An Ultrastructural Study of the Embryogenesis and Seed Maturation of Capsella bursa-pastoris (L. Medic.) with an Experimental Approach to Morphogenesis of the Chloroplast.

A thesis submitted for the degree of Doctor of Philosophy in the Faculty of Science in the University of London

by

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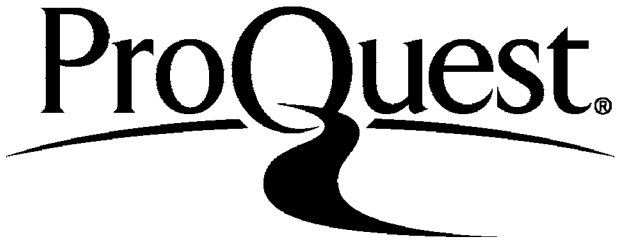

DECLARATION

The work presented in this thesis has not been accepted in substance for any other degree and is not concurrently being submitted in candidature for any other degree.

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Date 22. 7. 1977

This is to certify that the work here submitted was carried out by the candidate himself. Due acknowledgement has been made for any assistance received.

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Supervisor.

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ABSTRACT.

The literature relating to ultrastructure studies of angiosperm embryos up to seed maturity is reviewed. Ultrastructural changes in the cotyledon of the embryo of Capsella bursa-pastoris from the torpedo stage of embryogenesis to the mature seed are described. A revised classification of the developmental stages of the embryo is devised which recognises 11 stages, of which the final 8 stages are examined in the present study: the earlier 3 stages have already been studied by others. Particular reference is made to the development of the chloroplast. The chloroplasts increase in number, size, starch content and lamellar complexity as the embryo develops up to the mature-green stage, after which starch disappears abruptly. The deterioration and disruption of chloroplast lamellae begin after mature-green stage and are completed in the ivory-white and dry cotyledons. Some organelles (Golgi bodies, endoplasmic reticulum and nucleus) are recognizable in the dry cotyledon while other organelles are poorly defined or missing. The cotyledon develops storage tissue with numerous oil bodies and protein bodies in each cell. A single type of protein grain (aleurone grain) is formed within vacuoles. Oil bodies begin to appear from torpedo stage onward and reach a maximum in the ivory-white cotyledon. Ribosomes are dispersed freely in the cytoplasm.

Literature on the effect of light and darkness in chloroplast morphogenesis in higher plants is reviewed. Development of the etioplast is studied in the embryos and leaves of whole plants subjected to darkness. Etioplasts of the embryo in the ovule contain large crystalline prolamellar body after 3 days dark treatment. Plastids of leaves subjected to darkness do not form prolamellar bodies but instead an extensive development of very variable, irregularly-shaped, stroma lamellae with characteristic ring- or cup-shaped or looped lamellae. The extensive development of the abnormal lamellar system with such peculiar arrangement is

hitherto unknown in the plastids of normal higher plants.

The transformation of the etioplast into chloroplast under illumination is investigated utilising embryo culture techniques as it was not practicable to observe such development in embryos from whole plants. The problem of excessive accumulation of starch in plastids was encountered but solved by reducing the sucrose concentration in the growth medium to 0.5% from the 2% used by others. A 1% sucrose concentration in the growth medium gives overall maximum growth of the embryo. After 4 days in darkness the etioplasts of cultured embryos contain well-formed crystalline and concentric prolamellar bodies. Treatment with 3 hours of continuous illumination with white light of low intensity completes the transformation of etioplast into chloroplast. Preliminary studies on the effect of light of different wavelengths on the etioplast transformation are also presented.

Etioplasts in cultured embryos show typical protuberances of the jacket which are regarded as peripheral reticulum. The possibility of the formation of mitochondria from such protuberances is noted.

Some novel structures developed in plastids are reported. Two types of tubules, distinct from the tubules forming prolamellar bodies, are produced in the embryo. They appear as rows of small circular tubules and a group of long parallel tubules. The production of tubules, possibly the result of a reaction to the changed environmental conditions, are regarded as a sign of degeneration rather than a normal stage of development. Another novel structure reported in the etioplasts consists of a complex network of intermingled lamellae.

It is concluded that the use of embryo culture technique in relation to experimental morphogenesis of organelles offers a promising field of study which will contribute also to our knowledge of embryo physiology and development.

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INTRODUCTION.

The structure and development of plant embryos has been the subject of study for many years (Hanstein, 1870; Souges, 1914, 1919; Johansen, 1950; Maheshwari, 1950; Wardlaw, 1955; Davis, 1966). These studies have mainly been descriptive morphological accounts of the sequence and plane of cell divisions during development. Some more recent studies have been directed towards the nutritional requirements of young embryos (Raghavan, 1976) and ovules (Beasley and Ting, 1973, 1974; Jensen *et al.*, 1977) by removing them from the plant and culturing them on artificial media.

Geneticists and plant breeders have exploited the immense potentialities of embryo culture techniques in rearing embryos to maturity from several otherwise unsuccessful crosses. A common problem in plant hybridization is the abortion of the embryo following degeneration of the endosperm, the immediate food supply of the young embryo. This has been overcome in some cases by embryo culture, in this way nourishing the embryo successfully from a source apart from its endosperm.

It is thus well established that a very young embryo may be grown *in vitro* provided they are supplied with a complex medium that includes certain vitamins and hormones and a source of carbohydrate, and that older embryos can be cultured on a much simpler mineral medium.

The embryos of *Capsella bursa-pastoris*, as is probably the general rule, possess chloroplasts from the very beginning and are green to the unaided eye. There is little information in the literature that bears directly on the importance to the embryo of these chloroplasts, although it seems clear from culture experiments that the small green embryos, which are capable of sustaining growth on a medium without sugar, are autotrophic, or capable of being so, from an early age.

The hiatus in development which accompanies dehydration of the

embryo in the seed is characterized by important changes in the cellular organization. Most of the usual complement of cell organelles seem to disappear as the cells become reorganized to accomodate massive reserves, in the case of Capsella consisting of oil and protein. The mobilization of the reserves and reappearance of normal organelles have been more frequently studied than has the ultrastructural changes that accompany maturation of the embryo.

The developing green embryo within the ovule receives as much light as deep seated tissues in the leaf or stem and is capable of photosynthesis. Photosynthesis within the embryo surely contributes to a great extent to the accumulating food reserves as the embryo matures although this aspect of the embryo's nutrition awaits proper study. The role of light in photomorphogenic responses in the embryo is another question that arises. It is as a contribution towards further study of this question planned in this laboratory that information was desired on the effects of light on the embryonic chloroplasts. No previous studies have been made on the embryonic chloroplasts to see if their photoresponses are similar to those described from experiments with leaves and similar tissues.

The investigations reported in this thesis fall into two main categories:

1. the description of the ultrastructural changes in cells of the cotyledons as the embryo develops from the early torpedo-shaped stage through to the dry ivory-white embryo of the seed. This complements the descriptions by other workers of the earlier stages of embryo development. Special reference is made to the chloroplasts.

2. an experimental study, with ultrastructural observations on the chloroplasts of the embryo, with particular reference to the formation of etioplasts with prolamellar bodies and the reorganization of the lamellar system upon re-exposure to light.

PART I.

ULTRASTRUCTURE OF THE ANGIOSPERM EMBRYO.

REVIEW OF ULTRASTRUCTURAL STUDIES ON PLANT EMBRYOS.

The literature on the ultrastructure of cytological changes during embryogenesis is discussed below. A beautiful ultrastructural account of early embryogenesis of Gossypium hirsutum was presented by Jensen (1963). Pollock and Jensen (1964) compared the cell number, cell size and distribution of cell divisions of Capsella bursa-pastoris and Gossypium hirsutum embryos during early embryogenesis. They concluded that the general pattern of embryo growth is essentially the same in both embryos and except for the early cleavages in the embryo, the dynamics of cell development in both plants follows the same pattern.

The application of electron microscopy and refinement of techniques for fixation of plant tissues expanded the numerous detailed studies on the cellular organelles development which were initiated with the light microscope, and revealed other details that were not within the resolving power of the optical microscope. Ultrastructural work on early embryogenesis is presented below in tabulated form (Table 1).

TABLE 1. Ultrastructural studies of early embryogenesis.

Species	References	Notes
Aquilegia formosa (Ranunculaceae).		
	Vijayaraghavan *et al.*, 1972.	The synergids.
Calendula officinalis (Compositae).		
	Godineau, 1969.	The synergids.
Capsella bursa-pastoris (Cruciferae)		
	Schulz and Jensen, 1968a.	The synergids.
	Schulz and Jensen, 1968b.	The egg, zygote up to 3-celled embryo.
	Schulz and Jensen, 1968c.	The early embryo, up to heart stage.
	Schulz and Jensen, 1969.	The suspensor and basal cell.
	Schulz and Jensen, 1971.	The antipodals and chalazal proliferating cells.
	Schulz and Jensen, 1973.	The central cell.
	Schulz and Jensen, 1974.	The endosperm.
Cichorium intybus (Compositae)		
	Godineau, 1969.	The synergids.
Crepis tectorum (Compositae).		
	Godineau, 1969.	The synergids.
Epidendrum scutella (Orchidaceae).		
	Cocucci and Jensen, 1969a.	Mature megagametophyte.
	Cocucci and Jensen, 1969b.	Megagametophyte following fertilization.

Gossypium hirsutum (Malvaceae)

Jensen, 1963.	Synergids, zygote and embryo up to globular stage.
Jensen, 1965a.	The nucellus.
Jensen, 1965b.	The synergids.
Jensen, 1965c.	The egg and central cell.
Jensen, 1968a.	The zygote.
Jensen, 1968b.	Endoplasmic reticulum - tube containing.
Jensen, 1968c.	Polysome (long helical) in the zygote.
Jensen and Fisher, 1967.	Double fertilization.
Jensen and Fisher, 1968 .	Pollen tube entrance and discharge.
Fisher and Jensen, 1969.	Degenerate synergid.
Jensen *et al.*, 1977.	Embryo sac development (endosperm development and synergid changes) in cultured unfertilized ovule.
Schulz and Jensen, 1977.	Endosperm development.

Helianthus annus (Compositae)

Newcomb and Steeves, 1971.	Wall projections of embryo sac.
Newcomb, 1973a.	Embryo sac before fertilization.
Newcomb, 1973b.	Embryo sac after fertilization up to heart stage embryo and suspensor.

Hibiscus costatus (Malvaceae)

Ashley, 1972.	Zygote shrinkage and embryo up to heart stage.

Hordeum vulgare (Gramineae)	
Norstog, 1972.	Embryo up to heart stage.
Ipomoea purpurea (Convolvulaceae)	
Ponzi and Pizzolongo, 1972.	The suspensor.
Ponzi and Pizzolongo, 1973.	Suspensor, unusual type of plastids.
Jasione montana (Campanulaceae)	
Berger and Erdelska, 1973.	Cell walls of embryo sac.
Linum catharticum (Linaceae)	
D'Alascio-Deschamps, 1973.	The embryo sac.
Linum usitatissimum (Linaceae)	
Vazart and Vazart, 1966.	The embryo sac.
Deschamps, 1969.	Early embryo up to heart stage.
Vazart, 1969.	The embryo sac.
Myosurus minimus (Ranunculaceae)	
Woodcock and Bell, 1968 .	Female gametophyte.
Pelargonium x *Hortorum* (Geraniaceae)	
Khera and Tilney-Basset, 1976.	Cotyledons, radicle and suspensor haustorium.
Petunia hybrida (Solanaceae)	
van Went and Linskens, 1967.	Embryo sac, egg apparatus.
van Went, 1970a.	The synergids.
van Went, 1970b.	The egg and central cell.
van Went, 1970c.	Changes in embryo sac during and after fertilization (the zygote).
Picris echiodis (Compositae)	
Godineau, 1969.	The synergids.
Pisum sativum (Papilionaceae)	
Marinos, 1970.	The suspensor (unusual type of plastids).

Plumbago capensis (Plumbaginaceae)	
Cass, 1972.	The filiform apparatus.
Plumbago zeylanica (Plumbaginaceae)	
Cass and Karas, 1974.	The egg.
Quercus gambelii (Fagaceae)	
Mogensen, 1972.	Egg apparatus before and after fertilization.
Mogensen, 1973.	Nucellus and integuments.
Singh and Mogensen, 1975.	Zygote up to late globular stage embryo.
Stellaria media (Caryophyllaceae)	
Newcomb and Fowke, 1973.	Endosperm development.
Newcomb and Fowke, 1974.	Suspensor development.
Torenia fournieri (Scrophulariaceae)	
van der Pluijm, 1964.	Filiform apparatus before and after fertilization.
Triticum aestivum (Gramineae)	
Mares *et al.*, 1975.	Endosperm development.
Tropaeolum majus (Tropaeolaceae)	
Nagl and Kuhner, 1976.	Suspensor and embryo proper (cotyledons, meristem of plumule and radicle).
Nagl, 1977.	The suspensor.
Zea mays (Gramineae)	
Diboll and Larson, 1966.	Mature megagametophyte.
Diboll, 1968.	Megagametophyte following fertilization.

The Synergids.

Little was known about the ultrastructure of the synergids until 1964. A few papers have been published (van der Pluijm, 1964; Pritchard, 1964; Jensen, 1963, 1965b; Diboll and Larsen, 1966; Diboll, 1968; Schulz and Jensen, 1968a; Godineau, 1966, 1969; van Went, 1970a; and Mogensen, 1972), showing that synergids are highly differentiated cells with a complex structure. Two synergids generally flank the egg cell at the micropylar end of the embryo sac in most plants, like Capsella bursa-pastoris. Most workers proposed that synergids produce and secrete substances which direct the growth of the pollen tube (Vazart, 1958; van der Pluijm, 1964; Diboll and Larson, 1966; van Went, 1970a; Mogensen, 1972). Pritchard (1964), Jensen (1965a) and Schulz and Jensen (1968a) suggested a nutritional function to the synergids as their dense cytoplasm contains many dictyosomes, plastids with starch, numerous mitochondria, dense amounts of endoplasmic reticulum, numerous ribosomes and vesicles as compared to the egg before fertilization, suggesting that synergids are metabolically more active than the relatively quiescent egg cell.

Moreover presence of numerous plasmodesmata between the synergids, egg cell and central cell at the early stage provide an additional route for free flow of metabolites. Thus synergids of Stellaria media (Pritchard, 1964), Gossypium hirsutum (Jensen, 1965b) and Capsella bursa-pastoris (Schulz and Jensen, 1968a) do the function of absorption and transportation of substances from the cells of surrounding integuments to the egg cell and to central cell. Such nutritional function seems unlikely for synergids of other plants like Zea mays (Diboll and Larson, 1966), Petunia hybrida (van Went, 1970a), Quercus oambelii Mogensen, 1972), since the plasmodesmata in the walls of egg apparatus and numerous starch grains in synergids which have been taken as

evidence for the absorption and translocation and storage of metabolites by synergids could not be found in the latter species. van Went (1970a) and Mogensen (1972) propose a possibility of entering the food material to the embryo sac from chalazal end in Petunia hybrida and Quercus gambelii, while Diboll and Larson (1966) attribute this function to antipodals in Zea mays.

The Filiform Apparatus.

Strasburger's view (1884) that the filiform apparatus is an elaboration of the synergid cell wall is held true now for all the plants studied with the electron microscope. A prominent filiform apparatus which increases greatly the surface area of the plasma membrane, is present at the micropylar end of both synergids. The filiform apparatus is shaped in the form of a number of finger-like projections that extend into the cell. Their wall structure is unusual as it contains a number of elements including a twisted cylindrical unit surrounded by a puffier wall material. The absorptive function of the filiform apparatus is accentuated by the lining of plasma membrane around its lobes in Gossypium hirsutum (Jensen, 1965b), Capsella bursa-pastoris (Schulz and Jensen, 1968b), Epidendrum scutella (Cocucci and Jensen, 1969a), Petunia hybrida (van Went, 1970a) and Aquilegia formosa (Vijayaraghavan et al., 1972). The filiform apparatus in Petunia hybrida (van Went and Linskens, 1967) is less elaborate and more fleeting in appearance, developing only late in the maturation of the cell and decreasing after fertilization. Mogensen (1972) reported a uniform distribution throughout the cytoplasm of mitochondria and dictyosomes in Quercus gambelii synergids, rather than being concentrated near the filiform apparatus as found in Linum usitatissimum (Vazart and Vazart, 1966; Vazart, 1969), Gossypium hirsutum (Jensen,

1965b), Crepis tectorum (Godineau, 1966), Petunia hybrida (van Went and Linskens, 1967), Zea mays (Diboll, 1968; Diboll and Larson, 1966), Capsella bursa-pastoris (Schulz and Jensen, 1968a), Epidendrum scutella (Cocucci and Jensen, 1969a), Aquilegia formosa (Vijayaraghavan et al., 1972), Linum catharticum (D'Alascio-Deschamps, 1973) which hints at the role of these cells in the absorption of nutrients and translocation. In general it has been concluded that the filiform apparatus probably serves as a pathway for diffusion fluxes. The egg apparatus of Plumbago zeylanica (Cass and Karas, 1974) lack synergids, but the micropylar wall of the egg generates and looks similar to the filiform apparatus and perform all similar functions as the filiform apparatus in the synergids.

The Synergid Wall.

The synergid is surrounded by walls that become progressively thinner from the micropylar to the chalazal end. In some plants like Gossypium hirsutum (Jensen, 1963, 1965b), Torenia fournieri (van der Pluijm, 1964), Zea mays (Diboll and Larson, 1966; Diboll, 1968), Linum usitatissimum (Vazart, 1969), Petunia hybrida (van Went, 1970a) and Quercus gambelii (Mogensen, 1972), the wall disappears about halfway along this distance and the chalazal end of the cell is surrounded only by a plasma membrane and not an additional wall. But in the case of Capsella bursa-pastoris (Schulz and Jensen, 1968a) and Epidendrum scutella (Cocucci and Jensen, 1969a), the wall is present around the entire cell but becomes honeycombed at the chalazal end and thus appears unique in having a very thin wall at the chalazal pole. The absence of a wall presumably facilitates the movement of sperm nuclei into egg and central cell. The pollen tube enters into one of the synergids through the filiform apparatus at the time of fertilization. How the pollen

tube is drawn to one of the synergids is not known. There is no apparent structural difference between the two synergids. Possibly each synergid is equally capable of receiving the pollen tube (Mogensen, 1972). Just before fertilization the synergid which receives the pollen tube gets degenerated. Recently Jensen et al. (1977) while studying the embryo sac development of unfertilized cultured ovules of Gossypium hirsutum reported that one of the synergids shows degenerative changes after two days in culture similar to those reported in fertilized ovules induced by presence of pollen tube (Jensen, 1965b). These changes are the collapsing of vacuole, increase in electron density followed by an irregular darkening and thickening of membranes surrounding mitochondria and plastids, a swelling of rough endoplasmic reticulum and finally the disappearance of the membranes of rough endoplasmic reticulum and plasmalemma. Starch persists in the degenerate plastids during these changes.

The Egg Cell.

Ultrastructurally the egg of the angiosperm appear more variable than the synergids. The cytoplasm of Gossypium hirsutum (Jensen, 1965b) and Petunia hybrida (van Went, 1970b) is highly vacuolated with few mitochondria, amyloplasts and dictyosomes. Ribosomes are few in number and not aggregated into elaborate polysomes. Endoplasmic reticulum is also scant and lie nearer to plasma membrane. But the egg of Epidendrum scutella (Cocucci and Jensen, 1969a) has numerous small vacuoles around a centrally located nucleus , but there is apparent lack of dictyosomes. The mature egg of Zea mays (Diboll, 1968) also has relatively few, small vacuoles and a rich, dense cytoplasm filled with numerous mitochondria, plastids and possess many dictyosomes unlike Epidendrum scutella. The mature egg of Capsella bursa-pastoris

(Schulz and Jensen, 1968b) and Linum usitatissimum (Vazart, 1969) are more or less intermediate in having prominent vacuoles but the cell is not as highly vacuolate as it is in Gossypium hirsutum. In the mature egg of Capsella bursa-pastoris (Schulz and Jensen, 1968b) the plastids are aggregated around the nucleus, the cytoplasm is packed with ribosomes with little or no aggregation into polysomes, numerous mitochondria, few dictyosomes and scanty endoplasmic reticulum. The egg of Quercus oambelii (Mogensen, 1972) seldom has plastids, but contain numerous mitochondria, ribosomes both free and attached to endoplasmic reticulum and characteristic lipid bodies. In Capsella bursa-pastoris (Schulz and Jensen, 1968b) egg and central cell a few dense lipid bodies appear in small groups which are used up in early stages of development following fertilization, and these represent early stages of the larger, less dense lipid bodies seen in zygote and early embryo. The egg and central cell of Capsella bursa-pastoris always have more starch and lipid than in the synergid while in Gossypium hirsutum synergids store larger amounts of starch than the egg cell (Schulz and Jensen, 1968c). In the egg cell of Linum usitatissimum (Vazart, 1969) and Linum catharticum (D'Alascio-Deschamps, 1973), plastids, endoplasmic reticulum and lipid droplets are concentrated around the nucleus with the ribosomes occupying a thin layer of cytoplasm surrounding the vacuole. Woodcock and Bell (1968) reported cup-shaped organelles resembling plastids, ribosomes, helical polysomes in the egg of Myosurus minimus. As in synergids the egg is also surrounded by a wall at the micropylar end, and either no wall at the chalazal end or a wall which is honeycomb-like in appearance. Only the egg of Epidendrum scutella (Cocucci and Jensen, 1969a) has a thick wall around it. The absence of wall presumably facilitate the entry of sperm during fertilization and also help in maintaining contact with the nutritive substance of the central cell.

The Central Cell.

The wall around the central cell is quite complex and variable. The wall is generally smooth as in Gossypium hirsutum (Jensen, 1965b) but in Capsella bursa-pastoris (Schulz and Jensen, 1973) the central cell has extensions from the wall that are like small filiform apparatus projections. The central cells of some other plants also have massive membrane-lined wall projections (Vazart and Vazart, 1966; Newcomb, 1973a; Newcomb and Steeves, 1971; Newcomb and Fowke, 1973; Berger and Erdelska, 1973) which strongly resemble the "transfer cells" described in various plant parts (Pate and Gunning, 1972). These wall ingrowths provide an efficient means of absorption and translocation of metabolites from the nucellus and integuments to the embryo sac.

The cytoplasm of central cell contain reserve food materials in the form of starch grains, protein bodies and lipids (Jensen, 1965c; Diboll, 1968; Diboll and Larson, 1966; Newcomb, 1973a; Schulz and Jensen, 1973). The central cell is metabolically very active as the cytoplasm is rich in endoplasmic reticulum and ribosomes and contain numerous well developed mitochondria and chloroplasts (or amyloplasts) and dictyosomes in some cases like Gossypium hirsutum (Jensen, 1965c), Zea mays (Diboll, 1968; Diboll and Larson, 1966), Epidendrum scutella (Cocucci and Jensen, 1969a), Linum usitatissimum (Vazart, 1969; Vazart and Vazart, 1966), Petunia hybrida (van Went, 1970b), Helianthus annus (Newcomb, 1973a) and Capsella bursa-pastoris (Schulz and Jensen, 1973). In the central cells of some species the organelles glyoxysomes (in which the enzyme of glyoxylic cycle are localized) have been reported (Newcomb, 1973a; Schulz and Jensen, 1973) suggesting their role in nutrition possibly by supplying required energy to the egg to be used for absorption of nutrients.

The Antipodals.

The ultrastructure of antipodals have been studied in few plants as they are often not present in the mature embryo sac as they usually get obliterated before fertilization as in Gossypium hirsutum. Three antipodals in Capsella bursa-pastoris (Schulz and Jensen, 1971) remain intact and their cytoplasm is packed with dense ribosomes but contain relatively few organelles like mitochondria, plastids(cup shaped), little endoplasmic reticulum and virtually no recognizable dictyosome suggesting that these cells are not highly synthetic and have no specific nutritive function. In Zea mays (Diboll, 1968) the antipodals continue to divide for some time, thus increasing in number up to about twenty and they have a regular developmental sequence through which they progress. These antipodal cells in Zea mays have a well developed endoplasmic reticulum and a large number of active mitochondria and dictyosomes suggesting that these antipodals may have a nutritional function similar to that of the synergids (Diboll, 1968). The presence of digitate wall projections on the inner face of antipodals walls bordering the nucellus in Zea mays (Diboll, 1968) also suggests their role in nutrition of embryo sac. The antipodals in Epidendrum scutella (Cocucci and Jensen, 1969a) are small with relatively thick walls and a dense cytoplasm filled with ribosomes. The antipodals of Helianthus annus (Newcomb, 1973a) after their differentiation embark on a synthetic phase characterized by an increase in nucleic acid and organelle components of the cytoplasm. Moreover the presence of plasmodesmata in their end walls facing the central cell, suggest that material synthesized in the antipodals are probably channelled to central cell and egg apparatus preparing for fertilization.

The Zygote.

The ultrastructure of the zygote either changes dramatically (Jensen, 1968a; Schulz and Jensen, 1968b; Diboll, 1968) or slightly (van Went, 1970) during the early development. The zygote nucleus in Gossypium hirsutum (Jensen and Fisher, 1967) has only two nucleoli, one from the egg and one from the sperm. In Capsella bursa-pastoris the nucleolus of sperm fuses with that of the egg so that the zygote has only a single new nucleolus (Schulz and Jensen, 1968b). After fertilization the size of the vacuole decreases temporarily, but the size of the zygote remains approximately the same in most plants like Zea mays (Diboll, 1968), Epidendrum scutella (Cocucci and Jensen, 1969b), Petunia hybrida (van Went, 1970c), Quercus gambelii (Mogensen, 1972). But in the case of Gossypium hirsutum (Jensen, 1968a) the size of the zygote is reduced to almost half the size of the egg. A similar shrinkage of zygotes (self fertilized) to about 50% of the volume of the unfertilized egg has also been reported in Hibiscus costatus and to about 20% in hybrid zygotes (Ashley, 1972). Such zygote shrinkage is related to the fact that the egg is highly vacuolate cell, and such shrinkage is based on presumed osmotic gradient induced by the initial rapid growth of the endosperm that would cause water to move from the vacuole of the zygote to the endosperm (Jensen, 1968a). Zygote undergoes extensive reorganization during this shrinkage period. This shrinkage and rearrangement of the zygote seems important, not as preparation for subsequent division, but for differentiation of the developing embryo as Ashley (1972) has shown that if the zygote of Hibiscus costatus fails to shrink in size, a haphazard pattern of cell divisions results, which causes the embryo to abort. The egg cell in unfertilized cultured ovules of Gossypium hirsutum (Jensen et al, 1977) does not shrink or divide or undergo the structural changes

characteristic of the Gossypium hirsutum zygote (Jensen, 1968a). In Capsella bursa-pastoris (Schulz and Jensen, 1968b) the zygote is slightly smaller than the egg which soon increases in length before it divides as the small micropylar vacuole begins to enlarge. In Gossypium hirsutum (Jensen, 1968a) very conspicuous changes take place in the zygote as at the chalazal end organelles like plastids, mitochondria, endoplasmic reticulum and ribosomes get segregated and soon rearrange themselves in a ring around the nucleus. In the eggs of Capsella bursa-pastoris (Schulz and Jensen, 1968b) and Epidendrum scutella (Cocucci and Jensen, 1969b), the differences in organelles distribution before and after fertilization are not so clear. The zygote of Quercus gambelii (Mogensen,1972; Singh and Mogensen, 1975) in contrast to others like Zea mays (Diboll, 1968), Gossypium hirsutum (Jensen, 1968a), Capsella bursa-pastoris (Schulz and Jensen, 1968b), Epidendrum scutella (Cocucci and Jensen, 1969b), Petunia hybrida (van Went, 1970c), and Hordeum vulgare (Norstog, 1972), is almost devoid of plastids and starch. It is in zygotes of most species that mitochondria reach their highest degree of differentiation. But it is not clear as what factors in terms of enzymes, substrate or precursors in the fertilized egg cause mitochondria to divide and elaborate more cristae.

Starch and Lipid in the Zygote.

Most of the plastids become concentrated in a shell around the outside of nuclear membrane in the zygote of most plants that have been studied. The number of starch grains in them increase markedly. The presence of starch in the zygote of Gossypium hirsutum and Capsella bursa-pastoris and its gradual depletion during the early stages of embryogeny has been used to implicate the participation of starch in

the nutrition of the zygote and early embryo. But we have not yet been able to account clearly for the storage of starch in the plastids of the egg following fertilization. A small number of lipid bodies occur already in the zygote of Capsella bursa-pastoris (Schulz and Jensen 1968b), Petunia hybrida (van Went, 1970c), Quercus gambelii (Mogensen, 1972), and Linum catharticum (D'Alascio-Deschamps, 1973) and their number increases. An increase in lipid bodies after fertilization is a feature not observed in other plants and very little changes after fertilization are noticed in these plants. In Capsella bursa-pastoris these lipid bodies are larger (0.5 μm) and less dense than those in the egg and occur in aggregate of up to 35 droplets in the zygote, but the number of lipid droplets reaches a peak in the terminal cell in the 3-celled embryo and rapidly decreases with the subsequent divisions. Lipids in suspensor and basal cells persist longer than in terminal cells (Schulz and Jensen, 1968c). In Quercus gambelii (Mogensen, 1972), however, the number of lipid bodies increases considerably and which is retained through the early globular stage until the lipid bodies disappear at the late globular stage. Since the Quercus gambelii zygote is devoid of starch, these lipid bodies might play a role in the nutrition of the zygote and early embryo, although the most active utilization seems to be at the late globular stage.

Ribosomes in the Zygote.

Ribosomes are free in the cytoplasm of mature eggs of Gossypium hirsutum (Jensen, 1968c), Capsella bursa-pastoris (Schulz and Jensen, 1968b), Epidendrum scutella (Cocucci and Jensen, 1969b), and Linum usitatissimum (Deschamps, 1969), but after fertilization they strikingly group to form polysomes. Thus aggregation of the ribosomes is

first seen within the cytoplasm of the zygote and increase in polysome is considered to be an important feature in the formation of zygotes as large polysome complexes have been found in the zygotes of most plants that have been studied. In Gossypium hirsutum, Jensen (1968c) reported striking changes in ribosomal aggregation and distribution at time of fusion of egg and sperm nuclei, when ribosomes becomes arranged into helical polysomes containing as many as 30-40 individual ribosomes and appear associated with the membranes of plastids and occasionally mitochondria. Additional ribosomes, a second generation of ribosomes, appear in the zygote before its division but then the helical polysomes gradually disappear and additional ribosomes appear as individual ribosomes or as small polysomes and exist side by side with large polysomes. These polysomes can be traced through the early stages of embryo development and are present in the young globular embryo but disappear at the differentiation of the heart stage. In Zea mays (Diboll, 1968), however, less striking changes are reported in the zygote as helical polysomes present in the egg increase in length after fertilization. In Capsella bursa-pastoris (Schulz and Jensen, 1968b) polysomes appear after fertilization although in the early zygote they are less conspicuous, but ribosomal aggregation become more prominent in the late zygote and two-celled embryo. In Quercus gambelii, however, no polyribosomal changes were recorded by Mogensen (1972) in any part of the egg apparatus either before or after fertilization.

Endoplasmic reticulum in the Zygote.

An unusual type of endoplasmic reticulum which contains numerous tubules is found in Gossypium hirsutum (Jensen, 1968b) during the period of zygote shrinkage but before it divides. Such tube-containing

endoplasmic reticulum is also found in small amounts in the egg (Jensen, 1965). These endoplasmic reticulum tubules which are slightly larger in diameter than the microtubules found free in the cytoplasm of the zygote nearer the periphery are wavy in appearance and branch and fuse. They are seen connected with the membrane of the endoplasmic reticulum and have even been seen in the nuclear membrane. Such endoplasmic reticulum tubules have not been found in other zygotes of plants studied with the electron microscope and no function could be ascribed to these microtubules in the endoplasmic reticulum. A marked increase in the amount of endoplasmic reticulum after the fertilization was reported in Gossypium hirsutum (Jensen, 1968b) and Zea mays (Diboll, 1968). Mogensen (1972) did not notice any marked increase in endoplasmic reticulum in Quercus gambelii after the fertilization but endoplasmic reticulum changes from rough to mostly smooth type.

The dictyosomes become active and a wall around the entire zygote is formed. The synthesis of additional cell material at the micropylar end and elaboration of a new cell wall at the chalazal end usually take place as a result of increased activity of dictyosomes reported upon fertilization of eggs of Gossypium hirsutum (Jensen 1968a) and Capsella bursa-pastoris (Schulz and Jensen, 1968b).

Two-Celled Embryo.

With very few exceptions the first division of the angiosperm zygote is transverse and unequal and results in the formation of a larger vacuolate basal and a smaller densely cytoplasmic terminal cell (Maheshwari, 1950). The small terminal cell, in most cases produce the embryo proper through repeated cell divisions, while the large basal cell divides to form the long suspensor. Schulz and

Jensen (1968b) reported more pronounced aggregation of ribosomes and more amount of starch and lipid in two-celled embryo than the zygote in Capsella bursa-pastoris. In most plants studied, the histochemical staining shows that the small terminal cell stains darkly for protein and nucleic acids while the larger basal cell stains relatively lighter (Jensen, 1968a). But in Capsella bursa-pastoris (Schulz and Jensen, 1968b) a higher concentration of protein and nucleic acids was noticed in the basal cell than in the terminal cell for a short period, but the situation is soon reversed after the next division of the basal cell.

There are marked differences in the electron density of the two cells at the ultrastructural level too, since the terminal cell appears to contain more ribosomes than the basal cell. In Capsella bursa-pastoris (Schulz and Jensen, 1968b) the degree of aggregation and ribosomal density is the same in both cells. Singh and Mogensen (1975) reported a very dense cytoplasm with high concentration of cytoplasm ribosomes in the basal cell of two-celled embryo of Quercus gambelii. The terminal cell also contains less endoplasmic reticulum than the basal cell in most plants studied and in Gossypium hirsutum (Jensen, 1968a) most of the tube containing endoplasmic reticulum is found in basal cell rather than the terminal cell but in Capsella bursa-pastoris the amount of endoplasmic reticulum remains about the same in both cells as it was in the zygote (Schulz and Jensen, 1968b). The nucleus of the terminal cell which is smaller than the basal cell nucleus, in most cases, is surrounded by a shell of plastids and mitochondria, while the arrangement in the basal cell is more usual. In Capsella bursa-pastoris (Schulz and Jensen, 1968b) the plastids and starch and lipid bodies are more abundant in the basal cell while the greater number of plastids and mitochondria has been reported in the terminal cell

of Gossypium hirsutum (Jensen, 1963) and Quercus gambelii (Singh and Mogensen, 1975). Both cells have many small vacuoles. The vacuoles in the basal cell enlarge considerably restricting the cytoplasm to the thin peripheral area. Many wall ingrowths which increase in size and number with the development of the embryo are also found at the micropylar end of the basal cell (Schulz and Jensen, 1968b). These wall projections increase the absorptive surface and have numerous mitochondria and dictyosomes associated with them (Schulz and Jensen, 1968b, 1969). These ingrowths strongly resemble those described in the transfer cells of Pate and Gunning (1972). In most plants studied, many plasmodesmata appear on the end wall separating the terminal and basal cell and these plasmodesmatal connections maintain the cytoplasmic continuity between the basal cell and embryo proper.

Three-Celled Embryo.

The basal cell divides next to give rise to a 3-celled linear embryo, the outermost cell of the three is suspensor cell. The terminal cell of Capsella bursa-pastoris (Schulz and Jensen, 1968b) stains slightly more intensely for protein and nucleic acids than the suspensor or basal cell as a result of increase numbers of ribosomes and thus terminal cell appears more electron dense in the 3-celled embryo. Schulz and Jensen, (1968c) have also reported in Capsella bursa-pastoris, the increase in size and number of wall projections at the micropylar end of the basal cell and also on lateral walls of basal cell; and like most other plants the more abundant endoplasmic reticulum with wider cisternal phase in the suspensor and basal cell than in the terminal cell. However, in Gossypium hirsutum (Jensen, 1963) endoplasmic reticulum of embryo cells contains tubes. The starch is equally abundant in all the cells of the embryo of Capsella

bursa-pastoris and reaches a maximum in the embryo at this stage; lipid droplets reach a peak in the terminal cell of three-celled embryo of Capsella bursa-pastoris and rapidly decrease with subsequent divisions. Schulz and Jensen (1968c) give the following account of embryogeny in Capsella bursa-pastoris. The first division of terminal cell is in longitudinal plane (onagrad-type, Johansen, 1945 or crucifer-type, Maheshwari, 1950 embryonomy). The formation of longitudinal wall is associated with increased dictyosomal activity and the incorporation of small vesicles. Plastids are still oriented around the nucleus but with marked decrease in the starch contents. Starch and lipid deposits which accumulate in the terminal cell disappear almost completely after its first division. Starch is resynthesized and reappears in almost mature embryos, while lipids appear only in the late torpedo stage of development. The accumulation of starch in the terminal cell and its rapid disappearance during the first 2 divisions of the cell suggest its use as a necessary energy supply for the first critical division of the embryo. Lipid metabolism follows a similar pattern and therefore may also act as an energy reserve for the first cleavage of the terminal cell. Jensen (1968a) reported in Gossypium hirsutum that abundant starch in the zygote surrounding the nucleus decrease as the zygote divides and after subsequent divisions the amount of starch per cell and size of starch grains decrease further until at the full globular stage where starch could not be detected. In Quercus gambelii, Singh and Mogensen (1975) reported marked appearance of plastids which are mostly undifferentiated, having electron-dense matrix at two-celled stage of embryo which gradually increase in number through the 6-12 celled stage. Plastids are lobed and generally contain starch grain. A number of lipid bodies occurs in the zygote are retained through the early globular stage until they finally disappear in the late globular stage. Lipids are

probably more important as a nutrient source in the zygote and early embryo, while starch becomes more important at a late stage in the case of Quercus gambelii. Mitochondria are numerous and appear active at all stages. Dictyosome and endoplasmic reticulum are in abundance (Singh and Mogensen, 1975).

The Globular Embryo.

Once again, the detailed ultrastructural study of embryo development is that of Capsella bursa-pastoris where the embryo development was studied from the terminal cell of the 3-celled embryo through a globular embryo consisting of some 32-64 cells. In this case the terminal cell divides further to give quadrant, octant,16- and 32- and 64-celled embryo. Cell size and number of organelles per cell decrease with repeated divisions through the formation of the globular embryo and then remain relatively constant through the formation of the torpedo stage in Capsella bursa-pastoris (Schulz and Jensen, 1968c). This is also true for Gossypium hirsutum (Jensen, 1963) embryo, where the cell size is drastically reduced during early embryogenesis. At the early globular stage the embryo of Capsella bursa-pastoris stains intensely for protein and nucleic acids, but surprisingly there is no apparent ultrastructural and histochemical difference between the component cells of embryo (protoderm, procambium and ground meristem) until the formation of heart-shaped embryo although some of these now constitute protoderm (Schulz and Jensen, 1968c). The cells at this stage contain few dictyosomes and little endoplasmic reticulum but are rich in ribosomes grouped into small polysomes. Same is true of Gossypium hirsutum (Jensen, 1963) but the large polysomes that were present in the zygote persist through the first several generations of cell divisions and then gradually disappear. The density of ribosomes is

greater in the cells of embryo than in those of suspensor at the octant and later stage. High amount of dictyosomes are reported in Quercus gambelii (Singh and Mogensen, 1975) at this stage.

Heart-Shaped Embryo.

In the globular embryo of dicotyledons, localised cell division at two centres of growth result in outgrowth of two cotyledon primordia. In this heart-shaped stage in Capsella bursa-pastoris the cells are densely packed with ribosomes. The whole embryo also stains intensely for protein and nucleic acids. The cells at the tip of cotyledons even stain more intensely for nucleic acids and proteins. The embryo becomes green at this stage. Ultrastructural differences are noticeable between the component cells at this stage. The plastids in the cells of ground meristem and protoderm begin to form fused lamellar system by invagination of inner membrane. Plastids in procambium remain relatively undifferentiated (Schulz and Jensen, 1968c). The cells of procambium and ground meristem are more vacuolate than those of the protoderm. There is an increase in the plasmodesmata in the end wall of suspensor cell at this stage.

As the embryo develop beyond the heart-shaped stage, the number of ribosomes increase, and they become less aggregated and more random in orientation. The significance of change in ribosome pattern beyond the heart-shaped stage, from an aggregated one to a random one similar to that in egg, is not clear (Schulz and Jensen, 1968c). In addition to the increased density of the ribosomes at this stage the cell matrix also becomes very dark in Capsella bursa-pastoris.

Most of the ultrastructural studies of the developing embryo which passes successively through globular, heart-shaped, intermediate, torpedo-shaped, walking-stick-shaped, inverted U-shaped and mature

stages have been restricted up to the classical heart-shaped stage of the embryo. Many other workers have been more interested in the development of food reserves and have therefore, restricted their attention to later stages of development after the maturity of the embryo. Few attempts have also been made to study the desiccation physiology of the embryo and such studies are restricted mainly to dry embryo. Few attempts have been made to study the ultrastructure of cytological changes after the classical heart-shaped stage up to the beginning of the maturity of the embryo.

The Basal and Suspensor Cell.

The ultrastructural study of the basal and suspensor cell has remained neglected and received only passing mention by most workers who have concentrated on the ultrastructure of cotyledons. One series of studies has been carried out on Capsella bursa-pastoris (Schulz and Jensen, 1968c, 1969) which is the traditional material for plant embryologists. In this plant the basal and suspensor cells divide further forming a suspensor up to ten cells which connects the developig embryo at chalazal end of the suspensor to the large basal cell at its micropylar end. The basal cell is much larger than the other cells and contains a huge vacuole bordered by a thin, peripheral cytoplasm. The chalazal end walls of the basal cell is perforated by many endoplasmic reticulums containing plasmodesmata which maintain continuity between the cytoplasm of the basal cell and the suspensor. The micropylar wall projections which begin to develop in the zygote and young embryo (Schulz and Jensen, 1968b) increase in size and number and appears to be a smaller version of the filiform apparatus of the synergids (Schulz and Jensen, 1968a) as both structures have

a structural similarity in having a central electron-dense core bordered by electron-transparent periphery, numerous mitochondria and dictyosome and as both increase the absorptive surface they are clearly the transfer cells of Pate and Gunning (1972). Many large, round or rod-shaped or cup-shaped plastids are present around the nucleus of young basal cell. These plastids have a dense matrix, single lamellae, osmiophilic droplets and frequently contain starch. But in the older basal cell at globular embryo stage when cytoplasm matrix of the cell darkens, the nucleus becomes deeply lobed and the plastids get concentrated between the lobes of the nucleus and have many vesiculate lamellae but no starch. In similar basal cells of other species like Phaseolus coccineus (Clutter and Sussex, 1968), Phaseolus vulgaris (Nagl, 1969) and suspensor cells of Phaseolus coccineus (Nagl, 1974) and Tropaeolum majus (Nagl, 1976) it has been shown that the nuclei are highly polyploid and chromosomes actually seem to be in polytene situation. From the general size and staining of the nucleus of the basal cell of Capsella bursa-pastoris, Jensen (1974) also concluded reasonably that it too is highly polyploid. The basal cell remains active after the suspensor cell cytoplasm has degenerated. In Quercus gambelii (Singh and Mogensen, 1975) the basal cell with large vacuole on micropylar end has numerous mitochondria and plastids near the nucleus and electron dense lipid bodies in the parietal cytoplasm. These plastids have no thylakoid lamellae but contain a single starch grain.

The cells of the suspensor in Capsella bursa-pastoris (Schulz and Jensen, 1969) are conspicuosly more vacuolate and contain more endoplasmic reticulum and dictyosomes, but fewer ribosomes and stain less intensely for protein and nucleic acid than those of the embryo. Numerous plasmodesmata are reported on the end walls of the suspensor cell while there are no plasmodesmata in the walls separating the

suspensor from the embryo sac. The lower suspensor cells fuse with the embryo sac wall, and a lateral wall of the lower and middle suspensor cell produce finger-like extensions into the endosperm (Schulz and Jensen, 1974). The rod-shaped plastids with single vesiculate lamellae and electron dense matrix containing osmiophilic droplets and possibly starch encircle the nucleus and often constricted in the middle and appear to be dividing. Mitochondria are rod-shaped having short vesiculate cristae and also appear to be dividing. At the heart stage of embryo development the suspensor cells begin to degenerate and gradually lose their ability to stain for protein and nucleic acid, but plastids show no sign of degeneration and stain intensely for protein but starch completely disappears. These cells still have many mitochondria which appear intact, but lipid bodies are completely absent. Schnepf and Nagl (1970) examined the ultrastructure of young and old suspensor cells of Phaseolus vulgaris which also show wall protuberances, highly endopolyploid nuclei and plastids of varying, often bizarre shapes showing unusual internal structures. These cells also contain large amounts of smooth tubular endoplasmic reticulum. Marinos (1970)also reported the abundant occurrence of unusual types of plastids in the suspensor cell of Pisum sativum. These plastids contain spherical bodies consisting of intertwined bundles of tubules and these tubular complexes are not typical prolamellar bodies as the arrangement of tubules is quite different to any reported form of prolamellar bodies and moreover no transformation of this complex into grana-like structure could be observed. Marinos (1970) reported the resemblance of this tubular complex of the plastids with the structure of protein-P in developing phloem cells (Cronshaw and Esau, 1967) (although in the latter case the tubular aggregations are found free in cytoplasm and not within organelles) and concluded that these plastids may function as accumulation sites of specific proteins that are subsequently

utilized in the growth of the embryo. The function of suspensor plastids in the nourishment of the embryo has also been envisaged in the recent years for Ipomoea purpurea (Ponzi and Pizzolongo, 1973), Helianthus annus (Newcomb, 1973b), Stellaria media (Newcombe and Fowke, 1974), and Tropaeolum majus (Nagl and Kuhner, 1976). Plastids with such unusual internal structures have also been reported in Phaseolus vulgaris (Schnepf and Nagl, 1970) and Ipomoea purpurea (Ponzi and Pizzolongo, 1972).

In Tropaeolum majus (Nagl and Kuhner, 1976) the early embryogenesis is characterised by the development of a large, tripartite suspensor many times larger than the embryo proper, and storing cotyledons. This suspensor differs from that in other species with the suspensor-cotyledo way of development (Nagl, 1976) in as far as it is a highly differentiated organ that develop two haustoria (carpel and placental haustorium) into the carpel tissue. The organization of plastid in this suspensor is quite different. Chloroplast which rarely form the grana occur in the elongated cells of embryo-suspensor as well as suspensor cells adjacent to the embryo proper and thus the embryo-suspending thread of the suspensor are green. Leucoplasts with an electron-dense matrix and electron-transparent tubes occur in the basal cell mass of embryo-suspensor. Undifferentiated leucoplasts occur in carpel haustorium, which get transformed into electron-dense plastids during autolysis of the suspensor. Etioplasts develop in several cells of placental haustorium at late heart-like stage while during earlier stages undifferentiated leucoplast are reported from placental haustorium of the suspensor.

Singh and Mogensen (1975) have also given a brief account of the ultrastructure of suspensor cell in Quercus gambelii embryo which is similar to that of the basal cell. Khera and Tilney-Basset (1976) have investigated in detail the subcellular structure of suspensor along

with cotyledons and radicle in Pelargonium X Hortorum from heart-shaped embryo stage up to maturity. These suspensor cells have basically the same ultrastructure as that of the suspensor cells of Capsella bursa-pastoris, and the basal cells of earlier embryo. The suspensor haustorium of Pelargonium X Hortorum at three week stage when embryo is fully formed, show big lipid droplets, unexpectedly minute mitochondria (half the diameter of typical cotyledon and radicle cell mitochondria) which have very long cristae which cross the centre of the organelle. On the other hand chloroplasts are mostly elongated with as many as 40 thylakoids comprising the large grana. Some of the plastids have abnormally large osmiophilic deposits.

The ultrastructural evidence supports the view that both the suspensor and the basal cell function actively in absorption and transport of nutrients from endosperm and integuments. The suspensor can be regarded as a specialized transport mechanism for the embryo which, on the basis of the presence of wall ingrowths in Capsella bursa-pastoris increases the efficiency of absorption from endosperm and other nutritive tissues rejecting the traditional view that the suspensor pushes the embryo into the developing endosperm and thus aids the absorption of material from the endosperm by the embryo. Recent studies on Phaseolus coccineus (Alpi et al., 1975) shown that suspensor plays a role in embryogenesis by acting as a site of synthesis of growth regulators especially gibberellins and their transport to the embryo where these are needed. This view has been strongly supported by the in vitro experiments on Phaseolus coccineus embryos (Cionini et al., 1976).

The suspensor cells retain the potentiality for growth. When the developing embryos of Eranthis hiemalis (Haccius, 1965) were selectively killed in an experiment the surviving suspensor cells regenerated

a new embryo. The formation of more than one adventive embryo in this way was reported.

Maturing and Quiescent Embryo. (Desiccation Physiology of the Embryo).

After fertilization the ovule undergoes changes leading towards the development of the seed. The stages in seed formation mainly consist of 1. synthesis of storage reserves and 2. maturation dormancy (Bain and Mercer, 1966). Some seeds store reserve food material during ripening as well as during maturation stage, whereas other seeds store all the food material during the ripening phase. However, activity of most seeds ceases at maturity at a critical tissue moisture level. The moisture content, which in young developing embryo may be as high as 80-85%, may drop below 10% during maturation. It is imperative to argue that desiccation (dehydration) of seeds beyond a particular moisture level might inhibit protein synthesis in the embryo. Inhibition in the synthesis of protein during moisture stress is assumed to be due to the inactivation or loss in the structure of protein synthesizing units (Klein and Pollock, 1968). Restriction in moisture availability during the maturation phase of the seeds determines the disappearance of the short-lived mRNA, which is necessary for binding monosomes to polysomes. The other possibility exists that degradation of polysomes may be induced by moisture stress (Nir et al, 1970). Polysome loss during plant desiccation is not due to ribonuclease (Dhinsa and Bewly, 1976).

As a matter of fact, the seeds of almost every flowering plant, experience the impact of dehydration during maturation phase and thus pass through a phase of a very low physiological activity which coincides with a period of severe desiccation. Such a drastic dehydration other than during maturation phase of seed, proves deleterious to plants, because even a mild water-stress in plants causes destruction of cell structures and metabolic processes (Nir et al, 1970). If the embryo is dried quickly at the ripening phase, it will not survive

the effect of desiccation (Klein and Pollock, 1968). If the embryonic axis is intact with the seed during maturation, the same level of desiccation is tolerated with a potentiality to resume germination. It is assumed that the embryo acquires a strong resistance to desiccation prior to the loss of moisture. Klein and Pollock (1968) suggested that cessation of physiological activities in the maturing seeds is a pre-requisite for the embryos to become resistant towards desiccation rather than water loss being the cause of seed inactivity. This limited information on the maturation of seed embryo does not provide clarity on the physiological and structural changes in the embryo during different phases of seed ripening and maturity.

A study of dormant or quiescent embryo is of interest because they have a minimal rate of metabolism and are highly resistant to adverse conditions of the environment (Paulson and Srivastava, 1968). It is pertinent to enquire, if there is any structural basis for these qualities. Little is known about the ultrastructure of dormant embryos owing to the technical difficulties of preparing and sectioning desiccated storage tissue of seed in a manner appropriate for electron microscopy.

A number of ultrastructural studies have been made of dormant seed structures and the subsequent structural changes that occur during the germination processes. Some of the important studies are presented below in the tabulated form.(Table 2).

TABLE 2. Ultrastructural studies of mature embryo and germinating seed

Species.	References.	Notes.
Arachis hypogea (Papilionaceae)		
	Bagley *et al*, 1963.	Cotyledons from seeds (unsoaked and soaked) and seedings from resting through various phases of germination (protein bodies).
Avena sativa (Gramineae)		
	Hinchman, 1972.	Shoot tissue (coleoptile plus the enclosed leaf) plastids.
Bidens cernua (Compositae)		
	Simola, 1971.	Cotyledon and radicle at the resting stage (protein bodies).
Bidens radiata (Compositae)		
	Simola, 1969.	Cotyledons at the resting stage (protein bodies, spherosomes and chloroplasts).
Carthamus tinctorus (Compositae)		
	Englebrecht and Weier, 1967.	Chloroplast development in cotyledons of mature seeds and during germination in dark and light.
Clarkia elegans (Onagraceae)		
	Dengler, 1967.	Radicle and hypocotyl (axis) at the resting stage (seed maturation).
Crambe abyssinica (Cruciferae)		
	Smith, 1974.	Cotyledons at the resting stage (spherosomes and oil bodies).

Cucumis sativus (Cucurbitaceae)	
Butler, 1967.	Cotyledons during aging and senescence.
Trelease *et al.*, 1971.	Cotyledons during various phases of early germination in light and dark.
Cucurbita maxima (Cucurbitaceae)	
Lott, 1970.	Cotyledons through various phases of germination (plastids and chlorophylls).
Lott *et al.*, 1971.	Cotyledons from dormant seed (protein bodies).
Harnischfeger, 1973.	Cotyledons during aging and senescence (chloroplast degradation).
Glycine max (Papilionaceae)	
Treffry *et al.*, 1967.	Cotyledons during early germination and subsequent seedling growth.
Gossypium hirsutum (Malvaceae)	
Yatsu, 1965.	Cotyledons at the resting stage (dormant seed).
Engleman, 1966.	Cotyledons of maturing seed (aleurone grains).
Hordeum vulgare (Gramineae)	
Nieuwdorp, 1963.	Scutellum- epithelial cells.
Nieuwdorp, 1964.	Scutellum- epithelial cells during germination.
Paleg and Hyde, 1964.	Mature dry aleurone cells from aleurone segments (by removing the embryo and basal end of endosperm).
Jones, 1969.	Aleurone cells from aleurone layers of dry and imbibed seeds.
Abdul-Baki and Baker, 1971.	Floret parts (thin slices).

Kochia childsii (Chenopodiaceae)	
Marin and Dangler, 1972.	Cotyledons of dry embryo (granal plastids).
Lactuca sativa (Compositae)	
Paulson and Srivastava, 1968.	The radicle, hypocotyl, shoot apex and cotyledons of dry embryo.
Srivastava and Paulson, 1968.	Embryo- from resting through various phases of germination.
Oryza sativa (Gramineae)	
Öpik, 1972.	Shoot ends (coleoptiles) from dry grains.
Harris and Juliano, 1977.	Endosperm (protein bodies).
Pelargonium X *Hortorum* (Geraniaceae)	
Khera and Tilney-Basset, 1976.	Cotyledons from resting through various phases of germination (embryo with normal and mutant plastids).
Phaseolus lunatus (Papilionaceae)	
Klein and Ben-Sheul, 1966.	Rootlets and hypocotyl (seed axes) from resting through early germination.
Klein and Pollock, 1968.	Cotyledons and embryonic axes at the resting stage (seed desiccation).
Phaseolus vulgaris (Papilionaceae)	
Öpik, 1966.	Cotyledons from resting through various phases of germination.
Öpik, 1968.	Cotyledons at the resting stage (ripening seed).
Pinus banksiana (Pinaceae)	
Durzan *et al.*, 1971.	Whole embryos excised from dormant and germinating seeds

Mia and Durzan, 1974.	Shoot apical meristem in dry and germinating embryos.
Pinus niora (Pinaceae)	
Nikolic and Bogdamovic, 1972.	Cotyledons of dark grown seedlings (plastid differentiation).
Pinus sylvestris (Pinaceae)	
Simola, 1974.	Cotyledons, rootlet and endosperm from resting through various phases of germination.
Pisum sativum (Papilionaceae)	
Varner and Schidlovsky, 1963.	Cotyledons (protein bodies).
Perner, 1965a, b.	Radicle and hypocotyl cells from air dried dormant seeds. A true study of dry seed.
Setterfield *et al.*, 1959.	Whole embryo moistened on damp filter paper for 4 hours.
Bain and Mercer, 1966.	Developing cotyledon up to maturity.
Perner, 1966.	Radicle and hypocotyl from resting through various phases of germination.
Yoo, 1970.	Radicle from dormant and soaked seeds, and during germination.
Mollenhauer and Totten, 1971.	Cotyledons during late maturation and early germination (lipid vesicles).
Secale cereales (Gramineae)	
Hallam, 1972.	Mature dry embryo (lateral root primordia with coleorhiza).
Hallam *et al.*, 1972.	Root primordia during resting and germination stages.
Hallam *et al.*, 1973.	Root primordia of non- viable embryos (unimbibed and wetted).

Setaria lutescens (Gramineae)	
Rost, 1972.	Caryopsis (embryo- dormant, non-dormant and endosperm) (protein bodies and lipids).
Sinapis alba (Cruciferae)	
Rest and Vaughan, 1972.	Cotyledons at the resting stage (protein and oil bodies).
Werker and Vaughan, 1974.	Hypocotyl and cotyledons, changes during germination (aleurone and myrosin cells).
Triticum aestivum (Gramineae)	
Swift and O'Brien, 1972.	Scutellum of air dried grains.
Triticum durum (Gramineae)	
Grahm *et al.*, 1962.	Endosperm (protein bodies).
Triticum vulgare (Gramineae)	
Grahm *et al.*,	Endosperm (protein bodies).
Buttrose, 1963a.	Mature grain (aleurone cells).
Buttrose, 1963b.	Endosperm.
Nougarede and Pilet,1964.	Scutellum.
Setterfield, 1959.	Whole embryo moistened on damp filter paper for 4 hours.
Tropaeolum majus (Tropaeolaceae)	
Nougarede, 1963.	Cotyledons from resting through various phases of germination.
Vicia faba (Papilionaceae)	
Briarty *et al.*, 1969.	Cotyledons from mature seed (protein bodies).
Briarty *et al.*, 1970.	Cotyledons from resting through various phases of germination (protein bodies).

Vigna unguiculata (Papilionaceae)	
Harris and Boulter, 1976.	Cotyledons at the resting stage (protein bodies).
Yucca schidigera (Liliaceae)	
Horner and Arnott, 1965.	Ungerminated seed- perisperm and embryo tissue (seed protein).
Horner and Arnott, 1966.	Ungerminated seeds and seeds from seedlings.
Zea mays (Gramineae)	
Khoo and Wolf, 1970.	Endosperm (protein bodies).
Deltour and Bronchart, 1971.	Root cells of embryo from resting through early germination.

The remarkable feature of seed maturation is the rates of development between one tissue and another within the same seed after the cell division is completed, and these differences are accompanied by variations in the integrity of organelles and properties of membranes. For instance, according to Abdul-Baki and Baker (1970) the proplastids in the embryonic axis of developing and mature Hordeum vulgare seeds do not develop into organized chloroplasts that are capable of photosynthesis until after the seed germinates and the shoot and root resume growth. Proper light conditions are apparently required for the formation of the lamellar system. In contrast, the proplastids of the pericarp and seed coat differentiate into chloroplast which give the developing seed its green colour at the milk stage. By the time seed matures and dries, the chloroplasts of the pericarp and coat become non-functional and their membrane system break down (Abdul-Baki and Baker, 1970). Plastids in the cotyledons of Pisum sativum (Bain and Mercer, 1966), Phaseolus vulgaris (Öpik, 1968) follow a developmental pattern similar to that in the pericarp of cereals.

During early development of the various species studied, cells of the cotyledons undergo hardly any major change in organelle components. At this stage as usual they have a thin wall, well developed mitochondria, Golgi bodies, rough endoplasmic reticulum and large nucleus. The oil and protein bodies and large central vacuole is lacking at this stage. During later phase, the cells of cotyledons (Bain and Mercer, 1966; Klein and Pollock, 1968; Öpik, 1968) and endosperm (Buttrose, 1960, 1963; Abdul-Baki and Baker, 1970) show the formation of chloroplasts containing numerous tightly packed stacks of lamellae and electron-dense grana. The newly-formed chloroplasts, which lack starch at an early stage, soon appear to have one or more starch granules (late torpedo or walking-stick stage) whose size continues to increase until the cotyledons or endosperm are mature. The protein and oil

bodies also appear at about this stage. Simola (1971), however, reported small protein bodies in the cytoplasm of young cell of Bidens radiata embryo. Cells of embryonic axis also resemble cotyledons during these stages of development (Klein and Ben-Shaul, 1966)

During maturation, the moisture content drops rapidly to 10-15% while dry weight continues to increase slowly. The most striking changes in organelles have been reported in this period when metabolic activity is reduced to a very low level characteristic of the quiescent dry seed. The growth of embryonic axis remains arrested, whereas growth of cotyledons and endosperm continue during this period. Cells of embryonic axis still have thin walls, a central nucleus and large number of oil bodies aligned along the cell wall. All organelles maintain intact external membranes. But in the embryonic axis of mature Phaseolus lunatus, internal membranes of mitochondria appear poorly organised and rough endoplasmic reticulum and Golgi bodies are hard to find (Klein and Pollock, 1968). The cytoplasm remains rich in free ribosomes, protein bodies, and lipid bodies, but lacks starch grain and central vacuoles.

