SUMMARY

The final stages of Helleborus pollen-grain ontogeny, which culminate in maturation and germination of the grain, have been investigated at the ultrastructural level. Following the deposition of primary and secondary exine, and during the early stages of intine formation, the microspore passes through a vacuolate phase, in which the cytoplasm appears devoid of most organelles other than the prominent nucleus. The formation of the vacuole results in the displacement of the nucleus to one side of the pollen grain. The vacuole quickly disappears and a number of organelles reappear in the cytoplasm, in particular the dictyosomes and strands of endoplasmic reticulum, with associated grey bodies. Following mitotic division of the pollen grain, the first signs of the generative cell wall appear as a pair of tightly appressed unit membranes in the narrow strand of cytoplasm separating the two newly formed generative and vegetative nuclei. As development proceeds, the space between the two membranes gradually fills with an electron-transparent material similar to the substance found in the numerous dictyosome-derived vesicles which, together with the endoplasmic reticulum, are both closely associated with the developing cell wall. The generative cell wall fuses with the cellulosic intine, which has gradually increased in amount during these stages, and the cell division is complete. The smaller generative cell contains a prominent nucleus and a small amount of cytoplasm devoid of plastids and most other organelles. The larger vegetative cell also contains a prominent nucleus and a large amount of cytoplasm containing amyloplasts, mitochondria, dictyosomes and endoplasmic reticulum, and abundant ribosomes, many of which are in a polysome configuration. The final stages in development are characterized by a progressive decrease in the amount of starch in the vegetative cell and an increase in the size of grey bodies, many of which are invested in multilayered shrouds of endoplasmic reticulum. The generative cell wall disappears and a multivesicular/granular body gradually appears at the periphery of the pollen grain. The granular-vesicular material, which is formed from the dictyosomes and/or the degenerating plastids, is thought to represent metabolic reserves necessary for pollen-tube formation. One or more pollen tubes emerge from the apertural sectors of the pollen grain, and maturation of the grain is complete.

INTRODUCTION

A description has already been given of the ultrastructural changes which take place during the early stages of development of the pollen grain of Helleborus foetidus (Echlin & Godwin, 1968a, b, 1969). This study attempts to illustrate the changes which occur within the pollen grain from the time of intine deposition through the formation of the generative and vegetative nucleus, to the initial stages of the germination of the pollen grain and the formation of the pollen tube.

A large number of papers have already been written on the light-microscopical aspects of this phase of angiosperm pollen-grain development. For further details of
this earlier work reference should be made to the publications of Maheshwari (1950), Vazart (1958), Foster & Gifford (1959) and Vasil (1967). The ultrastructure of the final stages of pollen-grain development has been examined in only a few plant species. These include the work of Bopp-Hassenkamp (1960) on *Fritillaria* and *Lilium*, Diers (1963a, b) on *Oenothera*, Sassen (1964) on *Petunia*, Dexheimer (1965) on *Lobelia*, Maruyama, Gay & Kaufman (1965) on *Tradescantia*, Larson (1965) on *Parkinsonia*, Jensen, Fisher & Ashton (1968) on *Gossypium*, Angold (1968) on *Endymion*, Heslop-Harrison (1968) on *Dactylorchis*, Hoefert (1969a, b) on *Beta*, Vazart (1969) on *Linum*, Mepham & Lane (1970) and Mepham (1970) on *Tradescantia* and the recent study by Sanger & Jackson (1971a–c) on *Haemanthus*. This present work continues our studies on the ultrastructure and ontogeny of *Helleborus* pollen and although confirming several facets of development which have already been reported in other species reveals some important differences. A description will be given of these differences within a general discussion of pollen-grain development, and while for the sake of completeness it would have been interesting to have made a more detailed study of pollen-tube formation and even fertilization of the embryo by the male gamete, our preliminary work indicates that there is little new to add to the already burgeoning literature on the subject. For details on the ultrastructure and physiology of pollen tubes, reference should be made to the review articles by Linskens (1967), Rosen (1968, 1971) and to a number of papers presented on the subject at a meeting held in Pullman in 1969 (Heslop-Harrison, 1971).

