

CONTRIBUTIONS TO THE MORPHOLOGY OF *EPHEDRA FOLIATA*, BOISS.¹

I. The Development of the Male and Female Gametophytes.

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Received February 23, 1935.

1. Introduction.

THE Gnetales have long been attractive to the morphologist because of their intermediate position between the Angiosperms and other Gymnosperms, and recently this claim has again been emphasised by Hagerup (1933, 1934), who has made an extensive study of the organogeny of the flowers in the Coniferales, Gnetales and some Angiosperms. Although this relation has never been definitely established, investigations dealing with them will continue to be of interest for a long time to come.

Four years ago, the author had access to some plants of *Ephedra foliata*, grown in this Department from seeds obtained from the Punjab, and some material was collected in January-March, 1931, and sectioned with a view to prepare some slides for class use and to examine the plant regarding its suitability for investigation.

Due to the small amount of work done on Indian Gymnosperms, I was prompted to keep on the collections with the object of working out the complete life history and anatomy of this species. As the full work will still take some time to be completed, it is proposed to deal with it in parts:—

- (1) The development of the male and female gametophytes.
- (2) Pollination, fertilisation and embryogeny.
- (3) Anatomy of the staminate and ovulate strobili.
- (4) Anatomy of the vegetative organs.
- (5) Anatomy of the seedling.

A general discussion of the results obtained will be given in the last paper.

2. Historical.

Very few species of *Ephedra* have been studied with any degree of completeness from the point of view of the developmental stages in the life

¹ Some authors have subdivided this sp. into three varieties, in which case the one described here should be called *Ephedra foliata* Boiss. var. *ciliata* (C. A. Mey) Stapf.—see Florin, 1933, p. 39.

history. A complete bibliography has recently been given by Markgraf (1926), Pearson (1929) and Schnarf (1933), and I have not considered it advisable to lengthen my paper unduly by covering the same ground again.

The fullest work is that of Land (1904), who first gave an account of the development of the gametophytes and later, in 1907, described the process of fertilisation and embryogeny in *E. trifurca*. Berridge and Sanday (1907) traced oogenesis and embryogeny in *E. distachya*, and Berridge (1909) gave an account of fertilisation in *E. altissima*. The late Miss Herzfeld (1922) described *E. campylopoda* laying special stress on the development of the female flowers and fertilisation. She demonstrated clearly that in several cases one of the male nuclei fuses with the ventral canal nucleus, while the other fertilises the egg.² More recently Geitler (1929) has made some interesting observations on the cytology of two species of *Ephedra*, and Florin (1932) has made an important contribution on chromosome numbers in the Gnetales. There have been other investigations also, to which I shall call attention in the text.

3. Material and Methods.

As stated in the beginning, most of the material was fixed from plants growing in the Agra College Botanic Gardens, and formalin-acetic-alcohol was used as the killing fluid. Chrom-acetic acid and Corrosive sublimate-formalin-acetic-alcohol were also tried, but they were in no way superior to the first. The bracts were always removed from the female strobili except in the youngest stages, when they were too delicate to be handled without injury; in older flowers even the outer integument³ was dissected away. This was found to be quite easy as it is absolutely free from the inner integument and comes away without much difficulty. Ovules killed in this way were found to be well-fixed and gave no trouble in cutting, which is otherwise bound to occur, when the outer envelope becomes hard.

Later, in March 1933, a visit was made to Chhanga Manga (Punjab) and some material was fixed from plants growing in their native habitat.⁴

² This was regarded by her as the equivalent of "double fertilisation," but see Review by Chamberlain (1923).

³ Since there is a good deal of discussion about the morphology of these structures in the Gnetales, I prefer the non-committal terms "envelope" or "investment", and these will, therefore, be used throughout the rest of the paper.

⁴ I wish to record here my deep sense of gratitude to the late Prof. S. R. Kashyap of Lahore, who provided facilities for the trip and told me where to look for the plants. I am also indebted to Mr. Pran Nath Mehra of the Government College, Lahore, for having accompanied me on the trip and helped me in the collection.

As only one collection could be made, it was not possible to get all the stages from this material, most of it showing gametophytes with free nuclei or archegonia in different stages of development. Comparison with the Agra material, however, indicates that the development proceeds in the same way in both places.

Some material was also fixed with the late Prof. S. R. Kashyap's permission from plants cultivated in the Government College Botanic Gardens, Lahore, and my friend Mr. A. C. Joshi, now of the Benares University, kindly sent me four tubes of material, that he had previously fixed from the same place in Flemming's fluid and Bouin's fluid.

The usual methods of infiltration and imbedding were followed and the blocks were sectioned at 5—10 microns. Haidenhain's iron-alum hæmatoxylin proved to be the best stain, with picric acid as the destaining agent (Maheshwari, 1933). Safranin and crystal violet, and safranin and Fast Green were also tried in a few cases. Newton's crystal violet-iodine method, which was also used, did not give satisfactory results with this material.

4. *Distribution and External Morphology.*

E. foliata is occasionally met with in the wild state in the Southern parts of the Punjab plains and may extend southward to parts of Rajputana (Brandis, p. 686 ; Parker, p. 536). The plants that I saw at Chhanga Manga were scandent shrubs, climbing over small trees, especially *Prosopis spicigera*, which is very common in that area. Some specimens were found to reach a height of more than 20 feet. Their general appearance is very much like that of certain leafless Asclepiads, which are also found in dry places, so that in the absence of flowers one may not be able to recognise the plant without previous experience.⁵

5. *The Staminate Strobilus.*

The staminate strobili like the ovulate are borne in whorls round the nodes of branches, in leaf axils. The plants are strictly dioecious and I did not come across a single bisporangiate strobilus, although such have been reported previously in some species. The inflorescence consists of an axis bearing decussately arranged pairs of bracts, which are fused in the lower part. The first one or two pairs may be sterile, but each of the rest has one male flower in its axil.

The male flower is composed of a sheath consisting of two primordia and enclosing within it the antherophore (Fig. 1). The latter outgrows the sheath and protrudes out at maturity, bearing usually three anthers on its top.

⁵ Details about the distribution of all Indian spp. of *Ephedra* and their ephedrine content have been given recently by Krishna and Ghose, 1931.

As the development is continuous and there is no resting period, it is easy to secure all the stages from a comparatively small number of collections. The fact that there is also a gradation in the development of the flowers (the youngest being at the apex) makes it possible to observe a good many stages in a single preparation.