The Chloroplast.

According to Bain and Mercer (1966) cells of fully mature cotyledons of Pisum sativum continue to accumulate starch in plastids, protein in protein bodies, and lipid in spherosomes, as reflected by the increase in their dry weights. The increase in size of starch grain within the plastid of maturing Pisum sativum cotyledons (Bain and Mercer, 1966) leads to disruption of lamellae and by the end of this stage all that could be seen from the plastids are a few fragments of lamellae. Plastids with some lamellae have also been reported in the dry cotyledons of Gossypium hirsutum (Yatsu, 1965), root tips of

Pisum sativum (Perner, 1965). Paulson and Srivastava (1968) recognised bare (without outer membrane) plastids with little or no lamellae in dry cotyledons of Lactuca sativa. Öpik(1968) in mature dry Phaseolus vulgaris cotyledons found no plastid membrane around starch grains which are suspended in clear, vacuole-like spaces and lamellar system was very less extensive in small surviving plastids. Klein and Pollock (1968) reported that disorganization of lamellae in plastids of mature Phaseolus lunatus cotyledon does not seem to be caused by rupture of chloroplast membrane by the growing starch grains, as was suggested by Bain and Mercer to be the cause in mature Pisum sativum cotyledons.

The Mitochondrion.

Mitochondria with normal cristae have been reported in the dry embryo of Pisum sativum (Perner, 1965), Gossypium hirsutum (Yatsu, 1965) and Hordeum vulgare (Abdul-Baki and Baker, 1970). Setterfield et al. (1959) mentioned that they are recognizable in Pisum sativum and Triticum vulgare embryos. Mitochondria seem to have lost some of their internal membrane in the mature cotyledons of Pisum sativum (Bain and Mercer, 1966), Phaseolus lunatus (Klein and Pollock,1968) Phaseolus vulgaris (Öpik, 1968) and Lactuca sativa (Paulson and Srivastava, 1968). Mitochondria could not be seen in Hordeum vulgare grains (Paleg and Hyde, 1964) or Arachis hypogea seeds (Bagley et al., 1963).

The Endoplasmic Reticulum.

Endoplasmic reticulum is either missing or infrequently found, when infrequent then it appears only in short fragments in dry and mature embryos. The endoplasmic reticulum is poorly represented in dry

embryos of Pisum sativum (Perner, 1965), Gossypium hirsutum (Yatsu, 1965), Lactuca sativa (Paulson and Srivastava, 1968). Endoplasmic reticulum becomes fragmented and disperse in the cytoplasm in the form of short vesicles in drying cotyledons of Pisum sativum (Bain and Mercer, 1966), Phaseolus lunatus (Klein and Pollock, 1968), and Phaseolus vulgaris (Öpik, 1968). A disappearence or vesiculation of the endoplasmic reticulum has been reported in Pisum sativum radicle (Perner, 1965). However, Klein and Ben-Sheul (1966),in contrast, showed well-defined endoplasmic reticulum in cells of embryonic axis of Phaseolus lunatus after one hour of soaking, which reflects the situation obtained in the dry embryo. Perner (1965), however, could see abundant endoplasmic reticulum, both smooth and rough, in embryo root tissue of the dry seed of Pisum sativum fixed over several weeks in OsO_4 fumes. To our knowledge Perner's observation was the first cytological evidence of the existance of abundant endoplasmic reticulum in dry seed.

Polysomes.

Certain organelles in the embryonic axis and cotyledons of dry seeds have been referred to as "disorganized", as in the case of internal membranes of mitochondria and plastids (Paulson and Srivastava, 1968); others, such as Golgi bodies and polysomes, are missing (Yatsu, 1965; Klein and Ben-Sheul, 1966; Paulson and Srivastava, 1968; Öpik, 1968; Yoo, 1970). Thus ribosomes lie free and are generally not aggregated into polysomes; only very exceptionally chains or spirals of polysomes could be seen (Öpik, 1968). During reimbibition, synthesis of mRNA and polysomes proceed rapidly. Polysome loss during embryo desiccation is not due to ribonuclease (Dhinsa and Bewly, 1976). Thus polysomes are common in developing embryos, rare in maturing, missing in dormant or quiescent embryos and common in germinating embryos. Yatsu (1965)

speculated that stack of membranes that form the Golgi bodies get so compressed due to dehydration that they become hard to recognise. It is also possible that membranes of Golgi bodies and endoplasmic reticulum become more fragile in dry tissue and therefore, become hard to preserve through conventional procedures of fixation that were developed primarily for tissues with high moisture contents.

Storage Bodies.

Mature endosperm of Hordeum vulgare (Buttrose, 1960; Abdul-Baki and Baker, 1970) and Triticum vulgare and Triticum durum (Graham et al., 1962; Buttrose, 1963) exhibit a more advanced degree of senescence than mature cotyledons of Pisum sativum, Phaseolus vulgaris and Phaseolus lunatus. Except for a layer of aleurone cells (2-3 cells thick) which remain intact by late maturation, cells of this tissue become filled with starch and, to a lesser extent, with lipid and protein bodies. The cytoplasm and its component organelles become compressed by these storage bodies and ultimately disintegrate and disappear among the extensive mass of starch (Abdul-Baki and Baker, 1970).

The cells of most mature oil seeds embryos contain two main types of food reserves, ie. protein and oil. In all oil seeds embryos, oil is the major storage product while protein is the secondary storage product. Protein from mature and dormant seeds has been studied more extensively than oil bodies.

Protein Bodies.

The major site of storage protein in seeds are the protein bodies, also referred to as protein granules or aleurone grains. Protein bodies may be composed of amorphous proteins, or, in addition, have various

types of inclusions; globoids which are calcium and magnesium salts of phytic acid, crystalloids of proteins, and even inorganic crystals, such as those of calcium oxalate (Altschul et al., 1961; Bagely et al., 1963; Mayer and Poljakoff, 1963).

In the embryo of Glycine max, Bils and Howell (1963) have described the cytoplasmic protein globules in association with a ribosome-rich endoplasmic reticulum, with no mention of the presence of a membrane around the protein. In the embryo of Tropaeolum majus (Nougarede, 1963), the storage protein granule occurs within a vacuole bounded by a single membrane, and thus this presents a classical example of storage protein granule. In the mature embryo of Pisum sativum (Bain and Mercer, 1966 ; Yoo, 1970) the storage protein granules appears as granules within both vacuoles and endoplasmic reticulum cisternae and as needle-like crystalline structure in the cytoplasm. According to Engleman (1966) the protein bodies in the mature embryo of Gossypium hirsutum are derived from secretion of protein into cisternae of the endoplasmic reticulum. Ultrastructural studies over a wide range of embryos have shown, in general, that protein is accumulated in vacuoles bounded by a single membrane, although the precise origin of the vacuole and method of protein accumulation are not clear until now (Harris and Juliano, 1977). In cotyledons of developing embryos of various species, numerous small vacuoles coalesce to produce one large vacuole which later on again subdivide. Each of these vacuole subdivision become filled with protein resulting in a single protein grain as has been reported in Ricinus communis (Dangeard, 1921), Phaseolus vulgaris, (Öpik, 1968), Sinapis alba (Rest and Vaughan , 1972), Crambe abyssinica (Smith, 1974). The contents are initially flocculent but as the embryo matures they become dense and membrane bound. Thus the storage function of the embryo of various species is taken up by vacuoles which operate as organizational centres for the formation of protein bodies. In Gossypium hirsutum (Engleman, 1966) and in Zea mays (Khoo and Wolf,

1970) the vacuolar origin of protein is believed rather than its plastid origin.

Recently Harris and Juliano (1977) have generalized that whatever the precise original formation of the protein body, the extent of its development may be shown by the intensity of electrondensity; the early stage of protein deposition is marked by most electron-transparent stage; followed by a stage where the central part is electron -dense but the double membrane feature is still apparent and finally a completely dense body bounded by a single membrane. They also reported that in the endosperm of Oryza sativa increase in total protein with maturity is the result of increased number of protein bodies rather than an increase in size.

In the cotyledons of developing embryo of Vicia faba (Briarty et al., 1969) the earliest rudiment of the protein body is deposited around the margins of vacuoles. These deposits increase in size, filling small vacuoles and spreading around the periphery of larger ones. Large masses of protein are formed by the fusion of individual protein bodies with another. In this way, large proteins are formed until they become closely pressed together and are bounded by a single membrane. The development of protein bodies in the embryo of Bidens carnua (Simola, 1971) is different. In the early stage of its embryo development, small vesicles which are initials of globoids are seen associated with protein masses at the edge of vacuoles and finally they are evenly distributed in the vacuoles.

Horner and Arnott (1965, 1966) have reported two morphological types, meshwork and core types, of membrane-bound protein bodies in the embryo of Yucca schidigera. In the meshwork type, there are electron-dense and electron-transparent regions in which are embedded birefringent bodies. In the core type, which is less frequent, there is a core surrounded by a matrix in which birefringent bodies are embedded.

Paulson and Srivastava (1968) have reported protein bodies in the embryo of Lactuca sativa which are clearly similar to the meshwork and core types found in Yucca schidigera.

In some oil seed plants, embryos contain two types of reserve protein grains of vacuolar origin (with or without inclusion) each restricted to separate cells. Rest and Vaughan (1972) used the term aleurone grain for protein granules with the inclusion of globoids and myrosin grains to describe those without inclusions and filled with homogeneous fibrillar material in the embryo of Sinapis alba. Aleurone grains are weakly refractive and somewhat angular while myrosin grains are highly refractive and spherical. Protein granules occupy all the cell space not occupied by oil bodies.

Cotyledons of mature embryo of oil seed plant like Gossypium hirsutum (Yatsu, 1965; Engleman, 1966; Lui and Altschul, 1967), Arachis hypogea (Dickert et al, 1962), Bidens radiata (Simola, 1969), Pinus banksiana (Durzan et al, 1971), Sinapis alba (Rest and Vaughan, 1972; Werker and Vaughan, 1974), Crambe abyssinica (Smith, 1974), Pinus sylvestris, (Simola, 1974) have protein bodies containing globoids. The globoid-cavity of Crambe abyssinica seem to be surrounded by a membrane (Hofsten, 1973). Globoids have also been reported from monocot grains as in Triticum vulgare grain (Poux, 1973) and Hordeum vulgare aleurone cell (Nieuwdorp, 1973; McLeod et al., 1964; Jones, 1969) but Jennings et al., (1963), in Triticum vulgare and Triticum durum and Oray and Henningsen (1969) in Hordeum vulgare could not see any globoid. Jacobsen et al(1971) demonstrated that protein, phosphate (phytin) and lipid are present in the globoid. Globoids of Gossypium hirsutum embryos contain large amount of phytic acid (Lui and Altschul, 1967).

Another type of protein grains which are without globoid are mostly spherical and scattered throughout the tissue. These are the myrosin grains and although they appear most commonly in cruciferous plants,

Metcalfe and Chalk (1950) have mentioned their presence in Capparidaceae, Resedaceae and Tropaeolaceae. Myrosin idioblast have been reported as early as in 1884 by Heinricher, and 1893 by Spazier. Giugnard (1890) however, equated them with myrosin tubes found in Cruciferous plants and from that time they have been called Myrosin cells. After the detailed study of myrosin cells in Brassica napus and Brassica montana plants by Sharma (1971) this type of protein grain has been reported from the embryos of crucifers like Sinapis alba (Rest and Vaughan, 1972; Werker and Vaughan, 1974), and Crambe abyssinica (Smith, 1974).

Leguminous protein bodies, however, do not have any special structure (Varner and Schidlovsky, 1963; Bain and Mercer, 1966 ; Öpik, 1966; Tombs, 1967; and Briarty et al., 1970). Not infrequently, protein bodies may serve as major sites of storage of specific protein such as the globulin in legumes (Altschul et al., 1961; Varner and Schidlovsky, 1963; Ericson and Chrispeels, 1973). The protein bodies in the embryos of Ricinus communis (Orey et al., 1968) and Cannabis sativa (St. Angelo et al., 1968) contain a crystalloid particle which in Cannabis sativa is formed by the main storage protein, edestin. The nature of crystalloid structure found in aleurone grains of Crambe abyssinica embryos is not known (Smith, 1974). In the embryo of Setaria lutescens (Rost, 1972) protein bodies are devoid of any inclusions whatsoever. According to Lott et al. (1971) the variability in the structure of protein bodies of embryos might be accounted for by the type or duration of fixation.

Oil Bodies.

Spherosomes are abundantly present in oil seeds (Engleman , 1966; Orey et al., 1968). Spherosomes are now regarded as a heterogeneous

collection of objects that float around in the cytoplasm (Misra and Calvin, 1970; Sorokin and Sorokin, 1966) usually with a spherical shape which arises by minimization of their surface area by force of surface tension. A spherosome is considered to be the direct precursor of an oil body by some workers (Frey-Wyssling and Muhlethaler, 1965). The best account of the formation of oil bodies from spherosomes, which themselves are derived from the endoplasmic reticulum, is given by Frey-Wyssling et al. (1963) and Schwarzenbach (1971). Sorokin (1967) has shown, however, that spherosomes and oil bodies are separate entities, and spherosomes are commonly found in most vegetative cells of higher plants, even in cells which do not produce oil. On the other hand, oil bodies are found only in oil producing cells. Spherosomes have a limiting membrane, while oil bodies do not have any (Sorokin, 1967). Recently Smith (1974) while working on Crambe abyssinica embryo has supported Sorokin (1967) as he noticed the presence of spherosomes in the cells of the earliest stage embryo development, when the cells are meristematic with no storage oil bodies. He has also shown how the spherosome is developed from a terminal vesicle formed on endoplasmic reticulum as shown by Frey-Wyssling et al. (1963) in Allium cepa. Oil bodies develop at a later stage, 8-10 days after petal fall in Crambe abyssinica, and only spherosomes are present in the cell up to that stage (Smith, 1974). The oil bodies increase rapidly in number after this and by 25 days after petal fall all cell spaces apart from occupied by protein body, chloroplast, nucleus is densely packed with reserve oil droplets. These oil bodies in mature seeds are localized mostly at the periphery of the cell.

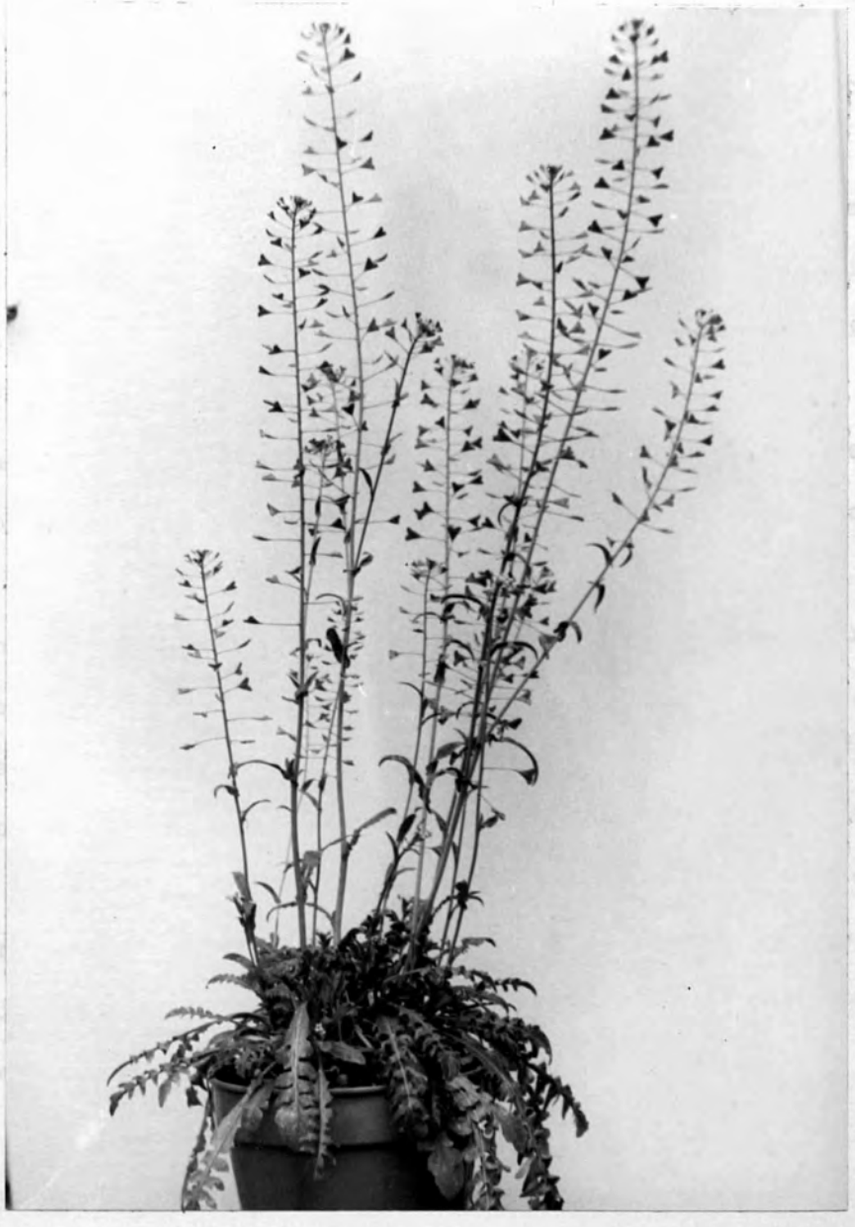
Oil bodies, appear initially as a small electron-dense particulate mass and are the last of the original seed reserves to be utilized completely. Smith (1974) has described the formation of oil bodies. The majority of oil bodies lose their original circular or spherical

form due to their tight packing within the cell at maturity but still retain individuality. The exact nature of the limiting boundary is not yet known (Smith, 1974).

These ultrastructural studies described in brief, in this review, are only modest beginnings into a promising field of future investigations, for in no other tissues in plants do we find such dramatic changes in growth, differentiation and tissue formation crowded into a relatively brief span of time as in the embryos.

Plate 1. Plant material used in the present thesis.

Fig. 1. A healthy plant of Capsella bursa-pastoris growing in a pot in the green house.



MATERIALS AND METHODS.

1. Plant Material

The plant material selected for the present study is Capsella bursa-pastoris L. Medic, commonly known as Shepherd's Purse, which belongs to the family Cruciferae. This is a small herbaceous plant (up to about 60cm) growing profusely as a common weed during spring and summer (Fig. 1). This choice was based on the following considerations.

The plant material is readily available throughout the year as these plants flower for six months at a stretch and during this time yield all embryonic stages. The plants grow well under controlled conditions. They are autogamous, so no problem of pollination occurs.

The embryo of Capsella bursa-pastoris is one of the most intensively studied of angiosperm embryos and is a classic example of the dicotyledon embryo.

In the inflorescence the flowers and fruits are arranged regularly along the axis in acropetal sequence, the younger one at the top, the older ones near the base thus facilitating the search for specific stage of embryo development. Each fruit (silicle) contains about 20-25 ovules in which the embryos are of approximately equal size. This is useful in culture experiments since sufficient number of embryos of one size can usually be excised from one silicle for immediate transfer to the culture medium.

2. Growing Conditions

Plants of Capsella bursa-pastoris were grown from seeds collected at the Botanical Garden, Bedford College, London during summer of 1974 and 1975 from the plants growing in the greenhouse. Seeds were sown in flats in a mixture of John Innes seed compost and placed either in

greenhouse (during summer) or in a growth cabinet (during late autumn and winter) under long day photoperiods (14 hours). Day temperatures were 25°C and night temperatures about 18°C. Light intensity at the level of plants was about 7500 lux.

Seeds started germinating after about 7-8 days. Single plants were transferred to 9cm. and 15cm. pots in the John Innes Compost mixture No. 1 about two weeks after germination. Plants were watered daily. Under these conditions plants flower at about 6 weeks and the first sample of early developmental stages of embryo could be collected at the end of the seventh week. Ultrastructural, developmental and experimental studies of embryo were made separately with plants grown either in growth cabinet or in greenhouse. Embryos from both the sources had essentially the same results.

In order to reduce the variations of the results, the plant material for study was brought together in the following way

a) only one plant was used for each experiment

b) The number of inflorescences that were used was limited as much as possible.

c) Care was taken that in each culture experiment embryos of almost identical initial length (torpedos) were homogeneously distributed over the culture media.

3. Embryo Excision Techniques

(a) Collection of silicles

Individual silicles were tagged on the day of petal fall. Excess floral branches were pruned off, especially in smaller plants to prevent any axis bearing an excessive load. For the initial study of the classification of developmental stage, silicles were removed at 1-day intervals

after petal fall up to 10 days after petal fall, and then at 2-day intervals until the maturity of the seed. Embryos were excised, measured under a binocular microscope equipped with an eyepiece graticule, and the stages of development thus determined. Embryo measurement at each developmental stage has been done from the tip of radicle up to the extreme tip of cotyledons excluding the length of the suspensor (Fig. 2).

(b) Opening of silicle

The selected silicles from the cut inflorescences of Capsella bursa-pastoris kept in a beaker of water, were cut in the region of placenta, and the ovules were exposed by pulling apart the two halves of the silicle with forceps.

(c) Embryo location in ovule

To find out the location of the embryos of different developmental stages in the ovules, which ultimately was very helpful for embryo dissection, whole ovules were dipped in 5% KOH for about 4-5 minutes. The positions of developing embryos, which were usually bright green, were observed through the cleared wall of the ovules. Some whole ovules were also cleared by warming in chloral hydrate for 1-2 minutes. The former method, however, gave satisfactory results.

(d) Embryo dissection

Embryos were excised from the ovules in a drop of sterile distilled water on the stage of the microscope. Embryo dissection for the ultra-structural developmental studies (Part I) and other preliminary studies was carried out in the laboratory, while the embryo dissection for the

culture studies (Part II) was carried out aseptically in a sterile room. Ultraviolet-Sterilamp was switched on in the dissection room for one hour before the start of dissection. Selected silicles in the desired stage of development were surface sterilized by wiping them with cotton ball dipped in alcohol. All instruments were sterilized by heating or by wiping with alcohol.

Embryos were excised from the ovules by dissecting them under the binocular microscope which was left in the sterile room when the ultraviolet-Sterilamps were on. This binocular was also wiped with alcohol for complete sterilization. Sterilized glass-distilled water was used for the dissection of embryos for the culture experiment. To prevent any infection during these dissections, our hands and clothes were fully sterilized with alcohol before the start of dissection. A mild spray of alcohol was done in the sterile room to prevent the infection.

With the help of sharp, mounted blade, the ovule was split longitudinally to isolate the embryo-containing half which appears green. By further manipulations with surgical blades (Swan-Morton No. 11) it was possible to separate completely the embryo from the surrounding ovular tissue. Embryos in early and late torpedo-shaped stages could be removed and collected by this method. A small incision in the ovule, followed by slight pressure with a blunt needle was enough to free the embryos in advanced stage of development from the ovule (Raghavan and Torrey, 1963). Dissected embryos floated freely in the sterile glass-distilled water. Next, the excised embryos of almost uniform length, were transferred by means of a sterilized needle with flat tip, and placed in a circle on the culture medium (about 25ml) in 10cm petri dishes. Usually 12, sometimes more (up to 20 or so) embryos of desired stage were transferred to the culture medium in one Petri dish. The

Petri dishes were sealed with masking tape to prevent desiccation of the cultures.

Embryo dissection from the ovular tissues is a somewhat painstaking procedure, especially when the embryo dissection was carried out under a dim green safelight in the case of the dark-treated whole plants for ultrastructural developmental studies. But after adaptation of our eyes to the safelight, the excision of embryos was possible.

4. Culture Medium

Many different types of culture media have been developed for the culture of plant embryos. The basal medium used in the present investigation is the same as used by Raghavan and Torrey (1963) for culturing young Capsella embryos, but with variable concentrations of sucrose and also an addition of more agar. As most successful embryo cultures appear to have been carried out on agar surfaces, although some succes has also been reported with liquid cultures, more (1.2%) agar was added to the basal medium as compared with 0.9% of Raghavan and Torrey (1963) for the present investigation. The basal medium used in the present investigation consisted of macronutrient salts, trace elements and vitamins in similar proportions as used by Raghavan and Torrey (1963). The basal medium used in the present investigation contained either various concentrations of sucrose or with no sucrose at all. The basal medium containing 2% sucrose was tried as recommended by Raghavan and Torrey (1963) for culturing very young embryos (globular stage) of Capsella. But the satisfactory results of plastid development could not be achieved with this concentration of sucrose which proved excessive. The sucrose percentage was then reduced to 1% and 0.5% in the basal medium. For all our subsequent experimental studies the basal medium contained 0.5% of sucrose, which proved satisfactory.

The composition of the basal medium is as follows:

1. Macronutrient salts (mg/litre):

(a) $Ca(NO_3)_2.4H_2O$	480
(b) $MgSO_4.7H_2O$	63
(c) KNO_3	63
(d) KCl	42
(e) KH_2PO_4	60

2. Micronutrient salts (mg/litre):

(a) H_3BO_3	0.56
(b) $MnCl_2.4H_2O$	0.36
(c) $ZnCl_2$	0.42
(d) $CuCl_2.2H_2O$	0.27
(e) $(NH_4)_6Mo_7O_{24}.4H_2O$	1.55
(f) Fe.EDTA (sequet)	10.00

3. Vitamins (mg/litre):

(a) Thiamin (Aneurine) hydrochloride	0.1
(b) Pyridoxin hydrochloride	0.1
(c) Niacin (Nicotinic acid)	0.5

4. Sucrose (g/litre):

20

10

5

5. Agar (g/litre):

specially purified 'oxoid' No. 3	12

6. Glass distilled water to one litre.

All the components of the medium were mixed together and sterilized for 15 minutes at 15 lb/in^2. The cultures were kept in the culture room at 22 $\pm$ 1^{o}C where they needed the various light and dark treatments.

5. Light Sources

(a) In the culture room

The white light source was a single Philips cool-white 'Day Light' fluorescent tube of 80W giving 430 lux at the level of the cultures on the table. The distance between the level of cultures and light source was 170cm.

Red light was provided in boxes covered by 2 layers of Cinemoid (Strand Electrics) No. 14 Ruby. This gives almost total absorption below 600nm. apart from slight transmission in the blue region (375 nm.). The blue light was also provided in boxes covered by 2 layers of Cinemoid No. 19. In this case the peak transmission was 450 nm. (approximate limit 380-530 nm.). The distance in each case between the level of cultures and light source was 100 cm.

(b) In the growth cabinet

White light was provided by cool-white fluorescent tubes giving 7500 lux, at the level of the growing plants.

Green safelight

'Day Light' fluorescent tube (20 W) wrapped with 3 layers of Cinemoid No. 39 Primary Green and a black electrician's tape wrapped around the ends of the tube to check the emission of any far-red light.

All dark cultures were maintained in an incubator at 23 ± 1^{o}C which was kept completely dark except for brief periods of opening for examination in dim green safelight.

6. Electron Microscopy

(a) Fixation

Embryos from whole plants were fixed immediately after excision; excised cultured embryos were transferred directly into the fixative after various treatments. Leaf tissue not more than 1 mm. cube were cut with a sharp blade from the middle of the young leaf on a sheet of dental wax in a drop of fixative. The cut tissue blocks of leaves were then transferred directly into the fixative. All the fixing operations were done at room temperature.

Tissue containing air, especially the leaf tissue and older embryos (all stages after the young torpedo stage) did not sink to the bottom of the vial containing fixative until the vials were aspirated several times under a vacuum pump.

Fixative

The primary fixative used was glutaraldehyde, obtained from TAAB laboratories as a 25% solution. All fixatives were buffered with 0.066, 0.1, or 0.15 M phosphate buffer at pH 6.8 or 7.2.

Fixation procedures

Two fixation procedures were used.

(1) The specimens were fixed for 2-4 hours in buffered 6% glutaraldehyde buffered by 0.1/0.15 M at pH 7.2. Then the specimens were washed very thoroughly through several rinses of 0.1/0.15 M buffer at room temperature to remove excess glutaraldehyde with a minimum of four rinses in one hour and then were left overnight in the last rinse at $4^{o}C$. The specimens were next post-fixed for one hour in 1% osmium tetroxide buffered

to pH 7.2 at room temperature. 1% osmium tetroxide was prepared in the same buffer from 2% solution supplied by Messrs. B.D.H. in 10 ml. vials. This method was used for leaf tissue and some late stages of maturing embryos.

(2) The embryos were otherwise fixed for four hours in 6% glutaraldehyde buffered by 0.066 M phosphate at pH 6.8 at $4^{o}C$, then washed very thoroughly with four changes of 0.06 M phosphate buffer to remove excess glutaraldehyde. The embryos were next post-fixed with 2% unbuffered osmium tetroxide containing 4% sucrose for 15 hours at $4^{o}C$ (Schulz and Jensen, 1968).

(b) Dehydration

After treatment with osmic acid the specimens were then rinsed thoroughly in distilled water with a minimum of two rinses, the first one rapid and the second for 15 minutes. Then the specimens were passed through grades of acetone series followed by propylene oxide as follows:

30% acetone	15 minutes
50% acetone	15 minutes
70% acetone	15 minutes
90% acetone	15 minutes
100% acetone, two changes	30 minutes each
50:50 propylene oxide: acetone	10 minutes
100% propylene oxide, two changes	15 minutes each

Propylene oxide was added gradually to the specimens in 100% acetone, and brought to a concentration of about 50% propylene oxide over a period of about 10-15 minutes.

The embryos fixed by fixation method No. 2 were allowed to remain overnight in 70% acetone to which 1% uranyl nitrate was added (Schulz

and Jensen, 1968).

(c) Infiltration and embedding.

Resin mixture

The embedding resin introduced by TAAB laboratories (Reading, England) was selected for all experiments. This is a developed epoxy resin without any published composition. This resin is claimed to have the following valuable characteristics:

1. relatively low viscosity
2. good cutting characteristics
3. can be used with uncoated grids
4. their thermostability with little or no shrinkage upon polymerization

A wide range of hardnesses could be obtained by using different proportions of the hardners, DDSA and MNA. BDMA was used as the accelerator. For most of the work in the present investigation the two resin mixes used were made up according to the following schedule:

	No. 1	No. 2
TAAB resin	20 g.	25 g.
DDSA	10 g.	13.7 g.
MNA	10 g.	11.3 g.
BDMA	0.8 g.	1.0 g.

Mixture No. 1 was used for the leaf study and No. 2 (soft) was used for the embryo studies.

Process of infiltration

After two changes of propylene oxide, the resin mixture was added to the tube containing specimens according to the following schedule:

10:1 propylene oxide : resin	overnight (on rotator)
50:50 propylene oxide : resin	4 hours "
100% resin	4 hours "
100% resin-specimens removed to 100% resin	overnight "
Fresh resin	One day "

Fresh resin was filled in embedding moulds, specimens were placed in the centre of fresh resin in embedding moulds with desired orientation of specimens.

Polymerisation

The moulds filled with resin and containing specimens were placed in an oven at 60°C for 40-48 hours.

The specimen blocks which were released by flexing the moulds were later cut out and stuck to resin blanks with araldite adhesive for mounting in the microtome.

(d) Sectioning

Sections were cut with a glass knife on a Huxley ultramicrotome. Glass knives used were made on a LKB knife maker. Sections were floated on distilled water. Sections which showed silver/gold to grey interference colours were picked up on copper grids (150 and 200 mesh) coated with formvar film. Some sections were also picked up on freshly-cleaned uncoated copper grids (200 and 300 mesh). Sections showing grey to silver interference colours were generally used, but difficulty in handling old, starchy or protein-filled material occasionally necessitated the utilization of pale gold sections.

Dry seeds were imbibed in water for short time to make sectioning

less difficult. One set of dry seeds were imbibed in water for two hours at room temperature, while other set of dry seeds were imbibed at $4^{o}C$ to allow water uptake with minimum metabolic activity.

(e) Staining

The sections were stained on the grid, usually with lead citrate (Reynolds, 1963) for 2-10 minutes,

i) The sections of embryos fixed in fixative No. 2 and pre-stained with 1% uranyl nitrate in 70% acetone were stained with lead citrate only for 2-3 minutes.

ii) Other sections were stained only with lead citrate for 5-10 minutes.

All staining was carried out by floating grids face downwards on drops of stain placed on a dental wax slab, kept in a covered Petri dish whose floor was covered with filter paper either soaked in saturated solution of sodium hydroxide or contained a reservoir of sodium hydroxide pellets. The staining with lead citrate in the presence of sodium hydroxide was carried out to achieve an atmosphere of low CO_2 tension, which might otherwise lead to contamination with lead carbonate which appears as black insoluble deposits in sections.

After the staining, the section-containing grids were rinsed thoroughly with distilled water, usually 20-30 gentle squirts from a wash bottle were sufficient for proper washing of sections, after which the grids were touched on the edge of a filter paper disc to drain them.

(f) Examination in microscope

Sections were examined in an AEI (Associated Electrical Industries) EM6B and Corinth 275 microscopes. A lot of preliminary breakdown occurred with these microscopes especially during 1975 as a result of which the work was ofter held up.

Photographs were taken on Ilford N50 plates and Ilford N5 50 film in AEI-EM6B and Ilford N4 E 50 in Corinth 275 microscopes and films were processed in Ilford Bromophen and Hypam.

7. Light Microscopy

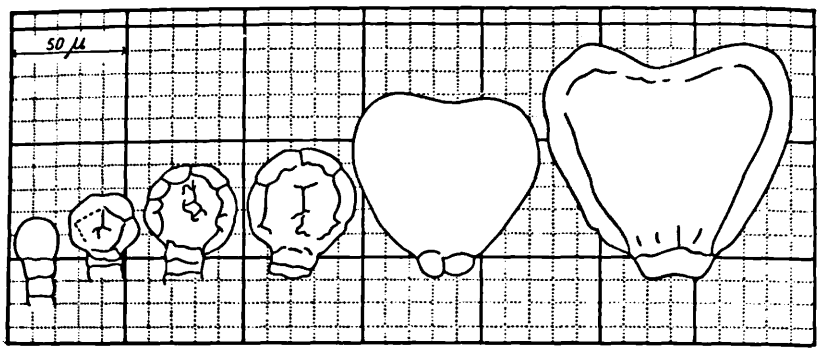
Sections for light microscopy of whole embryos of various developmental stages, embedded in TAAB resin were cut at 1-2 μm thick with glass knives on a Huxley ultramicrotome. Sections were flattened as usual with chloroform vapours, picked up in a drop of water by means of a fine, clean copper loop, and dried at 60°C in a drop of distilled water on a slide. The sections were routinely stained for survey work in a mixture of 1% toluidine blue O in 1% borax solution (O'Brien et al, 1964), which stains well the materials embedded in epoxy resins. Other stains used for specific localization of starch, protein and oil were 1% methylene blue containing 1% borax and 1% Azure II, dilute iodine solution, Sudan III and IV, etc. (Table 5).

Sections were examined and photographed in a Zeiss photomicroscope.

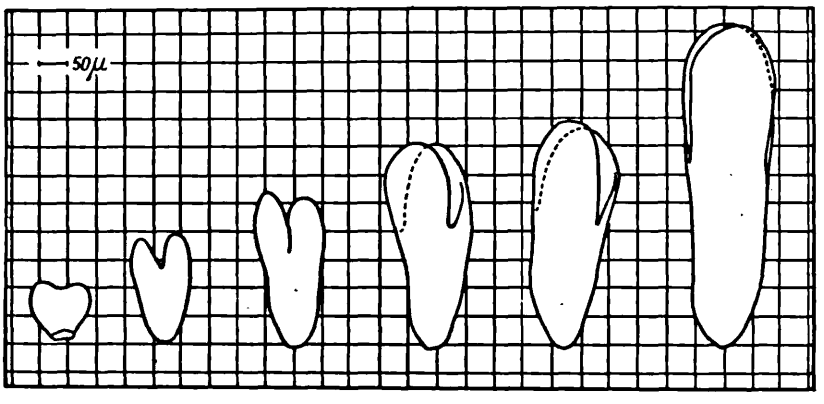
Some squash preparations of cotyledons of mature embryos were made and stained with lactophenol-aniline blue for one hour and mounted in pure lactophenol (Werker and Vaughan, 1974) for observing the myrosin cells. Squash preparations of the cotyledons of different age and from different sugar experiments were stained with light iodine in potassium iodide solution (KI_2) for comparison of starch accumulation in plastids.

Plate 2. The Stages of the development of the embryo.

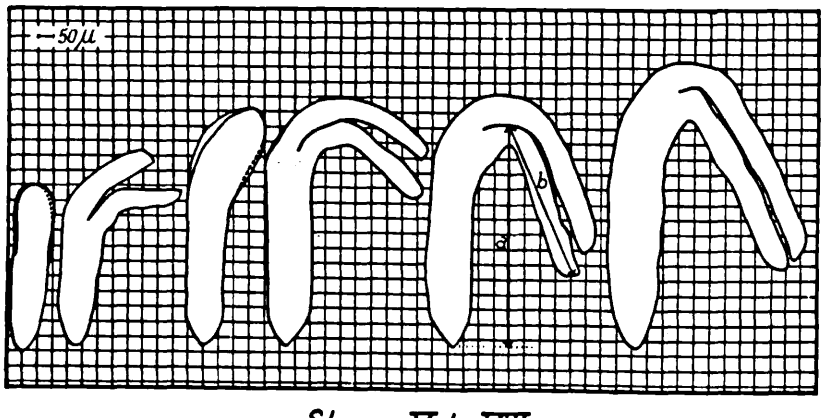
Fig. 2. The stages of the development of the embryo as described by Rijven (1952). Stage I- globular, Stage II- reversed trapezium, Stage III- heart, Stage IV- intermediate between III and V, Stage V- torpedo, Stage VI-"walking stick", Stage VII- upturned U, Stage VIII- fully grown embryo. (From Rijven, 1952).



Stage I to IV



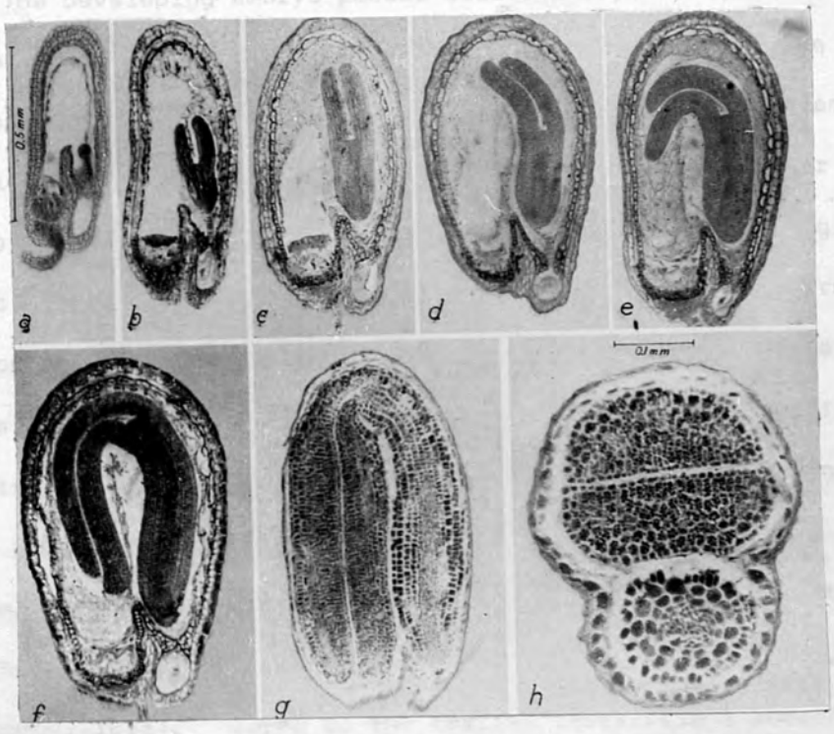
Stage III to VI



Stage V to VIII

Plate 3. Histological sections through ovules of different stages.

Fig. 3. Micrographs of histological sections through ovules of different stages. a-f: developing ovules; g and h mature seeds. All reproductions at the same scale except h, which shows the cross section of the seed with abundant aleurone grains in the embryo. Staining with Gentian violet. (From Rijven, 1952).


0.5mm
a
b
c
d
e
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f
g
h

OBSERVATIONS.

1. Developmental Stages of the Embryos.

The embryo of Capsella bursa-pastoris is one of the most intensively studied mainly because of the conspicuous regularity of the divisions that give rise to young embryo. The early cleavage pattern in Capsella bursa-pastoris is extremely regular until the formation of the globular embryo. The developing embryo passes successively through many stages until it matures. The terminal cell divides both by transverse and longitudinal walls to give quadrant, octant, 16- and 32-celled embryo. The globular embryo consists of some 32-64 cells. The globular embryo finally forms the primordia of two cotyledons and cotyledons begin to develop at the distal region and the spherical embryo now appears more or less cordate (heart-shaped). During further development the cotyledons and hypocotyl elongate (torpedo-shaped) and the shoot apex is begun to be organized in the depression between the cotyledons. A different pattern of distribution of cell division, accompanied by cell elongation, is probably responsible for the change in shape of the embryo from the heart-shaped to the torpedo-shaped stage (Pollock and Jensen, 1964; Wochok, 1973). Owing to the spatial restrictions inside the ovule the embryo becomes a curved structure (walking-stick-shaped) and finally assumes the shape of a horse-shoe (inverted U-shaped) and retains this shape until it dries. The cleavage planes occur with great regularity up to the globular stage of development. In Gossypium hirsutum, however, the divisions are almost random in the early globular embryo and no two embryos look identical (Jensen, 1963).

Rijven (1952) classified 8 stages of embryogenesis of Capsella bursa-pastoris on the basis of the shape and length of the embryos (Figs. 2, 3). Raghavan and Torrey (1963) modified this classification by adding

"early globular" and "early heart-shaped" stages to the classification of Rijven (1952), and their measurements of the embryo included the suspensor. On the basis of our observations we have devised our own classification which is the modification of the above two classifications and which also includes days after petal fall as a visible indicator of the seed age. In plants with small flowers like Sinapis alba (Rest and Vaughan ,1972) and Crambe abyssinica (Smith, 1974) where hand pollination is impracticable and in Capsella bursa-pastoris which is self-pollinating, the number of days after petal fall is a useful visible indicator of seed maturity. Days after petal fall for different developmental stages (on the basis of embryo shapes) have been worked out for Capsella bursa-pastoris to enable us to collect embryos of desired stage at desired time for experimental work.

Since the initial length of the embryo was variable, embryos were categorized on the basis of their developmental stages, that is, with specific shapes which are attained after the specific number of days after petal fall (Table 3).

A range of embryos assuming different shapes during development between 5-28 days after petal fall was obtained. A wide range of developmental stages was recorded up to 9th day after petal fall, when the embryo starts maturity (Table 3). From 10 days after petal fall until 28 days after petal fall only maturity of the embryo takes place and all the embryos between 9 and 28 days after petal fall were in one or the other maturing stage. Therefore, in the present investigation, from 5 to 9 days after petal fall embryo shapes are used rather than age as the criterion of development and from 10 days after petal fall to 28 days after petal fall the age (days after petal fall) is used as the criterion to study the sequential ultrastructural changes.

Since it takes about 20 days to get a completely mature and dry seed starting from the maturation phase when the embryo is green, the

maturation phase of the embryo has been divided into four sub-stages for studying the sequence of ultrastructural changes. Even the mature green stage is further divided into two separate stages, as there are ultrastructural differences between early maturing green embryo (mature-green embryo stage) on 9th or 10th day after petal fall and the fully mature green embryos on 15-16 days after petal fall when it is still green but harder (fully mature-green embryo stage). The stage of embryo when it loses pigmentation and moisture at 18-20 days after petal fall has been termed mature ivory-white embryo stage. Complete dry seeds which are hard and shrunken with brown testa and creamy-white cotyledons are produced 26-28 days after petal fall and have been termed mature-dry embryo stage. All these maturity stages of developing Capsella bursa-pastoris embryo show sequential ultrastructural differences.

In plants with bigger flowers like Phaseolus vulgaris (Öpik, 1968a) the age of seed development is counted in days from the anthesis. While in Pisum sativum (Bain and Mercer, 1966) days from fertilization has been taken as a criterion for measuring the age of the seed. Klein and Pollock (1968) used moisture percentage (fresh-weight basis) as the measure of seed maturity in Phaseolus lunatus, but because these plants are compltely indeterminate in their growth habit and only a small proportion of blossom set seed, it was impossible to tag blossom to determine the chronological age of seed as in the first two cases. Harris and Boulter (1976) also used increase in fresh weight of seed as an index of working out the sequence of ultrastructural changes in Vigna unguiculata and considered this parameter as the most reliable in determining the stage of development. Khera and Tilney-Basset (1976), however, have investigated embryo development by electron microscope in Pelargonium X Hortorum at weekly interval from week one to week four. At one week — the heart stage embryo in which the cotyledons are just beginning to form; at two weeks — when the embryo is expanding rapidly

and cotyledons are quite elongate; at three weeks — when the embryo is fully formed and fully expanded; and at four weeks — when the plastids have lost their pigments and the hard shrunken seed is ready for dispersal.

Harris and Juliano (1977) have used the days after flowering as a criterion for measuring the age of grain in Oryza sativa for studying the sequence of ultrastructural changes in the endosperm protein bodies of the developing grains. Individual grains were tagged at anthesis and harvested at 2-day intervals after flowering to 10 days after flowering and then at 5-day intervals until mature.

TABLE 3. The developmental stages of Capsella bursa-pastoris embryo.

Developmental stages	Length (Without suspensor) (μm)	Days after petal fall.
Early globular	18-50	2
Late globular	51-80	$2\frac{1}{2}$
Heart	81-200	3
Early torpedo	201-400	5
Late torpedo	401-700	6
Walking-stick-shaped	701-1000	7-8
Inverted U-shaped	1001-1700	8-9
Mature-green	1701-1800	9-15
Fully mature-green	> 1800	15-17
Mature ivory-white	> 1800	18-20
Mature-dry	> 1800	26-28

TABLE 4. Changes in cell dimensions, cell organelles, cell reserves of developing cotyledons of Capsella bursa-pastoris.

Embryo stages	Cell			Organelles			Cell wall	Cell reserves			
				Nucleus	Chloroplast			Starch	Protein	Oil	
	*L (µm)	*B (µm)	*Diam (µm)	*L (µm)	*B (µm)	Thickness (µm)		*L (µm)	*Diam. (µm)	*Diam (µm)	Number (per cell)
Early torpedo	16.8	7.5	5.3	-	-	0.04		-	-	0.40	4
Late torpedo	20.0	9.0	6.5	1.60	0.80	0.06		0.80	-	1.13	16
Walking stick-shaped	17.0	7.0	6.4	2.00	0.80	0.08		1.22	-	1.40	20
Inverted U-shaped	17.0	11.0	6.0	2.65	0.90	0.22		1.30	-	1.81	34
Mature-green	14.0	7.2	5.4	3.00	1.25	0.20		1.80	-	2.40	40
Fully mature-green	20.0	14.5	4.0	2.70	1.23	0.22		-	-	2.65	70
Mature ivory-white	30.0	14.0	3.6	2.00	0.80	0.30		-	3.0-8.0	3.00	260
Mature-dry	14.4	7.0	3.3	1.00	0.80	0.30		-	3.0-5.0	2.00	200-250

*L= Length; *B= Breadth; *Diam.= Diameter.

Note: 1. Epidermal and subepidermal cells have been excluded from measurements. Cell diameter have been obtained from photomicrographs of gluteraldehyde and TAAB embedded tissues. Both photomicrographs and electron micrographs have been used for measurement.

2. Average of 100 cells in the first three stages and of 50 of other stages has been reported.

2. Ultrastructure of the Embryo of Capsella bursa-pastoris.

In the present study the ultrastructure of cytological changes in the embryo of Capsella bursa-pastoris are followed, from early torpedo-shaped embryo stage to the complete seed maturation phase. The ultrastructural study of early embryogenesis in Capsella bursa-pastoris giving detailed account of cytological changes up to heart-shaped embryo stage has already been made by Schulz and Jensen (1968). The electron microscopic work described in the present investigation starts from a point where Schulz and Jensen's published description ends (c.f. Figure 6 herein, which is similar to their Figure 17 of torpedo-shaped embryo stage). For the most part, the cells of cotyledon and radicle develop similarly and are considered together. Unless otherwise specified the observations in the present study relates to the cotyledon cells of Capsella bursa-pastoris. Most of the electron micrographs represent sections through the inner parenchyma cells of cotyledons. The epidermal and subepidermal cells which are comparatively smaller than those of bulk parenchyma, have been excluded. The size of the cell and its nucleus varies up to the maturity of the embryo when the cells become filled with storage reserves and the embryo dries. These storage reserves also show variation in their size and number.

Cotyledonary cell size increases up to late torpedo-shaped embryo stage and this can mainly be accounted for by increased vacuolation (Table 4). From torpedo-shaped embryo stage onwards, there is a little or no increase in cell dimension, but the embryo shows marked growth over this period which can be accounted for by increase in cell number. The cells apparently stop dividing at about 15 days after petal fall during the mature stage at which time there is an expansion of cells until the ivory-white stage. The maximum length of a complete mature cell at this stage reaches 30 μm. By this time cells are completely

filled with reserve protein and oil bodies. There is no further increase in cell size or storage materials. The oil bodies gradually increase in size and number from torpedo-shaped embryo stage up to 15 days after petal fall, when embryos are in mature stage but still quite green, though firm, after which there is a 5-6 fold increase in the number of oil bodies (Table 4). It takes about 26-28 days after petal fall to produce a complete dry seed (Table 3).

The size of the nucleus seems to increase gradually up to torpedo-shaped embryo stage and then remains almost constant (6.0 µm) until after the cell division ceases. The size of nucleus decreases gradually after mature-green embryo stage and is about 3.6 µm at mature ivory-white embryo stage. At mature dry embryo stage the nucleus is further reduced to 3.3 µm (Table 4). The nuclei become irregular in outline and are not often observed in the later two stages.

(a) Torpedo-shaped embryo.

Early torpedo-shaped embryos (201-400 µm) develop 5 days after petal fall, while late torpedo-shaped embryos (401-700 µm) develop 6 days after petal fall. Early torpedo-shaped embryos which have also been called "intermediate stage embryos" by earlier workers (Rijven, 1952; Raghavan and Torrey, 1963) are characterised by longitudinal growth of the cotyledons and of hypocotyl to a lesser extent. In late torpedo-shaped embryos cotyledons are flattened against each other and are half the length of the embryo, the other half of embryo is formed by hypocotyl and radicle together (Fig. 3).

At this stage, the embryos are still suspended in liquid chlorophyllous endosperm and still held by suspensor (Fig. 2). The thin delicate cotyledons are bright green. The cells of cotyledon at this stage are fairly homogeneous with respect to cytological structures

(Fig. 4). Almost all the cells of embryos are meristematic and dividing. In some cells very small, possibly submicroscopic, vacuoles are found scattered throughout the cytoplasm. Many cells are in the process of coalescing to form large vacuoles. The epidermal and sub-epidermal cells of early torpedo-shaped embryos show very little vacuolation as compared with that of late torpedo-shaped embryos which show one small irregular vacuole developing at either end of the cell (Fig. 4). The cell walls are thin (about 0.06 μm) with plasmodesmata (Fig. 8) but devoid of intercellular spaces (Fig. 4). Nuclei which are large in proportion to cell volume, are circular to ellipsoidal lying in the centre of cell (Figs. 4,5,7). The nuclei of early torpedo-shaped embryos are about 5.3 μm in diameter, while those of late torpedo-shaped embryos are about 6.5 μm in diameter. Each large centrally placed nucleus includes a large, prominent nucleolus and a few small dense chromatins distributed near to nuclear membrane and also near to nucleolus. Each nucleolus which appears as a homogeneous circular body is without a delimiting membrane. Most nucleoli contain a clear central area similar to those obtained in the embryos of Pisum sativum (Setterfield et al., 1959) (Fig. 7). The double nuclear membrane shows very clearly the nuclear pores filled with an electron opaque material in few cells (Fig. 7). Nuclei also contain ribosome-like particles in the nucleoplasm (Figs. 6,7). The cytoplasm is densely populated with free ribosomes, while polyribosomes are scarce. The density of ribosomes increases at this stage as compared to that at heart-shaped embryos, and most ribosomes appear to be randomly dispersed as compared to that of aggregated distribution of heart-shaped embryos (Fig. 8). This random arrangement of ribosomes is just similar to that in the egg of Capsella bursa-pastoris. The endoplasmic reticulum which appears to be mainly of the rough type with relatively short elements, lies mainly near the plasmalemma (Figs. 5,9). In early torpedo-shaped embryos, the endoplasmic reticulum is not well developed but scattered irregularly

throughout the cytoplasm and at places in the form of small vesicles; it does not show any connection with developing vacuoles or Golgi bodies. The Golgi bodies are frequently present consisting of four to seven cisternae with a few vesicles (Figs. 6,8) but are not very conspicuous in many cells. Plastids and mitochondria, though numerous, are highly differentiated in inner cells than in the outer non-vacuolated cells.

The mitochondria vary considerably in size and form, probably depending on the plane of the section (Figs. 5,6). At this stage most of them appear oval or circular and of variable sizes. Few of them are also elliptical or elongated with irregular substructures. The cristae are very well developed with their membranes slightly swollen. Some mitochondria exhibit very few cristae, while others none at all. Sometimes it is difficult to distinguish between early developing plastids and mitochondria without cristae; many small profiles could equally be interpreted as to whether the mitochondria or small plastids. The mitochondrial matrix is, however, less electon-dense than the matrix of plastids. Some mitochondria which have electron transparent matrix areas do show thin thread like 'immature' mitochondria of young meristematic cells.

Many different stages of early development of chloroplasts are met with in different cells (Figs. 6,7,9). In early torpedo-shaped embryos many proplastids of different shapes are lying nearer the large central nuclei with very little or no lamellar differentiation. Occasionally chloroplasts from late torpedo-shaped embryos contain a single, small starch grain of about 1.0 μm (Fig. 9). Such starch containing chloroplasts which are more or less elliptical in shape, are more common in the cells of the hypocotyl. Chloroplasts without starch grains have an irregular amoeboid outline and contain few lamellae with occasional stacking (Fig. 5). Chloroplasts in early torpedo-shaped embryos show hardly any advancement over those in the heart-shaped embryos. Many

chloroplasts are still under-developed or undifferentiated while others form a fused lamellar system, probably by invagination of the inner membrane. Such chloroplasts with 2-3 lamellae and occasional stacking have earlier been reported in heart-shaped embryos of Capsella bursa-pastoris (Schulz and Jensen, 1968c). Chloroplasts from late torpedo-shaped embryos show little lamellar differentiation, but lack grana formation (Figs. 6,9). Their stroma contain ribosome like particles. Dividing chloroplasts are not uncommon at this stage. The maximum length of chloroplasts with definite shapes reaches up to 1.6 μm.

Oil bodies are altogether absent from heart-shaped embryos but are occasionally present at early torpedo-shaped embryo stage, and appear in greater number in the cytoplasm at late torpedo-shaped embryos either singly or in small groups with maximum number of sixteen per cell in a section. Oil bodies are spherical, greyish white, moderately electron-dense, with a maximum diameter of up to 1.13 μm. They have indistinct boundary which cannot be compared with the tonoplast. They normally do not coalesce with adjacent oil bodies in the cell. These oil bodies do not have any oensely staining limiting membrane but the fact that individual oil bodies do not coalesce in the cell indicates that a limiting membrane of some nature may be present. Most of them are distributed throughout the cytoplasm with a few lying in the immediate vicinity of the cell wall (Fig. 8).

(b) Walking-stick shaped embryo.

About 7-8 days after petal fall, embryos take the shape of a walking-stick which is caused by the turning back of the cotyledons at an angle to hypocotyl (Fig. 3). The embryos with curved cotyledons are still attached to the endosperm at the chalazal end of the ovule (Fig. 3). Cells still show signs of current divisions as cell plate formation is

occasionally seen (Fig. 12). Moreover they are of smaller length (17.0 μm) and breadth (7.0 μm) as compared to those at the last stage (Table 4). There is a little change in cytoplasmic fine structures from torpedo-shaped embryo onwards (Fig. 11). Vacuolation at this stage of development is variable. Some cells are devoid of them whereas other cells have many small vacuoles which in some cells coalesce to form large vacuoles (Fig. 10). Other cells have one or two larger vacuoles appearing on either at the end or in the centre of cells. Cell wall still remains very thin (up to 0.08 μm) without any intercellular spaces but with many plasmodesmata. Endoplasmic reticulum, Golgi bodies, mitochondria, nuclei and oil bodies remain almost unchanged with a slight variation in diameter of oil bodies which are up to 1.4 μm with a maximum number of 20 per cell in a section (Fig. 11). The density of ribosomes which are free and randomly dispersed is increased and cell matrix has become darker. Small nucleolar vacuole and granular and fibrillar zones constituting the dense material, are seen in the nucleolus (Fig. 11). Nuclear pores and the inner and outer envelopes are resolved in some electron micrographs.

The lamellae of chloroplasts in some cells are more differentiated (a stack of 3-4 lamellae) as compared to that of the torpedo-shaped embryos (Fig. 13). The size of chloroplasts has also increased, with maximum length up to 2.0 μm as compared to torpedo-shaped embryos. Occasionally a chloroplast contains a single but small starch grain, with a maximum diameter of 1.22 μm; although most starch grains are smaller about 0.50 μm in diameter (Fig. 13). Chloroplasts in some cells are still at different developmental stages (Fig. 10). Most of the chloroplasts are now more or less regularly shaped, with greater lamellar development which is at different aggregation stages; some lamellae show overlapping, whereas others show invagination at few places resulting in sparse granal stacking to form small grana.

(c) Inverted U-shaped embryo.

With further growth of cotyledons, 8-9 days after petal fall, the embryo assumes an inverted U-shape in which one leg is formed by the hypocotyl and radicle and the other leg by the flattened cotyledons (Fig. 3). This enlarged embryo almost fills the seed cavity with no liquid left at this stage (Fig. 3). Most of the increase in size is due to the expansion of central parenchyma cells of cotyledons, which seem to have stopped dividing and their volume is increase as some cells are up to 21.0 µm long and 11.0 µm broad. Other cells still show the signs of recent divisions. The increase in cell volume of central parenchyma cells is also associated with an increase in the total amount of cytoplasm, endoplasmic reticulum and increase in other organelles. Vacuolation is still variable like that of the walking-stick shaped embryos, but the vacuoles in some cells have enlarged, whereas in some other cells large vacuoles have become central (Fig. 14). Cell wall thickness is increased to 0.22 µm, with numerous but conspicuous plasmodesmata (Fig. 15).

Intercellular spaces have appeared at a few places (Fig. 14). Nuclei are almost circular like that at earlier stages with roughly spherical central nucleolus and few dark chromatin masses near to nuclear membrane, suspended in the nucleoplasm (Fig. 15). The inner and outer membranes of the nuclear envelope are clearly resolved and the outer membrane bears ribosomes, like the endoplasmic reticulum (Fig. 15). The nucleolus exhibits differentiation into a granular region and a region composed of closely packed fine fibrils and has small nucleolar vacuole. The nuclei in other cells have very little chromatin material left in the form of 1 to 3 dense granular bodies either near to nucleolus (Fig. 14) or near to nuclear envelope (Fig. 18). In the cells having larger vacuoles, nuclei migrate nearer to the plasmalemma. Double membranous nuclear envelopes have conspicuous nuclear pores (Fig. 15). Endoplasmic reticulum which is of rough type has become more extensive with

vesicles scattered throughout the cytoplasm, but concentrate at the plasmalemma (Fig. 15). Thus, the cells at this stage are more regularly lined with rough endoplasmic reticulum (Fig. 19). Golgi bodies are prominent throuhout this stage, and an electron-dense material is present in their loops and in adjacent cytoplasm. This electron-dense material is progressively decreasing in density from torpedo-shaped embryo stage through walking-stick shaped embryo stage up to this stage, suggesting that the activity of the Golgi bodies gradually becomes less as the embryo progresses towards maturity. The density of ribosomes which are not aggregated, is increased (Fig. 16) and some adjacent cells show differences in ribosome density, as if indicating varying degree of differentiation (Fig. 14). The mitochondria which are circular and elongated and lie usually nearer the nuclei (Fig. 15), are clearly distinguishable (Fig. 16). The chloroplasts and starch bodies in them have enlarged with maximum length of chloroplast up to 2.65 μm. Chloroplasts show greater differentiation of lamellae, resulting in the formation of many small grana with maximum stack of 8 lamellae in one granum (Fig. 16). Chloroplasts which have definite shape contain mostly one starch grain (2-3 in a few chloroplasts, Fig. 19). Starch grains are bigger with a maximum length of 1.3 μm and are either central or marginal (Fig. 17) in position occupying half the volume in many chloroplasts (Fig. 16). Chloroplasts are clearly grouped around the nuclei in most cells (Fig. 18). Many smaller chloroplasts do not form starch, but their lamellar system remains intact, and other are still in developing stage (Fig. 15). Possibly there are two types of chloroplast present at this stage. Chloroplasts with or without starch contain a varying number of grana which are not fully developed in some cells, while in other chloroplasts they are fully developed, containing stack of very electron-dense lamellae. Such fully developed grana do not appear

compressed or distorted by the presence of starch at this stage. Since the chloroplasts increase in size more rapidly than the starch grains, their dimensions are not altered by the enlargement of starch grains. Some connections between grana lamellae are found to develop. The stroma of chloroplasts contain ribosome-like particles and other small electron-dense particles.

