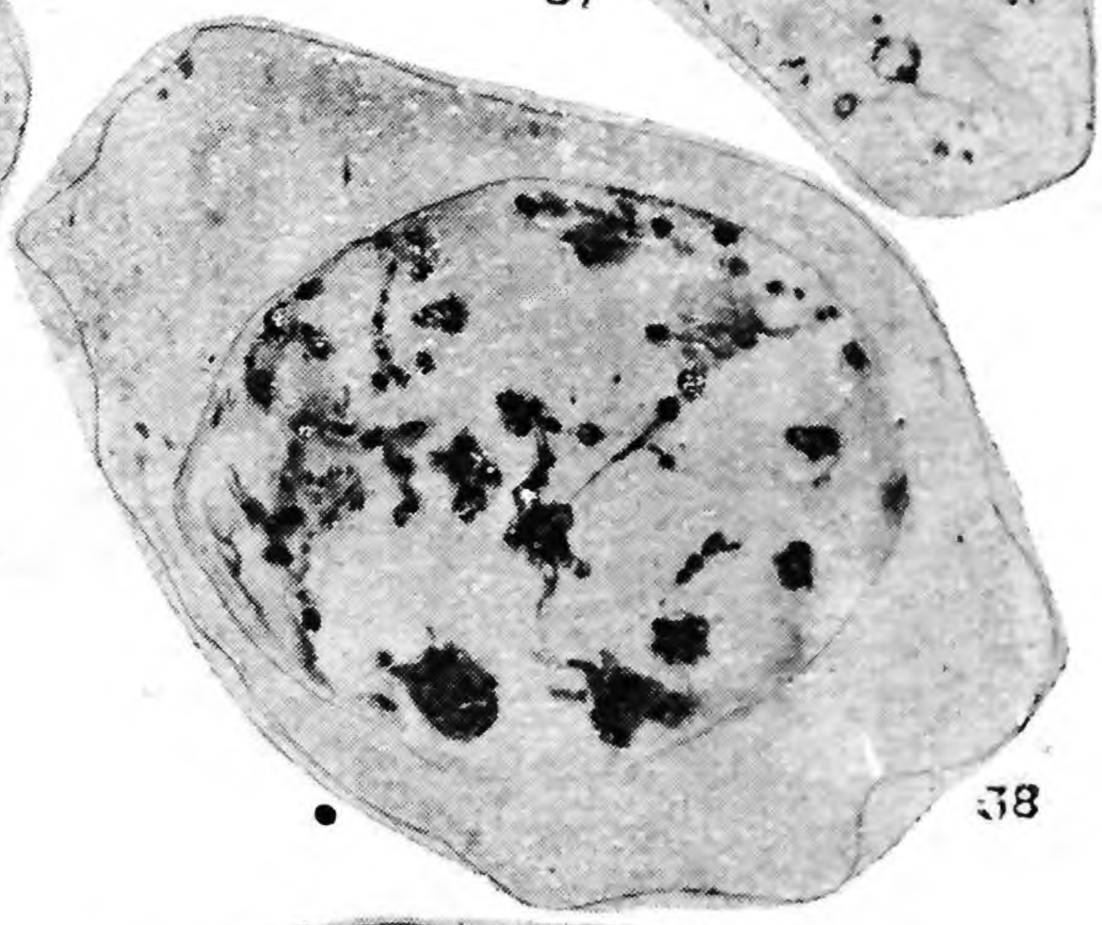

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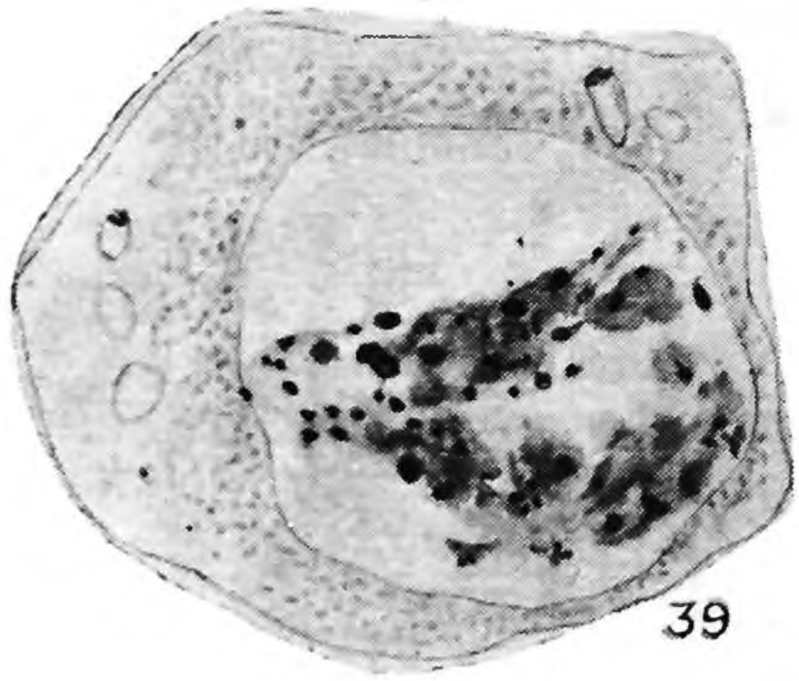

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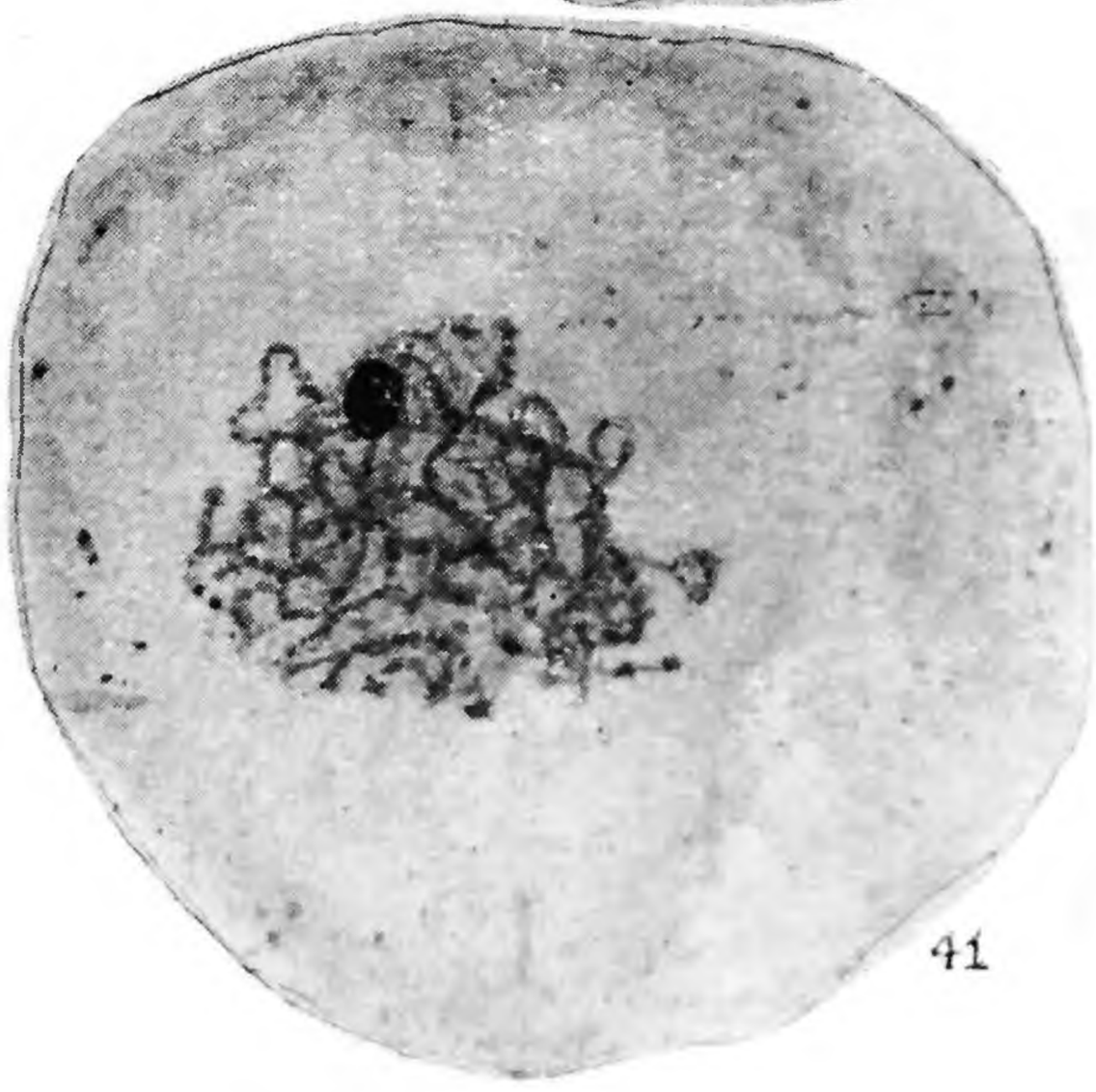

