STUDIES IN THE PROTEACEÆ

VII. The Endosperm of Grevillea robusta Cunn., with Special Reference to the Structure and Development of the Vermiform Appendage

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Introduction

In two recent studies on the embryology of Grevillea robusta Cunn. and G. Banksii R. Br., the writer (Kausik, 1938 a and 1939 a) has described the occurrence of a coiled worm-like haustorial structure, the vermiciform appendage, which develops after fertilization as a part of endosperm from the lower portion of the embryo-sac. A detailed investigation of this structure was very recently made, and the several stages in its development and also its behaviour during seed formation are now given in the following paper. A preliminary note has already appeared some time back (Kausik, 1941).

Methods

The material for this investigation was killed as usual in Bouin's fluid and was subsequently dehydrated slowly in a weak solution of glycerine. After this, chloroform was used for infiltration instead of xylol. Sections were cut ranging in thickness from 16 to 24 μ for younger stages, and from 30 to as much as 40 μ in the case of older material in order to get the endosperm and the appendage as completely as possible in one or only a few sections. The sections were all stained in a 0.5% Heidenhain's iron-alum hematoxylin. Destaining was generally done in a saturated solution of picric acid (Maheshwari, 1933), but in some cases a 2% iron-alum solution was also used.

In addition to the microtome sections, many entire preparations of the endosperm were also used for studying the development of the vermiciform appendage. These preparations were made according to the dissection methods already described elsewhere (Kausik, 1939 b). Further, while one
set of such entire preparations were made into permanent whole mounts, after killing and staining, to study the interesting details, another set was used for observing the appendage in the fresh living condition as soon as the preparations were dissected out of the seeds.

The Embryo-Sac

The development of the embryo-sac conforms to the normal type (Brough, 1933; Kausik, 1938 a). The synergids become definitely beaked in appearance with a clear lateral indentation (Figs. 12 and 13; Pl. X:1), so that the apex in each synergid is distinct from the basal part where a large vacuole is seen. Further, the apex is characterized by the possession of a number of minute striations which converge towards the tip (Fig. 13) to form the "filiform apparatus". The two polar nuclei are found together close to the egg apparatus (Figs. 11-13) and remain without fusing till the actual entry of the pollen tube into the embryo-sac. At the lower end of the sac there are three prominent antipodal cells (Figs. 11 and 15) which persist as such for a long time after fertilization and are seen even during later stages of endosperm formation (Figs. 2, 3, 4, 17, 21, 34, 37; Pl. XII: 12).

The cytoplasm in the embryo-sac is highly vacuolate (Fig. 11; Pl. X: 1) and includes innumerable starch grains which are distributed throughout, except at the micropylar end. This fact is probably to be accounted for as follows: All the cells of the nucellus contain starch, and since the destruction of the nucellar cells begins first all round the lower portion of the embryo-sac (the nucellus being fairly intact at the micropylar region) where again starch is especially abundant, it appears that the starch contained in these cells is passed into the embryo-sac, probably in some diffusible form. The absence of starch in the embryo-sac in the earlier stages of its formation when the surrounding nucellus is but slightly encroached upon seems to lend support to this surmise.

During the final stages of its organization, the embryo-sac enlarges considerably at the expense of the surrounding nucellus, a feature which becomes especially marked later during all subsequent development when the nucellus suffers an almost wholesale destruction on account of a tremendous increase in the size of the sac with the endosperm tissue developing rapidly inside. Nevertheless, even such an increase in size "fails to keep pace with the space vacated by the nucellus" (Brough, 1933), and thus there always remains an unoccupied peripheral region at every stage during the development of the seed.
FIGS. 1-10. Camera lucida drawings of entire living preparations of endosperm of Grevillea robusta Cunn. showing the development of the vermiform appendage. The appendage shows the formation of cross partitions in Fig. 4, and cell organization in Fig. 5; Fig. 5a is a portion in Fig. 5 (marked a) enlarged; some of the cells show two nuclei. In Fig. 6 the cell formation in the appendage is more progressive. Figs. 8 and 10. Decline of the appendage when it becomes crushed. Figs. 1, 7 and 9 show outlines of seeds in different stages. The embryo is shown black in Figs. 3, 4, 6, 7 and 8. Tn., Pad of tannin tissue at the base of seed: V.B., vascular bundle.
Fertilization

In a number of preparations the pollen tube was seen inside the micropylar part of the embryo-sac, either immediately after entering and discharging its contents, or some time afterwards. Thus it is clear that normal fertilization occurs in Grevillea robusta. Further, the entering pollen tube forms a clear canal-like passage (Figs. 17, 18c and 28) in the glandular apical tissue of the nucellus overlying the embryo-sac. Mauritzon (1939) has recorded the presence of such a canal in the case of Sonneratia acida.