Oil bodies have greatly increased in number (about 34 per cell in a section). The diameter of oil bodies has also increased up to 1.8 μm.

(d) Mature-green embryo.

Maturation of the embryo starts on the 9th day after petal fall and continues for about 6 days remaining green till then. The cotyledons of fully mature embryos have thickened considerably to fill the seed completely (Fig. 3). The cell divisions seem to have ended by this stage as no evidence of cell divisions is found during this and subsequent maturation stages.

At 10-12 days after petal fall the embryo is still soft as it is not fully mature and is brighter green. By this stage, each cell has a large central vacuole produced by the coalescence of smaller vacuoles of early stages and pushing cell contents nearer to the cell wall (Fig. 20). Vacuoles are more or less electron-transparent regions surrounded by a unit membrane. These irregular vacuolas contain homogeneous substances which are probably developing protein bodies (Fig. 20). The quantity of cytoplasm has clearly increased and the volume of cell organelles has also increased. Intercellular spaces are clearer (Fig. 21) and more numerous (Fig. 20) than in the inverted U-shaped embryos. Cell wall thickness has not increased much with less conspicuous plasmodesmata. Electron-dense middle lamella can be marked out from electron-

transparent primary walls (Fig. 21). Proliferation of endoplasmic reticulum at this stage is less elaborate than at the initial stages. The density of ribosomes is increased in the cytoplasm and the cell matrix has become dark. Mitochondria which are spherical to elliptical in section, are clearly distinguishable at this stage with well defined outer membranes, swollen tubular cristae and electron dense matrix (Fig 21). Some mitochondria show more electron transparent regions which lack cristae but have fibrillar inclusions. Mitochondria, the number of which is increased at this stage, occur either in groups (Fig. 20) or singly (Fig. 21) close to the nuclear envelope. Although the mitochondria are widespread within the cells, they often occur in localised groups while larger areas of the cell contain none of them (Fig. 20). The large nuclei in a few cells are still present in the centre of the cell with (Fig 21) or without (Fig 20) a large electron-dense circular nucleolus and small chromatin. In other cells with large central vacuoles, the nuclei are either present adjacent to cell wall or cannot be seen clearly. Many nuclei have lost their circular form and are becoming lobed, and most organelles tend to congregate around them (Fig. 20). Golgi bodies are not as frequent as during early stages.

Cells of cotyledons continue to accumulate the storage reserves ie. starch (in the plastids) and lipids (in oil bodies). Protein, the other main storage reserve, has started accumulating homogeneously in the vacuoles (Fig. 20).

Most of the chloroplasts which have increased in size with maximum length up tp 3.0 μm containing one big (or two), starch grain (Fig. 20) up to 1.6 to 2.0 μm in length filling almost half the volume of chloroplast and compressing the lamellae and stroma against the limiting membrane. The synthesis of starch grain (one per plastid) does not seem to occur more rapidly than plastid growth, thus does not alter the shape and structure of the chloroplasts. Starch grains have never

been found in contact with the plastid membranes at any stage of their existance at this stage of embryo development. Few of the fully developed chloroplasts have not formed any starch and their lamellar system is intact with fully developed grana and intergrana lamellae (Fig. 21). Possibly two types of chloroplast are present at this stage also like the earlier inverted U-shaped stage. Chloroplasts are fully developed with a varying number of well developed grana with large stacks of electron dense lamellae. Occasionally starchless chloroplasts have got small electron-dense spherical bodies in the stroma called osmiophilic plastoglobuli.

Oil bodies which by this stage are arranged parallel to cell wall, have increased a little in number per cell up to 40 per cell per section, while the diameter reaches up to 2.4 μm.

(e) Fully mature-green embryo.

By 15 days after petal fall the embryo becomes fully mature, when it is dense green and hard. At this stage cell division has completely ended but cell expansion still continues. The large central vacuoles have been subdivided into many small vacuoles which are more or less of uniform size and separated by cytoplasmic strands in many cells (Fig. 22). These vacuoles show various stages of their contents which are beginning to turn into reserve protein bodies. Different stages of filling can be seen within one cell and between neighbouring cells. Some of the vacuoles show very electron dense homogenous contents, whereas other vacuoles show flocculated contents in them (Fig. 22). Such deposits of electron dense protein are formed within the vacuoles, first accumulating at the periphery of the vacuoles (Figs. 21,22). But in most vacuoles such protein deposits which appear to concentrate at the inner surface of the tonoplast (Fig. 22), contain small particles, the globoids,

surrounded by clear areas in the protein matrix. The tonoplast, which later surrounds the mature protein body, is rarely seen with sufficient clarity (Fig. 22). It is normally composed of a single membrane as in the case of the lysosome and microbody. The well developed rough endoplasmic reticulum which surrounds the protein vacuoles in most plant embryos studied, could not be traced clearly at this stage as it appears to be greatly reduced to a few disappearing remains and tiny profiles.

There is a considerable increase in the number of oil bodies at this stage of maturity, reaching up to 70 per cell in a section, and these appear to occupy most of the cell area unoccupied by protein vacuoles and other cell organelles. They obviously occur more frequently in the peripheral cytoplasm along the cell wall. These spherical oil bodies which are homogeneous, show insignificant increase in their size at this stage. Starch grains have grown considerably, distorting the inner plastid structure in some cases (Figs. 24,25), probably due to more rapid synthesis of starch grains not accommodated by an enlargement of chloroplasts. Some small round electron-dense bodies, possibly the osmiophilic plastoglobuli are seen in the stroma of some chloroplasts (Fig. 24). These elctron-dense bodies appear free in the stroma lying nearer to the lamellae and occur singly at this stage. By this stage almost every chloroplasts seems to have developed a single (rarely two) well developed starch grain (Fig. 22).

Vague round nuclei without nucleolus but with irregular aggregates of chromatin are visible in a few cells (Figs. 22,23). One or two very dense spherical bodies are found attached to the inner nuclear membrane probably representing the left-out nuclear chromatin. Mitochondria which are very clearly distinguishable are small and circular (Fig. 23). Golgi bodies are not traced at this stage.

(f) Mature ivory-white embryo.

By 18-20 days after petal fall, the embryo becomes fully mature,ie. becomes hard, and turns ivory-white by the loss of chlorophyll. The cotyledonary cells at this stage continue for some time to expand, and maximum expansion takes place during this stage when cell reaches up to 30 µm in length. The ultrastructure of cell shows how extensively the cotyledons and radicle have become transformed into storage organs, packed with lipid and protein and without starch. These cells continue to accumulate protein reserves which are laid down uniformly throughout the cotyledon (Figs. 26, 27), except the epidermal and subepidermal layers. Starch abruptly disappears at this stage. The amount of protein formed within the vacuole during last maturation stage, gradually increases until the vacuole is fully occupied by a protein matrix with evenly distibuted globoids in it, and it is recognizable as aleurone grain (Figs. 26, 27). Two types of inclusion may be found in the same aleurone grain.

A limiting membrane could not be resolved around these bodies, though it may be present. The larger of the two inclusions appears as one or more almost spherical, transparent regions which may sometimes contain a darkly stained body and referred to as globoids (Figs. 26, 27). The second type is represented by small crystalline inclusion (Fig. 28). These crystals are probably crystalline protein, which is known to occur in the aleurone grains of many seeds (Smith, 1974). Both types of inclusion are distinguished from the rest of the protein body, which appear moderately electron-dense and amorphous in texture when stained either with lead citrate only, or with uranyl nitrate with lead citrate. Globoids are not stained with these stains (Paulson and Srivastava, 1968), and their presence can be inferred only by the presence of electron transparent areas with sharply defined edges (Figs. 26, 27).

Crystalloids appear as highly electron-dense and often irregular in shape and commonly occur in areas interpreted as globoids (Fig. 28). Sometimes they are found in proteinaceous matrix, possibly because they are dislocated from their natural position during sectioning. The number of globoids in aleurone grains is variable, those with higher accumulation of protein have more globoids. Some globoids are larger and take on an angular shape. The number of globoids is much greater in Capsella bursa-pastoris as in other cruciferous embryos studied. These aleurone grains of Capsella bursa-pastoris vary considerably in size, shape and number per cell. They are maximum up to 5.5 µm in length (Fig. 28) and are spherical, irregular, angular or oval in outline, possibly due to pressure caused by increasing number and proliferation of oil bodies (Figs. 26, 27), or sectioning at various levels through spherical protein bodies. In these embryos, aleurone grains (2-15 per cell) are arranged in a regular fashion forming a ring or two around the nucleus. Some of these look homogeneous while most of them contain inclusions and each aleurone grain retains a single membrane.

The second type of protein grains which are called myrosin grains and are without any inclusion (globoid or crystalloid) and probably complete their development before the aleurone grains (Rest and Vaughan, 1972) could not be traced in these embryos. Myrosin grains are characteristic of most crucifer embryos and were frequently found in Sinapis alba (Rest and Vaughan, 1972) and sometimes in some parenchyma cells of Crambe abyssinica embryos (Smith, 1974).

Oil is generally abundant in the Capsella bursa-pastoris seed like most oil seeds in comparison with protein. There is a rapid increase in the number of oil bodies in the mature ivory-white embryo, maximum up to 260 per cell in a section, which occupy all the cell space unoccupied by aleurone grains, nucleus and residual cytoplasm. Owing to their increased number, the oil bodies get arranged in more than one layer

around the periphery of the cell or in clear band like formation and some of them are pressed together and so lose their spherical shape and have become elongated or irregularly shaped. Many of them are seen in a ring around each aleurone grain in each cell. These oil bodies are surrounded by single membranes and are smaller on the average than the protein bodies. Oil bodies as seen in sections fixed in glutaraldehyde-osmium tetroxide, appear homogeneous in structure and are light grey in colour. It appears that there is a gradual decrease in electron-density of oil bodies from torpedo-shaped embryo stage to complete dry embryo. There is little increase in the size of the oil bodies since only a few of them appear bigger (0.3 μm), the increase in oil content of the seed is brought about by the increase in number of oil bodies.

Because of the great number of protein and oil bodies within each cell, it becomes difficult to recognise any organelle. However, organelles seem very few and far between among the reserve granules and are recorded only in the residual cytoplasm. The cell wall becomes thicker with clear electron-dense middle lamella and electron transparent primary walls (Fig. 27). Most of the bigger cells become hexagonal or irregularly polygonal in section. Nuclei become highly lobed, more dense with projections, penetrating between reserve bodies and joining with residual cytoplasm (Fig. 26). Irregularly outlined nuclei are present in the centre of many cells and appear to be surrounded by oil bodies (Fig. 27). A faint nuclear membrane is also seen in some nuclei (Fig. 29). Residual chromatin which is darker is present in most nuclei (Figs. 26, 29).

The mitochondria appear to have lost their elongated shapes, some of their internal membranes and the electron density of the matrix. They have become rounded with very less electron-dense matrix than the cytoplasm with hardly any recognizable cristae. Ribosome density has

faded; endoplasmic reticulum has still further fragmented and mostly disappear and Golgi bodies also seem to have disappeared abruptly during the maturation. A general loss of contrast of electron micrographs is noticed at this stage, particularly in the membranes of nuclei, mitochondria, chloroplasts and endoplasmic reticulum. Some irregularly shaped organelles could not be identified (Fig. 30).

Chloroplasts can still be identified but with little (Figs. 31, 33) or complete absence (Fig. 32) of lamellar structure. The two membranes of their envelope come very close together leaving a very narrow lumen. Chloroplasts gradually become rounded and assumes bell shapes or globular form or become irregular (Fig. 32). The internal structure of chloroplast is breaking down. Lamellae have separated and typical stacking into grana is partially or completely gone. A certain degree of shortening and swelling of lamellae in the grana is seen in few chloroplasts and only a few very dense and thick grana like structures are recognizable in the centre of such elongated chloroplasts (Fig. 33). In other irregularly shaped chloroplasts few disorganising (fading) elongated lamellae are seen in their centre and all internal structures appear as if macerated (Fig. 31). During early maturation, the grana appear as distinct units, but as maturation proceeds the lamellae appear to become progressively less electron-dense. Such degenerating chloroplasts contain many small, electron-dense spherical bodies- the osmiophilic plastoglobuli, in their stroma arranged either singly or in groups. In some chloroplasts plastoglobuli are found in very close association of degenerating grana and appear attached to them (Fig. 33). Most of the chloroplasts appear dominated by plastoglobuli at this stage (Fig. 32).

(g) Mature-dry embryo.

A dry seed with brown testa forms within about 26-28 days after petal fall. The pale coloured embryo has become dehydrated and the dry cotyledons are creamy white and hard. Most of the central cells assume a hexagonal shape (Fig. 34). The cell walls appear thicker and are 0.3 to 0.4 µm wide with an obvious middle lamella (Fig. 40). The cell walls are more thickened at the corners of the cells with larger intercellular spaces (Fig. 34). Lipid materials accumulate locally in some intercellular spaces (Fig. 34). Very little residual cytoplasm with few free ribosomes can be seen between oil bodies. Endoplasmic reticulum and Golgi bodies are still preserved at this desiccated stage. Endoplasmic reticulum lamellae get concentrically arranged in the residual cytoplasm (Figs. 38, 39). Many stacks of Golgi bodies with swollen vesicles are seen in the cytoplasm surrounded by big oil bodies (Figs. 36, 37). Such fine and well preserved stack of membranes forming Golgi bodies along with big vesicles and also such typical concentrically arranged endoplasmic reticulum lamellae have hardly been reported from other dry seeds studied so far, and thus these structures form an outstanding feature of the dry cotyledons of Capsella bursa-pastoris.

There is a general shrinkage of most of the organelles, as in the case of the internal membranes of mitochondria and chloroplasts, and they are referred to as 'disorganized'. The chloroplasts especially become much shrunken (Fig. 40). Mitochondria are not readily distinguishable at this stage except that few roundish bodies left in residual cytoplasm without any structural organization may be regarded as mitochondria. Lobed nuclei with few dark spherical bodies in each lobes are recognizable in a few cells with indistinct nuclear membrane (Fig. 40). One of the electron micrograph shows a large nucleus with densely stained nucleolus and chromatin (Fig. 41).

The plastids are not so poorly defined as mitochondria. They show

distinct outer envelope but lack any lamellae in them. The details of stroma are obscure, but several small electron-dense spherical bodies, the osmiophilic plastoglobuli, are present, thus distinguishing chloroplasts from the surrounding cytoplasm containing disorganising organelles. A distinctive feature of these plastids is the presence of one or more highly electron-dense inclusions in the stroma bigger than plastoglobuli. These inclusions are usually spherical in sectional view, and heterogenous in structure. They stain positively with uranyl nitrate and osmium tetroxide. The embryos of mature seed show a fairly uniform population of aleurone grains with respect to size, although approximately 8-10 percent of aleurone grains are irregular in shape in comparison to normal spherical shape. The apparent size differences shown in many electron micrographs seems to be to sectioning at various levels through spherical protein bodies. Few of them are seen elongated and contain few globoids and/or crystalloids (Fig. 35). In general aleurone grains appear smaller compared to those at the earlier stage.

Oil bodies do not increase much in number from the last stage but some of them are enlarged, possibly through the coalescence of the smaller droplets. All cells look completely full of oil and protein. Oil bodies may get compressed due to their large number with the maximum diameter of 2.0 μm. More smaller oil bodies have been concentrated adjacent to cell wall. A distinctive feature in dry Capsella bursa-pastoris cotyledons is the local accumulation of lipid material in some intercellular spaces (Fig. 42). This is presumably due to migration of excess lipid from the adjacent cells.

3. Notes on Light Microscopy.

Sections 1-2 um thick were prepared covering all the stages and stained with different dyes for light microscopy. Torpedo-shaped embryos when stained with toluidine blue O have dark blue stained central nuclei lying almost in the centre of cells along with greenish stained cytoplasm. Cells of inverted U-shaped and mature-green embryos show positive reactions for starch with KI_2.

Cells of mature ivory-white stage and dry embryo stage contain large amount of reserve material which occur as discrete bodies. Table 5 summarizes the histochemical tests for their identification. One type of material gives a positive test for protein with toluidine blue O (Fig. 43b). Protein bodies appear as clear spherical areas stained light brown with Sudan IV (Fig. 43a) and greenish yellow with Azure II (Fig. 44). The second type gives a positive test for lipids with Sudan III, IV (all saturated solutions of 70% alcohol), in tissue which is either fresh or fixed in glutaraldehyde-osmium tetroxide as the latter show very well preserved structures of lipid bodies, which do not get extracted during processing for electron microscope studies. Other fixatives do not properly preserve the lipids. Oil bodies appear homogeneous in structure and are smaller than protein bodies and are seen very clearly in cells stained greenish yellow with Azure II (Fig. 44). Sudan III and IV stain oil bodies more clearly in fresh cotyledonary cells as compared to cells of cotyledon fixed in glutaraldehyde-osmium tetroxide (Fig. 43a).

Protein bodies vary considerably in size and are spherical or irregular in outline (Fig. 43b). Some protein grains appear as homogeneous while others contain inclusions of two types. The larger of the two appear as one or more almost spherical, transparent regions, which may sometimes contain densely stained bodies called "globoids". The second

type is represented by small crystalline inclusions which are occasionally stained red with toluidine blue O and is referred to as "crystalloid" (Fig. 4b).

Squash preparations of dry cotyledons stained with aniline blue for one hour and mounted in lactophenol, probably show positive test for myrosin protein as dark blue stained structures. When stained with orcein, myrosin protein also seem to give a positive test, but such myrosin grains could not be traced during electron microscopic studies. Their presence appears doubtful at present although more histochemical tests might eventually confirm their presence in Capsella bursa-pastoris embryos.

TABLE 5. Staining reactions of reserve materials with various dyes.

Material	Stains	Reaction				
		Starch	Protein	Lipid	Nucleus	Cytoplasm
Fixed with glutaraldehyde OsO_4	Toluidine blue O	-	Bright blue	-	Blue	Greenish
	Sudan III, IV	-	-	Brownish yellow	Dark brown	Brownish
	Sudan black	-	-	Brownish grey	Dark brown	Brown
	Azure II	-	-	Greenish yellow	Dense blue	Greenish yellow
	KI_2	Blue	Brown	-	Dark brown	Light brown
Fresh sections/ squash preparations.	Sudan III	-	-	Yellowish brown	-	-
	Sudan IV	-	-	Orange	-	-
	Toluidine blue O	-	Blue	-	Blue	-
	Orcein	-	Pink (myrosin only)	-	-	-
	Anilin blue	-	Dark blue (myrosin only)	-	-	-
	KI_2	Blue	Brown	-	Dark brown	Light brown

DISCUSSION.

The ultrastructural development of early embryonic stages of Capsella bursa-pastoris through to the heart-shaped stage was described by Schulz and Jensen (1968a, b, c). The present work is an extension of their work in the sense that it deals with the later stages of embryogenesis from the early torpedo-shaped embryo to the mature seed. The sequential development of these embryonic stages is completed between 5-28 days after petal fall. Our observations for embryo development in Capsella bursa-pastoris fit very well into the general sequence of events that has emerged from other studies.

Initially the cotyledonary cells of Capsella bursa-pastoris undergo changes common to many cruciferous embryo cells during early growth and differentiation. At the ultrastructural level, there is some simil - arity between cell contents at various stages of Capsella bursa-pastoris embryo, found in the present investigation, and similar stages of Sinapis alba embryos observed by Rest and Vaughan (1972). Meristematic cells similar to those observed in the early stages of embryonic development are observed in torpedo-shaped embryos of Capsella bursa-pastoris. These cells have thin cell walls, well developed mitochondria, Golgi bodies,rough endoplasmic reticulum, non-aggregated ribosomes, proplastids and large central nucleus. The cells lack intercellular spaces, a central vacuole, oil bodies and protein bodies. A few oil bodies appear in some cells slightly later. Subsequent growth of Capsella bursa-pastoris cotyledons, as in the case of Pisum sativum (Bain and Mercer, 1966) and Phaseolus lunatus (Klein and Pollock, 1968) is accompanied by a comparatively thicker cell wall, appearance of smaller intercellular spaces, large central vacuole, proliferation of endoplasmic reticulum into many long cisternae, the formation of chloroplasts with tightly packed stacks of lamellae and electron-dense grana.

The newly formed chloroplasts, which lack starch at an early stage, soon appear to have one or more small starch grains, which continue to increase in size until the cotyledons are mature. During the development and maturation, like other seeds, food reserves, in the form of carbohydrate, lipid and protein are laid down in the cells of the cotyledon. Thus along with the accumulation of starch grains during development, oil bodies gradually increase in quantity with subsequent growth of the embryo. Fully developed protein bodies appear during late maturation stage (mature ivory-white embryo stage), when starch grains completely disappear. The increasing importance of storage is reflected by the frequency of starch grain during early stages, the widespread occurence of lipid and the deposition of protein inside the vacuoles. The synthesis of storage products is completed by the green maturation stage of embryo. Though the origin of organelles and the means by which their number per cell increases, remain unsettled issues, the concept of dynamic organelle with a continuously changing membrane system has been well established (Setterfield et al., 1959; Hrsel et al., 1961). For instance, cell elongation and differentiation bring about increases in the surface area of the plasmalemma and an organized complexity in the internal membranes of mitochondria and chloroplasts. Likewise, daily increases in oil and protein of storage tissue like cotyledons lead to the increase in number and size of oil bodies and protein bodies.

Maturation begins after the embryo (seed) has reached maximum fresh weight and during maturation the moisture contents drop rapidly from 65% (moisture contents during ripening decreases from 85% to 65% approximately) to about 15% with almost no increase in dry weight (Klein and Pollock, 1968). Most striking changes in organelles have been observed during this period in the present investigation.

Ribosomes.

Polsomes are common in young developing embryos, rare in maturing embryos, missing in the dry embryos, and common in germinating seeds. The predominance of polysomes over monosomes up to the heart-shaped embryo stage and complete absence of polysomes beyond heart-shaped stage is very significant in Capsella bursa-pastoris embryos. This early return to a random distribution of the ribosomes in Capsella bursa-pastoris embryo may be related to a change in nutritional and metabolic relationships between the embryo and its environment at this time (Schulz and Jensen, 1968c). It is at this stage that the initiation of endosperm breakdown and resorption starts, oil bodies begin to appear and starch accumulation starts in the plastids.

Plant tissues subjected to drought or water-stress lose polysomes (Bewley, 1972; Hsiao, 1970). A decrease or loss of polysomes appears to be a general phenomenon induced by dehydration of the plant tissues. It has been reported during maturation of seeds (Klein and Pollock, 1968; Opik, 1968a) during desiccation of germinating embryos (Chen 1968; Chen et al, 1968; Sturani et al., 1966) during water stress of isolated root tips (Nir, 1969; Nir et al., 1969; Nir et al., 1970). However, the loss in polysomes has never been observed before in plants at such an early stage as in the present study of Capsella bursa-pastoris embryo, when its tissues still retain high percentage of water. This loss of polysome from very early stages of Capsella bursa-pastoris embryo which continues to grow up to the maturity of seed demands further investigations and explanations.

Polysomes which are considered to be held together by a messenger of ribonucleic acid (mRNA) molecule (Warner et al., 1962) appear as thread-like strands in the cytoplasm or, attached to the endoplasmic reticulum to give it the rough surface appearance as it is seen in

metabolically active cells where protein synthesis is taking place. The loss of polysomes in Capsella bursa-pastoris embryo may be due either to some defect in the ribosomes themselves or to the lack of supply of new mRNA. A cessation of RNA synthesis and disappearance of polysomes has been reported during mitosis (Prescott and Bender, 1962; Scharf and Robbins, 1966). The cessation or decrease, in the formation of new mRNA, results in a decrease in the polysome population in the protoplasm and, thus interferes with normal protein synthesis (Nir et al., 1970). Sturani et al. (1966) suggested that the restriction of water uptake determines the disappearance of short-lived mRNA able to bind monosomes to synthesize polysomes. Primary cause of polysome loss during desiccation according to Dhinsa and Bewley (1976) is that ribosomes run off from mRNA coupled with a failure to re-form an initiation complex, rather than the increased activity of ribonuclease (Genkel et al., 1967).

If the polysome-endoplasmic reticulum complex is indeed needed for rapid accumulation of protein, its disappearance may be correlated with the decreased rate of protein formation towards the beginning of torpedo-shaped embryo stage in Capsella bursa-pastoris and continues until the mature-green embryo stage. There is no indication of an increase in endoplasmic reticulum during the period of maturity of Capsella bursa-pastoris embryo; on the contrary a reduction in endoplasmic reticulum units occur when the embryo is fully mature. This may suggest that in the absence of membrane formation the protein formed by the polysome-endoplasmic reticulum complex during early stages (heart-shaped embryo stage) may be used principally for storage (Klein and Pollock, 1968).

Some reports suggest that all the mRNA of a ripening seed is not lost as the tissues dry. The entire apparatus for protein synthesis is present in the ungerminated Triticum vulgare embryos (Marcus and Feeley, 1964), and that only the activation or formation of mRNA during

imbibition is required for protein synthesis (Marcus and Feeley, 1966). Dure and Waters (1965), demonstrated the persistance of stable mRNA in the mature Gossypium hirsutum embryo and dry seed. They also reported that the level of polysomes, in dry Gossypium hirsutum embryo, is as high as at any point during the first few days of germination. Chen et al., (1968) using hybridization technique have elucidated the presence of mRNA which becomes activated in dry Triticum durum embryo during germination.

These few studies provide conclusive evidence that polysomes exist in dry seeds. It is possible that the polysomes are damaged during isolation or fixation either mechanically or chemically through the degradation of mRNA molecules by ribonuclease (Loening, 1968). Hence, their absence from electron micrographs of mature and dry embryos does not necessarily prove that they are not present in the tissue; but their absence during the early stages of Capsella bursa-pastoris embryo still remains inexplicable.

Chloroplast and Starch grains.

Plastids follow normal course of progressive development from an early torpedo-shaped stage until the mature green embryo stage of Capsella bursa-pastoris embryo. Cotyledonary cells at early stages show few proplastids along with other developing chloroplasts. Starch which is present during the very early stages of development of Capsella bursa-pastoris embryo and disappears after or during 3-celled embryo stage (Schulz and Jensen, 1968a, b, c), reappears during torpedo-shaped embryo stage, when it begins to accumulate in occasional plastids as a very small starch grain. Starch occurs in the cotyledonary cells at later stages of embryos as single but large grains whose size continues to increase attaining its maximum at mature green embryo stage, but after which it disappears abruptly. During maturation there

appears to be little increase in the number of starch grains, although there is an increase in their absolute size. Thus, the frequency of starch grains per cell in the mature green embryo stage is approximately the same as that of plastids in the cells of earlier stages like late torpedo-shaped embryo stage, suggesting that little or no plastid division has occurred during maturation and increase in total starch is due to increase in grain size rather than number. The accumulation of starch in this embryo from the egg cell stage and its disappearance during the first two divisions, accounts for the necessary energy supply required during the critical divisions of embryo. Starch accumulation during the later stages of embryo development might serve as one of the storage reserves. Its abrupt disappearance after mature green embryo stage can be accounted for the appearance of another type of storage reserve in the form of protein bodies. The significance of two types of plastids- starch containing and starchless, during inverted-U-shaped embryo stage and mature green embryo stage is not understood.

In some cases bigger starch grains during mature green stage of Capsella bursa-pastoris embryo disrupt the inner plastid structure. Bain and Mercer (1966) also reported such disruption of lamellae in dry Pisum sativum cotyledons by the growing starch grain which ultimately results in the rupture of chloroplast membrane. Such loss of chloroplast membrane is, however, not observed in the present investigation. The internal structure of the plastid during late maturation stages shows breaking down, as lamellae get separated and typical stacking into grana is partially or completely lost. As some of the plastids show certain degree of swelling of lamellae in the grana, the loss of grana in them may be ascribed to progressive disruption and swelling of lamellae (Khera and Tilney-Basset, 1976). But the real chloroplast deterioration is experienced by cotyledonary cells of

Capsella bursa-pastoris embryo, approaching late maturity at ivory-white embryo stage not by rupturing of chloroplast membrane or disruption or swelling of lamellae. But there is decrease in electron density of the grana (which up to green mature embryo stage appears as tightly packed stack of thylakoids) as the embryo loses some moisture. Thus, the change leading to the disappearance of grana is due to gradual fading of the density of the thylakoids and concomitantly with loss. Similar chloroplast deterioration has been described in Phaseolus lunatus cotyledons (Klein and Pollock, 1968). Chloroplast deterioration in Capsella bursa-pastoris embryo appears to be a different process from that of the leaves, where chloroplast disintegration usually takes place by swelling of thylakoids and disruption of grana (Wrischer, 1965). Plastids from dry cotyledons show a distinct outer envelope with no lumen left between the two membranes of the envelope and with no lamellae, but with one or more highly electron dense inclusions in the stroma like those found in dry cotyledons of Lactuca sativa (Paulson and Srivastava, 1968). These inclusions appear to be some kind of a reserve lipoprotein (Paulson and Srivastava, 1968).

While working on the development of embryos of Pelargonium X Hortorum containing normal plastids and mutant plastids, Khera and Tilney-Basset (1976) observed that the development of embryo containing mutant plastids, proceeds in a similar manner of those with normal embryos, indicating that the changes in the plastids do not seriously affect the functioning of other organelles. On the basis of these observations they made a probable conclusion that green chloroplasts, as found in normal embryos, is not absolutely indispensible. This idea needs further investigation and experimental verifications as it is probable that the chloroplast in situ is a real aid and really helps in a quantitative way to bring higher proportion of embryo to good mature seeds than is possible without chloroplast.

Osmiophilic Plastoglobuli.

Osmiophilic plastoglobuli are a characteristic feature of chloroplasts fixed with osmium tetroxide, and appear as round, electron-dense bodies in the stroma of mature-green embryo stage chloroplasts of Capsella bursa-pastoris. These plastoglobuli are not found in the chloroplasts of earlier embryonic stages of Capsella bursa-pastoris embryos although these lipid-containing bodies are regarded as a normal feature of the chloroplast stroma (Granick, 1961; Menke, 1962, 1966), occurring at all stages of the growth and differentiation of plastids (Lichtenthaler, 1968), but appearing in large numbers at some stages while at other stages they reach an especially large size (Falk, 1960; Price et al., 1966). They appear free in the stroma, occurring singly as well as in clusters in the chloroplasts of mature-ivory-white embryos and mature-dry embryos of Capsella bursa-pastoris as in old maturing cotyledonary chloroplasts of Cucumis sativus (Butler, 1967) and Glycine max (Treffrey et al., 1967). At the mature-green embryo stage of Capsella bursa-pastoris the plastoglobuli appear smaller in size and fewer in number while at the mature-ivory-white embryo stage these globuli appear in their maximum number and of almost one size. At the mature-dry embryo stage, when the chloroplasts are shrunken, the number of globules also appears less. Lichtenthaler (1968) suggested that plants can be divided into two groups according to whether the older senescing chloroplasts show a large number of plastoglobuli with little increase in size or a similar number of much larger plastoglobuli. The chloroplasts of Capsella bursa-pastoris embryos clearly belongs to the first group like the chloroplasts of many other annuals as proposed by him. The number and size of plastoglobuli have been observed to increase according to the age of mature, non-degenerating chloroplasts (Greenwood et al., 1963; Lichtenthaler, 1969a,b). The chloroplasts

of Capsella bursa-pastoris embryo during the last two maturing stages are completely dominated by these lipid globules which have increased in number and these chloroplasts are almost degenerated: the grana lamellae have separated and collapsed accompanied by the disappearance of stroma. Such breakdown of the chloroplasts resulting in a spectacular accumulation of osmiophilic globules have also been reported in the cotyledons of Cucumis sativus (Butler, 1967), in the senescing leaves of Ginkgo biloba (Toyama and Ueda, 1965), Phaseolus vulgaris (Barton, 1966), and Betula verrucosa (Dodge, 1970). As the thylakoids consist of 50% lipid and 50% protein (Lichtenthaler, 1968) the globules found in the present study could be an accumulation of membrane breakdown products, an interpretation favoured by Ikeda and Ueda (1964), and by Barton (1966), is that part of the lipids available from the breakdown of the thylakoids is deposited in the plastoglobuli. In our present studies of Capsella bursa-pastoris embryos some chloroplasts from the mature ivory-white embryo stage show a close association between the globules and degenerating grana and some globules appear attached to grana like those also reported earlier (Barton, 1966; Dennis et al, 1967) and it may well be concluded that these globules are derived from thylakoids. This hypothesis is strengthened when the thylakoid development was prevented by lack of nutrients or by treatment with chemicals (Heslop-Harrison, 1962; Thomson et al , 1964) or when thylakoids break down, there is an increase in the number or volume of the plastoglobuli. Moreover lamellar lipids synthesized in the dark are deposited in the plastoglobuli lying near to prolamellar bodies and not in thylakoids which are absent (Present study part II; Lichtenthaler and Peveling, 1967). The present study on Capsella bursa-pastoris embryos (Part II) like earlier studies (Lichtenthaler, 1968) show that a rapid synthesis of thylakoids follows illumination, while the prolamellar bodies break up and the number of plastoglobuli is reduced. Part of the lipid stored

during the dark in the plastoglobuli is evidently used in the synthesis of the thylakoids. Possibly the osmiophilic plastoglobuli serve some function in the formation of lamellae, as precursors, or reserve or waste material, especially as they appear first near the prolamellar body, a site of membrane formation (Wettstein, 1959; Greenwood et al., 1963; Bailey et al., 1966; and present study part II). Thus plastoglobuli of Capsella bursa-pastoris as in other plants act as a general store or reservoir of excess lipids and other substances (Lichtenthaler and Peveling, 1967). Physiological conditions, besides the age of the plant, also affect the size and number of the plastoglobuli. Adams and Strain (1969) while working on Cercidium floridum, a drought-deciduous plant, found that the chloroplasts in the rather ephemeral leaves appearing after the rainfall contained starch similar to other higher plants. The chloroplasts in the green bark tissue, resemble with those in the ephemeral leaves and other plants, except they lacked starch and have numerous large plastoglobuli. As the green bark tissue evidently provide a major source of photosynthetically fixed carbon to the plant, Adams and Strain (1969) have suggested that the plastoglobuli may represent a form in which photosynthetically fixed carbon is stored in these chloroplasts subsequent to further utilization in the carbon economy of the plant. Thus these plastoglobuli may have the same general role as starch in most other plants. This attractive hypothesis which has been put forward for plants where limited water may be available for metabolic processes, stands true for the maturing embryo of Capsella bursa-pastoris with maximum number of plastoglobuli at mature ivory-white embryo stage and mature-dry embryo stage when the embryo is experiencing a'drought' situation (desiccation stage) with minimal water available to them. This seems to be an efficient energy conservation and utilization device in tissues experiencing dehydration. Ljubesic (1970) , however, suggested that these globules

might in fact be fixation artifacts.

Mitochondria.

The mitochondria in the developing embryo of Capsella bursa-pastoris have a highly organized membrane system which reflects high metabolic activity. The system gets somewhat less organized during maturation and drying. Mitochondria are not readily distinguishable in dry cotyledons of Capsella bursa-pastoris. It appears inescapable to conclude that as the tissue dries the mitochondria become more easily damaged during preparation for the electron microscope studies and this fragility may partly explain the difficulty in preserving the membrane systems of organelles when dry tissue are fixed. Mitochondria appear normal in some dry embryos like Pisum sativum (Perner, 1965); Gossypium hirsutum (Yatsu, 1965); while in Pisum sativum and Triticum vulgare, Setterfield et al. (1959) mentioned that they are recognizable like those in the present study of Capsella bursa-pastoris. Mitochondria however, could not be recorded in dry Hordeum vulgare (Paleg and Hyde, 1964) and Arachis hypogaea (Bagley et al., 1963) embryos. Reduction of mitochondrial cristae has been reported in the cells of maturing embryos of Phaseolus lunatus (Klein and Pollock, 1968) and Lactuca sativa (Paulson and Srivastava, 1968), where the cells are dehydrated and their overall metabolism limited. This reduction of mitochondrial cristae has been regarded as visible indication of decreased respiration activity and such respiration inhibition in plants suffering from water deficit has been confirmed many times (Kramer, 1969). The mitochondria of the dry embryo of Arachis hypogaea have a respiratory chain deficit in cytochrome C (Wilson and Bonner, 1971), which presumably is an adaptation developed by mitochondria to prevent uncontrollable loss of substrate during the quiescent period of the embryo.

Golgi bodies and Endoplasmic reticulum.

There is a general reduction in Golgi bodies and endoplasmic reticulum units during development of embryo of Capsella bursa-pastoris as has been described in the case of Pisum sativum cotyledons (Bain and Mercer, 1966), and Pisum sativum radicle (Perner, 1966), where endoplasmic reticulum becomes fragmented and disperse in the cytoplasm in the form of vesicles. Endoplasmic reticulum is poorly represented and Golgi bodies are lacking in the cells of most mature and dry seeds (Yatsu, 1965; Klein and Ben-Sheul, 1966; Öpik, 1966; Paulson and Srivastava, 1968). However, in one case (Perner, 1965) radicle tissue portions from dry Pisum sativum seeds were fixed with osmium tetroxide fumes over a period of several weeks and the tissue appeared to contain well preserved endoplasmic reticulum of rough and smooth types along with general shrinkage in size of other organelles due to low tissue moisture. Yatsu (1965) also applied the procedure used by Perner on cotyledons of dry Gossypium hirsutum seeds. In the dry tissue, he found plastids, mitochondria and nuclei that possessed normal double membranes, similar to those found in fully hydrated plant cells. However, he could not recognise any Golgi bodies or endoplasmic reticulum. He, along with others, (Paulson and Srivastava, 1968; Yoo, 1970) concluded that Golgi bodies are missing from the cotyledons of dry seeds. Yatsu (1965) speculated that the lamellae that form the Golgi bodies get so compressed due to dehydration that they become hard to recognize. It is also possible that the membranes of Golgi bodies and endoplasmic reticulum become more fragile in dry tissue and therefore, are difficult to preserve through conventional procedures of fixation that were developed primarily for moist tissues. A few Golgi bodies have, however, been reported from the dry aleurone of Hordeum vulgare (Paleg and Hyde, 1964).

In our studies, we occasionally were able to find Golgi bodies and endoplasmic reticulum in dry Capsella bursa-pastoris cotyledons. Although we do not claim that our techniques are ideal for fixing dry tissues, we are able to obtain dictyosomes with vesicles and endoplasmic reticulum with well preserved membranes. Our findings suggest that, by modifying the fixation techniques we might be able to preserve the integrity of some organelles, such as Golgi bodies, which other investigators have not been able to envisage in the dry tissue, and as well as other organelles, which usually appear less organized than those in developing tissues. In our standard procedure dry tissues were imbibed in water for two hours at 25° C to make sectioning less difficult. (see Methods). Some seeds were also imbibed at 4° C to allow water uptake, with minimum development or metabolic activity. The organelles appeared identical in both experiments. Therefore, in our studies, as well as in studies of others (Setterfield et al., 1959; Horner and Arnott, 1966), the dry tissue was imbibed in water for a short time which did not seem to effect a change in organelle before fixation (Bagley et al., 1963).

Frequently, the arrangement of the remaining endoplasmic reticulum in the dry seed differs from that in actively germinating tissues (Perner, 1965; Klein and Ben-Shaul, 1966; Öpik, 1966). Our electron micrographs of Capsella bursa-pastoris embryos indicate that reduction in endoplasmic reticulum starts during the mature-green stage after which it cannot be traced until in the complete dry cotyledons when endoplasmic reticulum lamellae are found to be arranged in definite concentric rings. Coleoptile vascular bundle cells of ungerminated Oryza sativa grains show peculiar whorls of rough endoplasmic reticulum associated with smooth unit membranes of unknown nature (Öpik, 1972). Such an orientation of endoplasmic reticulum lamellae in the dry cotyledon cells of Capsella bursa-pastoris seems to be connected with

certain usually unfavourable physiological conditions such as desiccation and anaerobiosis etc. Hrsel (1966) proposed the same view for parallel orientation of long and frequently straight units of rough endoplasmic reticulum in plant tissues experiencing such stresses. Recently Ciamporova (1976) has also supported this view, when he found that under conditions of lethal water deficit, endoplasmic reticulum produced closely adjacent, concentric formation of rough endoplasmic reticulum profiles in the epidermal cells of primary root of Zea mays.

Parallel endoplasmic reticulum participates in autophagic processes of cells in anoxia (Coulomb et al., 1972). The degradation of cell components is expected to ensure, in this way, decreased energy and metabolite consumption and consequently, the chances of cell survival during anoxia (Coulomb and Coulomb, 1972). Ciamporova (1976) thinks that this mechanism may also function in the case of water deficit or other unfavourable conditions that provoke development of parallel endoplasmic reticulum formations.

Dexheimer (1966), reported the formation of concentric whorls of endoplasmic reticulum, interleaved with smooth membrane, similar to those found in ungerminated Oryza sativa grains (Öpik, 1972), in the germinating pollen of Lobelia erinus exposed to chloramphenicol suggesting a response to inhibition of protein synthesis. Both desiccation and cessation of synthetic activity occurs as the embryo in the Capsella bursa-pastoris seed becomes dormant like most other embryos.

It is interesting to note that formation of concentric endoplasmic reticulum also exists in the dormant buds of the Solanum tuberosum tuber Shih and Rappaport, 1971) and during development this concentric arrangement opens coincident with increasing RNA synthesis (Rappaport and Wolf, 1968). Hallam et al.,(1972) also agreed to this explanation for the crescents of rough endoplasmic reticulum present in cry embryo of Secale cereale, which develop to the expanded state within 5 hours

of imbibition.

But the significance of endoplasmic reticulum assuming such configuration is not understood.

Protein bodies.

Reserve protein in cotyledonary cells of Capsella bursa-pastoris embryo becomes visible quite late than the starch (though some synthesis may have occurred before it shows up on electron micrographs) during mature-green embryo stage, and fully developed protein grains are seen in mature ivory-white embryo stage, when starch disappears abruptly. The development of protein body in the cotyledonary parenchyma cells of Capsella bursa-pastoris follows closely the development pattern of protein bodies as in other plant specimens, like Ricinus communis (Dangeard, 1921), Phaseolus vulgaris (Öpik, 1968), Sinapis alba (Rest and Vaughan, 1972) and Crambe abyssinica (Smith, 1974). Numerous small vacuoles appear during torpedo-shaped embryo stage coalesce to produce a large central vacuole during walking-stick shaped embryo stage and inverted U-shaped embryo stage. Such large central vacuoles get subdivided during maturing green embryo stage, when protein starts appearing as homogeneous mass, resulting in several aleurone grains in the cell during mature ivory-white embryo stage. Protein bodies in Gossypium hirsutum embryo are derived from vacuoles which do not fuse and subdivide (Engleman, 1966). In Zea mays endosperm the protein grain develops within vesicles originating from the endoplasmic reticulum (Khoo and Wolf, 1970). In Pisum sativum (Bain and Mercer, 1966) and Vigna unguiculata (Harris and Boulter, 1976) cotyledons, two modes of protein body formation occur, that is by subdivision of the large cytoplasmic vacuole(s), and by enlargement of newly-formed cytoplasmic vesicles which may develop from Golgi cisternae. It is now an accepted

fact that the vacuoles of plant cells arise from the endoplasmic reticulum (Dainty, 1968). The analogy between aleurone grains arising directly from the endoplasmic reticulum or within the vacuoles which themselves are derived from the expansion of endoplasmic reticulum cisternae, has been indicated by Öpik (1968a). Cotyledonary cells presumably secrete protein which is probably sequestered from the cytoplasm in a vacuolar phase.

Some association of protein secretion has been correlated with the abundance of endoplasmic reticulum (Schnepf, 1963), but there are no connections existing between endoplasmic reticulum and vacuole. Bailey et al. (1970), however, have shown by autoradiography that rough endoplasmic reticulum is involved in protein synthesis in Vicia faba. In Vigna unguiculata (Harris and Boulter, 1976) and Pisum sativum (Bain and Mercer, 1966) a pattern of multi-vesiculation in the cotyledonary cells with protein deposition in vesicles occur at the same time as a massive increase in cytoplasmic rough endoplasmic reticulum and Golgi body production. This strengthens the possible role of rough endoplasmic reticulum in protein synthesis. The problem regarding the origin of protein bodies still remains unsolved. Electron micrographs of Capsella bursa-pastoris cotyledon cells do not show how the protein enters the vacuoles. It is unlikely that protein synthesis is actually taking place in the vacuole as no ribosomes are found attached to the tonoplast. Reserve protein in Capsella bursa-pastoris is first observed within the tonoplast as lumps of electron-dense material associated with the presence of globoid inclusions. Such lumping of protein in developing protein grains were first reported by Engleman (1966) in Gossypium hirsutum embryo and later by Rest and Vaughan (1972) in Sinapis alba embryos. The effect of different aldehyde fixatives upon the development of protein body has also been observed by Engleman (1966) and Rest and Vaughan (1972). When aldehyde fixatives are used,

there is correlation between uniform electron density and the absence of globoid inclusions. Lumping of protein occurred when inclusions were present. The mechanism of origin of globoids, which appear in the vacuole as spherical bodies within a clear angular area of the protein matrix, is not understood. The number of globoids increase with the increase in amount of protein matrix. Many legumes do not have any globoids or only a single larger one in their aleurone grains (Bain and Mercer, 1966 ; Orey et al, 1969). These observations do not support the theory that globoids precipitate before the protein in the vacuole (Frey-Wyssling and Muhlethaler, 1965). However, the presence of protein, phosphate (phytin) and lipid is demonstrated in the globoids by Jacobsen et al. (1971).

Oil bodies.

The oil bodies in Capsella bursa-pastoris are present as early as from the egg cell stage up to three celled embryo stage like that of starch grains (Schulz and Jensen, 1968). Oil bodies disappear at three celled embryo stage and starts reappearing as a few spherical bodies with stainable contents at early torpedo-shaped embryo stage, and increase gradually up to mature ivory-white embryo stage. There is small increase in the number of oil bodies up to inverted U-shaped embryo stage, and from then up to full maturity all the cell space not occupied by protein bodies becomes filled with oil bodies. There is little change in the size of oil bodies during later developmental stages and the increase in the amount of oil may be entirely by the synthesis of further oil bodies as has been suggested for Sinapis alba cotyledons (Rest and Vaughan, 1972).

No evidence could be collected in Capsella bursa-pastoris for a tripartite membrane or a densely staining limiting membrane surrounding

the reserve oil body, but a sharply defined interface with the cytoplasm can be seen as suggested by Harwood et al. (1971). The fact that they do not appear to coalesce with adjacent oil bodies in the cell, indicates that a limiting membrane of some nature may be present as described for Crambe abyssinica (Smith, 1974) oil bodies. This possibility takes the form of a monomolecular layer of phospholipid as proposed by Rest and Vaughan (1972) for Sinapis alba or the unique single membrane described by Yatsu and Jacks (1972).

The oil bodies of Capsella bursa-pastoris appear to arise from the cytoplasm. There is no evidence to suggest that oil bodies arise either from endoplasmic reticulum (Frey-Wyssling et al., 1963) or from spherosomes (Schwarzenbach, 1971). Smith (1974), however, proposed that in Crambe abyssinica cotyledons spherosomes and oil bodies are separate entities supporting the view of Sorokin (1967). According to him, spherosomes are found in most vegetative cells of higher plants, and even those that do not produce oil and spherosomes (not oil bodies) are found at the earlier stages of embryo oevelopment when the cells are meristematic with no storage oil bodies. Oil bodies present during the earlier stages (egg stage to three-celled embryo) of Capsella bursa-pastoris (Schulz and Jensen, 1968) probably need further investigation and experimental verification in the light of recent work of Smith (1974), as at this stage of development the cells are basically meristematic cells, with presumably no storage oil body. Schulz and Jensen (1968) have accounted for these oil bodies as necessary energy supply required during critical divisions of the embryo of Capsella bursa-pastoris.

Oil bodies of Capsella bursa-pastoris embryo appear to have lost some of their stainable contents as the embryo approaches maturity. At mature ivory-white embryo stage oil body contents appear completely electron-transparent, but with a dark single line bounding each body. As new bodies are formed after the existing ones begin to lose their

stainable contents, it would seem that the loss of stainability represents a change in the nature of oil connected with the developmental age of the embryo, rather than a necessary stage in the formation of oil bodies (Rest and Vaughan, 1972).

Intercellular lipid.

The accumulation of lipid in the intercellular spaces or even on or across the cell walls in the cells of dry cotyledons of Capsella bursa-pastoris is difficult to understand, and no special transport mechanism could be detected. No functional significance can be suggested for this behaviour of lipid. Lipid material is present in especially large amounts in the intercellular spaces of stigma of Petunia hybrida (Konar and Linskens, 1966) and also in seedlings of some leguminous species and in Avena sativa coleoptiles (Scott, 1950; Scott and Lewis, 1953; Sorokin, 1967) and also in small amount in the intercellular spaces of dry Bidens cernua embryo (Simola, 1971). The mechanism of lipid transport in plants is virtually unknown.

The general conclusion that can be drawn from this discussion suggests that the organelles in developing embryo are continuously changing, and most of the changes are 'adaptive' in the sense that they are responses to changes in the internal environment of the cell. The structure of dry cotyledons, have been interpreted with some caution, for imbibition with liquid during fixation may have obliterated structural features peculiar to desiccated stage. Lack of well established procedures for fixing tissues of low moisture, limit cytological progress and weakens

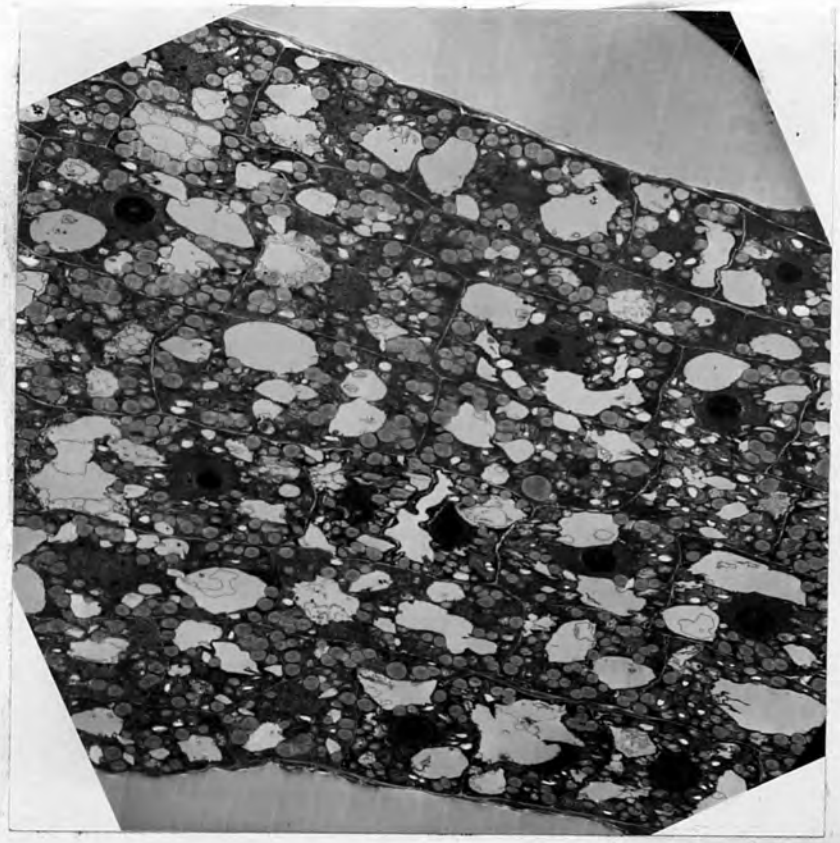
our confidence in interpreting the findings. Many organelles that appear disorganised or missing in the dry cotyledons, might be present in some cases and their disappearance or disorganisation occurs during fixation. Better fixation techniques are a prerequisite to any cytological progress. Dry cotyledons are characterised by general lack of membrane clarity, absense of some organelles normally present in metabolically active plant cell and presence of rather large amounts of reserve food material. Although the dry embryo has ample food reserves for resumption of growth, most of this reserve is not readily available. It has to be hydrolysed, released from limiting membranes and transported to the sites where it is needed.

Priorities for cytological investigations must be established for better and organised ultrastructural cytological studies. Attention should first be focussed on those organelles responsible for multiple functions such as chloroplast, mitochondria, polysomes, Golgi bodies, endoplasmic reticulum and plasmalemma. However, inclusions such as oil bodies and protein bodies should not be ignored.

Plate 4. Cell structure of late torpedo-shaped embryo.

Fig. 4. Longitudinal section through the cotyledon of late torpedo-shaped embryo showing the general plan of cell arrangement and structure. x 1,640

Fig. 5. Enlarged view of a cotyledon parenchyma cell of late torpedo-shaped embryo. Two vacuoles are developing on either end of the cell. A central nucleus, oil bodies, developing mitochondria (M) and chloroplast, endoplasmic reticulum lying mostly near to plasmalemma and several plasmodesmata are seen. x 10,800



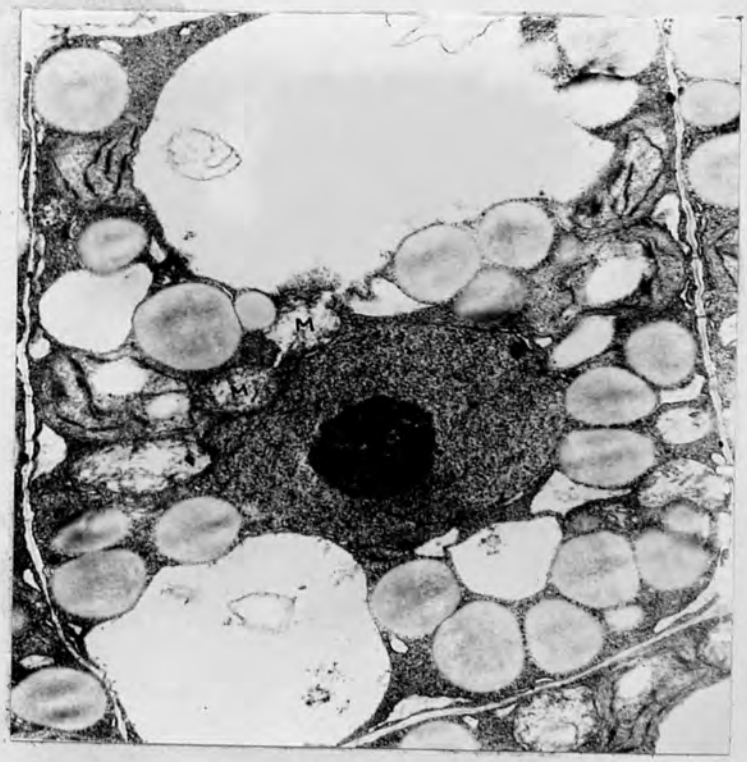
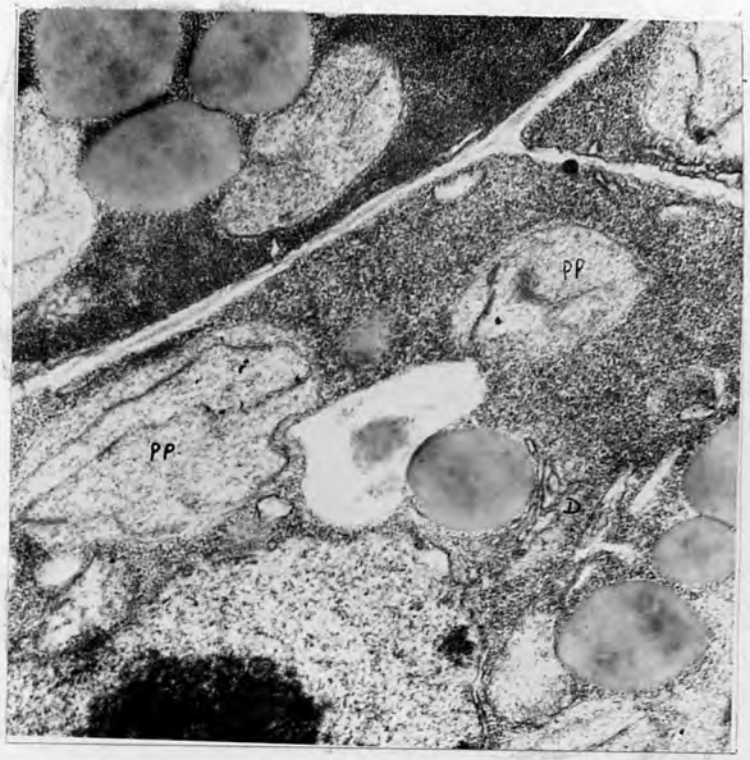

M
M

Plate 5. Cell structure of early torpedo-shaped embryo.

Fig. 6. A young cotyledon parenchyma cell of early torpedo-shaped embryo. The cell matrix is very dark. The cell possesses a large nucleus, few oil bodies, proplastids (pp) and a plastid showing the development of lamellar system, dictyosome (D), mitochondria with cristae, and endoplasmic reticulum. x 21,000

Fig. 7. Young elongated cotyledon parenchyma cells of early torpedo-shaped embryo. The central nucleus occupies a large proportion of the cell volume and the nucleus is very conspicuous. The double membraned nuclear envelope shows many nuclear pores. The dense cytoplasm contains many ribosomes, a few small vacuoles and oil bodies, proplastids, developing mitochondria, and endoplasmic reticulum. x 10,500


PP
PP
D

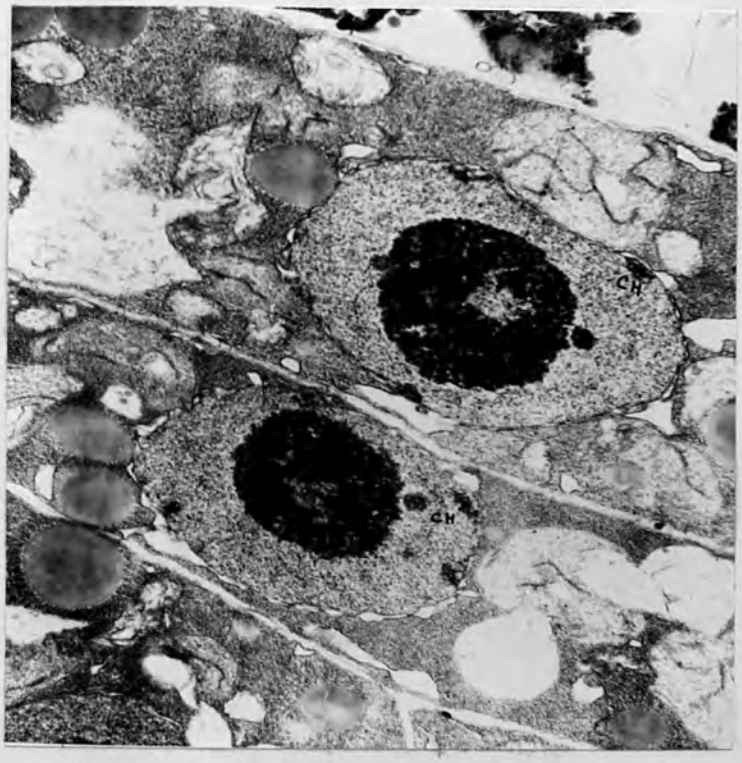

CH
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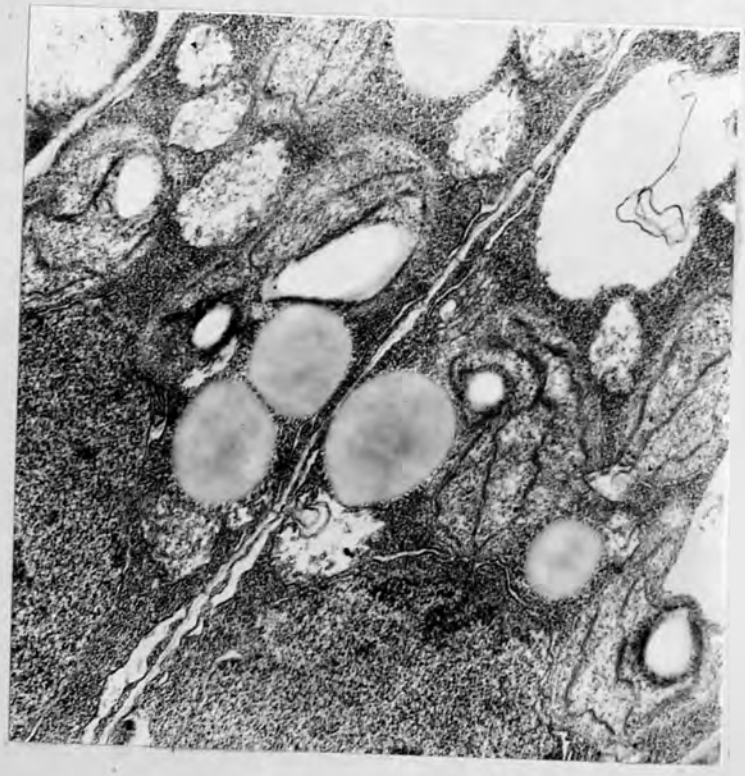
Plate 6. Developing organelles in torpedo-shaped embryo.