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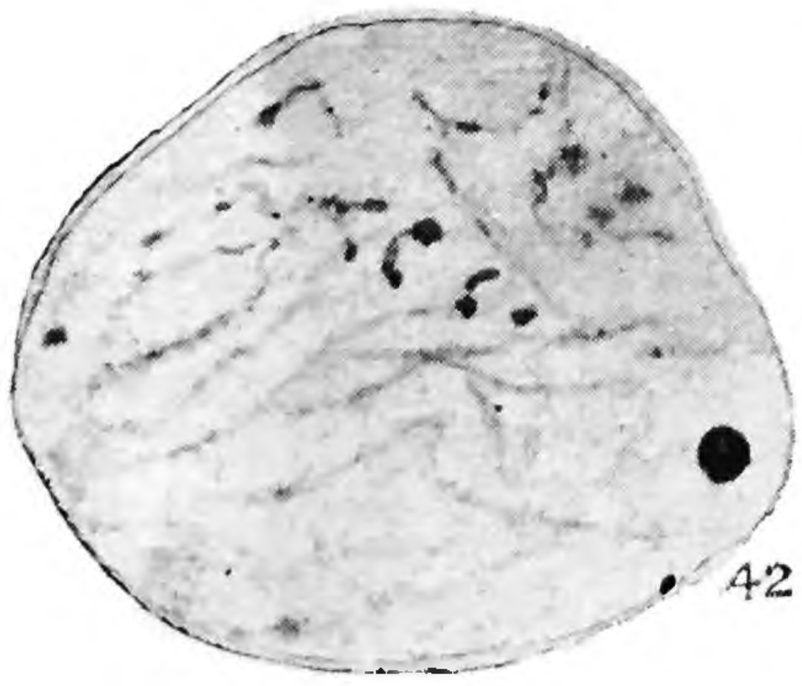

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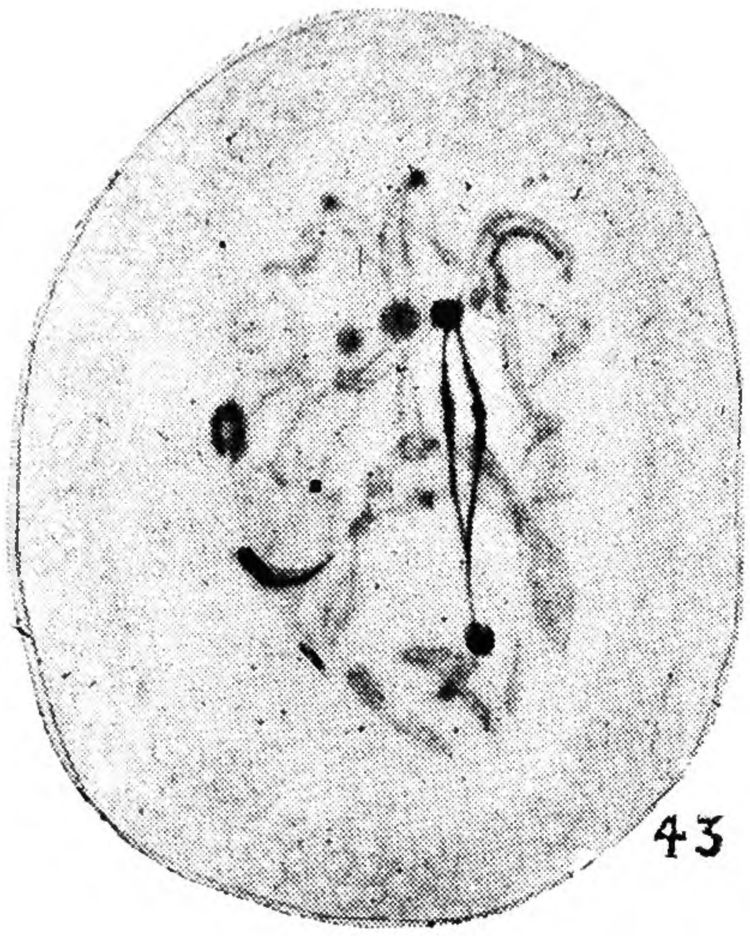
Figs. 35—42.

