R E A D I N G T H E S I S

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STUDIES IN THE CYTOLOGY AND PHYSIOLOGY OF POLLEN

PART I

Major contributions to pollen studies to the end of the nineteenth century.

The structure and function of pollen have long been subjects of curiosity and speculation. Even before the concept of sexuality in plants was generally accepted (see Wodehouse, 1935), natural scientists such as Nehemiah Grew and Marcello Malpighi had recorded observations on pollen morphology and hinted at some possible functions of pollen. Wodehouse (1935) quotes from Grew's "Anatomy of Plants", published in 1682, and from Malpighi's Opera omnia (1687), pointing out the comparatively high degree of accuracy in their descriptions of pollen, but at the same time revealing their uncertainty at its "higher purpose". A few sentences from these works serve to illustrate attitudes to botanical investigation at that time. Describing pollen grains, Grew says:

"The Particles of these powders, though like those of Meal or Dust, they appear not easily to have any regular shape; yet upon strict observation, especially with the assistance of an indifferent Glass, it doth appear, that they are a Congeries, usually of so many perfect Globes or Globulets; sometimes of other Figure, but always regular. That which obscures their Figure is their being so small: In Dogs-Mercury, Borage, and very many other Plants, they are extremely so. In Mallows, and some others, more fairly visible.

"The Colour of these small particles contained in the Theca is also different. But as that is usually white or yellow, so are these: sometimes Blewish; but never Red. And sometimes not of the same Colour with that of the Theca. Which further shows how scrupulous Nature is in differentiating the Tincture of the several parts."

Although not as detailed, Malpighi's descriptions were essentially similar to those of Grew. However, he tended to interpret

the function of pollen in terms of human physiology:

"The pollen dust is likewise a mere secretion...prior to the maturation of the ovum...and may be compared perhaps to the menstrual discharge of women."

Few advances in the study of pollen were made during the next century and a half. Further work was forced to wait upon improvements in microscopy made towards the turn of the eighteenth century.

Early botanists devoted much attention to the morphology of the pollen wall, possibly because it was easy to observe. Turpin in his Essai d'une iconographie végétale (1820) stated that the pollen wall consisted of two layers, which he denoted "exhyménie" and "endhyménie". Purkinje, a Bohemian physiologist, discussed the possible taxonomic significance of pollen wall structure in De formis granorum pollinis relate ad familias naturales adnota (1830). This was the second part of his major work on pollen. He too recognised the composite nature of pollen walls, and described some in detail, including observations on the spines and pores of the external coat, and the apparent protrusion of the inner coat at points of weakness. The French botanist Brongniart included in his investigations the structure and development of pollen grains, and although many of his hypotheses proved to be quite erroneous, the plausibility of his ideas is emphasized by the fact that the Paris Academy of Sciences awarded him a prize in experimental physiology. The paper which earned this acclaim, Mémoire sur la génération et le développement d'embryon dans les végétaux phanérogamiques, was published in 1827. Brongniart described the pollen wall as consisting of two layers, but mistakenly believed segments of the reticulate thickening on the surface of certain pollen grains to be individual cells. He also believed that the function of all pores and furrows in the wall was to permit the passage of "spermatic granules" into

and ultimately out of the pollen grain.

Brongniart's work, firmly backed by the Paris Academy, influenced subsequent investigation to such an extent that few pollen researchers of the time were able to approach problems of pollen structure with originality and absence of prejudice. Von Mohl, a German botanist generally highly regarded for his individuality, was unable to free himself sufficiently from the current influence to make any real innovations in the study of pollen surface structure, as was revealed by his major pollen work, Über den Bau und die Formen der Pollenkörner (1834). A contemporary, Fritzsche, was more successful in this respect. He produced four important works in five years: Beiträge zur Kenntnis des Pollen (1832), his doctoral dissertation De plantarum polline (1833), Über den Pollen der Pflanzen und das Pollenin (1834), and Über den Pollen, which was read before the Academy of Science in St. Petersburg in 1836. This last work exposed von Mohl's misconceptions of pollen structure, and its publication may well have been the factor which discouraged von Mohl from further work in pollen morphology. Fritzsche was a chemist by training, and much of his work on the pollen wall dealt with the chemical nature and reactivity of the intine and exine - terms which Fritzsche himself introduced.

Pollen studies increased in popularity during the middle and later years of the nineteenth century. Nägeli contributed much to the understanding of pollen development through his work on pollen of five different genera. In Zur Entwickelungsgeschichte des Pollens bei den Phanerogamen (1842) he described the growth of the intine around each microspore, and the appearance of the exine with its modifications for the outgrowth of pollen tubes. Schacht (1860), Strasburger (1889), and Mangin (1889) made further important observations on wall development (see Wodehouse, 1935). Fischer (1889) investigated the structure and chemistry of the exine, and included his

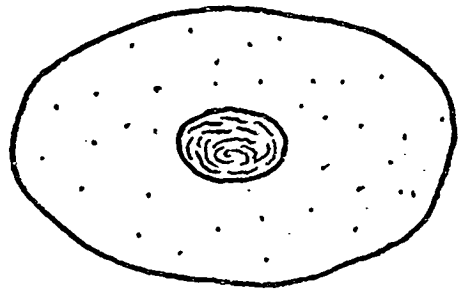
findings in his doctoral thesis Beiträge zur vergleichenden Morphologie der Pollenkörner. He stated that the exine "cuticle" showed similar reactivity to that of proteins, but differed from most of these in its insolubility in alkali, differing also from cutin and suberin in this respect. The exine itself he found to be insoluble in concentrated nitric acid, hydrochloric, or sulphuric acid, and resistant to "gastric digestion". He reported that exine did react with sodium hypochlorite solution and with chromic acid, but to markedly different degrees in different species. These observations showed an awareness of the exceptional properties of exine material. Fischer also commented on the higher refractive index of the inner layer of exine, and the stronger affinity of the outer layer for aniline dyes. His studies of the pollen of more than two thousand species led him to conclude that the evolutionary trend had been towards a strengthening of the exine, particularly by means of raised appendages. The patterns appeared to be most complex in the Dicotyledons.

Although pollen wall morphology continued as a major field of research in the early twentieth century, botanists at this time were beginning to appreciate the importance of studying developmental processes, and found pollen to be well suited to investigations of this kind. The contributions of Sachs, who introduced several important concepts in his developmental study of pollen, will be discussed in Part II.

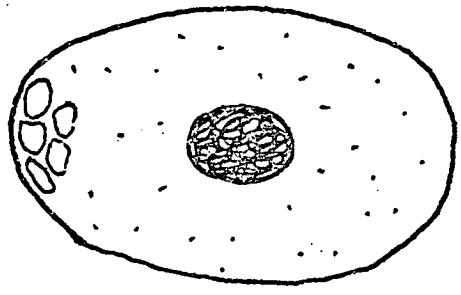
Introduction to Part II.

It would seem appropriate to introduce Part II with a generalised description of Angiosperm pollen grain development, so that the following reviews of cytological, physiological and biochemical phenomena can be related to the developmental sequence. Most pollen work has been carried out with Angiosperms, and the wide variation in gametophyte forms among the Gymnosperms precludes a generalised account of their development. The relatively small amount of relevant work on Gymnosperm microspores will therefore be referred to in passing.

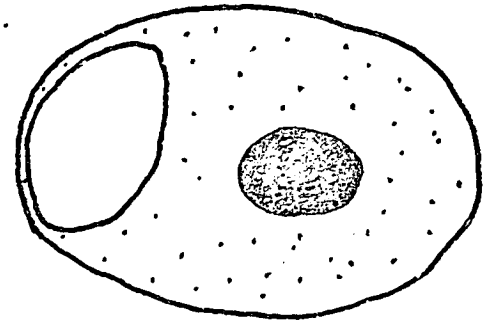
The microsporangia or pollen sacs of Angiosperms are grouped together in anthers, usually four at a time. Octosporangiate anthers are known, and bisporangiate types occur as a result of precocious lysis of the intersporangial septum. The microspores develop from sporogenous cells which divide initially by mitosis for several generations, the final division giving rise to the pollen mother cells. Each pollen mother cell develops a thick callose wall, the "special" mother cell wall, and the nucleus divides by meiosis to form four haploid nuclei. Cytokinesis may occur in one of two ways, by simultaneous or successive cleavage. Simultaneous cleavage produces a tetrad of microspores as soon as the four nuclei are formed, while successive cleavage first produces diads and forms the tetrads by subsequent deposition of another wall. Each cell of the tetrad begins to develop an exine within the "special" callose wall. The callose is eventually degraded, and the microspores are released into the anther loculus. After liberation, the exine usually undergoes further development. A cellulosic intine is secreted beneath it some time before anther dehiscence. The major nuclear and cytoplasmic changes which usually occur during pollen maturation are represented diagramatically below:-



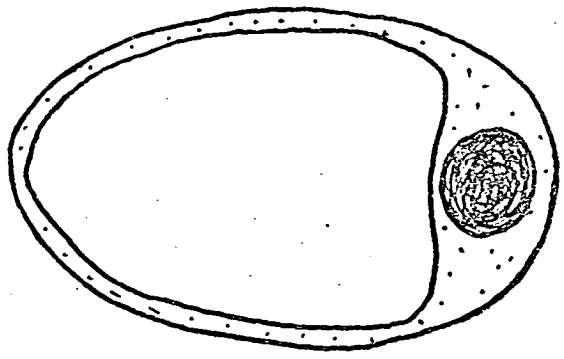
After release from the callose mother cell wall, the microspore has a central nucleus, and the cytoplasm is not very dense. The nucleus enlarges as it approaches S-phase, and during it. The timing of S-phase, however, may not be correlated with the same cytological events in all species.



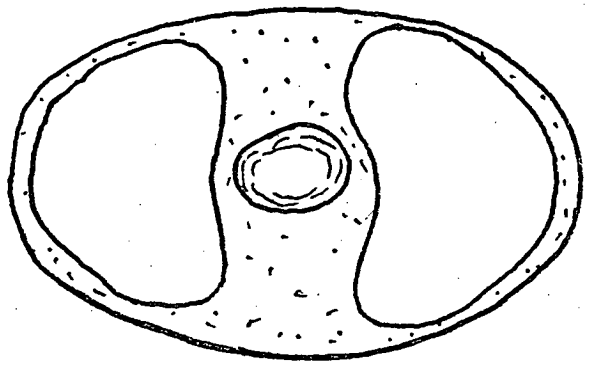
Small vacuoles usually develop at one pole in the cytoplasm.



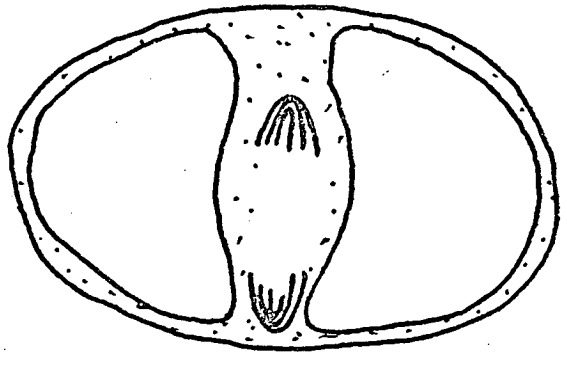
The small vacuoles coalesce to form one large vacuole, which displaces the nucleus from its original position.



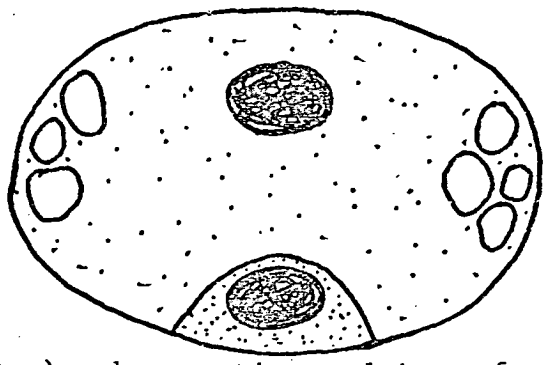
The vacuole enlarges, taking up most of the cell.



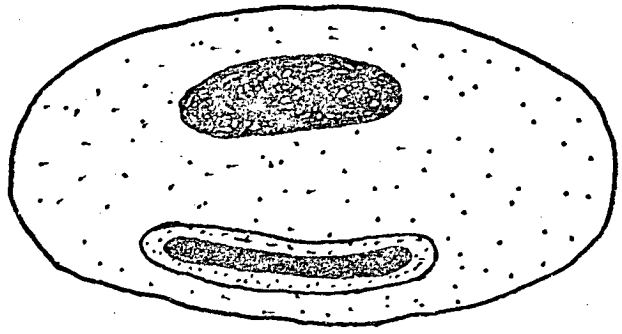
The vacuole divides, and the nucleus moves back to central position in the bridge of cytoplasm.



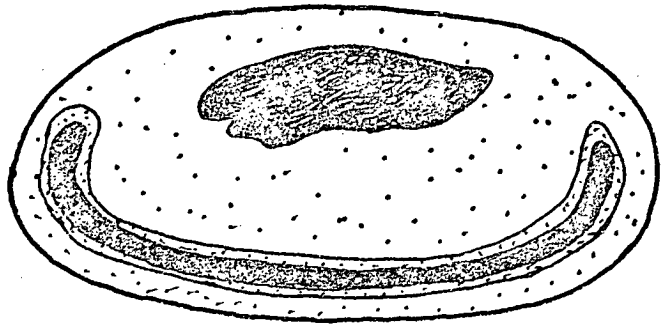
The mitotic spindle is formed asymmetrically, in the bridge of cytoplasm. Pollen grain mitosis occurs.



The vegetative (top) and generative nuclei are formed. A wall is secreted, enclosing the generative nucleus in a little cytoplasm within the vegetative cell. Initially both nuclei are similar in appearance.



The vegetative nucleus becomes diffuse (euchromatic), whereas the generative nucleus elongates and becomes dense (heterochromatic). The generative cell is now independent of the vegetative cell wall. The generative cell wall is lost, prior to this, permitting shape change and migration of cell.



The mature pollen grain in most species is binucleate at anthesis. In some species the generative nucleus has already divided into two sperm nuclei before dehiscence. A few species shed uninucleate pollen, i.e. anthesis occurs before pollen grain mitosis.

The pollen grains are shed at anthesis, and if pollination is successful and incompatibility factors do not interfere, pollen tube growth will follow. Under appropriate conditions fertilisation occurs, sometimes within hours of pollination, sometimes not for months. It is probable that the tube produces enzymes which enable it to penetrate the tissues of the stigma and style. While the tube grows towards the ovary, the generative nucleus divides to form two sperm nuclei, if it has not already done so before anthesis. One sperm nucleus fertilises the egg, and the other combines with the "fusion nucleus" to initiate the endosperm.

PART II

Studies since 1900.

A. The Pollen Wall

"It is the aim of investigations of pollen wall growth to elucidate the devices through which the intricate detail of different components is molded in faithful conformity with genetical instruction. There is no doubt that any evidence gained must contribute, in equal measure, to our understanding of plant growth and morphogenesis in general, since so many manifestations of differentiation and development in plants do concern the cell wall."

Heslop-Harrison (1968)

Interest in pollen wall development grew during the middle years of the nineteenth century. Sachs recorded his studies of pollen in his "Textbook of Botany", the third edition of which appeared in English in 1875, and included the observation that microspores bore sculptured exines even before their release from the callose mother cell wall. This fact received scant attention until the publication of Fitting's work (1900)

on the development of spores in *Isoetes* and *Selaginella*. Dispute arose over the matter of control of exine formation, since Fitting had observed that exines developed normally on many aborted spores. This was widely regarded as evidence for control over exine secretion by the anther tapetum - for how could non-functional cells produce their own walls? On the other hand, if the tapetum was responsible for deposition of exine on the naked surfaces of microspores, how should one interpret Sachs' observation of exine inside the callose mother cell wall, in physical isolation from the tapetum? The rôles of the tapetum and the pollen protoplast were obviously far from clear.

Among the early workers who studied this problem were Tischler (1908, 1915) who found well-developed exines on aborted microspores of *Mirabilis*, and Kosmath (1927) and Übisch (1927) who independently showed that in some Angiosperms the tapetum produced microscopic bodies of a chemical nature similar to that of the exine. These are commonly known as "Übisch bodies", although they were first described by Rosanoff in 1865. At the time, their presence was almost universally accepted as proof of Fitting's contention that the tapetum controlled exine synthesis. However, Beer (1911) had already suggested that early exine patterning may be initiated by the pollen protoplast, but that subsequent wall development came under the influence of the anther tapetum. This was also proposed by Drahowzal (1936). Sachs' important observations were apparently either ignored or forgotten.