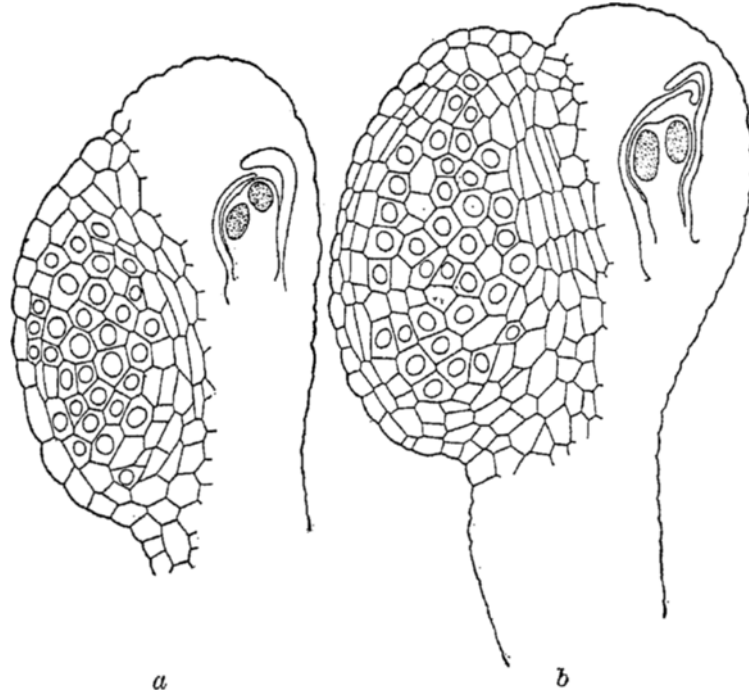


FIG. 1 (*a* and *b*).

FIG. 1 (*a*).—L.S. young staminate flower showing mass of archesporial cells. $\times 265$.

FIG. 1 (*b*).—Same; slightly older stage; showing the sporogenous tissue and two wall layers, the inner of which is the tapetum. $\times 265$.

The small inset in each figure is a diagram of the entire male flower. $\times 45$.

There is no evidence that the archesporium originates from a single hypodermal initial cell. As shown in Fig. 1*a*, there is a group of such cells, which becomes clearly recognisable in each lobe only after the differentiation of the perianth. A primary parietal layer is cut off as usual, and this by tangential divisions gives rise to one wall layer and the tapetum⁶ (Figs. 1*b*, 8, 9). As a rule there are no further periclinal divisions, although some anticlinal divisions do take place to keep pace with the growth of the anther. During the further growth of the sporogenous cells, the wall layer becomes flattened and crushed, leaving only a thin band of granular substance lying

⁶ Land (1904) states that "in *E. trifurca* numerous instances were observed in which individual tapetal cells were not distinguishable from adjacent mother-cells. This seems to indicate that the tapetum is potentially sporogenous, and by virtue of its position has become sterile."

against the epidermis. The tapetal cells, however, increase in size and stain densely.

6. *Tapetum*.

In the meantime the sporogenous cells divide and form a considerable mass of cells—the microspore mother cells (Fig. 8). When these are in the prophase of the first reduction division, the tapetal cells become 2–4 nucleate (Fig. 9). According to the notions prevalent at that time, I and thought that this was due to amitotic divisions, but Geitler (1929) has recently demonstrated that in *E. maior* these divisions are mitotic. It is also reported that sometimes the two spindles of the second division fuse together to give rise to tetraploid nuclei. These phenomena are exactly comparable to those found in several angiosperms (Cooper, 1933).

In *E. foliata* I have seen undoubted mitotic divisions in the tapetal cells of several anthers, and I think that at least the first nuclear division is always karyokinetic. After this is over, the two daughter nuclei sometimes lie very close to each other (thus giving the appearance of amitotic division) and may later fuse together to form a tetraploid nucleus, or their spindles may join together in the next division to give rise to two tetraploid nuclei. In some tapetal cells, one large and two smaller nuclei were seen, which gives rise to the suspicion that originally four were formed, but two fused together; or, this 3-nucleate condition may result from the division of only one of the two nuclei formed after the first division. It also happens that towards the close of activity in the tapetum, all four nuclei in a cell sometimes fuse together into a densely staining irregularly lobed mass.

There is no evidence whatever of the formation of a periplasmodium, and the tapetal cells begin to degenerate soon after the reduction divisions are over, leaving only the epidermis in the mature anthers. Some of the cells in the sporogenous tissue, however, begin to degenerate at an early stage (one such is seen in Fig. 8). Evidently these are tapetal in function and serve to nourish the remaining mother cells. It is needless to consider this point in detail, as this is a common feature of many large sporangia in vascular plants.

7. *Formation of Microspores*.

The reduction divisions go through in the normal way. The microspore mother cells retain their polygonal shape for a long time, but their protoplasts begin to round up at the edges after synizesis and the space at the corners becomes filled with a clear homogeneous substance, staining very lightly or not at all with hæmatoxylin. At diakinesis (Fig. 10) the cytoplasm contracts round the nucleus and becomes very dense, the space between this and cell-wall beginning to be filled with the same substance. Usually

no cell-plate appears after the first reduction division (Fig. 11), but in a very few cases a very delicate membrane *was* observed. This is, however, not seen in later stages.

The two spindles in the second division are usually at right angles to each other, but sometimes parallel or even in an intermediate plane. This division is accomplished very quickly and the four grand-daughter nuclei arrange themselves near the periphery of the mother cell protoplast, the fibres connecting them disappearing very soon after the division is over.

Ephemeral cell-plates were also seen in a few instances after this division, but they fade away and I have no doubt that they have nothing to do with the division of the cell. Quadripartition of the mother cell is effected by furrowing (Fig. 12), as has now been shown for a large number of Angiosperms.

The mother cell-wall is no longer clearly distinguishable, but the space between this and the protoplast becomes filled with a gelatinous substance (now seen very clearly in preparations stained with Safranin and Fast Green but scarcely noticeable when iron-haematoxylin alone is used), which advances centripetally and separates the four microspores (Fig. 13).

8. *The Male Gametophyte.*

The only clear account of the development of the male gametophyte is that by Land (1904) and the stages, I have obtained, are almost exactly similar to those figured and described by him. The microspores after freeing themselves from the jelly-like matrix, already mentioned above, become oval in outline (Fig. 14) and the nucleus travels down to one of the pointed ends. The first division cuts off a prothallial cell (Fig. 15), marked off from the remaining nucleus by a clear hyaline space. The second division (Fig. 16) cuts off another prothallial nucleus, but this time no separating membrane is formed and its cytoplasm is not distinguishable from the general cytoplasm of the pollen grain (Fig. 17). Both the prothallial nuclei stain very densely and begin to degenerate soon after they are formed. The remaining nucleus now enlarges very much and divides to form the tube and antheridial⁷ nuclei (Fig. 18). The former usually lies at the end opposite to the one in which the prothallials are situated, but in some cases it was found to have shifted itself laterally. The antheridial nucleus becomes surrounded by a denser zone of cytoplasm (Fig. 19), and one sterile and another spermatogenous nucleus, which result from its division, lie within the same cytoplasmic sheath (Fig. 20). The mature pollen grain is thus 5-nucleate at the time of shedding. It differs in this respect from both *Welwitschia* and *Gnetum*, which have advanced to the tri-nucleate condition.