Plate VII.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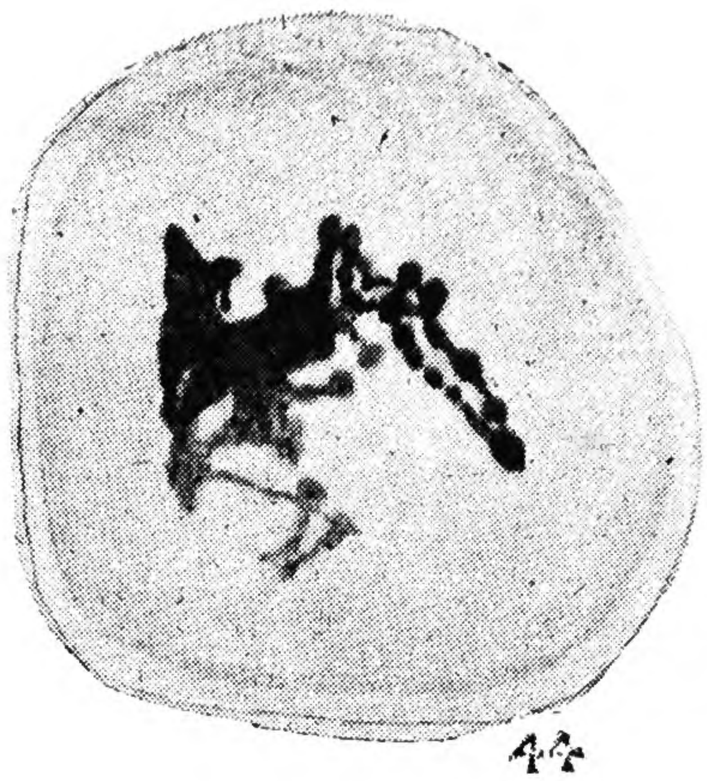
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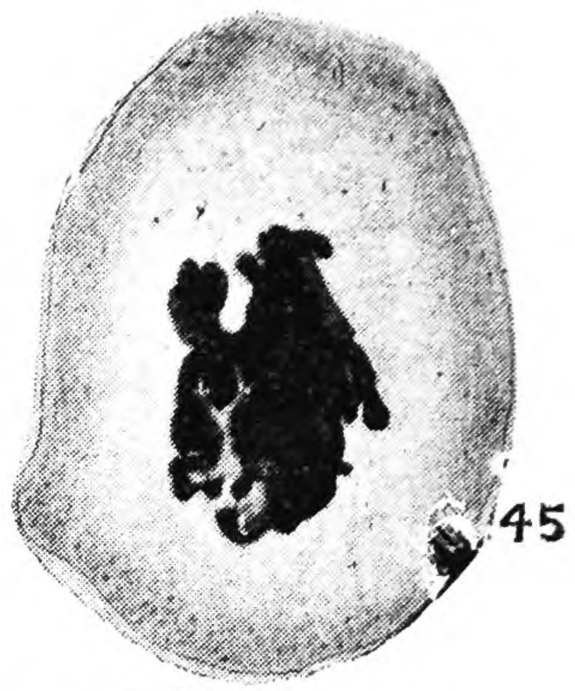
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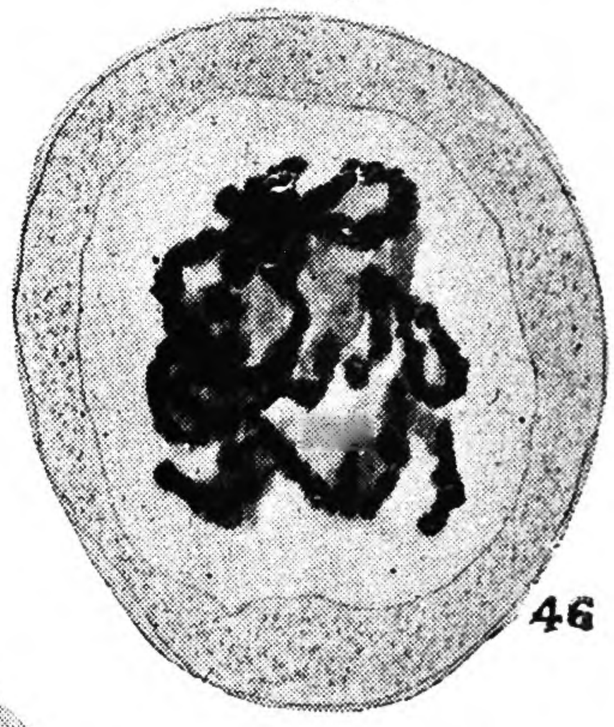
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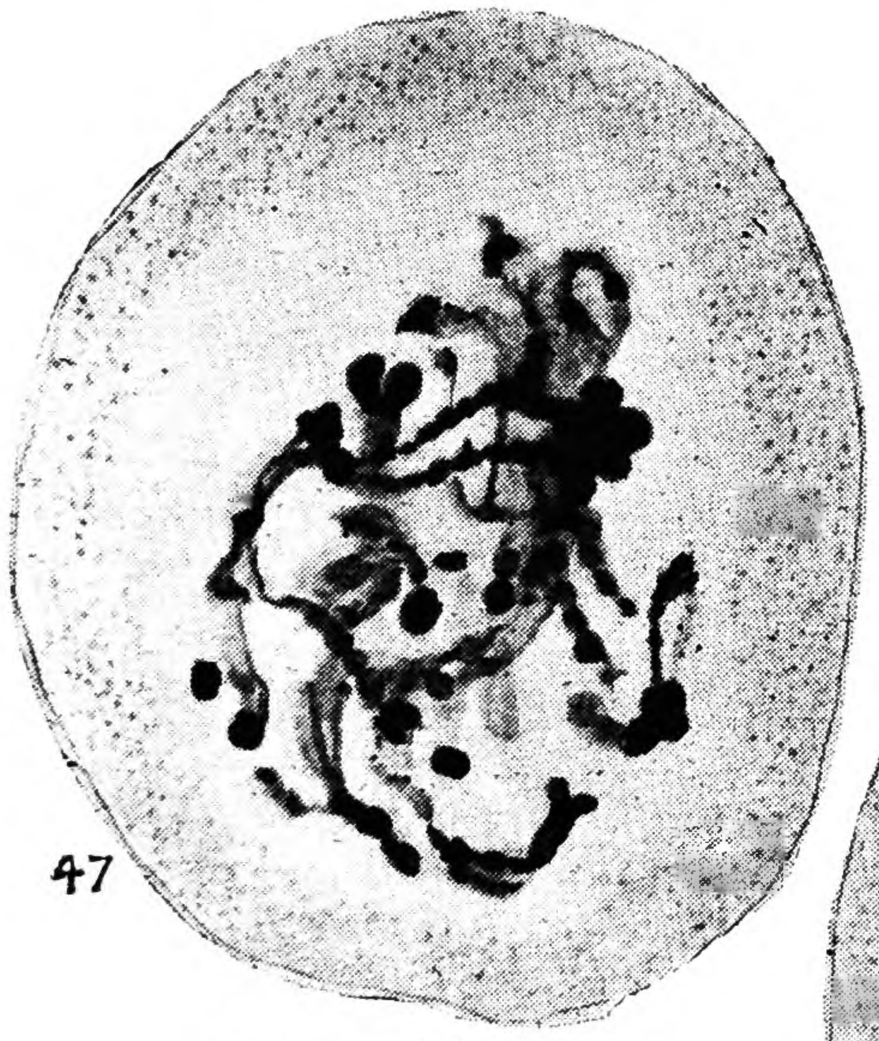
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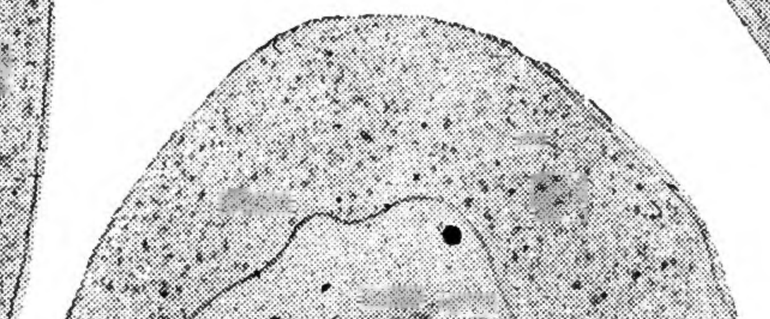
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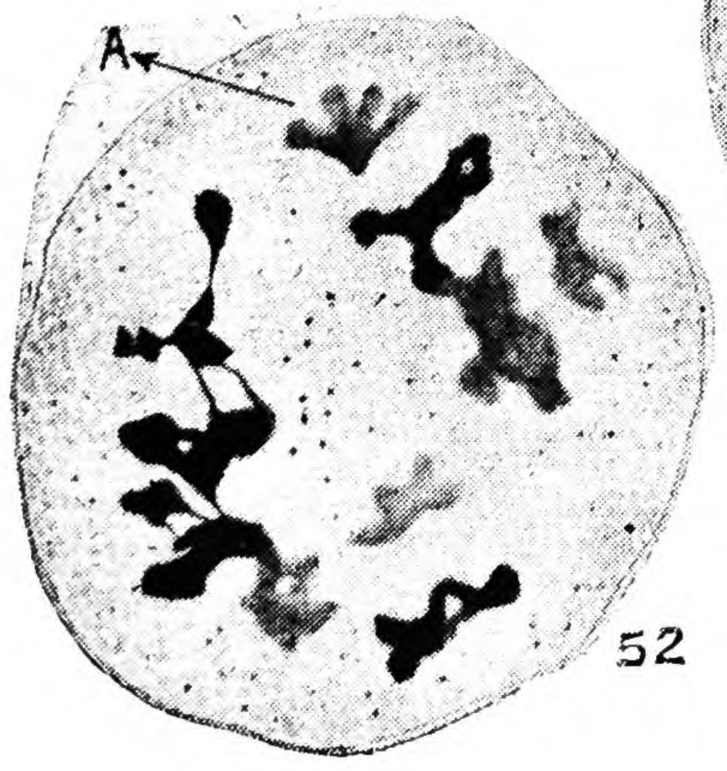
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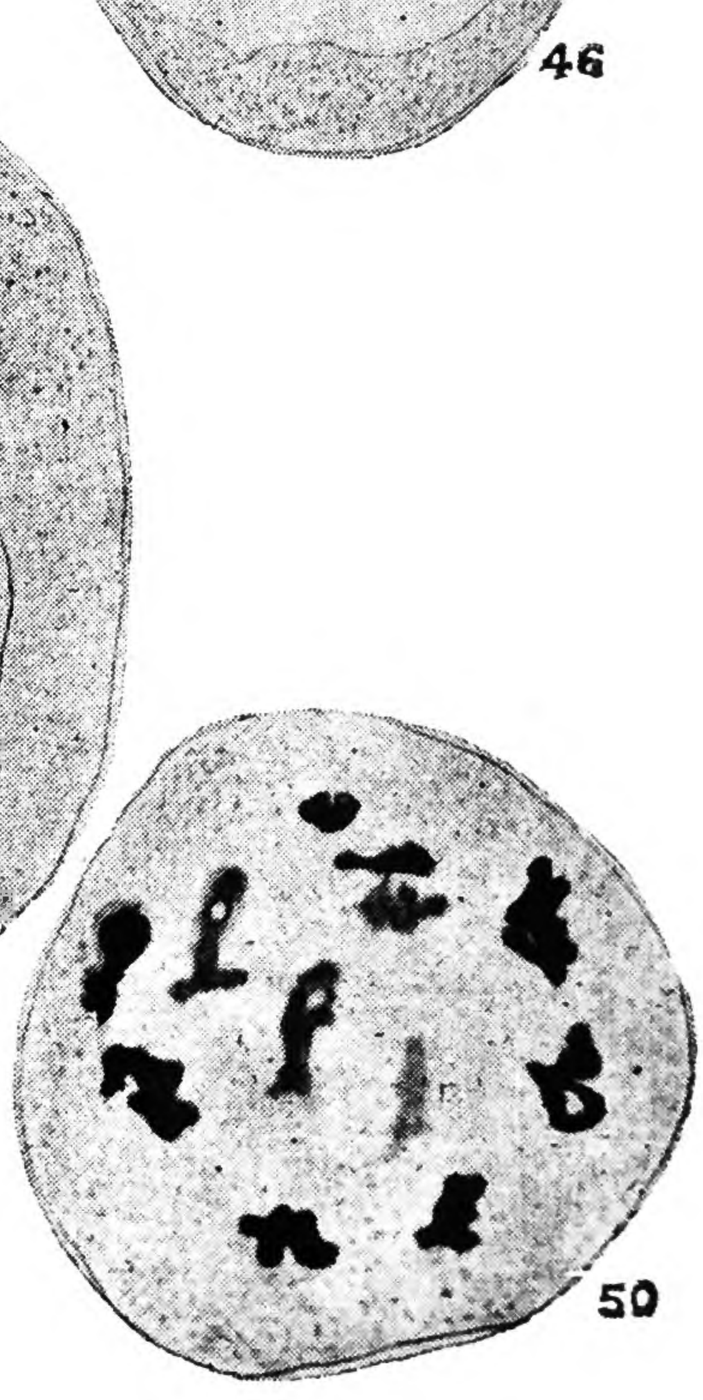
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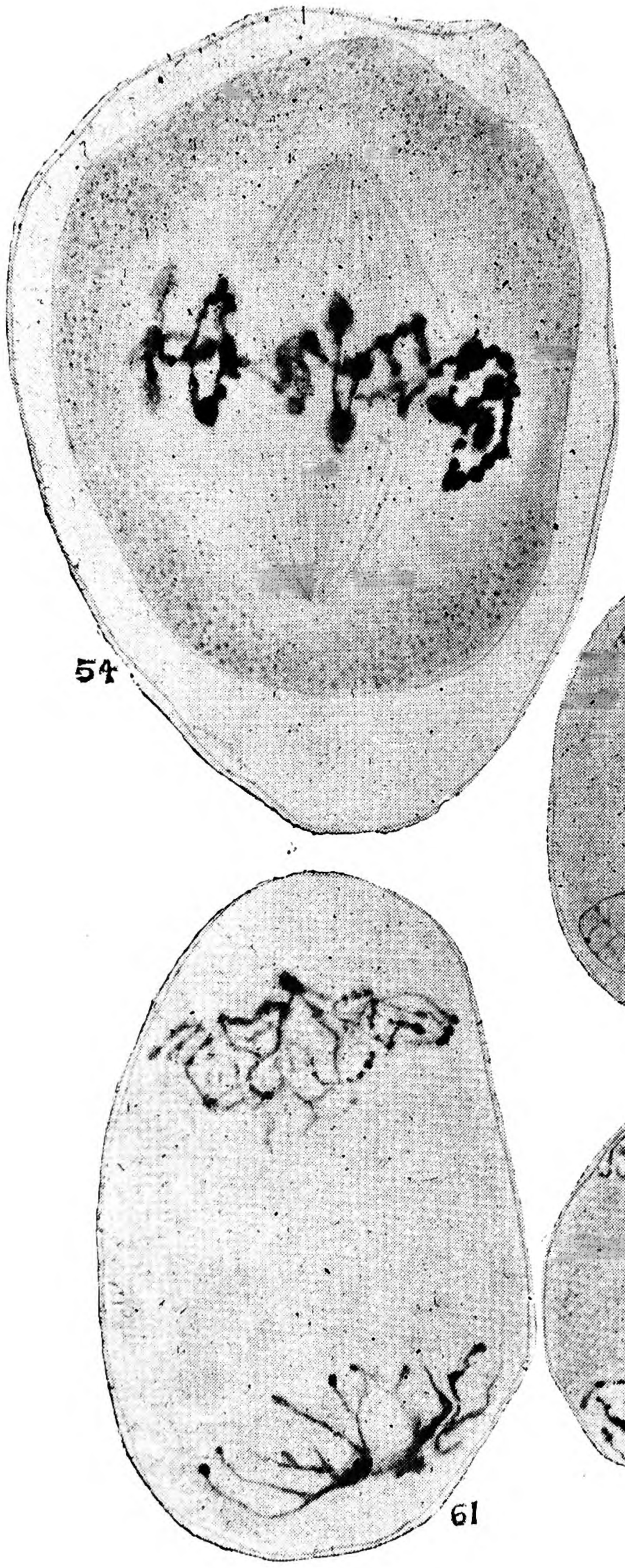
Figs. 43—50, 52.