The chemistry of the exine attracted attention from early workers concerned with wall development. The first detailed analyses for a wide range of species were made by Zetzsche (1932), who proposed a general formula for a material which he named "sporopollenin", the main component of all exines. Sporopollenin appeared to be a highly unsaturated polymer

characterised by resistance to acetolysis and to non-oxidative chemical attack (cf. observations of Fischer, 1889). The nature of the polymer remained an open question for some years, until Swiss chemists (Karrer *et al.*, 1935, 1949, 1950) resurrected the work of Bertrand and Poirault (1892) who had observed the accumulation of carotenoids in both anthers and pollen. Karrer was concerned to discover whether carotenoids were implicated in the synthesis of sporopollenin, since their unsaturated nature suggested that they may be a suitable precursor. However, nothing definite emerged from these investigations. Studies into the chemical and physical properties of sporopollenins, largely sponsored by the oil industry, intensified towards the end of the sixties. At an international symposium in 1970 Brooks and Shaw presented convincing evidence that sporopollenins largely consist of polymers formed from carotenoids and carotenoid esters. While a few workers still believe there to be a refractory component other than sporopollenin, the work of Brooks and Shaw is generally accepted as the best analytical study to date.

Work on pollen wall structure continued sporadically throughout the forties and early fifties, but it was the development of electron microscopy which provided the techniques needed to observe the mechanisms of wall secretion. Following the development of the carbon replica technique for studying pollen surfaces (Mühlethaler, 1955) a stream of papers appeared describing the topography of hundreds of types of pollen. In this context the contributions of Afzelius, Erdtmann, Mühlethaler, Frey-Wyssling, Sitte, Faegri, Fernández-Morán and Dahl are important, but the significance of their work was largely taxonomic and contributed little to the understanding of exine development *per se*. At this time, published works were purely descriptive, and electron microscopy did little more than substantiate the findings of light microscopy. With improvements in techniques there was a growth of interest in the potential use of pollen

as a material for studying several fundamental morphogenetic processes. Ehrlich (1958) voiced this interest in the introduction to a paper on pollen walls of *Saintpaulia*:

"The ontogeny of the pollen grain, especially the development of its characteristic wall, poses a number of basic problems in the cytology of growth. Among these are the relationship between pollen wall formation and ploidy or genetic control, and the degree to which the protoplasm is involved in pollen wall development." In the first truly ontogenic study, Rowley (1959) revived the old controversy regarding the rôle of the anther tapetum in exine secretion. As the most convincing evidence for tapetal control had come from studies of aborted pollen, he paid special attention to sterile grains of *Tradescantia* and was able to confirm at the electron microscope level that their exines were in fact perfectly formed. He suggested however that the presence of a well developed exine on aborted pollen may be due to the late death of the protoplast, rather than direct tapetal activity.

Rowley's investigation was concerned with sequential changes in the pollen wall, and made no mention of changes in the cytoplasm of either the tapetum or the pollen protoplast. To elucidate the fine-structural mechanism of exine secretion it is clearly necessary to study the cytoplasm responsible, and the first productive moves in this direction were made by Heslop-Harrison. In his paper "Origin of Exine" (1962) he stated that microspores of *Silene* and *Cannabis* did not actually bear an exine at the tetrad stage, but that exinous material, formed in mitochondria, accumulated on the microspore surfaces. In contrast to Rowley, he regarded the exine on aborted pollen as evidence for tapetal control of exine deposition. At the First Internation Symposium on Pollen Physiology and Fertilisation at Nijmegen he presented the keynote paper (Heslop-Harrison,

1964) in which he reported the observation of protoplasmic continuities between sporopollenin "plaques" (Übisch bodies) at the tapetal surface, and the exine. This phenomenon he regarded as part of a series of constantly changing interconnections between sporophyte and gametophyte, and it appeared to strengthen his argument for tapetal control of exine deposition. In another paper (1963) Heslop-Harrison suggested a possible rôle for the endoplasmic reticulum of the pollen protoplast in determining the wall pattern. He proposed that parts of the ER close to the plasmalemma might become oriented in a particular way to assist the formation of the "primexine", a term he coined for the initial patterned wall which he had observed within the callose mother cell wall. He believed the primexine to be cellulosic in nature (although no convincing evidence was presented to support this contention) and therefore did not regard it as a "proper" exine. He suggested that the primexine became progressively impregnated with sporopollenin, eventually forming the acetolysis-resistant wall which appeared after the dissolution of the callose. In this way, the pattern of the exine was supposedly determined by that of the primexine. As Heslop-Harrison had stated only the previous year that a true, patterned exine was apparently <u>not</u> present at the tetrad stage, the concept of a "primexine" may well have developed as a face-saving device in the light of new data - although it became widely accepted at the time.

In the same year, Rowley (1963), who had investigated the development of Übisch bodies in *Poa*, proposed that the tapetum was the source of monomer precursors for sporopollenin and that polymerisation could occur on any suitable surface. So far, Übisch bodies had only been detected in plants with the "secretory" type of tapetum, and Rowley suggested that, among sporophyte tissues, only the degenerating cells of a "secretory" tapetum provided a suitable surface. The newly formed microspores provided another such surface. The precise nature of the

surface was not known, nor was the precursor. All that was known was that sporopollenin accumulated at specific sites at specific stages of development. The fact which led Rowley to believe the tapetum to be the sole source of precursors was the current belief that "true" acetolysis-resistant sporopollenin was synthesized in the anther only after release of the microspores from the tetrads. Both exine and Übisch bodies were known to develop after this.

The physical structure of exines was clarified to some extent about this time. By the sixties it had been confirmed that most exines consisted primarily of two layers, an outer ornamented ektexine and an inner endexine (using Faegri's terminology) or a sexine and nexine respectively (using that of Erdtman). Improvements in ultramicrotomy led to the detection of both lamellate and amorphous components in exines, the sexine being characteristically amorphous, while the nexine invariably displayed both structures. Gullvåg (1966) discussed the significance of the two components in relation to taxonomy and function, and Skvarla and Larson (1966) reported the association of membranes with developing exines and Übisch in *Zea*, an important step forward in the understanding of lamellate structures. It was postulated that Übisch bodies might be formed around pieces of membrane-bound cytoplasm deriving from ruptured microspores. This was followed shortly by the recognition that lamellate exine was produced by deposition (polymerisation?) of sporopollenin on membranes in spores of *Anthurium* and the liverwort *Scapania* (Rowley and Southworth, 1967). Lepousé and Romain (*Oenothera*, 1967), Godwin *et al.*, (*Ipomoea*, 1967), and Angold (*Endymion*, 1967) were able to confirm this. Rowley and Erdtman (1967) observed the progressive accumulation of sporopollenin on membranes in the space between the plasmlemma and the sexine, giving rise to the lamellate component of the nexine on microspores of *Populus* and *Salix*. Dickinson and Heslop-Harrison (1968) asserted (contrary to Godwin

et al., 1967), that "all stratified exine originated on or near lamellae which are produced near the plasmalemma." They emphasized the rôle of the plasmalemma, from which they believed the lamellae arose, in determining exine patterning (cf. Heslop-Harrison, 1963). In another study of Übisch body development Echlin and Godwin (1968a) described "pro-Übisch-bodies" originating in close association with the ER of tapetal cells in *Helleborus*. They appeared to be membrane-bound structures with lipoid content. Once lysis of the inner walls of tapetal cells had begun, these bodies passed through the plasmalemmae and developed acetolysis-resistant properties only after liberation into the thecal fluid. Echlin and Godwin suggested that Übisch bodies might develop from fragments of degenerating tapetal cytoplasm which still retained some synthetic capacity. They noted the presence of "white lines", apparently membrane profiles, in both Übisch bodies and in mature lamellate nexine. The fact that sporopollenin always developed extra-cellularly, in situations between the plasmamembranes of microspores and the cells of the tapetum, they regarded as evidence for the tapetal origin of precursors - a conclusion which cannot have been very carefully considered.

It should be noted that most publications of the time referred to "the" exine, as though all exines were identical, and it is clear that many workers expected to discover a universal system governing the detailed structure, and mode and phasing of synthesis of all exines. Nowhere was this attitude more prevalent than at Cambridge, where Echlin (1968), having summarised the developments of the past few years, asserted confidently: "The results of other investigators...together with our own work indicate that there appears to be a common sequence of developmental events during pollen maturation", and also: "it would be wise to seek a general ontogenetic scheme that brings Übisch body and exine development into a basically similar pattern, and we believe that...the *Helleborus* system may

prove to be a general one" (Echlin and Godwin, 1968a). Nevertheless Mepham and Lane (1968) questioned certain assumptions implicit in current reports. Their own work with *Tradescantia bracteata* suggested that the exine was not in fact deposited through direct activity of the tapetum in this plant, but that it was "wholly a secretion of the pollen protoplast", an acetolysis-resistant exine being formed in quantity prior to loss of the special mother cell walls. They found no evidence that sporopollenin was formed in tapetal mitochondria, as had been suggested by Heslop-Harrison (1962), and in fact found no evidence at all of sporopollenin in the tapetum of *Tradescantia*, a plant with an "amoeboid" tapetum. Their work was the first ultrastructural study of such a system. They proposed that the breakdown of cell walls in the "amoeboid" tapetum was not a degenerative process, preferring to regard it as a process of constructive reorganisation. In a later paper (1969a) they described evidence for continuing synthetic activity in the tapetal periplasmodium until shortly before anthesis (e.g. mitochondrial phosphorylation, and a late phase of starch production) and also reported the formation of apparently fully developed exines with apertural regions, inside callose mother cell walls (cf. Rowley, 1959). While these findings were readily accepted, they initially attracted criticism from members of the Cambridge school, who disputed the validity of some observations. Godwin (1968b) was not prepared to accept the presence of "true" exine inside the special mother cell walls, despite an amount of published evidence, and despite the fact that he had alluded to its presence himself in an earlier paper (1968a).

The problem of participation of the microspore nucleus in the control of exine development was also a matter of contention at this time. Echlin and Godwin (1968b) took the view that available evidence favoured genetic control by the haploid nucleus of the microspore, and Heslop-Harrison (1968a) stated that, while the matter was unsolved and the balance of

evidence favoured sporophytic control, there was no evidence to completely exclude participation of the microspore nucleus. The most persuasive evidence against microspore nuclear control has come from studies of genetically deficient microspores. Savage (1956) working with *Pulmonaria* observed proper development of exine on "miniature" microspores. These arise at telophase II when simultaneous cleavage provides a mass of cytoplasm around each nucleus, whether the nucleus is complete or consists only of one or more chromosomal laggards. In Savage's plants, which were polyploids, laggards frequently led to the formation of such "micronuclei", which then became centres for the development of "miniature" spores. It is difficult to conceive that in all "miniature" pollen grains the very limited genome would always carry the genes necessary to direct exine development. If it is accepted that exine does in fact develop inside the callose mother cell walls, then one must conclude that the cytoplasm of the pollen mother cell is already "programmed" to produce exine at the right time. This hypothesis was restated following observations with *Linum* by Rogers and Harris (1969), and again by Mepham (1970) who had used triploid clones of *Tradescantia* and observed perfect exines on genetically unbalanced and deficient microspores, using scanning electron microscopy to support transmission work. In this paper Mepham states that there is plenty of evidence to show that microspore protoplasts can and do synthesize exine, and that it is not necessary to invoke tapetal synthesis to explain any phenomena observed to date. He summarises the conditions probably required for sporopollenin production: a surface for polymerisation, enzymes to carry this out (possibly attached to the surface), a supply of precursors, and suitable environmental conditions such as pH and ionic concentration. He believes that precursors may well arise in both tapetum and sporogenous tissue (cf. Echlin, 1968, who recalled the common origin of tapetal and sporogenous cells, suggesting that both may have the potential for

sporopollenin precursor production). The enzymes necessary to effect polymerisation may also arise in both tissues. If these are present, but environmental conditions are not conducive to polymerisation, no exine or Übisch bodies can develop. A sudden change, such as a pH or ionic change, could induce deposition of sporopollenin on suitable surfaces. Mepham suggests that the enzymes may be of two types: those that are membrane-bound, which initiate polymerisation, and soluble enzymes which continue polymerisation once it has begun. He explains that the outward growth of sexines, and the increase in girth of Übisch bodies after liberation into the thecal cavity, may be attributed to activity of this second type of enzyme. The increase in thickness of nexines by lamellar apposition of sporopollenin may be due to the development of successive layers of membranes from the plasmalemma which carries the initiator enzyme. Mepham points out that two-enzyme systems of this type are not uncommon, and are involved in the synthesis of several polysaccharides such as glycogen and some starches (although there is no certain evidence that any particular enzyme in these cases is membrane-bound). His ideas provide one possible explanation of the observation, so frequently made, of membranes associated with newly formed sporopollenin.

The appearance of sporopollenin at sites other than exines and Übisch bodies was first described by Banerjee (1967), who found that a fenestrated membranous structure developed over the inner surface of the tapetal cells in certain grasses. This structure, which was acetolysis-resistant, bore projections reaching into the thecal cavity among the pollen grains, which often appeared entangled in the network. Heslop-Harrison (1969) then located a sporopollenin "membrane" around the <u>outside</u> of the tapetum in some Compositae with an "amoeboid" tapetum. The entire tapetal periplasmodium and microspores were therefore contained in a sporopollenin "sac". Similar extratapetal structures have subsequently been demonstrated

in other species (e.g. Dickinson, 1970a) and they appear to be a widespread phenomenon among plants with "amoeboid" tapeta. Where a fenestrated structure develops inside the thecal cavity, as described by Banerjee, it is likely that polymerisation occurs over the surfaces of the exposed plasmalemmae of the tapetal cells, once their inner walls have been lost. The sporopollenin projections may perhaps develop around finger-like projections from the tapetal protoplasts.

Some of the most productive recent work has been that of Waterkeyn and Bienfait (1970) and Dickinson (1970b), aimed at uncovering the precise mechanism of exine patterning. Interpreting data obtained from *Lilium*, Dickinson suggests that the pattern is established by outgrowths of the plasmalemma into a fibrous layer between the callose wall and the microspore protoplast. In fact, it appears from his micrographs that the fibrous layer may be an area of callose dissolution. Waterkeyn and Bienfait, using phase-contrast and fluorescence microscopy, observed a pattern on the inside of the callose wall, which appeared to act as a hollow template for exine deposition in *Ipomoea purpurea*. It seems likely that both papers are describing the same phenomenon: the formation of a hollow template by dissolution of patches of the callose wall, into which the microspore plasmalemma protrudes. The shape of the plasmalemma may then determine the eventual shape of the exine.

Whether or not contributions are ever made to the exine by adherence of Übisch body material (perhaps in a plastic state) is still a matter for debate. It is clear that in some species most, if not all of the exine develops inside the tetrad wall, whereas in *Sorghum*, for example, it appears that very little exine is present until after dissolution of the tetrads (Christensen *et al.*, 1972). Certainly in some species the main exine development occurs <u>after</u> the tetrad stage, but whether tapetal

material in the form of polymerised sporopollenin is actually involved in this growth, is not yet clear. The consensus is that Übisch bodies do not contribute in this way, but Risueño *et al.* (1969) and Banerjee and Barghoorn (1970) have presented contrary views. The latter paper suggests that new spinules may be added to the ektexine by Übisch bodies. It is quite possible that this method of exine growth does occur at times in certain species.

In summary, it would appear that the "primed" pollen mother cell cytoplasm, bequeathed to the microspores within the tetrad, is the controlling factor in exine synthesis. The tapetum may be implicated in the transportation of sporopollenin precursors, but it is likely that the microspore itself can synthesize these from basic materials supplied via the tapetum. Evidence from "miniature" grains suggests that the microspore genome plays no major part in exine formation.

B. The Pollen Protoplast

Pollen has become increasingly popular as a material for studying the mechanisms involved in many fundamental cellular processes. It is readily obtainable, and can be stored in the viable state for relatively long periods, but its main advantage is that it provides a simple haploid system, the developmental fate of which is well known. The development and differentiation of pollen can be easily studied, because an anther contains a population of pollen grains developing in relative synchrony, particularly in the early stages. Being small and easy to handle in quantity, pollen has many of the advantages which have made bacteria so popular in biochemical and cytological research, with the added advantage of being eucaryotic. Its major disadvantage is that, being a gametophyte, it is only part of a life-cycle, and there is at present no

knowledge of the proportion of its genome that is expressed during its life.

