Development of the Vegetative Cell in the Pollen Grain

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In Angiosperms, after meiosis and the formation of microspores, these develop a series of processes leading to maturation. It may be said that one of the first important steps towards maturation is taken when the haploid nucleus undergoes post-meiotic mitosis to give rise to two cells, the vegetative and the generative, which are formed with marked spatial polarity, dividing the cytoplasm very unequally (Vaznart 1963), so that the generative cell possesses little cytoplasm while the vegetative occupies most of the pollen grain. The former undergoes a mitotic division, and produces two spermatic cells which later effect the double fecundation characteristic of Angiosperms, while the latter forms the pollen tube which is to carry the spermatic cells towards fecundation.

The subjects of the study carried out by us were the various changes observed mainly in the vegetative nucleus and the evolution of the membrane systems from cytoplasmic organules in the course of development and maturation of the pollen grain.

Material and method

The development of the microspores was studied in material from *Scilla peruviana* and *Allium cepa.* One anther from each flower was taken as control and observed under the light microscope to identify the stage reached by the microspores, while the remaining anthers were fixed in permanganate at 2% for two hours at room temperature and the pollen sacs were cut into three or four fragments.

The fixed material was dehydrated in the acetone series, and included in Durkupan ACM (FLUKA). During dehydration the material was stained during the night with lead-uranyl-acetate according to the technique of Giménez Mart'n *et al.* for contrast. The sections were obtained with an Ultratom LKB and studied under the electron microscope with a Siemens Elmiskop I.

Observations

Nuclei

The pollen grain is remarkable for the degree of cell differentiation that takes place in the development of its vegetative and generative cells. The nuclei of the two cells, produced from the same nucleus by mitosis, have the same genic content, but it would seem that it is the considerable difference between the amounts of cytoplasm in the two cells that is the key to their