During fertilization one of the synergids is destroyed, while the other persists for a little time after this. It appears probable that the minute striations at the apex of the synergids which form the filiform apparatus have an important rôle in fertilization by secreting some substance and thus directing the course of the pollen tube inside the embryo-sac (cf. Kausik, 1939 a), for, the filiform apparatus becomes somewhat distorted in appearance (Fig. 16) soon after the entry of the pollen tube, while the basal part of the synergid still remains more or less intact.

During fertilization the first male nucleus fuses with the egg (female) nucleus and the second unites with the secondary nucleus formed by the union of the two polar nuclei near the egg apparatus (Fig. 14). At this time there is usually some accumulation of starch grains all round the egg nucleus, and also to some extent near the secondary nucleus.

Development of Endosperm

After fertilization the primary endosperm nucleus formed by triple fusion moves down slightly from its former position near the egg apparatus (Fig. 16), and after the lapse of some time gives rise to a number of free endosperm nuclei (Figs. 17 and 18). These nuclei are found mostly in the upper two-thirds of the embryo-sac and are especially concentrated in its micropylar portion where a dense accumulation of cytoplasm is seen. The remains of the pollen tube and a densely staining disorganizing body, which by its position and form appears to be the surviving synergid, are seen at this stage (Fig. 18c), and even later when the development of the endosperm tissue is initiated at the micropylar end of the embryo-sac (Fig. 22).

When the free endosperm nuclei are formed the embryo-sac tends to become slightly zig-zag in appearance (Fig. 17), a tendency which was already foreshadowed even earlier (Fig. 15; Pl. X: 1), but which becomes more marked a little later when the endosperm tissue is just formed (Figs. 2, 20, 21; Pl. X: 2; Pl. XI: 6–9). It is interesting to compare now the free nuclear stage of the endosperm in Fig. 17 with one of Brough's (1933) figures (cf. Fig 70, here reproduced as Fig. 19). The two figures agree closely
except for the fact that the embryo-sac in Fig. 17, especially in its lower part, seems to be narrow, but this is due to shrinkage caused by the fixing fluid in spite of careful treatment. It appears that Brough’s figure is drawn slightly diagrammatic so as to give an idea of the general outline of the embryo-sac at this stage, and I say this because of the difficulties I have experienced in getting microtome sections of the embryo-sac with its wall quite intact at this stage; the wall is rather delicate, and there being no tissue around (the surrounding nucellus is almost completely gone in the vicinity of the sac) to support it, nor enough cytoplasm inside to keep it stretched, this part of the embryo-sac becomes easily distorted on account of the action of the fixing fluid. However, by modifying Fig. 17 slightly as shown in dotted outline, which also then corresponds closely to the form of the embryo-sac in dissected preparations (Pl. XI: 6), the resemblance between this and Brough’s figure (here Fig. 19) becomes very close.

Subsequent to the free nuclear stage, the inception of the cellular endosperm takes place, and in this it is only the nuclei in the micropylar accumulation of cytoplasm that participate (Figs. 2, 2a, 21, 22; Pl. X: 2; Pl. XI: 7–9), while the rest of the embryo-sac still continues to have only free endosperm nuclei and becomes conspicuous as a coenocytic and definitely worm-like structure (Pl. XI: 9). Further, it is found in entire living preparations at this stage that the early endosperm cells in the micropylar part have their outer free walls bulging out rather conspicuously (Figs. 2, 2a; Pl. XI:8) which is presumably due to the pressure on them from inside on account of the rich cell contents. As further development proceeds, the endosperm tissue becomes larger and larger in size with increase in the number of cells, and concomitant with this, the lower coenocytic portion of the embryo-sac becomes more strikingly worm-like so that it may be referred to hereafter as the **vermiform appendage** of the endosperm (Figs. 3, 26; Pl. X: 3; Pl. XI: 10).