A basis for understanding the problem of genetic control of cellular growth and differentiation was provided by the Watson and Crick model for the structure of DNA (1953), a proposal which has been substantiated with only very minor modifications. Nevertheless, it is only a basis and many far-reaching problems remain to be investigated. These include the influence of the internal cellular environment on gene function; the influences exerted on a cell by its external environment, including that constituted by neighbouring cells; the detailed mechanism of protein synthesis, which is still poorly understood; the interrelationships between biochemical pathways and their feedback mechanisms; and the exact structure and function of sub-cellular organelles. To be acceptable, any model of gene action must embrace all observed phenomena, biochemical, biophysical, and cytological, and this means that apparent anomalies such as extranuclear DNA must be included. The complexity of the problem has increased rather than decreased since the historic paper of Watson and Crick.

Contributions made by pollen workers to the understanding of fundamental processes will be discussed in Parts IIB and C.

(i) Synthesis of nucleic acids and nucleoproteins

Nucleic acid metabolism in pollen has attracted much attention during the past three decades. The most extensive early work was carried out by von Euler *et al.* (1945, 1948) who determined the DNA and RNA contents of pollen of *Betula pubescens* and other plants, and by Sosa-Bourdouil (1949, 1952, 1954) who undertook comparative biochemical studies of the pollen of several higher plants. Biochemical estimation methods are limited in that they can only give an "average" picture of the nucleic acid content of many nuclei, and workers at the time tried to

adapt newly developed microphotometric methods to use instead of, or in conjunction with biochemical analysis. For example, Caspersson (1936) developed a photometric technique which made use of the high absorption of nucleic acids in the UV region. Pollister and Ris (1947) employed a technique in which nucleic acids were reacted with selected compounds to produce insoluble coloured derivatives with absorption peaks in the visible part of the spectrum. The principal advantage of these techniques is that they permit the examination of individual nuclei, and comparisons can be made between them. Errors arise due to heterogeneous distributions of chromophore, but these can be reduced and standardised by making absorption measurements on each nucleus at two wavelengths, and processing results mathematically. However, much of the early work is now known to be unreliable, involving errors of 30% or more, and all work is strictly comparative. Bryan (1951) chose microspectrophotometry to investigate DNA and nucleoprotein synthesis in pollen of *Trandescantia paludosa*, from the tetrad stage to the mature gametophyte. In comparing his data with results obtained by Ogur *et al.* (1951) for *Lilium longifolium*, using biochemical extraction and UV-absorption methods, he claimed good agreement on relative estimates of changes in levels of DNA per nucleus, at most stages of development, but his technique was essentially unreliable for quantitative evaluations. The results of Bryan, and Ogur *et al.*, contrary to those of Swift (1950) indicated that the vegetative and generative nuclei came to contain the diploid amount of DNA, although their chromosome complement was apparently only haploid. The increase in total DNA just before anthesis, reported by all three parties, was attributed by Swift to the expected replication of DNA prior to generative cell mitosis. Bryan did not agree with this interpretation, as he regarded the generative nucleus as already diploid with respect to DNA. Although the morphology of the differentiating generative nucleus made it impossible to measure DNA levels with any degree

of accuracy, Bryan concluded that the observed increase in DNA before anthesis was entirely associated with the vegetative nucleus. On the other hand, Ogur *et al.* assumed that the increase was shared by both nuclei. Bryan found that the rate of nucleoprotein synthesis was much faster than that of DNA. He reported a phase of rapid protein synthesis in the vegetative nucleus just after pollen grain mitosis, but did not suggest a reason for this activity, and in fact questioned the plausibility of unequal nucleoprotein levels in nuclei having equal DNA content. The inherent inadequacies of the photometric technique at the time, and the possibility that unknown contamination from tapetal nuclei may have affected the quantitative estimates, leave the results open to doubt. Bryan was well aware of this, and stressed that the data obtained could not be regarded as definitive. Perhaps the most important point arising from this is that one should not expect all pollen to behave in the same way. In some species DNA replication may occur simultaneously in both nuclei; in others it may be staggered.

Taylor and McMaster (1954) used autoradiography and microspectrophotometry to determine phosphorus incorporation into DNA, and from this obtained reputedly quantitative data on changes in the DNA content of pollen during anther development in *Lilium longiflorum*. Their purpose was to test the hypothesis that DNA incorporates phosphorus only when the amount of DNA per cell is increasing. Their technique permitted the simultaneous estimation of ^{32}P incorporated and the amount of DNA per nucleus of microspores at equivalent stages of development. Autoradiographs showed that incorporation of ^{32}P into DNA occurred during three separate interphases: before pollen mother cell meiosis, microspore mitosis, and generative cell mitosis, in three "S" phases in fact. Feulgen photometry indicated within its limits of accuracy that the amount of DNA in each nucleus was constant at all stages of development, except for the "S"

phase periods, when it doubled, and during nuclear division, when the expected halving occurred. Moses and Taylor (1955) also used ^{32}P-autoradiography and Feulgen microspectrophotometry to investigate DNA synthesis during microsporogenesis in *Tradescantia paludosa*. Their "class values" for DNA support the work of Swift (1950), but conflict to some extent with Bryan's values. The autoradiographic data did not permit them to decide conclusively whether ^{32}P incorporation preceded or accompanied DNA synthesis. One important point raised by Moses and Taylor was the possibility of variation from species to species, and even from organism to organism, in the precise time of DNA synthesis. They found that synthesis in *Tradescantia paludosa* was not necessarily fixed to a clearly defined period, observing that it occurred in early prophase of meiosis, in late interphase preceding microspore mitosis, and in mid-interphase preceding generative cell mitosis. It has been known for some time that the length of the various stages of nuclear division tends to vary under different environmental and experimental conditions, and it would therefore seem rather futile to attempt to assign a fixed time in the cycle to DNA synthesis, as some workers have done (Moses and Taylor refer to Pasteels and Lison, 1950, and Thoday, 1954, in this respect).

During the fifties, the general consensus among biologists working on DNA turnover was that synthesis only occurred in cells preparing to divide. Later studies, however, gave evidence of DNA synthesis in non-dividing cells. Working with tube (vegetative) nuclei of germinating pine pollen, Stanley and Young (1962) used autoradiography to investigate the incorporation of labelled nucleosides (thymidine -^{3}H) into DNA. From their data it would seem that DNA turnover was in fact taking place in the vegetative nucleus, although the authors themselves admitted uncertainty as to whether end-group fixation or short nucleotide synthesis may have occurred rather than actual incorporation. They

questioned the general phenomenon of DNA synthesis in a non-dividing cell, suggesting two possible reasons for such a synthesis:

i) the vegetative nucleus, being derived from the same parent cell as the generative nucleus, may retain totipotency and therefore not only replicate DNA but also divide under certain conditions.

ii) the large amounts of enzymes produced by the rapidly growing pollen would require a considerable amount of mRNA, and possibly extra DNA template would be needed to cope with the increased demands.

These findings lead to speculation as to whether or not Stanley and Young were observing the synthesis of "metabolic" DNA. This term was coined by Pelc, and describes a DNA fraction which can be manufactured or lost without affecting the basic genetic complement of the cell. Pelc (1972) suggests that this DNA fraction comprises reproductions of the cistrons that are active in a cell at particular stages of development, and that these copies carry out the "metabolic" functions of the genes, e.g. RNA transcription. They deteriorate while in use, and are replaced by further synthesis. Pelc puts forward the hypothesis that, at least in eucaryotes, the production of extra copies of appropriate cistrons is the first step to follow activation of a gene. In this way, the "hereditary" DNA is protected from the repeated risk of damage occurring while in the single-stranded state. Roels (1966) has reviewed photometric investigations up to the early sixties which suggested the presence of "metabolic" DNA, but the information obtained from these has been superseded by more recent autoradiographic data, particularly that of Pelc and La Cour (1959) with differentiating root cells of *Vicia faba*, Sampson and Davies (1966) also with *Vicia*, Owen (1963) and Owen and McPherson (1963) with osteocytes, Pelc and Viola-Magni (1969) using adrenal medulla cells of rats, and Lima-de-Faria *et al.* (1968) with insect oögonia. Synthesis of "metabolic" DNA appears to be common during cellular differentiation, and losses have

been observed during periods of high metabolic activity. Stanley and Young may well have observed the incorporation of precursors into "metabolic" DNA in the vegetative nucleus in *Pinus*. In a later paper (Young and Stanley, 1963) they indicate an awareness of certain similiarities with events described in other organisms, referring to the work of Pelc (1959) with seminal vesicle cells of mouse.

Steffensen (1966) studied RNA synthesis in *Lilium longiflorum* pollen and observed that major syntheses occurred just before pollen grain mitosis in G_2 and after this division. Column chromatography indicated that most of the RNA synthesized at these times was of the ribosomal type. Steffensen was not able to detect the presence of nucleoli and rRNA in germinating pollen of *Lilium*, although active ribosomes have been seen in tubes of other species (see p. 71). Mascarenhas (1966a) demonstrated the incorporation of labelled nucleosides into unspecified RNA by both the vegetative and generative nuclei in germinating pollen of *Tradescantia paludosa*, substantiating the evidence of Young and Stanley (1963). Treatment with actinomycin-D failed to inhibit germination and initial elongation of pollen tubes, implying that the requisite RNA for these processes must have been synthesized before anthesis, and must therefore be of the "long lived messenger" type of RNA. Actinomycin-D inhibited subsequent RNA synthesis, preventing further tube elongation and generative cell mitosis. Moss (1967) and Moss and Heslop-Harrison (1967) undertook cytochemical studies of DNA, RNA and protein levels in the developing anther and spore tissue of *Zea mays*, using spectrophotometric techniques. They intended to investigate the possibility that the anther tapetum supplied nucleic acids to the developing microspores. Their data for microspore DNA content and timing of synthesis substantiated the evidence of Taylor and McMaster (1954), but did not support the hypothesis that tapetal nuclei provide discrete DNA, RNA and protein for the sporogenous

tissue or the microspores. However, it was considered possible that the tapetal cells supplied soluble DNA and RNA precursors to the sporogenous tissue during early anther development, from degeneration of tapetal protoplasts. It is now recognised that microspores have the capacity to synthesize their own precursors, and the rôle of the tapetum is believed to be little more than that of a transport tissue for basic materials.

Improved techniques have enabled workers in the last few years to obtain more reliable data on nucleic acid synthesis in pollen. Mascarenhas and Bell (1969, 1970) have provided further evidence that extensive rRNA synthesis ceases well before anthesis in *Tradescantia* pollen, and Mascarenhas (1971a, b) has also produced evidence for absence of transfer-RNA synthesis after the obset of germination. The function of small molecular weight RNA synthesized in pollen tubes is still under investigation (Mascarenhas and Goralnick, preliminary report 1971). Mascarenhas and LaFountain (1972) have developed a method of separating vegetative and generative nuclei, and have used ^{32}P-autoradiography to demonstrate qualitative and quantitative differences in the RNA's synthesized by the two nuclei. Although the separation method has not yet been widely applied, results to date indicate it to be a promising new approach. Linskens, van der Donk and Schrauwen (1971) studied RNA synthesis during pollen germination in *Petunia*, using ^{14}C-orotic acid, and found that germination and early tube growth were under the control of reactivated long-lived RNA carried by the pollen grain, cf. evidence of Mascarenhas (1966a) and Young and Stanley (1963). A minor synthesis of RNA occurred during germination, but its precise type and function were not known. Sauter (1971a) has stressed the importance of understanding the limitations of techniques currently employed in the analysis of nucleic acids, in the light of somewhat contradictory data. Nevertheless, he feels that there is general agreement on several aspects of phasing of

nucleic acid and nucleoprotein synthesis during pollen development. In discussing the meiotic period he refers to increasing evidence of a "late" replication of DNA occurring during prophase 1, which appears to be involved in the subsequent pairing of homologous chromosomes and in chiasma formation. This late-replicating DNA may be "satellite" (highly repetitive) DNA, a nucleic acid species studied intensively since its detection during the early sixties in CsCl density gradient analyses. Specific physical properties suggest that it consists of large numbers of short repeated polynucleotide sequences, and it appears to be localised in homologous chromosomes in regions of "constitutive heterochromatin". Blocks of repetitive sequences are found on either side of centromeres and reputedly at the telomeres, at the nucleolar organising region, and occasionally in other areas of chromosomes. Observations suggest that "satellite" DNA represents parts of chromosomes which are not transcribed into RNA for protein synthesis, but which have structural and protective functions, including those referred to by Sauter (see Yunis and Yasmineh, 1971). Sauter's own cytochemical work with vegetative and generative nuclei in *Paeonia* pollen (reviewed, 1971b) tentatively supports the theory that histones, the basic proteins associated with nucleic acids in eucaryotes, may have some regulatory rôle in the activation and deactivation of genes. He found that chromatin in the active vegetative nucleus was relatively poor in histones, whereas the heterochromatic generative nucleus contained greater amounts of histones. However, this is almost certainly overstating the case; the rôle of histones is probably only a gross one. It is true that active chromatin is less rich in basic proteins than heterochromatin, but it is widely agreed that histones could not provide the necessary specificity to operate any kind of fine control.

(ii) Amino acids and proteins

Aqueous and saline extractions of pollen show considerable complexity. Among the compounds present are free amino acids, polypeptides, and proteins that are either free or linked to carbohydrates or pigments. Most of the early work on pollen proteins and amino acids was carried out by people not directly involved in botanical research: apiarists have attempted to analyse the amino acid composition of various types of pollen, in the search for satisfactory substitutes for use in bee nutrition, and allergists have been concerned with the isolation of pollen allergens for diagnosis and treatment of pollen sensitivity reactions. In the past decade there has been a growth of interest in the immunological aspects of pistil-pollen incompatibility, and this field is still under intensive investigation.

The development of suitable chromatographic techniques has facilitated the qualitative analysis of amino acids in pollen. It has been found that all the essential amino acids are present, either in the free state or bound in protein (Stanley, 1971). Levels of free amino acids fluctuate during pollen development, and it has been shown that long-term storage of mature pollen causes a marked decrease in unbound amino acid content in many species. Total crude protein percentages vary considerably among the pollen types studied (11%-30% of dry weight) but the ratios of essential amino acids bound in the protein are similar. Efforts to establish free amino acid content of pollen as a taxonomic indicator have been singularly unsuccessful, as have attempts to relate this content to growth potential (Stanley, 1971).

Investigations have shown that most of the labile proteins in pollen are associated with the wall (Knox and Heslop-Harrison, 1970), and it is these that are believed to be implicated in allergenicity and

incompatibility reactions. Knox, Heslop-Harrison and Reed (1970) used immunofluorescence techniques to localise antigens in the pollen walls of *Gladiolus gandavensis* and *Ambrosia trifida*. Earlier cytochemical tests had indicated that antigenic substances may be incorporated into the walls, and Knox *et al.* were able to substantiate this with their own results. The intine in particular was found to be the site of heavy labelling in the fluorescence micrographs, indicating the presence of considerable amounts of antigen. However, proteins which were at the same time inevitably extracted from the pollen protoplast during the leaching process, did not appear to be significantly allergenic.

Studies on the mechanisms of pistil-pollen incompatibility are being carried out by many workers, building on the important investigations of Lewis's group in London during the fifties and sixties. The techniques of electrophoresis and immunofluorescence (see Hagman, 1964) have been applied to the incompatibility problem with considerable success. The recent work of Knox *et al.* (1972) with two species of poplar has provided experimental support for the hypothesis that "self-recognition factors", protein in nature, are involved in certain types of incompatibility mechanisms. These proteins, like those involved in allergenicity, appear to be localised in the intine. They are released in the presence of moisture and diffuse through the exine and germination apertures. Their precise rôle in the failure of pollen to bring about fertilisation, has yet to be determined. A general discussion of incompatibility will be included in the section on pollen morphogenesis.