⁷ The term "generative nucleus" has been avoided for reasons given ahead.

The exine, which begins to be laid down immediately after the microspores have freed themselves from the mother cell-wall, is quite well-developed and has, on an average, about 16 ribs running longitudinally from one end to the other. These are best seen in a section passing at right angles to the long axis of the grain (Fig. 22). Herzfeld (1922) remarks that in *E. campylopoda* the intine is also similarly ribbed and there is a space between this and the exine, which is either filled with air or some other non-stainable substance. I have not noticed this peculiarity in *E. foliata*.

In several pollen grains another dark-staining nucleus (a sixth one) with a flattened outline was found lying closely attached to the second prothallial nucleus. At first I was doubtful about its method of origin and considered this to be the sterile nucleus ("stalk nucleus") that had pressed itself down on the second prothallial nucleus, but appearances like those shown in Fig. 21 leave no doubt that the latter itself occasionally fragments into two. What significance this particular abnormality has, I am not yet prepared to say, but similar phenomena are also known to occur in some Conifers.

One case was found of a double pollen grain, which was cut at right angles to the long axis and was dumb-bell shaped in outline (Fig. 23).

Attention must be called here to the confusion that exists in literature on the names given to the nuclei in these pollen grains. This is due to the attempts made by certain authors to homologise the pollen grain of Gymnosperms with the antheridium of Pteridophytes, and by others to compare the further development of the microspore with that of the megaspore. The following table summarises the nomenclature used by various authors at present:—

Strasburger, 1879	prothallial cells	tube nucleus	spermatogenous cell	sterile sister cell	antheridium mother cell	generative nuclei
Coulter and Chamberlain, 1917	"	"	generative cell	stalk cell	body cell ⁸	male nuclei
Schürhoff, 1926	"	"	spermatogenous cell	"Stielzelle"	antheridium mother cell	sperm. nuclei
Herzfeld, 1927 Wettstein, 1933	"	vegetative nucleus	antheridial cell	"Wandzelle" ⁹	spermatogenous cell	generative nuclei
Terminology used here	"	tube nucleus	"	sterile (spermatogenous) cell	"	sperm. nuclei

⁸ German authors have translated this as "Korporzelle".

⁹ Goebel (1932, p. 1800) calls this the "Dislokatorzelle".

Due to the different names used to designate the same nucleus, it is sometimes quite difficult to extract a clear idea of the structure of the male gametophyte from the descriptions of several authors. It seems, however, that Jaccard (1894) did not see the prothallial cells and found only the tube, sterile and spermatogenous nuclei in *E. helvetica*. Fig. 2 of Berridge (1909) indicates that the male gametophyte of *E. altissima* is similar to that of *E. trifurca* and *E. foliata*. Among recent investigators, Herzfeld (1922, p. 254) writes that in *E. campylopoda* :—

“Die Anlage der Mikrosporen wurde nicht untersucht; doch konnte beobachtet werden, dass die reifen Pollenkörner schon während ihres Aufenthaltes in der Anthere keimen, das heisst eine sterile Prothalliumzelle, Pollenschlauch- und Antheridialzelle entwickeln. Land beobachtete bei *Ephedra trifurca* zwei Prothalliumzellen übereinander an jenem Ende der Mikrospore, welches dem Pollenschlauchkern gegenüber liegt; bei *E. campylopoda* konnte an dieser Stelle nur eine sterile gesehen werden, doch waren ab und zu seitlich von der Antheridialzelle 1 bis 2 kleine Zellen sichtbar, die als Wandzellen gedeutet werden müssen (Taf. II, Fig. 38 b). Meist sind alle Zellwände sichtbar—ab und zu wird eine Wand nicht angelegt.”

This account is very brief and meagrely illustrated. Only two figures of the pollen grain have been drawn and they do not bring out the points stated above.

There have been some other investigations on the structure of the mature pollen grain, but these are of a doubtful nature and have therefore been left out of consideration.¹⁰

The time taken by the events already described is perhaps the shortest among Gymnosperms. In the Cycadales, Ginkgoales and Coniferales this period usually extends from several months to more than an year. In *E. trifurca* (Land, 1904) the staminate strobilus was first recognisable (in New Mexico) in the 1902–1903 season in December and the pollen was shed in April. From observations made at Agra for the last five years, I can say that in *E. foliata* the strobili are not recognisable before the first week of January and the development goes on continuously, as the climatic conditions do not necessitate any resting period. Some of the male flowers are ready to shed their pollen even in the second week of February, but more continue to develop so that mature anthers can be seen on a plant right up to the end of March. Indeed, the only approach to this condition is *Gnetum*, where also the whole development is completed in a few weeks (Thompson, 1916).

I believe that the shortening of the period of flowering and fruiting is correlated with the external conditions and is not an index of phylogenetic

¹⁰ I am trying to obtain material of some other species of *Ephedra* and examine the structure of the male gametophyte in these as soon as opportunity permits.

advance. In support may be cited the case of *E. gerardiana* Wall., which flowers in its native habitat in the Himalayas (8–14,000 ft.) in May and the seeds ripen towards the end of September (before the beginning of the severe winter), but due to the milder climate, the plants in cultivation at Lahore, begin flowering much earlier and the seeds are set by the middle of May (Mehra, 1934).

9. *The Ovulate Strobilus.*

The female inflorescence is usually made up of four pairs of decussate bracts, which are fused below, forming cup-like structures. The lowest of these is very small, the upper two progressively larger, and the fourth is the largest and contains within it the two ovules. The stem-tip is continued as a small projection between the two ovules (Fig. 3). Occasionally one of these gets aborted so that the strobilus is left with only one ovule.

10. *Formation of Megaspores.*

The earliest stages show the nucellus to be a mass of homogeneous cells without any differentiation of the integuments. Fig. 24 shows a longitudinal section of a slightly older ovule with two hypodermal archesporial cells lying side by side. As I have only one preparation showing this stage, I cannot say if this is the usual number; succeeding events give the impression that in most cases there is only one.