The **vermiform appendage** grows to a length of about 3 to 5 mm., or may even be slightly longer sometimes. Its lower end, where the three antipodals are seen prominently (Figs. 3, 34, 37; Pl. XII: 12), is generally slightly pointed. The wall of the **appendage** appears to be distinctly lamelllose (Fig. 33), and within this the cytoplasm is peripheral with a large central vacuole (Fig. 26; Pl. X: 3), which, however, is here and there traversed by strands and plates of cytoplasm (Fig. 33). The presence of the large vacuole, together with the fact that the peripheral cytoplasm may be in some places quite thin, or even almost absent, indicates that the quantity of cytoplasm is here rather insufficient for the large size attained by the **appendage** during its rapid development, and this is also the reason why it collapses frequently in fixed material.
Figs. 11–23.—Fig. 11. Embryo-sac just before fertilization showing the destruction of the surrounding nucellus and the presence of starch inside the sac (from 3 sections). Fig. 12. Egg apparatus enlarged from Fig. 11. Fig. 13. Egg apparatus from another preparation; in this
and in the previous figure the synergids show the beaked appearance with the filiform apparatus and the egg cell is slightly masked by the synergids. Fig. 14. Fertilization; note starch grains round the egg nucleus and near the secondary nucleus. Fig. 15. Part of ovule in longitudinal section showing embryo-sac immediately after fertilization; the sac is slightly curved at the lower end where the three antipodals are seen. Fig. 16. Micropylar part of same enlarged; the pollen tube is partly masking the egg cell. Fig. 17. Embryo-sac showing free endosperm nuclei which are concentrated in the micropylar part; the lower part of the sac is shrunk and the dotted outline represents its true form (from 4 sections). Fig. 18 a, b, c and d. The four sections used for previous figure drawn separately on a slightly larger scale. The canal formed by the pollen tube in the apical part of the nucellus is seen in Fig. 18 c. Fig. 19. Embryo-sac with free endosperm nuclei (from Brough, 1933, drawn here for comparison with Fig. 17). Fig. 20. Longitudinal section of seed showing embryo-sac forming early endosperm cells at the micropylar end, the lower part remaining free nuclear; note the zig-zag nature of the sac and the destruction of the surrounding nucellus. Fig. 21. Same stage from another section (from 2 sections). Fig. 22. Micropylar part of sac from previous figure enlarged to show early endosperm cells. Fig. 23. Antipodal end of sac from same enlarged to show the three antipodal cells. Figs. 11, 18, 22. x450; Figs. 12, 13, 16, 23. x900; Fig. 14. x1800; Figs. 15, 17. x200; Fig. 19. x180 (as given by Brough, but here reduced to half); Fig. 20. x80; and Fig. 21. x160.

The vermiciform appendage invades the large mass of nutritive tissue formed, as already described elsewhere (Brough, 1933; Kausik, 1938 a), by the remarkable meristematic activity in the chalazal region of the seed, and the appendage seems, by its peculiarly tortuous course, almost to wander, as it were, in search of nutriment for the needs of the future embryo which develops at the micropylar end (Fig. 41). Thus the appendage exhibits a definitely haustorial rôle of a very aggressive nature, and finally brings about a thorough and complete destruction of all the adjoining cells (Figs. 26, 29, 41; Pl. X: 3) by dissolving away even their cell walls so that an immense cavity begins to be formed inside the seed.

While the vermiciform appendage is thus growing and exhibiting a haustorial rôle, certain interesting facts require to be mentioned in connection with the development of the endosperm tissue above. In this tissue a distinction becomes soon apparent between two regions, an upper one with small and rather compactly arranged cells (Figs. 3, 38; Pl. X: 4; Pl. XI: 10), and a lower one with larger and also more or less loosely arranged cells (Fig. 39). Further, a cell of the endosperm tissue may here and there contain two nuclei at first, but becoming uninucleate later by the formation of a wall.