Plate VIII.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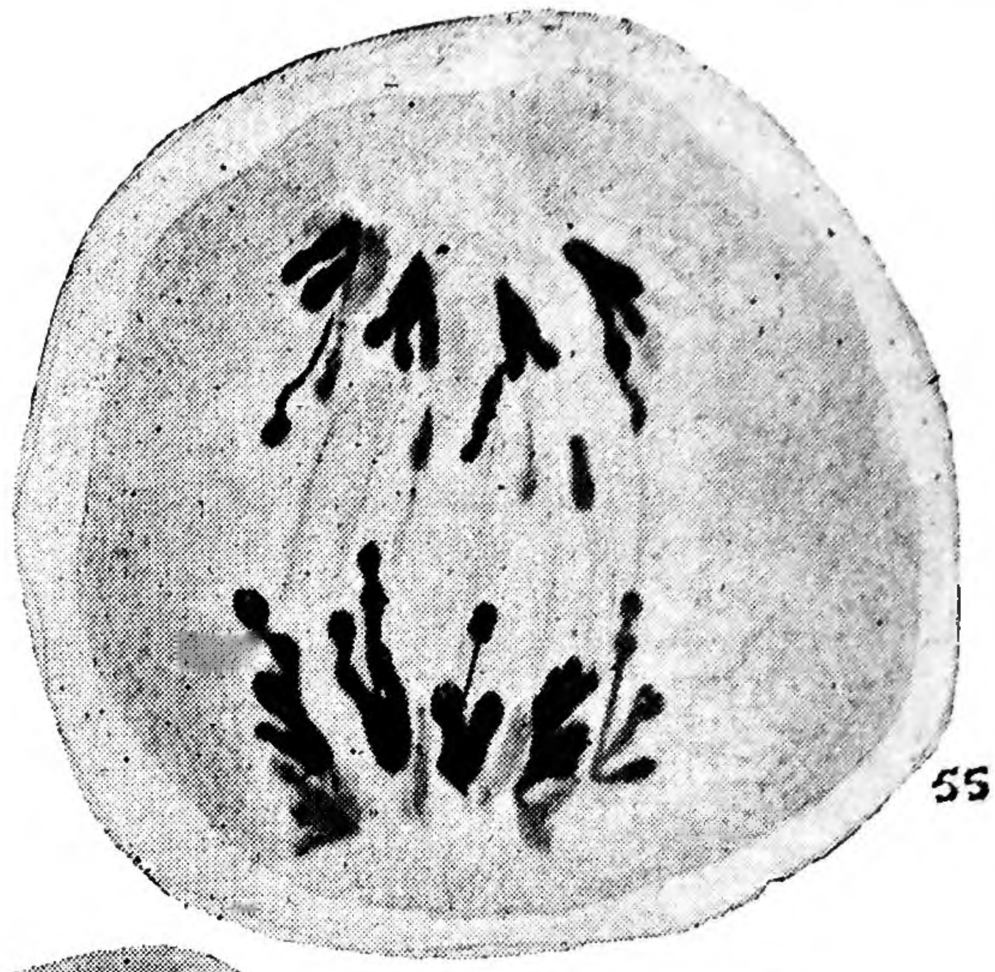
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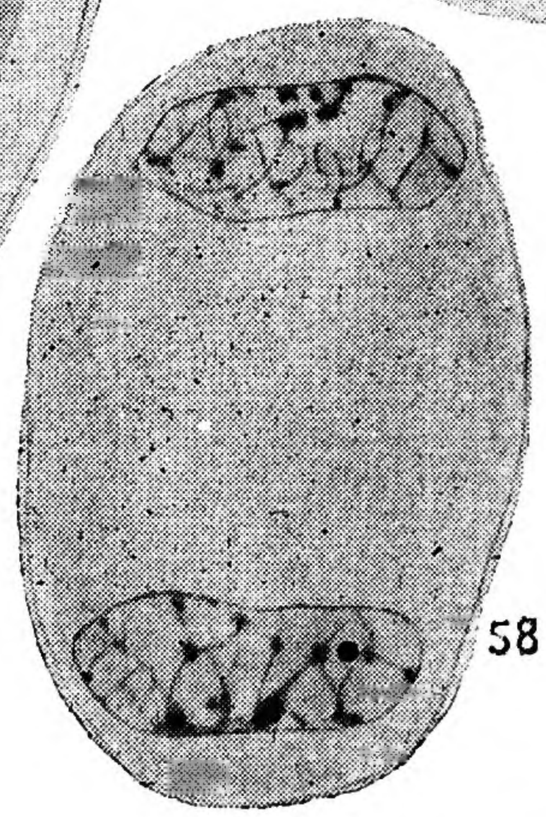
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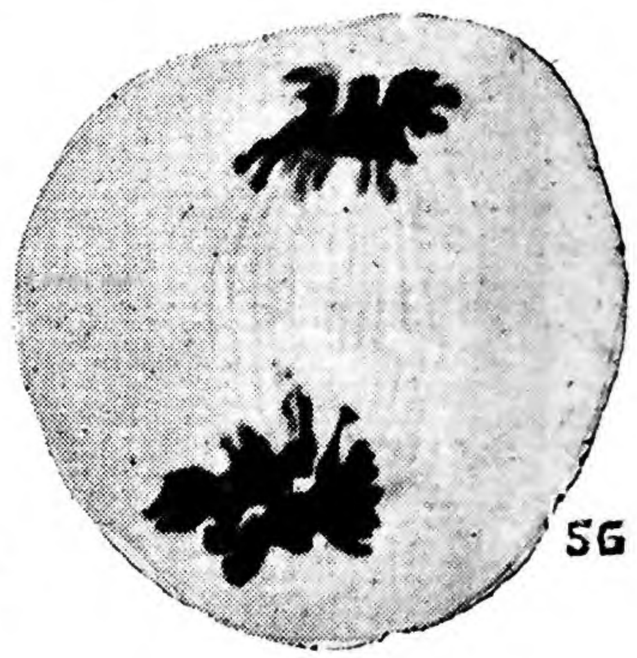
56