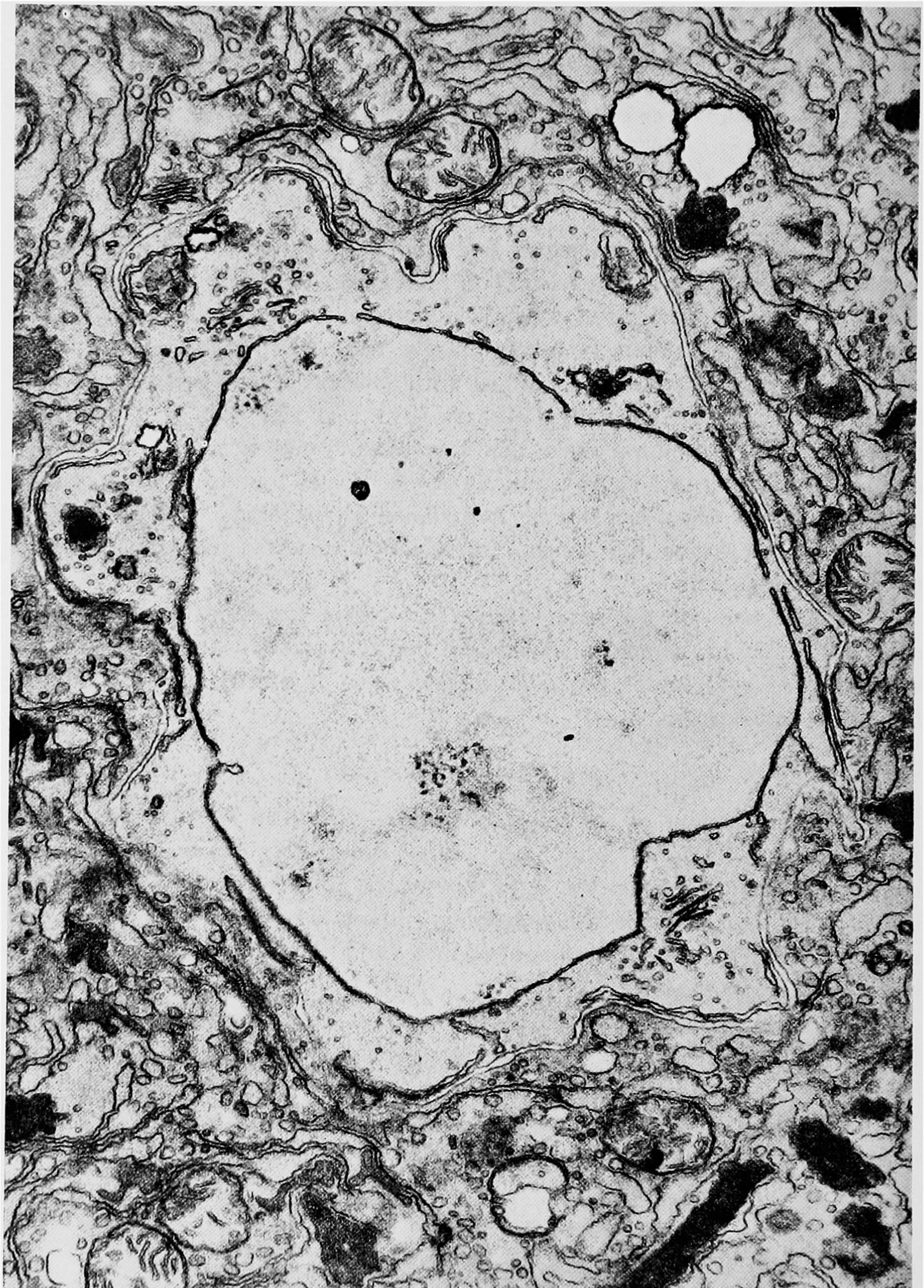

Fig. 1. Generative cell of the pollen of *Scilla peruviana* during germination, showing the nucleus in interphase.

intense differentiation. The cytoplasm surrounding each of the two nuclei, both spherical and having similar characteristics in the early stage of formation, is divided off from that of the other by a membrane.

The nucleus of the generative cell shows few modifications, as far as can be observed, during the development of the microspore, being to all appearances in a state of "resting" to judge by its volume, its double membrane and its general characteristics, throughout the period prior to germination (Fig. 1).

The nucleus of the vegetative cell, on the other hand, undergoes considerable evolution. To begin with, its volume increases rapidly, and the nucleolus grows to a remarkable size, eventually assuming proportions similar to those of the entire generative nucleus. Then the nucleus beging to show lobulations all round, which become more and more marked as it develops, eventually lending it a highly complicated outline, with deep cytoplasmic inlets and nuclear salients, and a very characteristic appearance. The ultimate result is a considerable increase in the surface of the nucleus. The nucleolus does not remain fixed in a particular area, but extends into the lobulations, following their ins and outs, until it become difficult to discern its content. The content of the nucleus appears to be heterogeneous, unlike that of the generative nucleus, and when it is fixed with MnO_4K one can make out granules about 150 Å thick, distributed all over the nucleus and forming more or less compact groups (Figs. 2 and 3).

During this period, the vegetative nucleus appears to be engaged in intense functional activity. The double membrane, besides assuming its characteristic shape, is seen to have its two component units running parallel in the initial stages of its development, but as lobulation proceeds to outer component shows active formation of vesicles (Fig. 2), and is seen to expand at various points all along its surface. This process of vesiculation seems to occur during the early period of lobulation, and the two components forming the membrane later appear parallel again, with wide open spaces in it and pores 400 to 500 Å in diameter (Fig. 3).

When the process of lobulation is finished, sacs of ample proportions are to be seen attached to the double nuclear membrane or its outer unit, establish a connexion between the space inside the membrane (the perinuclear space) and the content of the sacs (Fig. 3).

The changes undergone by the vegetative nucleus during the maturation of the pollen grain take place "pari passu" with the intense production of membrane systems in the vegetative cytoplasm, which is thus endowed with the "machinery" required for the active functions which it will have to carry out during germination and fecundation.

Cytoplasm

The cytoplasmic components which appeared in the form of primitive organules and rudimentary cell systems in the meiocytes and in the first stage