The upper and lower cellular regions in the endosperm tissue are not, however, separated by any clear boundary line; on the other hand, the change from one region to the other is a very gradual one. In this connection it must be mentioned that the writer (Kausik, 1938 a) has elsewhere described some "horn-like" cells at the base of the endosperm tissue where it abuts on the proximal end of the vermiciform appendage. These cells are only some of the very large endosperm cells at the lower region. In some dissected
Figs. 24-31.—Fig. 24. Part of chalazal region of young seed showing the nutritive tissue formed by the chalazal meristematic zone which is above the pad of tannin cells (shown black); the embryo-sac in Fig. 21 is drawn from this preparation; the antipodal end with the antipodal
cells is seen invading the nutritive tissue which forms a shallow pocket. Fig. 25. Longitudinal section of older seed showing embryo-sac with upper endosperm tissue and the lower vermiform appendage; the dotted line indicates that these parts of the appendage shown here are taken from two additional sections, while the continuous line shows the endosperm and the appendage (part) appearing in one section. Fig. 26. The endosperm and the appendage enlarged from previous figure. Fig. 27. Part of appendage from the same seed as above but seen in the next section. Fig. 28. Apical glandular tissue of nucellus in young seed showing the canal-like passage formed by the pollen tube; the basal part of the micropyle and the inner layer of tannin cells of the inner integument (shown black) are also seen. Fig. 29. Part of appendage to show the dissolving away of the walls of the surrounding cells of nucellus which thus become separated. Fig. 30. Upper part of appendage where it is in contact with the base of the endosperm tissue; note that some of the cells are conspicuous and it is these that become "horn-like" and referred to in the text. Fig. 31. Part of appendage, later stage showing the beginning of cross wall formation. Figs. 24, 28, 30. x450; Fig. 25. x20; Figs. 26, 27. x160; Fig. 29. x400; and Fig. 31. x200.

Out preparations these cells were generally about four in number, but varied in other preparations. Similarly, their shape, often strikingly horn-like as described, may also vary to a certain extent (Figs. 34-36). Further, they may not develop prominently at all times, but may be even completely absent in some cases (Figs. 3, 33). When present, however, they are rather conspicuous being sharply marked off from the other large endosperm cells by their densely staining cytoplasm containing one or more nuclei and also by their curious outlines as shown in Figs. 34-36.

It is probable that the conspicuous appearance of these cells is due to the fact that they are situated just at the junction of the two well-marked regions of the embryo-sac, namely, the upper containing the endosperm tissue and the lower forming the vermiform appendage, and thus all absorbed nutriment seems to pass through these cells to the upper micropylar region where the embryo develops. Further, these cells are perhaps also of the nature of what may be termed local haustorial structures for absorbing nutriment from some of the nucellar cells in the immediate neighbourhood.

During subsequent development, the growth of the endosperm tissue is very rapid, particularly in the direction of the chalaza and in the antero-posterior plane of the seed with the result that it becomes large and also flattened (Figs. 6, 8; Pl. XII: 13, 15, 17). This growth in the antero-posterior plane is shared by the nucellus, as also by the other component parts of the ovule and seed, and as the sides of the broadening endosperm tissue begin to extend steadily across on either side, the cells of the nucellus are attacked layer by layer successively so that finally a few peripheral layers of the nucellus alone persist immediately within the seed-coat (Fig. 41; Pl. X: 4). The destruction of the nucellus in the antero-posterior plane seems to be hastened up when an intimate contact is established between it and the sides of the endosperm tissue, especially near the base of the latter where
Figs. 32–37.—Fig. 32. Outline camera lucida drawing of permanent whole mount of endosperm and the _vermiform appendage_. Fig. 33. The _appendage_ from previous figure under very high magnification to show nuclear divisions. The spindles were located with the help of a binocular microscope under oil as all parts of the _appendage_ cannot appear in one and the same focus. The entire _appendage_ was drawn in four parts marked A, B, C and D corresponding to
that the lamelllose wall of the appendage. Fig. 34. \textit{Vermiform appendage} from another permanent whole mount with free nuclei (this precedes the stage shown in Fig. 33 where the cross partitions are already seen). The upper part of appendage shows some "horn-like" cells. Figs. 35 and 36. The different forms of the "horn-like" cells (see text). Fig. 37. The lower end of the appendage in Fig. 34 enlarged to show the three prominent antipodal cells; the cytoplasm at the tip is slightly shrunk. Fig. 32. \( \times 50 \); Fig. 33. \( \times 270 \); Fig. 34. \( \times 40 \); Figs. 35, 36. \( \times 80 \); and Fig. 37. \( \times 200 \).