**MATERIALS AND METHODS**

Whole flowers of *Helleborus foetidus* L. (Stinking Hellebore) were removed from plants grown in a Cambridge garden, and the stage of development of the anthers established by light-microscopic examination of acetocarmine squashes. Intact anthers of the required stage of growth were removed from the flowers and immediately cut in half in either 2.5 % glutaraldehyde in 0.1 M Sorenson's phosphate buffer, pH 7.0, or 2.5 % glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.0, containing 0.01 M CaCl₂. The anther halves remained in the fixative for 15 h at 4 °C. The tissue was then thoroughly washed in the appropriate buffer and postfixed for 2 h at 4 °C in 1 % osmium tetroxide in either the phosphate or cacodylate buffer at pH 7.0.

The tissues were then briefly rinsed in buffer and dehydrated in a graded ethanol series, passed through 3 changes of 1 : 2 epoxy propane and embedded in Araldite without plasticizer. Thin sections were cut, briefly stained with lead citrate and/or uranyl acetate, coated with a thin layer of evaporated carbon and examined in an AEI EM 6 electron microscope.

Samples of anther segments were acetylated using a mixture of 1 part concentrated sulphuric acid and 10 parts glacial acetic acid. Small aliquots of pollen were placed in this mixture for periods between 15 s and 1 h and at temperatures ranging from 15 to 100 °C. After treatment, the samples were washed in distilled water and placed in 4 % aqueous osmium tetroxide for 90 min at room temperature. The dehydration and subsequent embedding followed the method outlined above.

Pollen grains from freshly picked flowers were dusted on to the surface of a solution of 0.001 % H₃BO₃ in 10 % sucrose and 0.01 % Difco yeast extract. Samples of the germinating pollen were fixed after 15, 30 and 180 min, using buffered glutaraldehyde for 30, 45 and 75 min at room temperature; all subsequent treatments followed the schedule as outlined above.
RESULTS

The final stages in the development of the hellebore pollen grain will be more clearly understood if they are related to earlier ontogenetic events, such as the deposition of intine, which Echlin & Godwin (1969) were able to show occurs towards the end of secondary or endexine formation. It is apparent that intine deposition is closely linked with the formation of the generative cell wall which, following mitosis of the pollen grain nucleus, separates the smaller generative cell from the larger vegetative cell.

At the time of intine deposition, the pollen-grain cytoplasm, although relatively electron-dense, contains few readily recognizable cell organelles other than the prominent nucleus and nucleolus (Fig. 1). Sporopollenin deposition has finished, and although the exine is thickest in the region of the furrow, it is composed of a relatively loose aggregation of material. This feature has an important bearing on the subsequent increase in size of the pollen grain during the phase prior to germination and formation of the pollen tube.

As development proceeds, the pollen grain passes through an abrupt yet brief phase of vacuolation (Fig. 2). The large nucleus, with the chromatin condensed into compact electron-dense masses, occupies one half of the cell, while the other half is largely taken up by a vacuole. The nucleus usually occupies the region of the cell nearest a pore, although this configuration is difficult to establish exactly in a tricolpate pollen grain. The principal organelles which are recognizable at this stage are a few small electron-dense mitochondria and a few larger plastids which contain considerable starch deposits. At one or two places around the cell periphery it is possible to see dictyosomes and associated vesicles and occasional strands of endoplasmic reticulum. Gentle plasmolysis of cells at this stage of development fails to reveal any connexions between the pollen-grain cytoplasm and the pollen-grain wall other than in the region of the aperture (Fig. 2). The vacuolation phase of the pollen grain corresponds to the stage in tapetal development where there is a complete loss of any recognizable cell walls, although the general outline of the cells is still recognizable.

At a slightly later developmental stage, when the pollen grain is less vacuolate and chromatin condensation is more marked, the first signs of intine deposition may be seen at the periphery of the pollen-grain cytoplasm. As has been shown in other species, the pollen-grain nucleus divides mitotically, and while the larger vegetative nucleus remains roughly in the centre of the pollen grain the smaller generative nucleus remains towards one side, midway between the regions of the furrow.