The study of pollen enzymes has been of considerable importance to the field of enzymology in general. As early as 1894, Green reported enzyme activity in pollen preparations. He tested pollen of thirteen species, and found that the extracts liquefied starch paste. Pollen of

five other species was found to release invertase into a sucrose solution. Elser and Ganzmüller (1930) investigated the occurrence of saccharase, catalase and amylase in three species, and Sosa-Bourdouil (1939) also published a review of saccharase and amylase activity in pollen of several species. Von Euler *et al.* (1945, 1948), and von Euler and von Euler (1948) studied cozymase and catalase in various pollen types, and the activity of succinic and lactic acid dehydrogenases in *Salix* pollen. Important work on pollen respiration was carried out during the early forties by Okonuki (1942, 1943) who determined the content and activity of certain respiratory enzymes in nine species. Phosphatase activity in pollen was studied by Haeckel (1951) who found it comparable to that in seeds of the same plants. Nakamura and Becker (1951) obtained purified phosphatases from *Ambrosia* pollen and investigated the kinetics of these enzymes, and Palumbo (1953) made an extensive study of levels of acid and alkaline phosphatases, succinic dehydrogenase and adenosine triphosphatase in developing pollen of *Tradescantia paludosa* and *Lilium longiflorum*.

In the early sixties Tsinger and Petrovskaya-Baranova (1961) identified the intine as the location of many wall-bound enzymes. Since then many papers have been published on the identification of these enzymes and their rôle in germination, early pollen tube nutrition and penetration of the stigma. Knox and Heslop-Harrison (1969) used cytochemical techniques and electron microscopy to localise and identify various hydrolytic enzymes in the pollen walls of ten species of higher plants, detecting acid and alkaline phosphatases, ribonuclease, esterase and amylase. They believe the enzymes to be products of the microspore, incorporated during intine development, and discount the possibility that the tapetum is the source of these enzymes. In their more detailed investigations (1970) they studied pollen of fifty Angiosperms, one Gymnosperm, and the spores of a

species of *Equisetum* and two ferns. Enzyme activity (acid phosphatase, ribonuclease, esterase, amylase and protease) was detected in the walls in all cases except the ferns. Their statement on the relationship between enzyme localisation and wall ontogeny is of interest:

"Developmental study showed that the enzymes are incorporated in the intine during the early period of wall growth following the release of the spores from the meiotic tetrads. During this period, stratified ribosomal endoplasmic reticulum lies adjacent to the inner spore wall over the areas of incorporation. In *Cosmos bipinnatus*, a composite, the material is incorporated as ribbons or leaflets, which interleave with cellulose lamellae. In other species the wall protein may takethe form of granules, tubules, or vesicles, embedded in the intine cellulose. At maturity the intine is separated from the spore cytoplasm by an intact plasmalemma, so the wall enzymes are to be regarded as being extracellular."

Other work on pollen enzymes by Knox and Heslop-Harrison has included a study of acid phosphatase in the intine of *Crocus vernus* (1971a), and an investigation of the relationship between cytoplasmic RNA levels and lysosome enzymes (particularly acid phosphatase and ribonuclease) during meiotic prophase in *Cosmos* (Knox, Dickinson and Heslop-Harrison, 1970).

Brewbaker (1971) has produced a review of work on pollen enzyme identification. He states that most plant tissue enzymes have been detected in pollen or pollen tubes, with the exception, under normal circumstances, of catechol oxidases, enzymes associated with plastids and plant pigments (although some pollens are in fact pigmented), maltase, lipase, betagluconuridase, arylsulphatase, para-diphenyloxidase, "zymase", and several of the group of pyrophosphorylases. Gel electrophoresis has shown that pollen has characteristic isoenzymes in each category of activity, differing from those in the seed or sporophyte

tissues. Stanley and Search (1971) studied short-term elution products of germinating pollen and reported on the proteins identified, which included the enzymes cellulase and pectinase. Dickinson and Davies (1971a) investigated the rôle of nucleoside diphosphate kinase in rapid growth of plant tissues, using pollen of *Lilium longiflorum*. The reaction catalysed by this enzyme converts ATP produced by mitochondria to CTP, UTP or GTP, which are essential in many metabolic processes; the enzyme is therefore important in high-energy transfer reactions. Dickinson and Davies found that the greater part of the enzyme fraction was soluble, only about 4% being associated with mitochondria. They suggest that a high level of the soluble enzyme may be characteristic of rapidly growing plant tissues. Larson and Lonergan (1972) have described a successful pollen enzyme extraction method which uses only distilled water. They extracted glucosyltransferase from pollen of *Zea mays*, intending to purify the enzyme and use it to study control mechanisms involved in the biosynthesis of anthocyanin pigments. The fact that the pollen itself lacks such pigments stimulates speculation about the rôle of this enzyme in pollen metabolism - presumably it transfers glucosyl moieties to more than one acceptor, and it would therefore be involved in the metabolism of several types of compounds other than anthocyanins.

It is possible that the pollen grain at anthesis is already "programmed" with m-, r-, and tRNA and ribosomes needed for appropriate enzyme synthesis during germination. Another possibility is that the pollen grain contains certain enzymes in inactive form at anthesis, and they become activated upon germination. However, it seems likely that enzyme protein synthesis may in fact occur *de novo* in the pollen tube (Mepham, personal communication), although it has yet to be demonstrated. Further consideration will be given to the activity of tube enzymes in the section on pollen morphogenesis.

(iii) Carbohydrates

Among the major components of pollen, carbohydrates tend to be the most variable within and between species. At anthesis, corn pollen, for example, may contain more than twice the amount of carbohydrate (36-40% of dry weight) than the pollen of other Angiosperms at the same stage, while most Gymnosperms appear to be relatively deficient. The carbohydrates occur as free soluble sugars in the cytoplasm and as insoluble polysaccharides such as the structural pectins, celluloses and callose. Some types of pollen also contain starch. It has been shown that pollen is able to metabolise many types of sugars which are not present in the cytoplasm. *Pinus ponderosa* pollen, for example, has the capacity to produce enzymes to metabolise a wide variety of sugars from the external environment, an important aspect of tube growth.

The more common sugars detected in the pollen of various species include sucrose, raffinose, stachyose, rhamnose, glucose, fructose, arabinose, xylose and galactose. The rarer sugars lactose, turanose and nigerose have also been found, possibly the result of fragmentation of polysaccharides. There is some variation in the type of sugar that predominates: in many Gymnosperm pollens, sucrose represents over 93% of the free sugar content, whereas in Angiosperms sucrose varies from 20-50% of the total soluble sugars (Stanley, 1971). Free sugar content also varies with methods of handling and storage time, as might be expected. Bee-collected pollen, which is kept moist by bee secretions and nectar, contains large amounts of reducing sugars; mechanically collected pollen, which is drier, contains more non-reducing sugars and is relatively deficient in reducing types. Soluble sugar content generally diminishes with increasing storage time, possibly due to respiration.

In vitro investigations have been made of the stimulation and inhibition of pollen germination by different sugar solutions. Studies such as those of Hrabětová and Tupý (1964) have provided comparative data for many species, and show that the sugar substrate giving best growth response is often the endogenous sugar present in greatest quantity, i.e. specific growth effects are closely linked to the internal metabolism of the germinating pollen. In this respect it is important that the osmotic balance is right - plasmolysis of the pollen grain must obviously be avoided if germination is to occur.

Starch content varies considerably, not only among different pollen types but at different stages of development. Mature *Typha latifolia* pollen has about 13% of its dry weight in the form of starch. *Zea mays* pollen, depending on the variety and handling methods, contains 12-30% starch, whereas pollen of *Pinus sabiniana* contains only 2.2% starch when shed (Stanley, 1971). During germination, some pollens absorb exogenous sugars and convert them to starch for storage as a reserve material. This process has been demonstrated for several species. The probable utilisation of endogenous starch material in the production of the intine has been reported by Mepham and Lane (1970) for *Tradescantia*.

Cellulose, the polysaccharide present in many plant cell walls, is the main component of pollen intines. It has a characteristic microfibrillar structure and in at least some types of pollen the layers of cellulose alternate with layers of protein (Knox and Heslop-Harrison, 1971b). The pollen tube wall, which is contiguous with the intine, is also partially cellulosic.

Pectins, polymers of galacturonic acid-methyl esters, are associated with cellulose in the intine and tube wall. Pectins and hemicelluloses (lower D.P. polysaccharides, derived from glucose and other

sugars such as arabinose, xylose, mannose or galactose) are the main materials deposited at the growing tip (Stanley, 1971). Van der Woude *et al.* (1971) have presented evidence which suggests that pectin precursors and other polysaccharides are transported in dictyosome-derived vesicles to the tube apex, where they are added to the wall by fusion of the vesicle membrane with the plasmalemma. This method of wall material transport, which has been described by a number of investigators for different species, is discussed in more detail in Part IIC. Pectin synthesis in pollen tube membranes has been studied by Stanley and Loewus (1964). They reported that synthesis of basic units of pectin was boron-dependent, the boron possibly acting as an enzyme co-factor in the process.

Callose, a beta-1, 3-glucan with no microfibrillar structure, is an important and relatively widespread plant carbohydrate. It is associated with pollen at different developmental stages: it covers the naked pollen mother cells after wall loss, forms the initial walls of the tetrads, and the transient wall of the generative cell, where its reappearance is an early sign of germination in *Tradescantia* (Mepham, personal communication). Callose is also one of the components of the pollen tube wall, and a callose "plug" forms at the upper end of the tube, separating the cytoplasm of the tube from the pollen grain. Small breaks which may occur in the tube wall during germination are also sealed off with callose. Its ability to be formed and destroyed rapidly makes it an extremely useful material.

It is only in recent years that some insight has been gained into the chemical nature and physiological functions of callose. In 1957, when Currier published his extensive study of "callose substance" in different plant cells, little was known about the material. In his review

of previous studies, Currier cited the early work of Mangin (1889), who was apparently the first to record the presence of callose in pollen grains, tube walls, and in tube plugs. Using fluorescence microscopy, Currier confirmed and extended previous visible staining localisation of callose in various cell types, including pollen. His observations enabled him to make tentative statements about the nature and functions of callose. He suggested that several kinds of callose may exist, and regarded the substance as "a characteristic material in the same generic sense that is applied to starch and cellulose", developing in a variety of circumstances. He stressed that callose was not necessarily an "abnormal" material, in the sense of a response to plant tissue injury. In many locations it appears to be a normal cell wall constituent, especially in the pollen tube. He also suggested that callose may be an intermediary substance in the synthesis and break-down of cell wall materials.

Heslop-Harrison (1966a) regards the callose tetrads walls as an important factor in the expression of genetic individuality in the microspores:

"..it may be surmised that up to the meiotic divisions themselves there is a requirement for a substantial sharing of materials between meiocytes, but that thereafter the need is for isolation of the products of meiosis, from each other, and from the enveloping parental tissue - an isolation established by the total investment of the spores in the callose wall of the tetrad." This implies that callose is virtually impermeable, a property which has frequently been ascribed to this polysaccharide. However, in a paper published a little later McKenzie and Heslop-Harrison (1967) suggest that the permeability properties of callose may not be the main factor in preventing exchange between the microspores and their environment. The importance of the callose may be its rôle as a

temporary physical barrier to growth of the microspore, thus controlling movement of materials at a vulnerable stage of development. They felt it was unlikely that an apparently unstructured polysaccharide material should have such marked impermeability. Walker (1957) and Mepham (1970) have also demonstrated that callose may not be as impenetrable as is generally believed.

Other studies of the occurrence of callose in pollen include those of Waterkeyn (1962) with special mother cell walls in *Helleborus* and *Tradescantia*; the work of Górska-Brylass (1967a, 1967b, 1968, 1970), and of Waterkeyn and Bienfait (1970, 1971). Górska-Brylass investigated the temporary wall around the generative cell in five types of Angiosperm pollen (1967a, b), detecting the presence of callose in all cases by visible staining methods and fluorescence microscopy. She regards the presence of this wall as an essential factor in the individual differentiation of the generative and vegetative cells. This investigation was extended to include four Gymnosperm species (1968), and it was found that thin layers of callose separated the prothallial cells, the generative cell, and later the stalk and body cells, providing further support for the suggestion that callose walls play an important rôle in differentiation within the male gametophyte. In a later paper (1970) Górska-Brylass summarises her previous work and discusses in detail the significance of the transitory callose wall. Like Currier, she regards the formation of callose walls in pollen as a "perfectly normal development", and rejects the pathological derivation theory in this particular instance. Waterkeyn and Bienfait have investigated the importance of the callose mother cell wall to early exine patterning. They suggest that the characteristic pattern on the inside of each tetrad quadrant may function as a "negative template" for exine deposition. Although the callose pattern is not the exact inverse of that subsequently formed by the exine, it may well be regarded as "a kind of mould for the primexine matrix", as the authors suggest.

(iv) Lipids

Not a great deal has been reported on pollen lipids. Early investigations showed that lipid content varies widely among species, standard ether extractions producing values from 1 - 20% of dry weight, (Lundén, 1956). Fatty acids are usually found in the form of esters linked to sugars, phosphates and other groups. Stanley (1971) reports that Standifer (1966) found a high fatty acid content in numerous pollen types, the most common being linoleic, linolenic and palmitic acids. Ching and Ching (1962) detected large quantities of linolenic acid in conifer pollen. Hoeberichts and Linskens (1968) have published a study of lipids in ungerminated pollen of *Petunia*. Shaw (1971) suggests that certain types of fatty acids may be involved in sporopollenin synthesis. Analyses of the non-saponifiable fraction of various pollen lipids have indicated the presence of hydrocarbons, higher alcohols, and sterols, some of which have been isolated and identified.

There has been some dispute about the origin of lipid materials which are found in the exine. Some workers regard these lipids as exclusive products of the tapteum. Others, such as Mepham and Lane (1968, 1969a), have provided evidence for the exudation of lipids by the pollen protoplast, and claim that these can be distinguished from the lipid material originating in the tapetal periplasmodium.

(v) Vitamins

The vitamin content of pollen has been assayed for many species. Since 1918, when Dutcher published his study of the curative effect of corn pollen, honey and nectar on avian polyneuritis, many qualitative and quantitative analyses have been made and useful comparative data is available. Most pollens appear to be consistently rich in water-soluble

B-group vitamins but low in the fat-soluble vitamins. A table based on information from Lundén (1956) and Stanley (1971) is presented (p. 41) for comparison of vitamin content in a range of species.

The vitamin level required for germination and pollen tube growth is present in the pollen grain at maturity, or in the pistil tissues penetrated by the tube. The vitamins frequently appear to function as enzyme co-factors (Stanley, 1971). The occurrence of inositols in pollen has a particular significance. Myoinositol is often found as a free compound, also occurring as phosphoinositol. In addition to its function as an enzyme co-factor, inositol is incorporated into pectin in the growing pollen tube wall (Stanley and Loewus, 1964, and Kroh and Loewus, 1968).

TABLE 1. VITAMIN CONTENT OF POLLEN

Vitamin	Assays in µg/g of pollen								
	Pinus Montana	Alnus incana	Zea Mays	Dactylifera palmae	Various species	Various species	Various species	Various species	Various species
thiamine	-	-	-	4.91-5.63	6.31-10.8	-	-	6.0	1.4-7.9
riboflavin	5.6	12.1	5.7	6.38-6.59	16.3-19.2	-	-	16.7	-
nicotinic acid	79.8	82.3	40.7	79.3-87.5	132-210	-	-	100	-
pyridoxine	3.1	6.8	5.9	-	-	-	-	9	-
pantothenic acid	7.8	5	14.2	-	16-27.6	-	22-51	27	-
biotin	0.62	0.69	0.52	-	-	-	-	0.25	-
folic acid	-	-	-	-	-	3.4-6.8	-	-	-
inositol	range: 3 -	30 mg/g pollen		-	-	-	-	-	-
ascorbic acid	-	-	-	530-640	152-176	360-590	-	-	-
vitamin A	-	-	-	0	0	-	-	-	-
vitamin D	-	-	-	-	(0.2-0.6 (IU/g fat	-	-	-	-
vitamin E	-	-	-	0	(0.32 (mg/g fat	-	-	-	-
vitamin K	-	-	-	-	0	-	-	-	-

1) Data from Nielsen, Grommer and Lundén (1955).

2) Data from Vivino and Palmer. Main sources: dandelion, plum, apple, clover, goldenrod, aster. (1944).

3) Data from Weygand and Hoffman (1950). 8 species.

4) Data from Pearson (1942). Various species.

5) Data from Kitzes, Schnette and Elvehjem (1943). Mixed pollen.

6) Data from Sagromsky (1947). various species.