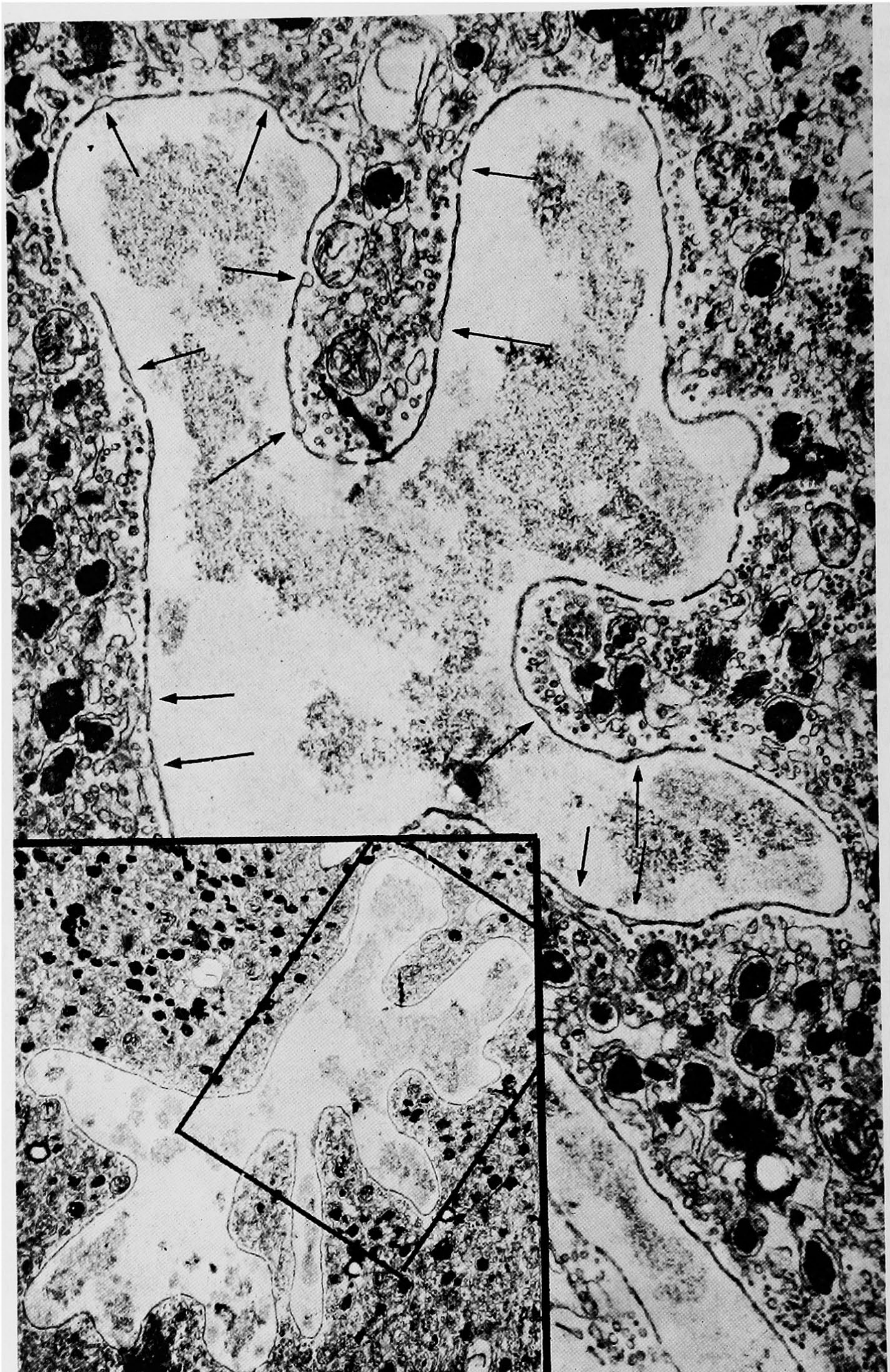

Fig. 2. Nucleus of the vegetative cell of *Scilla peruviana*; the stage of this nucleus is parallel to that of the generative nucleus in Fig. 1. Note the strong lobulation of the nucleus left bottom and the active formation of vesicles by the external membrane of the double nuclear membrane right enlarged photo.

Fig. 3. Vegetative nucleus with intense lobulation. The nuclear membrane lacks lobulations. Note the reticular sacks joined to the double nuclear membrane and the nuclear pores. The cytoplasm contains a large quantity of active membrane systems.

of the microspore's formation now begin to develop intensively "pari passu" with the variations observed in the vegetative nucleus (Fig. 4). The ground