Some of the peripheral endosperm cells tend to bulge out rather prominently and thus form a number of small invading processes (Figs. 40, 41; Pl. XII: 15, 17). These remind one of the peripheral cells of the foot in the sporophyte of \textit{Anthoceros} (cf. Kausik, 1938 a).

Now turning our attention again to the \textit{vermiform appendage}, we notice that some very important changes have already been initiated in it at the time when the upper endosperm tissue has become a prominent structure inside the young seed. After the appendage has assumed an aggressive haustorial role with a steady increase in the number of nuclei, and after a further period of its activity when it dissolves away the cells of the surrounding nutritive tissue (Fig. 41), it becomes septate by the formation of cross partitions here and there along its length (Fig. 4). These partitions seem to arise definitely in relation to the cytoplasmic strands traversing the large vacuole in the appendage (Fig. 31). There is, however, no regular sequence in the appearance of these partitions; consequently, the appendage forms a number of chambers which are of different sizes (Fig. 4) with the separating walls either transverse or slanting at various angles, especially in slightly later stages (Fig. 33; Pl. XII: 13, 14) when they are also oblique and often curved in an intersecting manner.

The chambers formed in the appendage contain a varying number of nuclei (Figs. 4, 33) which subsequently show a further increase in number by regular mitotic divisions followed by wall formation. This mitotic activity seems to proceed gradually and in orderly fashion so that one may see almost all division stages from the most initial in the lower portions of the appendage to the most advanced in its upper portions (Fig. 33; Pl. XI: 11). Further, since the dividing nuclei are generally restricted only to such portions in the appendage where the cytoplasm is rather dense, cell formation which is, in later stages, a very conspicuous feature, seems to be at first more or less confined only to these portions. The result is that some portions of the appendage remain, either completely or almost so, without progressing far in this cell organization. In these portions the cytoplasm becomes rather thin and the nuclei begin to degenerate later. In many preparations these portions were seen sharply marked off from the rest of the appendage
Figs. 38–44.—Fig. 38. Upper part of endosperm tissue; the young embryo is seen at the top.
Fig. 39. Middle part of endosperm tissue; note that the cells are slightly larger and also
irregular here than in the previous figure: some of the cells also contain two nuclei. Fig. 40. Basal part of endosperm tissue, at a later stage, to show the peripheral invading cells. Fig. 41. Part of young seed in longitudinal section (outer integument is not shown) to show the endosperm tissue and the coils of the appendage in situ (from a few sections); note the cells in the appendage in the upper part; the embryo is shown black. Fig. 42. A group of cells in the appendage formed following cross wall formation. Fig. 43. A much later stage of appendage showing the groups of cells becoming very progressive and appearing like clusters (from a living preparation, later killed and mounted). Fig. 44. A group of cells from a microtome section. Figs. 38, 39, 44. ×450; Figs. 40, 43. ×80; Fig. 41. ×40; and Fig. 42. ×200.

where cell formation proceeds rapidly (Figs. 6, 8, 10; Pl. XII: 15, 16, 17). These empty portions begin to shrivel up later in slightly older material.

As cell formation proceeds in the appendage as noted above, there result finally groups of small cells, which in several preparations in slightly later stages appear rather like loosely arranged clusters with the peripheral cells bulging out prominently (Figs. 43, 44; Pl. XII: 16). This feature is especially clear in fresh living preparations. In these cell groups a cell may here and there show occasionally two nuclei before finally becoming uninucleate (Fig. 44).

When this cell formation progresses further, the appendage begins to show distinct regions with clearly defined cell groups interrupted here and there by portions which remain empty and structureless (Figs. 6-10, 42; Pl. XII: 15, 16). The latter begin subsequently to collapse considerably and shrivel up almost completely in later stages. This is due, as we shall see presently, to certain changes that are initiated in the upper part of the embryo-sac where the endosperm tissue and the embryo are developing.