(vi) Growth regulation substances

Two groups of growth regulation compounds have been detected in germinating pollen: acknowledged plant growth substances such as IAA, auxin inhibitors, gibberellins and kinins, and the animal hormonal substances, the steroids. It has been known for some time that pollen of some species synthesizes or induces a substance which inhibits the formation of the peduncle abscission layer in pollinated flowers. One of the first sources of auxin was orchid pollen (Laibach, 1932), and Michalski (1967), using chomatographic separation, has found IAA, auxin inhibitors and gibberellins in Gymnosperm (pine) pollen. The exact rôle of these substances in germination and development is not well understood. Some have been extracted from stylar tissues as well as pollen tubes, but the complex nature of pollen -style interactions makes meaningful observation exceptionally difficult. Experiments designed to demonstrate hormonal stimulation and inhibition of tube growth in artificial media do not necessarily give a valid picture of growth regulation *in vivo*. Advances in the understanding of plant hormone function in general will hopefully help to define the rôle of these substances in pollen germination.

Botanists have long been interested in the effects of steroids on growth of plant tissues. Oestrogenic substances were first detected in *Salix* pollen by Skarzynski (1933) and in *Phoenix dactylifera* pollen by Hassan & Aboul Wafa (1947) and Ridi & Aboul Wafa (1947). Since then, several steroids have been isolated from pollen of various species. Bennett *et al.* (1966) investigated the sterol fraction of *Phoenix* pollen, using thin layer chromatography to separate oestrone, confirming the identity of the compound by infra-red spectrometry. Cholesterol, the precursor of steroid hormones in animals, was also isolated. The occurrence of both cholesterol and oestrone in pollen suggests that, in

plants as well as animals, these substances may be biosynthetically related. Bennett *et al.* stressed however that the species distribution and physiological activity of steroidal substances in plants remained open to intensive investigation. Standifer *et al.* (1968) undertook a mass-spectrographic survey of sterol fractions from pollen of fifteen species, and identified the three main compounds as cholesterol, 24-methylene cholesterol, and beta-sitosterol. There was no evidence of a taxonomic relationship between the pollen sterols and plant families. Stimulation of pollen tube growth by certain steroids has been demonstrated by several workers. Stanley (1971) reports that cholesterol at fairly low concentrations stimulates germination of pear pollen. Matsubara (1971) tested several steroid hormones and phytosterols for *in vitro* growth response in germinating pollen of *Chrysanthemum leucanthemum*, obtaining a positive response with some compounds from each group. As with the auxins, kinins and gibberellins, the function of steroids in pollen metabolism is poorly understood.

(vii) Inorganic requirements

Modern techniques such as autoradiography and x-ray microprobe analysis have greatly facilitated estimation and localisation of inorganic substances in pollen. Early reports were based largely on information obtained from pollen ashed in concentrated acid, and comparative data may often be misleading. For example, the possibility of volatilisation during the ashing is an important consideration for elements such as boron and chlorine. One of the earliest mineral analyses of pollen was made by von Planta (1886) for *Pinus sylvestris*. Anderson and Kulp (1922) analysed *Zea mays* pollen, adding magnesium and chlorine to the list of minerals already found in various species, which included iron, calcium, potassium and phosphorus. Lundén (1956) has briefly summarised the data available

up to the mid-fifties. It should be noted that the wide range of values obtained for a particular element may be due not only to intrinsic differences in inorganic requirements among the species analysed, but also to widely varying mineral levels in different environments. As well as those mentioned above, the elements silicon, copper, manganese, sulphur, aluminium, nickel, titanium and zinc have been found in pollen in different chemical forms.

The specific mineral requirements of pollen are quite marked. Some elements, such as potassium, are maintained at levels similar to those found in leaves and roots, while others such as sulphur and phosphorus occur in levels five to ten times higher, on a percentage of dry weight basis, than in other tissues of the plant (Stanley, 1971). Boron is of great significance in the germination of many types of pollen, (Schmucker, 1932; Visser, 1955; Vasil, 1964; Fähnrich, 1964; Stanley and Loewus, 1964; Stanley, 1971). Numerous hypotheses have been put forward for the functions of boron in pollen growth and development. The element is believed to play an important rôle in the absorption and mobilisation of sugars during germination; Vasil (1964) refers to the work of Gauch and Duggar (1953), Linskens (1955), O'Kelley (1957) and Tupý (1960) in this respect. Stanley and Loewus (1964) report that boron is important in the synthesis of pectins for the pollen tube wall. Schmucker (1932, 1933, 1935) and Gauch and Duggar (1954) have presented evidence for a relationship between boron deficiency and bursting of pollen grains and tubes due to excessively rapid water intake. Boron may certainly be involved in these phenomena, and may function in ways not yet suspected. The extent of its influence is not clear. Vasil's final statement in his paper on boron and pollen tube growth (1964) is still valid: "Although more than a dozen different and often conflicting roles have been postulated thus far to explain the role of boron in plant growth and

metabolism, it is still far from clear and the explanation of its stimulatory effect on pollen germination and pollen tube growth thus must await further intensive work. In fact, boron presents a stubborn challenge to the plant physiologists to clarify and explain its exact mechanism of action and function in the living plant."

The function of the calcium cation in pollen germination has been investigated both as an individual factor and in conjunction with boron and other substances. In a comprehensive paper on cellular chemotropism Rosen (1962) reviews earlier work, including the observations of Sachs (1882), Brink (1924), Beck and Joly (1941a, b), Iwanami (1953), Miki (1954), and his own studies (1959, 1961) - all of which provided evidence for the presence of a chemotropic agent in the tissues of the gynoecium in many plants. Mascarenhas and Machlis developed a highly successful bioassay method for detecting chemotropic responses in pollen tubes (1962a), and were able to show that tubes of *Antirrhinum majus* exhibited positive tropism towards a calcium source (1962b). Several different inorganic and organic calcium compounds were tested by them, and were found to be successful in inducing the response. A fairly high level of calcium was detected in the gynoecium tissues of *Antirrhinum*, which also stimulated chemotropic activity, whereas the petals and stamens, found to be low in calcium, did not induce a response. Mascarenhas and Machlis suggested that the reported ability of various other substances to induce chemotropism (egg albumin, diastase, compressed yeast, sodium malate) could perhaps be explained by the undetected presence of sufficiently high levels of calcium in these preparations. However, the universality of calcium in pollen tube chemotropism was questioned by Rosen (1964), who confirmed the calcium response in *Antirrhinum* but failed to obtain a similar response with *Lilium* pollen. He was therefore less inclined to accept the suggestion that calcium may be the chemotropic agent for pollen tubes in

all flowering plants, but considered the possibility that other substances may block a potentially positive response under certain conditions. Brewbaker and Kwack (1964) found that calcium reduces or blocks the effect of a number of pollen tube growth inhibitors. They also showed that calcium is almost exclusively bound in tube wall pectins.

Further work by Mascarenhas and Machlis (1964) has supported their identification of calcium as a widely-distributed chemotropic agent involved in pollen tube growth. They state: "Calcium, which has been found to have a tropic effect in addition to a growth effect, appears to possess all the properties of the tropic substances for pollen tubes that have been reported in the literature." They were able to show that boron enhanced the chemotropic effect of calcium, but did not attempt to explain the phenomenon. Mascarenhas (1966b) studied the distribution of ionic calcium in the gynoecium of *Antirrhinum*. The low overall level of calcium and apparent lack of a concentration gradient in the stigma led him to suggest that some other chemotropic factor may be active in this region. The greatest concentration of ionic calcium was found in the placental cells and the cells lining the inner ovary wall. The concentration in the cells of the ovule, micropyle and embryo sac was found to be relatively low. Mascarenhas suggested that, in view of the reduced calcium level in these regions, some other chemotropic agent(s) may also be involved in the final stages of pollen tube growth. Glenk *et al.* (1971) were unable to demonstrate calcium-induced chemotropism in *Oenothera* pollen tubes, and agreed with Rosen that calcium was unlikely to be the universal factor in pollen chemotropism in higher plants.

De Bruyn (1966) studied *in vitro* germination of *Setaria sphacelata* pollen, observing the effects of different concentrations of calcium, magnesium, potassium and sodium at low, "optimal", and high

levels of boron. The monovalent cations potassium and sodium did not significantly affect germination or pollen tube growth at any level of boron. At high concentrations they displayed an inhibitory effect in some cases. Calcium at all concentrations was unable to stimulate germination and tube growth in the absence of boron, but tube growth was markedly increased by medium calcium concentrations at "optimal" boron levels. The inhibitory effect of high boron concentrations was counteracted by calcium. Lower levels of magnesium stimulated germination and tube growth at "optimal" levels of boron, and counteracted the inhibitory effect of high boron concentrations. A high magnesium level was strongly inhibitory. Sen and Saini (1969) investigated the elongation of *Lilium regale* pollen tubes under the influence of growth regulation substances and the cations of calcium, potassium and magnesium. The results of experiments with optimum concentrations of cations, and with White's nutrient solution*, are given below:

TABLE 2. Tube-lengths of pollen grown in inorganic cation solutions.

Solution	Tube-length x 20μ	average obtained from no. of experiments given in brackets
dist. water	22.4 ± 1.5	(60)
5 ppm Ca	39.9 ± 2.2	(30)
85 ppm K	37.8 ± 1.2	(20)
5 ppm Mg	40.0 ± 1.0	(20)
White's Soln	77.2 ± 2.8	(20)

*Composition:

$MgSO_4$	360 mg/litre	sucrose	20 g/l
$Ca(NO_3)_2$	200 mg/l	gylcine	3 mg/l
Na_2SO_4	200 mg/l	nicotinic acid	0.5 mg/l
KNO_3	80 mg/l	pyridoxine	0.1 mg/l
KCl	65 mg/l	thiamine	0.1 mg/l
$NaH_2PO_4.H_2O$	16.5 mg/l	$ZnSO_4$	1.5 mg/l
$Fe_2(SO_4)_3$	2.5 mg/l	H_3BO_3	1.5 mg/l
$MnSO_4$	360 mg/l	KI	0.75 mg/l

Rather more informative is the data obtained from experiments which tested the combined action of four growth regulation substances and the cations:

TABLE 3. Tube-lengths of pollen germinated in solutions containing different inorganic cations and growth regulators (x 20μ)

water	38.5 ± 1.6 (30)	42.0 ± 1.0 (30)	38.8 ± 1.5 (20)	48.0 ± 1.0 (30)
5 ppm Ca	40.0 ± 0.9 (20)	48.8 ± 0.8 (20)	42.0 ± 0.8 (20)	40.0 ± 1.0 (20)
85 ppm K	43.9 ± 1.6 (15)	50.0 ± 1.8 (15)	39.1 ± 1.3 (15)	37.5 ± 1.2 (15)
5 ppm Mg	43.4 ± 1.3 (15)	48.0 ± 1.8 (15)	39.3 ± 1.2 (15)	39.3 ± 1.4 (15)
White's Soln	88.0 ± 2.3 (10)	102.5 ± 3.1 (10)	95.0 ± 2.8 (10)	57.2 ± 1.9 (10)

Comparing tube-lengths in Tables 2 and 3, it will be seen that growth was stimulated in all IAA combinations except with calcium, where growth was negligible. All GA_3 combinations markedly stimulated growth. TIBA-K and TIBA-Mg growth stimulation was not significant, and TIBA-Ca stimulation was only small. Growth was substantial in water-TIBA and White's Solution-TIBA. ABA, in combination with the cations and White's Solution, appeared to have an inhibitory effect on tube elongation. This particular set of experiments gives only the barest information about the physiological effects of interaction between growth regulators and inorganic substances. Extensive testing of different combinations would be required before any definite relationships could be determined.

(viii) Pigments

Carotenoids and flavonoids are the main classes of pigments found in pollen. There is a wide range of colour gradation, from almost white through blue and grey to dark brown, but most pollens (about 80%, according to Möbius, 1923) are within the yellow range. The principal

carotenoids are alpha- and beta-carotene, lycopene, xanthophyll, and zeaxanthin, and small amounts of crocetin have been found (Lundén, 1956, and Stanley, 1971). Lundén refers to the early analytical works of Vivino and Palmer (1944), von Euler *et al.*(1945), Karrer (1950), and Tappi (1949-50), who identified and estimated carotenoids in the pollen of various species. Carotenes are mostly found in the lipid fractions of the exine and cytoplasm, and also occur in surface oils in some species. The main flavonoids of Angiosperm pollens appear to be quercetin, kaempferol, and isorhamnetin. Naringenin is prominent among Gymnosperms, with considerably smaller amounts of kaempferol and quercetin (Stanley, 1971). Isorhamnetin has apparently not yet been found in Gymnosperm pollen. Flavonoid glucosides can be isolated from some species after the carotenes have been removed, and flavones, which are water-soluble, can be removed without difficulty.

The function of pollen pigments has been a matter for much dispute. It has been suggested that some pigments may protect the gametophyte from genetic damage by their UV-screening action, but it is doubtful whether even anemophilous pollen would be in serious danger of UV radiation damage. It has also been suggested that different pigmentation in anemophilous and entomophilous pollen ensures the appropriate mode of distribution, but the validity of this hypothesis is also open to doubt. Certain pigments are believed to be important in fertilisation. Tsinger and Poddubnaya-Arnoldi (1954) found that carotenoids in orchid pollen tubes played some rôle in compatibility reactions. Samorodova-Bianki (1956) studied the relationship between carotenoid levels and fertility in the anthers of various plants, and observed that the normal accumulation of carotenes during microsporogenesis did not occur in sterile plants. The possible function of carotenoids as enzyme co-factors is suggested by *in vitro* studies in which carotenes stimulated pollen tube growth. Minaeva and Gorbaleva (1967) reported that

flavonoids also stimulated tube growth. Pollen pigments clearly have a number of possible functions, but their precise rôles have yet to be clarified.

C. Pollen Morphogenesis - A developmental study

Three major nuclear events occur during pollen development: the meiotic division giving rise to microspores; pollen grain mitosis, producing the generative and vegetative nuclei; and generative cell mitosis, which produces the sperm nuclei. For convenience, each of these will be discussed separately, with the accompanying and subsequent cytoplasmic changes.

The meiotic period

The transition from diploid sporophyte to male haploid gametophyte is achieved through the process of meiosis in the anther. The characteristic pattern of development of the gametophyte differs markedly from that of the sporophyte, and it would seem that the typically sporophytic parts of the haploid genome are in some way repressed during gametophyte development. The question is, therefore, what occurs during and immediately after meiosis that encourages the expression of the gametophytic part of the genome? During the past fifty years or so, many workers have studied the cycle of nuclear and cytoplasmic events which constitute meiosis in pollen mother cells. Light microscopy provided basic information on the behaviour of chromosomes and cytoplasmic organelles during meiotic divisions (Guilliermond, 1920, 1924; Py, 1932; Wagner, 1927; Frankel, 1937), but modern concepts of the meiotic process in different plants are largely based on more detailed information obtained from electron microscopy. The actual nuclear events are usually very similar, and some cytoplasmic similarities have emerged, but the universality of these is still disputable.

It should therefore be stressed that, contrary to the expectations (implicit or explicit) of numerous cytologists, a generalised picture of nuclear-cytoplasmic relations during meiosis in Angiosperms has not developed. At the present stage of knowledge the cytology of meiosis can only validly be discussed on the basis of observations in individual species.

Heslop-Harrison and co-workers have studied in detail the nuclear and cytoplasmic changes occurring during meiosis in several plants (Heslop-Harrison, 1966a, b; MacKenzie, Heslop-Harrison and Dickinson, 1967; MacKenzie and Heslop-Harrison, 1967, Dickinson and Heslop-Harrison, 1970a, b; Knox, Dickinson and Heslop-Harrison, 1970). Much of this work, and also that of Linskens (1966), Moens (1968), Walters (1968), Das (1965), Mather (1965), Hotta *et al.* (1963, 1966), and Maruyama (1968) is reviewed by Heslop-Harrison (1971a). In the introduction he states that "there must be some rather far-reaching cytoplasmic reorganisation during the meiotic period, to prevent the carrying over of extranuclear diplophase information and to permit the institution of a new environment favourable to the expression of gametophyte functions." He supports this statement with data from a number of species. The most striking changes involve ribosomes, nucleoli, mitochondria and plastids. Intercellular cytoplasmic connections also undergo marked changes. In *Lilium henryi* substantial quantities of what are described as free ribosomes and ribosomal ER are present at the beginning of Prophase 1 (leptotene). Extraction techniques and cytophotometry both show that the zygotene/pachytene interval is characterised by a sharp fall in ribosome numbers, although electron micrographs indicate that elimination is not complete. Heslop-Harrison regards the observed rise in lytic enzymes at this time as further evidence for destruction of ribosomes and breakdown of cytoplasmic RNA. Ribosome levels rise again

during Metaphase II- Telophase II, just before the microspore tetrads are complete. Studies with *Trillium erectum* (MacKenzie, Heslop-Harrison, and Dickinson, 1967), *Cosmos bipinnatus* (Knox, Dickinson and Heslop-Harrison, 1970) and *Paeonia tenuifolia* (Sauter and Marquart, 1967) tend to confirm the evidence obtained from *Lilium henryi*, and are in agreement with the earlier observations of Py (1932), who noted a loss in affinity of the prophase cytoplasm for basic dyes, and Painter (1943), who linked this with a fall in RNA levels.