Fig. 8. Part of a cotyledon parenchyma cell of late torpedo-shaped embryo. The cell matrix is very dark due to great density of ribosomes. The dense cytoplasm contains stacks of 6-7 cisternae called dictyosomes, endoplasmic reticulum, and oil bodies. x 45,000

Fig. 9. Cotyledon parenchyma cells of late torpedo-shaped embryo. The dense cytoplasm contains numerous ribosomes, abundance of endoplasmic reticulum most of which lies parallel to the plasmalemma, a few small vacuoles and oil bodies, proplastids and developing mitochondria. Starch grain appears in some plastids. The inner and outer membranes of the nuclear envelope are resolved. The cell wall in this section shows about 5 plasmodesmata. x 18,000



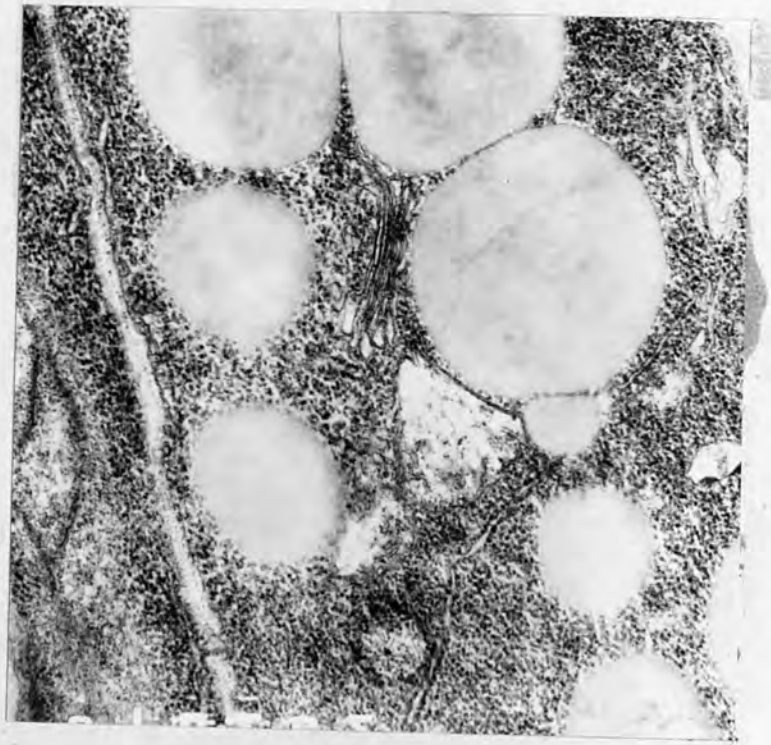
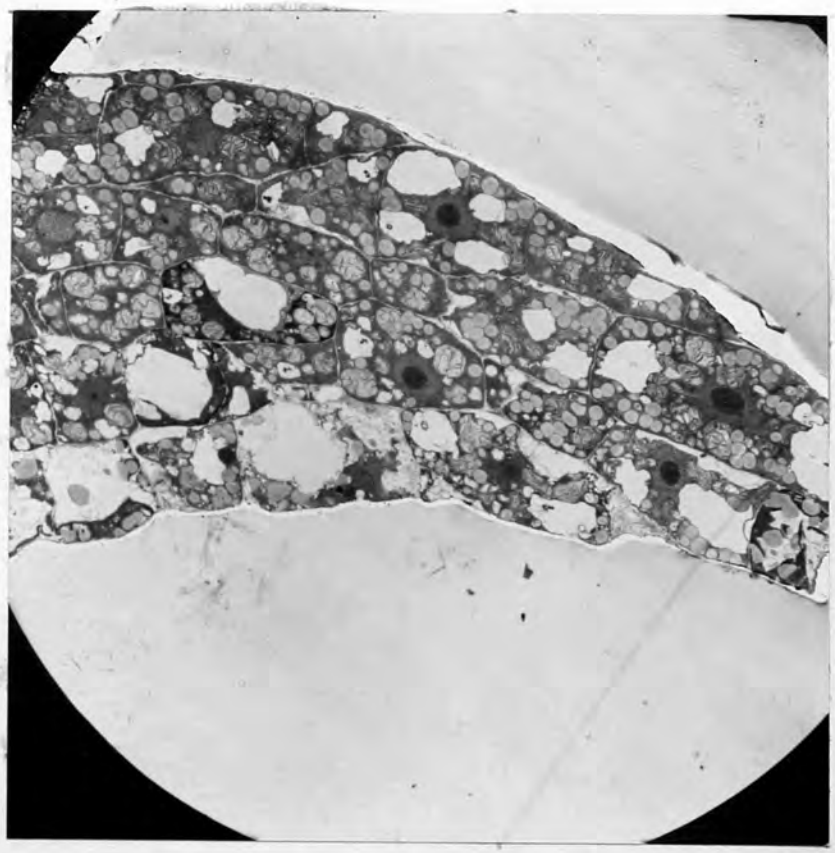


Plate 7. Cell structure of walking-stick-shaped embryo.

Fig. 10. Longitudinal section through the cotyledon of walking-stick-shaped embryo showing the general plan of cell arrangement and cell structure. x 1,640

Fig. 11. Enlarged view of a cotyledon parenchyma cell of walking-stick-shaped embryo. The cell possesses a prominent central nucleus with a prominent nucleolus, a darker cell matrix due to increased density of ribosomes, chloroplasts with differentiated lamellae and with occasional stack, mitochondria with well developed tubular cristae, and oil bodies which are more in number lying near to cell wall. x 10,500



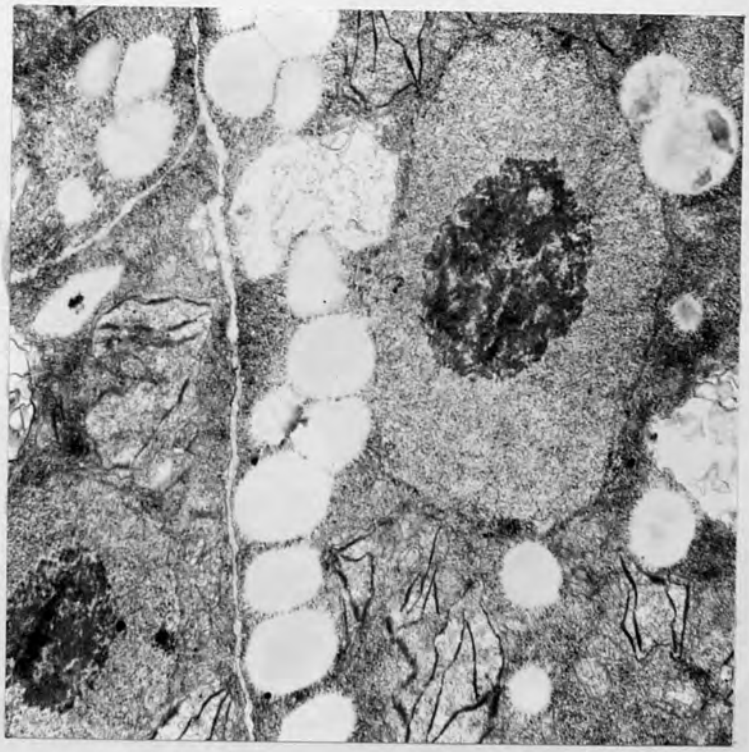
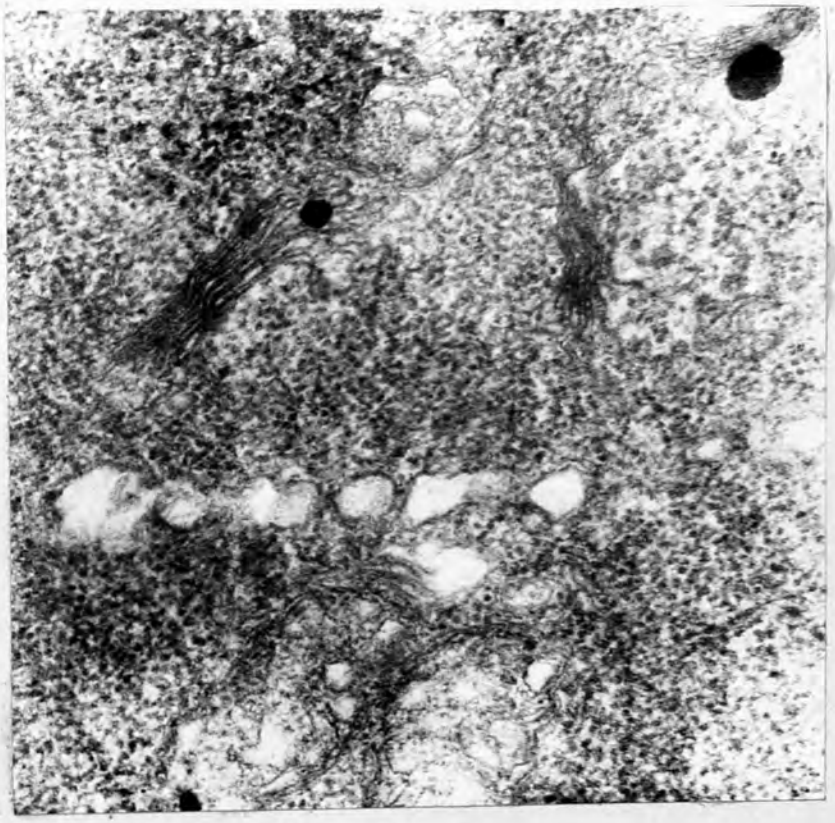


Plate 8. Cell plate formation and chloroplast structure in walking-stick-shaped embryo.

Fig. 12. Part of a cotyledon parenchyma cell of walking-stick-shaped embryo showing the cell plate formation. Abundant dictyosomes and randomly dispersed ribosomes are seen. x 16,000

Fig. 13. Enlarged view of a chloroplast in the cotyledon parenchyma cell of walking-stick-shaped embryo in which outer and inner membranes of chloroplast envelope are resolved and the differentiated lamellae show stacking, maximum up to 3 stacks to form primary grana. The chloroplast ribosomes which are smaller than the cytoplasmic ribosomes and a small transparent starch grain are also seen. x 72,000



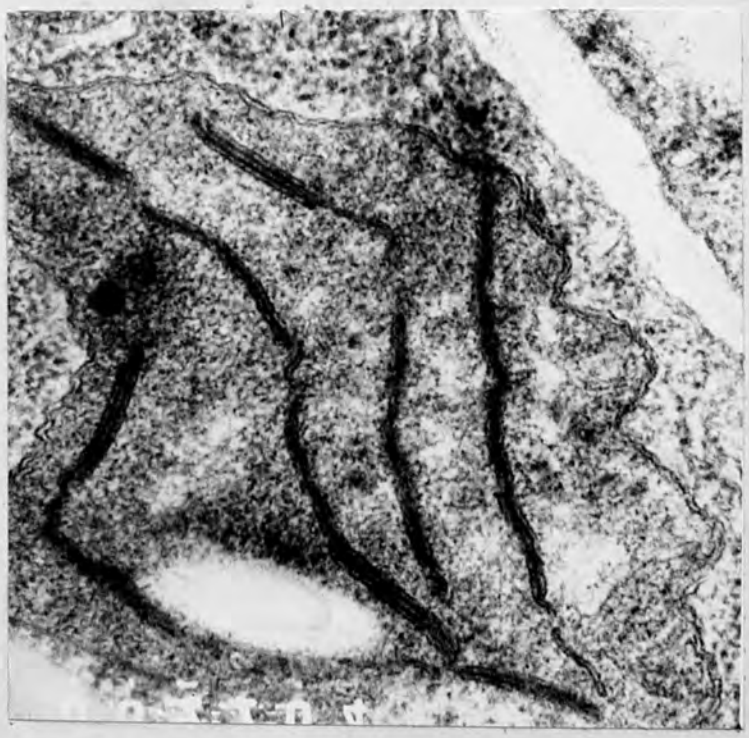
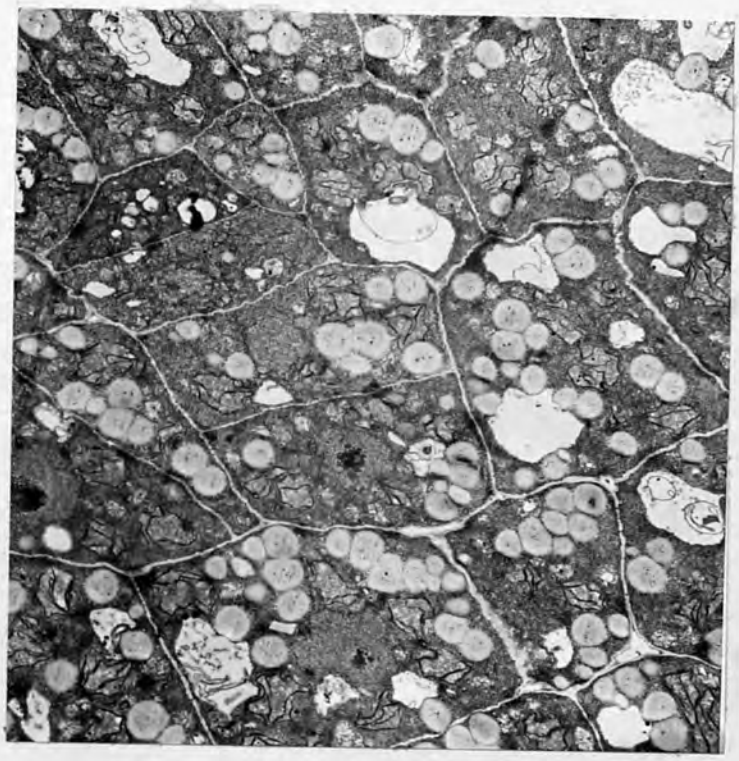


Plate 9. Cell structure of inverted U-shaped embryo.

Fig. 14. Longitudinal section through the cotyledon of inverted U-shaped embryo showing the general plan of cell arrangement and cell structure. x 3,500

Fig. 15. Enlarged view of a cotyledon parenchyma cell of inverted U-shaped embryo. The cell possesses prominent nucleus with prominent nucleolus. The outer and inner membranes of nuclear envelope are resolved, the outer like endoplasmic reticulum bears ribosomes(R). The cell matrix is dense due to increased density of ribosomes which are randomly dispersed. This cell shows many chloroplasts with few developing lamellae, some developing mitochondria and proplastids, abundance of rough endoplasmic reticulum, a large vacuole, and many plasmodesmata. x 14,000



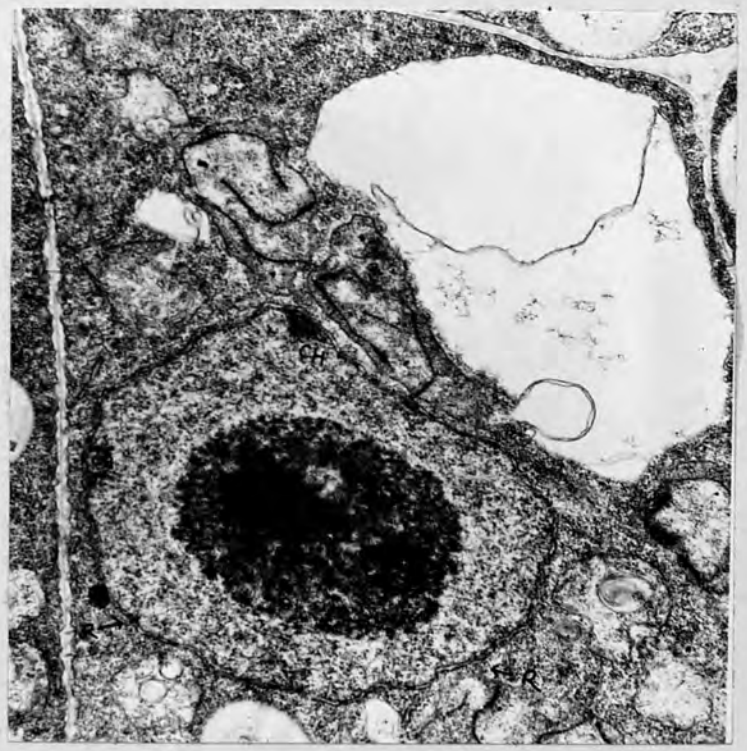
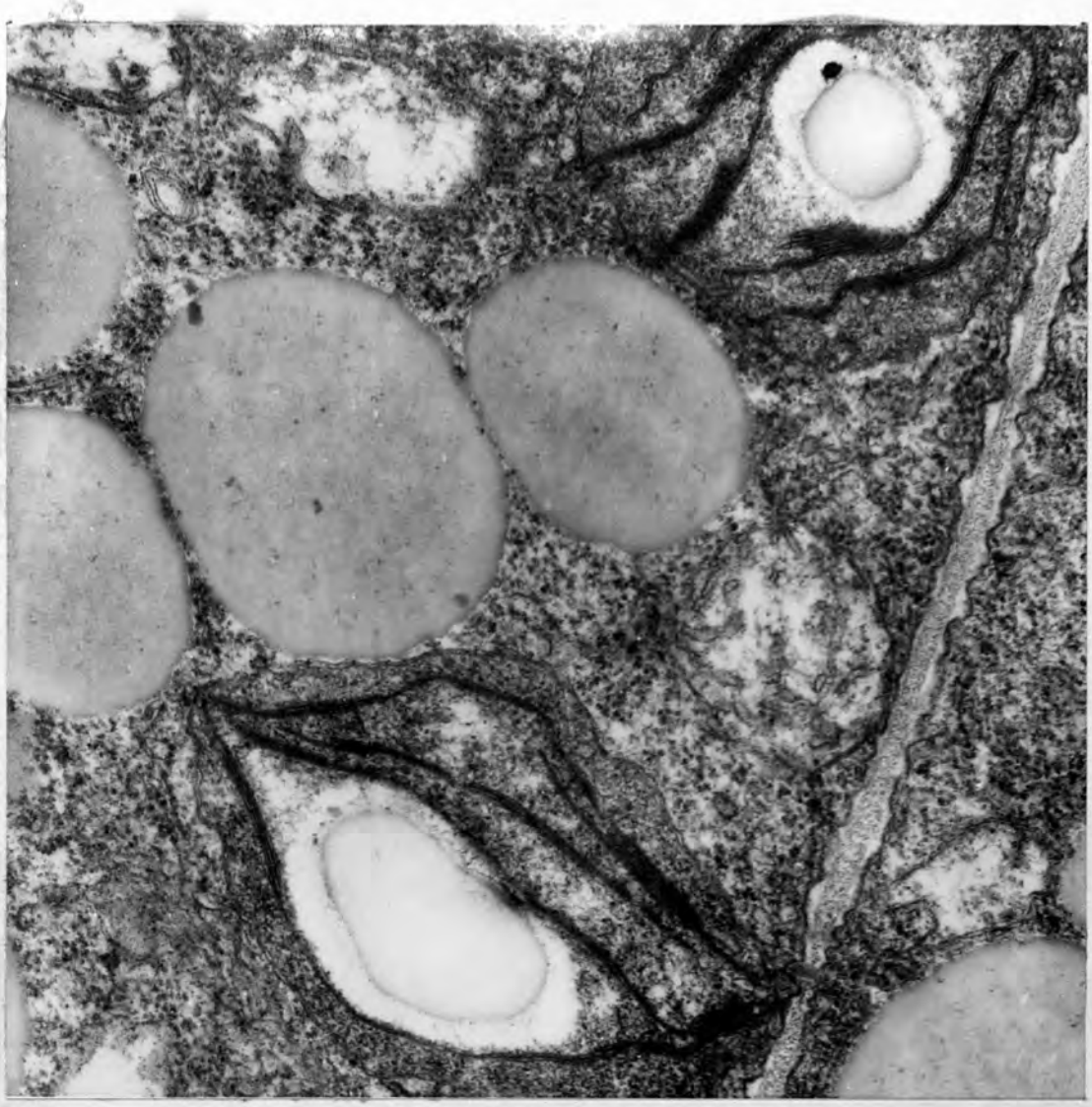

N
RER
SER

Plate 10. Chloroplast structure in inverted U-shaped embryo.

Fig. 16. Part of a cell from a cotyledon of inverted U-shaped embryo showing fully developed chloroplasts of various shapes. Each chloroplast has one starch grain which occupies one half of the chloroplast volume. The growth of starch grain compresses the well developed lamellae which stack to form well developed grana. The dense cytoplasm also contains numerous ribosomes, well developed mitochondrion and oil bodies. x 39,000

Fig. 17. Enlarged view of one of the chloroplast of the cotyledon parenchyma cell of inverted U-shaped embryo showing well developed grana and intergrana lamellae and a starch grain. Two membranes of chloroplast envelope are resolved. x 49,200



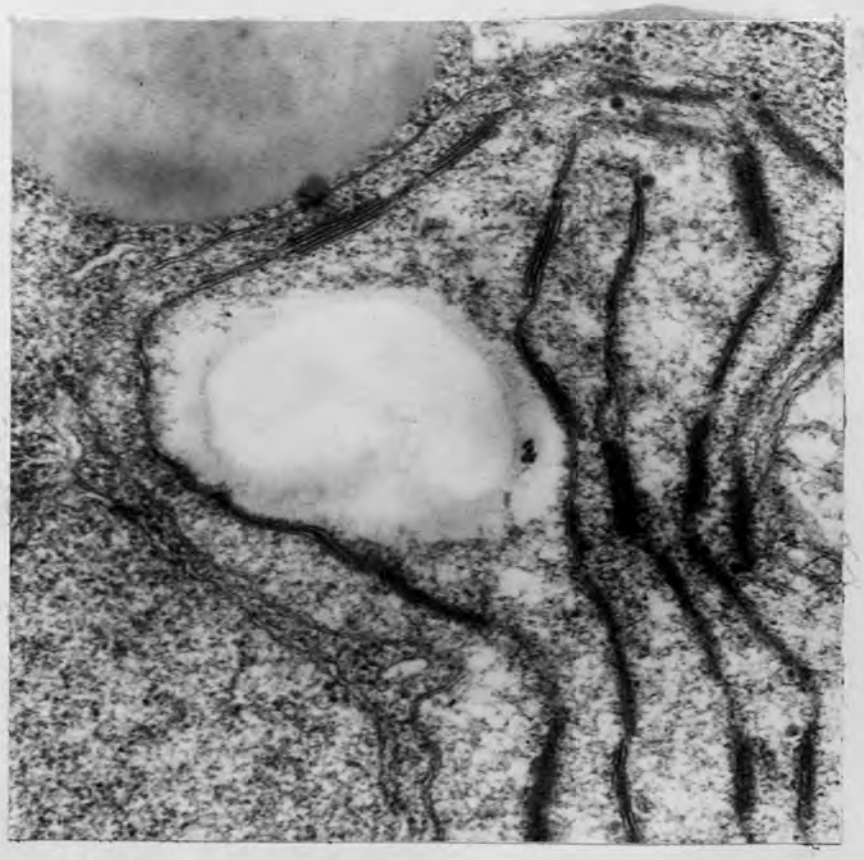
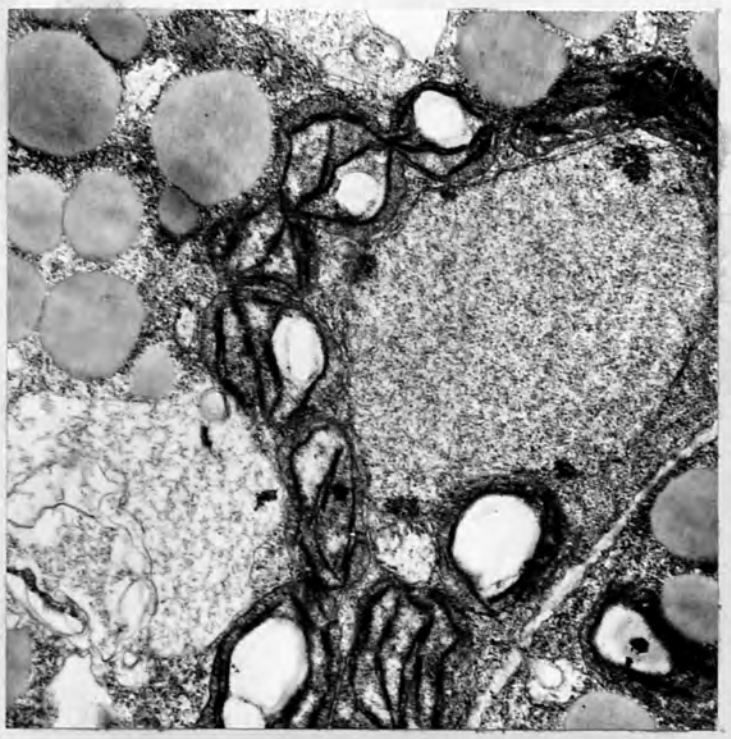


Plate 11. Chloroplast arrangement in inverted U-shaped embryo.

Fig. 18. Part of a cell from the cotyledon of inverted U-shaped embryo showing chloroplasts with well developed lamellar structures grouped around the nucleus. Most of the chloroplasts contain one starch grain each. x 10,500

Fig. 19. Part of 2 cells from the cotyledon of inverted U-shaped embryo. Starch grain fills most of volume of chloroplast and in one of the chloroplasts 2 big compound starch grains occupy the greater part of the volume and only few lamellae are seen near to the inner membrane of chloroplast envelope. Parallel alignment of endoplasmic reticulum near to plasmalemma is seen. x 16,400



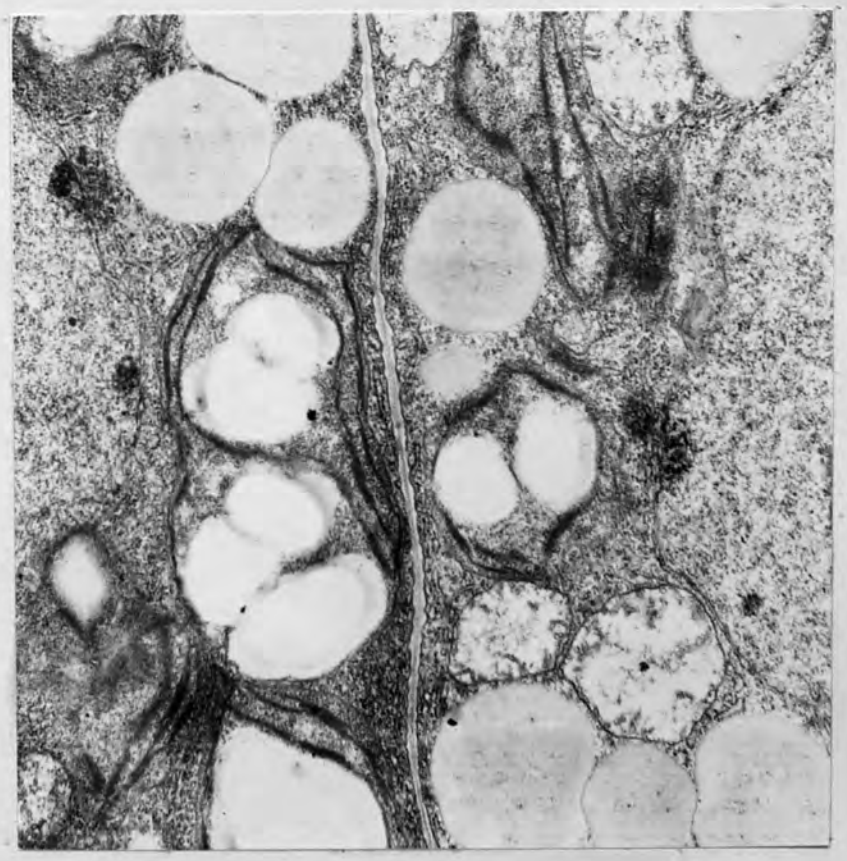
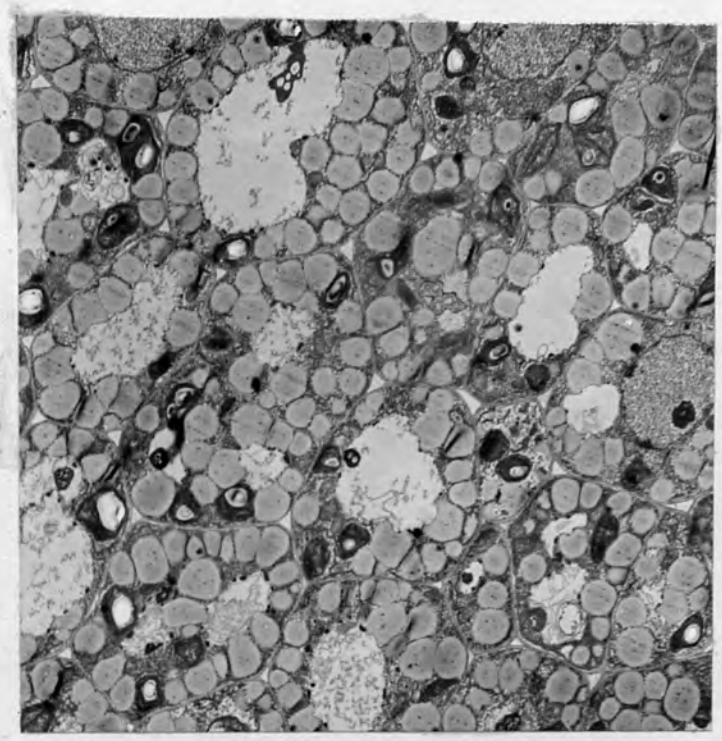


Plate 12. Cell structure of mature-green embryo.

Fig. 20. Longitudinal section through the cotyledon of mature-green embryo (9 days after petal fall) showing the general plan of cell arrangement and cell structure. Most cells contain a large central vacuole showing various stages of their contents which is the first stage of their turning into reserve protein bodies. Large number of oil bodies along with some densely stained chloroplasts containing one or two starch grains are arranged around the central vacuole. x 3,500

Fig. 21. Enlarged view of the part of cotyledon parenchyma cell of mature-green embryo marked by a cell wall showing electron dense middle lamella and an intercellular space. One of the cells possesses a vacuole with dense protein deposits starting accumulating at the periphery of vacuole at the inner surface of tonoplast. Fully developed but starchless chloroplasts, big and small oil bodies lined near to cell wall and fully developed mitochondria are also seen. x 21,000



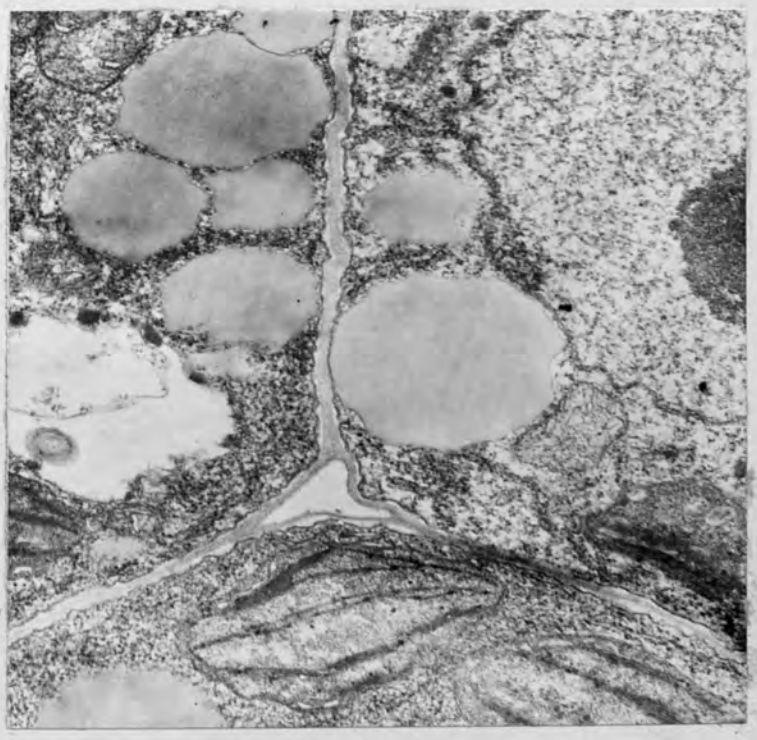
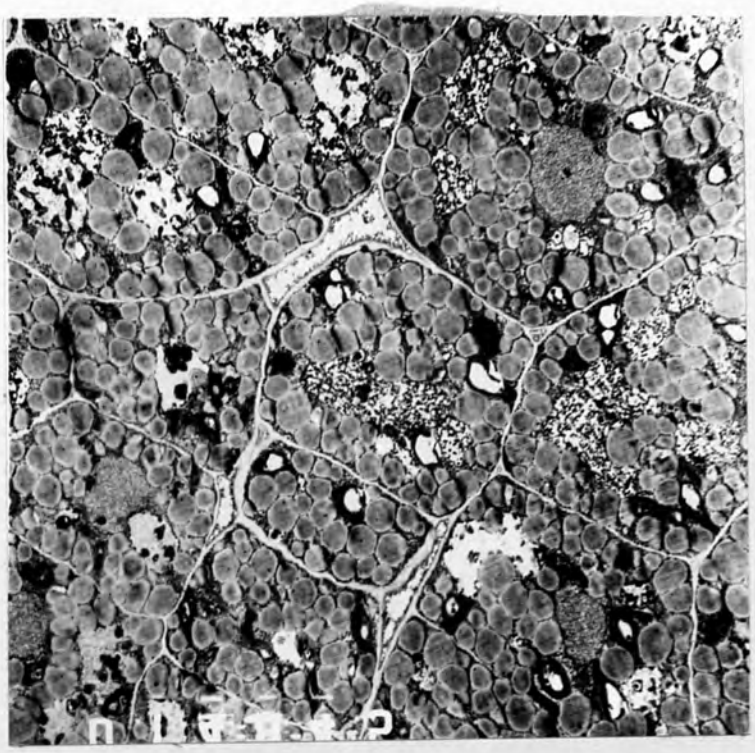


Plate 13. Cell structure of fully mature-green embryo.

Fig. 22. Longitudinal section through the cotyledon of fully mature-green embryo showing the general plan of cell arrangement and cell structure. All cells show protein vacuoles filled with flocculent or homogeneous protein. Some protein vacuoles have developed globoids. Oil bodies have increased several fold and few chloroplasts with one starch grain each are seen. x 2,700

Fig. 23. Enlarged view of a cotyledon parenchyma cell of fully mature-green embryo showing prominent central nucleus with prominent nucleolus even at this stage. The cell possesses large number of oil bodies and a few chloroplasts with one starch grain each. x 12,300



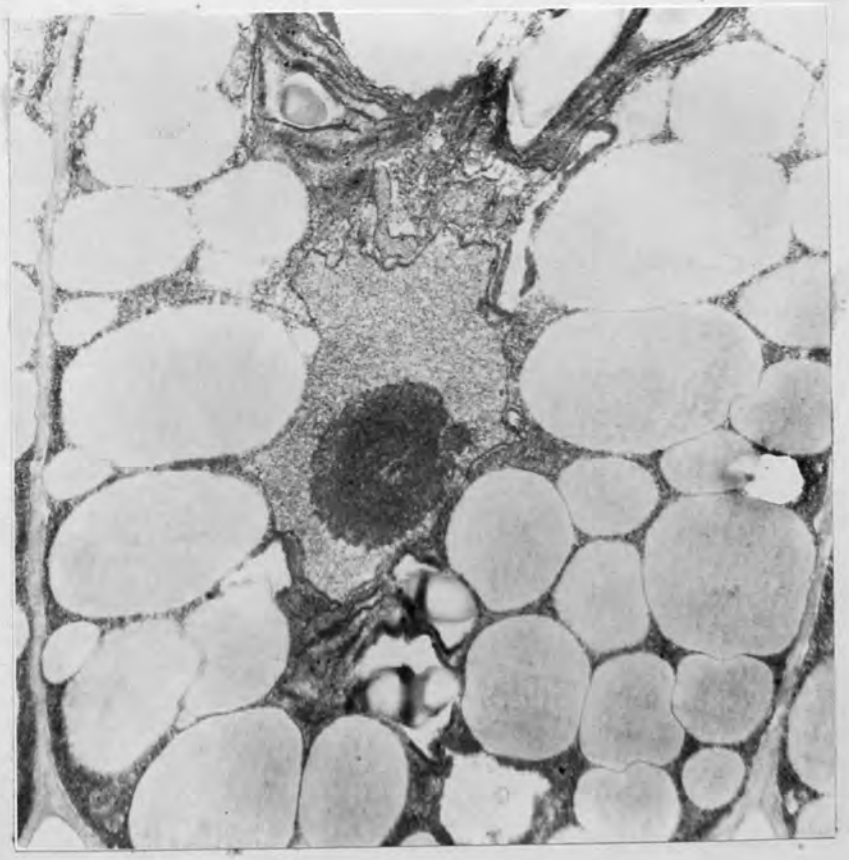
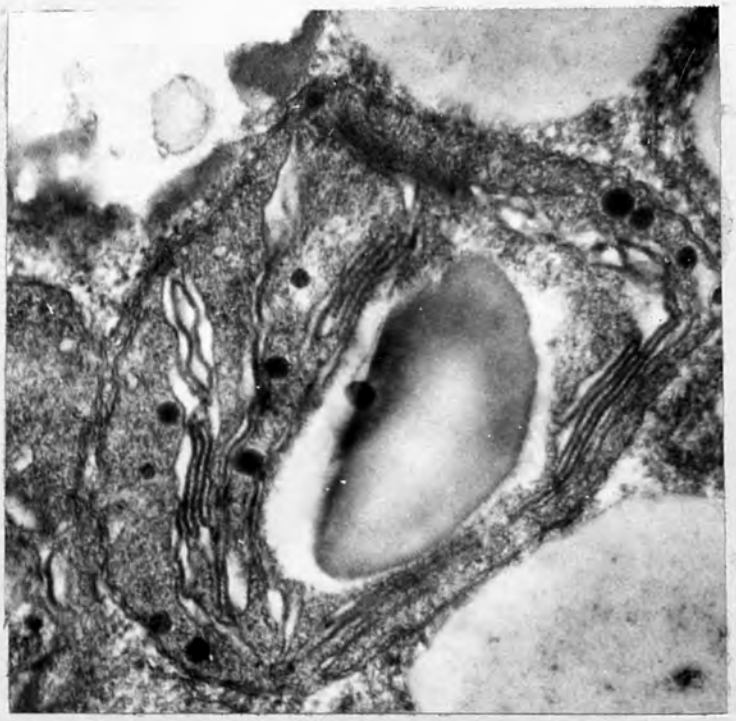


Plate 14. Chloroplast distortion in fully mature-green embryo.

Fig. 24. Enlarged view of a chloroplast in the cotyledon parenchyma cell of fully mature-green embryo in which growth of starch grain compressed the stroma causing disruption of lamellae. Osmiophilic platoglobuli are seen in the chloroplast. x 60,000

Fig. 25. Enlarged view of two chloroplasts in the cotyledon parenchyma cell of fully mature-green embryo with distorted lamellae. x 32,800



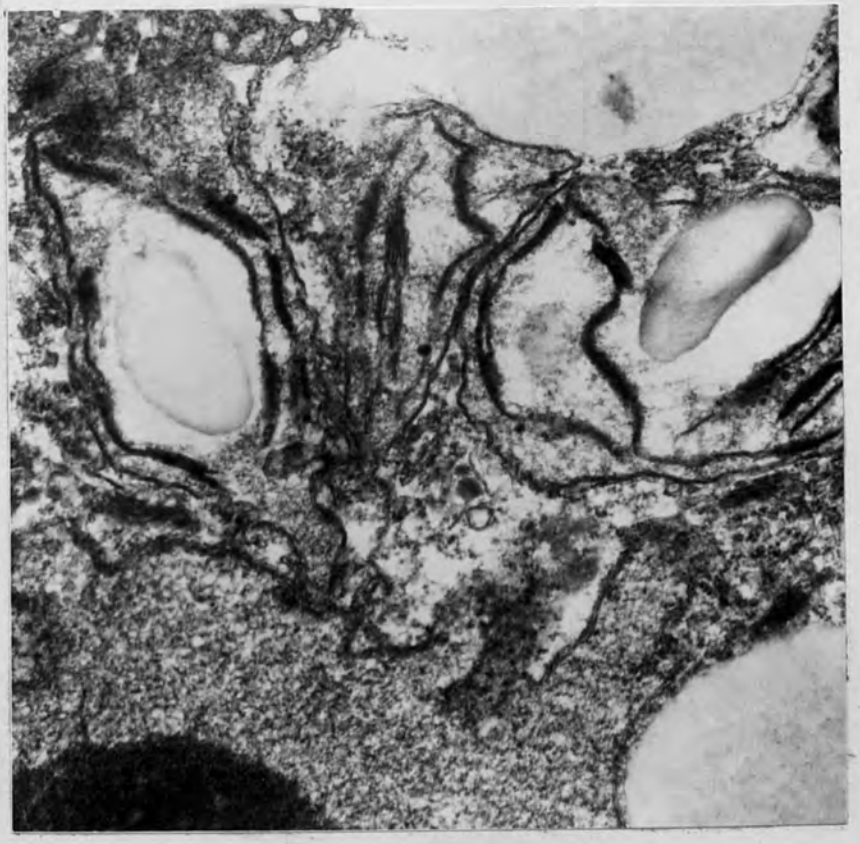
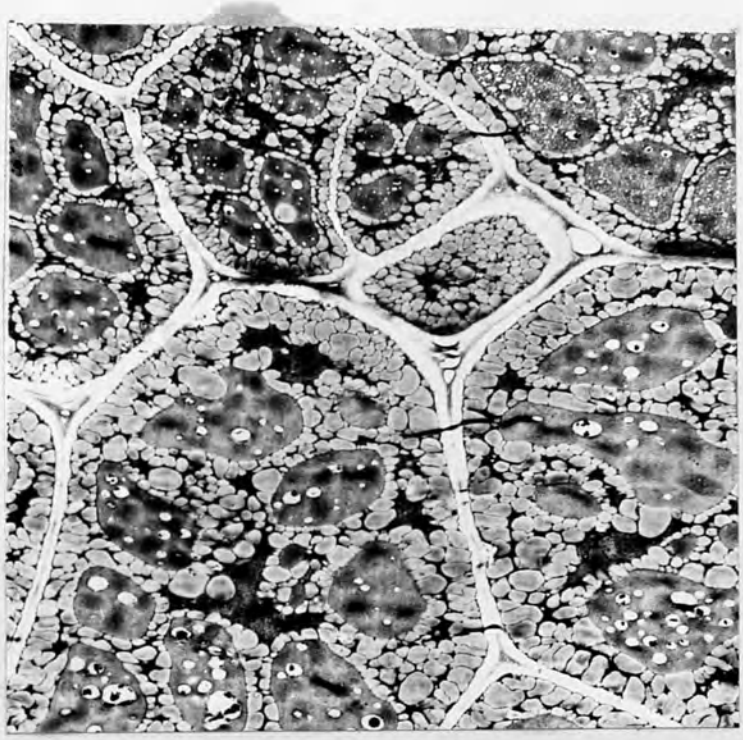


Plate 15. Cell structure in mature ivory-white embryo.

Fig. 26. Longitudinal section through the cotyledon of mature ivory-white embryo showing the general plan of arrangement of aleurone cells and aleurone grains in aleurone cells. The cells show a rapid increase in the number of oil bodies which occupy all the cell space not occupied by aleurone grains. x 4,500

Fig. 27. Enlarged view of a portion of cotyledon aleurone cell showing the arrangement of aleurone grains around the central degenerating densely stained nucleus. No other organelle is seen in this cell as all space is occupied either by oil bodies or protein grains. x 7,000



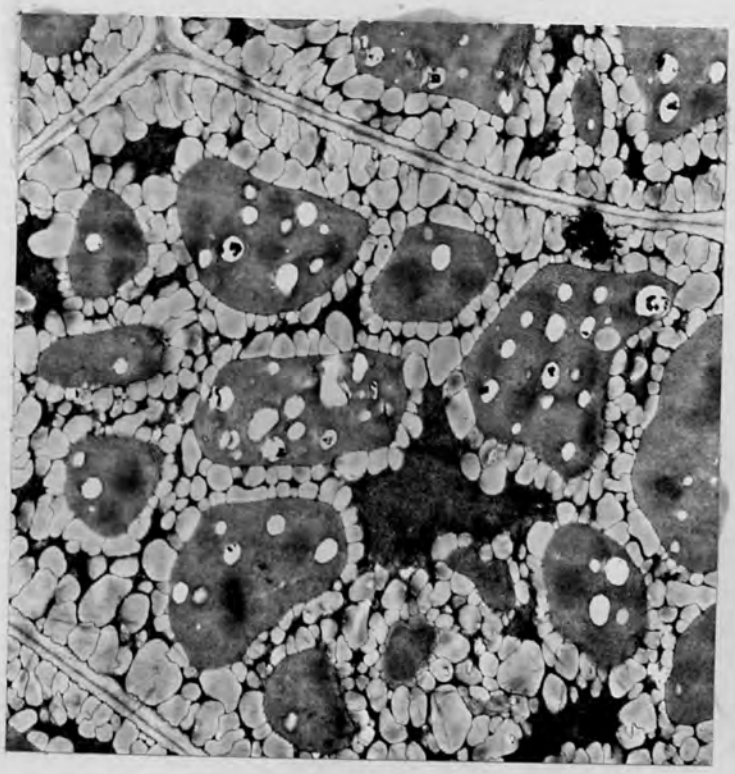


Plate 16. An aleurone grain and disorganising nucleus in mature ivory-white embryo.

Fig. 28. Enlarged view of an aleurone grain from the cotyledon parenchyma cell of mature ivory-white embryo showing globoid and crystalloid inclusions. The angular shape of the aleurone grain is probably due to the pressure caused by proliferating oil bodies. x 40,000

Fig. 29. Enlarged view of a disorganising nucleus from the cotyledon parenchyma cell of mature ivory-white embryo showing the general loss of contrast in the electron micrographs. Nuclear outline can be seen with a comparatively darker chromatin in nuclear lobes. Another disorganising organelle is possibly a plastid. x 26,000

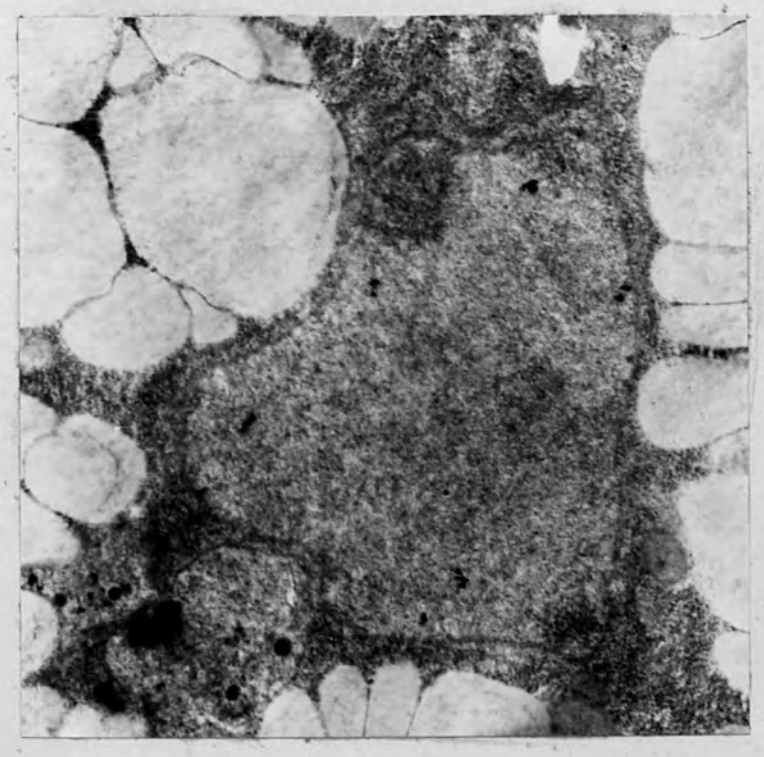
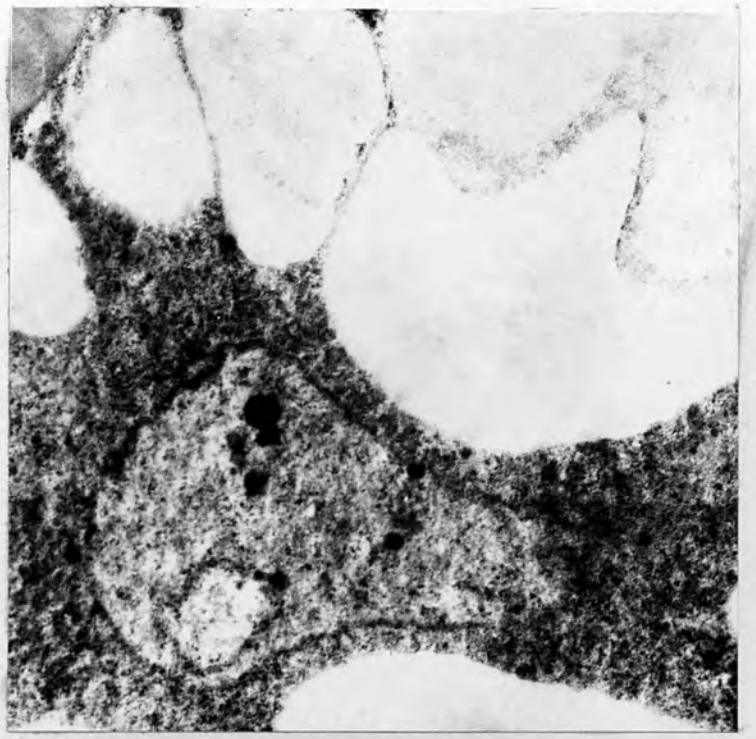


Plate 17. Disorganizing unidentified organelles in mature ivory-white embryo.

Fig. 30. Enlarged view of a portion of cotyledon parenchyma cell of mature ivory-white embryo showing disorganizing cell organelles in the residual cytoplasm and compression of oil bodies. x 56,000

Fig. 31. Enlarged view of a portion of cotyledon parenchyma cell of mature ivory-white embryo showing a disorganizing organelle possibly a chloroplast, surrounded by big oil bodies. These disorganizing organelles show a general loss of contrast in electron micrographs. x 65,000



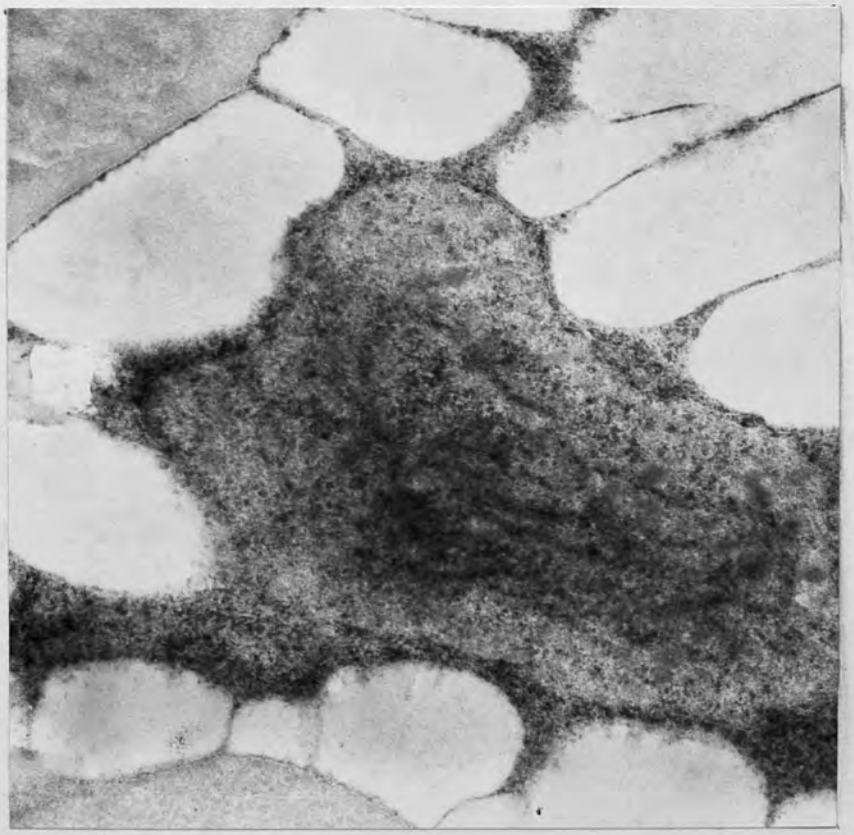


Plate 18. Disorganizing chloroplasts in mature ivory-white embryo.

Fig. 32 and Fig. 33. Enlarged view of a portion of cotyledon parenchyma cell of mature ivory-white embryo showing chloroplasts at various stages of disorganization. Lamellar structure is either completely lost or some lamellae form abnormal grana in the centre of chloroplast. Large number of densely stained osmiophilic plastoglobuli (o.g.) are seen in these chloroplasts which show general loss of contrast in electron micrographs. Large number of oil bodies around these organelles are seen. x 28,000


O.G.

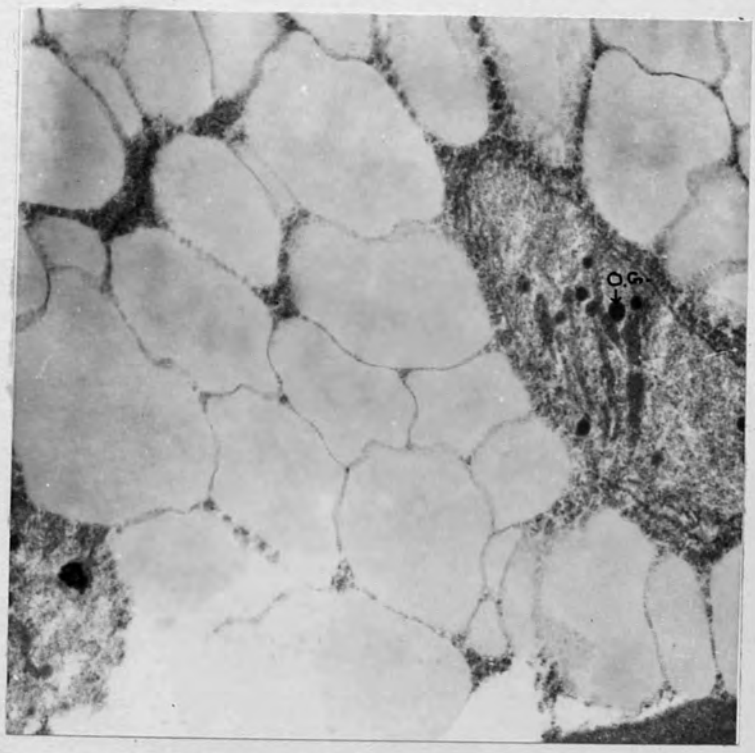

O.G.

Plate 19. Cell structure of mature dry embryo.

Fig. 34. Longitudinal section through the cotyledon of mature dry embryo showing general plan of arrangement of cells and cell structures. All cells are reduced in their dimension become hexagonal in outline filled with large number of oil bodies and some aleurone grains. A local accumulation of lipid in intercellular space and in cell wall can be seen. x 2,700

Fig. 35. Enlarged view of a portion of cotyledon parenchyma cells of mature dry embryo. The cells show the aleurone grains with inclusions. x 7,000

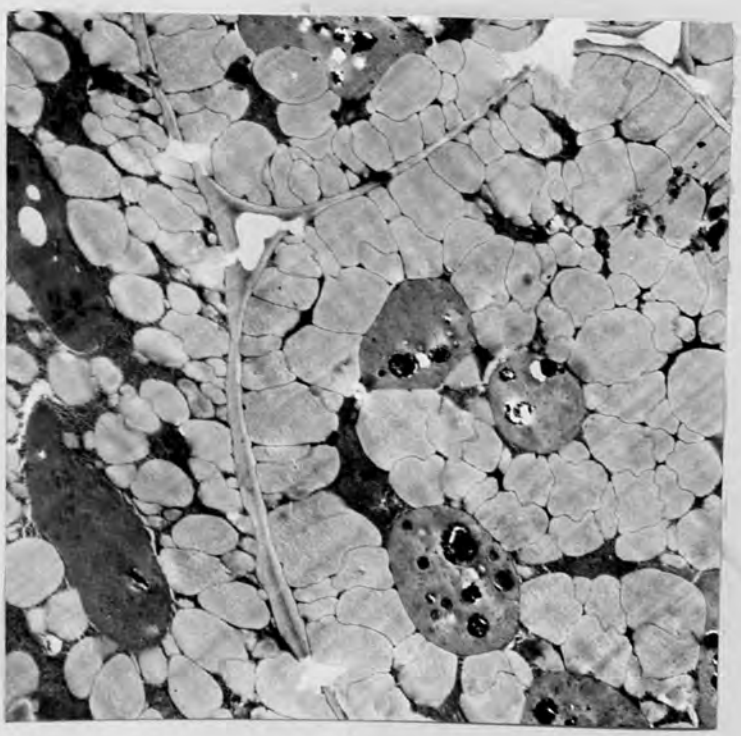
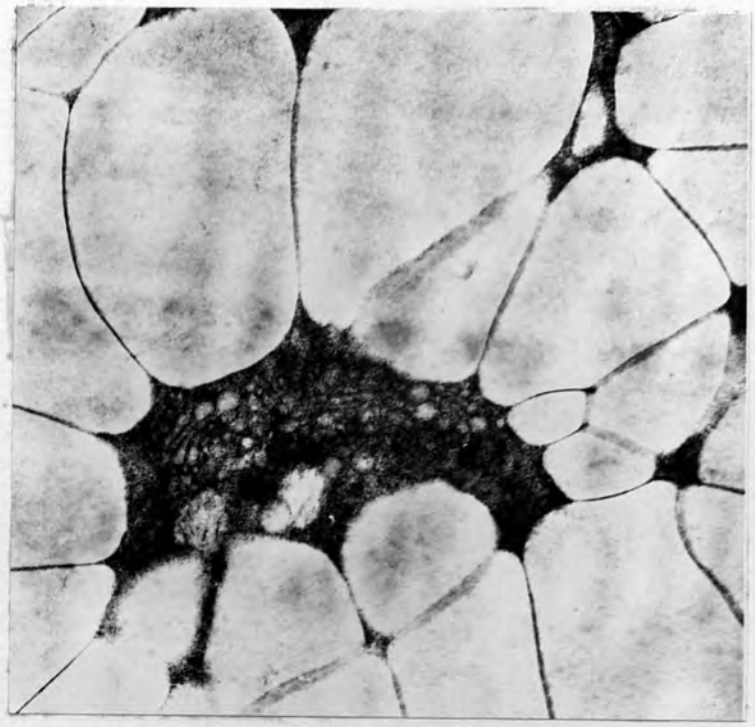


Plate 20. Golgi apparatus in mature dry embryo.