There are very few Gymnosperms (besides the Gnetales) in which a hypodermal archesporial cell has so far been reported.¹¹ The usual textbook statement is that the megaspore mother cell is deeply "buried" inside the nucellus from the very beginning. Strasburger (1879) concluded that in *Larix europæa* the megaspore mother cell arises by the divisions of a hypodermal archesporial cell. Saxton (1930) has recently reinvestigated some stages in the morphology of this plant and he states that "the most careful search failed to reveal the slightest trace of such cells or any cell which could be interpreted as a hypodermal archesporium". Land (1904) says that in *E. trifurca* the archesporium could not be definitely traced back to a single hypodermal cell, but he regards this as likely. The earliest stage, he figures, shows two large cells lying one above the other and separated from the epidermis by two wall layers. He regards the lower of these as an undoubted megaspore mother cell and the upper as a wall cell. As the figure is very diagrammatic, one cannot feel sure about this interpreta-

¹¹ So far as I know, this has been definitely recorded only in two plants:—*Zamia floridana* (Smith, 1910) and *Taxus canadensis* (Dupler, 1920). There are two other cases, where (judging from figures) its occurrence seems to be likely, though not yet actually demonstrated:—*Keteleeria fortunei* (Hutchinson, 1917) and *Athrotaxis selaginoides* (Saxton and Doyle, 1929).

tion; it appears equally likely that this is a case of two sporogenous cells lying one above the other and separated from the epidermis by a couple of wall layers. Herzfeld (1922, Plate I, Fig. 2) [shows a megaspore mother cell in synapsis, lying separated from the epidermis by a single wall layer. Sigrianski (1913) definitely reported a hypodermal archesporial cell in *E. helvetica* and asserts that this functions directly as the megaspore mother cell, although it becomes sunken in later stages by periclinal divisions in the nucellar epidermis. That the wall layers are all epidermal derivatives, seems to be rather striking, for this is the only known case of its kind among the Gymnosperms.¹²

From a comparison of the figures of other authors and my own preparations of *E. foliata*, it seems certain that a hypodermal archesporial cell will be found to be of a general occurrence in *Ephedra*, if the material is sectioned at a sufficiently early stage. The absence of "spongy tissue" which is of frequent occurrence in the Cycadales, Ginkgoales and Coniferales is noteworthy.

The next stage, represented in Fig. 25, shows an elongated megaspore mother cell with two wall layers separating it from the epidermis. Evidently the wall cells are the result of periclinal divisions of a primary parietal cell cut off from the archesporial cell. In later stages the epidermal cells also undergo many periclinal divisions and the parietal tissue has thus a double origin (Figs. 26, 27, 28, 30).

The first division of the megaspore mother cell is a reducing division. In one slide the nucleus was seen in the diakinesis stage (Fig. 26), but the preparation was not good enough to give a clear evidence of the exact number of chromosomes. Fig. 27 shows the close of the first division resulting in the formation of two daughter cells. In her Plate I, Fig. 1, Miss Herzfeld shows two nuclei formed as a result of the heterotypic division, but there does not seem to be any indication of a wall formed between them!

Several tetrads of megaspores were encountered. Usually all the four cells are arranged in a single linear row (Fig. 29), but occasionally the upper two may be placed obliquely (Fig. 28) and sometimes even at right angles to the lower two, resulting in T-shaped tetrads. In two cases a row of only 3 cells was seen, although a careful search was made for the fourth in adjacent sections. This is now regarded as a matter of frequent occurrence in both Gymnosperms and Angiosperms and does not seem to deserve special attention.

Herzfeld regularly found 4 megaspores arising from the mother cell, whether arranged in the form of a "T" or in a single row. In her Plate I, Fig. 3,

¹² See *Review* by Land (1915).

showing a normal tetrad of megaspores with the lowest enlarging, there is also a big cell on top of the tetrad, which I am inclined to regard as another sporogenous cell that has failed to divide (compare Land, 1904, Fig. 26).

Occasionally I found two megaspore tetrads lying side by side in the same nucellus. These are evidently due to the divisions of two hypodermal initials as seen in Fig. 24.

11. *The development of the female gametophyte.*

The lowest megaspore is the one that usually functions, but in one case the third was seen to enlarge (Fig. 2) and occasionally the third and fourth

were seen to grow together. Berridge and Sanday reported that in *E. distachya* the arrangement of megaspores appeared to be "tetrahedral and not linear" and the third megaspore functions. From their figure, however, it would appear more correct to regard this as a slightly disturbed T-shaped arrangement.

The only account that shows the most radical departure from the usual course of events is that of Sigrianski (1913), who states that in *E. helvetica* the four megaspore nuclei are not separated by walls and that all of them take part in the further development of the gametophyte. This, if correct, would correspond to the "Lilium-type" of embryo sac met with in several Angiosperms. It would be very desirable if this species is studied again by those who are more favourably placed regarding the collection of its material.¹³

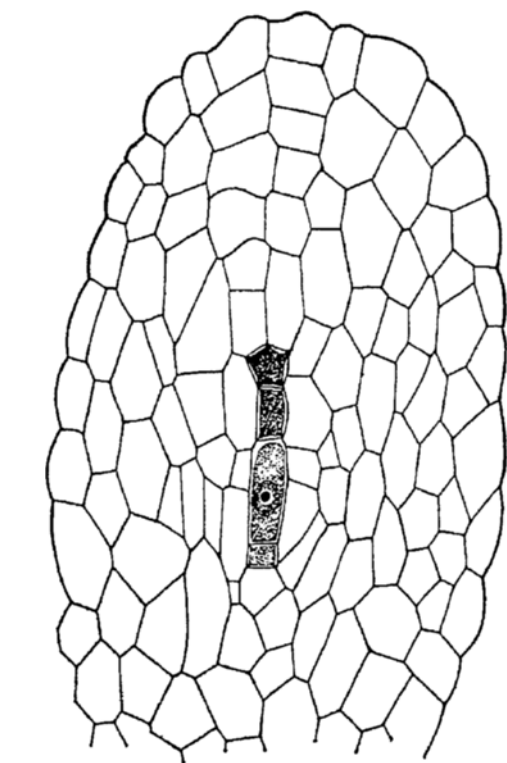


FIG. 2.—L.S. nucellus, showing tetrad of megaspores, of which the third has enlarged and the others have been suppressed. $\times 530$.

The growth and development of the "integuments" is shown by Figs. 3-7. The outer becomes several layers thick and at its upper end it sends out numerous short papillate outgrowths, which are directed inwards. The

¹³ Others giving similar reports have already been corrected. Cavara and Rogasi (1902) stated that in *E. campylopoda* all four nuclei take part in the formation of the female gametophyte. Herzfeld has, however, shown that 4 megaspores are formed as usual. See in this connection Schnarf (1933, 63-64).

inner is only two cells thick, but elongates more quickly and forms a long "micropylar tube", which is characteristic of the Gnetales.