Changes in ribosome numbers appear to be closely correlated with the meiotic nucleolar cycle. Nucleoli are small dense bodies associated with the nucleus, containing DNA, RNA, and protein, and appear to be involved in ribosome synthesis. They arise at specific chromosomal sites - the so-called nucleolar organiser regions - which appear as constrictions in mitotic chromosomes. In a karyotype these are found on specific chromosomes. At telophase, when nucleoli reform, each organiser may give rise to a small individual nucleolus, or they may all come together to form one large nucleolus. The latter case is probably due to preferential aggregation of "satellite" DNA (see p. 28), which is found on either side of each nucleolar organiser. The maximum number of nucleoli present in a cell at Prophase 1 is genetically determined, and varies from species to species, but as nucleoli may fuse the maximum number may seldom be seen. In cells with more than one, reduction in number may occur at the time of synapsis of homologous chromosomes. Lin (1955), while studying chromosomal control of nuclear composition, observed a volume increase in nucleoli after synapsis. Latter (*Lathyrus odoratus*, 1926), Frankel (*Fritillaria*, 1937), and Moens (*Lilium longiflorum*, 1968) reported a "flattening" of nucleoli towards one pole. By diplotene, however, normal spherical shape appeared to be restored. Loss of stainability for RNA and protein is noticeable in several species at the end of Prophase 1, and the pollen

mother cell nucleolus disappears by the time of formation of the Metaphase 1 plate. Frankel (1937) reported that the "parental" nucleolus does in fact persist throughout meiosis in *Fritillaria*. The autoradiographic studies of Das (*Zea mays*, 1965) have supported the general impression that RNA synthesis in nucleoli ceases after leptotene. In contrast, synthesis of chromosomal RNA increases during early Prophase 1, reaching its highest level in diplotene, but decreases again towards the end of the prophase. There are many reports of the appearance of nucleolus-like bodies in Telophase 1 and during the second division of meiosis. They have been described by numerous workers, including Latter (*Lathyrus*, 1926), Frankel (*Fritillaria*, 1937), Håkansson and Levan (*Pisum sativum*, 1942), Lindemann (*Bellevalia romana* and *Agapanthus umbellatus*, 1956), and Dickinson and Heslop-Harrison (*Lilium*, 1970a). In spite of their structural and cytochemical similarity to the "parental" nucleoli, the small "nucleoloids" described by the latter pair are apparently not formed at the established nucleolar-organiser regions of chromosomes - although bodies of this kind were observed in association with chromosomes during Anaphase 1 in *Lilium*. Similar bodies were seen in the Anaphase 11 - Telophase 11 interval. Biochemical extraction data for *Lilium henryi* suggests that a chromosome-associated synthesis of "ribosomal type" RNA occurs near the end of Prophase 1. Comparing this information with observed phenomena, Heslop-Harrison (1971) postulates that this synthesis may be concerned with the formation of the nucleoloids. *Lilium* gives evidence of a synthesis of ribosomal type RNA before the appearance of the Anaphase 11 - Telophase 11 nucleoloids, and data from the diplotene stage in *Zea mays* (Das, 1965) also seems to support the postulate.

There is some possibility that each nucleoloid is simply a mass of compacted ribosomes, or parts of ribosomes, released at the appropriate time to re-establish the ribosome level for post-meiotic

protein synthesis. This would appear to be the case at least in *Lilium henryi*. Evidence for a wider application of this process is found in meiosis in the oöcyte of certain amphibia (Gall, 1969), and it is possible that the mechanism described is one form of cytoplasmic "pre-programming" - if it is assumed the "message" is present in the ribosomes. Heslop-Harrison (1971a) suggests that the "late" replication of DNA reported by Gall in *Bufo americanus* and *Xenopus laevis*, and by Hotta *et al.* in Prophase 1 in *Lilium longiflorum* and *Trillium erectum* (1966) may in part be concerned with the subsequent production of ribosomal RNA for ribosome restoration, - although there is increasing evidence that late-replicating DNA is of the "satellite" type, which is apparently not involved in RNA transcription.

In line with the decline in ribosome numbers, Heslop-Harrison (1971a) reports marked changes in the membranes of the endoplasmic reticulum, between zygotene and Telophase 11 in *Lilium*. The normally plate-like profiles of sectioned ER are replaced by concentric groups of paired membranes, apparently part of spherical and not tubular or cylindrical structures, but the precise metamorphosis involved in this transition is not described. The membranes of these spheres lack ribosomes, but often enclose organelles such as plastids and mitochondria. This is referred to as the "period of compartmentation". The membranes gradually return to the more usual form as ribosomes are restored. At the diad stage, the cytoplasm in *Lilium* shows both plate-like and "compartmented" ER, but at tetrad dissolution only plate-like ER is seen. It remains to be seen whether this cycle of membrane changes is universal or only species-specific.

Several workers in recent years have studied the changes occurring in cytoplasmic organelles during microsporogenesis. In particular, mitochondria, plastids and the Golgi apparatus have been

investigated in some detail. The problem of multiplication of mitochondria and plastids should perhaps be considered first. There is still uncertainty about the genetic continuity of these organelles and the precise mode of reproduction. Bell and Mühlethaler (1964) produced evidence of loss of mitochondria in developing egg cells of the fern *Pteridium aquilinum*, followed by the formation of new ones from evaginations of the nuclear envelope. A similar method of *de novo* formation of plastids was later suggested by Bell *et al.* (1966), and again for mitochondria (Bell, 1972). (Note: The process of "nuclear blebbing" will be discussed in more detail in relation to intine formation, p. 62). Dickinson and Heslop-Harrison, however, (1970b) have published electron micrographs which suggest that plastids enter a division phase in the premeiotic period in *Lilium* and pass through a process of "de-differentiation and re-differentiation", regaining internal structure and starch from the tetrad stage on. Genetic continuity is implied in this sequence of events. In fact, Heslop-Harrison (1971a) states that, for *Lilium henryi*, "There clearly are no phases of elimination and subsequent restoration from the nuclear envelope as envisaged for the fern egg." As with so many ontogenetic processes it is highly likely that both hypotheses are equally valid, and apply to different plant types, or even to a given plant under different conditions. Investigation of these organelles during meiosis in a much wider range of species would be required to test the frequency and distribution of the two methods observed.

Mitochondria undergo a series of marked morphological changes during microsporogenesis. Maruyama (1968), building on the earlier work of Bal and De (1961), has used standard electron microscopy to demonstrate this cycle of *Tradescantia paludosa*. Before meiosis, mitochondria in the pollen mother cells appear to be elongated, (round in cross-section), up to 1.5μ in length and about 0.4μ in width. A few cristae can be

distinguished, but are not well developed. There may be a small number of vesicles or tubules. By pachytene the mitochondria are spherical - about 0.4μ in diameter - and appear to be without cristae, although some retain the tubular structures. As meiosis proceeds even the tubules decrease in number, and by the formation of the tetrads no more than three are found per mitochondrion. Similar changes have been described in *Gasteria verrucosa* by Willemse (1972), who was also able to show by statistical analysis that no increase in the number of mitochondria (or other organelles) occurs during meiosis in this plant. (Willemse had previously demonstrated the stability of meiotic organelle populations in *Pinus sylvestris*, 1971a, b). It should be mentioned that fixation methods can greatly influence the amount of detail seen in structurally complex organelles such as mitochondria. Although many workers other than Maruyama and Willemse have reported little internal structure in mitochondria during meiosis and early microspore development, others believe that improved fixation may reveal the presence of greater structural detail throughout the process.

Maruyama (1968) and Dickinson and Heslop-Harrison (1970b) have clarified some aspects of the behaviour of plastids during meiosis. At leptotene in *Tradescantia paludosa*, plastids in the pollen mother cells appear ovoid, measuring about 1.8μ in length and 0.8μ in width. Starch granules are gradually lost, and thylakoids and tubular structures regress. In *Lilium longiflorum*, constricted plastids, suggesting incipient division, are becoming less common by leptotene. During zygotene, most of the internal structure of the plastids is greatly reduced; ribosomes are lost from the stroma, and only "osmiophilic globuli" remain to assist their identification as plastids at this stage. Dickinson and Heslop-Harrison state: "Conceivably the globuli represent storage centres for the membrane lipids mobilised during the elimination of the lamellae." From pachytene

to Metaphase 1 the plastids remain in the proplastid state, only the globuli being visible. In this interval they are spheroidal or slightly elongated.

Changes occurring in the Golgi apparatus are of particular importance to the fine -structural study of pollen wall formation. Golgi bodies have long been implicated in secretory activities, and it is to be expected that these organelles would show a high level of activity during the period of callose and pollen wall formation. Maruyama (*Tradescantia paludosa*, 1965) and more recently Skvarla and Kelly (*Canna generalis*, 1971) and Willemse (*Pinus sylvestris*, 1971a, b, c; *Gasteria verrucosa*, 1972) have studied the behaviour of Golgi bodies, and have shown that the species observed exhibit numerous similarities in Golgi development during microsporogenesis. In the premeiotic period in *Canna*, each Golgi apparatus consists of several dictyosomes whose cisternae are interlinked by networks of tubules. These are often associated with smooth tubular ER. According to Skvarla and Kelly, secretory activity (i.e. vesicle production) by dictyosomes is not obvious until well after meiosis. They report a decrease in the number of "static" cisternae as microsporogenesis progresses, associated with a rise in vesiculation of the dictyosomes. Willemse also reports a paucity of vesicles in dictyosomes of *Gasteria* pollen mother cells, observing an increase during zygotene-pachytene, possibly associated with the secretion of the callose special mother cell wall. He suggests that the consistent location of the Golgi bodies near the region of the dividing nucleus may indicate a rôle for these organelles in reconstructing the nuclear membrane.

Heslop-Harrison (1964, 1966a, b, 1971a) has observed major changes in cytoplasmic interconnections during meiosis in *Cannabis sativa*, *Silene pendula*, and other species. In the premeiotic period pollen mother

cells are linked by plasmodesmata which become "massive cytoplasmic channels" in early Prophase 1, forming a type of syncitium (cf. protoplasmic connections between animal spermatocytes). He regards this as an important factor in maintaining synchrony in early meiosis. After the interconnections are severed in Meiosis 11, synchrony decreases, and the isolation and individuality of each daughter cell is reinforced by the formation of the callose tetrad walls. As mentioned earlier Heslop-Harrison suggests that interchange of materials via the larger channels may be useful prior to diad formation, but that the subsequent isolation of the haploid nuclei from one another is of prime importance in the development of genetically individual gametophytes.

Changes within the uninucleate microspore

The substantial cytoplasmic changes associated with meiosis are probably necessary for the ultimate expression of the gametophytic part of the genome. After the tetrad stage the young microspore undergoes another series of complex changes that convert it to a functional male gametophyte. The transition from microspore to gametophyte is strongly influenced by surrounding tissues: initially the anther tapetum, and later the gynoecium in which the pollen tube grows. Ribosome numbers rise during the final stages of meiosis, and the appropriate ribosome population for subsequent metabolic activity is restored by the time of tetrad formation. The microspore therefore begins its independent existence with the quantity of ribosomes for protein synthesis required in the growth period.

Both mitochondria and plastids show increasing internal structure during the uninucleate stage in *Beta*, and starch grains are found in the plastids (Hoefert, 1969). Maruyama (1968) also reports the

frequent occurrence of large starch grains in well developed plastids of young *Tradescantia paludosa* microspores. The plastids lose starch by the vacuolate stage, and show signs of incipient division. A little later, "proplastids" are seen, some of which contain starch. These reach full development, often with a single large starch grain, just before pollen grain mitosis. Mitochondria in *T. paludosa* microspores appear relatively undifferentiated before the vacuolate stage, and are recognisable only by analogy with bodies of a similar size in the tetrads. About midway through the vacuolate period, their internal structure reappears, and prior to pollen grain mitosis the mitochondria are large and well developed. Information from *T. bracteata* is essentially similar: plastids containing polysaccharide granules are present in the pre-mitotic cytoplasm, and mitochondria undergo morphological changes which suggest incipient division (Mepham and Lane, 1970a). Dickinson and Heslop-Harrison (1970b) have observed that the "membrane-particle associations" of Meiosis II within *Lilium* plastids regress after the tetrad stage, and redevelopment of internal structure follows rapidly. Ribosomes and starch granules reappear within them, and constrictions suggesting division can be seen. At the time of intine initiation the starch content of plastids is reduced; this may indicate mobilisation of carbohydrate reserves for cellulose synthesis, as pointed out by Mepham and Lane.

In the interval from the tetrads to pollen grain mitosis Golgi bodies show a high level of activity. The possible involvement of these organelles in early exine formation was mentioned by Heslop-Harrison (1968a). Vasil and Aldrich (1970) suggested that Golgi bodies may be active in exine deposition in *Podocarpus*, and Willemse (1972) has presented evidence which strongly supports this. Electron micrographs of *Gasteria* microspores imply that Golgi vesicles may transport material to the site between the plasmalemma and the callose tetrad wall. Willemse

suggests that the vesicles secrete a polysaccharide which forms a template for the exine. Some of this material appears to be included in the substance of the developing bacula, tectum and foot-layer, but the major wall component is formed at the plasmalemma from precursors probably originating within the microspore cytoplasm. Lipid globules are in abundance at this stage in *Gasteria*. Heslop-Harrison's concept of a "primexine" is recalled in Willemse's interpretation of the electron micrographs of *Gasteria:* "The suggestion can be made that the pollen wall consists of two kinds of material: less electron dense material and electron dense material, which may be the sporopollenin. The other possibility is that the sporopollenin is preceded by a less electron dense precursor". Another interpretation is that pieces of homogeneous material, which are present in different thicknesses in a section, will appear to have differential electron density.

The exine reaches its mature form while the microspore is still expanding. Several workers have investigated the problems of exine permeability and expansion, and have succeeded in abolishing the earlier idea of the exine as an impenetrable, inflexible barrier. Banerjee *et al.* (1965) studied exine plasticity during the maturation of *Sparganium androcladium* microspores. They concluded that the material is in fact capable of stretching and shrinking to accommodate for changes in protoplast volume, and accepted the possibility that each species has a characteristic exine size, to which the exine will revert if stretched during microspore maturation. Rowley *et al.* (1959, 1970, 1971a, b) observed structures that would presumably facilitate transfer of materials into the expanding pollen grain: cytoplasmic strands from the tapetum to the exine, and channels through the exine which appeared to be formed from plasma membrane evaginations. They were also able to show that the tracer lanthanum nitrate passed directly through the exine -

not only along the channels - and accumulated at the surface of the cytoplasm and in lamellae of the nexine. This presented a rather different concept of the permeability properties of exine, for, as pollen workers have long been aware: "the resistance of the exine to degradation and its relatively high mass offers a mental block to any thinking about transfer of materials through the material of the exine." (Rowley and Flynn, 1970). Mepham and Lane (1969a) also reported that tapetal membranes penetrate the exine in *Tradescantia bracteata*. In a paper published a little later (1970b) they suggested a special function for the sculptured exine of microspores in plants with an "amoeboid" tapetum. They noted the presence of wall projections in plant cells specifically involved in absorption and translocation of nutrients, such as "transfer cells" in leaf veins (Gunning *et al.*, 1968), and juncture cells between sporophyte and gametophyte in *Polytrichum* (Maier, 1967) and *Sphaerocarpos* (Kelley, 1969) - and suggest that the early appearance of exine projections associated with the tapetal periplasmodium may provide an efficient method of transport from sporophyte to microspore. For plants with a "secretory" tapetum (apparently lacking the close association between tapetal membranes and microspores) the significance of the exine projections to nutrient transport is open to question. Mepham and Lane, however, remark that improved research techniques may well reveal some form of association between the secretory tapetum and developing spores.