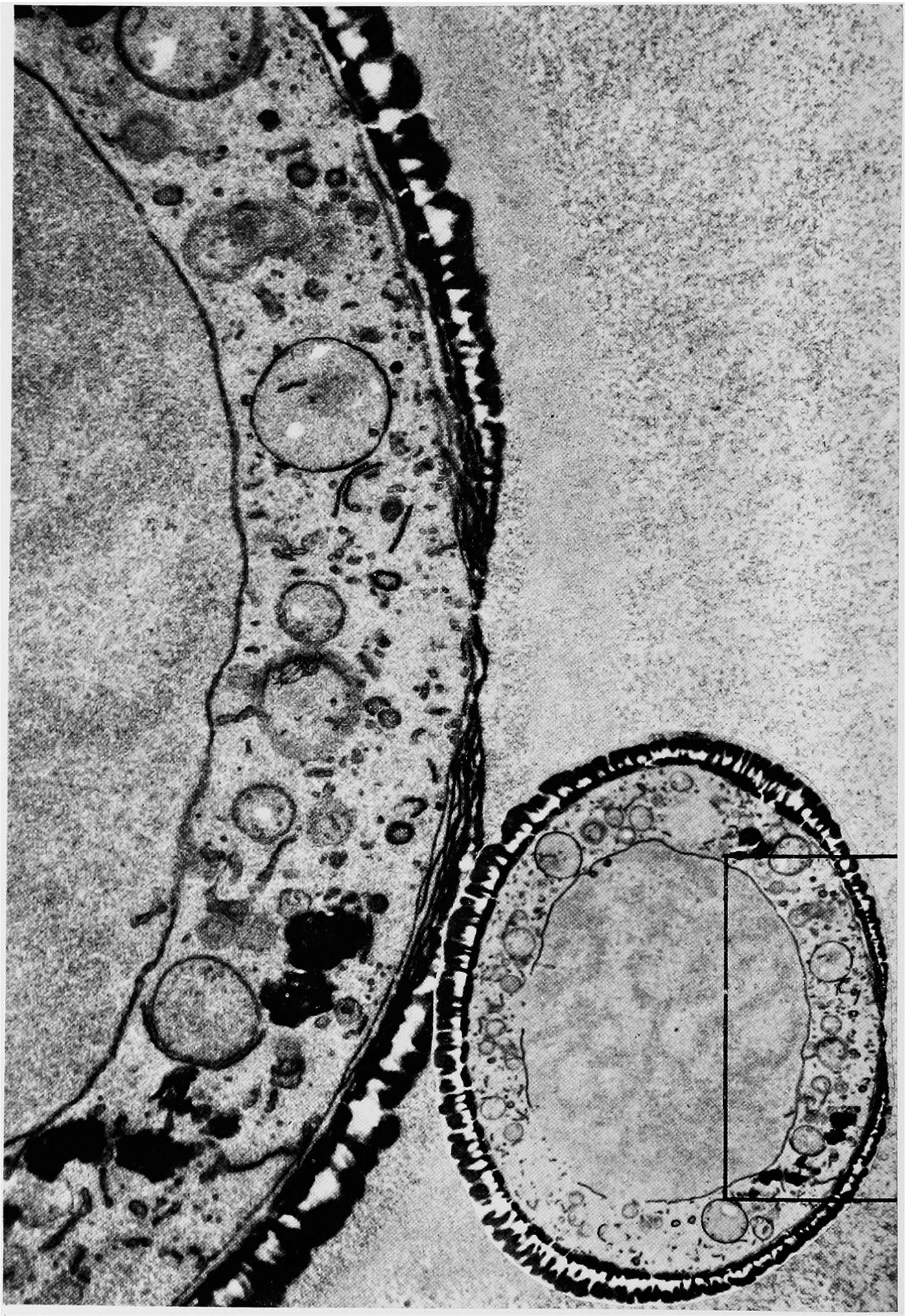

Fig. 4. A newly formed grain of pollen (a). Note the elementary stage of the membrane systems and the cytoplasmic organelles (G=Golgi; P=plastids; M=mitochondria).

cytoplasm, apparently structureless, which occupied most of the cytoplasmic space, is now replaced, in the course of this evolution, by a very highly developed membranous system in which the endoplasmic reticulum and the Golgi apparatus assume extraordinary dimensions.

Endoplasmic reticulum. During the development of the meiocytes and in the newly formed microspore the endoplasmic reticulum seems to consist of small tubules, of varying length but always short, rarely exceeding 8000 Å. In cross-section, they appear in the form of vesicles bounded by a unit membrane, with a diameter of 700 Å, according to the width of the tubules. These are found to be dispersed about the matrix of the cytoplasm during the early stages of development of the microspore, without any structural complication (Fig. 4). Further development then takes place, and before the post-meiotic mitosis, cisternae can be observed of about the same width as the tubules already mentioned.