The two nuclei formed by the divisions of the functioning megaspore move toward opposite sides and a vacuole arises between them (Fig. 30). The next stage with four nuclei is shown in Fig. 31. Free nuclear divisions continue for a long time. In all cases the cytoplasm and nuclei are limited to the periphery, while the centre is occupied by a large vacuole.¹⁴ In two or three cases I was surprised to find the whole sac full of cytoplasm with the nuclei scattered all over. Further examination revealed, however, that this behaviour was clearly abnormal and brought about by injuries caused by insects.

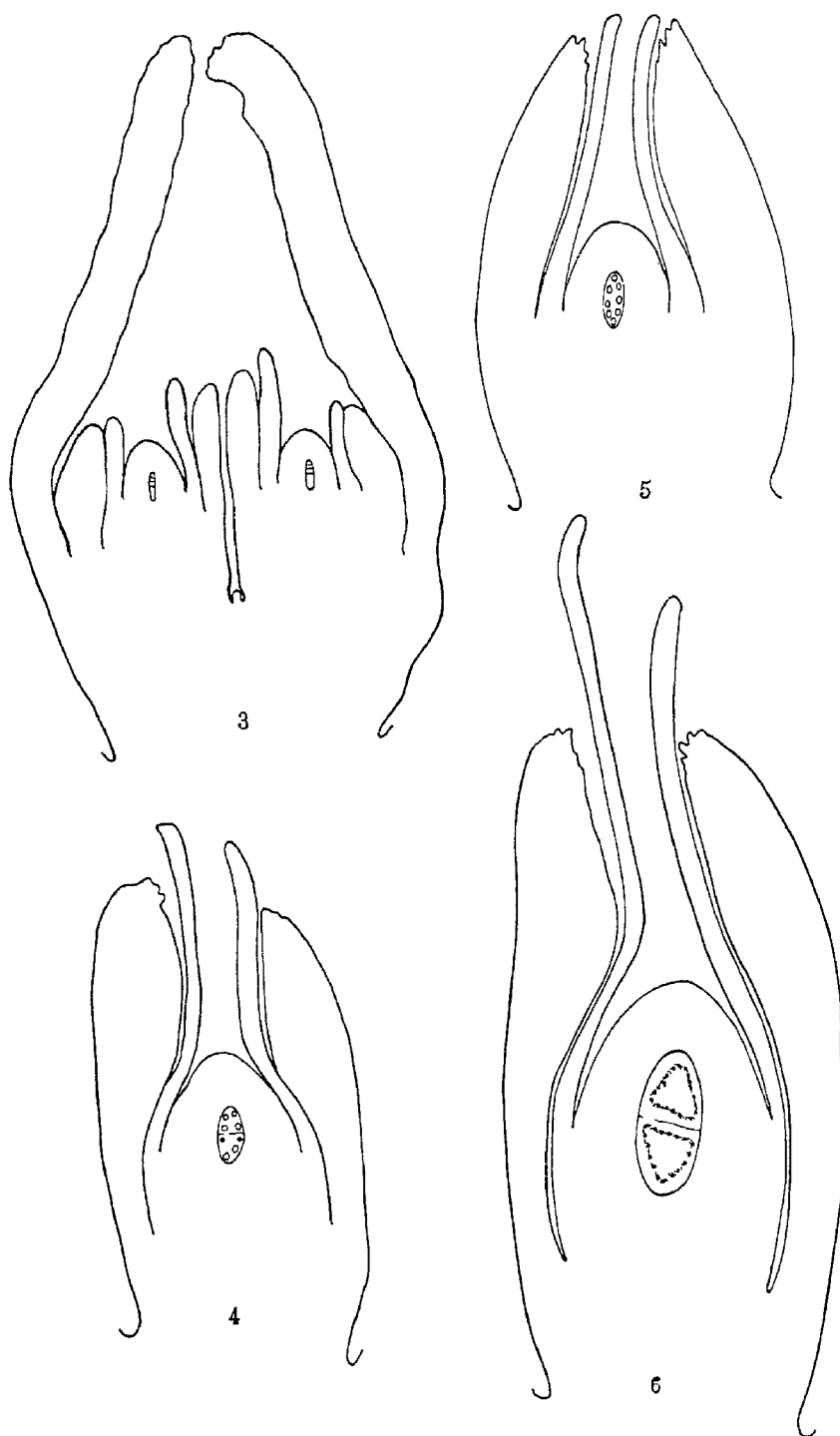
In several preparations two gametophytes were found lying one above the other in the same ovule, suggesting their derivation from sister megaspores of the same tetrad (Figs. 4, 6). Herzfeld once found three gametophytes in *E. campylopoda*. Other reports of a similar kind have been given by Saxton (1910) in *Callitris*, Lawson (1923) in *Pherosphaera* and Saxton (1934) in *Austrotaxus*. Perhaps the most striking case of this kind is that of *Taxus canadensis*, where Dupler (1917) found this kind of a thing to be of frequent occurrence and in some cases saw even four gametophytes in the same ovule.

Further examination revealed that in *E. foliata*, sometimes the upper gametophyte in an ovule enlarges and the lower is suppressed and sometimes it is just the reverse. Occasionally both were seen to grow together and even form archegonia.

The exact stage at which wall formation occurs could not be determined in my material. In preparations showing the largest free nuclear gametophytes I could count approximately 500 nuclei. Land (1904) says that in *E. trifurca* it occurs after 256 nuclei have been formed and Berridge and Sanday (1907, p. 129) state that in *E. distachya* this commences only after a thousand nuclei have been formed.

It is usually supposed that the free nuclear divisions are simultaneous and authors, who have reported the number of free nuclei in the female gametophytes of Gymnosperms and the endosperm of Angiosperms, have usually borne this fact in mind. Judging from the spindles seen in a couple of favourable preparations of this species, I am inclined to think that occasionally some of the nuclei lag behind in division. In one of these preparations most of the nuclei were in metaphase, but a few situated in a denser

¹⁴ Land (1904) writes, however, that careful staining showed the vacuole to be filled at all later stages with a delicate cytoplasmic structure, which gradually increased in density.



FIGS. 3-6.

- FIG. 3.—L. S. ovulate strobilus with two young ovules enclosed within the uppermost pair of bracts. $\times 68$.
 FIG. 4.—L.S. ovule with 2 gametophytes, both at the 4-nucleate stage. $\times 68$.
 FIG. 5.—Same; gametophyte with 8 free nuclei. $\times 68$.
 FIG. 6.—Two gametophytes within the same ovule; upper with about 64 free nuclei and lower with about 40. $\times 68$.