The first signs of the generative cell wall become apparent shortly after the nuclear division is completed. This structure appears initially as a pair of closely adpressed discontinuous membranes, which meander through the narrow region of cytoplasm separating the 2 nuclei (Fig. 5). The space between the membranes is not constant and shows periodic evaginations along its length, which are filled with electron-transparent material (Fig. 6). The 2 membranes are associated at various points with numerous ribosomes, fragments of endoplasmic reticulum and a few dictyosomes. The pollen-grain cytoplasm contains mitochondria, starch-containing plastids and several grey bodies (Fig. 5), similar to those seen at the earlier phase in development.
when Ubisch bodies are formed (Echlin & Godwin, 1968a). In some instances the grey bodies appear to be associated with the developing generative cell wall, but in most instances they are randomly distributed through the cytoplasm of both cells.

As development proceeds the space between the 2 membranes increases and gradually fills with an electron-transparent material (Figs. 7, 10). At a point half-way between 2 furrows it is possible to see the connexion between the newly formed generative cell wall and the intine (Figs. 3, 10). The electron-transparent intine and the generative cell wall contrast strongly with the pollen-grain cytoplasm which, at this stage, contains many ribosomes, mitochondria, and long strands of endoplasmic reticulum. There is little doubt that both the endoplasmic reticulum and the dictyosomes are associated with the formation of the generative cell wall. Both organelles are closely associated with this structure and the lumen of the dictyosome-derived vesicles and the intracisternal space of the endoplasmic reticulum contain material similar in structure and electron density to that making up the generative cell wall and newly formed intine (Fig. 10). At certain places within the generative cell wall, and particularly at the junction of this wall and the intine, it is possible to see small membranous fragments (Fig. 10). These are similar in form and thickness to the dictyosome-limiting membranes and it is suggested that they are the remnants of dictyosome-derived vesicles which have discharged their contents into the region of the developing generative cell wall.

Some of the material making up the first formed intine, which lies next to the exine, is distinctly fibrous in appearance, whereas there is only a little fibrous material in the generative cell wall (Fig. 10). A few microtubules may be found associated with intine deposition as well as with deposition of the generative cell wall (Figs. 6, 10), particularly where the generative cell wall fuses with the intine.

The end result of this spate of cellular activity is the successful division of the pollen grain into 2 dissimilar cells separated by a thin cell wall (Fig. 9). Once this wall is mature it is continuous and it has not been possible to find any cytoplasmic connexions between the 2 newly formed cells. The larger of the 2 cells, the vegetative cell, contains many organelles, including large plastids filled with starch grains, while the smaller, generative cell, has far fewer organelles and no plastids. The grey bodies are now closely associated with endoplasmic reticulum which, at this stage of growth, radiates from the structures like the spokes of a wheel, similar to the structures described by Echlin & Godwin (1968a) in the tapetal cells of *Helleborus*.

By now the tapetum has disrupted completely, and the pollen grains lie among the barely recognizable cellular debris. The pollen-grain wall, which was of medium electron-density at the initial stage of intine deposition, is now much less electron-dense, although the interbacular spaces are filled with electron-dense tryphine, one of the by-products of tapetal degeneration.

Following the deposition of the wall, which cuts off the generative cell from the larger vegetative cell, both nuclei become highly lobate and the chromatins more condensed. The generative cell nucleus appears much smaller in cross-section, and comparative light-microscope studies show that the cell nucleus does in fact become elongate and the generative cell itself assumes a crescent shape. Eventually the genera-
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tive cell wall pinches off its connexion with the intine, resulting in the complete isolation of this cell from the larger vegetative cell. It is a curious fact that this is the only stage in development at which the generative cell wall is such a prominent feature of the pollen-grain cytoplasm. For as development proceeds, the wall assumes the proportions of the 2 membranes from which it was originally engendered.

The cytoplasm of the vegetative cell becomes increasingly more active. The plastids and their attendant starch grains have nearly all disappeared, and those plastids which remain contain a few thylakoids in a densely staining matrix. As development proceeds, however, the remaining thylakoids become vesiculate and the whole plastid appears to be filled with a number of ill-defined spheres containing material of medium electron-density, similar in appearance to degenerating starch grains (Fig. 12). There is an increase in the number of mitochondria, which contain well defined cristae, and a dramatic increase in the number of dictyosomes. In a single 50-nm thick median section of a pollen grain at this stage, it is possible to count between 70–80 dictyosomes, compared with 40–50 dictyosomes per comparable section at the stage of intine/generative cell-wall deposition and less than 10 per section at an earlier stage corresponding to the termination of exine deposition.