As already mentioned, the upper endosperm tissue forms a broad and flattened structure on account of pronounced growth both in the direction of the chalaza and also in the antero-posterior plane of the seed. Subsequently, the endosperm fails to keep pace with the rapid growth of the embryo, which also becomes flattened in the antero-posterior plane and soon encroaches upon and increases very much in size at the expense of the endosperm tissue. As further growth of the embryo takes place, the endosperm tissue is pressed more and more from above (Pl. XII: 17) and this in turn causes considerable pressure on the haustorial vermiciform appendage. Consequently, the coils of the appendage become sharply folded together (Figs. 8, 10, 41, 45; Pl. XII: 17, 18) and the empty structureless portions of the appendage are almost crushed out of existence. The cell groups in the appendage become separated from one another, and as they are very loosely arranged, even the individual cells are frequently detached a little later with continued pressure from above. Finally, as the embryo grows further, there remain only some crushed and disorganized remnants of the vermiciform
Figs. 45-57.—Fig. 45. Longitudinal section of seed (cut at right angles to the antero-posterior plane, and so appearing flattened) showing the endosperm tissue in outline and the
coils of the appendage. The black area at the base of the seed is the tannin pad. Fig. 46. Part of the appendage with a group of cells enlarged from previous figure. Fig. 47. Lower part of seed, slightly tangential, to show the appendage at a very late stage when it is undergoing crushing; the coils of the appendage can be recognized here and there. The border with cross lines is the tannin layer of the inner integument. Fig. 48. Part of same on a larger scale to show the disorganizing cells of the appendage. Fig. 49. Part of inner integument showing the tannin layer (inner epidermis), the palisade-like arrangement of middle layers, and the outer epidermis. Fig. 50. Part of outer integument forming the wing; note the radial arrangement of cells which are formed by regular tangential divisions and the tannin cells near the inner border. A portion of the tissue of the wing between the inner and outer borders is not shown. Figs. 51-54. Stages in embryo development; note the pointed lower end and the broader upper end. In Figs. 33 and 54 the embryo is cut at right angles to the antero-posterior plane and so shows the initials of the cotyledons and the stem tip. Fig. 55. Late embryo showing the cotyledons with their basal lobes, auricles, and the pointed root-tip (dissected material). Fig. 46. Part of seed, micropyle turned down, showing the old embryo in situ; the endosperm and the appendage are completely used up at this stage (diagrammatic). Fig. 57. Embryo shown separately with the auricles partly sheathing the radicle (dissected material). Fig. 45. ×20; Figs. 46, 50. ×200; Figs. 47, 55. ×40; Fig. 48. ×100; Fig. 49. ×400; Fig. 51. ×900; Figs. 52-54. ×450; and Figs. 56, 57. ×3.

appendage and the endosperm tissue, and both of these together form an almost shapeless mass pressed closely against the tannin-tissue or the hypostase found at the base of the seed (Figs. 9, 47; 6). In still later stages, when the seed is passing through the final stages of development, even these crushed remains are more or less completely used up by the embryo, and thus they appear like a brownish highly corroded substance fringing the advancing margin of the embryo. These disorganized cells may be scraped out by means of a needle and examined under the microscope.

Embryogeny and the Structure of the Seed

Some stages in embryo development have already been given elsewhere by the writer (Kausik, 1938 a). Brough (1933) states that there is a gradual change in the shape of the embryo from an earlier oval to a later circular one. This, however, cannot be confirmed according to the observations now made and also previously. On the other hand, the embryo has a definite polarity at all stages; it clearly shows a broad and rounded distal (upper) portion, and a narrower and conical proximal (lower) end (Figs. 51-57).

The development of the embryo is accompanied by increased growth in the antero-posterior plane and thus the embryo becomes flat and disc-like. The cotyledons arise next distally (Fig. 53), and between them lies a central elevated mound of cells forming the stem-apex. These features can be easily seen in longitudinal sections of the seed cut at right angles to the antero-posterior plane. The cotyledons possess each two prominent basal lobe-like prolongations, the auricles, which are placed laterally, one on either
side (Figs. 8, 9, 55-57; Pl. XII: 17). According to Lubbock (1892), these are supposed to form a sort of padding tissue to fill up the space which would otherwise be left vacant all round the radicle. While this may be so, it is not clear if any other function may also be ascribed to these structures.

During development some important changes are seen in the structure of the seed. The nucellus is everywhere rapidly encroached upon and its cells become dissolved away by the enlarging embryo-sac, which, though slight in the early stages, becomes a very marked feature later when the endosperm tissue develops rapidly and the haustorial activity of the *vermiform appendage* reaches its maximum. At the same time, the large mass of nutritive tissue formed by the chalazal meristematic zone is also gradually invaded by the *appendage*. In this nutritive tissue an addition of fresh cells takes place as the cells in the immediate neighbourhood of the *appendage* break down (Fig. 24; Pl. X: 2), but after a time the meristematic activity declines and the whole of the nutritive tissue is used up as the seed reaches maturity (Figs. 41, 45). This wholesale destruction of the cells of the nucellus and the nutritive tissue results in the formation of a large cavity inside the developing seed. Within this cavity the endosperm and, later, the embryo are suspended freely.