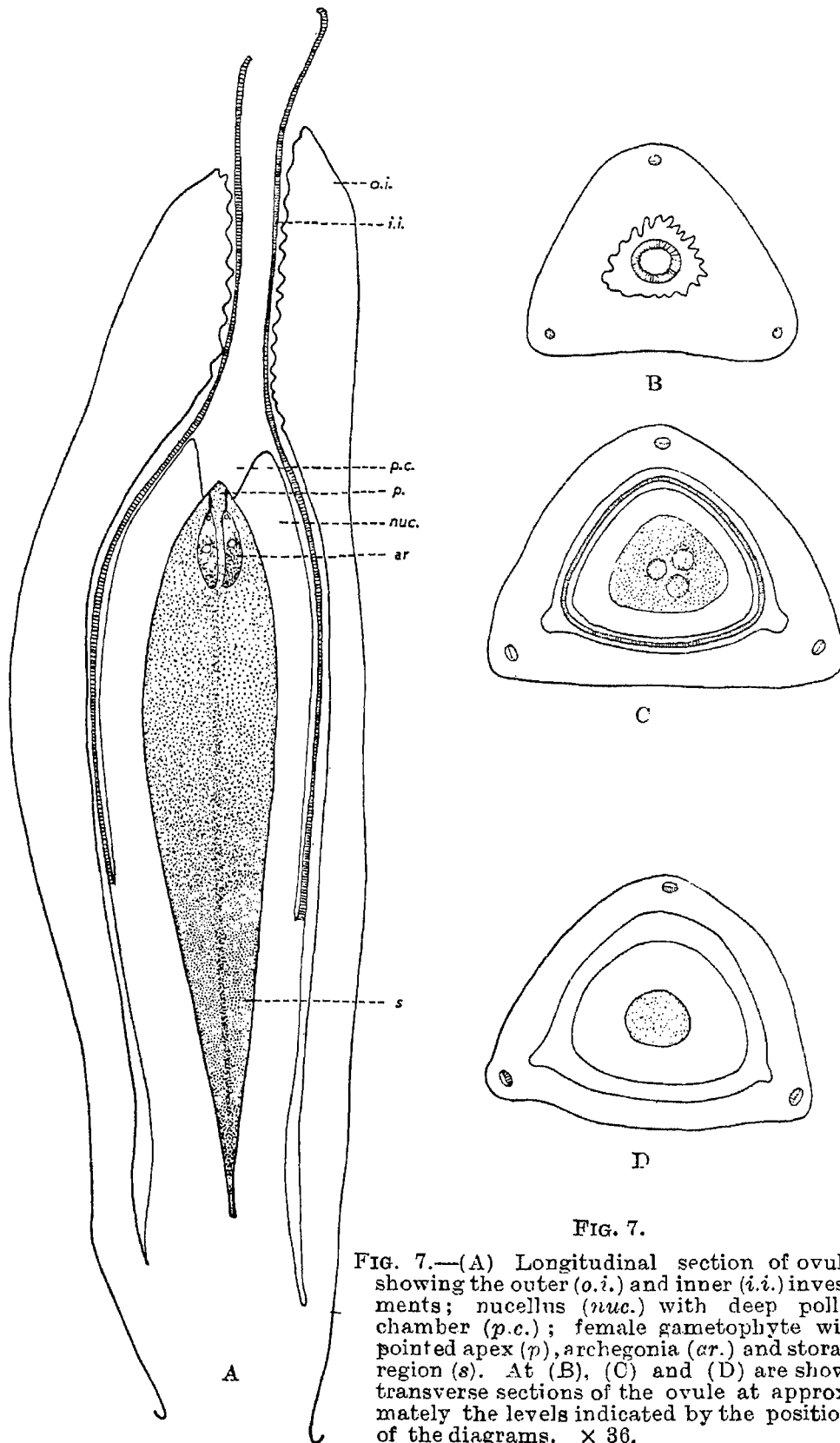


FIG. 7.

FIG. 7.—(A) Longitudinal section of ovule, showing the outer (o.i.) and inner (i.i.) investments; nucellus (nuc.) with deep pollen chamber (p.c.); female gametophyte with pointed apex (p.), archegonia (ar.) and storage region (s). At (B), (C) and (D) are shown transverse sections of the ovule at approximately the levels indicated by the positions of the diagrams. $\times 36$.

mass of cytoplasm at the chalazal end were in the prophase.¹⁵ It was also noted that some of the nuclei in some gametophytes were in process of degeneration and others already dead—looking like dense blobs, unable to pursue any further divisions.

Immediately after wall formation, the details of which I have not been able to make out clearly,¹⁶ the gametophyte begins to be differentiated into two regions, the micropylar and the chalazal. The former consists of very delicate cells with extremely thin walls; the latter has more solid-looking cells stored with food materials.

In the majority of cases the archegonia arise from superficial cells situated at the micropylar end of the gametophyte, and the most frequent number is 3, although 2 and 4 have also been seen. Their number is so variable in the different species that have been investigated, that I have not thought it necessary to bring together the literature on this point.

In early stages it is extremely difficult to distinguish the archegonium initials and the whole superficial layer of cells seems to be capable of producing archegonia (*cf.* Herzfeld, 1922). Some of the cells, usually three, divide periclinally into the primary neck cell and central cell (Fig. 32). The former undergoes several divisions (sometimes the first one is periclinal and sometimes it is anticlinal) to form a massive neck of about 30–40 cells at maturity (see Figs. 33–36). Exact counting of the cells composing the neck is impossible as they merge into the adjoining cells of the prothallus, but there is certainly no other genus among the gymnosperms which has such a massive neck.

The jacket cells take their origin from transverse divisions of cells similar and adjacent to the archegonium initials. Originally they have their longer axes at right angles to the archegonium, but later they become much elongated and their cell-walls become very thin and delicate. They are at first uninucleate, but later the number of nuclei increases. Geitler (1929) found this to take place by amitotic divisions. In one preparation, where I found them dividing, I saw aberrant mitoses in which the achromatic figure was poorly developed and the chromosomes were not sharply outlined. Normally each archegonium is bounded by one or two layers of jacket cells, but in one case I saw two archegonia lying in immediate contact with each other (*cf.* Sigrianski on *E. helvetica*).

¹⁵ I wish to mention here Weinstein's work on *Phaseolus vulgaris* (1926), where he came across nuclei in all stages of division as well as in the resting condition in the endosperm.

¹⁶ It must be pointed out that the gametophytes show a special tendency towards shrinkage at this stage, and this might perhaps explain the divergent accounts given by earlier authors about the method of cell-formation in Gymnosperms.