Fig. 36. Enlarged view of a portion of cotyledon parenchyma cell of mature dry embryo showing well organized Golgi apparatus showing several stacks of cisternae with swollen ends and many small vesicles. Several inflated extensions are observed along the periphery of the vesicles. x 35,000

Fig. 37. Magnified view of Golgi apparatus in Fig. 36. x 96,000



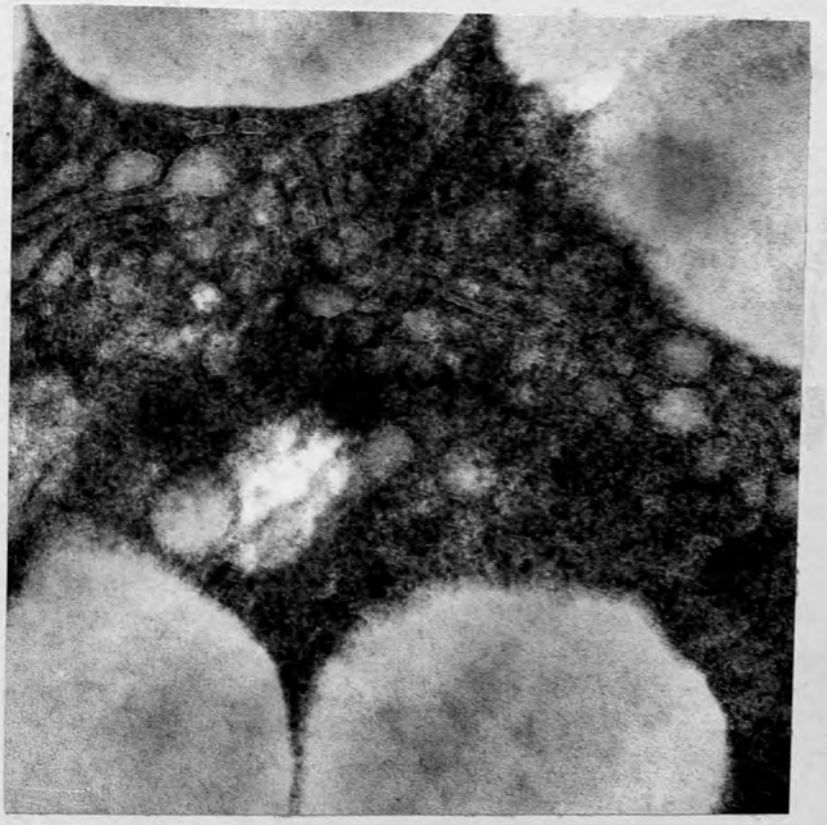
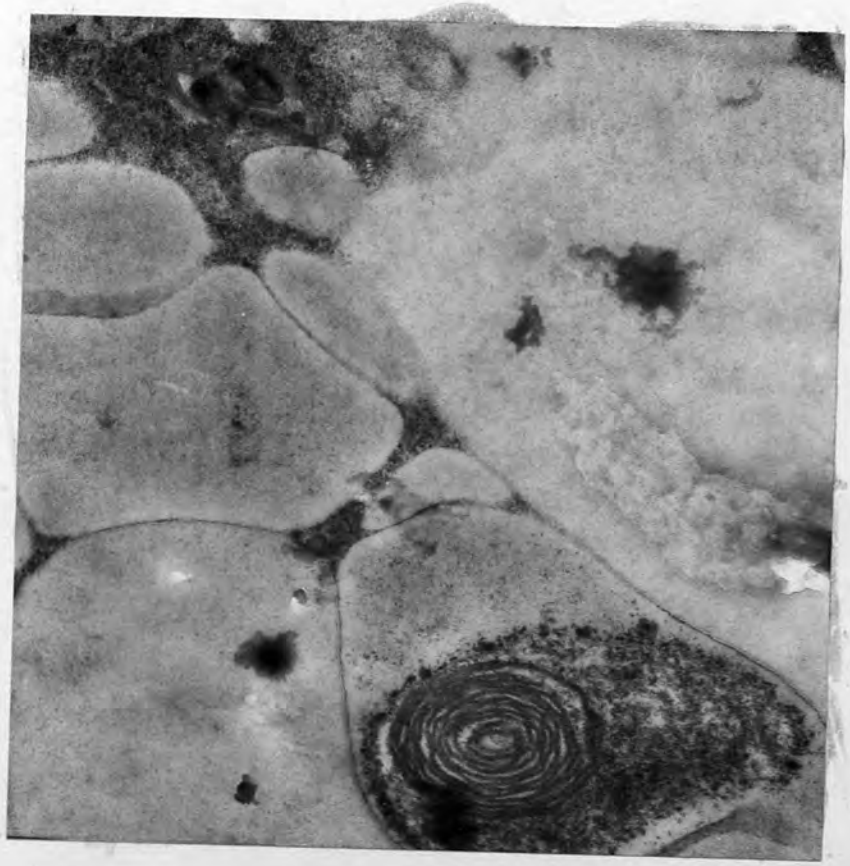


Plate 21. Concentric endoplasmic reticulum in mature dry embryo.

Fig. 38. Enlarged view of a portion of cotyledon parenchyma cell of mature dry embryo showing concentrically arranged endoplasmic reticulum in the residual cytoplasm. x 49,200

Fig. 39. Magnified view of endoplasmic reticulum in Fig. 38. x 96,000



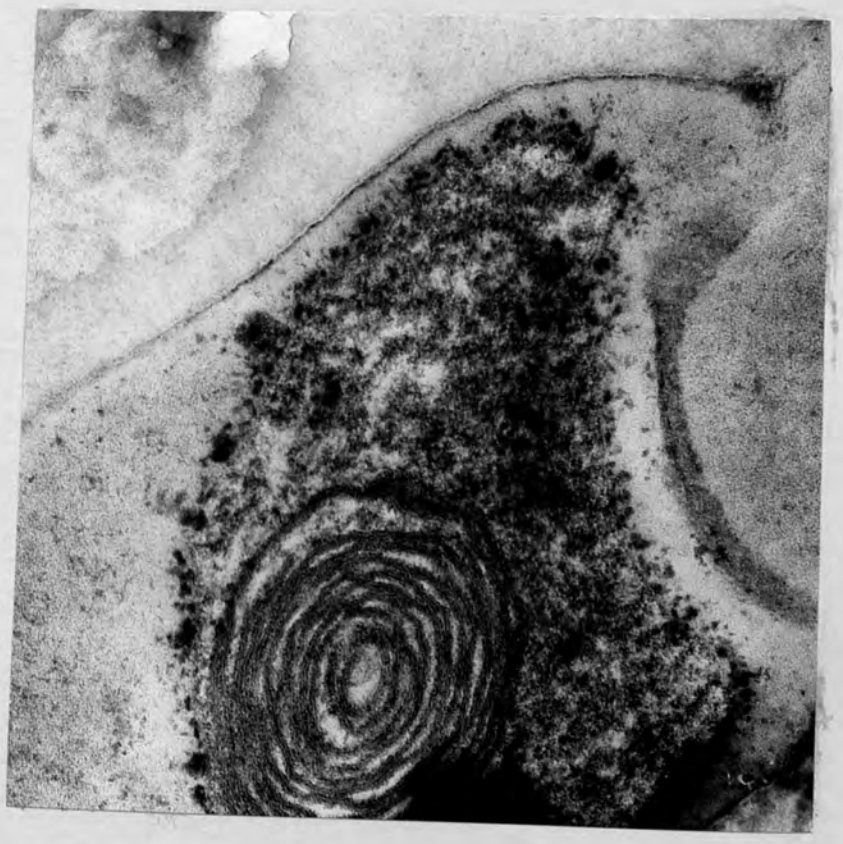
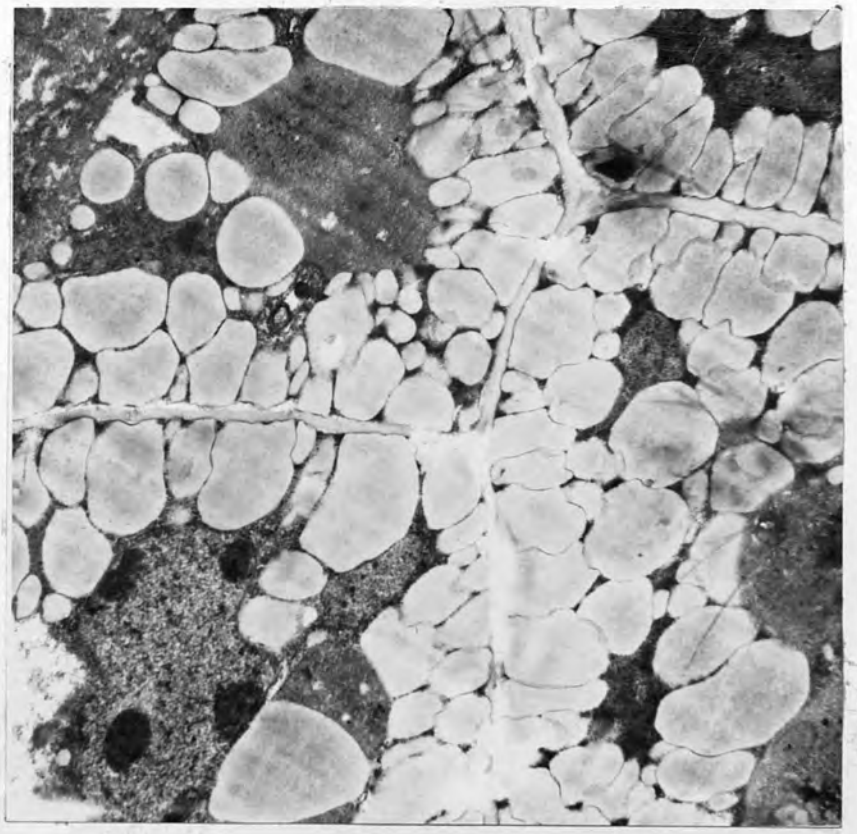


Plate 22. Disorganizing nucleus in mature dry embryo.

Fig. 40. Enlarged view of a portion of a cotyledon parenchyma cell of mature dry embryo showing disorganizing nucleus and other organelles. The nucleus with densely stained chromatin in four lobes can be seen. x 12,300

Fig. 41. Enlarged view of a portion of a cotyledon parenchyma cell of mature dry embryo showing disorganizing nucleus. In this large nucleus an electron dense nucleolus and some chromatin is seen. Small disorganizing plastids filled with plastoglobuli can also be seen. x 32,800



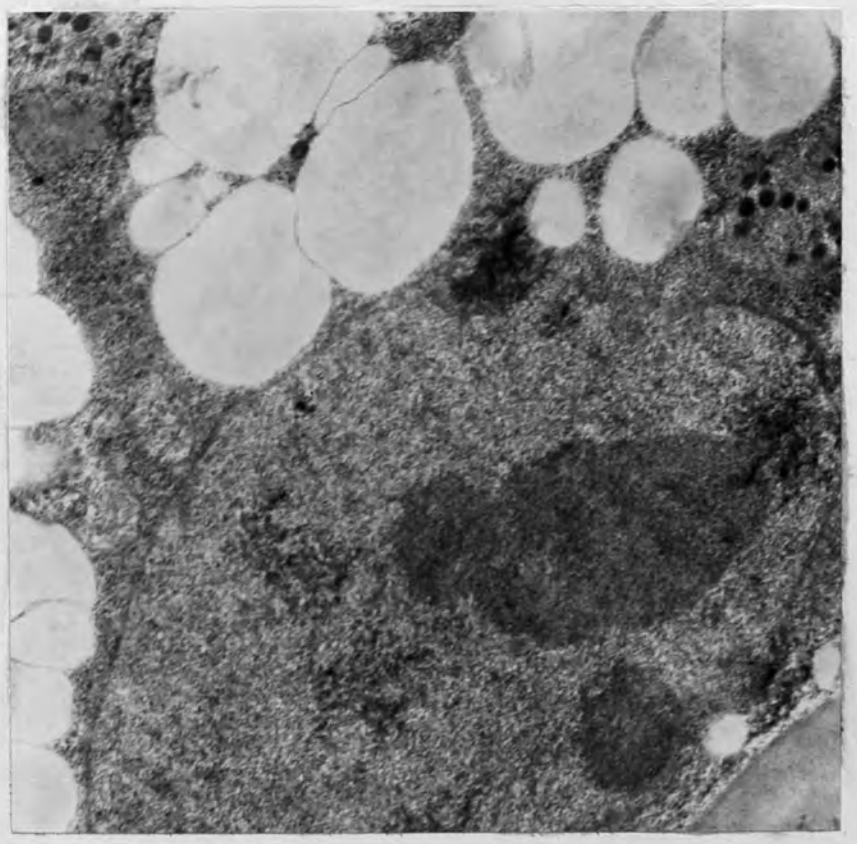


Plate 23. Intercellular lipid in dry embryo.

Fig. 42a. Enlarged view of a portion of a cotyledon parenchyma cell of mature dry embryo showing local accumulation of excess lipid in the intercellular space and also on cell wall. x 14,000

Fig. 42b. Enlarged view of a portion of a cotyledon parenchyma cell of mature dry embryo showing more accumulation of excessive lipid than in Fig. 42a. x 24,000

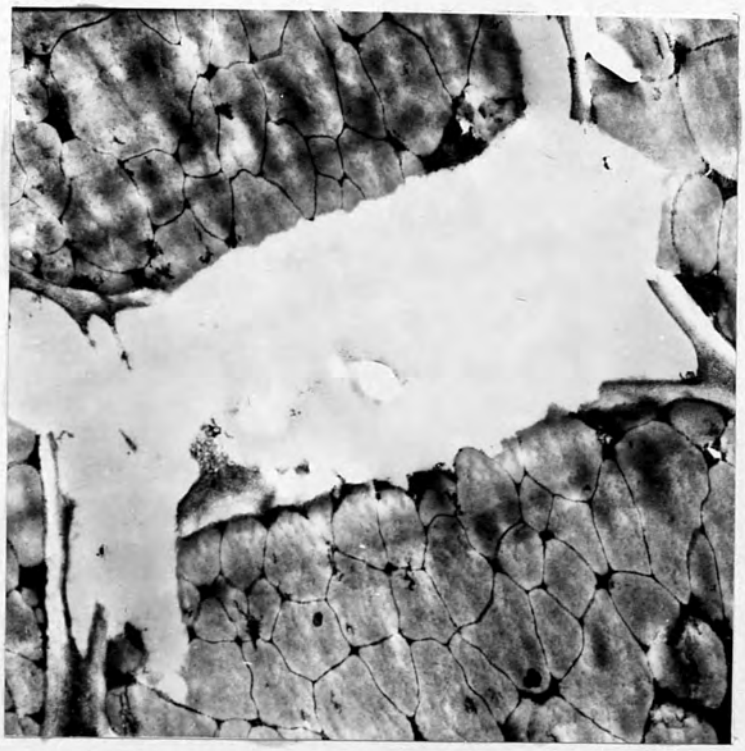
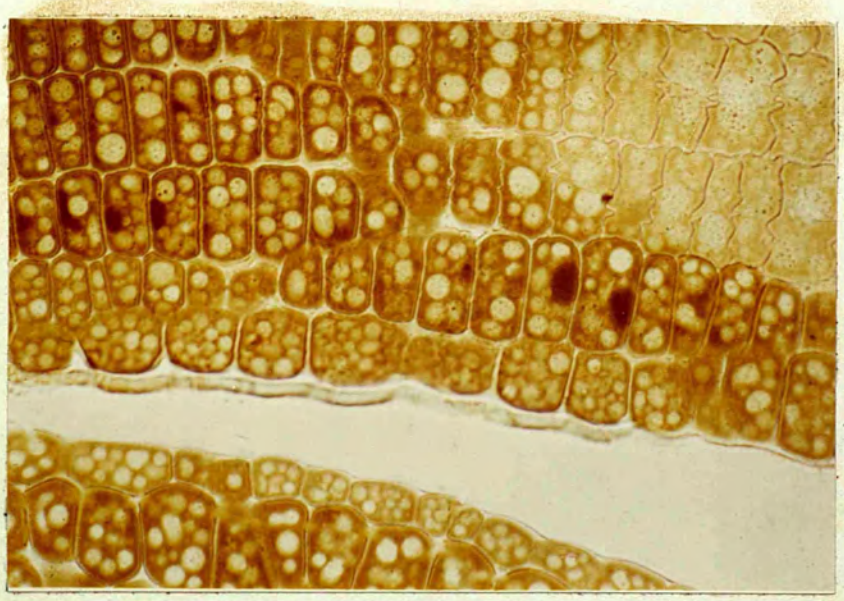


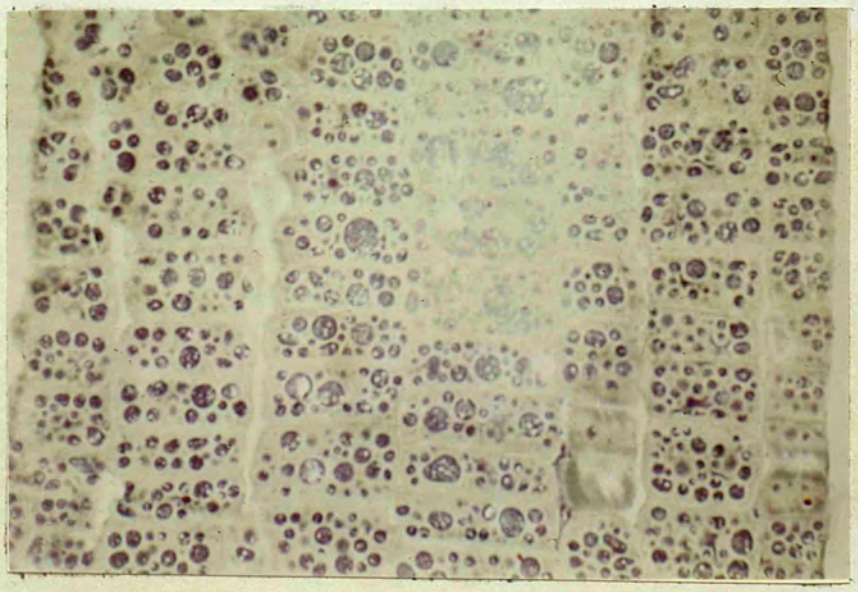
Plate 24. Light microscopy.

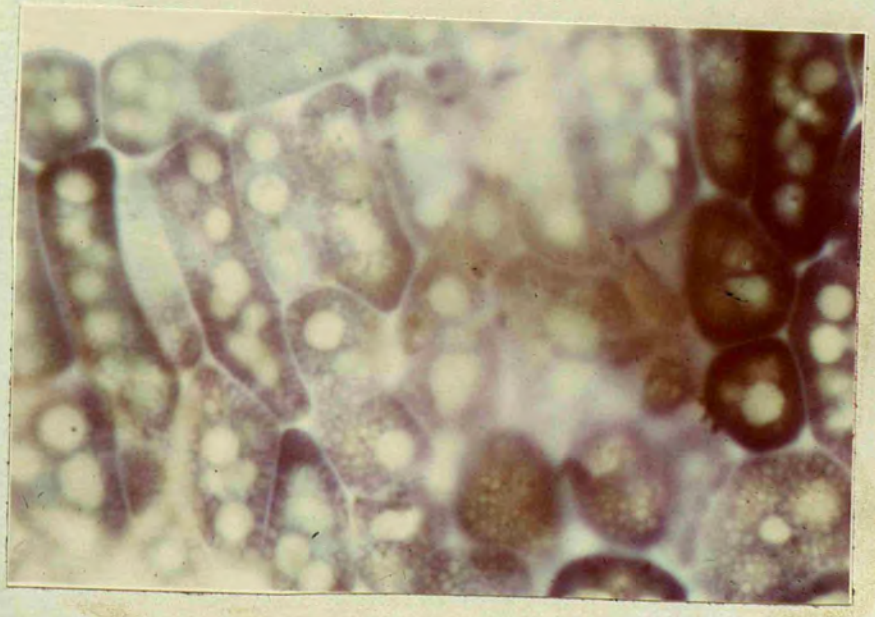
Fig. 43a. Micrographs of the longitudinal section through the cotyledon of mature ivory-white embryo showing general pattern of cells arrangement. The penetration of fixative is probably not complete in some mesophyll cells of central region of cotyledon and such areas do not show cellular details clearly. Epidermal as well as mesophyll cells are filled with large spherical protein areas and smaller oil bodies stained brownish yellow. Oil bodies are not very clearly seen as the cytoplasm is also stained brownish yellow. Some mesophyll cells also show darkly stained peripheral nucleus. Stained with Sudan IV. x 480

Fig. 43b. Micrograph of the same embryo material as in Fig. 43a. Cells contain larger and smaller protein bodies with clear inclusions. The protein bodies are stained bright blue with colourless inclusions in them. The cytoplasm in the cells is stained greenish. Lightly stained protein grains are seen in that area of cotyledon where fixative has not penetrated. Stained with Toluidine blue O. x 480

Fig. 44. Micrographs of the same embryo material as in Fig. 43a. Enlarged view of epidermal and mesophyll cells of the cotyledon. The cells are filled with smaller oil bodies and larger protein bodies which are not stained. The oil bodies are clearly seen as they appear as lighter or clear greenish yellow bodies. Protein bodies appear as large spherical protein areas. The cytoplasm in the cells is greenish yellow. Stained with Azure II. x 1,200







INTRODUCTION

Various types of plastids which differ in structure and function are present in a mature plant body. It is assumed that all these plastids are derived from the same origin, the proplastids, present in meristematic tissues. The proplastids which represent the juvenile stage have an envelope consisting of two distinct membranes and an absence of any other well defined fine structural details. During differentiation of the tissues, the proplastids differentiate into various types of plastids and one type of plastid may change into another. Plastids should, therefore, be regarded as polymorphous and multifunctional component of higher plant cells rather than being ascribed to particular group of organelles (Miyake and Maeda, 1976). Most work on plastids of higher plants has concentrated on chloroplasts in chlorenchyma of leaves. Chloroplasts are also found in outer tissues of the stem, green parts of flower like sepals, sepalloid perianth, stamens when green, ovary wall, ovules and developing embryo.

Comparatively little attention has been paid to the chloroplast structure and development in the reproductive parts of the flower, such as in the zygote and developing embryo, which is the ultimate source of all the cells of the mature plant. For the full understanding of various functions of the plastid, it is also desirable to know the structural variations of the plastids within a species.

Recent advances in electron microscopy have offered opportunities to examine plastids in various tissues where they may escape detection or unequivocal recognition by light microscopy. During the last decade ultrastructural work on the early and late embryogenesis of various species has included passing reference to the structure and development

of chloroplasts as well as to other organelles. The main effort in those studies has been directed towards the description of embryogenesis proper. So far as our knowledge goes, no special attempt has been made to study the differentiation of embryo chloroplast in the flowering plants.

It should be pointed out that our main concern here is not the differentiation of the organelles at the time of rehydration and germination of a dry resting embryo. It has been shown in several studies that the plastids along with other cytoplasmic features /organelles reappear at that time. Although it clearly follows that considerable loss of cytoplasmic organization occurs in the embryo as it dries out in the seed, few studies have paid attention to these changes or to the changes in the chloroplast prior to the maturation of the embryo, as noted above.

Plastids in embryogenesis with special reference to Capsella bursa-pastoris.

Plastids are present in most embryos from the very earliest stage of development. In the egg cell of Capsella bursa-pastoris (Schulz and Jensen, 1968b) the plastids are usually concentrated in a shell around the nucleus and in profile they appear rod-shaped (1.5 x 0.5 μm) or spherical (0.5 μm) and some have cup shaped invaginations. The characteristic egg plastids of Capsella bursa-pastoris form vesiculate lamellae by invagination of the inner membrane. These plastids usually contain an invariable amount of starch. The chloroplast matrix is dense probably because of the presence of clusters of dense droplets (440 Å) and ribosome-like particles (150 Å) in it. The synergids (Schulz and Jensen, 1968a) contain relatively few plastids with very

little starch as compared with the egg; even though the starch may be abundant in the egg and central cell of the same mega-gametophyte. Several synergid plastids were seen to be cup-shaped. The cytoplasm within the invagination contains fewer ribosomes than the neighbouring cytoplasm. This type of plastid is also common in central cell, egg, zygote and young embryo. The characteristic arrangement of plastids around the egg nucleus is also found in the endosperm and young embryo but not in the synergids. Each synergid plastid contains several single lamellae formed by the invagination of the inner membrane of double membraned plastid envelope. Some lamellae are quite vesiculate in appearance and only rarely are two fused. The plastid matrix is electron-dense similar to that of the egg probably due to the presence of clusters of dense, spherical (700 Å diameter) droplets and ribosome-like particles. There are few diffuse, light ares in the matrix of plastids of synergids and egg similar to those seen in the plastids of the very young leaves of Capsella bursa-pastoris (present study, Part II). The arrangement and structure of plastids in the zygote (Schulz and Jensen, 1968b) is almost similar to that of the egg. Starch grains which are conspicuosly present in the egg increase upon fertilization and during the first division of the zygote. In the zygote of Gossypium hirsutum , starch grains form a striking mass surrounding the nucleus, and their number decreases during subsequent divisions until they completely disappear in the mature embryo (Jensen, 1963). The total amount of starch in Capsella bursa-pastoris embryo which increases in the two-celled embryo reaches a maximum at this stage. The number of plastids and starch are more in the basal cell at the 2-celled stage. At the second division of the terminal embryonal cell, the starch grains completely disappear and are not synthesized until the embryo attains torpedo-shape. The plastids, which are mostly cup-shaped, still maintain their orientation around the nucleus at this 3-celled stage

and their number is also the same in all the three cells. The accumulation of starch in the terminal cell and its rapid disappearance during the first two divisions of the cell, prior to the formation of a whole embryo, suggest that it is metabolized as a source of energy for initiating division processes.

Octant and globular stages of the Capsella bursa-pastoris embryo possess relatively undifferentiated plastids. It is only at the heart-shaped stage that many ultrastructural differences are noticeable between cells of different regions of the embryo. The plastids in the cells of ground-meristem and protoderm begin to form fused lamellar system by invagination of the inner membrane, while the plastids in the procambium remain relatively undifferentiated even at this stage when the embryo appears green (Schulz and Jensen, 1968c). The embryo plastids at this stage never contain starch. The plastids in the region of hypophysis, at this stage of development, are of intermediate type between those of the embryo and the suspensor as they remain underdeveloped like embryo and retain starch like those of the suspensor.

The octant embryo has a 6-celled suspensor. The plastids in the suspensor cells at this stage of development are mostly rod-shaped encircling the nucleus with single plastid lamellae which is often vesiculate. The matrix is electron-dense and contains a few light areas, osmiophilic droplets, ribosome-like particles and, possibly, starch. Plastids are often constricted in the middle and appear to be dividing. The suspensor is 10-celled when the embryo is in the globular stage. Plastids at this developmental stage form cup-shaped invaginations which, in section, appear to have a piece of cytoplasm inside them. The plastids show no sign of degeneration when the cytoplasmic degeneration begins in the suspensor cells when they attain their maximum size at the heart-shaped embryo stage. During this degeneration phase of suspensor, the cup-shaped forms of plastids are no longer seen and

starch also completely disappears. Plastids still have single vesiculate lamellae and osmiophilic droplets but acquire some dense structures which are ordered in regular rows. The plastids of basal cells also have almost similar structural changes (Schulz and Jensen, 1969). Marinos (1970) reported the presence of an unusual type of proteinaceous plastid in the suspensor cells of Pisum sativum. These unusual plastids contain spherical proteinaceous bodies composed of bundles of tubules which do not form typical prolamellar bodies and are not converted into grana. Analogous plastids have also been found in the suspensor cells of Ipomoea purpurea (Ponzi and Pizzolongo, 1972, 1973) in the first stage of embryogenesis, and plastids with such unusual internal structures have been described by Schnepf and Nagl (1970) in the suspensor of Phaseolus vulgaris. In Ipomoea purpurea (Ponzi and Pizzolongo, 1972, 1973) the plastids present a system of short tubules in various forms: as irregular bent tubules, prolamellar bodies, flattened and sinuous tubules arranged at the periphery of the plastids, and bundles of large parallel tubules without interconnections. Besides these tubular formations, these plastids show large granules of starch, aggregates of osmiophilic globules, phytoferritin and dense proteinaceous bodies. The organization of plastids in the suspensor of Tropaeolum majus (Nagl and Kühner, 1976) is quite different. Chloroplasts, which rarely form grana, occur in the elongated cells of embryo-suspensor as well as the suspensor cells adjacent to the embryo proper and thus the embryo and the embryo-suspending thread of the suspensor are green. Leucoplast with an electron-dense matrix and electron-transparent tubes occur in the basal cell mass of embryo-suspensor. Undifferentiated leucoplasts occur in the carpel haustorium, which get transformed into electron-dense plastids during autolysis of the suspensor. Etioplasts develop in several cells of placental haustorium at late heart-shaped embryo stage while during earlier stages undifferentiated leucoplasts are

reported from placental haustorium of the suspensor. In the suspensor haustorium of Pelargonium X Hortorum (Khera and Tilney-Basset, 1976a) the numerous normal chloroplasts have thylakoids stacked to form many grana and have few osmiophilic plastoglobuli and no starch. Plastids at two week stage have swollen thylakoids which are loosely packed in the very long grana. At three week stage chloroplasts are mostly elongated with as many as 40 thylakoids comprising the large grana and they are more tightly packed than at two weeks. Some of the plastids have abnormally large osmiophilic deposits. It can, therefore, be said that plastids are capable of performing diversified functions according to the cellular physiological requirements, in order to assure the normal development of the embryo. The extensive development of chloroplasts in the suspensor of some species described above suggests that these chloroplasts contribute to the elaboration of different kinds of material available to the embryo and suggesting that such suspensors act as photosynthetic boosters for the developing embryo.

The chloroplasts in the early torpedo-shaped embryos (present study part I) show hardly any advancement over those in the heart-shaped embryos. Many chloroplasts still remain under-developed and undifferentiated while others form a fused lamellar system, probably by the invagination of inner membranes of plastid envelope. Chloroplasts from the late torpedo-shaped embryos show little lamellar differentiation, but lack grana formation. Starch seems to be most scarce at this stage so only few chloroplasts show the formation of one small starch grain each in them. At the walking stick-shaped embryo stage, chloroplasts show greater differentiation of the lamellae which are at different aggregation stages; some lamellae show overlapping growth whereas others show invagination at few places resulting in sparse granal stacking to form small grana. The chloroplasts reach their maximum length at inverted U-shaped embryo stage. Chloroplasts at this stage of development show more

differentiation of lamellae, the stacking of lamellae resulting in the formation of many small grana with up to 8 lamellae in one granum. Usually one, sometimes more than one, big starch grain is present in each chloroplast, although some of the chloroplasts do not develop starch. Chloroplasts are grouped around the nuclei in many cells.

The chloroplasts in the mature-green embryo stage are fully differentiated with a varying number of well-developed grana, and do not show any advancement over those in the inverted U-shaped embryo stage. Some fully developed chloroplasts do not form starch. Starch grains have never been found in contact with the plastid membranes at any stage of their existance during embryo development. Some chloroplasts show considerable distortion of lamellar structure due to growth of starch grains at the fully mature-green embryo stage.

The chloroplasts of the mature ivory-white embryo stage (present study part I) show complete deterioration as the internal structure of the chloroplasts start breaking down. The lamellae get separated and typical stacking into grana is partially or completely gone. A certain degree of shortening and swelling of lamellae in the grana take place resulting into few very dense and thick grana-like structures in the centre of chloroplasts which get elongated. Such degenerating chloroplasts contain in their stroma many small electron-dense spherical bodies, called the osmiophilic plastoglobuli, arranged either singly or in groups or in close association with degenerating grana. Some of the chloroplasts become irregular in outline having few disorganizing elongated lamellae and all internal structure appears as if macerated, but these do not contain any osmiophilic plastoglobuli. Chloroplasts from dry-embryo stage show some general shrinkage and appear as if disorganizing with indistinct outer envelope and with no lamellar structure. The details of stroma are obscure, but a few electron-dense small spherical bodies, the osmiophilic plastoglobuli, are present and thus they can be

distinguished from the other disorganizing organelles especially the mitochondria.

The aim of the present investigation has been to study normal plastid differentiation together with an experimental investigation of the changes in chloroplast structure induced by light and darkness. Excised embryos, grown in sterile culture media, as well as embryos from intact plants, have been used. All experimental studies are followed by the electron microscopic studies in order to compare the ultrastructural changes of plastids induced by the experimental treatments. Attempts have also been made in the present investigation to compare the photoresponses of in vivo embryo chloroplasts with those of the leaf chloroplasts of similar developmental stages at the ultrastructural level. The present in vitro study has exclusively been made on torpedo-shaped embryos.

In the present investigation, in vitro studies of the embryos are preferred over in vivo studies because in the embryo culture technique all embryos could be subjected to direct experimental control, to some extent at least free from the complex reactions occurring in the intact plants. Secondly the cultured embryos had the advantage of being supplied with its carbon source independantly of photosynthesis and this was a necessary condition for such experiments. Beside cultured embryos could be kept in the dark for indefinite (longer) period, a treatment which could not be given to intact plants with success, as the intact plants degenerated when subjected to a long period in the dark. Moreover embryos of uniform size could be excised and selected for the experiments, thus giving the uniformity of treatments. Thus a particular convenience of the culture technique is the high measure of control that can be exerted over the experimental material.

Attempts to obtain autotrophic tissue or cell cultures are still largely unsuccessful, but several laboratories are actively studying factors affecting chloroplast differentiation, at both the physiological

and ultrastructural level (Raghavan, 1976). To our knowledge, in vitro studies of chloroplast differentiation in embryos have not been initiated earlier, and thus the present study which is the first of its type, may be regarded as a significant contribution to our knowledge of chloroplast structure and development.

REVIEW OF EFFECTS OF LIGHT AND DARKNESS IN CHLOROPLAST MORPHOGENESIS.

The differentiation of proplastids into chloroplasts in the green tissues of plants grown under illumination and in dark grown plants subsequently exposed to light, has been the subject of various studies and reviewed by Kirk and Tilney-Basset (1967). Most studies on chloroplast development have employed dark-grown plants exposed to light. This technique is advantageous since chloroplast development is then accomplished in a matter of hours rather than days, as in the development in light-grown plants. It is also possible to regulate precisely the initiation of the developmental sequence and, since light is the inducing agent, it is relatively easy to regulate its quality and quantity. The disadvantage of this type of study is the speed with which the dark to light sequence of chloroplast development proceeds and important steps may proceed too rapidly for detection.

In general, angiosperm plants grown in darkness or in dim light fail to produce chlorophyll and the proplastids of their cells develop into "etioplasts", in the terminology of Kirk and Tilney-Basset (1967). On exposure to light, etioplasts develop into chloroplasts, undergoing ultrastructural changes which have been studied extensively in normal whole tissues, as well as in tissue cultures (Blackwell et al., 1969) and in isolated etioplasts (Wellburn and Wellburn, 1971; Wrischer, 1973; Kohn and Klein, 1976; Wellburn et al., 1977). The most conspicuous and characteristic structure of the etioplast is the prolamellar body; the process of formation and spatial organization of this structure has been the subject of several studies recently reviewed by Gunning and Steer (1975).

The Prolamellar Body.

The fine stucture of the prolamellar body in etioplasts of dark grown seedlings has been the subject of many investigations. According to

Wellburn et al. (1977), prolamellar bodies develop when lack of light interrupts the development of chloroplast, resulting instead in the formation of etioplasts. Usually one or two prolamellar bodies are found in each etioplast. On the other hand, von Wettstein (1958) recorded about 100 prolamellar bodies in the etioplast of cotyledon of Picea which was cultivated in the dark following the initial illumination. In the study of Wrischer (1966) many prolamellar bodies were also formed under dim light conditions (below 300 lux) and were called "weak light prolamellar bodies". Ikeda (1970) also observed the similar situation in the plastids cultured under the conditions of light-dark alternation. Each prolamellar body is a quasi-crystalline array of branched tubular membranes, with the etioplast stroma penetrating into the structure between the tubules (Wellburn et al., 1977). In thin sections these "crystalline bodies", with their numerous connecting tubules, present a mosaic appearance.

Under proper illumination the inner membrane of the plastid envelope forms sac-like compressed vesicles which have been termed "thylakoids" by Menke (1961). When developed in the dark, such lamellar systems are not formed, but instead, a number of such vesicles pinch off and aggregate to form a so-called prolamellar body (Hodge et al., 1956). This aggregate of vesicles was first observed originally in the proplastid under electron microscope by Leyon (1953) and described for the first time a year later (Heitz, 1954; Leyon, 1954). Such a vesicular structure has been referred to variously as the "dense core" (Leyon, 1954), the"primary granum" (Strugger, 1954; Strugger and Perner, 1956), the "prolamellar body" (Hodge et al., 1956), the "plastid centre" (von Wettstein, 1957; Lefort, 1959), the "vesicular body" (Gerola et al., 1960), the "vesicular centre" (Eilam and Klein, 1962), the "crystalline centre" (von Wettstein, 1958) and the"Heitz-Leyon crystal" (Menke, 1962). The term prolamellar body has gained wide acceptance and will be used in the present investigation.

The first observation of the prolamellar body under the light microscope was by Strugger, who in 1950 described a dense body in the centre of the etioplast of dark-grown seedlings and termed it the "primary granum". The early electron micrograph published by Leyon (1954), Heitz (1954) and Perner (1956) suggested this structure to be a three-dimensional array of beaded strands. The prolamellar body appears to be a complex network of interconnected tubular membranes having the appearance of a crystal lattice. It is therefore, referred to as crystalline-like or para-crystalline or quasi-crystalline. Several models have been proposed for the structure (mode of arrangement of the tubules) and process of formation of the prolamellar body. According to von Wettstein (1958) this is composed of interconnected tubules and he proposed a model of tubules arranged in a cubic lattice. The beads thus would represent points where six tubules meet. Granik (1961) also drew it as a three-dimensional cubic lattice of interconnected tubules. In contrast, Menke (1962, 1963) postulated that the prolamellar body of Chlorophytum comosum is a system of helically coiled, but not interconnected tubules. Schnepf (1964) has described the mode by which such tubules are brought into connection with double lamellae (thylakoids) in greening Avena sativa plastids, and surmised that the prolamellar body has a helically coiled structure. Gunning (1965) and Gunning and Jagoe (1967) presented a model of the prolamellar body in Avena sativa similar to that of von Wettstein's model with a three-dimensional lattice structure that is composed of interconnecting tubules lying in three major axes of a simple cube type of lattice (six tubules meeting at one point).

A somewhat complex structure for the prolamellar body has been suggested by Wehrmeyer (1965a, b, c) after examining serial sections through prolamellar bodies of Phaseolus vulgaris. In contrast to Gunning, he interpreted the major portion of the crystalline prolamellar

body structure as a six-sided crystal alttice comparable to the zinc-blende crystal lattice. He found concentric and non-concentric types in etiolated leaves of Phaseolus vulgaris. In the concentric type the basic units form a system of tetrahedrally branched tubules joined to five- and six-membered rings. The cnetre of the lattice has the shape of a pentagonal dodecahedron. The non-concentric prolamellar bodies follow either the crystal lattice of zincblende or wurzite or a combination of both. It is now agreed (Gunning and Steer, 1975) that cubic lattices are not very common and the majority of prolamellar bodies are undoubtedly based on tetrahedrally branched tubules arranged in one or other of the lattice-types described by Wehrmeyer (1965). Ikeda (1968) analyzed the fine structure of prolamellar bodies of Phaseolus vulgaris and confirmed Wehrmeyer's interpretation of prolamellar body as an aggregate of hexagonal units. He presented a model consisting of an aggregate of hexagonal units, as seen in surface view and concluded that the lattice structure was more complex than cubic model of Gunning (1965) but showed a close resemblance to the hexagonal model. Weier and Brown (1970) proposed a model of the prolamellar body of Phaseolus vulgaris which agrees closely with that of Wehrmeyer (1965a, b, c) and is identical to that described by Ikeda (1968). According to Weier and Brown (1970) the major portion of crystalline prolamellar body is constructed of tubules, equal in length. The basic structural unit is a six-sided star module with four tubules uniting at each of the nodes. From these models, it appears that the basic unit of prolamellar body is a tetrahedron (four tubules meeting at one point), with the tubules forming six-membered rings. The most common prolamellar body, therefore, contains hexagonal rings, but irregular patterns may also be found. The symmetry of prolamellar bodies may be affected by the plane of the section or may be altered when there is more than one centre of symmetry in a prolamellar body (Wehrmeyer, 1967).

Configuration of prolamellar body.

The configuration of the prolamellar body with irregular tubular pattern has been reported in early developmental stages of etioplasts from several species. For example "open prolamellar body" in Phaseolus vulgaris (Weier and Brown, 1970), "atypical lattice" in Avena sativa (Gunning, 1965; Gunning and Jagoe, 1967), "wide-spread tubules" in Hordeum vulgare (Henningsen and Boynton, 1969), the "non-crystalline" prolamellar body in Hordeum vulgare (Berry and Smith, 1971). The non-crystalline prolamellar body of Berry and Smith (1971) is clearly different from the transformed prolamellar body seen after exposure to light and most authors have regarded it as an alternative configuration of paracrystalline prolamellar body. According to Wehrmeyer (1967) it arises from a combination of five- and six-membered rings. But according to Henningsen and Boynton (1969) such prolamellar bodies differ from normal paracrystalline prolamellar bodies by virtue of wider spacing of the tubules in this configuration. Since these all appear to have the same structure, Weier and Brown's terminology of "open lattice" appears more appropriate. The proportion of prolamellar bodies and the time of their appearance in this configuration depend upon species and also on the physiological conditions of the seedlings.

Formation of the prolamellar body.

There is little information about the formation of prolamellar bodies during the development of the etioplast. Earlier it was generally agreed that the prolamellar body is formed by the fusion of vesicles derived from the inner plastid envelope and these vesicles accumulated in the centre of the etioplast before their fusion (Hodge et al., 1956; Mühlethaler and Frey-Wyssling, 1959; Röbbelen, 1959; von Wettstein, 1959;

Eriksson et al., 1961). According to Newcomb (1967) the prolamellar body is formed by the convergence of groups of tubules. Gunning and Jagoe (1967) did not find any stage of vesicle formation during prolamellar body formation and reported that the prolamellar body arises as connections are formed between thylakoids already present in the stroma. A similar formation of prolamellar bodies was also reported by Henningsen and Boynton (1969). They found three-armed tube elements and suggested that these could fuse with one another to form incompletely connected prolamellar bodies. Engelbrecht and Weier (1967) in the cotyledons of Carthamus tinctorius and Weier and Brown (1970) in the primary leaves of Phaseolus vulgaris rejected the earlier view of prolamellar body formation by the fusion of discrete vesicles derived from the inner plastid envelope. Weier and Brown (1970) concurred with the report of Engelbrecht and Weier (1967) that sheets of membranes, rather than vesicles, arise from the inner component of the plastid envelope. In Phaseolus vulgaris (Weier and Brown, 1970) these lamellae are porous and the prolamellar body arises through the contraction of these porous lamellae and the formation of interconnecting tubules linking each lamella to the one above it and the one below it. These authors have presented good evidence in support of their conclusion that these large porous membranes, rather than discrete vesicles, are involved in prolamellar body formation; they show gradations between the sheets and the paracrystalline prolamellar body indicating that the prolamellar body is formed by some type of contraction of these sheets. Thus it may be said that the prolamellar body is the result of an accumulation of membrane products due to the blockage of the light reactions in the normal process of greening of seedlings. The mechanism of its formation is not clear, but it seems to involve changes in a continuous membrane system rather than a breaking off of discrete pieces (vesicles) which migrate and then re-fuse to form a continuous membrane again (Weier and

Brown, 1970).

Prolamellar body transformation.

This term is usually used to describe either the loss of paracrystalline structure or the complete disappearance of the prolamellar body. The terms in general use are, transformation for the loss of paracrystalline structure; and the term conversion for the complete disappearance of the prolamellar body as it forms lamellae. The idea of von Wettstein (1958) regarding the transformation of prolamellar body in the light, that is, via a disruption into discrete vesicles with loss of continuity of the membranes has not been accepted, as all recent reports suggest that prolamellar body does not form vesicles upon illumination. Using glutaraldehyde fixation, a technique that appears to preserve certain cellular structures lost with other fixation techniques, Gunning and Jagoe (1967) reported that in Avena sativa prolamellar body transformation takes place via a direct transformation of prolamellar body into lamellar membrane without an intervening stage of vesicle formation. Thus according to them the tubules of the prolamellar body do not dissociate into vesicles which disperse and ultimately fuse to form new thylakoids. Rather the whole process is accomplished by a direct transition from the tubules into lamellar thylakoids. Thus, the break-up of prolamellar body to form primary lamellae is identical to what is generally referred to as the second step in lamellar construction and it has a high energy requirement (Bogorad, 1967). Prior to glutaraldehyde fixation techniques, the second step was described as the dispersal of vesicles into concentric layers. In this case of Avena sativa (Gunning and Jagoe, 1967), the prolamellar body loses its paracrystalline appearance and becomes a less ordered tubular network, with some connections between the tubules. In the cotyledons of Carthamus tinctorius

(Engelbrecht and Weier, 1967) using permanganate fixation and later in the leaves of Phaseolus vulgaris (Lemoine, 1968) using glutaraldehyde fixation, it has been reported that the prolamellar body was not transformed into vesicles.

Prolamellar body transformation from a paracrystalline to non-crystalline state has been reported to take place after a flash irradiation of only 1 msec. (Kahn, 1968). The prolamellar bodies in the etioplasts of mesophyll cells of Saccharum officinarum retain their paracrystalline structure even after one hour of light (Laetsch and Price, 1969). In fact, these etioplasts require up to 7 hours of light for this transformation even though etioplasts of the bundle sheath in the same species are transformed by very brief periods of light. In Avena sativa (Gunning and Jagoe, 1967), after five minutes of irradiation at 750 to 1000 f.c.(8,000- 10,700 lux), the regular crystalline arrangement of prolamellar body is lost and it appears as an irregular mass of connecting tubules. During this period of illumination all of the protochlorophyllide present is converted to chlorophyllide. Significantly, transformation of the prolamellar body is brought about most effectively by red and blue light, corresponding to the maximum absorption of protochlorophyllide, whereas green and far-red irradiation is relatively ineffective (Klein et al., 1964; Virgin et al, 1963). This agrees with the concept that protochlorophyllide is the photoreceptor responsible for these initial structural changes (Bogorad, 1967; Kirk and Tilney-Basset, 1967). The transformation of prolamellar body along with the simultaneous conversion of protochlorophyllide to chlorophyllide is sometimes referred to as the first step in the construction of a mature lamellar system (Klein et al., 1964; Virgin et al., 1963) and the energy reqirement for this is quite low (Bogorad, 1967).

Prolamellar body conversion and thylakoid formation (vesicle dispersal)

This stage when dispersal of vesicles from the prolamellar body takes place throughout the plastid has been described by von Wettstein (1958) and Virgin et al., (1963). These vesicles first become arranged in layers, and then fuse to form the several primary thylakoids of the plastids. This model shows the conversion of prolamellar body to thylakoid before the formation of extensive grana begins. Gunning and Jagoe (1967) have described the conversion of prolamellar body in etioplasts of Avena sativa leaves. According to them double-membraned sheets extend parallel to each other, out into the stroma from the prolamellar body. These are formed as some of the tubular connections are broken. The thylakoids formed in this way are perforated and if seen in cross-section, they have the appearance of rows of vesicles. They were the first to report the presence of perforated thylakoids during prolamellar body transformation and conversion. The first and obvious difference between the development of chloroplast and etioplast from proplastid is that in the etioplast the developing thylakoids are perforated (Weier and Brown, 1970; Klein and Schiff, 1972). The presence of perforated or reticulate sheets have also been reported by many workers (Engelbrecht and Weier, 1967; Lemoine, 1968; Weier and Brown, 1970; Klein and Schiff, 1972; Bradbeer et al., 1974a, b, c). These numerous perforations or depressed areas in the newly-formed thylakoids give the impression that they could consist of rows of vesicles. Perforations are seen during early stage of development but later disappear on continuous illumination. Gunning and Jagoe (1967) suggested that this disappearance of the perforations may correlate in time with the end of the lag period in chlorophyll synthesis. It appears that the prolamellar body is the sole contributor of building material to the primary thylakoids; that is, up to this point in

development little, if any, membrane material is produced de novo (Gunning and Jagoe, 1967; Kirk and Tilney-Basset, 1967). However, after the lag period there appears to be a good correlation between chlorophyll synthesis and membrane production (Virgin et al., 1963).

Thus, although it appears that upon illumination the prolamellar body does not break up into its individual vesicles, the mechanism by which the prolamellar body tubules form perforated sheets and finally smooth thylakoids is not understood. According to most authors some of the connections between tubules of the paracrystalline prolamellar body are broken, giving rise to the disordered appearance of the transformed prolamellar body. Some connections, however, remain intact and, perhaps, new fusions occur as the membranes extend outward from the prolamellar body.

The prolamellar body conversion stage has a much higher energy requirement than prolamellar body transformation. Depending on light intensity, this stage may be completed between a few minutes and several hours (Eriksson et al., 1961; Virgin et al., 1963). According to Henningsen (1967) only blue light is highly effective in bringing about this process. The light period required for the complete conversion of prolamellar body into primary thylakoids varies from 1-28 hours. This depends upon the intensity of light being used, cell type, and species. In some species (Englebrecht and Weier, 1967; Lemoine, 1968) reticulate sheets are seen after about 9 hours of illumination, the frequency and length of which increase and are joined to the prolamellar body at both ends. A complete conversion of prolamellar body is seen after 15 hours in the light, by that time extensive formation of grana has taken place. In other species (Bachman et al., 1968) prolamellar body was reported to be absent after only one hour of light. However, in Saccharum officinarum (Laetsch and Price, 1969) the prolamellar body may persist for at least 28 hours in the etioplasts of mesophyll cell.

The prolamellar conversion is closely related to the shift in the absorption maximum of the chlorophyll from 684nm. to 672nm. (chlorophyll-684 to chlorophyll-672) (von Wettstein, 1967; Henningsen, 1970). They found that if the etiolated leaves were illuminated briefly, then placed again in the dark for one hour at 0^{o} C or 10^{o} C, there was no special shift and no prolamellar body conversion. Both processes were acclerated when the temperature was raised from 23^{o} C to 30^{o} C.

Prolamellar body reformation.

If the leaves are etiolated again after a period of illumination, a new prolamellar body will be formed. In the leaves of Phaseolus (von Wettstein and Kahn, 1960) new prolamellar bodies are formed within 2-6 hours after the leaf was returned to darkness. Gunning and Jagoe (1967), however, observed in the seedlings of Avena sativa, a new crystalline lattice after 45 minutes in darkness. The situation becomes complex after prolonged exposure to light because then the plastids contain both thylakoids and prolamellar bodies. The thylakoids formed earlier are preserved in the state they reach before the leaves were returned to darkness (Mühlethaler, 1972). When etiolated leaves were greened under low light intensities, the prolamellar bodies were transformed, but within 4 hours they reformed a paracrystalline structure (Henningsen and Boynton, 1970).

Grana formation.

Grana formation is classified as a separate stage occurring after vesicle dispersal and fusion (Virgin et al., 1973). Most models of grana formation do not include any association of prolamellar body with, or its proximity to, grana which are forming. Thus prolamellar

body is not usually associated with grana formation.

Other reports indicate the association of prolamellar bodies with grana formation. In the etiolated cotyledons of Carthamus tinctorius, Engelbrecht and Weier (1967) have reported the presence of small grana after 3 hours of illumination. After 12 hours of illumination, large number of grana were seen and most were close to the prolamellar body. They have also reported that some grana formed or grew on the membranes extending outward from the prolamellar body, but their distance from it increased as the size of the prolamellar body decreased. Interestingly the plastids of etiolated leaves of Saccharum officinarum (Laetsch and Price, 1969) contained a halo of developing grana stacks around the still present prolamellar bodies even after 28 hours of irradiation.

The work of various authors suggest that the grana formation may take place by thylakoid invagination, overlapping growth, sliding growth, budding, or spiral growth of thylakoids or a combination of these (Wehrmeyer and Röbblen, 1965; Paolillo and Reighard, 1967; Saleme and Abreu, 1972). In general, in most species, after 4 hours of illumination stacks of 2 or 3 thylakoids can be observed, and after 10 hours stacks of 4 or more thylakoids are present (Gunning and Jagoe, 1967; Kirk and Tilney-Basset, 1967). Grana are clearly discernible following 20 hours of illumination and may contain as many as 8 thylakoids stacked one on top of the other somewhat similar to a pile of coins in appearance. The grana in many cases are connected to each other by bridging thylakoids. Finally, after approximately 45 hours of illumination, differentiation of the chloroplast is complete.

Prolamellar body in light.

There has been some question as to whether or not prolamellar bodies are formed during plastid development in the light. Laetsch and Price

(1969) found that the prolamellar bodies of both light and dark grown plastids of Saccharum officinarum persisted until the terminal phase of development. Some prolamellar bodies were also seen in the meristem of leaf base. All these had rudimentary grana but in that part of the leaf where chlorophyll had accumulated to about 50% of its maximum, both well-developed grana and paracrystalline prolamellar bodies were seen arrayed around the prolamellar bodies. They reported the increased number of grana and few prolamellar bodies which have lost their paracrystalline appearance at a stage when the leaf contained 75% of its final content of chlorophyll. Stetler and Laetsch (1969) reported poorly developed grana and several prolamellar bodies which were frequently connected to one or more small grana near the apex in the leaves of Nicotiana tabacum. They also reported the presence of prolamellar bodies in the leaf primordia even at relatively advanced stage when grana formation had proceeded quite far.

Paracrystalline centres were reported in mature plastids of leaves under low light intensity, lying always between grana in regions of stroma thylakoids and they completely disappeared after 15 minutes of exposure to strong light (Wrischer, 1966). When etiolated leaves were greened under low light intensity, the prolamellar bodies transformed, but within 4 hours they reformed a paracrystalline structure (Henningsen and Boynton, 1970).

Schötz et al. (1966) reported the persistence of the prolamellar body in the light in the presence of well-developed lamellar system. According to Bachman et al. (1968) prolamellar bodies reform in the light in a mutant of Zea mays. Paracrystalline prolamellar bodies were also seen when etiolated leaves were exposed to 24 hours of red light (Boardman et al., 1970) or far-red light (De Greef et al., 1971) or are greened under flashing light (Bradbeer et al., 1970). Prolamellar bodies which have been transformed in the light reform

paracrystalline prolamellar bodies if placed back in the dark but this is dependant on the age of seedling (Henningsen and Boynton, 1969).

Prolamellar body and chlorophyll.

According to Laetsch and Stetler (1967) there is a positive correlation between the presence and size of the prolamellar body and the rate of chlorophyll synthesis. Dark-grown buds induced from callus had larger prolamellar bodies and were dark green after 20-24 hours of light. But the dark-grown buds from the intact plant had smaller prolamellar bodies and needed 48 hours to synthesize the same amount of chlorophyll. Plastids of dark-grown callus, which had no prolamellar bodies, needed two weeks for complete differentiation. The view of Laetsch and Stetler (1967) gets support from the developing chromoplasts of ripening Capsicum (Spur and Harris, 1968) and Narcissus (Mollenhauer and Kogut, 1968) which have loosely aggregated interconnected tubular complexes along with newly synthesizing thylakoids. However, prolamellar bodies, or tubular complexes resembling disorganized prolamellar bodies may not always be associated with thylakoid formation. Newcomb (1967) has described a tubular complex which is attached to sacs involved in protein storage in root plastids of Phaseolus vulgaris. Marinos (1970) also reported a similar type of tubular complex in the plastids of suspensor cells of Pisum sativum to be involved in protein synthesis. Such tubular complexes appear to be quite different in their basic structure and their function although these complexes resemble superficially the prolamellar bodies of the leaves.

Prolamellar body transformation and protochlorophyllide reduction.

Protochlorophyllide appears to be associated with prolamellar body as it accumulates with the formation of paracrystalline prolamellar body

(Kahn, 1968.). Henningsen and Boynton (1969, 1970) found an accumulation of protochlorophyllide with the reappearance of prolamellar bodies when the etioplast was exposed to light for four hours. They also concluded that protochlorophyllide may be required for the formation of the paracrystalline prolamellar body. They confirmed their conclusion when they put back the etiolated leaves in the dark after a brief exposure to light and found a correlation between resynthesis of protochlorophyllide and reformation of paracrystalline prolamellar bodies. They also suggested that a change from open lattice prolamellar bodies to normal paracrystalline prolamellar bodies during growth in the dark may result from the rapid accumulation of protochlorophyllide occurring at the same time.

Although Henningsen and Boynton (1970) found the occurrence of paracrystalline prolamellar bodies and protochlorophyllide to be associated under certain conditions, there is really no direct evidence for a causal effect. There are examples where protochlorophyllide may be found in the absence of prolamellar bodies (as in the mutant of Zea mays by Millerd et al., 1969), and paracrystalline prolamellar bodies are found where chlorophyll rather than protochlorophyll is accumulating. When leaves of Phaseolus vulgaris were greened under flashing light (Bradbeer et al., 1970), the prolamellar bodies were found to retain their paracrystalline structure, even though rapid synthesis of chlorophyll and thylakoid formation took place. Boardman et al. (1970) reported small paracrystalline prolamellar bodies in the leaves of Phaseolus vulgaris greening under red light, even though there was as much chlorophyll present as in control greening under white light. But much of the chlorophyll present was in the unphylated form (chlorophyllide), suggesting a relationship between prolamellar body structure and phytylation. Treffry (1970) reached a similar conclusion after examining transformation of prolamellar bodies at low temperature.

It is a known fact, that lower temperatures (below 0^{o} C) inhibit the normal transformation of the prolamellar bodies in leaves in light, the prolamellar bodies remaining at these very low temperatures crystalline. Henningsen (1970) attributes this phenomenon to the inhibition of the *in vivo* transformation of chlorophyllide 684 nm. to chlorophyllide 672 nm., while Treffry (1970) ascribes it to the inhibition of the phytylation of the chlorophyllide molecules.

Virgin *et al.* (1963) reported that prolamellar body transformation and photoreduction of protochlorophyllide occur simultaneously. Even as short a flash of light as 1 millisecond is reported to be sufficient to convert the protochlorophyllide to chlorophyllide as well as to cause the transformation of prolamellar body (Kahn, 1968). Klein *et al.* (1964) found similar responses related to the total energy of light supplied by the red wavelengths.

According to Koski *et al.* (1951) the protochlorophyllide is a photoreceptor for its own conversion but the pigment involved in prolamellar body transformation is uncertain. Prolamellar bodies in a leaf need a low energy for their transformation (Virgin *et al.*, 1963). As either blue or red light is effective in the conversion, and since the effect is not reversible by far-red, it seems unlikely that phytochrome is the photoreceptor. The possibility thus exists that protochlorophyllide is the photoreceptor for prolamellar body transformation.

The pigment conversion and prolamellar body transformation were not necessarily associated (Weier *et al.*, 1970). They found that under low light intensity (about 100 f.c. or 1070 lux) protochlorophyllide reduction took place very rapidly (20 seconds) but prolamellar body transformation took 1-2 hours. After a few more hours, the prolamellar bodies reformed even though chlorophyll synthesis continued.

Conclusions and remarks.

The prolamellar body which was at one time regarded as an abnormality plays an extensive role in normal plastid development and is a temporary store of membranes produced during the limited development of plastids in dark grown green tissue of angiosperms. The development of etioplast stops when the etioplast and its prolamellar body or bodies reach a critical size, containing much less membrane material than the mature chloroplasts. It is not yet known what determines the critical size, but it is presumed that the supply of key enzymes or precursors run out, or become limited, and that illumination is a pre-requisite for their replenishment (Gunning and Steer, 1975). It is now known that lack of light does not of itself cause prolamellar body development, as they can also appear during chloroplast development in the light (Prioul and Bourdu, 1968; Henningsen and Boynton, 1969; Laetsch and Price, 1969; Weier et al., 1970). Another point worth mentioning is that the presence of protochlorophyllide (a yellowish pigment) in prolamellar bodies may not be a causal factor for their development, as they can appear when it is undetectable (Treffry, 1973). Thus, besides the major problem of determining the mechanism of prolamellar body formation, two other problems can be added: what causes it to grow, and what causes it to stop growing (Gunning and Steer, 1975).

Specific Light Effects.

Light exerts a decisive effect on the development of the plastid as pigment synthesis, membrane synthesis and synthesis of other plastid components can be induced by light. The formation of the chloroplast takes place only in light and occurs most readily in wavelengths which can be utilized in photosynthesis. The prolamellar body might serve in membrane storage under any conditions where extensive membrane would not be used, that is, where photosynthesis is limited. Light conditions which are unfavourable for photosynthesis, favour formation of a prolamellar body or of paracrystalline centres e.g. low light intensity. Higher light intensities which result in higher photosynthetic rates favour rapid conversion of prolamellar body. Also conversion of prolamellar body and formation of photosynthetic lamellae takes place most readily under red light, the wavelengths which are absorbed by chlorophyll in photosynthesis. Green light, which is not effective in photosynthesis, has no effect on conversion of the prolamellar body.

(A) Light Quality (Wavelength).

The effect of light on the development of chloroplast is dependant on the quality and intensity of light and the duration of exposure. With the introduction of new artificial light sources, it has become possible to study the effects of restricted bands in visible spectrum on plant growth and development revealing very profound effects. Light of restricted spectral range has mostly been used to extend a main light period in sunlight or in high-intensity artificial white light (Friend, 1968; Kadman-Zahavi and Ephrat, 1973). Only in a few studies have

plants been grown exclusively in coloured light, and because of low-light intensities and restricted space, these studies usually involve mainly small, short-lived herbaceous plants (Meijer, 1959; Friend, 1968).