There is also an increase in the number of grey bodies, and instead of the profiles of rough endoplasmic reticulum being arranged radially, as was the case during earlier phases of development, the endoplasmic reticulum is wrapped round these bodies in a circumferential pattern (Fig. 9). This endoplasmic reticulum, which may be several layers thick, is continuous with the abundant endoplasmic reticulum which ramifies through the cytoplasm at this stage.

The pollen grains are still in the anther at this stage, though they begin to show signs of dehydration because the tapetal contents have virtually disappeared. Following on from this stage of activity, the maturing pollen grain enters yet another phase, characterized by the presence in the vegetative cell of a large number of mitochondria, a decrease in the number of dictyosomes and the appearance of a new sub-cellular component (Fig. 4). There is a decrease in the number of grey bodies but those which remain show a considerable increase in size and are surrounded by many layers of circumferentially arranged rough endoplasmic reticulum (Fig. 8). Although there appear to be fewer dictyosomes (30–40 per pollen-grain section), they are nevertheless very active and a large number of vesicles are to be seen in the region surrounding each dictyosome. The vegetative nucleus continues to be a prominent feature of the pollen grain, remaining rather irregular in shape with most of the chromatin at the periphery (Fig. 13). Numerous nuclear pores may be seen, and there is an occasional association of chromatin on the nuclear side of the pore, and polyribosomes on the cytoplasmic side of the pore (Fig. 13). As mentioned earlier, the generative cell wall is reduced to a pair of membranes. The generative cell contains a prominent and highly lobate nucleus, and a thin layer of cytoplasm with occasional mitochondria, dictyosomes, and short profiles of endoplasmic reticulum.

The distinguishing feature of the vegetative cell at this stage is the large amount of what may be described as granular/vesicular material (Fig. 11). This material, which is of medium electron-density, is of a granular texture towards the centre and
fibrous to the outside and is arranged in whorls which in turn are packed within one area of the cell just below a germinal furrow. Each vesicle and each whorl is not limited by any recognizable membranous component, but the whole mass is clearly surrounded by a single continuous unit membrane. It is not certain where this material comes from, but 2 organelles are generally closely associated with its formation. The dictyosomes are active at this stage and some of the peripheral vesicles associated with this organelle contain material comparable in structure and electron-density to the material in the large mass. Yet this material is similar in appearance to the material found in the degenerating plastids (Fig. 12). It is important to note that no recognizable plastids may be found within the generative or the vegetative cell at this stage of development. If this mass of amorphous material does represent material derived from plastids, then the plastids themselves must have merged together, losing their identity yet retaining their rather aberrant contents.

The final stage in the development of pollen grains is the germination of the pollen grain and the emergence of the fragile pollen tube. The results show little that is not already known from the studies carried out on other plant species. One, or sometimes two, pollen tubes emerge from the pollen grain via the now considerably stretched aperture. It should be noted that during the developmental processes referred to in this paper the pollen grain shows a 2- to 3-fold expansion in volume, as reflected in an increase in the cross-sectional diameter of the grain. This particular expansion in the volume of the cytoplasm is not accompanied by a significant increase in the deposition of exinous wall material, although deposition of the mechanically weaker intine continues throughout most of this stage of development. This results in considerable stretching of the wall, and is best seen by comparing the region of the aperture, which has considerable endexine, and slightly more intine than is found in the interapertural region. At the termination of exine deposition the apertural region is the thickest part of the pollen-grain wall (Fig. 1), but because of the increase in size of the pollen grains at the time of germination the aperture is the thinnest part of the wall (Fig. 4) and it is through this region that the pollen tube emerges.

**Discussion**

The brief phase of vacuolation through which the pollen grain passes prior to mitosis has been observed by a number of workers, including Larson & Lewis (1962), Hoefert (1969a, b), and Mepham & Lane (1970). Sanger & Jackson (1971a) found that, just prior to mitosis in *Haemanthus*, the microspore nucleus is displaced from the centre of the pollen grain towards the side opposite the furrow and that a vacuole forms between the two.