The intine begins to form before pollen grain mitosis, during the period of volume increase and vacuolation. Heslop-Harrison (1968a) suggests that precursors may be transported by vesiculating dictyosomes lying close to the plasmalemma, but presents no direct evidence for this. The intine is initially laid down in the apertural region where exine is thin or absent, and extends over the plasmalemma surface. It appears to consist of microfibrillar cellulose in a pectic-hemicellulosic matrix,

with the inclusion of protein material (Knox and Heslop-Harrison, 1970a, 1971a). The cytoplasm of young microspores of several species studied shows various membranous and tubular structures which appear to be associated with intine production. Hoefert (1969), working with microspores of *Beta vulgaris*, reports a "contorted membrane structure" which she calls the "reticulum complex". This persists from the tetrads to the binucleate stage. Hoefert indicates that it may be involved in protein synthesis or transport of materials between the nucleus and the plasmalemma. A similar structure is described by Mepham and Lane (1970a) and is believed to be associated with intine secretion. Numerous cytoplasmic microtubules are visible prior to and during the vacuolate period in *Beta*, when in fact intine synthesis is taking place, but Hoefert does not suggest any connection. Heslop-Harrison (1971) has described similar microtubules adjacent to the plasmalemma during intine formation, but they were infrequent and he felt that their function was not clear. Rowley (1967), Angold (1967), and Mepham and Lane (1970a) have also reported the presence of microtubule systems in microspores of *Populus tremula*, *Endymion non-scriptus*, and *Tradescantia bracteata* respectively. They would therefore appear to be a fairly common phenomenon of this developmental stage. The function of general cytoplasmic microtubules (as opposed to cilia, flagella, and spindle tubules) is unknown, but they are frequently seen at times of wall synthesis and are known to penetrate the intine.

The association of nuclear evaginations with intine production has been discussed by Barth and von Rahden (for *Paeonia*, 1967) and Gullvåg (for *Lycopodium*, 1970). "Blebbing" of nuclear material has already been mentioned with respect to the formation of mitochondria and plastids (p. 55). During spore production in *Lycopodium* the nuclear envelope evaginates to produce vesicles which appear to empty their contents onto

the site of the developing intine. Gullvåg interprets this as transportation of the required genetic information for intine synthesis. Barth and von Rahden report a similar transfer of extruded nuclear material to the site of intine production, noting that the vesicles are carried via the ER, which appears to be continuous with the outer nuclear membrane. Nuclear "blebbing" may possibly have the basic function of packaging copies of parts of the genome which are needed to direct various cytoplasmic activities.

A possible function of the vacuolation occurring during microspore maturation should be mentioned briefly. The vacuoles, which appear to be derived from the ER, are often seen to contain plastids and mitochondria in various stages of disintegration, and it is possible that the cytoplasm may be removing "old" organelles through a process of lysis within the vacuoles. After pollen grain mitosis the vacuoles rapidly regress, perhaps indicating stabilisation of the cytoplasm of the two new cells (Mepham and Lane, 1970a). The derivation and possible lysosomal function of vacuoles and vesicles in different cell types has been referred to by several workers, including Pickett-Heaps (1967), Matile and Moor (1968), Robards and Kidwai (1969), Mesquita (1969), and Mahlberg (1972a, b).

Pollen grain mitosis and the binucleate stage

The final stage of microspore maturation is inaugurated when the nucleus divides mitotically to produce the vegetative and generative nuclei. Normally the mitotic spindle forms asymmetrically, probably influenced by earlier vacuole formation within the microspore cytoplasm (La Cour, 1949). The resulting cells are therefore unequal in size and shape. Formation of a symmetric spindle results in two equivalent cells,

and the pollen grain is sterile. The generative nucleus soon stains differently and its chromatin becomes highly condensed (heterochromatic). In contrast, the vegetative nucleus normally becomes euchromatic, and occasionally assumes bizarre shapes.

Larson (1963) studied "cytoplasmic dimorphism" in mature pollen of different species, and was able to demonstrate the presence of paired membranes separating the cytoplasm of the vegetative and generative cells in mature pollen. The existence of such membranes had been disputed not long before this by Venema and Koopmans (1962) but Larson's work confirmed earlier reports by Safijovska (1955) and Bopp-Hassenkamp (1960) that mature pollen was in fact bicellular. No evidence was found of an actual dividing wall in these studies, although such a wall has subsequently been observed in the immature pollen of several species. The presence of a transitory wall around the young generative cell had been suspected for many years before evidence was presented by a number of workers using improved electron microscopic methods, including Maruyama *et al.* (1965), Górska-Brylass (1967a, b), Heslop-Harrison (1968b), Angold (1968), Mepham and Lane (1970a), and Hoefert (1971). Maruyama and co-workers observed the fusion of electron-lucent vesicles to form a division between the vegetative and generative nuclei in *Tradescantia paludosa* pollen. This "wall", which appeared dome-shaped in cross-section, was in contact with the intine and gave cytochemical reactions which suggested the presence of cellulose and pectins. The wall later became very thin (less than 300 Å) and lost contact with the intine, permitting the vegetative cytoplasm to surround the generative cell completely. It was found to persist in this reduced form at pollen grain maturity. Maruyama *et al.* suggest that the formation of a true wall between the vegetative and generative nuclei may be an important factor in maintaining differential development of the two nuclei and

their accompanying cytoplasms. Górska-Brylass supports this view, although she differs in her interpretation of the chemical nature of the wall. Using two species of *Chlorophytum* and one each of *Hyacinthus*, *Tradescantia* and *Impatiens*, she has demonstrated the presence of a callose layer by two methods reputedly specific for this polysaccharide. The callose persists for only a short period - about twelve hours from the completion of pollen grain mitosis. The wall is between 1.5μ and 2.5μ in thickness, in most cases smooth and continuous but occasionally appearing scale-like. As the generative cell moves away from the pollen wall towards the centre of the grain, it becomes spherical and is completely enveloped in the callose wall. In essentials these observations for *Tradescantia* are confirmed by Mepham and Lane (1970a). Mepham (personal communication) has also observed plasmodesmatal connections through the wall between the two cells. After the disintegration of the callose, the generative cell slowly assumes its characteristic elongated shape. The presence of a transitory callose wall around this cell seems to be a widespread feature, and there is general agreement among palynologists that its main function is probably to achieve early separation of the vegetative and generative nuclei, permitting the development of individual potential. If the wall does not form, the two cells may fail to differentiate, and sterility results.

The marked developmental differences between the cells have been investigated in detail in recent years, and attempts have been made to relate the dissimilarities to differing rôles in the ultimate development of the gametophyte. The cells begin with apparently similar organelle complements, but proliferation of organelles has been shown to occur only in the vegetative cell. Larson (1963) observed that differentiation of the cytoplasms began soon after pollen grain mitosis. Plastids were found in both cells in most species studied, but those in the generative

cell were relatively small and immature and lacked starch, whereas the vegetative cell contained amyloplasts. Golgi bodies in the generative cell were few and inactive, and the ER was reduced. At anthesis, mature pollen of all species studied has shown negligible Golgi activity. Many Golgi bodies are present, but they remain dormant until water is taken up at the onset of germination. Larson found that differences in general cytoplasmic density were inconsistent, the vegetative cell being less dense than the generative in some species and denser in others.

Maruyama (1968) discusses in detail the overall phasing of changes in plastids and mitochondria during the binucleate stage in *Tradescantia paludosa*, but does not always distinguish clearly between the organelles of the vegetative and generative cells -- a fact which leads to occasional confusion. Mepham and Lane (1970a) report a proliferation of organelles in the post-mitotic vegetative cell of *Tradescantia bracteata*. Plastids accumulate plysaccharide, a mass of "rough" ER develops, dictyosomes increase in number (although no active vesiculation occurs), and the cell becomes packed with mitochondria. At pollen grain maturity the generative cell appears quiescent. Its organelle complement is essentially normal, but no increase in numbers occurs, and storage materials are not found. Hoefert's description of the binucleate pollen grain in *Beta vulgaris* (1969) is brief and sketchy, but in this plant also it is evident that the vegetative and generative cytoplasms become quite different. Willemse (1972) has found that the overall number of organelles per pollen grain increases after mitosis in *Gasteria verrucosa*, also in *Pinus sylvestris* (1971a, b, c). Mitochondria develop more cristae, but Golgi bodies do not appear very active and only limited vesiculation can be seen. Reserve material in the form of electron-transparent granules (presumably polysaccharide) accumulates in plastids until after mitosis, when the granules begin to disappear -

contrary to other reports, e.g. Hoefert (1969). Willemse's observations are of limited value, as he does not distinguish between the vegetative and generative cells in presenting either the morphological or quantitative data.

Oryol (1969) studied polarity and nuclear/cytoplasmic differentiation in *Zea mays* pollen, concentrating mainly on possible physical factors influencing movement of the vegetative and generative nuclei, and of the generative cell as an entity. A predictable pattern of movement was established, aligned with the polarity axis, and Oryol suggested that the presence of a gradient of some kind in the cytoplasm of the young microspore might predetermine the course of movement after mitosis.

It was believed for many years that vegetative nuclei were degenerate, showing little activity. Improved techniques have enabled workers to demonstrate that this is not so. The studies of Young and Stanley (1963) and Mascarenhas (1966a) on nucleic acid synthesis in vegetative and generative nuclei, mentioned above (p. 26), have shown that the vegetative as well as the generative nucleus actively incorporates labelled nucleosides. Larson (1965) modified his earlier views (1963) after producing electron micrographs which indicated that the vegetative nucleus was functional and not degenerate. Mascarenhas observed that treatment with actinomycin-D failed to prevent pollen tube germination and early growth, but that subsequent elongation and pollen tube mitosis were inhibited. This he regarded as evidence that the m-RNA and r-RNA required for initial tube growth are synthesized by the binucleate grain before anthesis. In a later paper supporting this hypothesis (Mascarenhas and Bell, 1970) he refers to the cytological work of Woodard (1958) which suggests that the vegetative cell in primarily responsible for the

observed synthesis of RNA. The significant level of organelle activity within the vegetative cytoplasm lends further support to the idea of a highly functional cell, at least in the binucleate period, and apparently also during germination and early tube growth. By comparison, the generative cell appears quiescent. The organelles are fewer in number and considerably less active, and ER is reduced. Towards pollen grain maturity the generative nucleus becomes highly condensed and appears to enter an extended prophase. Mitosis may occur before anthesis in some species, i.e. the mature grain is trinucleate, or, as in the majority of species, it may be delayed until after germination when the generative nucleus has entered the pollen tube. The number of nucleopores on the vegetative nuclear envelope increases markedly during the binucleate stage in *Tradescantia bracteata* (Mepham and Lane, 1969b). This appears to be another sign of increased cellular activity, as the pores are believed to form only when ribosomes are being extruded into the cytoplasm to direct protein synthesis. Towards anthesis, when cellular activity diminishes, pore numbers decrease. Few pores are seen on the generative nuclear envelope. LaFountain and LaFountain (1973) have supported the observations of Mepham and Lane with their own work on freeze-etched pollen of *Tradescantia paludosa*. They report that vegetative nuclear envelopes have about twice as many pores as those of generative nuclei, both in density and total pore number. This figure shows a good correlation with estimates of the amount of RNA synthesized by the two nuclei between pollen grain mitosis and anthesis, giving further support to the hypothesis of RNA extrusion via nucleopores.

Pollen Tube Development

Pollen germination and tube growth *in vivo* and *in vitro* have been the subject of many investigations, some as early as the nineteenth

century. Much has been published on the topic, and a complete review of surveys and results would go well beyond the limitations of this study. The discussion will therefore concentrate mainly on the large body of work carried out since 1960.

Uptake of water and activation or synthesis of enzymes appear to be the initiating mechanisms in pollen germination. The grain swells rapidly, and the tube tip grows through an aperture or break in the exine as a structure contiguous with the intine. Lytic enzymes in the intine are implicated in the dissolution of exine in apertural regions, prior to germination of the tube (Gherardini and Healey, 1969). As discussed above (p. 33) the cytoplasm of the pollen grain appears to be "pre-programmed" for production or derepression of enzymes required for germination and initial tube growth. The enzymes metabolise substrates both within the grain and possibly in the tissues of the gynoecium, providing precursors and energy sources for tube biosynthesis. Growth is limited to the tip, where elongation occurs by the deposition of pectic and hemicellulosic materials immediately behind the tip. The true cellulose component is probably incorporated after the initial wall is formed (Stanley, 1971). A layer of callose is generally deposited as a lining to the wall (excluding the tip) and callose plugs often appear at the grain end of the tube as a stopper device for the tube cytoplasm. Callose plugs may also be present in the wall, possibly as a response to wall injury.

Numerous investigators have shown that germinating pollen readily utilises endogenous and exogenous carbohydrates in the formation of structural polysaccharides, carbohydrate reserves and other materials. (Kessler, Feingold and Hassid, 1960; Stanley and Poostchi, 1962; Hrabětová and Tupý, 1963; Young *et al.*, 1966; de Bruyn, 1966b; Kroh *et al.*, 1971). There is some evidence that the materials required by the growing

tip are initially supplied in vesicles which accumulate internally stored compounds and move through the cytoplasm (probably under the influence of cyclosis) to coalesce at the tip region, eventually fusing with the wall (Rosen, 1964; Rosen *et al.*, 1964; Dashek and Rosen, 1966; Crang and Miles, 1969; Jensen and Fisher, 1970; Stanley, 1971). Polysaccharides and RNA appear to be the main contents of the vesicles. Rosen (1971a) has subsequently reconsidered modes of material accumulation, in the light of recent studies of fine structure *in situ*. It appears that tube wall growth after a successful pollination may begin in the way implied from *in vitro* studies, but may switch to a mechanism which permits direct incorporation of materials from stylar tissues. This will be discussed further below. The cyclitol compound myo-inositol is apparently an important precursor in pectin synthesis for wall formation (Stanley and Loewus, 1964; Young *et al.*, 1966; Kroh *et al.*, 1970; Roggen and Stanley, 1971), and the well-documented boron requirement of germinating pollen (see above, p. 44) is thought by many to be at least partially explained by the action of boron as an enzyme co-factor in myo-inositol incorporation.

There has been some confusion over the synthesis of ribonucleic acids in pollen tubes. The concept of cytoplasmic "pre-programming" by means of dormant messengers is gradually gaining wider acceptance, and the apparent reduction in m- and t-RNA synthesis can possibly be explained in these terms. Mascarenhas (1971a, b), Linskens (1971) and Tupý *et al.* (1965) have presented evidence which suggests that dormant-mRNA and tRNA are formed in the mature microspore cytoplasm, and are subsequently activated when germination begins. From this, it would appear that early tube growth is not dependent on new m- and tRNA, but a limited synthesis may be required for further elongation. Steffensen (1966, 1971) reported the apparent absence of *de novo* rRNA synthesis in *Lilium longiflorum* pollen

tubes, although functional ribosomes have been observed by Mascarenhas and Bell (1969), Crang and Miles (1969) and Rosen *et al.* (1964). Ribosomes may also be bequeathed in an inactive state by the pollen grain, becoming functional after germination. As most of the above studies were made with pollen germinated on culture media and with pollen extracts, they can give only limited information on the possible extent of *in vivo* activities. With this in mind, it should be noted that pollen germinating *in vitro* rarely elongates to the same extent as in the pistil tissues.

Increased activity of many enzymes during germination has been noted by several workers (see summary by Brewbaker, 1971).. The enzymes showing a significant increase include cell wall hydrolysing enzymes, amylases, β-fructofuranosidase, phosphorylases and transaminases. The wall softening enzymes cellulase and pectinase have been found in mature ungerminated pollen (Konar and Stanley, 1969) and are now believed to be part of the "pre-programming" mechanism activated during germination in many plants. It has been suggested that these enzymes assist in maintaining the plasticity of the tube tip by controlling the ratios of pectins, hemicelluloses and cellulose deposited there (Roggen and Stanley, 1969). An electron microscope study of germinating *Lychnis alba* pollen (Crang and Miles, 1969) revealed two features of importance to the understanding of enzyme behaviour. Highly organised crystalline bodies were seen in mature pollen grains and tubes, with lattice periods of about 80 Å. Some of the bodies were sites of positive tests for acid phosphatase, and the investigators suggested that the crystals may represent the inactive or "storage" state of certain enzymes which lose their crystalline structure upon activation. Acid phosphatase activity was also demonstrated in cytoplasmic vesicles in pollen tubes, suggesting the presence of lysosomes involved in the metabolism of exogenous and endogenous substrates. The problem of synthesis *vs.* activation of enzymes

at the onset of germination has interested several workers, but reports are conflicting. Some enzymes, such as the cellulases and pectinases mentioned above, are probably present in sufficient quantities in the microspore, but remain dormant prior to germination. Others may be present in limited quantities in the "pre-programmed" cytoplasm and further synthesis from long-term mRNA may be required during pollen tube growth. However, Brewbaker (1971) states: "The fact that many inhibitors of protein synthesis...fail to inhibit pollen germination implies that germination can proceed without net enzyme synthesis, at least in the binucleate pollen types which have been studied". Dickinson and Davies (1971b) reported no general synthesis or activation of enzymes involved in the production of carbon skeletons and energy for wall polysaccharide formation. Their data, obtained for *Lilium longiflorum*, implied that the required active levels of these enzymes were present prior to germination - but one could perhaps question the validity of this data, considering the rapid synthesis of much greater quantities of polysaccharide after germination. The possible rôle of certain enzymes and other proteins in incompatibility reactions will be discussed below.