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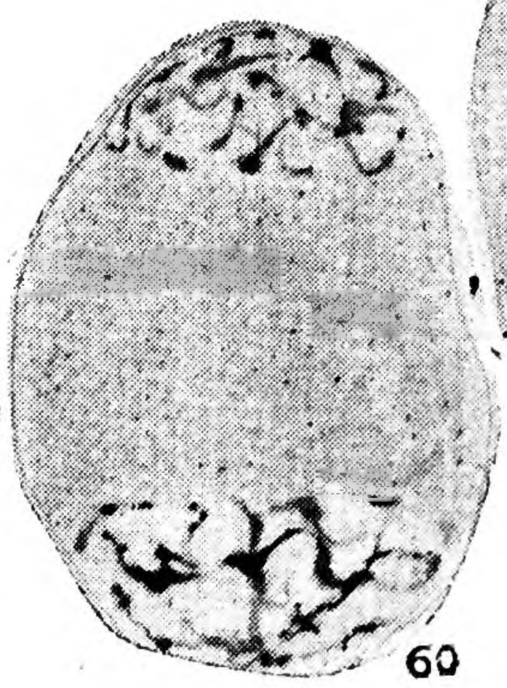
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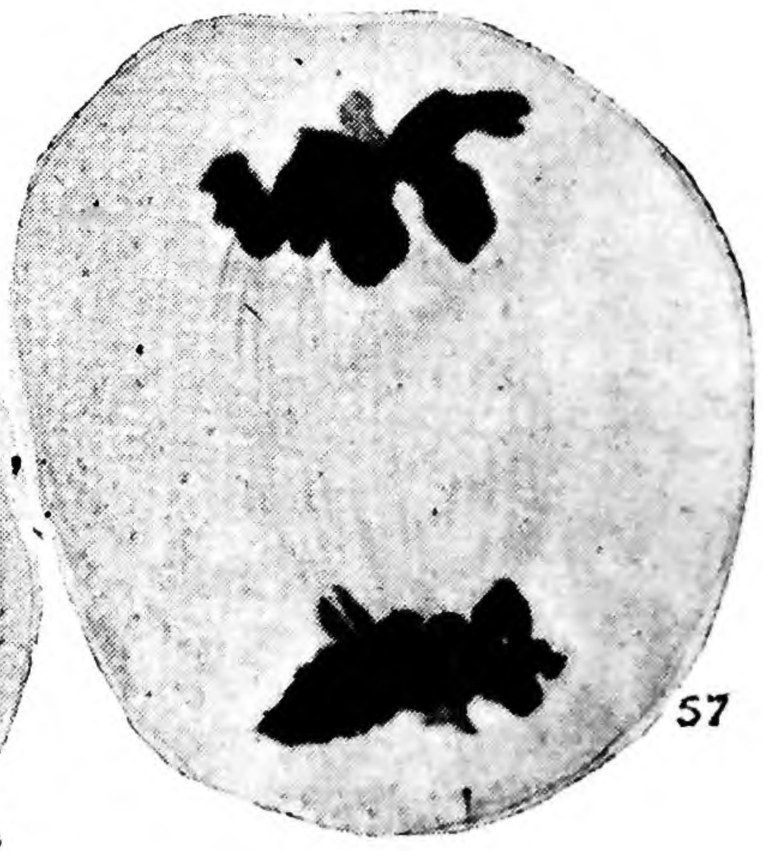
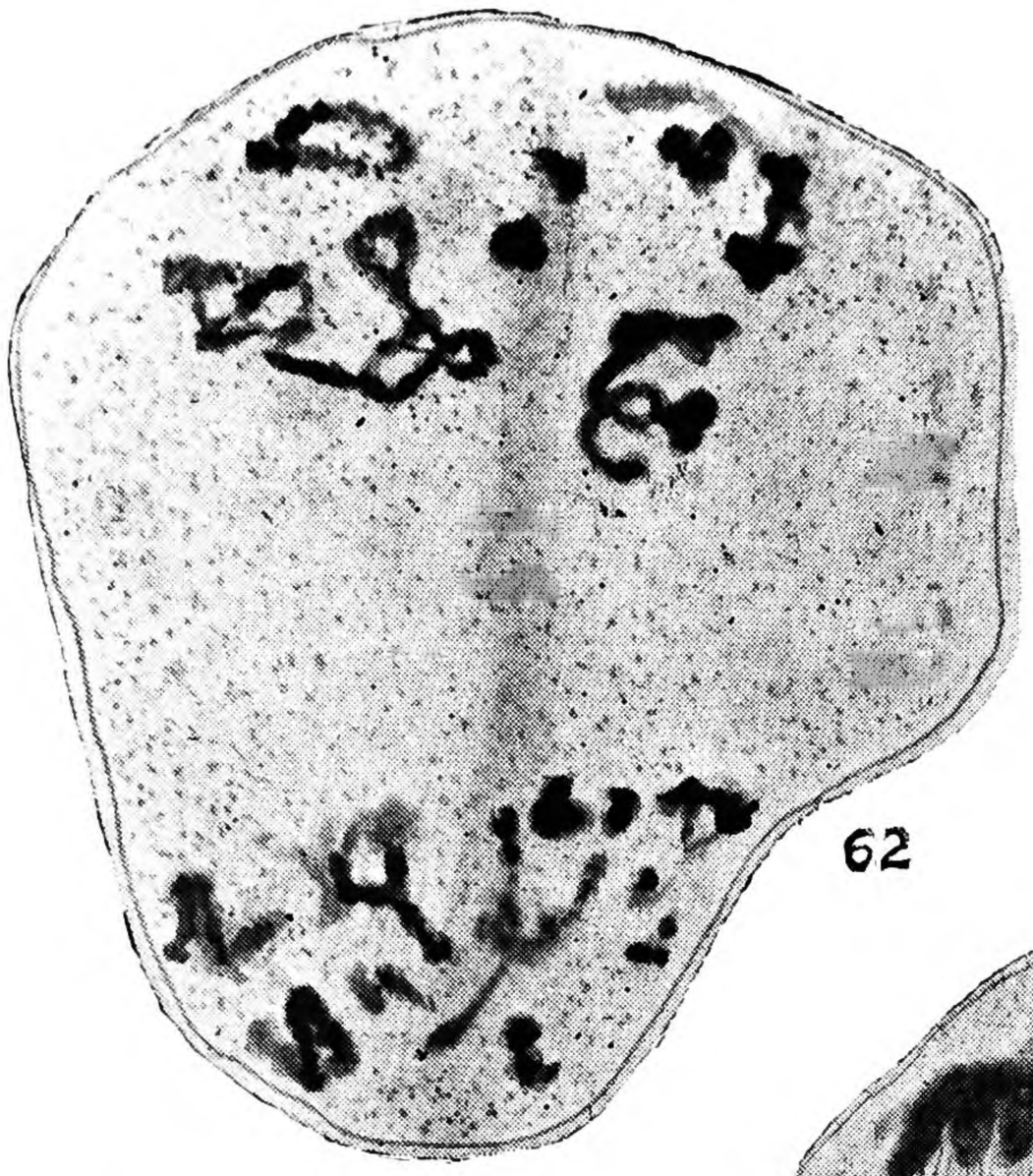

57

Figs. 54—58, 60, 61.