The cell system undergoes its greatest transformation in the pollen grain when it is constituted by the vegetative and the generative cells. During the process of maturation, the vegetative cytoplasm develops an extraordinarily complex reticulo-endoplasmic system. The small tubules in the meiocytes and the narrow cisternae of the microspore are replaced by spacious sacs of different lengths and widths, with a more electron-dense content than the original cytoplasm. Sections taken at different levels show connexions between one sac and another, thereby establishing a clear continuity between the components of the system within the vegetative cytoplasm. The cell organules, plastidia and mitochondria, and some areas of the basic cytoplasm, are surrounded by this system of membranes, which extends right through the cytoplasmic space, and gives the impressions of intense functional activity. The cisternae in the endoplasmic reticulum are observed to be noticeably distended through the accumulation of products inside, while the irregularity of the outline, the bifurcations and enlargements presented by it, lend the whole system the appearance of an extraordinarily complicated labyrinth (Fig. 5).

Golgi apparatus. The Golgi bodies in meristematic vegetable cells are seen to consist of a series of flattened sacs arranged in close parallel formation, as in animals, numbering generally from 4 to 6 cisternae.

In recently formed microspore, as in meiocytes, the Golgi bodies are characterized by their apparently elementary structure. Pairs of cisternae occasionally appear more or less closely joined, but in general they are dispersed about the cytoplasm, and we do not observe the typical vesicular formations at their edges, or there are very few (Fig. 1). The cisternae are observed to be completely open, like straight tubules in section, or in the form of rings with their ends closed and joined up (Fig. 1). During meiosis and in the early development of the microspore, the Golgi bodies seem to be rather inactive. Previously, during the mitosis following meiosis, the Golgi bodies acquire the dimensions and structure of meristematic cells, with the

Fig. 5. Grain of pollen in germination. There is a large quantity of vesicles in the cytoplasm and a notable development of the endoplasmic reticulum and the Golgi bodies.