In this present work on hellebore and in the light-microscope study by Oryol (1969) on *Zea*, the microspore nucleus is displaced prior to mitosis towards the side of the pollen grain nearest the furrow. It is while it is in this position that the first mitosis occurs, giving rise to the vegetative and the generative cell. The vegetative nucleus moves from the internal end of the pollen grain to the germinal pore and the generative cell after rounding off is also transferred to the pore.
Following on from the work of earlier light-microscopists, a number of functions have been ascribed to the vacuole and its formation. It has, for example, been suggested (Oryol, 1969) that it is a device for positioning the nucleus prior to mitosis, and there is general agreement that this is the probable function of the vacuole. The appearance of a vacuolate stage may also be a morphological representation of a thorough depletion of cellular reserves due to the formation of a rather large amount of exine. Maruyama (1966, 1968) had previously commented on this apparent depletion of organelles in *Tradescantia paludosa* and it is certain that subsequent metabolic events in the pollen grain are dependent on energy reserves from outside, the principal and most obvious source being the senescing tapetum.

Horner & Lersten (1971) in their study on microsporogenesis in *Citrus* found that the vacuolate stage prior to mitosis corresponds with tapetal lysis. In *Helleborus*, the tapetal cells begin to lose their integrity shortly after microspore vacuolation has commenced, and the whole thecal cavity gradually fills with cellular debris. It has already been suggested (Echlin & Godwin, 1969) that the loosely packed exine in the apertural region of the pollen-grain wall in *Helleborus* may well provide a passage into the pollen-grain cytoplasm.

There has, for some time, been controversy over the nature of the limiting layer between the generative and vegetative cells and the current status of the situation is admirably summarized in a recent paper by Gorska-Brylass (1970). Some workers such as Chardard (1958), Larson (1965) and Diers (1963a, b) showed that only a pair of membranes separated the 2 cells, while others, including Sassen (1964), Kroh (1967), and Maruyama et al. (1965) considered that a distinct wall-like structure separated the 2 cells. These apparently contradictory results have led some workers to consider the existence of 2 alternative situations in the limiting zone of the generative cell depending on the species concerned. However, Gorska-Brylass (1970) considers that the double-membrane and the wall-like structure are not due to differences in species, but reflect different developmental stages of the same generative cell. The evidence from our work on *Helleborus* would lend further support to her suggestion.

Because it has been difficult to establish exactly when and where the deposition of membranes starts, it is not known whether they develop centripetally or centrifugally. Angold (1968) found that the cell plate developed centrifugally, and the later work by Gorska-Brylass (1970) confirms that this is the process in most other plants. In *Helleborus*, once the membrane becomes readily apparent the subsequent development of the paired membranes is centrifugal, but following contact with the intine the widening of the space between the 2 membranes to form the generative cell wall occurs in a centripetal fashion. The origin of the small pieces of membrane at the junction of the intine and the generative cell wall remains uncertain but they are probably, as Sanger & Jackson (1971a) suggest, sites of membrane synthesis.

Gorska-Brylass (1967) reported the transitory occurrence of callose around the newly developed generative cell in a number of plant species which disappeared when the generative cell became spindle-shaped. The work of Angold (1968) on *Endymion* also showed that callose was present and he suggested it may be acting as a sealant allowing the generative cell nucleus to exercise a greater control over the small amount
of generative cell cytoplasm. However, Burgess (1970b), also working with *Endymion*, does not consider the generative cell to be completely isolated, as he found cytoplasmic connexions between the 2 cells.

On the basis of the morphological appearance of the intine and the generative cell wall it would appear that cellulose as well as callose may be present in *Helleborus* and *Endymion*. However, the cytochemical studies by Heslop-Harrison (1968) on orchids and Mepham & Lane (1970) on *Tradescantia* showed that although callose is present, no cellulose could be demonstrated, although it is present in the intine. In the initial stages of generative cell-wall formation the basic building blocks are most certainly 1–3 linked β-glucans (callose), and 1–4 linked β-glucans (cellulose) may only become involved at a later stage when connexion is made with the cellulose intine and even then be limited to the junction between the two. There is little evidence to support the suggestion that pectates are present in either the intine or the generative cell wall.

Some workers consider that the generative cell wall has a secretory as well as a partitive function isolating differentiating cells. Thus Horvat (1969a, b, 1970) using a modified Gomori technique found acid phosphatases associated both with the intine and the generative cell wall of *Tradescantia* and considered that this demonstrated the close similarity which exists between the 2 structures.