*Conclusions*

The synergids seem to be highly specialized with their apex forming the *filiform apparatus* with minute striations. These striations are also figured by Brough (1933; *cf.* Figs. 68, 69), but the general form of the synergids is rather different, and the lateral indentations described here and which give the synergids a distinctly beaked appearance are not shown by him. Beaked synergids have also been previously noted by the writer in *Grevillea Banksii* (Kausik, 1939 a) and *Hakea saligna* (Kausik, 1940). As already stated, it is probable that such synergids guide the pollen tubes into the embryo-sac.

A noteworthy feature met with during the development of the seed is the complete destruction of the nucellus and of the nutritive tissue, and this is due to the great increase in the size of the embryo-sac after fertilization when it forms the endosperm tissue and the *vermiform appendage*. The latter is a singularly striking structure met with in *Grevillea robusta* and also in *G. Banksii* (Kausik, 1939 a); its presence was also noted in yet another form, *G. Hilliana*. It is, therefore, highly probable that it is a characteristic feature of this genus, although no mention of it is made by Messeri (1928) in the case of *G. macrostachya* where, however, it appears likely that it has
escaped notice, just as Brough (1933) also overlooked its formation in the case of \textit{G. robusta} itself.

Further, it seems that the development of the \textit{vermiform appendage} has some correlation with the formation of an extensive nutritive tissue in the seed. A large nutritive tissue is also present in \textit{Macalamia ternifolia} (Kausik, 1938 b), but the \textit{vermiform appendage} is not seen. But here the haustorial lobes formed from the lower end of the embryo-sac after fertilization and containing free endosperm nuclei take up the function of absorbing materials from the nutritive tissue, and are thus clearly of the same nature as the \textit{appendage} in \textit{Grevillea}. It appears, therefore, quite reasonable to suppose that either the \textit{vermiform appendage} or any other similar or corresponding structure is likely to be met with in all cases in the \textit{Proteaceae} where a definite chalazal meristematic zone is present and where, consequently, an extensive nutritive tissue is also formed. In this connection it is significant to note that in the case of \textit{Hakea saligna} (Kausik, 1940) there is neither an extensive nutritive tissue (there being no meristematic zone), nor any haustorial structure corresponding to the \textit{appendage} or the embryo-sac lobes.

In the case of \textit{Protea Lepidocarpon} a chalazal meristematic zone is present according to Ballantine (1909), but it is not known if any haustorial structures are formed here. As Ballantine himself says, his work on \textit{Protea} is only a preliminary one, and the details, especially relating to the post-fertilization stages, are so meagre that it appears probable that a detailed study of this form will reveal some interesting features.

Coming to the nature of the \textit{vermiform appendage}, it is definitely to be regarded as a part of the endosperm in the manner of its origin, but is at the same time sharply marked off from it to assume a haustorial rôle. Further, it exhibits two distinct phases in its growth and development. The first phase is characterized by the presence of free endosperm nuclei which steadily increase in number as the \textit{appendage} assumes a more and more strikingly worm-like appearance. An examination of a number of living preparations revealed the fact that the cytoplasm in the \textit{appendage} exhibits a steady and gentle streaming movement in which the nuclei are also slowly carried along. This movement becomes less noticeable later on when cross partitions are formed in the \textit{appendage} and finally stops almost completely in still later stages.

After remaining thus for a time as a cœnocyctic structure, the \textit{vermiform appendage} enters into the second phase of its development with the formation of cross partitions in it. Subsequently cell formation begins and, as this
becomes more and more progressive, the appendage forms distinct groups of cells. These cell groups in the appendage constitute what may be termed a secondary endosperm tissue as contrasted with the primary tissue formed soon after fertilization in the micropylar part of the embryo-sac. In later stages both the first formed endosperm tissue and the secondary tissue formed in the appendage are rapidly encroached upon by the embryo and are finally completely used up in the mature seed. Thus the second phase in the development of the vermiform appendage comes to a close.