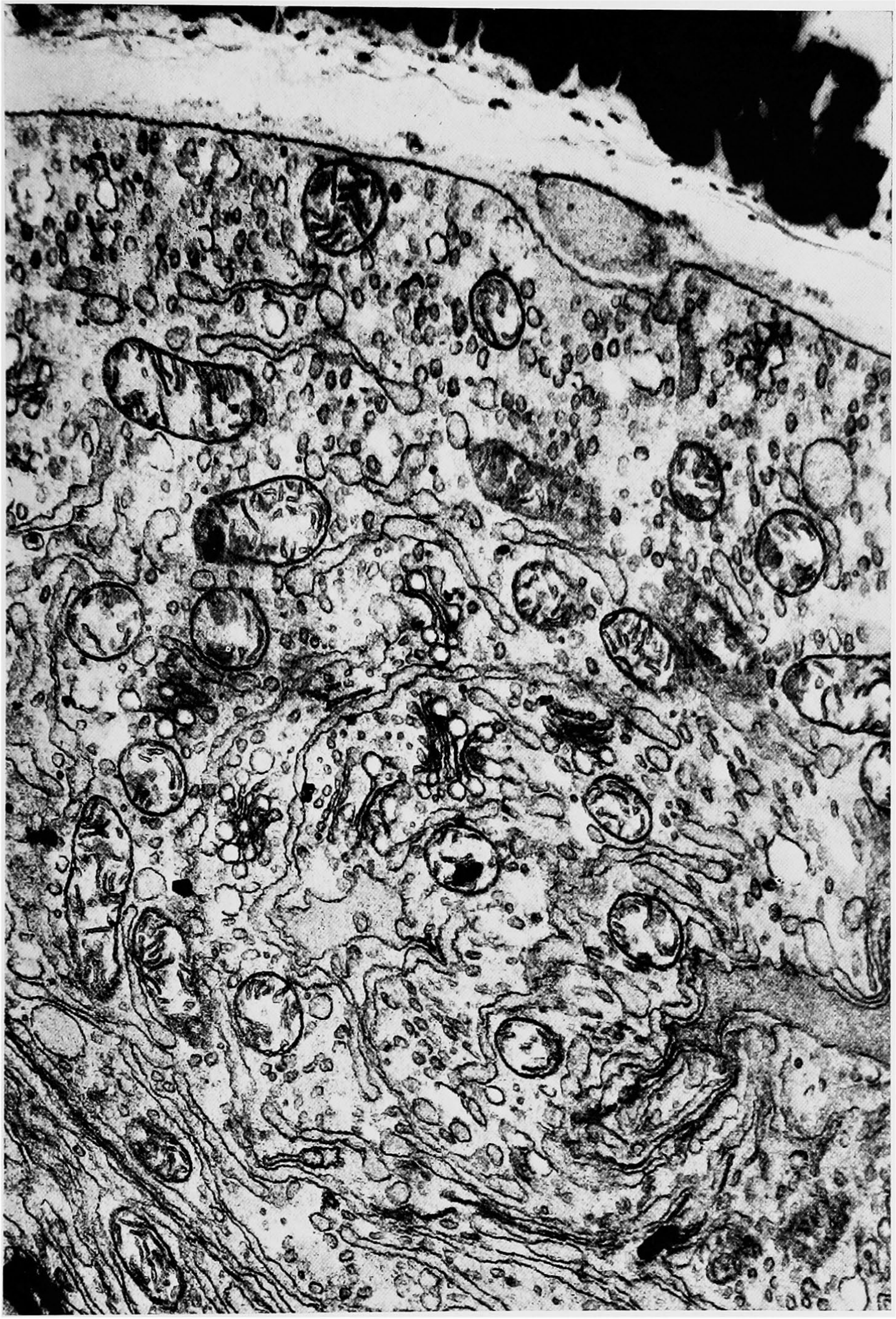

Fig. 6. Grain of pollen in germination. Note the large quantity of vesicles in the cytoplasm. The mitochodria can be seen with their cristae, had the Golgi bodies eliminating vesicles of two types.