The deposition of both the intine and the generative cell wall follows the well known pattern of wall deposition established by Northcote & Pickett-Heaps (1966) and Pickett-Heaps (1968). But whereas Sanger & Jackson (1971b) were able to show the apparent orientation of microtubules parallel to the presumptive long axis of the generative cell wall, only a few randomly arranged microtubules have been seen in *Helleborus*. As the *Haemanthus* generative cell elongates, the microtubules become oriented with their long axes running parallel to the long axis of the cell. Earlier studies by Burgess (1970a, b) had already shown a close connexion between cell shape and plane of cell division, and microtubule distribution and orientation.

The significance of the absence of plastids from the hellebore generative cell is not clear, but it may well be connected with the necessity of excluding from the generative cell all potential genetic material, other than that in the nucleus. Sanger & Jackson (1971a) consider that the large size of the organelle may preclude its inclusion in the generative cell. A more likely explanation is that the plastids rapidly develop into amyloplasts, providing food reserves which are vital to the subsequent development of the vegetative cell and eventually the pollen tube.

Both Maruyama (1966, 1968) and Sanger & Jackson (1971c) have examined the changes which occur in organelles during the final stages of pollen development. While there is little doubt that changes in patterns may be observed, the significance of such variation has yet to be realized. The vegetative cell is the active cell, while the smaller generative cell remains relatively quiescent. This is particularly noticeable in the appearance of ribosomes in the polysome configuration in the vegetative cell cytoplasm, a feature commented upon by Sanger & Jackson (1971c).

The presence of grey bodies in the vegetative cell was rather surprising, because previous studies (Echlin & Godwin, 1968a, b) had shown that these structures were associated with exine and sporopollenin synthesis. It is likely that the grey bodies are a
morphological representation of lipid reserves which are utilized in sporopollenin synthesis during earlier phases of development (Echlin, 1971), but which may reappear as food reserves at a later stage in development.

The tapetum has now reached the final stage of senescence, and it is assumed that this releases large amounts of low-molecular-weight compounds which may be utilized in the synthesis of new materials. At about the time of this final breakdown, the plastids in the vegetative cells of Helleborus begin to show an accumulation of starch granules. This represents another example of the 'ontogenetic drift' referred to in earlier papers in this series, and attests to the remarkable economy in the utilization of metabolites in biological systems.

The generative cell wall, which was such an obvious feature of the pollen-grain cytoplasm during an earlier stage of development, is now reduced to a pair of cell membranes. This feature is in agreement with the earlier findings summarized by Gorska-Brylass (1970) and confirms the work of Mepham & Lane (1970) who found that in Tradescantia as development proceeds the generative cell wall is reduced to the plasma membranes of the generative and vegetative cells.

The final stage of synthesis is the production of materials within the vegetative cell associated with the germination of the pollen grain. It is well known that pollen grains swell during the stage prior to the emergence of the pollen tube, and this is most certainly associated with a rehydration of the pollen-grain cytoplasm. The actual mechanics of pollen-tube emergence are still uncertain, but it is clear that a physical disruption of the thin wall in the region of the aperture is necessary before the fragile pollen tube emerges. It is suggested that the extra tension occasioned by the increase in the osmotic pressure of the pollen-grain cytoplasm would rupture the wall, which is thinner in the region of the aperture, and allow the pollen tube to emerge.

The increased osmotic pressure within the pollen grain may not be the only factor causing germination and a prior weakening of the thin wall may also be necessary. The work of Gherardini & Healey (1969) has demonstrated the apparent degradation of Pharbitis exine by species-specific stigmatic exudates. Knox & Heslop-Harrison (1969, 1970) have clearly demonstrated the presence of degradative enzymes in the intine, particularly in the region of the aperture where the exine is thinnest. The net effect of such degradative processes would be to weaken the exine either from without or from within.

The only new and unrecorded feature of the internal morphology of the cell at this stage is the appearance of the granular-vesicular material derived either from degenerating plastids or from the dictyosomes. It is unlikely that this material represents a fixation artifact as the other features in the cell at this stage are preserved so well.

The general appearance of the granular-vesicular material, and in particular the individual vesicles, is not unlike the structures described by Horvat (1970) in Tradescantia and Van Der Woude, Morré & Bracker (1971) in the germinating pollen of Lilium. These workers consider that these structures are associated with the formation of the pollen tube.