Thus the structural organization of the chloroplast and its functional activity in green plants can be changed with the aid of light of different spectral composition. Both blue and red light are active in these changes resulting in different responses of plants. The effect of spectral composition may change with light intensity (Meijer, 1959; Takimoto, 1967), and duration (Meijer, 1959; Lane et al., 1965).

So far very few studies have been made where plants grown under different wavelengths of light and their plastid fine structure then compared. Shakhov et al. (1967) treated the four-days old etiolated leaves of Zea mays with continuous light of different wavelengths for five days. After five days they noticed the formation of chloroplasts which were capable of carrying out photosynthesis under all wavelengths used, as they possessed some development of lamellar system. The white light resulted in the greatest degree of development of lamellae, but red light (600-700 nm.) also resulted in normal formation of the lamellar system. The chloroplasts in blue light (400-500 nm.) went through an early prolamellar body stage before forming lamellae. Thick "prolamellae" which formed normal thin lamellae but no clearly defined grana were formed under yellow/green light (490-600 nm.).

So far it is not very clear whether there are differences in the action of blue and red light on the formation of chloroplast fine structure during greening of etiolated seedlings. The limited number of experimental observations that have been carried out to date do not enable us to form a definite opinion of this question.

Red light and far-red light effects.

Some later studies have shown the substantial development of the lamellae under blue, red and far-red light. Seedlings grown under continuous far-red irradiation remain etiolated and accumulate little chlorophyll (Mohr, 1972) and at the ultrastructural level the structure of their chloroplasts differ from the normal. The chloroplasts of a seedling of Phaseolus vulgaris grown under continuous far-red irradiation have been reported to have the parallel formation of lamellar sheets, with no grana and a little or no fusion or overlapping of lamellae (De Greef et al., 1971). The prolamellar body remaims crystalline in this case. But in the cotyledons of Brassica, prolonged far-red irradiation results in the formation of crystalline prolamellar bodies in chloroplasts which show large amount of sheets without grana but in various degrees of appresion resulting in paired primary lamellae (Hacker, 1967; Kasemir et al., 1975). Kasemir et al. (1975) have also reported that in etioplast development in dark-grown cotyledons of Brassica the prolamellar bodies at first possessed a crystalline form which subsequently was lost. However, crystallization of the prolamellar body was reported to have occurred in response to either prolonged far-red or brief red irradiation, and to some extent in response to brief far-red irradiation, and was inferred by them to have resulted from the action of phytochrome. Klein et al. (1964) reported that 30 seconds of red light resulted in prolamellar body conversion even when followed by far-red light in the leaves of Phaseolus, indicating that red light effects on the membrane are not far-red reversible. Berry and Smith (1971) found that brief red-light treatments induced the crystallization of prolamellar bodies in the dark grown leaves of Hordeum vulgare but as there was no far-red induced reversal, they concluded

that phytochrome was not involved. They also reported that red light pretreatment stimulates the formation of granal lamellae upon subsequent transfer to continuous white light. When dark grown leaves of Avena sativa were given red-light treatment and then returned to darkness, prolamellar body reformation occurred to almost 100% crystallinity as is typical of fully dark-grown leaves (Gunning and Jagoe, 1967). In the dark-grown leaves of Hordeum vulgare (Berry and Smith, 1971) the etioplasts exhibit a relatively low frequency of crystalline prolamellar bodies (ca. 16-20%). Brief red-light treatments lead to rapid disruption of all prolamellar bodies followed by a slow reformation in the subsequent dark period. When several red-light treatments were given with intervening 3 hour dark periods, a marked increase in the proportion of crystalline prolamellar bodies was seen. Treffry (1973) reported a considerable reformation of prolamellar body during development, when treated with the high- and medium-intensity red light of the plastids in etiolated leaves of Pisum sativum. When etiolated leaflets were exposed to low intensity red or white light, the prolamellar body of the etioplast initially lost its regularity but was later reformed. Boardman et al. (1970) found that in the dark grown leaves of Phaseolus vulgaris and Pisum sativum the plastids developed in red light identically to those in white light during the first 8 hours of greening: prolamellar body dispersal, vesicle fusion and grana formation occurring at approximately the same time in both light treatments, although grana formation was slightly earlier in red light. Greening for longer than 8 hours in red light resulted in the appearance of small prolamellar bodies. Henningsen and Boynton (1969) irradiated the dark grown seedlings of Hordeum vulgare with red (650 nm.) and blue (450 nm.) monochromatic lights and found that transformation and conversion of the prolamellar body occurred under blue light at an intensity which converted all of protochlorophyllide; however, the structural changes were much slower

(21%) than with either red (59%) or white (69%) light.

Blue light effects.

For complete differentiation of the chloroplast and the synthesis of chlorophyll, blue light has been shown to be necessary in the following examples: excised roots of Triticum (Björn, 1967); starch-containing plastids in the parenchyma cells of the tuber discs of Solanum tuberosum (Berger and Bergmann, 1967); excised roots of Pisum sativum (Richter, 1969); excised root of cucumber and Pisum sativum (Björn and Odhelius, 1966); a cell clone derived from wound callus of Nicotiana tabacum Bergmann and Berger, 1966). Red light is not effective in the above cases, except for the roots of Triticum,where red light enhances, but does not replace, the blue-light effect. In excised roots of Solanum tuberosum, on the other hand, either blue or red light results in the synthesis of chlorophyll (although less than with white light), although blue light is comparatively more effective (Bajaj and McAllan, 1969).

In cell cultures of Nicotiana tabacum (Bergmann and Berger, 1966) and tuber discs of Solanum tuberosum (Berger and Bergmann, 1967) raised in the dark, greening under blue light promotes the formation of photosynthetically active chloroplasts which contain grana similar to those under white light. Red light, however, was no more effective than darkness, that is, it did not evoke chloroplast formation. In 14-18 day etiolated plants of Phaseolus vulgaris, Henningsen (1967) recorded a specific action on the part of blue light for the second light-dependant stage of chloroplast formation (the stage of vesicle dispersion according to von Wettstein). However, no differences were detected in the formation of chloroplast fine structure under red and blue light in 4-14 day etiolated seedlings of Zea mays greening under light of different spectral composition for periods of 20 (Shakhov and Balaur,

1969) or 24 hours (Farineau, 1968). Vlasova et al. (1971) concluded that blue light has a specific activating effect on the formation of lamellar structure of the chloroplasts in greening plants and that blue light also results in more numerous grana in the chloroplasts than treatment under red light. When 5-6 day etiolated sedlings of Pisum sativum were exposed to the action of red and blue light for periods of 4, 8, and 14 hours, formation of grana was faster under blue light in all cases. Accelerated granum formation under blue light was correlated with blue-light activation of protein and chlorophyll synthesis. Vlasova et al. (1971) later also concluded that precisely the quality of light (not its dose) determined the specific features of blue-light action on granum formation in the greening chloroplasts. They hypothesized that red and blue light act via different photoreceptors. They also reported that after 8 hours and especially 14 hours exposure to blue light, the number of chloroplasts containing prolamellar body turns out to be slightly higher than after exposure to red light for the same period of time. Shakhov and Balaur (1969) found prolamellar bodies in the chloroplasts of Zea mays after 30 hours exposure to the etiolated plants to blue light (but not after exposure to red). The high chlorophyll content under blue light was correlated with intensified protein synthesis and considerable numbers of prolamellar bodies under these conditions. It was also assumed that increase in the number of prolamellar bodies under blue light is a result of their de novo formation (Vlasova et al., 1971). Bradbeer et al. (1974a) however, noticed that in the primary leaves of dark-grown Phaseolus vulgaris, the prolamellar bodies retain their crystalline form, at least up to 30 days of dark, when senescence and death occur. Thus, they did not observe irradiation-induced prolamellar body crystallization in this plant and suggested a possibility that irradiation at an early stage of etioplast development might increase such crystallization.

Röbbelen (1968) has reported chloroplast development in the leaves of a mutant of Aradiopsis. Chloroplasts do not develop normally in this mutant. Blue light is, however, more effective in the formation of normal chloroplasts and red light is more effective in production of the abnormal mutant plastids. In the wild type, the chloroplast development is induced by red light.

Since the development of chloroplast is not wholly controlled by phytochrome, it is evident that the process requires both phytochrome and other photoreceptors. Björn (1967) has suggested from the action spectrum for the complete chloroplast transformation, that the photo - receptor may be a carotenoid or a flavoprotein, probably the latter.

(B) Light Intensity.

Little is known regarding the influence of light intensities on the fine structure of the chloroplast of developing and mature leaves of normal plants and the knowledge of such influence on the chloroplasts of the developing and mature embryo is negligible. The available data (published electron micrographs of chloroplasts) from plants growing under different light intensities show that at higher light intensities there appears to be less lamellae per chloroplast and a smaller proportion of lamellae occurs in the grana.

Bjorkman and Holmgren (1963) (using light microscope) reported that a higher light intensity destroyed the chloroplasts of shade ecotypes of Solidago virgaurea, whereas chloroplasts from exposed ecotypes were unaffected. They have shown that the photosynthetic capacity of a leaf may alter when it is transferred from one light intensity to another. The effect of light intensity on the fine structure of chloroplasts from pigment-deficient mutants of Helianthus annus (Walles, 1965), Nicotiana

tabacum (Schmid et al., 1966) and Lespedeza procumbens (Clewell and Schmid, 1969) was studied. Chloroplasts with no grana or those with rudimentary grana have been reported in mutants of Helianthus annus (Walles, 1965) and of Lespedeza procumbens (Clewell and Schmid, 1969) and in the seedlings of Zea mays grown under 4500 f.c. (48150 lux) (McWilliam and Naylor, 1967). Ballantine and Forde (1970) have found in the leaves of Glycine max grown in controlled environmental cabinet, that under higher light intensity (22 $mWcm^{-2}$ irradiation) their chloroplasts had very rudimentary or small grana, while under lower light intensity (9 $mWcm^{-2}$ irradiation) had chloroplasts with well formed grana. They found a striking contrast between the abundance of grana in leaves which developed under low light intensity, and the paucity of grana in leaves from light intensities similar to those outdoors and they noticed good agreement between chloroplast fine structure of cabinet-grown plants and those grown outdoors or in the glasshouse. They also noticed that chloroplasts from high temperature (27.5-22.5 ^{o} C) and high light had grana consisting of two or three appressed lamellae, while grana from lower temperature (20.0-12.5 ^{o} C) were confined to occasional lamellae overlap. Björkman et al. (1972) also reported similar findings in Atriplex patula grown under 20, 6.3, and 2 $mWcm^{-2}$ irradiations. In Glycine max (Ballantine and Forde, 1970), the number of chloroplasts in a leaf cross section was approximately the same at each light intensity but in Atriplex patula (Björkman et al., 1972) more chloroplasts were noted with increasing light intensity (in the light micrographs). Homann and Schmid (1967) reported that grana-less chloroplasts of a pigment-deficient mutant of Nicotiana tabacum with single, unfolded thylakoids were unable to carry out photosystem-II though they possessed photosystem-I activity. According to them a complete photosynthetic electron-transfer system required a close packing of at least two lamellae.

Plants grown in low light intensities show well developed chloroplasts with large grana. The extreme development of chloroplasts in plants grown in low light intensities has been reported in the deep shade plant Alocasia macrorrhiza (Anderson et al., 1973), which receives an average irradiation of 0.1 $mWcm^{-2}$ and possesses massive chloroplasts, each with a large amount of lamellae and very large grana stacks having high ratio of total length of grana to stroma. Chloroplasts of such plants contain more chlorophyll per chloroplast, an increased proportion of chlorophyll b to chlorophyll a and a big increase in the amount of grana lamellae per chloroplast as compared for example, with Atriplex patula (Björkman et al., 1972) or Phaseolus vulgaris (Ballantine and Forde, 1970). The extent of grana formation appears to be related to the total chlorophyll content of the chloroplast. Grana formation may simply be a means of achieving a higher density of light-harvesting assemblies and hence a more efficient collection of light quanta. The high chlorophyll content of the shade plant chloroplasts is no doubt a consequence of the need for these plants to capture all available light quanta reaching the leaves on the floor of the rain-forest. The shade plants are also enriched in chlorophyll b relative to chlorophyll a, as compared with sun species. This increases further the light-harvesting capabilities of the shade plant by extending the wavelength range over which quanta are absorbed (Anderson et al., 1973).

Bradbeer and Montes (1976) made a quantitative determination of the amounts of lamellae in 14-day-old dark grown leaves of Zea mays under three different light intensities: 5 (high light), 1.6 (intermediate light) and 0.16 $mWcm^{-2}$ (low light). They found an increase in the amount of lamellae per leaf section of chloroplasts with the increase in irradiation from low (0%) to intermediate (56%) to high intensity (65%).

Weier et al. (1967) unexpectedly found that low light intensity of even less than 100 f.c. (1070 lux) was able to bring changes in the

prolamellar body of 8-day dark-grown leaves of Phaseolus vulgaris. Weier et al. (1970) used the same low light intensity of 100 f.c. (1070 lux) and have described the effect on the fine structure of 8-day dark-grown leaves of Phaseolus vulgaris. In another experiment Weier and Brown (1970) have used fluorescent light of lower intensity ranging from 40-50 f.c. (500 lux approximately) to study the etioplast development in 7-8 days dark-grown leaves of Phaseolus vulgaris. In these experiments a time range from 20 seconds to 12 hours of irradiation was used. Bradbeer et al.(1974b) obtained similar results with their experiments on 14-day old dark-grown leaves of Phaseolus vulgaris and their interpretations were also similar to those of Weier et al(1970) except in one experiment where they used higher light intensity of 505 f.c. (5500 lux). While studying the ultrastructural morphology and ontogeny of the coleoptile of Avena sativa, Hinchman (1972) planted 18 hours dark cold treated seeds and harvested after 24, 48, 72 hours of planting and treated them with 720 f.c. (7704 lux) irradiation. This exposure to light was given in environmental chambers under fluorescent (standard cool white) and incandescent lamps with a wattage ratio of 2.2:1. He found that at 48 and 72 hours the coleoptile chloroplast and etioplasts were conspicuously different from the corresponding leaf plastids in the morphology and ontogeny but contained typical grana and prolamellar bodies. A unique chloroplast developed in the subepidermal ring of small parenchyma cell in the coleoptile after 24 hours in the light and coleoptile turned pale green in colour. Boardman et al. (1970) irradiated 10-15 days dark grown leaves of Phaseolus vulgaris and 8-days dark-grown leaves of Pisum sativum with Philips Daylight 40 W fluorescent tubes (800 f.c. (8560 lux) at level of plant), during their chloroplast development. They reported that the typical plastid, when greened for four hours under white light shows the fusion of vesicles to form lamellae and by 8 hours in white light extensive grana

formation takes place. Their data suggest that grana formation occurs between 4 and 8 hours of greening. Stetler and Laetsch (1969) placed 10 cm. stem segments of light grown plants of Nicotiana tabacum in the dark in a flask containing 20 ml. of Hoagland's solution. Etiolated shoots thus produced were then placed in white light (warm white fluorescent tube) at an intensity of 800 f.c. (8560 lux) at the tissue level, for the study of fine structural changes during chloroplast development in light grown plants and in etiolated tissue exposed to light. They found a single membrane-bound body in developing plastids of both light- and dark-grown tissue which in mature chloroplast contained a fibrillar network. They reported the presence of prolamellar body even in moderately well developed chloroplasts in light-grown plants and frequently a prolamellar body found connected to the membrane bound body as well as to the grana. They have also reported many dividing mature chloroplasts as well.

Boasson et al. (1972) have reported that changes in plastid structure during greening is accompanied by plastid replication, and thus for a fully quantitative study of membrane formation during greening requires the measurements of both plastid fine structure and plastid numbers. They irradiated etioplasts of dark-grown leaves of Nicotiana tabacum in a light intensity of 30 lu/mm^2 (provided by cool white fluorescent tubes with neutral density filters) for different periods ranging from 8 hours to 144 hours. They reported the loss of the paracrystalline structure of the prolamellar body after the dark-grown leaves had received 8 hours of white light and found an increase in number of plastid (after 24 hours of white light). Plastid division were recorded from 32 hours white light exposed tissue and continued for 96 hours. Chloroplasts in tissues which had received 144 hours light are mature having numerous well-developed grana and they ceased to replicate.

Another peculiar feature of plants growing under low intensities has been reported in recent years. Montes and Bradbeer (1976) reported peculiar chloroplasts which develop deep pockets containing mitochondria in the bundle sheath of Zea mays and in mesophyll of Hyptis suaveolens. They found mitochondria in deep invaginations, present near the periphery of the chloroplast. Such pockets were observed only when these plants were grown under low light intensities, 0.16 $mWcm^{-2}$ for Zea mays and 0.3 $mWcm^{-2}$ for Hyptis suaveolens. Mitochondria disappeared from the pockets when Zea mays plant was transferred to higher light intensity, (5 $mWcm^{-2}$). However, similar feature was first noticed by Ballantine and Forde (1970) in the leaves of Phaseolus vulgaris which had been grown under a low intensity diurnal illumination of 9$mWcm^{-2}$. They have reported the presence of mitochondria-containing invaginations in the chloroplasts. Wildman et al. (1974) have reported in 72 hour dark-treated leaves in Nicotiana excelsior (which also lost starch) a vigourous movement of mitochondria as a result of protoplasmic streaming. After 30 minutes illumination at 400 f.c. (4280 lux) in a few cases, mitochondria had become circular and were located beneath the concavity of a chloroplast.

OBSERVATIONS AND DISCUSSION.

1. Plastids in the leaf of light-grown and dark-grown seedlings.

To provide a basis for comparison with the chloroplasts of the embryos, the normal chloroplast structure in the light-grown leaf of Capsella bursa-pastoris was studied. The leaf chloroplast is compared also with the chloroplasts described from leaves of other species of plants.

An investigation of etioplast formation in darkened leaves, the prolamellar bodies, and their dispersal on subsequent illumination was also attempted. It will be shown, however, that formation of etioplasts does not occur in young leaves in the manner described for the commonly investigated species.

The chloroplasts in the young photosynthetic leaf grown in continuous light under controlled conditions are large, and possess the normal grana-fretwork system comprising the internal membrane system with lamellae stacked into large grana interconnected with intergrana (fret) membranes which are quite long in some chloroplasts. The stroma comprising the internal matrix material has a granular appearance, rather evenly distributed, presumably due to Fraction 1 protein which is the major constituent of proteinaceous stroma (Moyse, 1967). Most of the chloroplasts are very long (7 μm in length), appearing bi-convex, plano-convex or concavo-convex, in form and are surrounded by a 2-membraned envelope as usual. The stroma of these chloroplasts does not contain any starch, but contains about 10 to 20 densely staining osmiophilic plastoglobuli. The occurrence of plastoglobuli in the stroma of plastids during the development of the lamellar system has

also been reported for other species (Thompson, 1974), so that they are not always regarded as a disintegration product of lamellar structures as discussed in part I of the present thesis. Dividing chloroplasts by the formation of a constriction is also seen (Fig. 45). A group of membranes showing a complex arrangement not previously described in the literature, the exact morphology of which is unknown, is also seen in occasional electron micrographs (Fig. 46).

Plastid development in dark grown leaf.

Small seedlings grown in continuous light in the growth cabinet were kept in the dark for up to 8 days. The development of the plastid was followed from 4-days in dark up to 8th day when this extended dark period resulted in plastid senescence.

i) 4 days in dark

The fine structure of the plastids in 4-day dark grown leaf is similar to those of the light grown leaf showing normal grana-fretwork system with very well developed grana having maximum stack up to 20. Very long fret membranes connect the 2 grana especially near the inner membrane of the plastid envelope (Fig. 48). Plastids show various degrees of curvature of grana-fretwork system (Fig. 47). In profile, some are similar to the plastids of the light grown leaf, while in most of them the grana-fretwork system appears arc-shaped or deep arc-shaped, until it becomes 'U' shaped (Fig. 48) and 'S' shaped (Fig. 49). In most cases the double membraned plastid envelope has extended on one side resulting in a large space between the grana-fretwork system and the extended plastid envelope (Fig. 49). This space is filled with stroma which has a granular appearance and may be called 'stroma space'.

Densely stained plastoglobuli exist in the stroma as well as attached to the lamellae. No starch grain is seen in the plastids of either the

ordinary epidermal cells or the mesophyll cells, but the plastids in the guard cells are full of starch grains (Fig. 50).

The plastids from the 4-day dark grown leaf when given a 5 minutes exposure to white light show a group of electron opaque particles in the short stroma space in each plastid which are possibly phytoferritin, an iron-protein compound (Hyde et al., 1973). This intrinsic electron opacity is apparently due to the higher concentration of iron in the particles. This phytoferritin in the stroma space exists in crystalline arrays consisting of particles aligned in parallel, curved or straight rows, as a more irregular paracrystalline aggregation (Fig. 51). In some plastids phytoferritin exists as a cluster of particles. The plastids of the guard cells are full of starch grains (Fig. 52) which are more numerous (up to 6 in a plastid) and larger in size (1.5 μm long) than the starch grains of the guard cells of the 4-day dark grown leaves (1.0 μm long). No other plastids contain any starch grain but they do contain densely stained osmiophilic plastoglobuli.

ii) 5 days in dark

The plastids of the 5-day dark grown leaf show the start of the development of one or two stroma lamellae, which are irregularly-shaped and ring-shaped in the large or small stroma space (Fig. 53). In other plastids few grana and intergrana lamellae have developed in the stroma space (Fig. 54). Some plastids show the formation of small vesicles or loops formed by the incurving of a fret membrane to join again at the other end of the granum. Other plastids show only bladder-like protrusions of flat lamellae (Fig. 54). Plastids, which in this profile view appear as oblong or biconvex, have no starch grains but have a few small

densely stained osmiophilic plastoglobuli. An occasional plastid shows a small beaded lamella (Fig. 54). Possibly some sort of protusions of flat lamellae may appear as small beads or rings in cross section.

iii) 6 days in dark

The plastids of 6-day dark grown leaf show the development of many more stroma lamellae in the stroma (Fig. 55) than the plastids of 5-day dark grown leaf. The lamellae arise irregularly in the stroma forming vesicles, hooks and loops, either by curving inward (Fig. 55) and/or joining the other end of granum (Fig. 56). In some plastids transverse interconnections between two grana are also seen. Few bridge-like connections between lamellae are also seen (Fig. 56). The lamellae forming bridge-like connections turn by about 90^{o} above and below the respective juncture. These connections between lamellae reveal a characteristic alternation in the course of lamellar direction. In one of the plastids which show "top" view of the grana, a small network is seen in the stroma close to one of the grana (Fig. 55).

iv) 7 days in dark

The plastids of 7-day dark grown leaf are characterized by the extensive development of stroma lamellae, possibly as a result of the formation of lamellar sheets by the inner limiting membrane. Different stages of the formation of these irregularly-shaped stroma lamellae are seen in different plastids (Fig. 57). Some plastids show the very early stage (beginning stage) of the formation of a loop and vesicles from the fret membrane arising from the granum (Fig. 59), while other plastids show an extensive develpment of a fret membrane arising from the granum to an extent that it forms a 'U'-shaped structure through a deep invagination in the middle(Fig. 60). The formation of a parallel sheet of lamellae by the inner membrane of the plastid envelope is also seen in Fig. 59 with a small network joining together in the stroma.

Groups of membrane showing a complex arrangement, the exact morphology of which is not known, but also observed in the light grown leaf

(Fig. 46) are also seen lying near the plastids (Fig. 57). Some of these extensively developed stroma lamellae lie parallel to the inner limiting membrane and divide the interior of the plastid into bowl-shaped regions (Fig. 61). In most plastids these lamellae form loops, hooks, vesicles and bladder-like protrusions in places but some plastids do not show the formation of such structures (Fig. 58). Many inter-connections formed by the fret lamellae either between a bridge-like connection and a granum or between two grana lying side by side are seen in which case the connecting fret lamellae appears arc-like (Fig. 58).

Several of the stroma lamellae join laterally or terminally in the stroma resulting in bridge-like connections which are variously shaped and slightly more densely stained than the stroma. Such lamellae forming bridge-like connections between them usually turn by about 90^0 above and below the respective juncture and bridge-like connections get variously shaped. The resultant junctures are mostly hexagonal, star-shaped or triangular or irregularly-shaped depending upon the number and mode of stroma lamellae joining to form that juncture (Fig. 64). Some plastids also show one small beaded lamella interconnecting the 2 grana (Fig. 58) which appear possibly as a result of cross section of some protrusions of flat lamellae. The stroma lamellae develop so extensively in certain plastids that, along with the loops or vesicles formed by the fret membranes of grana, they push the grana-fretwork system towards the one side of the inner limiting membrane of plastid envelope (Fig. 64). As a result of this, in some plastids, only a few grana and intergrana lamellae are present near the inner limiting membrane of the plastid envelope and all the rest of the space in the plastid is occupied by the loops and vesicles and rings formed from the stroma lamellae (Fig. 63). A small network is seen in Fig. 63 near to the inner limiting membrane in between two grana. A similar group of cells is also seen in another plastid of the same electron micrograph and possibly both groups consist-

ing of more or less hexagonal cells are the cross section of long cells.

A group of small hexagonal cells, possibly tubules (10-20) arranged either regularly in 2-3 rows (Fig. 62), or irregularly, is seen cut in various planes in the stroma of plastids at a point where several stroma lamellae meet. Usually one such group of hexagonal cells is present in that part of most plastids dominated by stroma lamellae. This appears to be a rudimentary form of prolamellar body. Prolamellar body formation has not been observed even in these plastids of leaf kept in dark for 7 days. However an occasional plastid shows a non-crystalline form of prolamellar body and these show less lamellar structure (Fig. 65). Other electron micrograph shows a very rudimentary form of prolamellar body like structure but such plastids contain a very well developed grana-fretwork system and stroma lamellae (Fig. 66).

Most of the plastids at this stage are filled with an irregularly shaped lamellar system, mostly consisting of ring-shaped lamellae and several short hollow cylinders of lamellae. Some lamellae fuse in the formation of these hollow cylinders. Several bridge-like connections can be seen in each plastid. The grana-fretwork system is very scanty. The appearance of irregularly-shaped lamellar system which starts in the plastids of 5-day dark grown leaf reaches its maximum development at this stage and the appearance of this system probably indicates the degree of senescence experienced by the plastids rather than a stage towards the formation of a prolamellar body.

v) 8 days in dark

The plastids of the 8-day dark grown leaf are full of large densely stained osmiophilic plastoglobuli probably resulting from the collapse of the lamellar structure. The formation of small vacuoles in the stroma region is followed by the collapse of lamellar structure. The lamellae get deeply curved, concentrically or irregularly arranged (Fig. 67).

Various stages of lamellar collapse can be seen in which the plastids are also considerably shrunken. Some plastids also show disintegration of the plastid lamellae leading in turn to an accumulation of dense plastoglobuli in the stroma. A group of electron opaque particles, which is possibly phytoferritin, is also seen in some of these senescing plastids.

Comments

The chloroplasts in the leaf are very long with larger grana and very long fret membranes as compared to those of mature green embryos where they are mostly biconvex and contain large starch grains. Starch grains were not seen in the chloroplasts of the leaf except in the guard cells.

The typical prolamellar body, which has been interpreted as a symmetrical crystal lattice whose basic structural unit is a "six pointed star" (star module) with four tubules fusing at each of the nodes (Weier and Brown, 1970) is not formed even in the 7-day dark grown leaves, while in young embryos of intact plants, the prolamellar body formation in the etioplasts occurs even after 3 days in dark as described in the next section. Several dark periods were tried but only a small non-crystalline type of a prolamellar body is seen in occasional plastids of 7-day dark grown leaves or a small network (10-20) also loosely arranged in two rows can be seen in a few plastids representing a rudimentary prolamellar body.

Gunning (May 1977, personal communication) has, however, commented on our observations as follows. If a seedling is germinated in the light and then transferred to darkness, the chloroplasts that were in existence at the time of transfer are not expected to develop prolamellar bodies. If the seedlings are either i) sufficiently healthy to continue growing (utilising food reserves) then the new growth might contain etioplasts, or ii) if the existing green tissue (leaves or cotyledons) is young enough to continue lamellar synthesis, then again prolamellar bodies might develop. We could thus explain the formation of only very rudimentary prolamellar bodies or no prolamellar body at all in the chloroplasts of the leaves of our young seedlings, which were germinated in the light and then transferred to the darkness. As the plastids

start showing senescence after a few days in the darkness, it is clear that the seedlings were not healthy enough to continue growing in darkness possibly either due to the inability of seedlings to utilise food reserves when transferred from light to darkness or due to certain other environmental factors not clearly known at present. Our observations show that the lamellar synthesis was probably stopped in the chloroplast as soon as they were transferred to the darkness and thus resulting in the formation of irregularly shaped lamellar system rather than the formation of prolamellar bodies. Further experiments with low light intensities or utilizing leaves in the extreme apical region might give some useful information.

An extensive development of stroma lamellae as a result of the formation of lamellar sheets by the inner limiting membrane of the plastids takes place in the leaves of dark grown young seedlings. This extensive development of an irregularly-shaped lamellar system of Capsella bursa-pastoris leaves, showing peculiarly interesting structures has rarely been reported earlier in the normal plastids of higher plants. However, Schötz et al. (1968) have reported a somewhat similar irregularly-shaped lamellar system in the chloroplasts of the Oenothera hybrids (lamarckiana x hookeri) as a result of a disharmony between genom and plastom. This disharmony between genom and plastom does not only prevent unspecifically the chloroplast differentiation but it may also lead to a shape of the lamellar system which is exceptional for higher plants but common in the cells of various Cyanophyceae. A characteristic ring-shaped, cup-shaped, bladder-like, or loop-like lamellae, or the lamellae forming large bowl-shaped regions, or the lamellae connecting together forming bridge-like connections in the plastid stroma of the dark grown leaves of Capsella bursa-pastoris are similar to those as has been reported in the plastids of Oenothera hybrids. Diers and Schötz (1969) used serial sections to elucidate the three-dimensional configuration of the lamellar system

which are the basis for ring-shaped lamellae often seen in the electron micrographs of plastids. They have shown that these configurations can be more or less deep, cup-shaped single lamellae or stacks of lamellae. The lamellae either occupy only the small parts of stroma space or sometimes they divide the interior of stroma space in large, bowl-shaped regions (Fig. 61). The bridge-like connections between lamellae as seen in most of the electron micrographs of the present investigation actually reveal a characteristic alteration in the course of the lamellar direction (Schötz et al., 1968); it turns by about 90° above and below the respective juncture. This seems to be a more generally occurring type of lamellar structure found in the dark-grown leaf plastid in the present investigation. Some short hollow cylinders of lamellae formed by the fusion of lamellar membranes in the chloroplasts similar to those observed in the present investigation (Figs. 63, 64) have also been reported (Diers and Schötz, 1969). Such exceptional lamellar system might have developed in the normal plastids of the leaves of normal dark growing seedlings of Capsella bursa-pastoris due to some disharmony resulting from an incipient senescence.

Laetsch and Stetler (1967) reported the formation of an extensive stroma lamellae in the 28 days dark cultures of Nicotiana tabacum somewhat similar to those observed in the present experiment. They have also reported the absence of prolamellar body in the etioplasts of these cultures similar to the present experiment. Clewell and Schmid (1969) have reported a chloroplast from a yellow plant of Lespedeza procumbens in which the secondary multiplication of thylakoids has produced what appears to be channels. Such channels have also been reported earlier in normal green leaves of Cassia obtusifolia (Homann, 1967) and Rhodopseudomonas palustris (Tauschel and Drews, 1967) in which a secondary lamellar multiplication has been reported. But none of these species show an extensive development of the abnormal lamellar

system.

The development of extensive stroma-space in the plastids of 4-day dark grown leaf of a young seedling is another feature which was not found in the plastids of embryo at any stage of development, either in light or in dark of the present investigation. Stroma space developed in the plastids of 4-day dark grown leaf resemble to some extent the intramembranaceous space formed by the moving apart of the outer and inner membrane of chloroplast envelope (Schötz and Diers, 1967) and filled with stroma-like substance. But the space produced in Capsella bursa-pastoris is not formed by separation of the two membranes of plastid envelope but by the extension of the plastid envelope as a whole. This extension of plastid envelope results in the formation of large space in the plastid which has been termed 'stroma-space' by us in the present investigation. This stroma space is filled with stroma like substance having granular appearance possibly due to Fraction 1 protein. The formation of stroma lamellae, which become more extensive with the increase of dark period, takes place in this stroma space of the plastids. The inner limiting membrane of the plastid envelope appears to be the starting point of differentiation of stroma lamellae which are later directed towards the relatively large stroma space of the plastids. There is however, little critical evidence on the ontogeny of this complex lamellar system.

This study has also shown that plastoglobuli exist in the stroma of young plastids of light grown leaves during the development of lamellar system. So the plastoglobuli are not always regarde as a disintegration product of lamellar structures. It seems that the formation of plastoglobuli is caused by metabolic imbalance between lipid synthesis and lamellar formation, and that they play a significant role in the storage of excessive lipid materials as has been discussed in detail in part I of the present thesis. The validity of such speculation may be support-

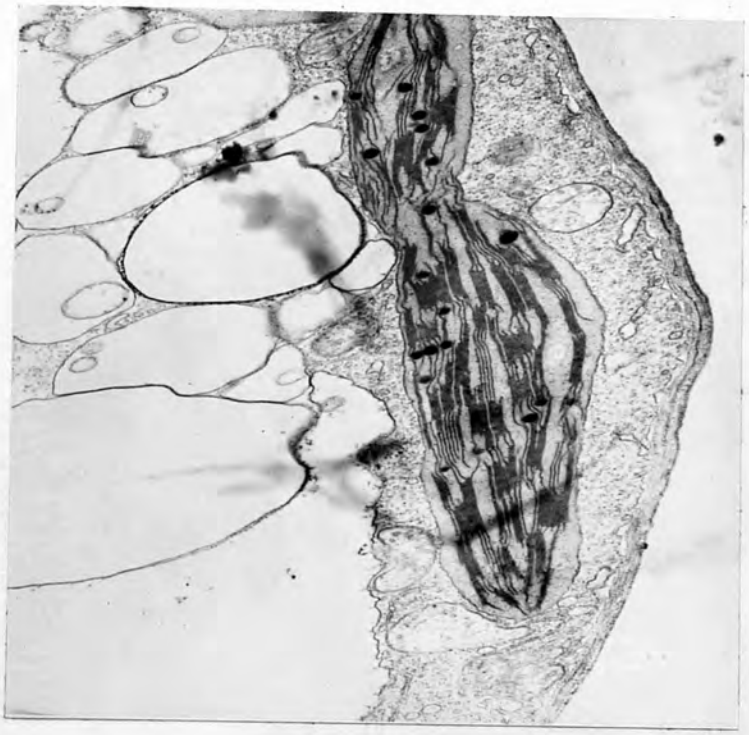
ed by the facts that the plastoglobuli also occur in plastids of seedlings grown in the dark, and that they decrease in number rapidly in light leading in turn to the formation of lamellar structure.

Granules interpreted as phytoferritin are found in the stroma of differentiating plastids in the leaves of Capsella bursa-pastoris but have not been seen in embryo plastids. Phytoferritin has been found most commonly in proplastids and differentiating plastids or in senescing chloroplasts and developing chromoplasts (Hyde et al., 1963). As phytoferritin occurs in proplastids and differentiating plastids and because in mature chloroplasts iron-containing compounds such as ferredoxin are essential for photosynthesis, Hyde et al. (1963) suggests that phytoferritin represents a reservoir of stored iron in the form of a non-toxic iron-containing protein. The phytoferritin particles usually appear electron opaque apparently due to high concentration of iron in the particles. The electron-opaque particles represents the iron-containing core and the electron-translucent region around the particles the proteinaceous shell (Robards and Robinson, 1968).

Plate 25. Chloroplast from a light-grown primary leaf.

Fig. 45. Section through the primary leaf of a young seedling grown in light. A dividing chloroplast showing a well developed grana and fret membranes and few osmiophilic plastoglobuli. x 19,500

Fig. 46. Section through the primary leaf of a young seedling grown in light showing a group of membranes with a complex arrangement. x 19,500



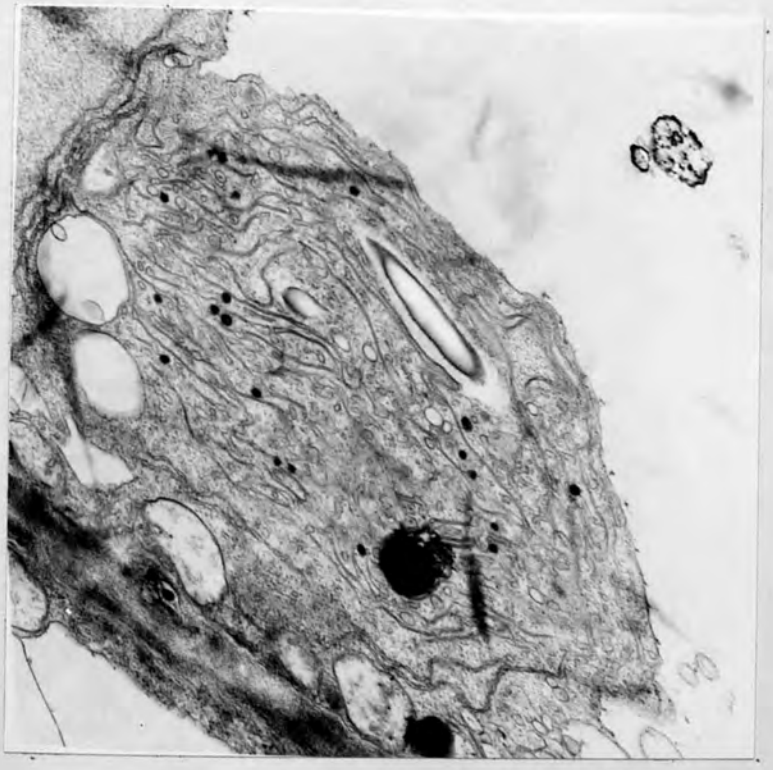


Plate 26. Shape and variations of grana and fret-work system in plastid from 4-day dark grown primary leaf.

Fig. 47. Section through the primary leaf of a light grown seedling transferred to dark for 4 days. A mesophyll cell showing general arrangement of plastids which show various shapes of grana fret-work system and a large stroma space. x 4,750

Fig. 48. A higher magnification of a plastid section from the same leaf material. The grana fret-work system appear'U'-shaped in which very long fret membranes are seen near to the inner membrane of plastid envelope. Few densely stained osmiophilic plastoglobuli are also seen. x 26,000

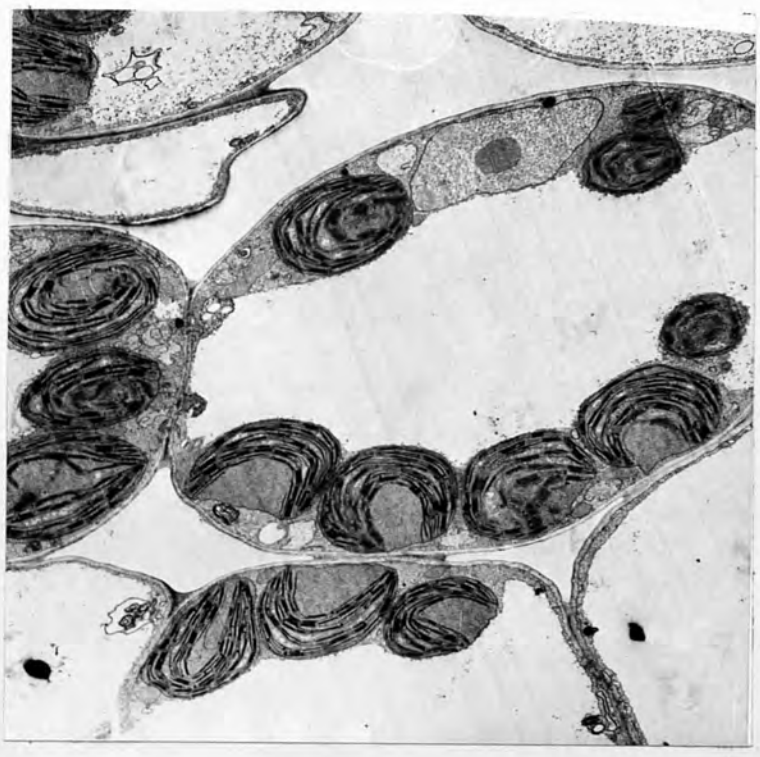
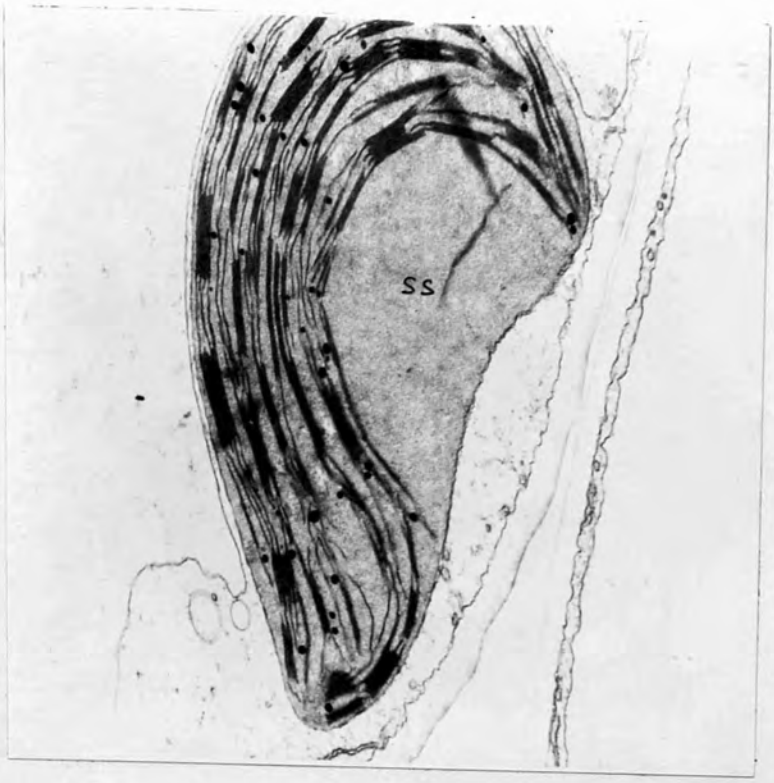


Plate 27. 'S' shaped grana fret-work system in plastid from a 4-day dark grown primary leaf.

Fig. 49. A plastid section of the primary leaf of a light grown seedling transferred to dark for 4 days. The grana fret-work system in this plastid assumes a 'S' shape resulting in a large stroma space (ss). x 19,500

Fig. 50. Section through the epidermal layer of primary leaf of light grown seedling transferred to dark for 4 days. The plastids containing starch are present in guard cells. x 6,500


SS

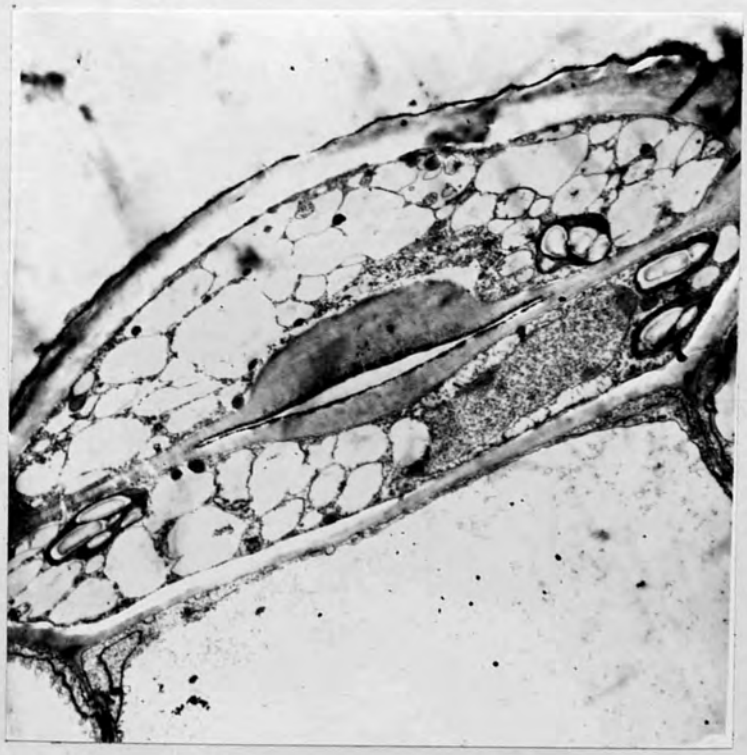
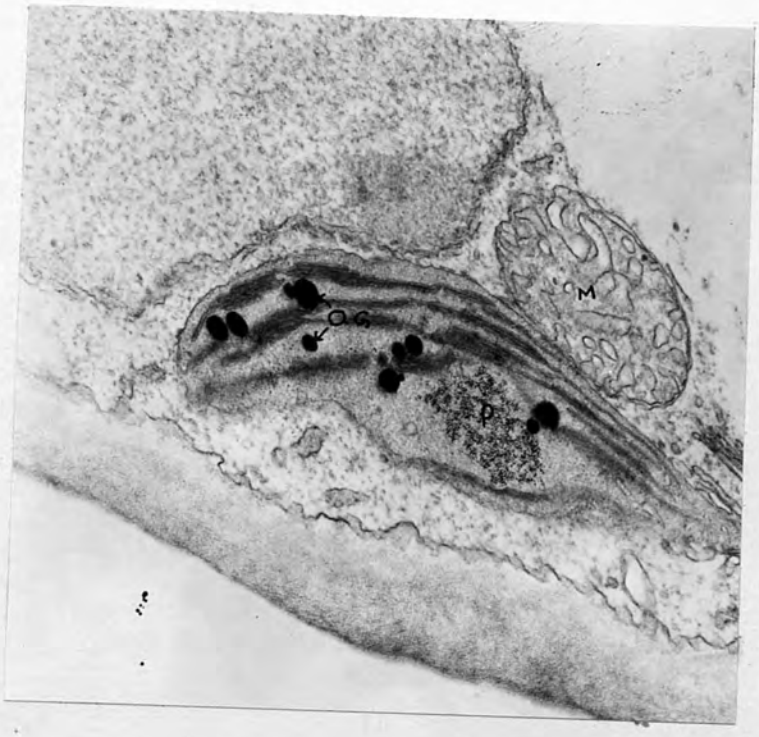


Plate 28. Plastids from 4-day dark grown leaf exposed to white light for 5 minutes.

Fig. 51. Section through the primary leaf of light grown seedling transferred to dark for 4 days and then exposed to white light for 5 minutes. Plastid shows paracrystalline form of phytoferritin (P) and large sized osmiophilic plastoglobuli (O.G.). x 32,500

Fig. 52. Section through the epidermal layer from the same leaf material as Fig. 51. Two guard cells show plastids completely filled with starch grains and few osmiophilic plastoglobuli. x 6,500


M
O.G.
P

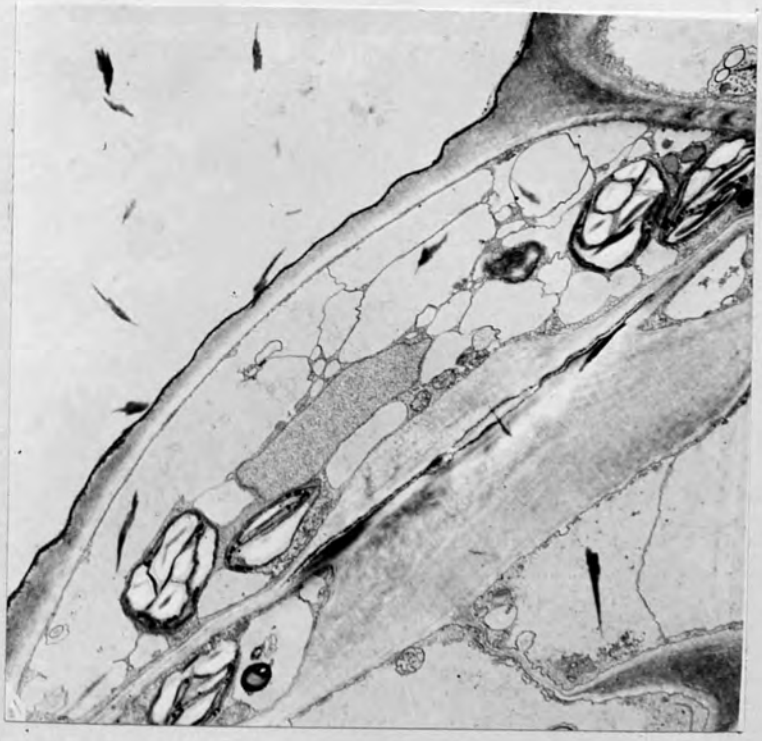
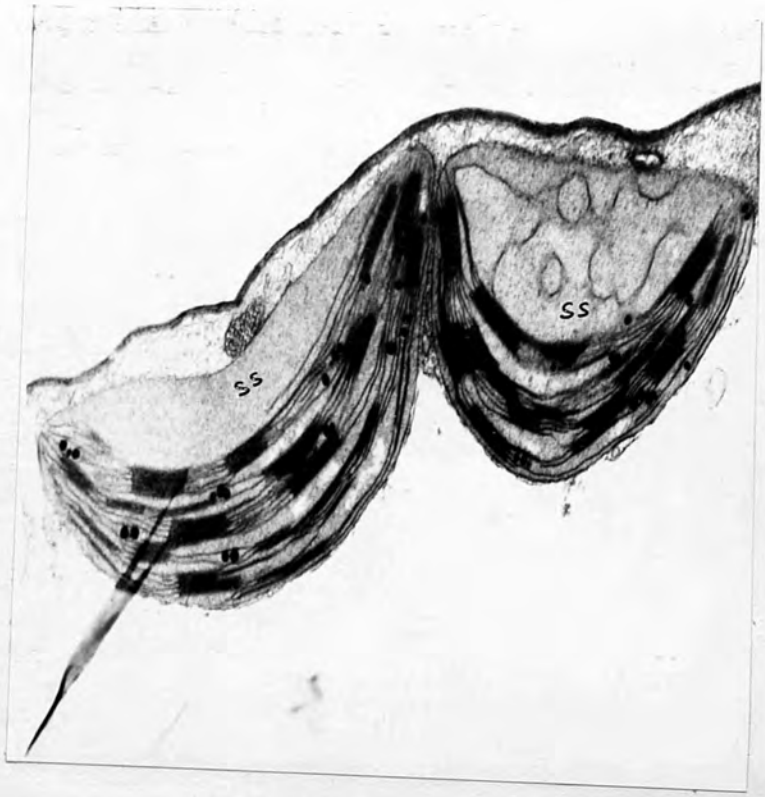


Plate 29. Plastids from 5-day dark grown primary leaf.

Fig. 53. Plastid section of the primary leaf of light grown seedling transferred to dark for 5 days. Irregular-shaped stroma lamellae start appearing in the stroma space (s.s.) at this stage. x 19,000

Fig. 54. Plastid section from the same leaf material as in Fig. 53. Plastid shows bladder-like protrusion (→) of flat lamellae, a beaded lamella (⇉) and the development of grana and intergrana lamellae in the stroma space. x 19,500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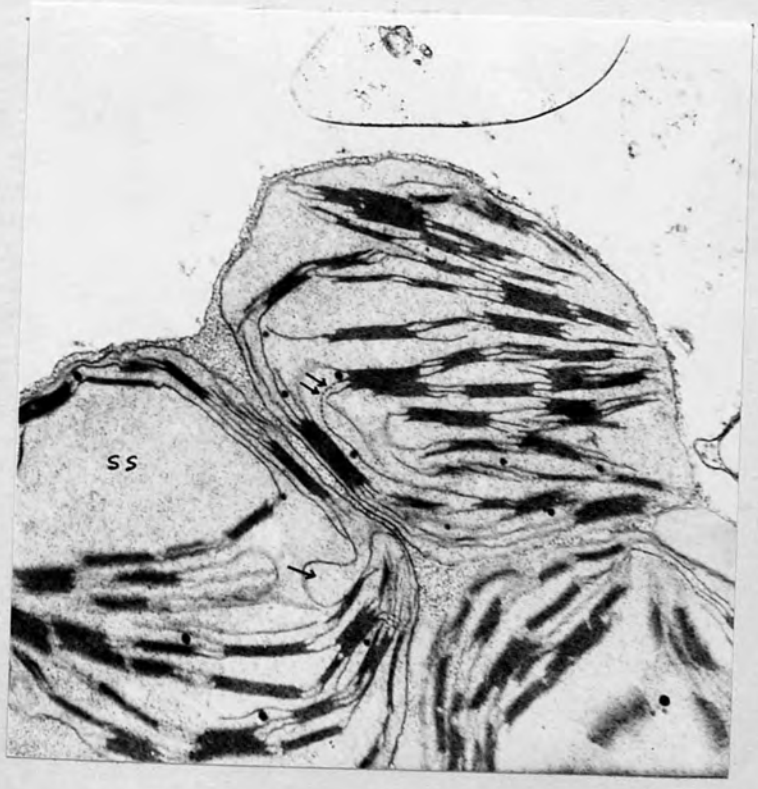
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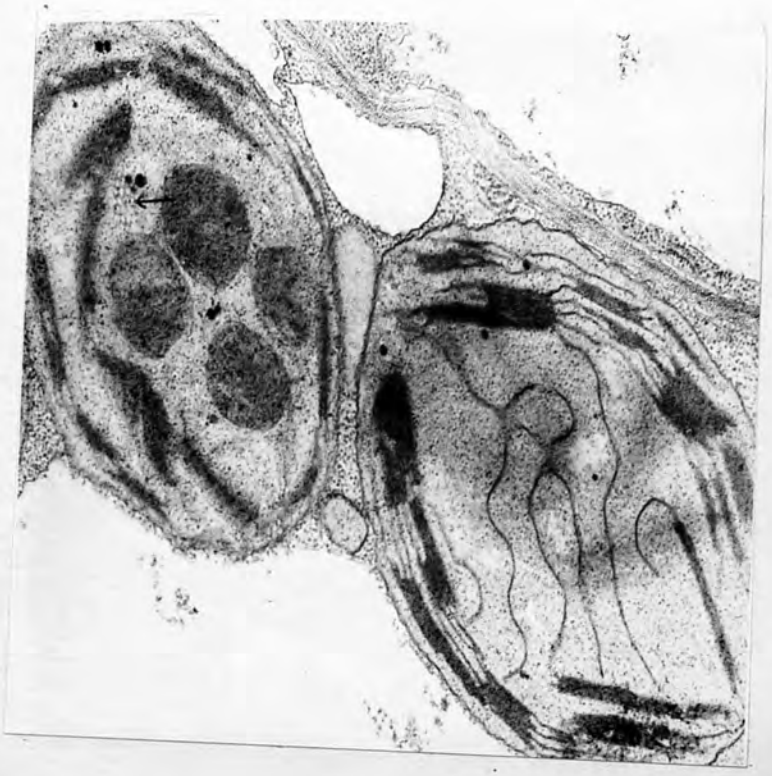
Plate 30. Plastids from 6-day dark grown primary leaf.

Fig. 55. Plastid section of the primary leaf of light grown seedling transferred to dark for 6 days. Plastid shows the formation of irregular lamellae showing various stages of "loops" formation. The adjacent plastid shows grana mostly in "top" view and a (→) (in cross section) joining one of such granum. x 28,500

Fig. 56. Plastid section from the same leaf material as in Fig. 55. Plastid shows vesicle (bladder)-like protrusion (→) of flat lamellae, bridge-like connections between lamellae. Adjacent plastid shows "top view" of grana and oblique view of fret membrane. x 26,000



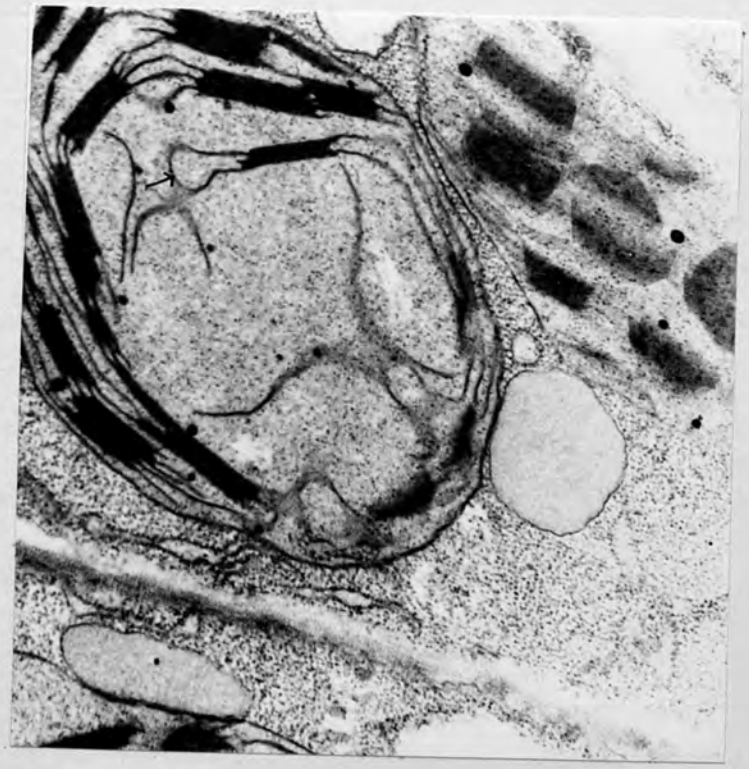
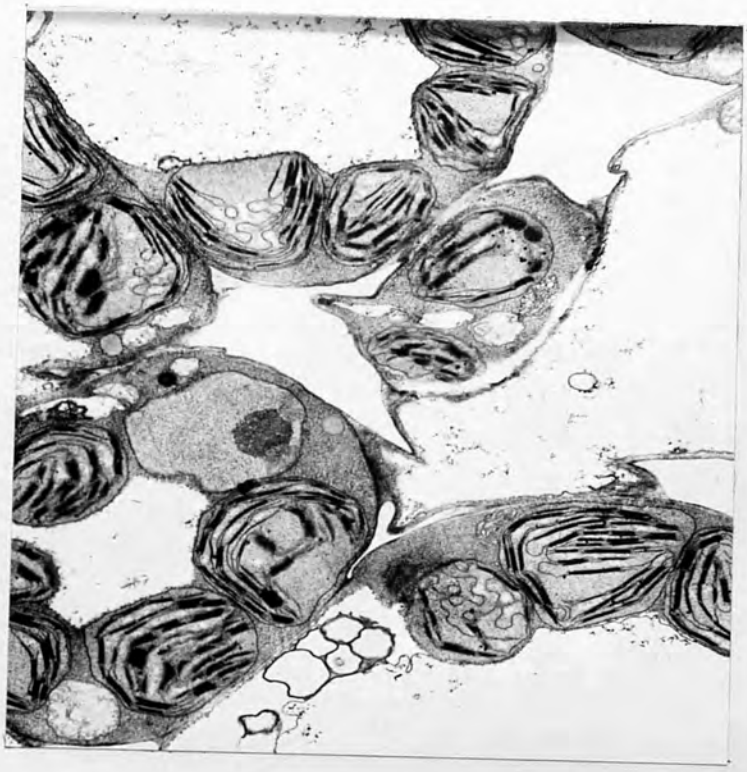


Plate 31. Irregularly-shaped stroma lamellae in plastids from 7-day dark grown primary leaf.

Fig. 57. Section through the primary leaf of light grown seedling transferred to dark for 7 days. Plastids show various stages of development of irregularly-shaped stroma lamellae. x 6,500

Fig. 58. Plastid section from the same leaf material as in Fig. 57. This plastid does not show any formation of loop or vesicle from the flat lamellae but instead flat membrane develop transverse connection between two grana. A small beaded lamella (→) connecting two grana and many bridge-like connections between lamellae are also seen. x 26,000



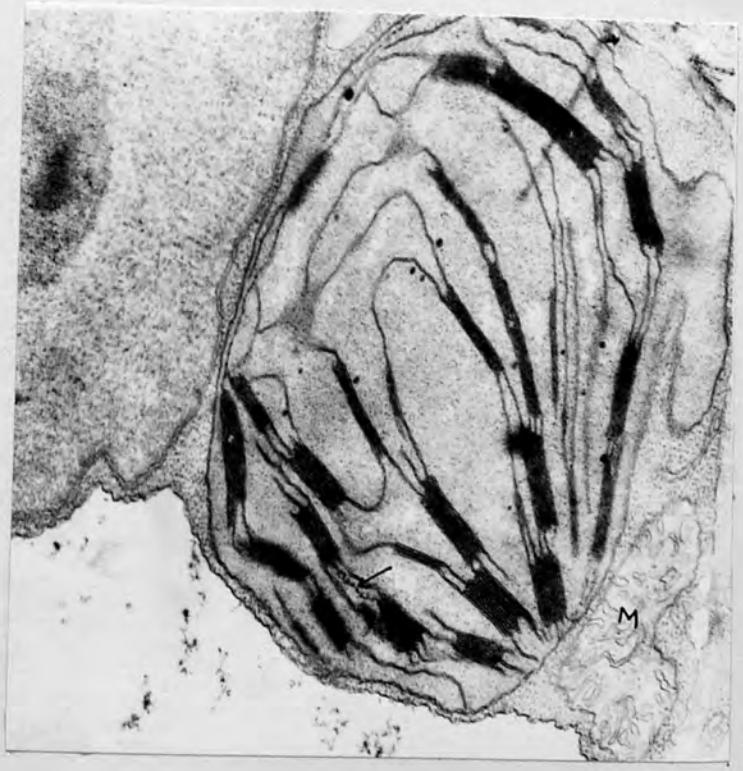

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Plate 32. Loops and vesicles in plastid from 7-day dark grown primary leaf.