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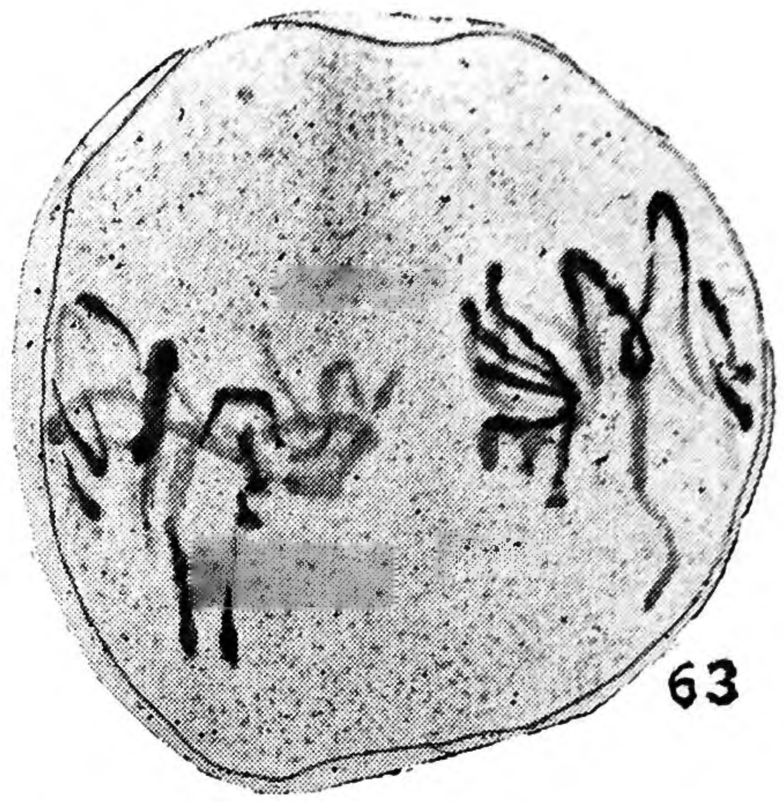

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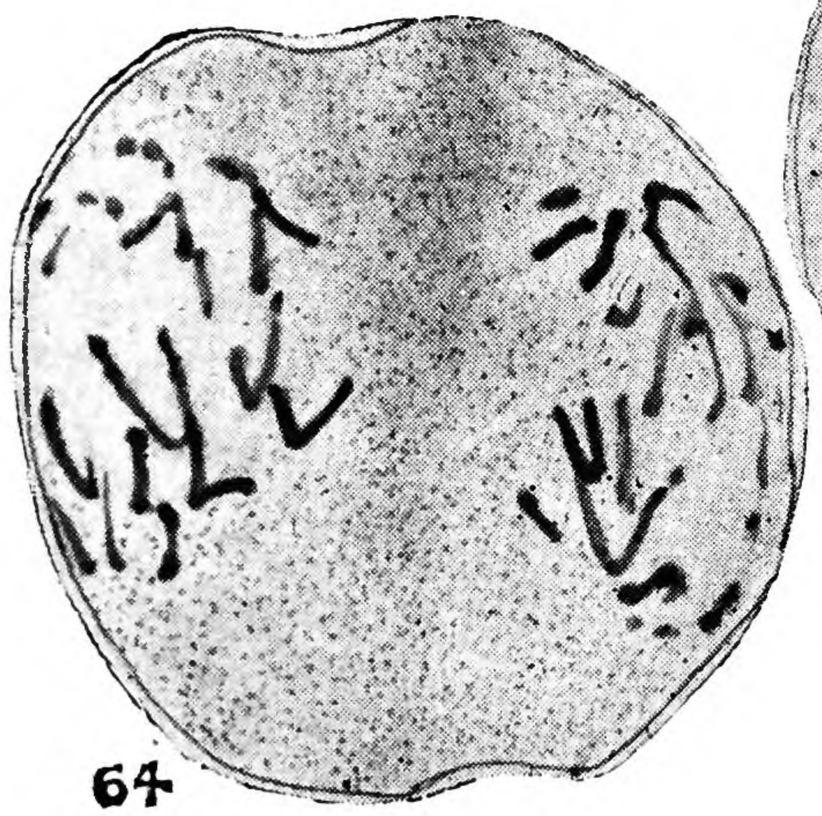

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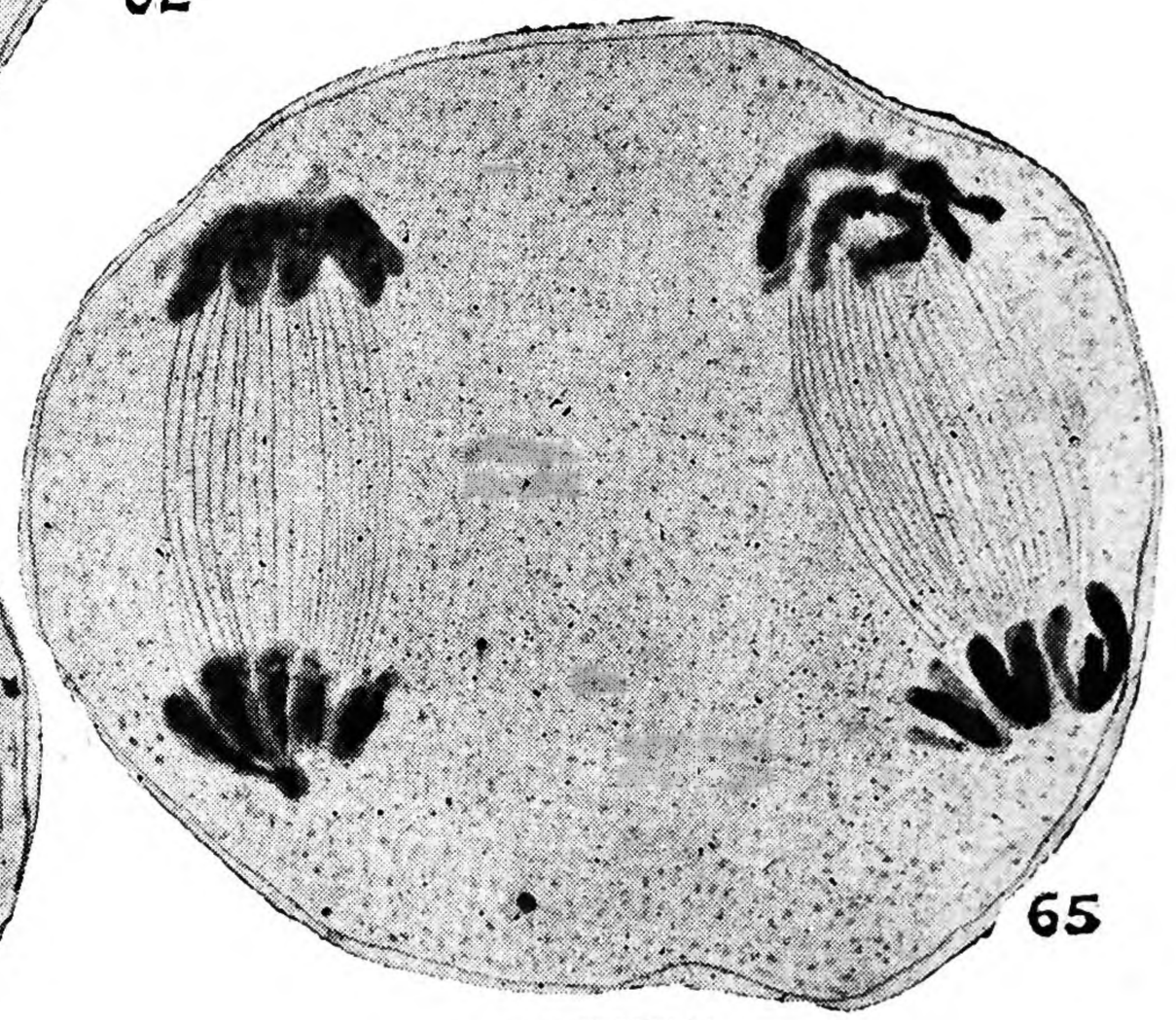