Pollen tube fine structure has received much attention, mainly because of certain differences between tubes growing *in vitro* and those observed from excision of stylar tissues. Ultrastructural dissimilarities undoubtedly reflect differences in the metabolic mechanisms of pollen germinating *in vivo* and *in vitro*, and comparative studies should reveal important information on the environmental requirements for successful germination. In a recent paper Rosen (1971a) has summarised the difficulties involved: "A problem which has bedevilled students of pollen growth has been that of achieving, *in vitro*, growth which equals that which must be accomplished in the pistil if fertilisation is to occur... A fruitful approach to the development of improved pollen tube growth

media would be to go directly to the pistil and to extract from it, and identify, those soluble components which promote pollen tube growth on a minimal medium...We must also, however, be attentive to the physical environment. Growth requirements, physical as well as chemical, may change significantly during growth. Indeed, the general failure to achieve growth in culture which approaches growth in the pistil may come from a failure to recognise changing growth and osmotic requirements at different stages of tube development". *In vitro* studies by Rosen and co-workers (1964), Rosen and Gawlik (1966), and Dashek and Rosen (1966) with pollen of *Lilium longiflorum* showed distinct cytoplasmic differences between the non-growing region of the tube and the growing tip. Mitochondria, amyloplasts, ER, Golgi bodies, lipid globules and vesicles of various sizes were found in abundance in the area behind the tip, whereas the tip region was characterised by a marked increase in the number of vesicles and an almost complete absence of organelles, lipid and starch. Cytochemical tests revealed a similar lack of "total" protein in the tip region, but an abundance of RNA and protein in the vesicles. Other ultrastructural studies of tube cytoplasm and walls have presented basically similar pictures of pollen germinating *in vitro* (e.g. Larson and Lewis, 1962, Larson 1965, Crang and Miles, 1969) with only minor variations which may be species-specific. Three recent papers on the fine structure of tubes growing *in vivo* are those of Kroh (1967), Jensen and Fisher (1970) and Rosen (1971a, mentioned above). Kroh found that the state of cytoplasmic organelles in *Petunia* pollen was much the same whether germination occurred in culture media or in the pistil. The one striking difference was in the structure of the tube wall. The outer layer of the wall *in vivo* appeared irregular in outline and somewhat diffuse in structure, possibly an advantageous feature for tubes growing in a less homogeneous environment where penetration of materials could be relatively difficult. In contrast, walls of experimentally germinated tubes were more regular

and compact in structure, possibly reflecting the ease of penetration of culture media solutions. Jensen and Fisher reported that cytoplasmic activities in *Gossypium hirsutum* tubes growing in the stigma and style were similar to those described by other workers for *in vivo* and *in vitro* germination, and stated that features of the wall were in agreement with the descriptions of Kroh for *Petunia*. Rosen's theory of a change-over in nutritional modes (see above, p. 70) is based on observations of tube wall structure *in situ*. He describes "irregular embayments" in the tip wall which appear to facilitate the entry of exogenous materials. He suggests that this ultrastructural feature may be connected with compatible pollination only, as the embayments apparently did not develop when pollination was incompatible, and the pollen tube ceased to grow after its internal substrates were exhausted. The embayments did not develop *in vitro* (cf. evidence of Kroh, Jensen and Fisher). Crang (1966) and Crang and Miles (1969) also implied that later tube growth may be at least partially achieved by "apposition".

Many workers other than Rosen have been concerned to define the appropriate conditions for successful germination and fertilisation. Rosen himself has been involved in a detailed examination of secretory cells of *Lilium longiflorum* in an attempt to determine their rôle in pollen germination (Rosen and Thomas, 1970; Dashek, Thomas and Rosen, 1971). Exudates on the stigma surface and in the stylar canal of various species appear to be involved in pollen tube nutrition, chemotropic activity, and incompatibility reactions (Welk *et al.*, 1965; Rosen and Gawlik, 1966; Kroh *et al.*, 1970; Kroh *et al.*, 1971; Ascher and Drewlow, 1971; Rosen, 1971b). Rosen and co-workers have suggested that the "secretion zone" of stylar canal cells in *Lilium*, and the embayments of the pollen tube tip, may be linked in an efficient transfer mechanism which is initiated by penetration of the pollen tube and discharge of

hydrolytic enzymes from the pollen into the stylar canal. Protein, pectin, cellulose, hemicellulose and lipid were detected in the cytoplasm and bordering wall of the "secretion zone", but the composition of the exudate itself was not determined. Jensen and Fisher (1969) studied the relationship between the pollen tube and the pistil tissues of *Gossypium*, a plant which does not have a stylar canal. Instead, the tube grows through a "transmitting tissue" of thick-walled cells, but does not penetrate the cytoplasm of these cells. Although there was no direct evidence of absorption of materials by the tube, the possibility was considered. Jensen and Fisher, however, preferred to regard the transmitting tissue as "a passive route for the growth of the tube", rather than a source of nutrients, wall precursors and other "active agents" controlling tube growth. Crang (1966) reported cytoplasmic disintegration of stylar cells in *Lychnis alba* as a direct response to tube penetration. Enzymes diffusing from the tube were believed to be responsible for the degradation. In this study Crang did not discuss the possible rôle of disintegration products in pollen tube growth. Martin and Brewbaker (1971) analysed the stigmatic exudate of species of *Petunia*, *Strelitzia*, *Zea* and *Ipomoea*, and suggested possible rôles for the various components. Lipids occurred as free fatty acids and esters, and appeared to function primarily in the prevention of desiccation. There was some possibility that they also affected the permeability of pollen tube membranes. (Roggen (1972) has suggested that heavier waxes on the stigmas of some species may be part of the incompatibility mechanism). Phenolics were also detected in the exudate, and it was proposed that they might act as inhibitors or stimulators of IAA-oxidase activity, thereby affecting the growth rates. Martin and Brewbaker reported that sugars and traces of free amino acids had been found in stigmatic exudates (Konar and Linskens, 1966), but that levels of enzymes and other proteins were very low in the species tested

(Martin, 1968; Konar and Linskens, 1966). The exact rôle of the exudates has yet to be determined.

Stanley and Linskens (1967) investigated oxygen tension as a possible controlling factor in pollen tube rupture. An oxygen gradient is known to exist in the style, approaching zero in the ovary, and Stanley and Linskens found that decreasing partial pressure of oxygen *in vitro* induced bursting of tubes in five species. The exact mechanism of rupture is not known, but it is possible that cytoplasmic streaming within the tube ceases as pO_2 approaches zero, creating wall stresses in the tip and eventually causing tube rupture and liberation of the sperm nuclei. Lytic enzymes such as cellulase, which are known to be less sensitive to low pO_2 than those involved in wall synthesis, may also be implicated. Not all pollen tubes burst at low partial pressure of oxygen, however, and other factors are undoubtedly involved. Nygaard (1969) studied growth conditions and the effects of pH and temperature changes on *in vitro* germination of *Pinus mugo* pollen, relating the growth rates to those known to occur *in vivo*. A pH optimum of 4.5-6.5 was recorded for successful germination. Initial tube growth was shown to be dependent on temperature: no germination was observed in cultures started at -10°C, less than 5% in cultures started at 10°C, and normal germination occurred at an initial temperature of 15°-18°C. Satisfactory growth rates were obtained when temperatures were maintained between 20°-37°C.

Arditti and Knauft (1969) prefaced their paper on post-pollination behaviour in orchid flowers with a list of important changes induced by the pollination process. These included: stimulation of ovule development, initiation of peroxidase activity in the ovule walls, starch accumulation at various sites, translocation of mitrogenous compounds, water, phosphates and carbohydrates from various floral parts to the column, development of

chlorophyll in the depleted floral parts, ethylene production, and numerous structural changes. An investigation into the effects of these changes on growth of the pollen tube itself, could provide much information on optimum conditions for germination. Pollen tube chemotropism and growth stimulation by various substances have been discussed in Part II (iii), (vi) and (vii) above.

The generative nucleus of binucleate pollen must undergo mitosis during tube growth, if fertilisation is to occur. The vegetative and generative nuclei move into the tube, probably to some extent influenced by cytoplasmic streaming. Following compatible pollination, mitosis occurs, giving rise to two haploid sperm nuclei. Limited tube growth and generative nuclear division may occur in some species even if the pollination is incompatible, but in such cases fertilisation does not occur. In many species the generative nucleus fails to divide following an incompatible pollination (Townsend, 1971). The vegetative nucleus appears to lose structure after formation of the sperm nuclei; it is possible that its final function is to influence mitosis in some way.

Incompatible pollination

Both interspecific (the inability to succeed in hybridisation) and intraspecific incompatibility (failure to set seed after fertilisation) have been investigated in some detail. Although the finer genetic and immunological mechanisms are not yet fully understood, several contributing factors have been revealed. As would be expected, different combinations of these factors influence incompatibility reactions in different plants, and it is extremely unlikely that a universal pattern of events will emerge. The possibility that proteins diffusing from pollen may be an essential factor in compatibility and incompatibility, had been suggested as early

as the nineteen twenties (East, 1929). Testing this hypothesis formed a major part of the work of Lewis *et al.*, and many others, in the fifties and sixties, but biochemical details were poorly understood prior to the adaptation of analytical techniques such as gel electrophoresis and serology. Valuable work on the analysis, localisation and properties of enzymes and other proteins potentially involved in incompatibility has been carried out in recent years by Stanley and Linskens (1964, 1965), Kendall and Taylor (1971), Stanley and Search (1971), Knox and co-workers (1969, 1970a, b, 1971a, b, c, d, 1972), Brewbaker (1971), and Pandey (1972). Pandey's investigations into temperature-induced inactivation of certain enzymes has provided support for the work of Hecht (1964), Bali and Hecht (1965), Ascher and Peloquin (1966, 1970), Leffel (1963) and Kendall and Taylor (1969). These workers obtained evidence that compatible pollinations can occur if certain proteins involved in incompatibility reactions are heat-denatured.

Incompatibility factors appear to be located in the pollen wall (Kwack, 1965; Knox and co-workers, 1969, 1970a, b, 1971a, b, c, d, 1972) in the stigmatic and/or stylar exudates (Pandey, 1963, Rosen, 1971b; Ascher and Drewlow, 1971), and possibly in the tryphine coating of pollen (Heslop-Harrison, 1968b; Dickinson and Lewis, 1973). Apart from specific enzymes and other proteins which may be either activated or repressed to achieve incompatible reactions, certain ions may be involved (Kendall, 1968; Kwack, 1965), and the stigmatic cuticle and epicuticular wax in some plants, e.g. *Brassica* and *Raphanus*, has also been implicated (most recently Ockenden, 1972; Roggen, 1972; Dickinson and Lewis, 1973). The genetic basis of incompatibility mechanisms has been the subject of controversy for many years. Since the 'S-gene' hypothesis was put forward by East and Manglesdorf (1925), many attempts have been made to fit experimental observations into this system, which proposes that

compatibility is controlled by a single multiallelic gene (see review of Townsend, 1971). The original hypothesis has been extended and modified in the light of new data, but no single genetic model has achieved general acceptance. It is likely that several or many models will be required to describe incompatibility mechanisms, even for the species studied to date.

Abnormal pollen development

Pollen abortion can result from disturbances in normal mechanisms at several developmental stages. Differentiation may be arrested as early as the pollen mother cell stage, before meiosis has even begun. In other cases the meiotic division itself may be abnormal in some way, and will give rise to various types of defective microspores. If development proceeds as far as pollen grain mitosis, the disturbance may occur at this division, and the mature grain will be non-functional. Prolonged cohesion of microspores within a tetrad (Rick, 1948), failure to form exine (Frankel, 1940), tapetal malfunction (Childers, 1952; Childers and Mclennan, 1960; Pandey, 1961; Singh and Hadley, 1961; Chowdhury and Das, 1968; Knox and Heslop-Harrison, 1966), and unsatisfactory anther development (e.g. absence of epicuticular wax; prevention of dehiscence by abnormal wall formation) are all known to affect pollen viability to a greater or lesser extent. The abortion rate in any plant may be genetically determined, as in the case of male sterile mutants, but is also influenced by environmental factors such as photoperiod, temperature, availability of nutrients, adequate water supply, and attack by plant parasites and micro-organisms.

Cytological studies of abnormal meiosis, such as those by Fabergé (1937), Rick (1944), Dodds and Simmonds (1946), Childers (1952), Bernardo (1957), and Bosemark (1957) have shown that disruption of normal nuclear and cytoplasmic events will result in different types of sterile

microspores, including the non-functional polyploids and multinucleates described by Giles (1939), and "miniature" grains resulting from chromosomal lagging. Disturbance of polarity in pollen grain mitosis will generally affect formation of the asymmetric spindle, which is essential for "proper" differentiation of the vegetative and generative nuclei. A symmetric spindle will give rise to two non-functional "vegetative" cells of equal size, forming a defective pollen grain which degenerates soon after mitosis. A mutant gene may be responsible for abnormalities of either meiosis or mitosis in the anther, but in cases where genetically based sterility can be ruled out the cause will undoubtedly be a particular environmental factor, or a combination of several factors.

Early studies of external influences on pollen fertility were carried out mainly for agricultural purposes. Kostoff (1930, 1933) and Kostoff and Kendall (1929, 1930, 1931) found that intergeneric grafting, arachnid parasites and viral disease affected pollen fertility levels in various plants. Howlett (1936) studied the effects of carbohydrate and nitrogen deficiency on microsporogenesis in tomato plants, and produced a wide range of results. Mild carbohydrate deficiency induced the formation of grains which appeared morphologically sound but failed to germinate either *in vivo* or *in vitro*. A more severe deficiency caused abnormal development ranging from failure of meiosis to occur in the pollen mother cells to degeneration of mature grains. Chromosomal lagging and other irregularities of division were common. A moderate degree of nitrogen deficiency did not appear to affect pollen development to any extent. Only a very marked deficiency resulted in a significant level of abortion. As under conditions of carbohydrate deficiency, degeneration occurred over the full range of pollen grain development. Giles (1939) investigated the effect of dehydration on microsporogenesis in *Tradescantia*, and found that water lack interfered with meiotic spindle and cell plate formation. This disturbance resulted in the production of

diploid, tetraploid, and even octoploid microspores, and bi- and quadri-nucleate cells. It was suggested that the effect of dehydration may be to increase the viscosity of the pollen mother cell cytoplasm, preventing normal spindle development and activity. The occurrence of natural polyploidy was discussed in relation to the results of this study. The length of the post-inductive photoperiod was shown by Nielsen (1942) to be an important factor in pollen abortion and failure of flowers to mature. Exposure of Biloxi soybeans to long photoperiods, following photoinductive treatments of two to twenty cycles, caused abortion at various developmental stages. Plants exposed to a low number of inductive cycles appeared to be affected at a much earlier stage of pollen development than plants which had received longer inductive treatment. The long post-inductive photoperiods produced a high level of abortion even in plants having ten inductive cycles.

Heslop-Harrison and co-workers have contributed much to the understanding of factors involved in pollen sterility, particularly the effect of photoperiod (Heslop-Harrison, 1959; Heslop-Harrison and Y. Heslop-Harrison, 1958; Knox and Heslop-Harrison, 1966). They have shown that photoperiodic effects on hormonal and metabolic activities can strongly influence tapetal function, a major factor in pollen viability. A study of cold-induced rice crop failure by Satake *et al.* (1969a, b; 1970) revealed that low temperatures affect the meiotic stage of pollen development, inducing a high level of abortion. Other affects include cessation of anther development, partial or no dehiscence of mature anthers, little or no pollination, and failure of mature pollen to germinate on the stigma. The delicate balance of factors in the environment can be seen to exert a strong influence on the success or failure of pollen development.

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