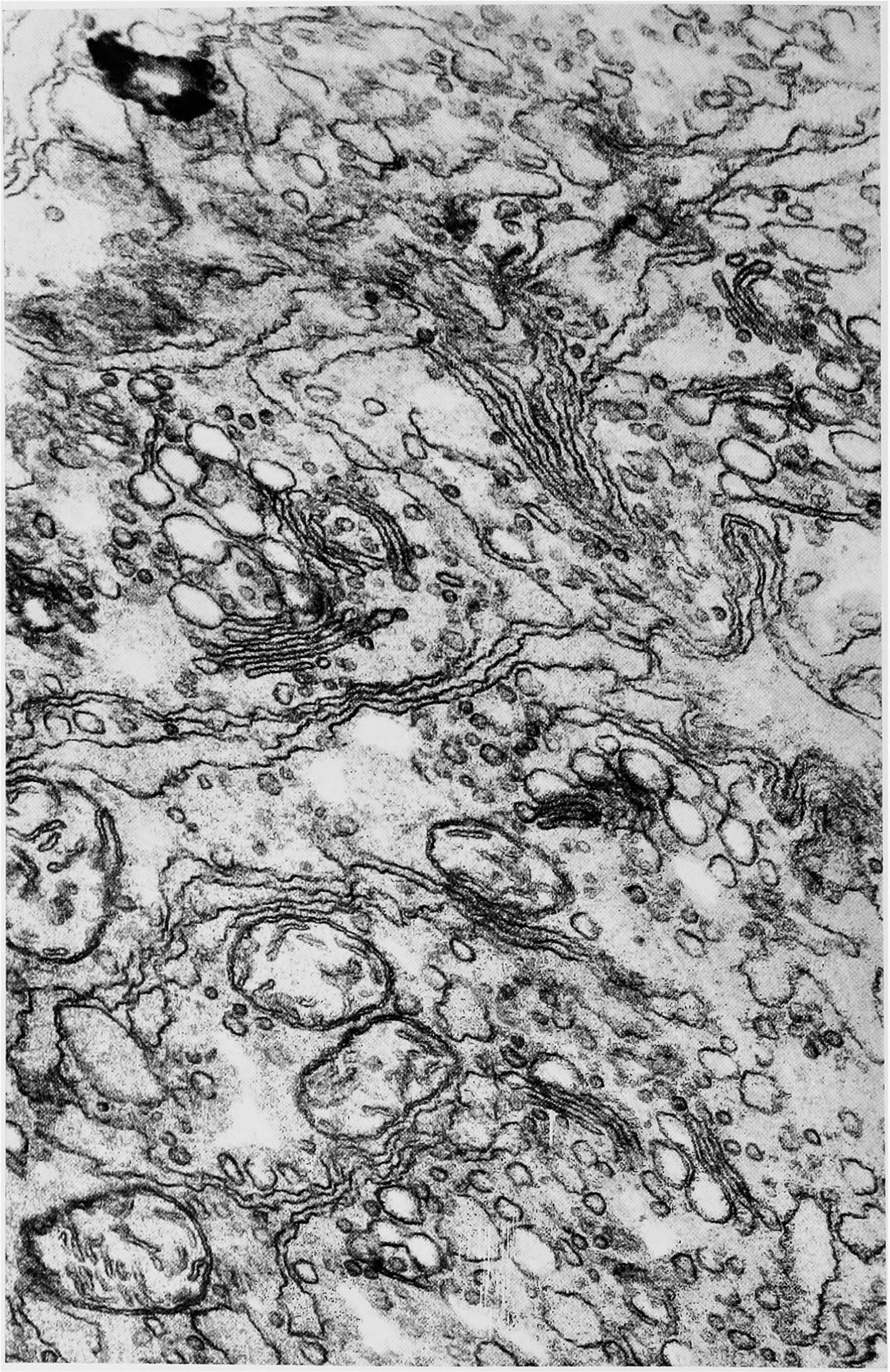

Fig. 7. The formation of two different types of vesicles in the Golgi bodies.

edges producing vesicles about 300–400 Å in diameter, roughly of the same size as those produced by the cell plate in mitotic division, but found in extraordinary proliferation in the cytoplasm of the vegetative cell before and during the germination of the pollen grain.

During these latter periods the Golgi bodies consist of 4 to 7 cisternae, all showing the formation of large vesicles on their edges, of a size far exceeding that of the vesicles produced when the cell plate in being formed, and with electron-permeable content. We can occasionally observe two types of vesicles produced in the Golgi body itself, some like those which form the cell plate, and others with a diameter of about 800–1000 Å (Fig. 6).

Unlike what we observe at the stage when the Golgi bodies appear, during the first period after the formation of the microspores, with little or no apparent activity, at the moment when the pollen grain germinates we observe a high degree of secretory activity, with a considerable number of vesicles being supplied to the cytoplasm.

Cytoplasmic organules. The types of cytoplasmic organules in plants, mitochondria and plastidia, also achieve a high degree of evolution in the course of microsporogenesis. Both types of organules are characterized, like the membrane systems already described, by their rudimentary structure during the meiocyte stage and in the early microspore stage (Fig. 1).

The mitochondria of plant cells are characterized by the possession of invaginations in their inner membrane consisting of "tubuli" of varying sizes and frequency; but the mitochondria of the germinating pollen grain are characterized, like those of animal cells, by the presence of "cristae" inside them resembling those of animal tissues, with intense metabolic activity (Fig. 5).

The plastidia, found in meiocytes and young microspores as proplastidia, are the organules which seem to develop least as the pollen grain evolves, either in size, in number, or in internal complexity. The thylacoids which appear in sketchy outline in some plastidia, are found also in others, in small numbers, but stretching right across the inner membrane from end to end (Fig. 5).

We have not observed any starch present in the plastidia of *Scilla peruviana*, but starch has been found in the vegetative cytoplasm of the pollen from *Scilla* non-scripta, and this would seem to indicate that its presence of absence depends on the reserve substances at the disposal of the cell rather than on the internal structure of its granules when it comes to effecting the synthesis.

Discussion

La Cour (1949) thought that the qualitative difference between the cytoplasm of the vegetative cell and that of the generative cell was the principal reason for the difference between the structures of the two nuclei. This idea was based on the common opinion suggested by the non-observation of any cytoplasmic organules in the generative cell. But the studies undertaken by Bopp-Hassenkam in *Lilium* and *Fritilaria* (1962) and by Diers in *Oenothera* (1962) provided evidence that the same systems and organules were present in the generative cytoplasm as in the vegetative, only in much smaller proportions. Geitler's study (1935) had already shown that the chromatids were evenly distributed in the mitosis following meiosis; so that it would seem that the quantitative difference between the cytoplasms of the two cells is to be considered as the cause of the marked and rapid differentiation between the two.