Summary

This paper is a detailed account of the structure and development of the endosperm and the vermiform appendage.

After fertilization some free endosperm nuclei are formed, and these are distributed in the upper two-thirds of the embryo-sac, with a strong accumulation at the micropylar end. The organization of cellular endosperm begins a little later, and in this only the nuclei in the micropylar end participate. The rest of the embryo-sac becomes zig-zag in its form and develops into a coenocytic haustorial structure with a strikingly worm-like appearance. This is designated the vermiform appendage which was discovered by the writer. The antipodal cells are seen persisting at this stage and also later.

The vermiform appendage shows two distinct phases in its growth and development; the first phase is characterized by the coenocytic nature of the appendage with free endosperm nuclei, and the second by the formation of cross partitions followed later by cell formation to give rise to a secondary endosperm tissue. In mature seeds both the appendage and the endosperm tissue are completely used up by the embryo.

The nature and origin of the vermiform appendage are discussed in the paper.

In conclusion, I wish to express my sincere thanks to Prof. B. Sahni, of the University of Lucknow, and Dr. P. Maheshwari, of the Dacca University, for kindly suggesting this work and for giving many valuable suggestions and timely advice. I am also grateful to them for the many kind courtesies extended to me during the course of this investigation. Further, I am also thankful to Prof. M. A. Sampathkumaran, Head of the Department of Botany, University of Mysore, for his keen interest in this work and for helpful criticism and advice given to me at various stages.
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LITERATURE

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EXPLANATION OF PLATES

PLATE X

1. Part of longitudinal section of ovule showing upper half of embryo-sac and the surrounding tissues (Fig. 13 is from this section). ×430.

2. Longitudinal section of seed with the embryo-sac showing the early endosperm tissue and the lower free nuclei; the antipodals are seen as a dark mass. Note the nutritive tissue below the antipodal end of the sac (Figs. 23 and 24 are from this section). ×104.

3. Part of longitudinal section of seed showing endosperm tissue and the major part of the appendage; note the large cavity formed inside the seed (Fig. 26 is from this section). ×104.

4. Later stage showing only the upper endosperm tissue; the young embryo is also seen here. ×130.

PLATE XI

5. Part of longitudinal section of seed showing one of the cell groups of the appendage (Fig. 42 is from this section). ×90.

6. Embryo-sac immediately after fertilization showing free endosperm nuclei and the fertilized egg cell (E.C.); note the slight curvature of the lower end of the embryo-sac. ×84.

7. Slightly later stage with early endosperm cells at micropylar end, while the lower part of the sac becomes zig-zag. ×84.

8. Same as above, but a little later; note that the outer walls of some of the endosperm cells are bulging. ×84.

9. A still later stage of the embryo-sac showing the worm-like appearance of the lower part. ×80.

10. A much later stage than before showing the large mass of endosperm and the tubular appendage; the tip of the appendage is not in proper focus. ×25.

11. Vermiform appendage showing the formation of cross walls and the nuclear divisions; many of the spindles are not seen in focus (Fig. 33 is from this preparation). ×100.
12. The lower end of the *appendage* from another preparation showing the three prominent antipodal cells (Fig. 37 is from this preparation). \( \times 220. \)

13. Endosperm and the *appendage* at a later stage showing the formation of cells in the *appendage*; the embryo is seen as a dark body at the top. \( \times 20. \)

14. Part of the *appendage* (marked in 13) enlarged to show the cells. \( \times 65. \)

15. A later stage than the one shown in 13, showing the groups of cells and the empty portions of the *appendage*; the embryo is seen here also. \( \times 15. \)

16. Part of *appendage* only at a still later stage to show that the groups of cells appear like loose clusters. \( \times 35. \)

17. Endosperm mass at a very late stage showing the *appendage* folded up and becoming pressed from above; the dark mass at the top is the embryo showing the cotyledon with the basal lobes on either side of the radicle (directed upwards); note the projecting cells of the endosperm at the base for invading the surrounding cells. \( \times 15. \)

18. The *appendage* at a very late stage with the loops sharply folded and portions shrivelling up.

(Note.—Photographs 1-5 are from microtome sections, and the rest are from entire preparations dissected out of the seeds; of these, 6, 7, 9, 10 and 13-18 are photographs of fresh living material, while 8, 11 and 12 are from killed and stained permanent whole mounts.)