It should be noted that the pollen grains are still in the anther at this stage, although they are somewhat dehydrated and free within the anther cavity. The granular-
vesicular material which does not lie below every germinal pore is not seen as such during the germination of pollen grains and one is tempted to suggest that this material which lies conveniently below the aperture may well represent a store of metabolites awaiting the initiating of germination. Craig & Miles (1969) working with germinated *Lychnis* pollen found cytoplasmic vesicles containing material of similar structure and electron density as the intine.

Other features which attest to the increase in metabolic activity of the pollen-grain cytoplasm are the progressive condensation of chromatin at the periphery of the nucleus, the more readily observable pores, and the presence of polyribosomes in the cytoplasm immediately adjacent to the nucleus. Vazart (1970) found that one of the many changes which occurred in the vegetative cell cytoplasm of developing *Linum* pollen grains was that ribosomes went into a polysome configuration at a stage prior to germination. Schrauwen & Linskens (1969) also found polyribosomes at the beginning of germination. These are all features which one would expect to see in cells where there is an active transference of information from the nucleus to the cytoplasm.

Finally this, the penultimate period in the development of the pollen grain of *Helleborus foetidus*, echoes and repeats the general phenomena found during earlier stages. The cytoplasm of the cell is continually alternating between stages of intense metabolic activity and stage of quiescence, and even in these final stages the autolysis of the tapetum makes an important contribution to the maturation of the pollen grain.

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**REFERENCES**


Helleborus pollen grain maturation


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The scale on Figs. 1-4 is equivalent to 50 μm, and on Figs. 5-13, 10 μm. All material was treated with cacodylate-osmium.

Fig. 1. Pollen grain at the end of exine deposition, showing large amounts of endexine below the furrow and a prominent nucleus and nucleolus. ×8000.

Fig. 2. Vacuolate stage preceding mitosis showing the nucleus displaced towards the furrow side of the pollen grain. The cytoplasm contains few organelles other than amyloplasts and mitochondria. ×7000.

Fig. 3. Generative (g) and vegetative (v) cells separated by a thin generative cell wall. The vegetative cell cytoplasm contains grey bodies, amyloplasts and mitochondria. There is active intine deposition in the peripheral cytoplasm immediately below the prominent pollen-grain wall. ×7500.

Fig. 4. Pollen grain prior to germination showing generative (g) and vegetative (v) nuclei together with the granular-vesicular material (gv) immediately below the aperture. The number of grey bodies has decreased, although they are bigger and invested with endoplasmic reticulum. ×4500.
Fig. 5. Pollen grain at the binucleate stage showing the thin membranous layer (arrowed) separating the vegetative (v) and generative (g) nuclei. A few remnants of the vacuolate phase still remain, and the presumptive vegetative cell cytoplasm contains mitochondria, amyloplasts, a few dictyosomes and abundant ribosomes. $\times 18,000$.

Fig. 6. Detail of a stage a little later than that in Fig. 5, showing the membranous layer (arrow) opening out to form the generative cell wall. $\times 46,000$.

Fig. 7. Details of generative cell-wall formation, showing a few microtubules and abundant ribosomes. The electron-transparent generative cell wall contains a few strands of fibrous material. $\times 35,000$. 
Fig. 8. Large grey body invested with many layers of rough endoplasmic reticulum on the vegetative cell cytoplasm. Note the dictyosome and associated vesicles together with some granular-vesicular material. $\times 50000$.

Fig. 9. Grey bodies with circumferentially arranged endoplasmic reticulum in the vegetative cell cytoplasm. Note the thin generative cell wall (arrowed) closely appressed to the generative cell nucleus. $\times 31000$.

Fig. 10. Details of the junction of the generative cell wall with the intine, showing the association of both the rough endoplasmic reticulum and the dictyosomes with this stage of development. A few membrane fragments (arrow) may be seen in the wall at its junction with the intine. $\times 64000$. 
Fig. 11. Details of the granular-vesicular material in the peripheral vegetative cell cytoplasm. Note the whorled arrangement of this material, together with the close association of dictyosomes with some of the smaller vesicles. $\times 35000$.

Fig. 12. Degenerating plastid containing granular-vesicular material prior to its association into larger masses as shown in Fig. 11. $\times 38000$.

Fig. 13. Vegetative nucleus showing chromatin associated with the nuclear pore and polyribosomes in the cytoplasm immediately adjacent to the nucleus. $\times 43000$. 