Fig. 59. Plastid section of the primary leaf of light grown seedling transferred to dark for 7 days. Plastid shows the formation of a small network (cross sectioned) in the stroma and early stages of the formation of loops and vesicle-like protrusions of flat (fret) lamellae. x 32,500

Fig. 60. Plastid section from the same leaf material as in Fig. 59. Plastid shows the formation of a 'U'-shaped structure through a deep invagination in the middle of frat lamellae. Bridge-like connections and a vesicle-like protrusion of flat lamellae are also seen. x 52,000

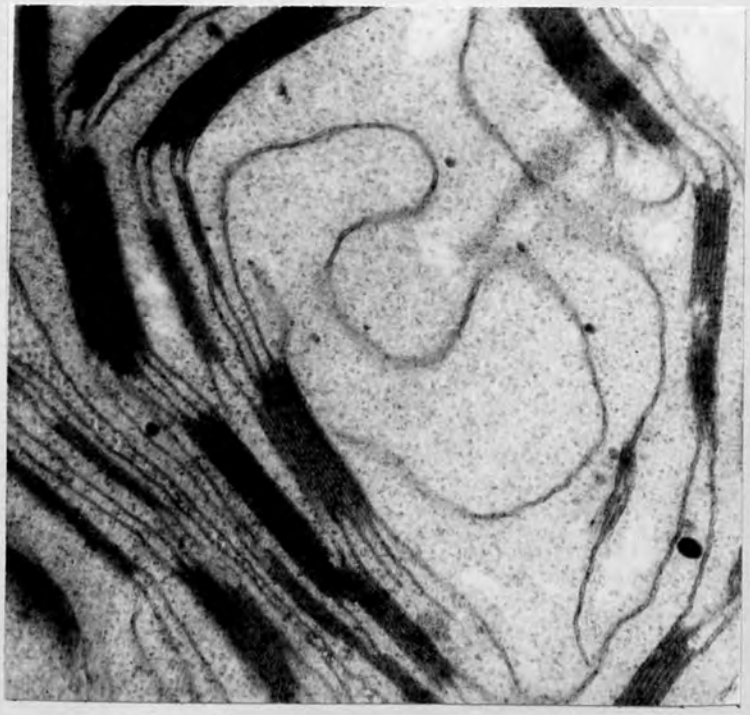
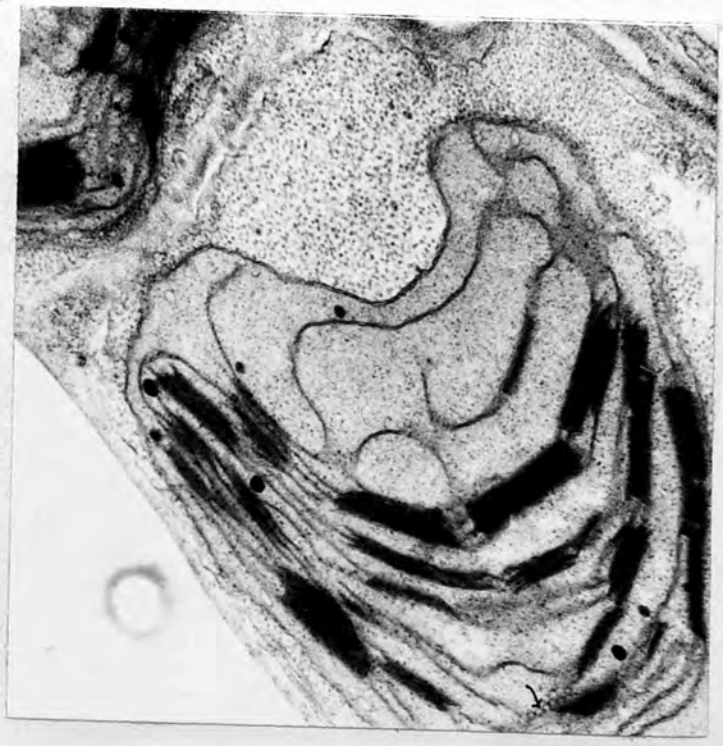


Plate 33. Bowl-shaped regions in plastids from 7-day dark grown primary leaf.

Fig. 61. Plastid section of the primary leaf of light grown seedling transferred to dark for 7 days. Some of the stroma lamellae divide some of the interior of plastid in bowl-shaped region. Plastid also shows a small network (in cross section) and bridge-like connections of stroma lamellae. x 32,500

Fig. 62. Plastid section from the same leaf material as in Fig. 61. Plastid shows a network (cross sectioned), "side" and "top" view of grana, system of fret membranes that interconnect the grana is better seen between grana in side view than between grana in top view where most of them are present in oblique or face view. x 39,000



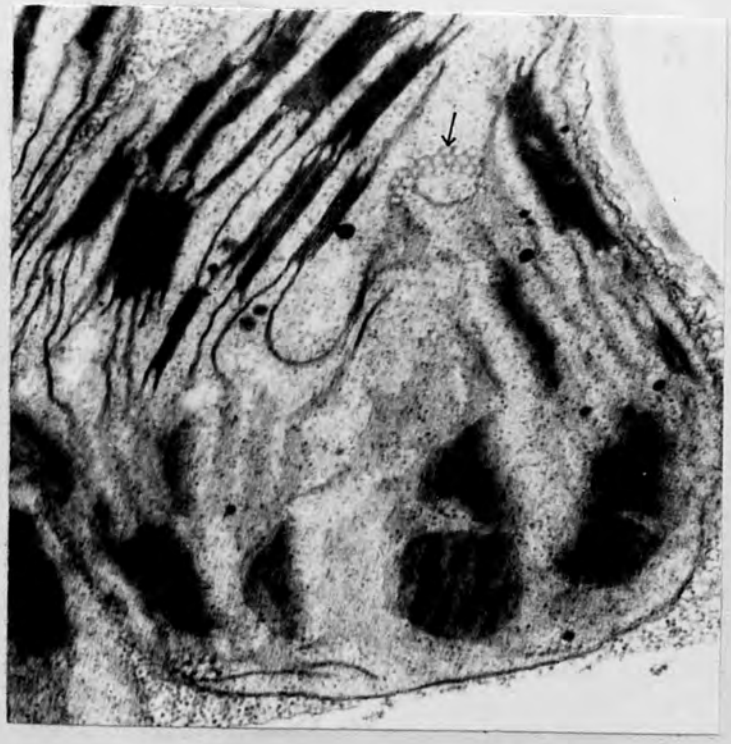
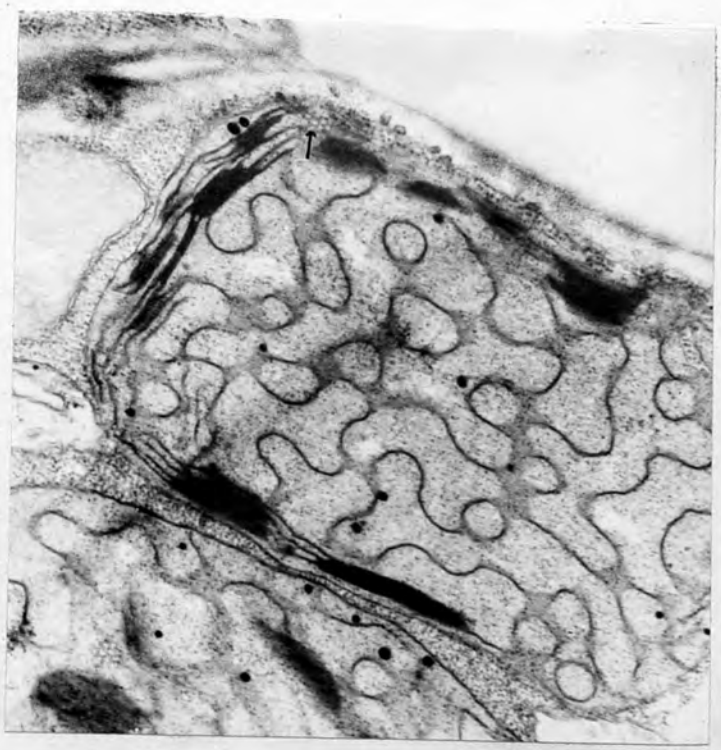


Plate 34. Extensively developed irregular-shaped and ring-shaped stroma lamellae in plastid from 7-day dark grown primary leaf.

Fig. 63. Plastid section of the primary leaf of light grown seedling transferred to dark for 7 days. Plastid shows an extensive development of irregularly-shaped and ring-shaped stroma lamellae forming bridge-like connections; the grana-fretwork system which is reduced to one layer lies close to the inner membrane of plastid envelope. Small networks are also seen (→). x 26,000

Fig. 64. Plastid section form the same leaf material as in Fig. 63. Plastid shows the shifting of grana-fretwork system on one side and extensive development of irregularly-shaped and ring-shaped stroma lamellae in the stroma forming bridge-like connections. x 39,000



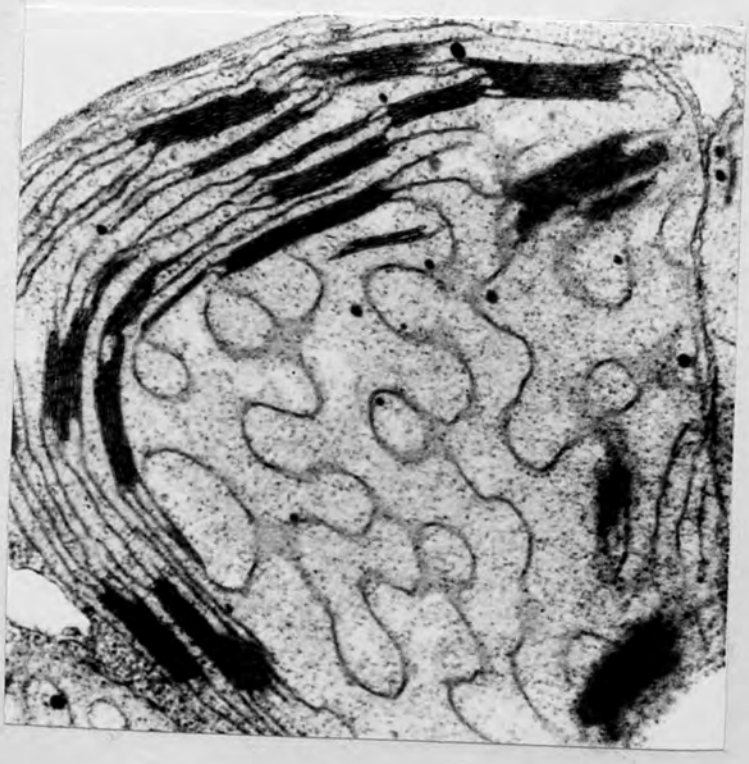
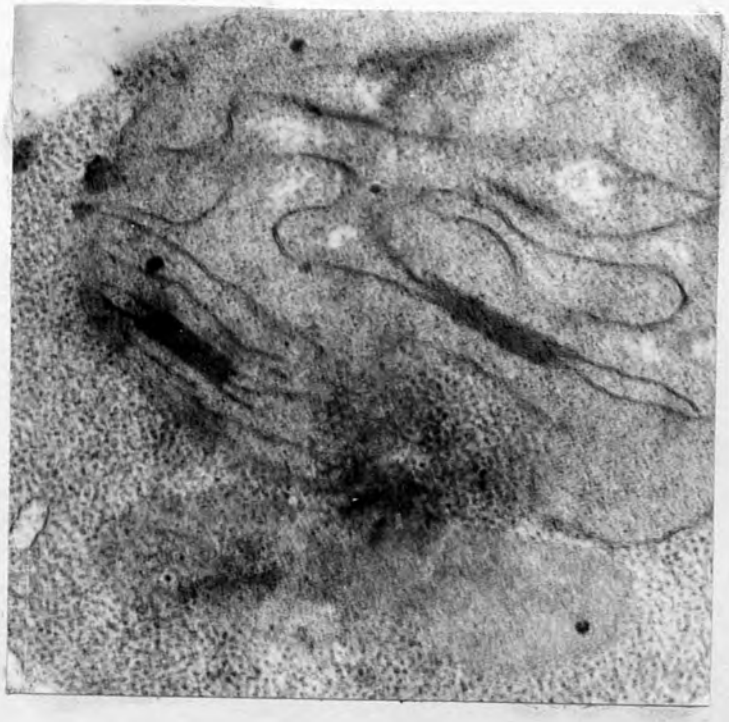


Plate 35. Prolamellar body in plastid of 7-day dark grown primary leaf.

Fig. 65. Plastid section of the primary leaf of light grown seedling transferred to dark for 7 days. A rudimentary prolamellar body and a few stroma lamellae with only two grana are seen. x 47,500

Fig. 66. Plastid section from the same leaf material as in Fig. 65. Plastid with rudimentary prolamellar body and well developed grana fret-work system and stroma lamellae. x 28,500



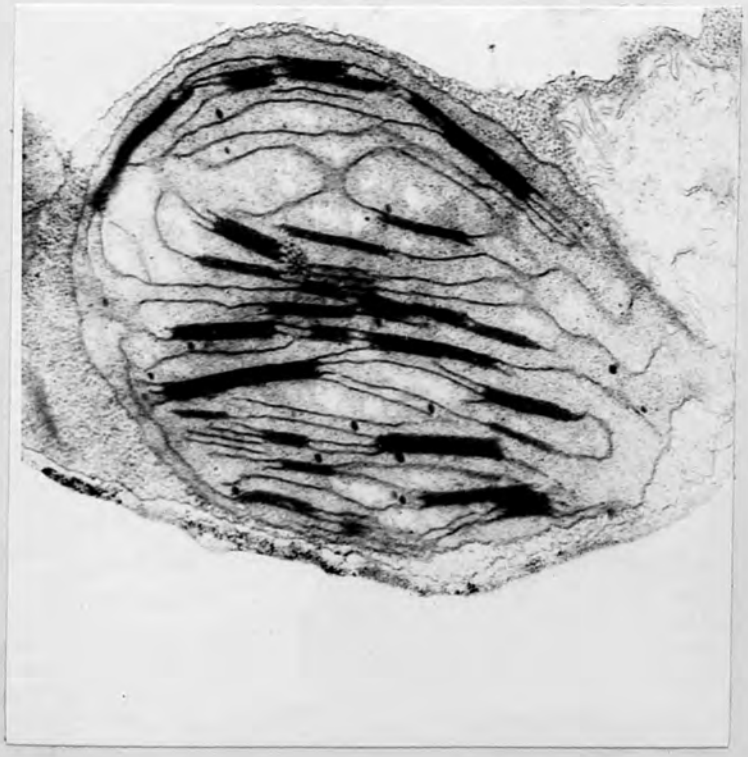
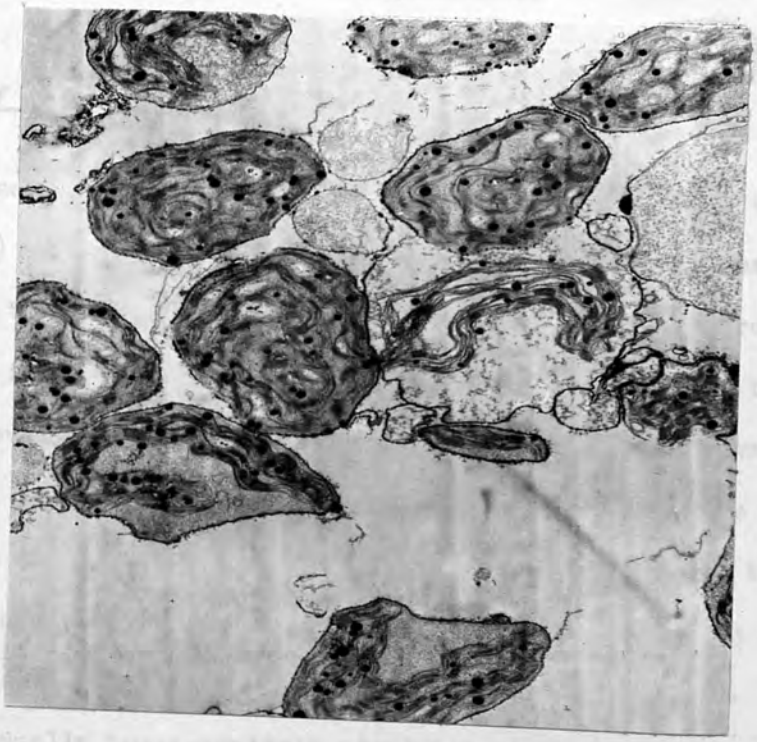
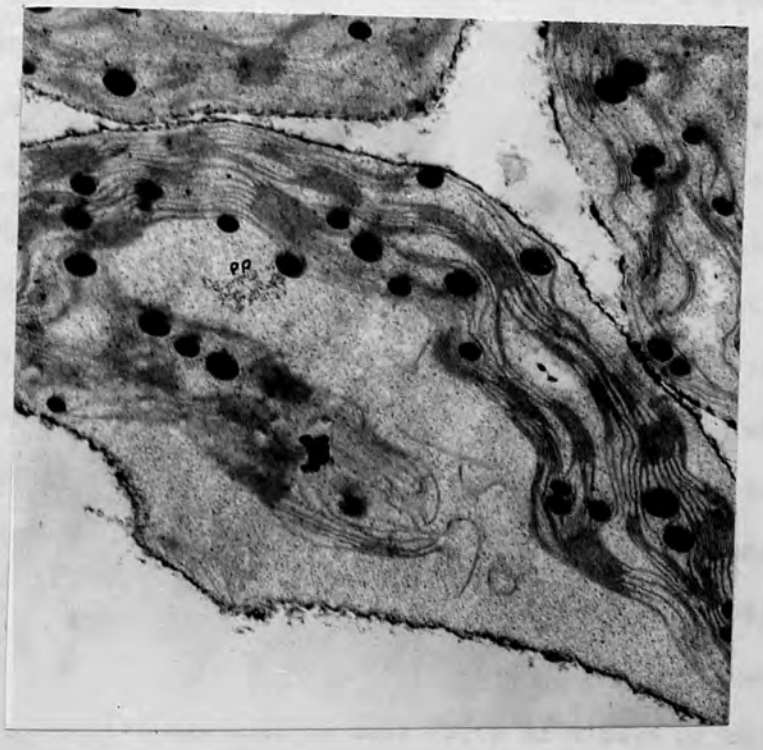


Plate 36. Plastids from 8-day dark grown primary leaf.

Fig. 67a. Section through the primary leaf of light grown seedling transferred to dark for 8 days. A group of shrunken plastids with disintegrating lamellae which are getting deeply curved and concentrically or irregularly arranged. Many large densely stained osmiophilic plastoglobuli are seen in all plastids. One plastid shows phytoferritin also. x 7,600

Fig. 67b. Plastid section from the same leaf material as in Fig. 67a. One of the shrunken plastid with disintegrating lamellae magnified. Lamellae are curved. Large osmiophilic plastoglobuli and a group of phytoferritin (PP) is seen. x 28,500

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2. Plastids in the embryo of dark-grown plants

Formation of etioplast

The development of the proplastids into chloroplasts of the embryo (cotyledons) of normal growing plants has been reported in part I of the present thesis. The development of plastids in dark grown plants as reported in the literature refers in almost all cases to the situation in young leaves taken from young seedlings grown from seed exclusively in the dark. Such conditions could not be employed for the present study of the development of plastids in the embryo, and for that, whole fully grown plants bearing inflorescence and already growing in the normal light conditions had to be transferred into the dark. Whole plants of Capsella bursa-pastoris can not be kept for a long period in the dark: by the 5th day in darkness most plant parts start to wither.

When the plants were kept in darkness for 3-5 days the plastids of cotyledons developed into etioplasts which are smaller and more spherical in shape than the normal plastids. The etioplasts usually contain one or two well organized crystalline prolamellar bodies of the six-edge star module type (Weier and Brown, 1970), composed of regularly cross-connected tubules. The electron micrographs show such tubules cut at various angles in different parts of the crystals in these etioplasts. The crystalline prolamellar body in the etioplasts appear after 3 days in dark, with their maximum growth after 4 days in dark and after 5 days in dark few very small prolamellar bodies are seen in the etioplasts, suggesting that under continuous dark, this crystalline organization is kept until the plants lose their turgescence (Wallas, 1965). Such etioplasts show very different lamellar structure organization as compared either with those kept in dark for 3 or 4 days or to normally growing green chloroplasts. In cases where only a few lamellae occur

in these etioplasts, they do not develop into a normal lamellar organization but have various irregular arrangements especially with thick (swollen) grana protuding from the prolamellar body.

(i) Whole plants in dark for 3 days

The etioplasts in the cotyledons of embryos on the normal growing plant kept in dark for 3 days usually contains only one small or sometimes two crystalline prolamellar bodies from which few lamellae extend outward into the stroma. Radial or irregular (Fig. 68) protusions of several poor incomplete primary layers with no differentiated grana are observed coming out from the periphery of the prolamellar body. The position of the prolamellar body in the etioplast is either central or marginal. Definite grana are occasionally observed along the peripheral lamellae radiating out from the crystalline prolamellar body. In occasional etioplasts few, thick, differentiated grana (up to 10 stacks) are observed around the crystalline prolamellar body and few lamellae of some such abnormally looking grana appear to be coming out of the prolamellar body.

Other etioplasts show two crystalline prolamellar bodies one at either end, and these are interconnected with lamellae extending out of them irregularly and some of which are stacked to form grana. Most of the crystalline prolamellar bodies are of a rectihexagonal type, in which each hexagon is equivalent in size within a prolamellar body exhibiting a characteristic honeycomb-like arrangement of tubules, as has been reported by previous workers (Gunning and Steer, 1975). In some thin sections, crystalline prolamellar bodies appear as zigzag lines and dots in linear fashion, which is formed as a result of breaking down of combination of hexagon. This breaking of hexagon combination is probably the result of thin sectioning (Weier and Brown, 1970). Some etioplasts have concentric type (Wehrmeyerm 1967) of prolamellar

body (Fig. 69). Occasional micrographs show etioplasts having two different types of prolamellar body at different stages of development, one of the prolamellar body which is fully developed is of concentric type and the other one is of non-crystalline type. Both these prolamellar bodies of different type are poorly interconnected with several protrusions of incomplete primary layers one of which connects with the inner membrane of the plastid envelope. Lamellae connecting with inner membrane of the plastid envelope are also observed in other etioplasts. Since the membrane surface is continuous both within the prolamellar body (the plastid stroma penetrates between its tubules) and outwards from it towards the envelope via these lamellae, the space enclosed within the two layers of the envelope, within the lamellae, and within the tubules of the prolamellar body itself is also continuous, and is not in direct contact with the plastid stroma (Gunning and Steer, 1975).

Dividing etioplasts are also occasionally observed exhibiting a baffle profile (Fig. 70). Such etioplasts may lie bisected by a baffle produced by an invagination of the inner membrane of the etioplast envelope. It is suggested that rupture of the outer membrane leads to the separation of the daughter etioplasts. Cran and Possingham (1972) have also reported such plastid division in mature organelles of spinach resulting in increase in chloroplast number.

(ii) Whole plant in dark for 4 days

Under our growth conditions the most rapid period of membrane synthesis occurs after 3 day dark growth. The etioplasts in the cotyledons of embryos of normal growing plants kept in darkness for 4 days show a large increase in size, and extensive prolamellar bodies of crystalline type are observed. All prolamellar bodies have lamellae radiating from their periphery which show stacking at places to form primary grana (Fig. 72), with a maximum stack made up of 8 grana lamellae; but no

definite grana are observed along the lamellae radiating out of prolamellar body, but at the juncture of the peripheral lamellae with the prolamellar body where adjacent membranes of the prolamellar body associate to form overlaps and small grana. Most of the etioplasts usually contain one prolamellar body but occasional etioplast shows more than one prolamellar body. In one etioplast 4 well-developed crystalline prolamellar bodies are present crosswise having lamellae radiating out from their periphery into the stroma and connecting with other prolamellar bodies (Fig. 73). The number of lamellae radiating out from the periphery of prolamellar body are more numerous than in 3-day etiolated embryos. In one of the micrograph of the etioplast a longitudinal row of about 8 small circular tubules is seen close to the side of the inner membrane of the plastid envelope (Fig. 74).

Etioplasts are frequently seen dividing, resulting in many smaller, darkly stained, plastids and frequently two adjacent plastids that appear to have undergone division are encountered and in many cases adjacent plastids have the same number of lamellae in similar arrangement within the plastid. The division of plastids is frequently seen in the young etiolated embryos ('torpedo') in this dark treatment. The etioplasts mostly divide by a constriction (fission profile) at their mid region (Fig. 71). Cran and Possingham (1972) have also reported that the most common way of increase in chloroplast number in cultured spinach leaf discs is by the formation of a median invagination of the chloroplast envelope (fission profile). Some adjacent plastids appear to be the result of division because of the position of the plastids and the number and arrangement of their lamellae. In any given cell the number of lamellae in the different etioplasts varies, and it is significant that two etioplasts similar in this respect are frequently adjacent while plastids to either side of the pair are markedly different. The actual process of chloroplast division has very seldom been watched in

higher plant materials, because plastid division is not frequently found in the chloroplasts of higher plants as in algae. Although we have not observed division of chloroplast in the embryos of normal light grown plants of Capsella bursa-pastoris (part I of the present thesis), plastid division is frequent in etiolated embryos. Somewhat similar findings have also been reported by Stetler and Laetsch (1969) who found many more dividing chloroplasts in the etiolated leaves of Nicotiana tabacum that has been placed in light than in the light-grown plants. It appears that plastid replication is independent of the size or specific internal morphology of the organelle, since replication continues after the chloroplast has stopped growing and has synthesized most of its chlorophyll (Stetler and Laetsch, 1969). It is intriguing that many of the plastids appear to divide almost equally. It is not at all clear what kind of mechanism might regulate this type of replication because there are no apparent morphological features involved in division of the organelles. Possingham and Smith (1972) reported that chloroplast replication proceeds in a similar way in culture discs and in intact leaves of spinach.

A second type of plastid division which begins with the growth of an invagination of the inner envelope membrane right across the chloroplast, partitioning it into two compartments, is not found in 4 day dark etiolated embryos but has been seen in the etiolated cotyledons of 3-day dark growing embryos. How this type of division is completed is still obscure.

Such newly formed plastids which are more or less circular in shape appear to have become very dense with large numbers of infoldings of small vesicles from the inner membrane of plastid envelope where these are mostly present (Fig. 75). Such invaginations of inner membrane of plastid envelope are seen mostly in all of the younger stages of plastid development and more in newly divided plastids. These vesicles do not

appear in the interior stroma region. These invaginations from the inner membrane of the plastid envelope are common, and the staining characteristics of these invaginations are characteristically different from those of stroma lamellae. The areas of low electron density are characteristic of the stroma in developing chloroplasts.

The majority of electron micrographs show etioplasts containing quasi-crystalline prolamellar body with attached lamellae radiating into the stroma. Since the formation of the prolamellar body is considered to result from the condensation of the lamellae, the fall in area of lamellae which occurs in most etioplasts can be accounted for by the parallel increase in the volume of prolamellar body. It is noted that the prolamellar bodies usually appear at the sites of intergrana lamellae, but not in the stroma of plastid. Some plastids show the development of small starch granules, which are mostly transparent and embedded in the stroma between the lamellae. As the lamellar system is not stable in these dark-grown plastids, there is a large space for the production of starch grains.

In order to clarify whether or not the prolamellar body is formed in fully mature plastids, some embryos at mature-green stage were fixed. The results have shown that the prolamellar body is not produced in the mature plastids, but only in growing plastids.

(iii) Whole plant in dark for 5 days

When normal growing plants were kept in dark for 5 days, the whole plant showed signs of desiccation and senescence. Thus all the electron micrographs of the etioplasts at this stage are of senescent cotyledons. Etioplasts show a large increase in their size with either complete loss of lamellae (fig. 76), or the presence of very few fenestrated (beaded) lamellae which are small and incomplete (Fig. 77). The size of

prolamellar body which is either non-crystalline or completely transformed, is reduced remarkably, probably due to the loss of turgescence, and in some etioplasts only a degenerating form of prolamellar body is seen. In some etioplasts both stages of prolamellar body, non-crystalline and completely transformed, are seen lying at some distance in the same etioplast and these are interconnected with poorly developed primary layers of lamellae radiating out of these prolamellar bodies (Fig. 76). Some prolamellar bodies show very poor radial protrusion of incomplete primary layers from the outer margin of prolamellar body, resulting in beaded structures lying in the etioplast (Fig. 77). The majority of etioplasts show the development of 1-3 small starch grains and also a few small densely stained osmiophilic plastoglobuli.

Some etioplasts are in dividing stage by median constriction as in 4-day etiolated embryos and many adjacent plastids appear to be the result of division, thus plastid number is also increased as some cells show 2-4 small spherical very darkly stained plastids with 1-3 small transparent starch grains of different shapes in each of them. Dividing and newly formed plastids also show infoldings of small vesicles from the inner membrane of plastid envelope.

Comments

Prolamellar bodies appear in etioplasts of 3-day dark grown embryos with maximum development in the 4-day dark grown embryos. Crystalline and concentric prolamellar bodies are seen in the etioplast. As the embryos lose their turgescence after 5-days in dark and the plants get dry, the etioplasts show senescing characters.

Etioplast division is very frequent and etioplasts divide both by constriction (fission profile) which forms a narrower and narrower waist in the middle region of plastid and also by the invagination of the inner envelope membrane right across the etioplast, partitioning it into two compartments (baffle profile). Plastid division is not frequently found in angiosperm plastids and so far very few reports are available.

Most of the younger plants show many invaginations from the inner membrane of the plastid envelope near which these are concentrated. It has been shown that these invaginations are contiguous with the stroma lamellae (Laetsch and Stetler, 1965) and that of stroma lamellae are contiguous with the tubules of prolamellar body (Stetler and Laetsch, 1969). Such vesicles which are regularly differentiated from the inner plastid envelope membrane of young plastids help in the formation of lamellae of the plastids as many such vesicles are occasionally seen contiguous with stroma and grana lamellae in the present investigation.

The occasional formation of a longitudinal row of small circular tubules close to the inner membrane of plastid envelope is noteworthy. The formation and the significance of such tubules is not understood at present.

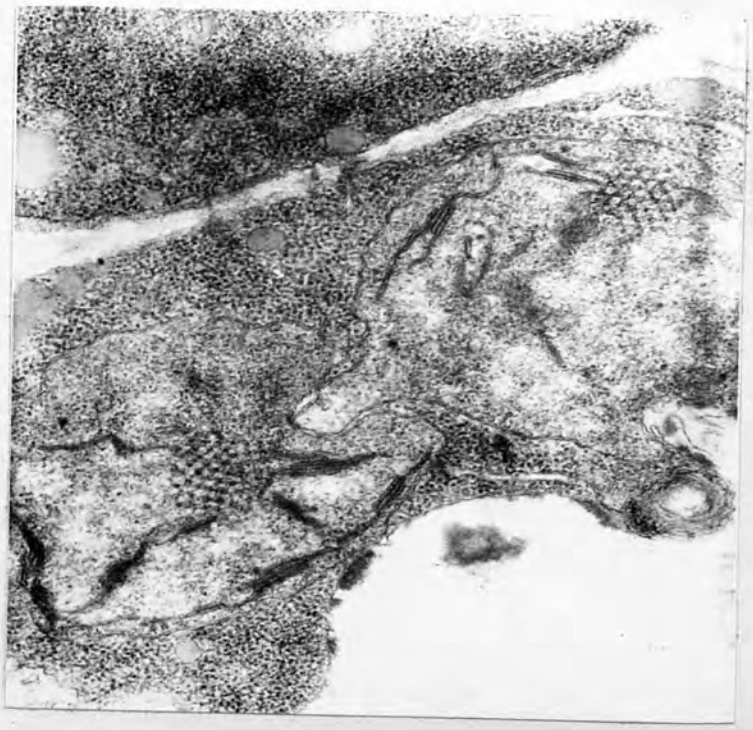
With the embryos on the whole plant it has been possible to obtain plastids with well developed prolamellar bodies after 4-days of dark treatment. It was not, however, practicable to observe any change resulting from subsequent re-illumination of the already senescing plants.

Conditions for the embryos can much better be controlled through culture techniques and this is the subject of the following section.

Plate 37. Etioplasts in embryos from 3-day dark grown plants.

Fig. 68. Section through the cotyledon of young embryo from normal growing plant transferred to dark for 3 days. Spherical etioplasts with poorly developed crystalline prolamellar bodies. Some irregular protrusions of several poor incomplete layers are extending out from the periphery of the prolamellar bodies into the stroma. x 39,000

Fig. 69. Etioplast section from the same embryo material as in Fig.68. Spherical etioplast with a concentric and amorphous prolamellar bodies interconnected with incomplete primary layers. x 52,000



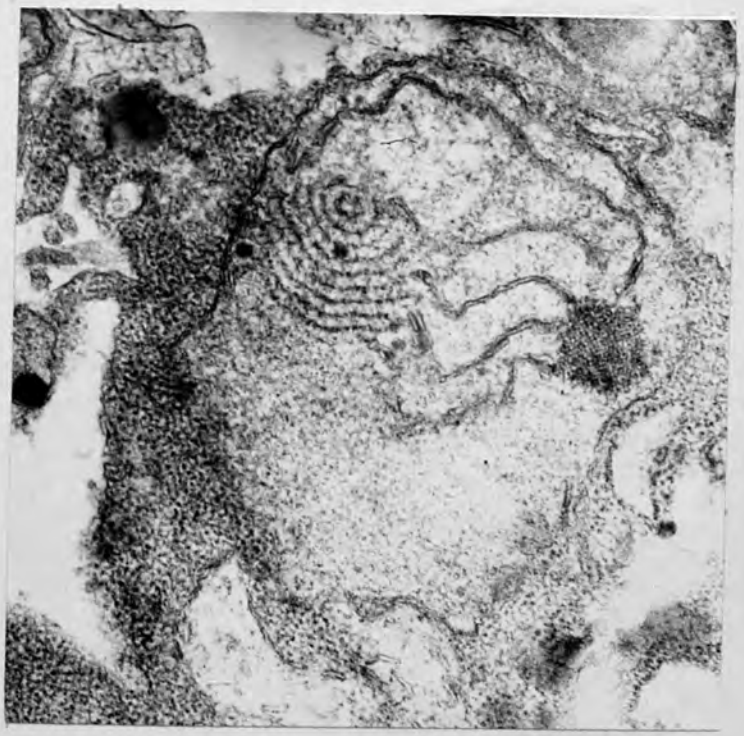
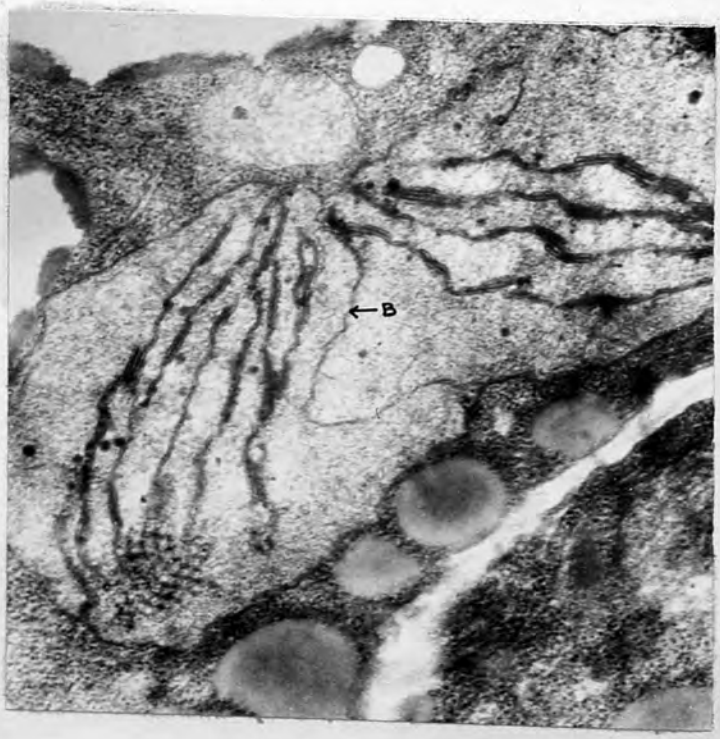


Plate 38. Dividing etioplasts in embryos from 3-day dark grown plants.

Fig. 70. Etioplast section from the cotyledon of young embryo from normal growing plant transferred to dark for 3 days. Etioplast exhibiting a baffle profile (β). The baffle is joined to inner membrane of etioplast envelope. x 52,000

Fig. 71. Etioplast section from the same embryo material as in Fig. 70. Dividing etioplast exhibiting a fission profile (→) (constriction formation). An invagination has been formed midway along the etioplast axis. x 52,000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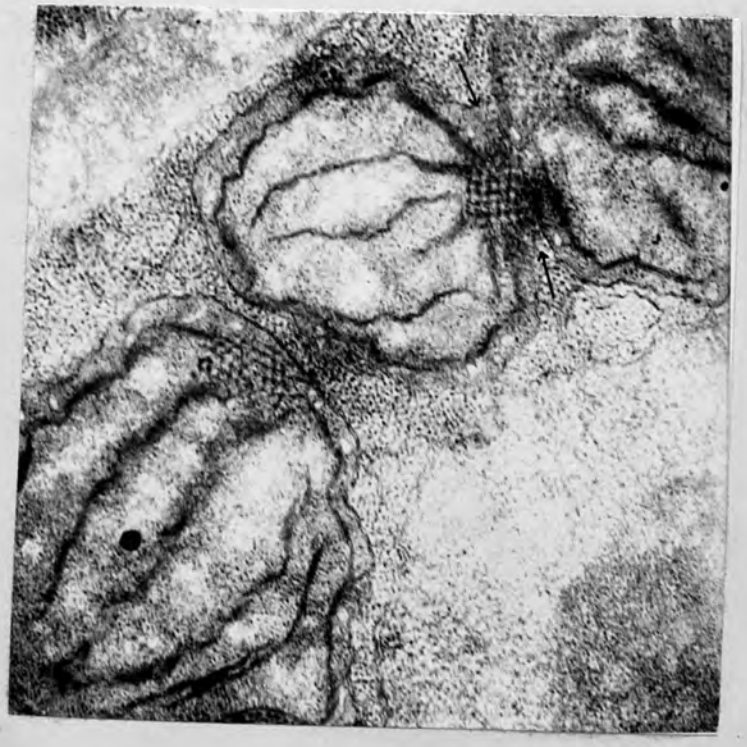
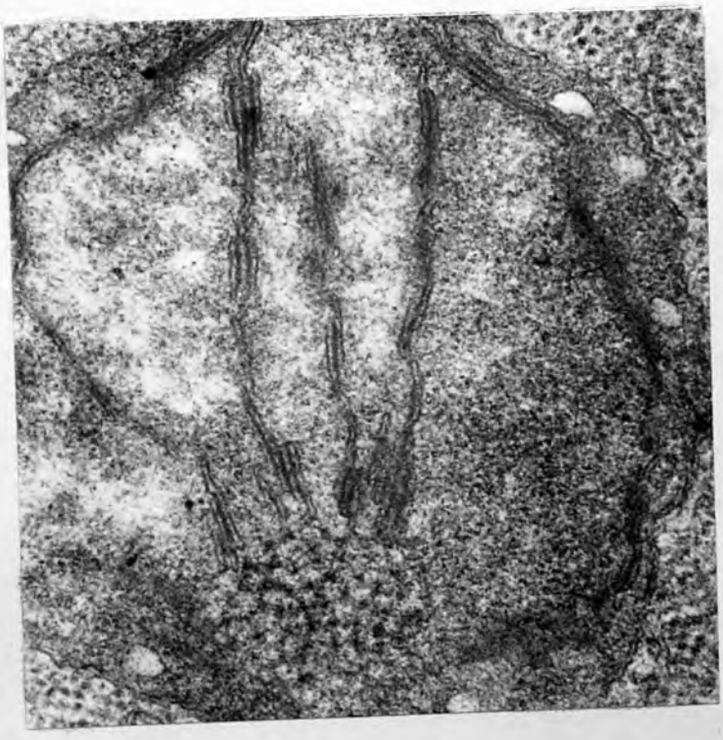


Plate 39. Etioplasts in embryo from 4-day dark grown plants.

Fig. 72. Etioplast section from the cotyledon of young embryo from normal growing plant transferred to dark for 4 days. Spherical etioplast with well developed crystalline prolamellar body with peripheral radiating lamellae into the stroma stacking at places to form primary grana. Vesicles formed from invagination of inner membrane of etioplast envelope are seen. x 78,000

Fig. 73. Etioplast section from the same embryo material as in Fig. 72. An etioplast with 4 crystalline prolamellar bodies interconnected with peripheral radiating lamellae. x 78,000



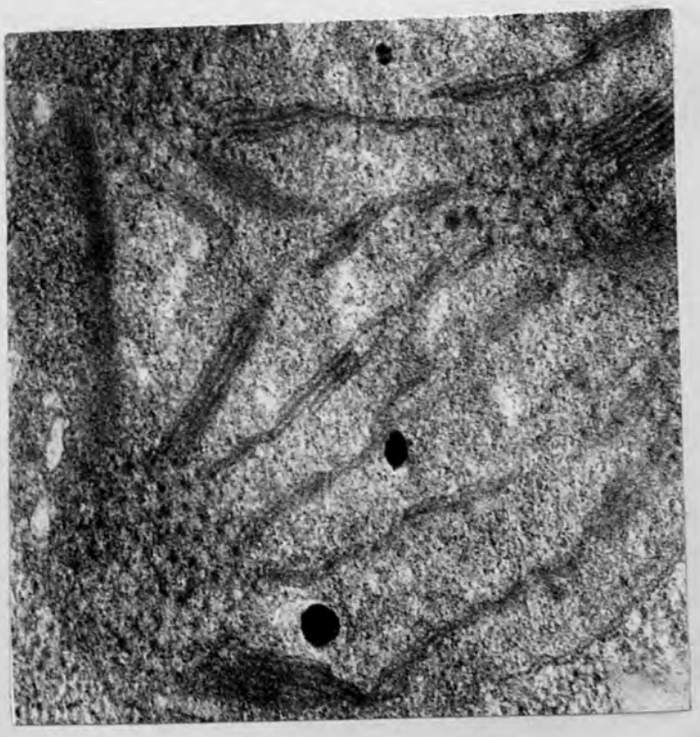
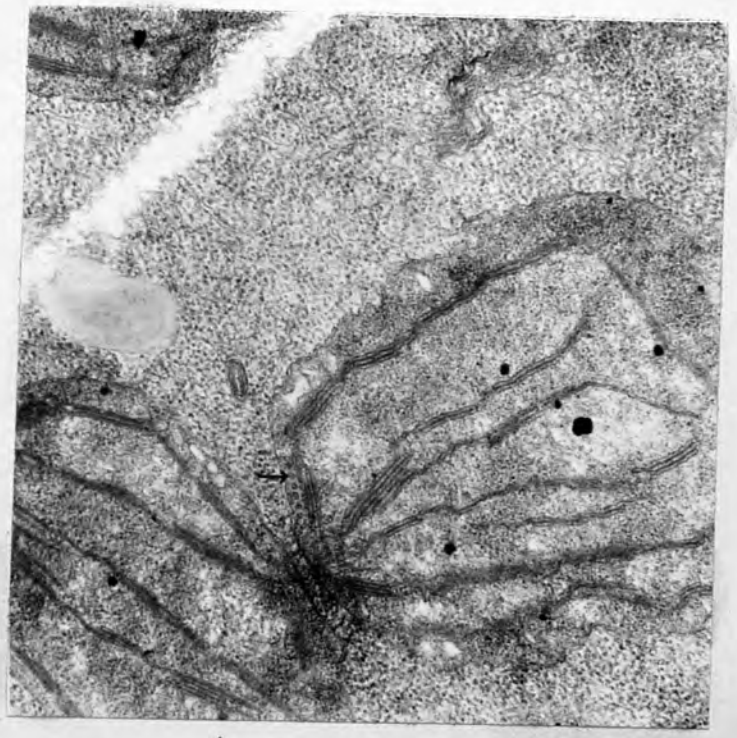


Plate 40. Divided etioplasts in embryo from 4-day dark grown plants.

Fig. 74. Etioplast section from the cotyledon of young embryo from normal growing plant transferred to dark for 4 days. One of the two etioplasts resulting from division shows a vertical row of 7 small circular tubules (→) close to the inner membrane of etioplast envelope. Few infoldings of small vesicles from the inner membrane of etioplast envelope are also seen. x 39,000

Fig. 75. Etioplast section from the same embryo material as in Fig. 74. Recently divided plastids with large number of infoldings of small vesicles (→) from the inner membrane of plastid envelope. The staining characteristic of these invaginations are characteristically different from those of stroma lamellae and these appear as areas of low electron density. x 52,000



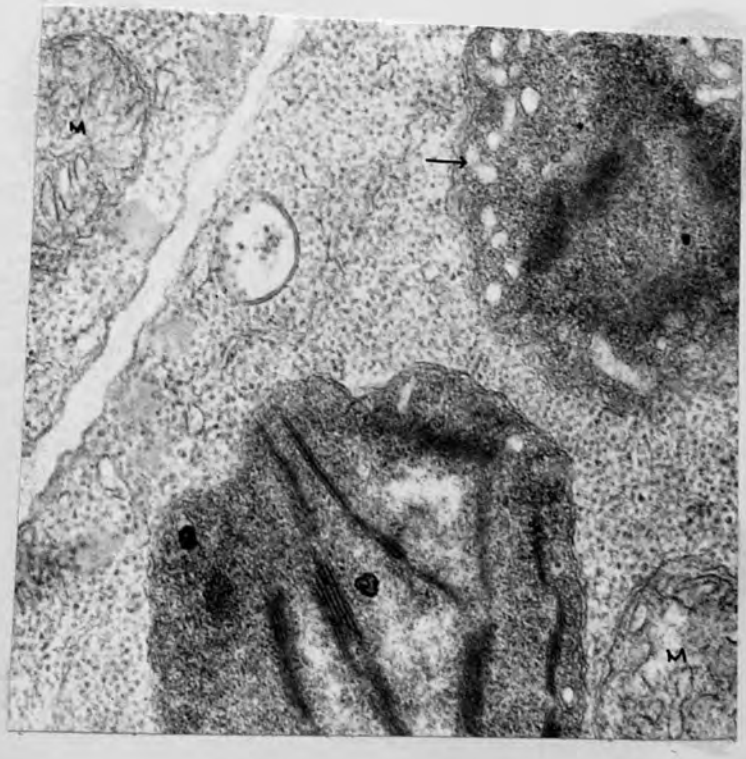

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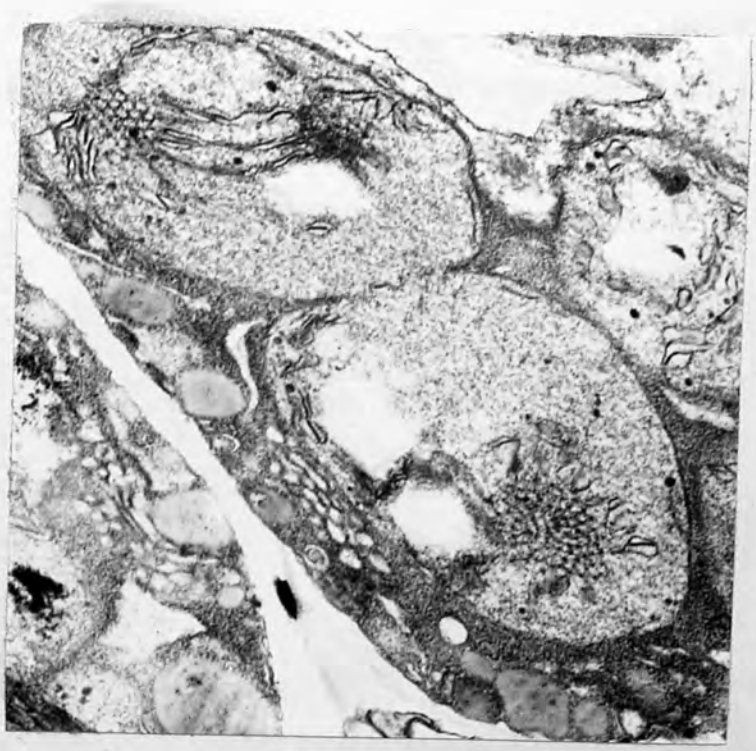
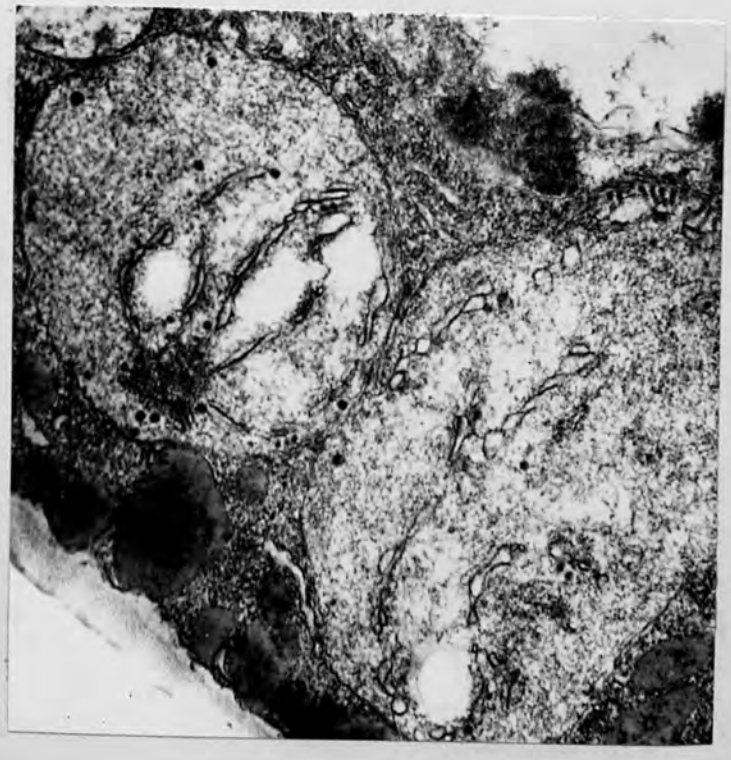
Plate 41. Etioplasts in embryo from 5-day dark grown plants.

Fig. 76. Etioplast section from the cotyledon of senescing embryo from normal growing plants transferred to dark for 5 days. Etioplasts with amorphous and non-crystalline prolamellar bodies with very poor radial protrusions of incomplete lamellae. x 32,500

Fig. 77. Etioplast section from the same embryo material as in Fig. 76. Etioplasts with no lamellae but some beaded lamellae and an amorphous prolamellar body. x 39,000





B. IN VITRO STUDIES.

INTRODUCTION.

Hännig (1904) was the first to succeed in cultivating immature embryos of Raphanus and Cochlearis in vitro in an artificial medium containing mineral salts and sucrose. Several others followed him, and in 1924 Dieterich used Knop's Solution for growing the immature embryos of several species and observed that the embryos tended to skip the stages of embryonic development and directly grew into seedlings, and found that embryos below a certain size could not be successfully cultured. The most significant contribution was that of Laibach (1925) who gave a valuable hint to plant breeders that when a cross does not yield viable seeds and the sterility is due to the mortality of the embryo, it is worthwhile to excise the embryo at an early stage and grow it in a nutrient medium away from the baneful influence of maternal parent. Laibach's work gave a tremendous impetus to the technique of embryo culture and during recent years the technique has been used in agriculture and horticulture to obtain viable hybrids from crosses which are otherwise unsuccessful and to overcome dormancy in seeds.

Despite many years of experimentation, the problem of culturing embryos does not appear to be solved. The younger the embryo, the more difficult it is to excise it under aseptic conditions and to design a suitable medium for its growth. The main problem is that young embryos have a tendency to germinate precociously resulting either in a malformation of the seedlings or necrosis of their parts. Young embryos are extremely susceptible to osmotic shock and a nutrient medium suitable for older embryos is often unsuited for younger embryos.

The fact that the research pertaining to the factors influencing embryonic growth did not make much progress, is primarily due to

unfamiliarity with the environmental demands of the embryo and moreover these requirements might be totally different for various species. Experiments with the culture of immature embryo revealed that they failed to grow even if many vitamins and growth substances were added to the nutritional medium. Therefore, it was realized that the younger embryos required certain substances which were supplied by the adjoining tissues in the seed but could not be replaced by any known chemical. Van Overbeek et al. (1942) overcoming this difficulty, made a breakthrough in embryo cultivation saying that the addition of coconut ~~oil~~ milk (unautoclaved) to Datura embryos causes a strong growth-stimulation and they called the factors responsible for stimulation, the 'embryo factors', the nature of which was not further investigated by them.

Later, much attention was paid by the experimental embryologists to the study of factors that control the progressive and orderly development throughout the ontogeny of embryos. The response, in terms of growth and development of the embryo, varies with the concentration of chemicals added to the nutrient media. It is now known that growth and differentiation of plant embryos may result from an induction by gradients of nutritional substances and hormonal factors in the immediate environment of the developing embryo. Embryos during the early stages of development are highly heterotrophic, that is the embryo develops at the expense of surrounding endosperm tissue and, therefore, all the attempts to culture very small embryos in chemically defined media outside plants, at globular and early heart-shaped stages have failed. The refinement in tissue culture techniques, has tempted many investigators to study the factors essential for the nutrition of immature embryos mostly in the early and late heart-shaped stages as the embryo is still heterotrophic at the globular stage. It is only in the late heart-shaped stage, with the beginning of cotyledonary development and the associated internal differentiation, that the embryo becomes sufficiently independent

and autotrophic possessing large and varied synthetic capacities, for it to be possible to remove it and culture it *in vitro* in a nutrient medium. Young embryos, thus pose various problems as compared to the older embryos because the nutritional requirements of young embryos are complex, and their response often highly variable but older embryos show less rigorous requirements and more definite responses to added nutrients in the medium.

Although nitrate, in general, has proved to be a satisfactory source of nitrogen for mature embryos, immature embryos and even mature embryos during the initial stages of germination profit from reduced forms of nitrogen. Sanders and Burkholder (1948) used casein hydrolysate (a complex of several amino acids) or equal parts each of cystein and tryptophan as present in casein hydrolysate and obtained appreciable growth of the embryo of *Datura*. Lofland (1950) found that high percentage of embryo of cotton grew in lower concentrations of casein hydrolysate. The work of Rijven (1952) on heart-shaped embryos of *Capsella bursa-pastoris*, and of Mauney (1961) on those of *Gossypium* have also shown that casein hydrolysate strongly supports the growth of young embryos. Rijven (1952), however, observed that the growth-promoting influence of glutamine on the embryo of *Capsella bursa-pastoris* surpassed that of a complex mixture of amino acids. Asparagine did not prove stimulative. Further, he reported considerable starch formation in the young embryos grown on a medium containing asparagine, while with glutamine there was scarcely any starch. Thus Rijven (1952) and later Raghavan and Torrey (1963) demonstrated that amino acids are important nutrients for the *in vitro* growth of the embryo of *Capsella bursa-pastoris* excised from the ovule during their development. Norstog (1961) grew the 60 μm long (smallest embryo cultured so far) embryo of *Hordeum vulgare* to maturity on White's medium containing coconut milk.

The first published report of *in vitro* cultures of the embryos of

Capsella bursa-pastoris is by Rijven (1952). Later Raghavan and Torrey (1963) modified the culture medium used by Rijven (1952) for a successful culturing of immature embryos (globular stage) of Capsella bursa-pastoris. In most studies, the culture medium has included a balanced mixture of inorganic salts, including microelements. Sucrose has, in general, proved superior to all other carbohydrates tested, a high concentration of sucrose often proving particularly important in the culture of immature embryos as evidenced by studies with embryos of Datura stramonium (Sanders,1950;Rietsema et al,1953),Capsella bursa-pastoris(Rijven, 1952), and Hordeum vulgare (Ziebur and Brink, 1951; Norstog and Smith, 1963). The importance of high sucrose concentration to promote development and prevent precocious germination (particularly as indicated by cell enlargement) has been ascribed, at least in part, to the establishment of high osmotic value in the medium. The relation between the osmotic value of the medium and the growth of embryo of different ages, has been emphasized by many investigators (Raghavan, 1976). The need to provide culture media of high osmotic values to induce growth of embryos which usually do not grow even in the most complex media tried, has now been established. Rijven (1952) and Veen (1961, 1962, 1963) have used 12 and 18% sucrose in the liquid medium in attempt to culture torpedo-shaped and heart-shaped embryos of Capsella bursa-pastoris. Rijven (1952) found that for a good embryonic growth of 'torpedoes' an osmotic value of 8.4 atm. is necessary. So 'torpedoes' were cultured in a medium with 12% sucrose. Heart-shaped embryos were cultured in a medium with 18% sucrose because some phenomena had indicated 12% sucrose to hypotonic in case of heart-shaped embryos (Veen, 1961). A 12% of sucrose was also used in glutamine-coconut milk medium in which young embryos (60 µm long) of Hordeum vulgare were successfully cultured by Norstog (1961), because Rijven (1952) found that lower concentrations of sucrose in the medium tended to allow embryos to assume seedling characteristics prematurely.

Contrarily, Raghavan and Torrey (1963) have reported that the osmotic value of the culture medium is of relatively little importance in the morphogenesis of cultured embryos. They have, however, shown that the need for high osmolarity can be dispensed with by growing embryos in simple agar nutrient medium (i.e. a semisolid medium) containing usual mineral salts, vitamins (three B-vitamins) and only 2% sucrose. Even early heart-shaped embryos (81-150 μm long) of Capsella bursa-pastoris grew normally when cultured in this medium showing normal embryonic stages of growth without showing any sign of germination, and gave rise to small plantlets. But globular embryos (< 80 μm long) cultured on the basal medium failed to develop even after a long time. However, they successfully cultured globular embryos (60-80 μm long) only when they supplemented the basal medium with indole-3-acetic acid, kinetin and/or adenine sulphate. A combination of all the three adjuvants provided the best milieu for growth. Still younger embryos (down to 20 μm long) could not be cultured in such a medium (Raghavan and Torrey, 1964) whereas older embryos (heart, and torpedo, and walking stick embryos) prematurely developed into small plantlets in the absence of these hormonal factors. Thus the studies of Raghavan and Torrey (1963) have shown that globular embryos (80 μm long) of Capsella bursa-pastoris did not show an absolute requirement for a high osmotic concentration, although a high sucrose content (12 or 18%) or enhanced level of mineral salts (ten times that in the basal medium) partly replaced the effect of three growth substances in inducing their development, suggesting a controlling influence of the intracellular sucrose level in certain pathways of biosynthesis within the embryo. On the basis of these observations Raghavan and Torrey (1963) suggest that the growth and differentiation of excised embryos in cultures are controlled not so much by the osmotic value of the culture medium but by the availability of specific growth factors. This being circumscribed by the concept that the optimum growing

conditions for *in vitro* cultures of embryos is one which imitates closely the composition of endosperm or the milieu of the embryosac. The success in culturing very young embryos of *Capsella bursa-pastoris* (Raghavan and Torrey, 1963) might be due to the use of solid basal medium instead of liquid medium being used earlier (Rijven, 1952), but due to lack of experimental evidence, the role of high osmotic pressure in growth induction in the embryos of *Capsella bursa-pastoris* remains uncertain. It has been suggested (Ryczkowski, 1962) that a knowledge of the osmotic values of the embryo during its growth and development in the ovule would be helpful in designing a proper nutrient medium for its growth *in vitro*.

The roles of nutrients, 'growth factors' and plant hormones are still not sufficiently clearly understood to invoke them in a meaningful way either individually, or in combination or in sequence, in the progressive differentiation of plant embryos.