The generative cell gives rise to two spermatic nuclei by division and it is these which are to effect the double fecundation characteristics of the Angiosperms. The vegetative cell has to produce all the "machinery" needed to form and develop the pollen tube which is to carry the spermatic nuclei. For these different purposes each cell develops its own distinct activity during the maturation of the pollen grain. While the generative cell alone apparently doubles its genetic content, the vegetative cell undergoes considerable change in the content of its cytoplasm.

Photometric studies by Swift (1950) showed that the amount of DNA in the vegetative nucleus remained constant, while it increased considerably, by contrast, in the generative nucleus, and according to the quantitative results of Ogur *et al.* (1951) the RNA and protein content of the vegetative cell increases steeply, compared with the fixed quantity of nuclear DNA. These studies indicate a double direction in the duplication of the generative DNA required to form the two spermatic nuclei and in the massive production of proteins and of RNA in the vegetative cells, as these are needed for the rapid development of the pollen tube. This latter idea suggested a form of activity on the part of the vegetative nucleus designed to bring about a quantitative growth in the cytoplasmic systems of the vegetative cell.

The vegetative nucleus undergoes considerable changes in the course of the maturation process in the pollen grain. The large overall increase in volume, especially of the nucleolus, and the extraordinary increase in the area of contact with the cytoplasm through lobulation, are the most notable. The volume of the nucleolus, which eventually becomes as large as the whole generative nucleus, exhibits intense functional activity. Steffen (1952) reported that the nucleolus had not been observed in the nucleus of the vegetative cell in several pollen grains under the light microscope, but it is clearly present when observed with the electron microscope, and so is its considerable growth as it develops.

The physiology of the nucleolus, which is closely connected with the formation of the RNA in the ribosome (Brown and Gurdon 1964, Georgiew 1967, Liau, M. C., Hnilica, L. S. and Hurlbert, R. B. 1965), is linked up with the formation of proteins in the cytoplasm (Casperson 1950). The remarkable rate of growth of the nucleolus in the vegetative nucleus observed with the electron microscope and the results obtained by Ogur *et al.* (1951) relate the activity with the morphology of the nucleolus.

The form assumed by the vegetative nucleus during the maturation and germination of the pollen grain and the changes observed in the nucleolus are similar to those described in cells with intense metabolic activity, whose nuclei assume irregular shapes, increasing their surface area by the formation of lobules, sacculations, or even, in extreme cases, of complex branches ramifying through the cell. An extreme example of this is offered by the spinning glands of certain insect larvae (Lepidoptera Trichoptera) in which the nucleus, originally spheroidal, finally assumes a labyrinthine appearance with convolutions occupying a large area in the cell (Wilson 1928).

In the germination of the pollen grain, the pollen tube develops at a rate of 6 to 7 mm an hour, which presupposes functional activity of the nucleus and cytoplasm before and during this development to provide the cell with the material and mechanisms needed for this explosive growth. The extension of the nuclear membrane by lobulation and ramification of the nucleus gives it increased possibilities of contact with the cytoplasm all round and great intensification of nucleo-cytoplasmic relationships.

Cells in the course of active development and differentiation show marked functional characteristics in the membranes of their nuclei. In microspores vesicles have been observed in course of formation from the inner unit of the double nuclear membrane (Barth und von Rahden 1967), and the formation of evaginations from the double nuclear membrane into the cytoplasm has been connected with the origin of mitochondria "de novo" in animal cells (Hoffman and Grigg 1958) and of plastidia and mitochondria in plant cells (Bell and Mühlethaler 1962). The formation of vesicles in the shape of annular laminae from the outer membrane of the nucleus in various species of oocytes and amphibians (Hadek and Swift 1962, and Hsu 1963), and especially the evidence from studies carried out by Kessel (1962, '64, '65, '68) on the nuclei of oocytes from Necturus, on the production of annular laminar, their distribution about the cytoplasm, their alignment, and, lastly, their connexions with the endoplasmic reticulum, seem to point to a close connexion between the production of vesicles in the nuclear membrane and the membrane systems found in the cytoplasm. Ward (1965) connects the annular lamellae with the formation of Golgi bodies in the cytoplasm, and Kessel (1966) studies the interchanges between the nucleus and the cytoplasm with the supply of material from the nucleolus to the cytoplasm.

The intense development of membrane systems in the cytoplasm of the vegetative cell alongside the vesiculation of the nuclear membrane and the increase in its area seem to indicate a considerable increase in nucleo-cytoplasmic relations and an active functional role of the nuclear membrane in the organization of the cytoplasmic membrane systems.

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