I must call attention here to Land's (1907) statement that during embryo formation the gametophyte is stimulated to further activity and some of the outermost cells of the gametophyte forming the bottom of the pollen chamber divide repeatedly to form a plug, which effectively closes the pollen chamber. In *E. foliata* this "plug" develops much earlier and is already well-marked when the archegonia are not yet mature (Fig. 36). Judging from its appearance it seems to be similar to the "tent-pole" observed in the Cordaitales and in *Ginkgo*. A pointed apex in the gametophyte has also been seen in *Taxus canadensis* (Dupler, 1917; Fig. 53), *Torreya taxifolia* (Coulter and Land, 1905; Fig. 5), *Cephalotaxus pedunculata* (Sahni, 1921), and *Austrotaxus spicata* (Saxton, 1934). It is possible that in *Ginkgo* it exercises some physiological function in connection with fertilisation by swimming sperms (Herzfeld, 1927), but its absence in the Cycadales and its presence in some of the Conifers and *Ephedra* is hard to explain.

The central cell, which is extremely vacuolate in the earlier stages (Figs. 32-34), soon becomes filled with cytoplasm. Vacuoles are still present, but they are smaller in size except at the upper end of the cell just near the nucleus. The cell itself also greatly increases in size as can be readily seen by a comparison of Figs. 32-39, and a conspicuous kinoplasmic mass often differentiates in the middle or a little towards the upper end.

The mitosis leading to the formation of the egg and ventral canal nuclei was seen in three archegonia all belonging to the same ovule. One of these has been shown in Fig. 37. The chromosomes are extremely slender and stained deep black with hæmatoxylin, but the achromatic figure stained very lightly. It must be pointed out here that Berridge and Sanday (1907) suspected amitotic division of the central cell nucleus and Herzfeld (1922), who saw it in the diaster stage, did not observe any trace of the spindle fibres.

Counts of chromosomes made in the division of the central cell nucleus indicate that the haploid number is certainly not more than 7. This is also the number given by Mehra (1934) from counts in nuclear divisions of the male gametophyte. The same number is found in *E. maior* and *E. campylopoda* (Geitler, 1929), *E. americana*, *E. nebrodensis* var. *procera*, *E. equisetina*, *E. procera* var. *chrysocarpa* and *E. procera* var. *erythrocarpa* (Florin, 1932). In *E. gerardiana* (Mehra, 1934) and *E. distachya* (Florin, 1932) the haploid number is 14—just double of what is seen in the spp. mentioned above. This makes a strong case for taking 7 as the basic number in the genus *Ephedra* and even the whole of the Gnetales (see full paper by Florin, 1932), the only exception being *E. trifurca*, in which Land's count of the haploid number (12) appears to be short by 2.

No wall is formed between the egg and ventral canal nuclei, which lie free as in other species of *Ephedra*, on which a definite statement is available. The further behaviour of the ventral canal nucleus is extremely variable. It may either begin to degenerate soon after it is formed (Fig. 39), or it may continue to persist for a time (Fig. 38). The egg nucleus travels down to the centre and surrounds itself with a mass of cytoplasm staining more densely than the general cytoplasm of the egg and showing the presence of delicate strands radiating out from the centre in all directions (Fig. 39). This dense cytoplasm finally extends downwards to the base of the egg (Fig. 38). One exceptional case was observed in which the egg nucleus had passed down to the bottom of the cell and the ventral nucleus had travelled to the centre. The appearance of the latter indicated that it was very similar to the egg nucleus and could perhaps function in a similar way.

Fig. 7 shows the condition of the whole ovule at this stage. The inner investment protrudes out and has a funnel-like opening. The outer, which comes next, is quite free from the former, but it sends out certain processes towards the inner side, which effectively seal the space between the two. The pollen chamber extends right down to the base of the gametophyte with its cone-like apex. The gametophyte itself is distinguishable into a looser upper part consisting of more delicate cells and the lower tapering portion which is loaded with food materials. A row of thin-walled cells extending from the base of the archegonia also becomes distinguishable at this stage. These cells are richer in food materials and it is through this region that the embryo is thrust down by the elongating suspensor.

Several interesting abnormalities were met with regarding the arrangement and distribution of the archegonia. I am not including them here, as a mention of several of these would have to be made again in a subsequent paper, now under preparation.

12. Summary.

1. The male flower consists of a filament bearing three anthers at the top and a rudimentary perianth at the base consisting of two members.
2. A plate of primary archesporial cells appears in each anther lobe. A primary parietal layer, cut off at the periphery, divides periclinally to form one wall layer and the tapetum. The former soon becomes flattened and crushed, while the cells of the latter enlarge and become 2-4 nucleate.
3. Usually no cell-plates appear during the reduction divisions and quadri-partition of the mother cell occurs by furrowing.
4. The pollen grains are five-nucleate at the time of shedding and contain 2 prothallial nuclei, one tube nucleus, one sterile nucleus and the

spermatogenous cell. Occasionally the second prothallial nucleus fragments into two nuclei.

5. The ovulate strobilus consists of two ovules enclosed in four pairs of bracts. One of the ovules often aborts.

6. The archesporium arises in the hypodermal layer and a primary wall cell is cut off at the periphery. Periclinal divisions in this and in the epidermal cells bury the megaspore mother cell in the nucellus.

7. A tetrad of four megaspores is usually formed, of which the innermost functions. Sometimes two megaspores enlarge together and give rise to two gametophytes within the same ovule.

8. Wall formation does not occur until after 500 nuclei have been formed and after wall formation the gametophyte becomes differentiated into two regions.

9. The archegonium initials (usually 3 in number) are not clearly distinguishable till after the division into the primary neck cell and central cell has taken place. The primary neck cell divides to form a neck of about 30-40 cells. The central cell enlarges and gives rise to the egg and ventral canal nuclei.

10. The haploid number of chromosomes, as counted from the division of the central cell nucleus, is 7.

11. The upper end of the gametophyte forms a pointed apex that grows up into the pollen chamber.

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EXPLANATION OF PLATES.

PLATE LXI.

Microsporogenesis and Male Gametophyte.

- FIG. 8.—Part of anther, showing epidermis, one wall layer, tapetum and sporogenous cells.
 × 530.
 FIG. 9.—Same, older stage; many of the tapetal cells have become bi-nucleate and the
 microspore mother-cells are in synizesis. × 530.
 FIG. 10.—Microspore mother-cell in diakinesis. × 1400.
 FIG. 11.—First reduction division completed. × 1400.
 FIG. 12.—Second reduction division completed; nuclei arranged tetrahedrally. Note the
 furrows proceeding centripetally. × 1400.
 FIG. 13.—Tetrad of microspores. The thinly dotted area indicates the gelatinous matrix
 formed inside the mother cell-wall. × 1400.
 FIG. 14.—Microspore. × 1400.
 FIG. 15.—First prothallial cell cut off. × 1400.
 FIG. 16.—Same; the remaining nucleus dividing again. × 1400.
 FIG. 17.—Three-nucleate stage. × 1400.
 FIG. 18.—Pollen grain with 2 prothallial cells at the lower end, the tube nucleus at the upper
 end, and the antheridial nucleus in the centre. × 1400.
 FIG. 19.—Same; but the antheridial nucleus has surrounded itself with its own plasma. × 1400.
 FIG. 20.—Antheridial cell divided into a large spermatogenous and a small sterile nucleus,
 both enclosed within the same sheath. × 1400.
 FIG. 21.—Abnormal pollen grain; the second prothallial nucleus has fragmented into two
 nuclei. × 1400.
 FIG. 22.—Pollen grain transversely cut; there are 16 ridges on the exine. In Figs. 14-21,
 the exine has not been represented. × 1400.
 FIG. 23.—Double pollen grain cut transversely. × 1400.