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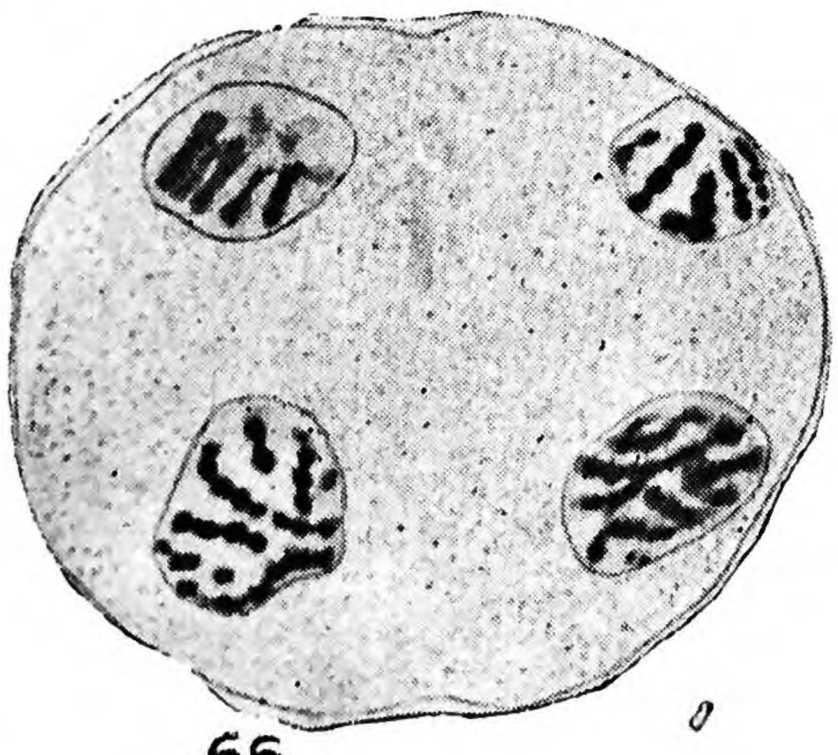