PLATE LXII.

Megasporogenesis and Female Gametophyte.

- FIG. 24.—L.S. nucellus with two hypodermal archesporial cells. × 400.
 FIG. 25.—Megaspore mother-cell, separated from epidermis by two wall-cells. × 400.
 FIG. 26.—Same, more advanced stage. × 400.
 FIG. 27.—First reduction division completed. × 400.
 FIG. 28.—Tetrad of megaspores; upper two are arranged diagonally. × 400.

FIG. 29.—Tetrad of megaspores, all arranged in one row; the upper three have already degenerated. $\times 400$.

FIG. 30.—Two-nucleate mega-gametophyte. $\times 400$.

FIG. 31.—Four-nucleate gametophyte. $\times 400$.

PLATE LXIII.

Development of the Archegonium.

FIG. 32.—Archegonium initial divided into primary neck cell and central cell. $\times 530$.

FIG. 33.—Primary neck cell dividing. $\times 530$.

FIG. 34.—Primary neck cell divided anticlinally. $\times 530$.

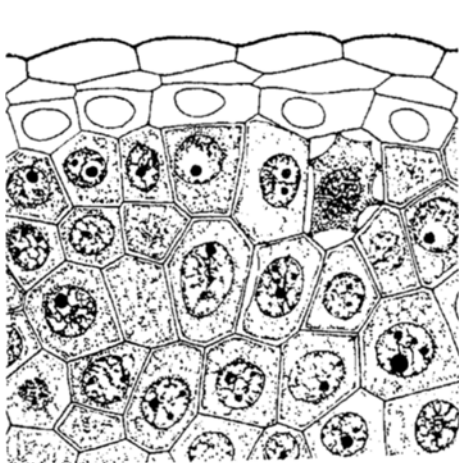
FIG. 35.—Young archegonium; the central cell has enlarged and the primary neck cell has undergone several divisions. $\times 350$.

FIG. 36.—Upper part of gametophyte, showing the pointed apex and two archegonia. Note the large kinoplasmic mass in each archegonium. $\times 230$.

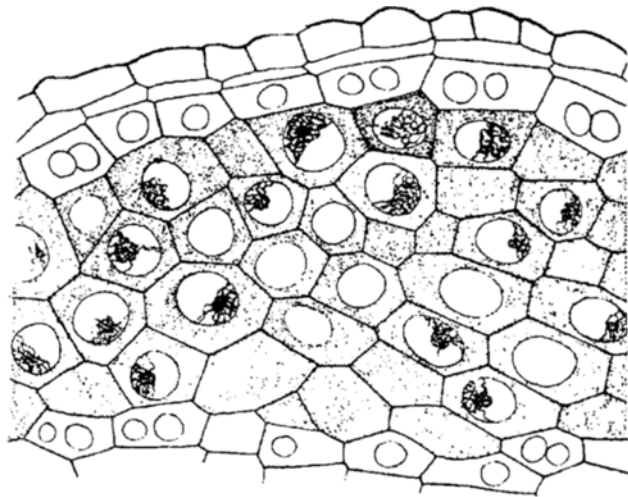
FIG. 37.—Central cell; nucleus in metaphase. $\times 230$.

FIG. 38.—Egg and ventral canal nuclei. $\times 230$.

FIG. 39.—Same; the ventral canal nucleus is degenerating. $\times 230$.



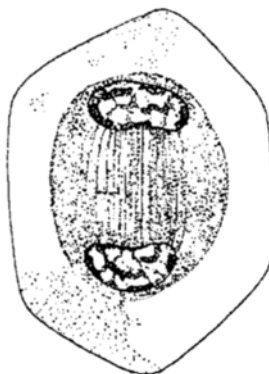
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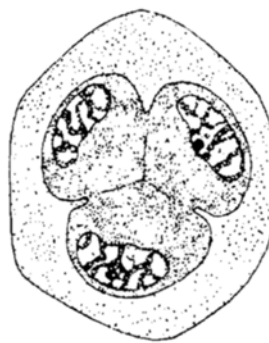
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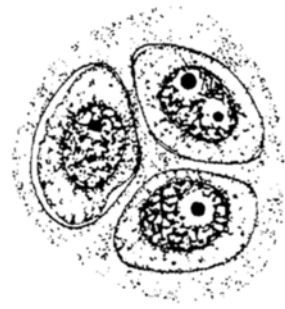
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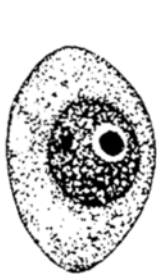
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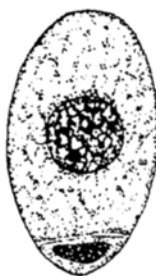
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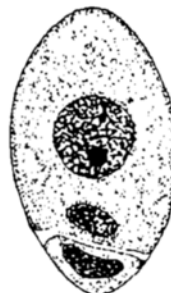
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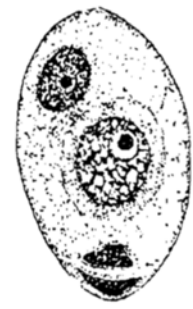
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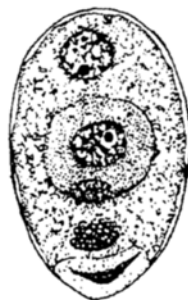
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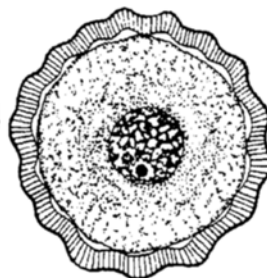
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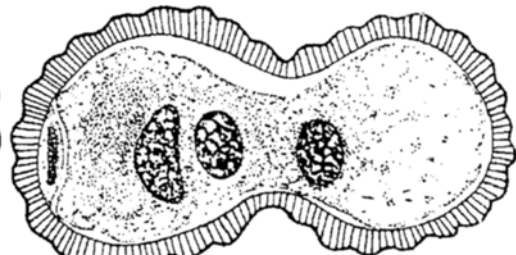
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