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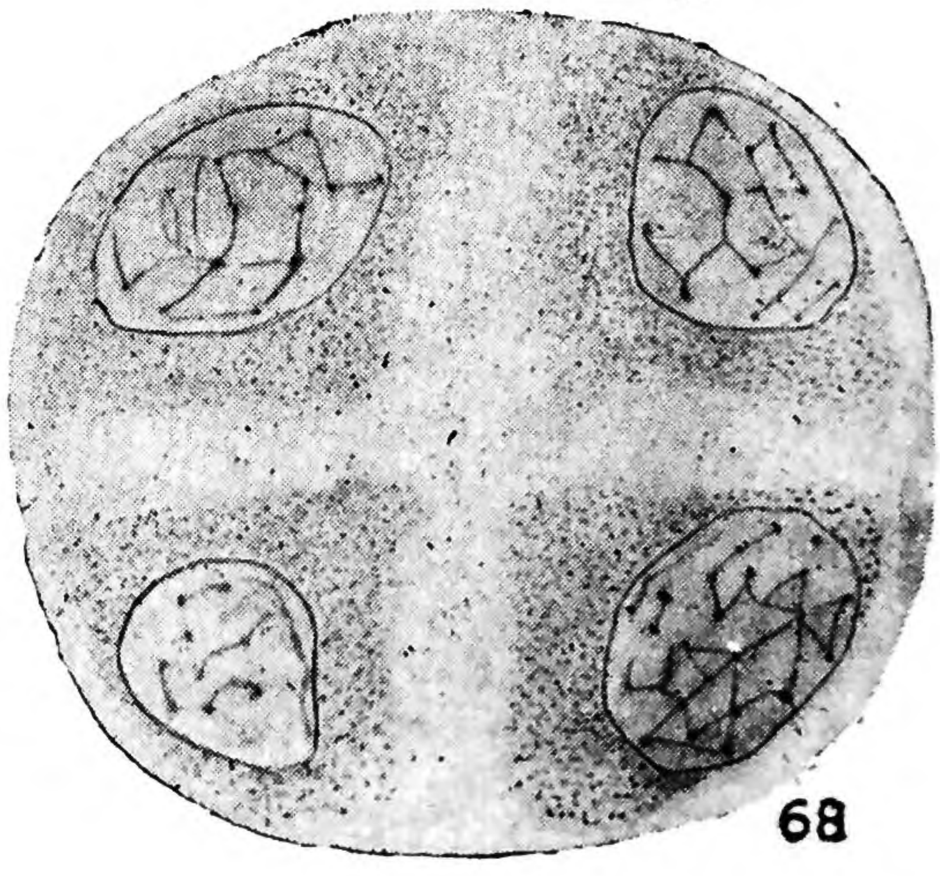
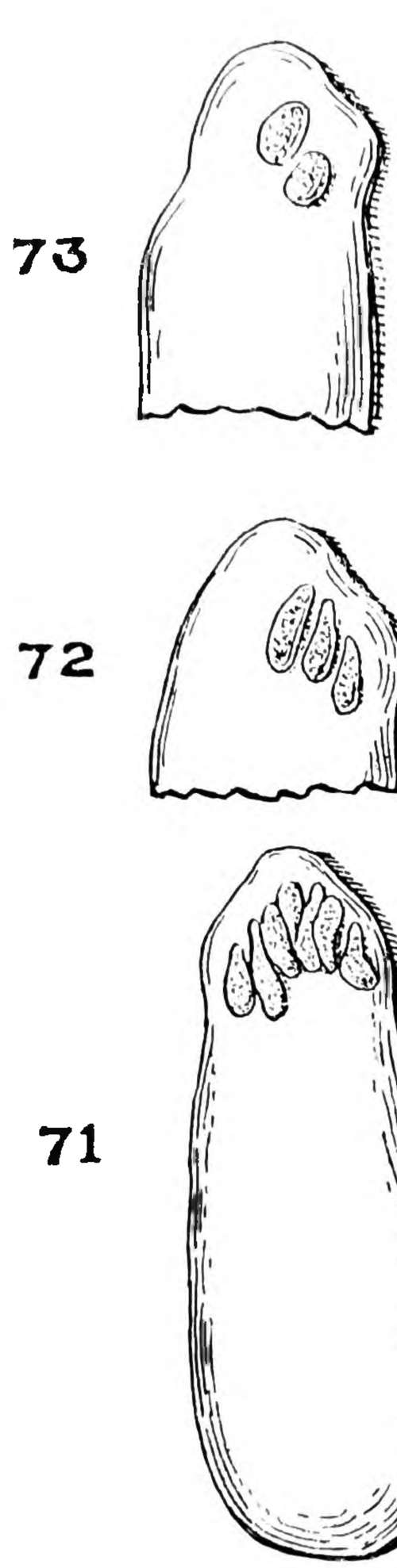
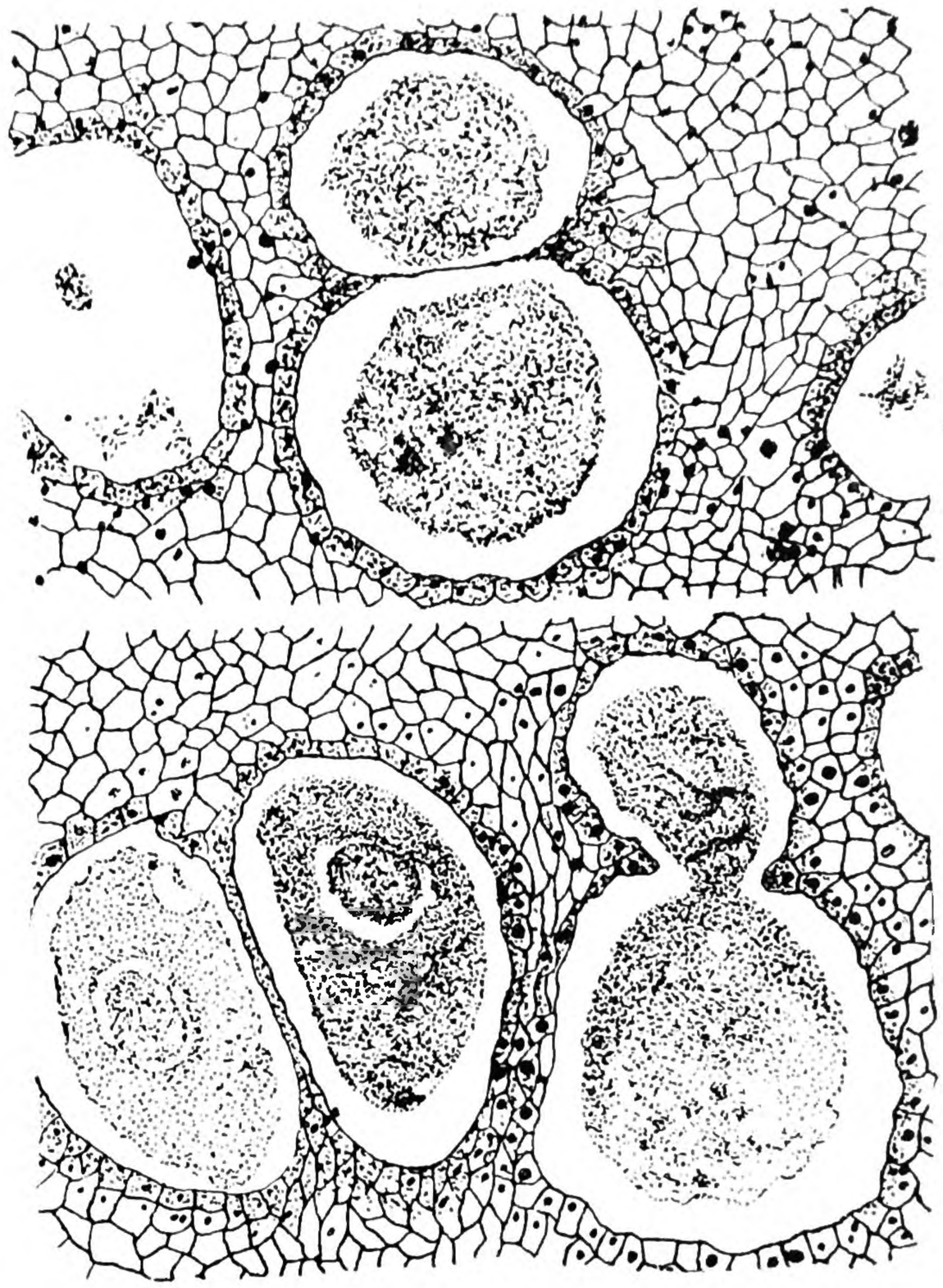
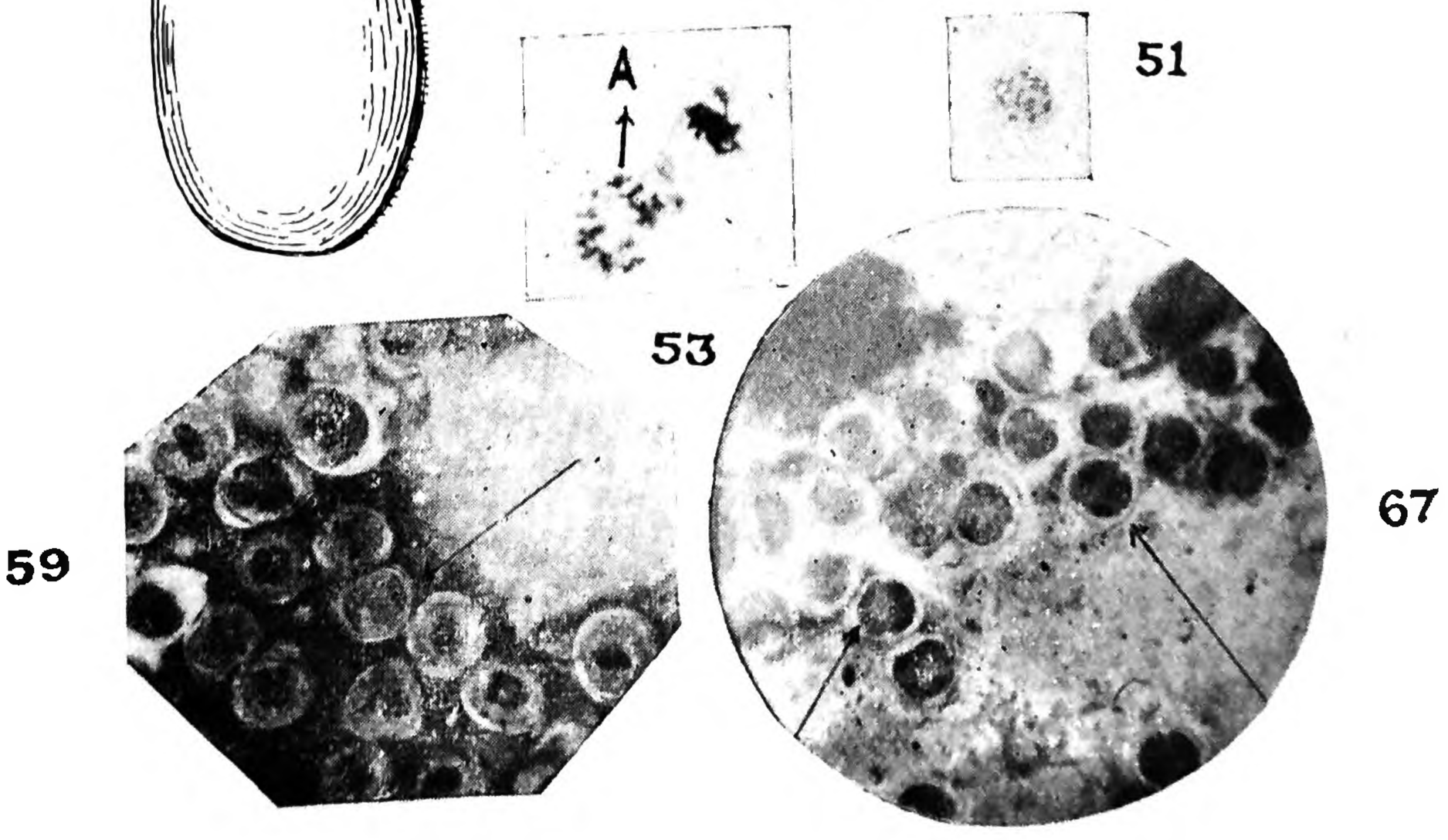


68

Figs. 62—66, 68.







Figs. 51, 53, 59, 67, 69—73.

Fig. 46. Recovery from second contraction. × 1690.
Figs. 47 and 48. Complete recovery from second contraction. × 2350, 2350.
Fig. 49. Bivalent showing univalents (strepsinema). × 2050.
Fig. 50. Diakinesis showing 12 bivalents. × 1690 (cf. photomicrograph in plate X Fig. 51).
Fig. 52. Diakinesis showing chromosome tetrads. × 1660 (cf. photomicrograph in plate. X Fig. 53,)

PLATE VIII.

Fig. 54. Metaphase just before anaphase. × 3325.
Fig. 55. Anaphase. × 2350.
Fig. 56. Late anaphase or early telophase. × 1690.
Fig. 57. Late telophase. 2350.
Fig. 58. Resting nuclei in the daughter cells at the end of the heterotypic division. × 1690.
Fig. 60. Early prophase of the second homotypic division. × 1690.
Fig. 61. Later prophase of the second homotypic division. × 2350.

PLATE IX.

Fig. 62. Prophase of the second division showing fully formed chromosomes. × 2350.
Fig. 63. Metaphase of the second division showing fully formed chromosomes. × 1690.
Fig. 64. Anaphase Ditto. × 1690.
Fig. 65. Early telophase Ditto. × 2350.
Fig. 66. Later telophase Ditto. × 1690.
Fig. 68. A tetrad. × 2050.

PLATE X.

Fig. 51. Photomicro. of the cell shown in Fig. 50.
Fig. 53. Photomicro. of the cell shown in Fig. 52.
Fig. 59. Photomicro. showing daughter cells at the end of the heterotypic division. No cellplate can be seen.
Fig. 67. Photomicro. showing mother-cells with furrows.
Fig. 69. T. S. Abnormal prothallus No. I. One archegonium has a bulge on one side.
Fig. 70. T. S. Abnormal prothallus No. I showing two archegonia separated by a thin membrane only.
Fig. 71. Abnormal female prothallus No. I. ×6.
Fig. 72. „ „ „ No. II. ×6.
Fig. 73. „ „ „ No. III. ×6.