1. Role of carbohydrates in the growth medium.

Intact plants meet their energy reqirement autotrophically via the photosynthetic fixation of carbon. When plant tissues are cultured, the normal functions of chloroplasts are frequently absent or blocked. Therefore, in plant cultures, it is usually necessary to substitute suitable carbon source for those carbohydrates that would otherwise have been generated photosynthetically in the intact plants. In plants, sucrose is the usual form of sugar exported by the leaf tissue, loaded into the cells of the phloem, translocated, and deposited in meristematic and storage tissues. It seems reasonable, then, that sucrose should serve best the energy needs of cells grown in culture, and sucrose is the most commonly used carbohydrate in plant embryo cultures. As noticed in previous section where it has been suggested that the level employed may play a role in preventing precocious germination in vitro. Sucrose was favoured over maltose, glucose, fructose, raffinose, lactose, mannitol, and glycerol by the embryos of Capsella bursa-pastoris, and very little success was obtained with the latter seven compounds (Rijven, 1952). Characteristic abundance of a particular carbohydrate in a species can, but need not, predicate its utilization. Thus although widely occurring, sugar alcohols are usually a poor energy source. When excised embryos of Hordeum vulgare were supplied with sucrose, they achieved a relatively high dry weight and showed the presence of abundant starch in their cells (Brown and Morris, 1890). So the important thing in the carbohydrate nutrition of embryo is the ability of embryos of some species to utilize substances such as starch which are not ordinarily metabolized by cultured tissues. It has been suggested that growth induction in proembryos of several species in artificial culture is achieved by the establishment of high osmotic value in the medium which

probably allows for an effective flow of metabolites (Raghavan, 1976).

Dieterich (1924) obtained better results with 5% sucrose than with 15%. Ziebur (1951) and Ziebur and Brinck (1951) found that the higher osmotic value obtained with casein hydrolysate prevented precocious germination of differentiated, excised embryos of Hordeum vulgare, although the growth of smaller embryos was inhibited. Substitution of 12.5% sucrose resulted in continuation of embryonic growth of such small embryos. Norstog and Smith (1963), after trying various sucrose concentrations in defined media used to culture small embryos of Hordeum vulgare , found a 9% solution of sucrose to be optimum. Rijven (1952) calculated the osmotic value for embryonic cells and determined that a medium containing 12% sucrose would prevent precocious germination of cultured embryos of Capsella bursa-pastoris. He stated that whenever one noted reports of precocious germination of cultured plant embryos, one could be sure, that the sucrose concentration used was too low. Raghavan and Torrey (1963), on the other hand have demonstrated that globular pro embryos ($<80\ \mu m$ long) of Capsella bursa-pastoris can be grown on media containing relatively low sucrose concentration (2%), providing a balanced supply of growth factors, kinetin, adenine and indole-3-acetic acid, is supplied to the medium. Interestingly enough, these growth factors could be replaced, in part, either by increasing concentration of sucrose to 12-18% or by increasing the level of macronutrient salts.

Paris et al. (1953) found that the optimum sucrose concentration for the growth of the embryos of Datura stramonium smaller tham 0.3 mm. was 8%, and that lower concentrations were better for the growth of older and larger embryos. Nearly mature embryos had an optimum sucrose requirement of 0.1% to 1.0%. They also found that mannitol could replace a portion of tho sucrose as a means of raising the osmotic pressure. The embryos of Datura stramonium larger than 0.35 mm. were able to initiate

growth on media containing 1% sucrose, but pre-heart stages of about 0.15 mm. size required sucrose level of 4-12%. Rietsema et al. (1953) also studied the effect of sucrose concentration on the growth of cultured embryos of Datura stramonium and reached similar conclusions. Amemiya et al.(1956) found that embyos of Oryza sativa responded variously to different concentrations of several sugars. Glucose was optimum at 2.5% for plant height and at 5% for root length.

While working on excised Datura stramonium embryos van Overbeek et al. (1944) concluded that sucrose was better than other sugars for their growth. Doerpinghaus (1947) found that various species of Datura responded differently to several carbohydrates. Lofland (1950) tested the effects of lactose, glucose, starch, galactose, arabinose, levulose, raffinose, xylose, and sucrose, and selected the sucrose for use in culturing embryos of Gossypium hirsutum. Radforth and Pegoraro (1955) noticed very little difference between the effects of sucrose and glucose on the growth of cultured proembryos of Pinus. Choudhury (1955) also reached the same conclusions concerning the effects of these two sugars on the growth of tomato embryos. In their work on embryo culture in Citrus, Ohta and Furusato (1957) reported that embryos of Citrus natsudaidai grew better on a non-sucrose medium than on a sucrose-containing medium. Brown and Gifford (1958) found a beneficial effect of sucrose on root growth and development of Pinus embryos when supplied through cotyledons. Ball (1959) compared the growth of Ginkgo biloba embryos on sucrose, glucose, levulose, and raffinose, and found activity in all, with sucrose yielding slightly better results in terms of shoot growth, and glucose producing the greatest increment in root. Mauney (1961) reported that embryos of Gossypium hirsutum grew better on sucrose media than on fructose-containing media. Cockerline (1961) investigated the effect of a wide variety of disaccharides, hexoses, and pentoses, as well as glycolytic intermediates, and reported that sucrose produced the

greatest growth of cultured barley embryos. The addition of sucrose above or below the optimum level in the cultured embryos of Elaeis guineensis (oil palm), have led to inhibition of growth of root and cotyledonary leaves (Raghavan, 1976). In the absence of sucrose, embryos of early ripening selection of peach appeared flaccid and transparent, and seldom formed roots and leafy shoots in cultures (Brooks and Hough, 1958).

2. Light and temperature requirements

(i) Light

Embryo cultures may be incubated in light or in the dark. Embryo cultures often grow best in the light. Chlorophyll and other pigments may develop with cultures grown in light (Vasil and Hildebrandt, 1966). The quality and quantity of light often influences tha amount of pigment formation as well as the differentiation of roots, stems, leaves and plants from undifferentiated cell cultures (Vasil and Hildebrandt, 1966). A combination of fluorescent and incandescent lamps has generally been used for this purpose.

The illumination of embryo cultures must be considered in terms of intensity, length of the daily exposed period and 'quality', that is, its spectral composition. It is evident that the light requirements of embryo cultures are not the same as those of growing on autotrophically developing intact plants. In embryo cultures, photosynthesis is not a necessary activity, since carbohydrate is adequately provided. Nevertheless, light is needed to regulate certain morphogenetic processes in the formation of shoot, the initiation of roots and in asexual embryogenesis (Haccius and Lakshmaran, 1965). The failure of embryo cultures is sometimes caused by the use of plant growth chambers of similar facilities where the light provisions have been intended for the development of intact autotrophic plants. Such failures of embryo cultures are due to excessively high light intensity, and sometimes the quality of illumination is also unsuited for embryo culture. The embryos of the intact plants receive light which is filtered as it passes through the green ovary/fruit wall. Excised cultured embryos in fact require light of low intensity for their normal growth since they are directly exposed to the light. In fact, a true comparison in the growth, particularly the

plastid differentiation, between the *in vivo* and *in vitro* grown embryos cannot be made accurately.

The light intensity varies with different species. Gautheret (1969) reported that maximum root initiation in *Helianthus tuberosus* tuber section cultures occur at an intensity of 5000 lux using white light and daily exposure period of 12 hours. Nebel and Naylor (1970) reported that shoot bud formation in moss *Phycomitrium turbinatum* was related linearly to light intensity, at least in the range 300-700 lux. They used incandescent lamps and provided light continuously. Optimum light intensity of 1000 lux with a daily exposure period of about 16 hours appears to be suitable for most tissue cultures, but for the embryo cultures, optimum light intensity would naturally be comparatively low. The requirement of prescribed periods of exposures to light each day has not been equitable with photoperiod requirements in the traditional sense. The main thing is the total radient energy of specified quality to which the culture is exposed. Hence it involves the combined influence of both intensity and exposure period, and for a given species, it is reasonable to expect varying optima in the length of daily exposure period, depending on the light intensity used. Rijven (1952) could detect no particular effect of light on embryonic growth. According to Rijven (1952) early and late torpedo stage embryos (over 250 μm) and over and not yet mature of *Capsella bursa-pastoris* are green when excised. He noticed the disappearance of green colour of these embryos when cultivated without light and embryos became white within the course of a few days. He also reported the inhibition of growth in length of the embryos. Working on the same plant Raghavan and Torrey (1963) used 12 hours illumination daily by a combination of cool-white fluorescent tubes and incandescent lamps giving ca. 50 f.c. (535 lux) at the level of cultures of young embryos of *Capsella bursa-pastoris* and reported the maximum growth of embryos of light-grown cultures. They also maintained parallel sets

of cultures in an incubator which was kept completely dark. Raghavan and Torrey (1964) reported that root elongation was inhibited in light, although hypocotyl and shoot grew better in light than in the dark, resulting in greater growth in length of embryos in light than in the dark of Capsella bursa-pastoris. The embryos of Haracleum sphondylium in the early stages of cultures accelerated their rate of growth when subjected to light as compared to those grown in the dark (Stokes, 1953). Light enhanced the formation of chlorophyll in the embryos of Pinus jeffreyi (Bogorad, 1950) and Gossypium (Lofland, 1950; Mauney, 1961).

Weis and Jaffe (1969) have indicated that the critical portion of light spectrum for shoot induction was the blue region. Red light (660 nm.) had no effect. Seibert (1973) confirmed these findings and reported that the most effective band was in the region of 467 nm. He further reported that blue light (419 nm.) also stimulated shoot initiation. Interestingly root initiation, in contrast to shoot initiation, is stimulated by red light but not by blue (Letouze and Beauchesne,1969).

Light and plastid pigments in culture

Tissue cultures have recently been used to follow the development of chloroplasts and photosynthetic capacity under the influence of light. Sunderland and Wells (1968) followed greening in cells of the endosperm of Oxalis dispar grown on 2% sucrose. Amyloplasts with extensive starch deposition were formed from proplastids in the dark. Chloroplasts developed when these cultures were transferred to light and such chloroplasts developed thylakoids between starch grains and starch disappeared, later grana developed with normal accumulation of pigments. A rapid decrease in the rate of cell division and expansion was noted and they suggested it was due to a diversion of raw materials into specific synthesis accompanying chloroplast maturation. While studying the effect of various auxins on pigmentation in cultures, they described that the

observation that the starch deposition and formation of lamellar structures from the inner plastid membrane occurring in the dark in Oxalis cultures was not affected by the presence of auxins, in contrast to thylakoid development, was tentative evidence that thylakoid membranes had a different ultrastructural origin from the membranes of amyloplasts.

In carrot cultures (phloem) the chlorophyll is formed in the light similar to that in mature leaves. The reason is, however, that in the appropriate medium and in the light, the plastids of cultured carrot form chloroplasts and the chlorophyll synthesis then follows upon the formation of the organelle (Steward et al., 1966). This means that chlorophyll synthesis is preceded by the organization. The synthesis of pigments is, in turn, determined by nutrition (Krikorian and Steward, 1969).

The calluses of many species (Sunderland and Wells, 1968) contained pigments of concentrations very similar to those in young leaf primordia in which the chloroplasts were rudimentary. Calluses contained fewer chloroplasts per cell on the average than the leaf, and chloroplasts of the callus did not have the complexity of structure now known to be associated with mature leaf.

(ii) Temperature

Most cultures grow well between 25-30° C. When extra light is used, it is essential to maintain the optimum temperature at the culture level. Most cultures are maintained in an environment in which the temperature is held constant and the temperature usually employed is about 25° C. This practice fails to recognize the temperature fluctuations, diurnally and seasonally, under which plants normally develop. While constant temperatures may be adequate for the culture of many annuals and tropical species, whose life cycles are completed during a period of relatively

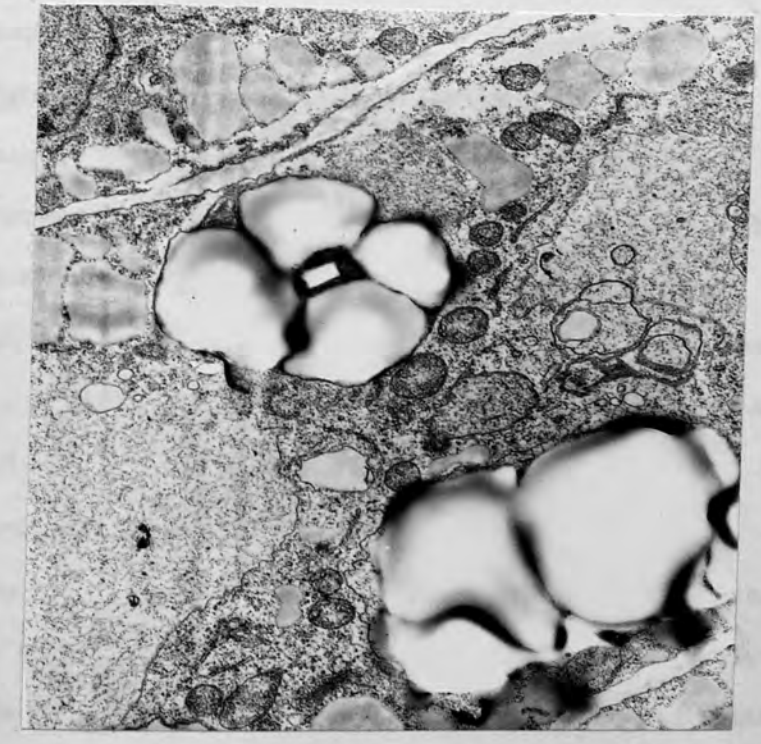
uniform temperature conditions, it may be desirable to explore the influence of periodically varied temperature on the behaviour of embryo cultures, especially plants adapted to more temperate and even desert climates. We did not experience any particular difficulty with our plant material as it grows and completes its life cycle during a period of uniform temperature of summer. All our cultures were maintained at a uniform temperature of 25 ± 1° C. Rijven (1952) used a temperature of 30° C while Raghavan and Torrey (1963) used a temperature of 25 ± 1° C for culturing young embryos of Capsella bursa-pastoris. Choudhury (1955) obtained the best growth of tomato embryos at 27° C, and found 32° C to be inhibiting. Haynes (1954) compared the growth of potato embryos at 15° C, 20° C and 25° C, and decided that 20° C was the best of the three temperatures. Matsubara (1962) cultured Datura tatula embryos at 30° C under dim light. Vanilla embryos grew best at 32°-34° C (Knudsen, 1950) and Gossypium embryos grew best in light at 30° C (Mauney, 1961). It appears that the optimum temperature range for the growth of embryos in culture is 27°-32° C (van Overbeek et al., 1944; Rijven, 1952; Choudhury, 1955). Although in Gossypium embryos in culture, the rate of growth increased with higher temperature (up to 43° C) but they tended to be spindly in appearance (Mauney, 1961).

It is now clear from the foregoing account that the maximum benefit from embryo cultures may be attainable only by fulfilling the precise temperature need of a plant. However, recent advances in understanding the relationships between light of different wavelengths and such phenomena as flowering, etiolation, and germination, suggest the need for a critical evaluation of light-related phenomena in plant embryo culture.

Plate 42. Amyloplast in 7-day dark cultured embryo (2% sucrose).

Fig. 78. Section through the cotyledon of embryo cultured in darkness for 7 days on a growth medium with 2% sucrose. A chloroamyloplast with a big compound starch grain, and few incomplete broken lamellae. x 76,000

Fig. 79. Amyloplast section from the same embryo material as in Fig. 78. Amyloplasts completely filled with compound starch grains. x 11,400



3) Starch accumulation.

In the present investigation, when young excised embryos of Capsella bursa-pastoris were cultured on a semi-solid growth medium containing 2% sucrose as a source of carbon, two major problems were experienced. The first, the excessive accumulation of starch in the chloroplasts and second, the bleaching of embryos. The intensity of bleaching was more in the dark grown embryos than in light grown embryos, and as well as in those embryos cultured on the growth medium containing no sucrose. This bleaching is probably due to the inhibition of chlorophyll synthesis.

Thorpe and Meier (1972) have studied the endogenous formation and utilization of starch reserves in Nicotiana tabacum callus. According to them conditions favouring bud formation greatly enhanced both build up and breakdown of starch. Cytokinins also stimulated starch accumulation according to them. An earlier starch accumulation was initiated by light, but both in light and dark, starch contents increases six-fold before it decreased. Thorpe and Meier (1974) reported higher specific activity of an appropriate anabolic and catabolic enzymes in the shoot-forming, compared to the non shoot-forming callus, suggesting the possible importance of starch for the differentiation process. It was proposed that high energy requirements for organogenesis favour starch utilization since its catabolism, compared to glucose, is energy-sparing (Thorpe and Murashige, 1968).

Electron micrographs of the present investigations actually show no chloroplasts but only plastids resembling chloroplast-like amyloplasts, with several large starch grains occurring in each plastid, in which a very weakly-developed lamellar system is present (Fig. 78). Due to large accumulation of starch in some amyloplasts, no lamellae could be seen at all (Fig. 79). To overcome this problem of excessive accumulation of starch in the plastids of excised embryos, when cultured for the study of plastid differentiation, various lower concentrations of sucrose

in the growth medium were tried. It was found that 0.5% of sucrose in the growth medium gives satisfactory results. The plastids of excised embryos cultured on the growth medium containing 0.5% sucrose had normal accumulation of starch similar to the embryos of intact plant. As a result, this concentration of sucrose (0.5%) was finally selected for conducting our experiments on the differentiation of the plastids of the embryos of Capsella bursa-pastoris. Experiments were also conducted using growth medium containing no sucrose, with the presumption that growing embryos on such medium might solve our problem of excessive starch accumulation in amyloplasts, formed eveidently by the presence of sucrose in the growth medium. But excised young embryos when grown on this medium showed retarded growth both in light and dark. Excised embryos on the growth medium with no sucrose showed no growth in the dark and presented various degrees of degeneration of their cellular contents, resulting ultimately in death with prolonged darkness. A growth medium with no sucrose was thus used for comparative studies but was not found useful for the experimental studies as the presence of sucrose in the growth medium, which is a source of carbon, is a necessity for the normal growth of embryos in cultures.

(4) Bleaching

During our in vitro studies on the photomorphogenesis of the embryos, most excised embryos cultured on the growth media containing sucrose (2%) or lacking sucrose showed some degree of loss of green colour. Cultured embryos, when grown in the dark, turned almost completely colourless within a few days. Cultured embryos, when grown in light, with or without sucrose, also lost their green colour, but gradually and to a lesser extent. According to Maretzki et al.(1974) when plant tissues are cultured, the normal function of the chloroplasts is frequently absent or blocked.

It appears that the inability to produce chlorophyll is neither necessarily the consequence of a repression of those genes controlling its synthesis nor simply a senescence phenomenon, but it might be expected to be related in some way to the presence of sucrose in the growth media and is also affected by light as well. Rijven (1952) has already reported that cultivation of the embryos of Capsella bursa-pastoris without light in a nutrient medium containing sucrose (12%) causes the green colour to disappear and the embryos become white within the course of some days. Chandler et al (1972) have succeeded in growing photosynthetically the two clones of Nicotiana tabacum in liquid suspension cultures by replacement of sucrose with 2% CO_2. The inhibitory effect of sucrose on chlorophyll synthesis in Daucus carota (Edelman and Hanson, 1972) suggests that the widespread use of concentrations greater than 2% sucrose in standard nutrient media may be detrimental for development of photosynthetic functions. The chlorophyll content of callus from six different species was higher when sucrose was replaced with coconut milk and other growth factors in the basal medium (Fukami and Hildebrandt, 1967), but growth and chlorophyll formation was poor, unless some form of exogenous carbohydrate was supplied (Vasil and

Hildebrandt, 1966).

Much work is being done by a Japanese school on the process of glucose bleaching in Chlorella protothecoides (Matsuka and Hase, 1965, 1966, 1969). Most of their studies have demonstrated that reversible degeneration and regeneration of chloroplasts are induced in the cells of Chlorella protothecoides by controlling nutritional as well as light conditions (Shihira-Ishikawa and Hase, 1964; Aoki and Hase, 1964; Aoki et al., 1965; Matsuka and Hase, 1965, 1966; Matsuka et al., 1966; Mihra et al., 1968; Oh-hame et al., 1968). When green algal cells are incubated in a medium containing a high concentration of glucose or some other utilizable organic carbon source (but no nitrogen source), they are strongly bleached simultaneously with disintegration of chloroplast structure and photosynthetic activity ("glucose bleaching"). It has been suggested (Matsuka and Hase, 1969) that assimilation of glucose into lipids is markedly enhanced in bleaching cells and, that when inhibitor is applied (cycloheximide) to green cells, the lipogenesis in question is probably suppressed, then keeping the cells green. They suggested that enzymes participating in lipogenesis may be inductively formed on addition of glucose, and that the enhanced lipogenesis may be related to the induction of algal cell bleaching. Sucrose, which is universally recognized as the best carbon source was added to our nutritional media and the bleaching of the embryos of Capsella bursa-pastoris can possibly be explained as described for Chlorella protothecoides.

The plastids of Capsella embryos grown in light without sugar perform their normal function of photosynthesis and produce the required carbohydrate needed for the normal growth of the embryo, as it is not available in the growth medium. So chloroplasts become functionless only if the embryos are grown on the medium containing sucrose, but behave normally if the embryos are grown on the medium containing no sucrose.

Brooks and Hough (1958) reported that in the absence of sucrose, embryos of early ripening selection of peach appeared flaccid and transparent and seldom formed roots and leafy shoots in culture. Hildebrandt et al., (1963) however, reported that the greatest concentrations of chlorophyll, in several different callus cultures, occurred in the culture medium devoid of sugars (supporting our ideas) or those which contained high sugar (8%) concentrations (Fukami and Hildebrandt, 1967).

Investigations of carbohydrate utilization and metabolism in embryo cultures have undergone a gradual change in emphasis. Some exceptions exist to the general rule that sucrose best meets the energy and carbon skeleton requirements for embryo cultures, and these should be exploited fully to investigate unknown, or incompletely known, pathways. This is the beginning of a new era and whereas the growth of excised embryos on synthetic nutrient media has yielded some results, much more remains to be achieved.

OBSERVATIONS AND DISCUSSION

Several preliminary experiments were performed on the culture of young embryos of torpedo stage on the growth medium used by Raghavan and Torrey (1963) for culturing young embryos of Capsella bursa-pastoris. The growth medium used by them contained 2% sucrose. This first culture experiment is performed on a growth medium containing 2% sucrose and on a growth medium lacking sucrose.

1. Plastid development in cultured embryos on a medium with 2% sucrose and a medium lacking sucrose.

(i) Formation of etioplasts in darkness.

When torpedo stage embryos were cultured in the dark on a growth medium containing 2% sucrose the formation of prolamellar body in the etioplasts of cotyledon was seen only in 6-day dark grown cultures. The prolamellar body appears of crystalline type but is not infrequently absent, most plastids being filled with large starch grains. Some electron micrographs show etioplasts with one large prolamellar body in a stage of losing regularity to become non-crystalline and large, abnormally condensed grana, which appear to be extending out from the prolamellar body. Some lamellae from such grana are lying around the big starch grain situated at one end of the etioplast (Fig. 80). At this stage, numerous vesicles resulting from the invaginations of the inner membrane of the palstid envelope are present at the periphery of stroma, close to the inner membrane of plastid envelope. Other etioplast sections show smaller prolamellar body at one end of the etioplast from which are radiating out long peripheral lamellae into the stroma, stacking at places forming grana, also containing 3-4 smaller starch grains. Many other plastids contain 3-4 big starch grains filling the whole

plastid, with scant lamellar system, showing few grana and intergrana lamellae present in the space between starch grains (chloro-amyloplast) (Fig. 81). Such chloro-amyloplasts also contain few densely stained osmiophilic plastoglobuli.

No prolamellar body is seen in the embryo plastids when the cultures are kept in dark more than 6 days but instead plastids full of several big starch grains. Some plastids have few internal lamellae and 2-3 small starch grains and in other plastids the production of starch grains has progressed to an extent that most of the plastid volume is occupied by big starch grains beside and between which are simple lamellae which may form grana. Starch grains in the plastids assume a variety of shapes but most of them are round, ellipsoidal, or kidney shaped (Fig. 81), showing variety of electron-densities having greater density at the periphery of the grain than in the centre. Since the number of starch grains in such chloro-amyloplasts is usually 2-3, it appears that the presence of sucrose in the medium has caused the increase in the size of the granules, rather than their number.

In one of the chloro-amyloplast of 7-day dark culture several starch grains (3-4) grow together into a compound starch grain which is almost transparent (Fig. 78). This amyloplast in particular and other amyloplasts of 6-day dark grown cultures are bounded by a double membraned envelope and here and there the inner of the two membranes is continuous with the internal membrane system which consists of a few incomplete broken lamellae lying on one side of the chloro-amyloplast.

In the 11-day dark grown culture (maximum dark period used in this experiment) all the cells of the cotyledon appear full of amyloplasts and oil bodies around the central nucleus (Fig. 82). These amyloplasts have bigger compound starch grains formed by growing together of many more starch grains as compared to 6-day or 7-day old dark cultures. In these amyloplasts neither the outer envelope nor the inner lamellar

structure is seen but some compound starch grains are still separated by narrow bands of stroma as found in the compound starch grains of Oryza sativa (Buttrose, 1960). Some of these compound starch grains present a beautiful structure (star-like) formed by the fusing together of several simple starch grains (Fig. 79). Some of these compound starch grains of 11-day dark cultures are transparent but others show variety of electron densities which is greater at the periphery than at the centre of the grain. Some compound starch grains are asymmetric with irregular lobes and branching. In all cases the excess sucrose in the growth medium appears to have caused an increase in the size of starch grains rather than their number.

When young embryos of torpedo stage are cultured in the dark on a growth medium lacking sucrose, degeneration of the cell organelles including plastids is seen to an extent that after 6-11 day growth in the dark they are difficult to fix and membrane structures are almost indistinguishable. Only a few oil bodies and nucleus can be seen in a few cells (Fig. 83).

(ii) Light grown cultures

When the young embryos of torpedo stage are cultured in light for 6 days on the growth medium containing 2% sucrose, all the cells are completely filled with many big starch grains , mostly simple but some compound. Many oil bodies are present around the nucleus in most cells. When cultured in the light on a growth medium lacking sucrose, moderately large plastids of variety of shapes are seen. The lamellar structural organization in them is normal and intact with well differentiated grana and intergrana lamellae and contain one or more starch grains.

Comments

This culture experiment has shown that embryos can be kept alive for up to 2 weeks in the dark on a growth medium containing sucrose. The results have also shown that the sucrose level (2%) used in the growth medium is not suitable for fine structural studies of the plastid, since it leads to an excessive accumulation of starch in the plastid, thus forming amyloplasts. The excessive accumulation of starch, causes either the destruction or complete disappearance of lamellar structure from the plastids and thus the desired developmental changes are not seen.

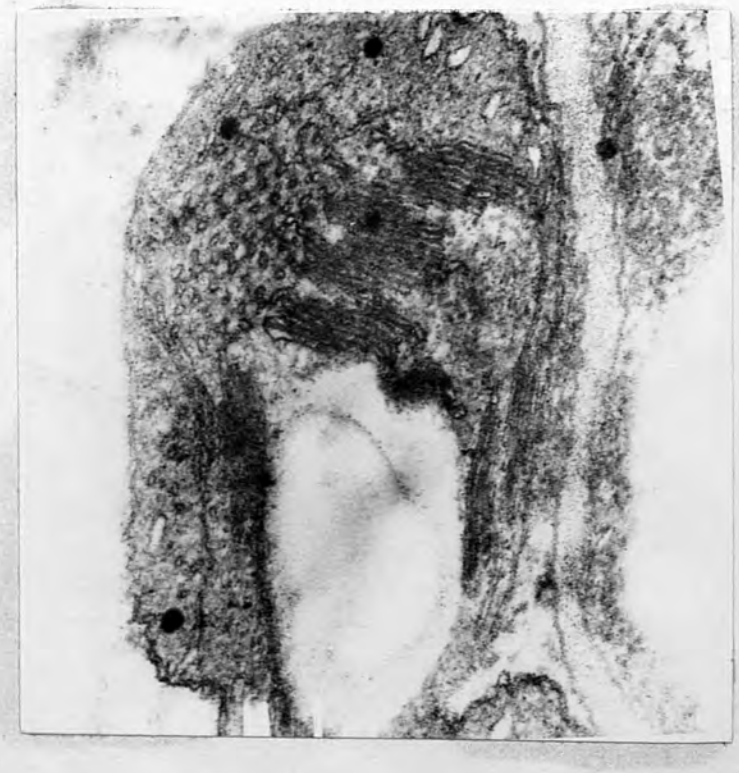
Since all the cells of embryos cultured in the dark on the growth medium without sucrose showed degeneration of almost all cell organelles including the plastids, the presence of sucrose is a basic requirement for the normal growth of the embryo in general, and plastids in particular, if cultured in the dark. Our main aim, therefore,would be to find out a suitable minumum concentration of sucrose, which should be just sufficient to keep the embryo alive in the dark and enable us to follow developmental changes in plastids.

When embryos are cultured on a growth medium without sucrose in the light, the plastids appear more or less normal in shape with normal lamellar structure and containing a reasonable quantity of starch. This suggests that plastids of such embryos which are grown under light can grow well on a growth medium with no external source of carbohydrate. The capacity of the embryo chloroplasts for normal photosynthetic activities seems therefore adequate to support the embryo's needs when excised from the ovule.

Plate 43. Plastid in 6-day cultured embryo (2% sucrose).

Fig. 80. Section through the cotyledon of embryo cultured in dark for 6 days on the growth medium with 2% sucrose. An elliptical etioplast with prolamellar body losing its regularity and show some loose tubules joining abnormal thick grana. Some infoldings of small vesicles from the inner membrane of etioplast envelope are seen. x 76,000

Fig. 81. Plastid section from the same embryo material as in Fig. 80. Spherical chloroamyloplast filled with starch grains with some lamellae and osmiophilic plastoglobuli. x 47,500



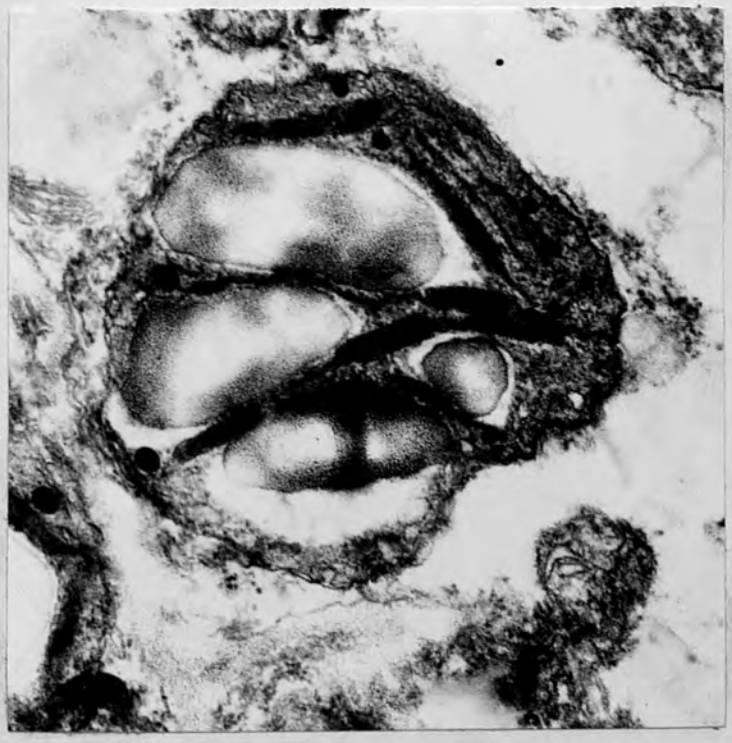
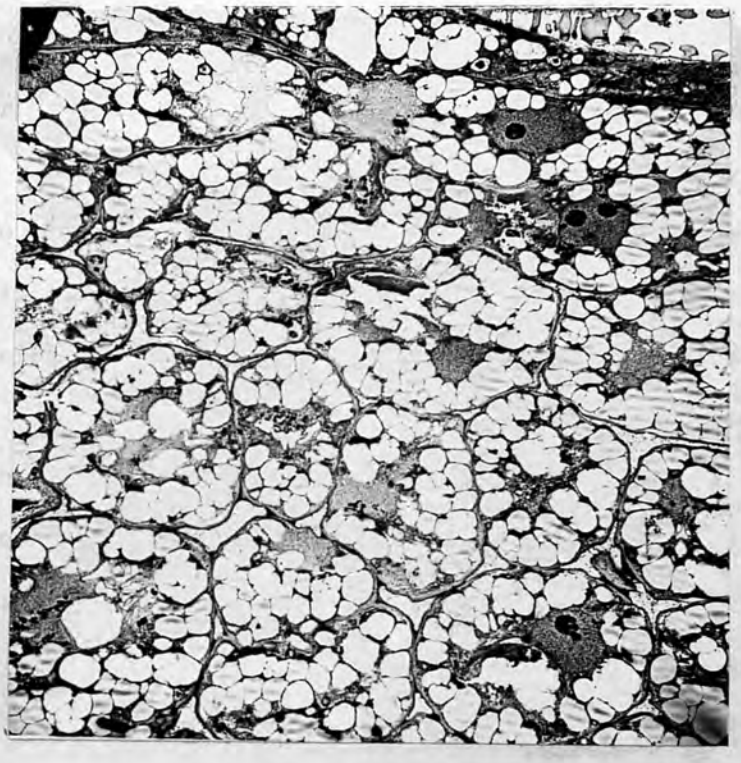
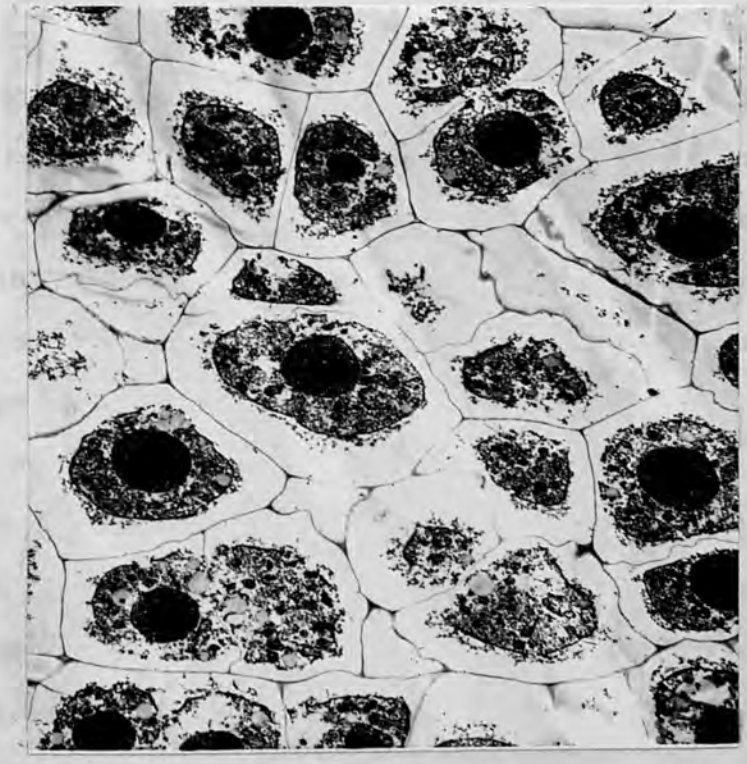


Plate 44. 11-day dark cultured embryo.

Fig. 82. Section through the cotyledon of embryo cultured in dark for 11 days on a growth medium with 2% sucrose. Cotyledonary cells are full of oil bodies and amyloplasts. x 1,900

Fig. 83. Section through the cotyledon of embryo cultured in dark for 11 days on a growth medium without sucrose. Cotyledonary cells show degeneration of cell organelles including plastids. x 2,850





2. Determination of suitable sucrose concentration in the culture medium.

This experiment was performed to find out the minimum sucrose concentration in the culture medium which would allow healthy growth of young (torpedo stage) embryos when cultured in the dark. We know from the preliminary growth and previous ultrastructural experiment of the present investigation that torpedo stage embryos do not show any growth in the dark when cultured on a growth medium lacking sucrose, but instead they show degeneration of tissues.

The previous culture experiment shows that when torpedo stage embryos are cultured in the dark on a growth medium containing 2% sucrose, they maintain good growth but with an excessive accumulation of starch in the plastids. This excessive accumulation of starch in the plastids is evidently due to the high level of sucrose used in the culture medium. This percentage of sucrose (Raghavan and Torrey, 1963) or even up to 12 or 18% (Rijven, 1952) has earlier been used for the growth studies of Capsella bursa-pastoris. The real problem arises for the study of plastid development, that is the formation of etioplast and development of etioplast under illumination at the fine structural level for which this concentration of sucrose in the medium is in excess of that actually required by the embryo. A lower concentration of sucrose in the culture medium should be sufficient for the normal and healthy growth of embryos (torpedo stage) in the dark but should not result in an excessive accumulation of starch in the plastids.

Two lower concentrations, 1% and 0.5% were tried in the growth medium. One growth study shows that torpedo stage embryos show a normal and healthy growth when cultured on a growth medium containing either 1% sucrose or 0.5% sucrose, both in light and in the dark (Table 6). The present study also recommends 1% sucrose in the culture medium for

for the growth studies of young (torpedo stage) embryos of Capsella bursa-pastoris as these embryos show maximum growth on the culture medium containing 1% sucrose as compared to either 2% (being used by earlier workers as well) or 0.5% sucrose in the culture medium (Table 6). The result of a squash test for starch, and electron microscopic studies of young torpedo embryos grown in these different culture media suggest the use of 0.5% sucrose in the culture medium. The starch accumulation in the plastids of embryos grown on a medium containing 0.5% sucrose is almost same when compared with the plastids of normal growing embryos. All further culture experiments on plastid development were therefore done using a culture medium containing 0.5% sucrose.

TABLE 6. Percentage growth in length of torpedo stage embryos of Capsella bursa-pastoris in the culture medium for 4 days containing different sucrose concentrations.

Sucrose concentration in medium	Percentage growth after 4 days	
	Light	Dark
2%	130.20	110.55
1%	219.06	160.98
0.5%	141.55	117.03

Comments

The results of the ultrastructural studies in the subsequent experiments show that this lower percentage of sucrose (0.5%) in the growth medium is very suitable for the study of plastid development as the fine structure of the plastids is very similar to those of the normal growing plants.

Prolamellar bodies appear in 4-day dark grown cultures of the embryo in this medium. This shows that the membranes of the chloroplasts behave in similar fashion to those of embryo chloroplasts in intact plants in which a 3-day dark period is required for the formation of prolamellar body. But in general, it is seen that the number of prolamellar bodies formed in *in vivo* studies are more, where they appear very well organized as compared to *in vitro* studies. However, the best growth of the prolamellar body is observed in 5-day dark culture experiment. While in the *in vivo* experiment the best growth of prolamellar body is seen in the etioplasts of the 4-day dark grown embryos. If the dark period is extended beyond 5 days, the etioplast develops many small circular tubules which are arranged in one row with no connection. So it is concluded that a 5-day dark period is the best for the study of development of etioplast of the embryo grown in a culture medium containing 0.5% sucrose.

3. Plastid development in cultured embryos on a medium containing 0.5% sucrose.

(i) Formation of etioplast and structure of prolamellar body

(a) 4-day dark culture

When young embryos of torpedo stage are cultured in the dark on a culture medium containing 0.5% sucrose, the formation of prolamellar body is first observed after 3 days (ie. 4 days in dark) in an occasional etioplast of the cotyledon. The prolamellar body is well organized and of the quasi-crystalline type. Majority of the prolamellar bodies are similar to the typical prolamellar body which has been interpreted to be a symmetrical crystal lattice whose basic unit is a "six-pointed star (star module) with four tubules fusing at each of the nodes" (Weier and Brown, 1970). The prolamellar body achieves maximum development and regularity after 4 days (ie. 5 days in dark) dark growth in culture.

(b) 5-day dark culture

The etioplasts of 5-day dark cultures demonstrate a large increase in the number of prolamellar bodies so that most etioplasts have an extensive prolamellar body. The prolamellar body consists of a central core built of an array of interconnected tubules which are arranged in the crystalline (Fig. 84) or concentric (Wehrmeyer, 1965) pattern. The prolamellar bodies in etioplasts show that the tubules that get branched and are regularly cross connected to form quasi-crystalline lattice are cut at various angles in different parts of the crystal. It is now known, that the pattern of tubules seen in sections of prolamellar body depends on the angle of sectioning and the thickness of the section, as

well as the regularity of the lattice (Ikeda, 1968; Weier and Brown, 1970). The effect of the thickness of section can be seen by comparing Fig. 84 with Fig. 86. In Fig. 86 and many other electron micrographs, some small circular profiles of interconnecting tubules appear to lack connection with other tubules. Such prolamellar body appear as zigzag lines and dots in linear fashion, which is formed as a result of breaking down of combination of hexagon (Ikeda, 1968). This pattern is explained as sections that are thin enough include only interconnecting tubules or only tubules which lie in the plane of peripheral lamellae (Weier and Brown,1970). In thicker sections these patterns are not seen but appear as rectihexagonal type, in which each hexagon is equivalent in size within a prolamellar body exhibiting a characteristic honey-comb like arrangement of tubules (ordered hexagonal). One of the electron micrographs (Fig. 87) demonstrates the combination of hexagons which are somewhat elongated to one direction (elongated hexagonal in one direction).

Majority of etioplasts show continuous or discontinuous lamellae radiating out into the stroma from the periphery of prolamellar bodies. Many of these peripheral radiating lamellae show stacking at places forming primary grana. These lamellae show at places short overlaps which at places appear as simple, short lamellae appressed to longer lamellae, or a single lamella may appear to double back on itself as in the formation of grana. Similar ways of grana formation has been reported in other species as well (Wehrmeyer and Röbbelen, 1965). These findings provide further evidence for the existance of extensive, if discontinuous, lamellar sheets in etioplasts. Concentric prolamellar bodies show very few or no lamellae radiating out from their periphery.

A dividing etioplast by the formation of a constriction in its middle is seen in which the two resultant etioplasts present either similar (Fig. 89) or an altogether different lamellar organization

(Fig. 88). In the latter case (Fig. 88) one of the resultant etioplasts shows a large starch grain, with normal flat lamellae and a few vesicles formed by the invagination of the inner membrane of the etioplast envelope. Second resultant etioplast contains a single crystalline prolamellar body of bi-pyramidal shape having equivalent hexagons exhibiting a honey-comb like arrangement. The peripheral radiating lamellae run from the periphery of the prolamellar body to the inner membrane of etioplast envelope and some of the radiating lamellae join it. These peripheral radiating lamellae alternate with a regular longitudinal row of small circular tubules (up to 10 in one row) with no interconnections. Such tubules have been recorded in earlier experiments but never in more than one row (up to 15) in an etioplast and these have never been seen alternating with flat lamellae as seen in the present experiment. Other undividing etioplasts also show similar structures (Figs. 85, 91). In one of the etioplasts peripheral radiating lamellae run around a big starch grain present at one end of the etioplast and a row of small circular tubules is present lying between the starch grain and lamellae surrounding it.

Longitudinal rows of small circular tubules alternating with flat lamellae radiating out from the periphery of crystalline prolamellar body are seen in some etioplasts (Figs. 85, 88). One of the electron micrograph demonstrate a prolamellar body which is half open and half crystalline with 3 peripheral radiating lamellae joining the inner membrane of etioplast envelope and alternating with tubules which are cut in different plane (possibly obliquely) and thus do not appear as circular structures. Another set of electron micrographs of etioplasts show an amorphous prolamellar body which is smaller, densely stained, almost circular in shape with peripheral radiating lamellae alternating with few small circular tubules (Fig. 90). Another electron micrograph demonstrate a completely transformed prolamellar body (reacted) with

peripheral radiating lamellae which alternate with small circular tubules are cut at two different angles and join other amorphous prolamellar body at other end. Some etioplasts which do not contain a prolamellar body demonstrate 2-5 long parallel rows of small circular tubules (up to 15 in a row) alternating either with single layered flat lamellae (Fig. 91) or with normally developed lamellar system in 4-5 rows. These small circular tubules when cut at different angle show cross connections with the lamellae with which they usually alternate in other etioplasts.

An electron micrograph of a recently divided etioplast (Fig. 84) demonstrates one large quasi-crystalline prolamellar body in each of the newly formed etioplasts with few peripheral radiating lamellae. Whole prolamellar body in both etioplasts shows a hexagonal outline which corresponds to the hexagonal arrangement of the tubules. One of the larger etioplasts shows the formation of large number of (15-16) long tubules cut lengthwise at one end away from prolamellar body. These tubules arise from the inner membrane of the etioplast envelope into the stroma and lie closely parallel to each other. Some of these tubules join either the outer periphery of the prolamellar body and/or the peripheral radiating lamellae. Some other etioplasts contain only such long tubules arising from the inner membrane of the etioplast envelope running deep into the stroma. Other lamellar structure is not seen in such etioplasts. It is presumed that such long tubules which are arranged parallel are produced either directly from the inner membrane of the etioplast envelope, or by the extensions of those vesicles produced by the invagination of inner membrane of etioplast envelope, as such vesicles are not found in that part of the etioplast where these long tubules are being produced and these vesicles are seen in other parts of the etioplasts where these tublules are not produced.

(c) 6-day dark cultures

The majority of etioplasts in 6-day dark grown embryo contain one ellipsoidal starch grain whcih show greater electron density at their periphery than at the centre and these starch grains are surrounded by electron-transparent haloes. Most etioplasts contain a well formed crystalline prolamellar body exhibiting a characteristic honey-comb like arrangement of tubules similar to those observed in the etioplasts of 5-day dark cultured embryos (Fig. 93). Several peripheral lamellae radiate out from the periphery of the prolamellar body into the stroma stacking at places forming primary grana.

A large number of infoldings of small vesicles invaginated from the inner membrane of the etioplast envelope are seen in many etioplasts scattered mostly near to the inner membrane of the etioplast envelope. One of the electron micrographs shows small vesicular invaginations and some flat finger-like invaginations which are not found commonly in plastids and both types of invaginations are contiguous to the grana and stroma lamellae (Fig. 92). Another etioplast of this micrograph shows the circular and dense protuberance of its envelope containing many vesicles produced from the inner membrane of the etioplast envelope (Fig. 92). This protuberance of the etioplast might give rise to mitochondria as this protuberance resembles mitochondria very much and thus supporting the observations of Wildman et al. (1962) that mitochondria-like bodies develop from the protuberances. Light microscopic and cinephotomicrographic studies of Wildman et al. (1962) also show the interconversions between chloroplasts and mitochondria-like bodies in living cells of mature leaf. Such plastid outgrowths is quite similar to the peripheral reticulum described by Laetsch and Price (1969) in the etioplasts of mesophyll cells of Saccharum officinarum; when they are illuminated for one hour, such etioplasts form lobes and protuberances

with a great deal of internal vesiculations.

Most etioplasts in 6-day dark grown embryos have one longitudinal row of small circular tubules (up to 15 in a row) present usually near to inner etioplast envelope membrane. Like microtubules, most of these small circular tubules show a dense wall and an inner transparent core. In some electron micrographs such tubules lying close to the inner membrane of the etioplast envelope appear to be cut obliquely to some extent and appear like several parallel lamellae arising from the inner membrane of the etioplast envelope and extending downward into the stroma of the etioplast (Fig. 92). This abnormally elongated etioplast does not show any other lamellar structure in it but a few osmiophilic plastoglobuli and some small vesicles and flat finger-like invaginations of the inner membrane of etioplast envelope are seen.

It is interesting to note that these circular tubules arranged in one vertical row in the etioplasts are more numerous in those etioplasts which have a well developed lamellar structure than from those etioplasts which contain prolamellar body. The relation between these small circular tubules (which are probably cross sections of long tubules and arranged always in a regular row not interconnected) with those of the interconnected tubules of a prolamellar body forming a lattice is not known. These etioplasts probably contain two types of tubules. One of the etioplast has a prolamellar body at one end and 4-5 small circular tubules at the other end lying close to the inner membrane of the etioplast envelope and this etioplast contains some lamellae as well stacking at places to form primary grana. The vertical row of small circular tubules is present on the outer face of a lamella facing the inner membrane of etioplast envelope and that part of the lamella is always thin or primary and never becomes stacked, at least in that part facing the row of tubules. The vertical row of small circular tubules is covered by very thin, incompletely formed lamellar structure formed

probably from the vesicles produced by the invagination of the inner membrane of etioplast envelope (Fig. 93).

(ii) Etioplast development under illumination

The 5-day dark cultured embryos were given brief, and longer, periods of illumination to white light to study the development of etioplast into chloroplast. The brief exposures are given with a hope to get some earlier stages of prolamellar body transformation before it is completely transformed.

(a) One-minute light

When 5-day dark cultured embryos are given 1-minute exposure to white light, most etioplasts of the cotyledons show a typical crystalline prolamellar body of hexagonal type. Occasionally the prolamellar body is in a stage of losing its regularity to become non-crystalline (Fig. 94). Thus the immediate effect of a brief white light illumination is the disruption of the prolamellar body, such that the tubules first become loosely and irregularly connected. Some etioplasts are dividing by constriction formation and others contain 3 vertical rows of small circular tubules alternating with flat normal lamellae of the etioplast similar to those observed in the etioplasts of 5-day dark grown embryos. Each etioplast may contain 1-3 transparent starch grains which are larger in some etioplasts.

(b) 15-minutes light

Most of the etioplasts of a 5-day dark cultured embryo when given 15 minutes exposure to white light show a well ordered hexagonal prolamellar

body exhibiting characteristic honey-comb like arrangement of tubules or different arrangememts as a result of thick or thin sectioning as has been observed earlier in the etioplasts of the cotyledons of 5-day dark cultured embryos. Other etioplasts show an almost transformed (reacted) prolamellar body containing loose tubules with all their connections broken (Fig. 95). Occasional etioplast shows only remnant of prolamellar body with well developed lamellae and big starch grains. Small circular tubules which are usually arranged in vertical rows alternating with flat lamellae in certain etioplasts are cut in a plane that they appear as small tubular structures connecting the flat lamellae on either side.

(c) 30-minutes light

Most of the etioplasts of 5-day dark cultured embryos when given 30 - minutes exposure to white light show the complete disruption of the crystalline prolamellar body resulting in a loose complex tangle of tubules (Fig. 96). The tubules become loose by the breaking down of all connections between them. Such prolamellar bodies have been designated as reacted prolamellar bodies by Weier and Brown (1970). Fig. 97 shows such tubules forming primary lamellae with occasional stacking. At the juncture of the peripheral lamellae with the prolamellar body, adjacent membranes of the prolamellar body associate to form overlaps and small grana. Both the membranes of the etioplast envelope are resolved in these etioplasts and meny vesicles are present near the inner membrane of etioplast envelope formed by the invagination of the inner limiting membrane. Some of the infoldings of small vesicles from the inner membrane of etioplast envelope are seen contiguous at places with the lamella and primary grana (Figs. 96, 97), suggesting that these are involved in the formation of plastid lamellae and grana. These etio-

plasts also contain several darkly stained osmiophilic plastoglobuli and most of them are attached to lamellae and primary grana suggesting that plastoglobuli are not always regarded as disintegration products of lamellar structure.

(d) One-hour light

Some of the etioplasts of 5-day dark cultured embryo, after one-hour exposure to white light, still show the prolamellar body in reacted state with several well developed peripheral radiating lamellae forming grana and also containing starch grains. Some reacted prolamellar bodies show only 4-5 tubules left as the others possibly have formed the lamellae. Few etioplasts show the remnant of a prolamellar body and well developed lamellar system. In other etioplasts a well developed lamellar system and starch grains similar to normal chloroplast is seen. Some electron micrographs show a peculiar way of etioplast fusion, where two etioplasts are fusing by the formation of a beak like projection formed by their envelopes (Fig. 102).

A conspicuous lobe or protuberance is formed in many etioplasts which have great development of internal vesiculation (Fig. 98) and is very much similar to the peripheral reticulum described for the etioplasts of Saccharum officinarum when illuminated for one hour with white light (Laetsch and Price, 1969). Another etioplast demonstrates two circular mitochondria like structures attached to the peripheral reticulum wall in a way as if they are being constricted from the peripheral reticulum (Fig. 99). A mitochondrion like outgrowth is seen attached to the jacket of one of the etioplasts (Fig. 100). This outgrowth which is full of vesiculations resemble very much to the mitochondria, suggesting the possible formation of mitochondria from chloroplast. Such possible interconversions of mitochondria and chloroplast has earlier

been suggested (Wildman et al., 1962; Vesk et al., 1965).

There is extensive formation of long tubules arranged in parallel order arising from the inner membrane of the etioplast envelope (Fig. 101). Such tubules are similar in number, length, and thickness, to those seen in the etioplsts which, after 2-minutes exposure to red light, were returned to dark for 2 hours. Some etioplasts are full of such long tubules and nothing else, and in such etioplasts long tubules arise from the inner membrane of plastid envelope, and extend through the stroma to the opposite end of the etioplast and some of these are contiguous with the inner membrane of other side. The formation of other type of tubules, that is, small circular tubules is not frequent. Occasional etioplast shows 2-3 vertical rows of such small circular tubules alternating with normal flat membranous lamellar sheets of the etioplast (Fig. 103) as observed in other treatments of the present study.

(e) Three-hours light

With continued illumination for about 3-hours the etioplasts have developed into the chloroplasts in which several adjacent lamellae associate to form extensive grana which look normal with respect to size and internal structure (Fig. 104). They possess grana stacks connected by stroma lamellae (fret membranes). These chloroplasts contain 1-4 small starch grains in the stroma in spaces between the lamellae. Remnants of the prolamellar bodies are also observed in occasional chloroplasts. Most chloroplasts are fully developed but instances of early stages chloroplast development are also seen (Fig. 105). Fig. 105 shows one chloroplast at very early stage of development showing several parallel sheets. Several incomplete lamellae are also being formed from the vesicles produced by the invaginations of the inner membrane of plastid envelope. Some of the lamellae (3-4) lie parallel to each other

in the centre of the chloroplast. This electron micrograph presents a very good evidence of lamellar formation from the vesicles being invaginated from the inner membrane of plastid envelope. Another plastid in the same electron micrograph shows only one or two parallel incomplete sheets of lamellae just near to inner membrane of chloroplast envelope. These developing chloroplasts also contain a few densely stained osmiophilic plastoglobuli which are either attached to the lamellae or primary granum or lie close to the lamellae. This suggests that plastoglobuli are not always regarded as disintegration product of lamellar structure. Some chloroplasts are seen dividing by the formation of constriction in the middle of chloroplast.

Comments

Before illumination the etioplasts of 5-day dark cultured embryo are more or less spherical in shape and contain well formed prolamellar bodies from which extend some single lamellae with occasional stacks. These lamellae of etioplasts have also been observed by other workers as has been summarised by Gunning and Steer (1975). As pointed out by Gunning and Jagoe (1967) when the etiolated leaves are illuminated, the prolamellar bodies become disorganized and the extending lamellae increase in number: this is also seen in the present results. But contrary to them, the tubules of prolamellar bodies do not dissociate into vesicles, as indicated in the present results similar to those reported by Weier et al. (1970). Our reacted prolamellar body is composed of tubules like that of crystalline prolamellar body before illumination. The whole processess, therefore, seem to be accompanied by the direct transformation of the tubules into the lamellae. Then, the primary grana are formed on the surface of extending lamellae by the appearance of 2 or more lamellae which are scattered around the reducing reacted prolamellar bodies. The prolamellar bodies of etioplasts in the present experiment are completely transformed after 3 hours of continuous illumination, when fully developed chloroplasts with well developed grana and stroma lamellae and some starch similar to the chloroplast of in vivo embryo chloroplast are seen. Although occasional chloroplast shows the remnant of transformed prolamellar body, the prolamellar body of the etioplasts are not retained in the present phase of plastid development under illumination. As plastid development proceeds, the plastids get transformed into the elongated ellipsoidal and lenticular shape. Both types are seen in single electron micrograph.

This study provides further evidence for the existence of extensive,

if discontinuous, lamellar sheets in etioplasts (Laetsch and Stetler, 1965; Laetsch and Price, 1969; Stetler and Laetsch, 1969; Weier and Brown, 1970; Weier et al., 1970; Boasson et al., 1972) although it does not support the view that vesicles form lamellar layers under the influence of light (von Wettstein, 1958; Virgin et al., 1963; von Wettstein, 1967). These lamellae increase in number after illumination, but do not appear to differ from those in the etioplasts.

The formation of longitudinal row of small circular tubules near to inner membrane of plastid envelope or outside the outer face of lamellae facing the inner membrane of plastid envelope, similar to those seen in the etioplasts of etiolated embryos of intact plants is noteworthy. Such vertical rows of small circular tubules are found more frequently in the etioplasts of this present in vitro experiment as compared to in vivo experiment. Like microtubules, many of the tubules show a dense wall and an inner transparent core. These structures appear to be cylindrical and the circular profile are probably cross sections of these long tubes. As these tubules are found usually in those etioplasts which do not contain prolamellar body, but lamellae, it can be presumed that these tubules are similar to those forming prolamellar body (crystalline aggregate of circular or hexagonal profiles) but are arranged in one row, not interconnected in any way to form a crystalline lattice as found in a prolamellar body. These tubules appear to be formed by the gradual contraction of extensive sheets of porous membranes into a regular ordering of tubules (Weier and Brown, 1970). This may be regarded as the first step in the formation of a prolamellar body, and the next step may be the development of interconnections of tubules forming a lattice. It is interesting to note that these tubules gradually disappear with the appearance of a prolamellar body in the same etioplast as in one micrograph of etioplast containing a small prolamellar body at one end, two to three such degenerating tubules are seen at other end near to inner plastid envelope membrane. On illumination,

the tubules disappear similar to those of prolamellar body and are used up to form lamellar system of the plastid. The tubules probably need a shorter period of illumination for their disappearance.

Results of the present experiment also show another interesting feature, that is, a mitochondria like protuberance of plastid jacket which appears to contain many small infoldings of vesicles produced by the invagination of the inner membrane of plastid envelope. Laetsch and Price (1969) have reported such lobes and protuberances with great deal of internal vesiculation formed from the etioplasts of mesophyll cells of Saccharum officinarum and called these "peripheral reticulum", as the protrusions arise from a network of peripheral tubules and vesicles. They found that the number of such lobes increased after the tissue has received one hour of illumination exactly similar to our results where many more instances of the formation of such lobes are observed in the etioplasts illuminated for one hour than in the etioplasts of 6-day dark cultured embryos. Laetsch (1970, 1971) described the peripheral reticulum as the only unique structure of the C_4-plant (plants which have high photosynthetic capacity and a low CO_2 compensation value) chloroplasts and may be associated with the carboxylation enzyme. Such reticulate system has also been reported in the chloroplasts of Zea mays, another C_4-plant (Schumway and Weier, 1967; Jacobson, 1968; Gracen et al., 1972a; Suzuki and Ueda, 1974).

It has been suggested that the peripheral reticulum is an adaptive character for free rapid transport of materials and related to transfer of energy from the granal membranes to the stroma and also to the cytoplasm (Laetsch, 1968; Rosado-Alberio et al., 1968). Since similar reticulate-system has also been observed in the chloroplasts of C_3-plants (Bisalputra et al., 1969; Hilliard and West, 1971; Taylor and Craig, 1971; Pallas and Mollenhauer, 1972; Gracen et al., 1972b),

we may postulate that the peripheral reticulum is not unique in the mesophyll chloroplasts responsible for C_4-pathway, and similar protuberance found in the etioplast of 6-day dark cultured embryo as well as in the etioplast of dark grown embryo illuminated for one hour, of Capsella bursa-pastoris (C_3-plant) may be regarded as peripheral reticulum. It remains to elucidate the function of peripheral reticulum in Capsella bursa-pastoris embryo and other C_3-plants. The chloroplasts either from the embryos of intact plants or from the leaves of Capsella bursa-pastoris do not show any peripheral reticulum at any stage of development. Gracen et al. (1972a, b) have investigated peripheral reticulum development in the leaves of C_3 and C_4 plants, and presume the involvement of the peripheral reticulum with the photorespiratory system and with the transport of photosynthate.

It was proposed that mitochondria arise from chloroplast by forming protuberances of the jacket of mature chloroplast (Wildman et al., 1962). The budding of material from the chloroplasts has been observed in the light microscope for many years (Wildman et al., 1962) and some evidence from electron microscopy on this apparent phenomenon has been obtained (Vesk et al., 1965).

According to Vesk et al. (1965), if chloroplasts give rise to mitochondria in mature leaf cells, then the organelles may exist in equilibrium, may have a dual potentiality at all stages of their existence, and may arise from a common precursor of dual potentiality: this is referred to as the "dynamic hypothesis".

It has been concluded by Vesk et al. (1965) that a "definite answer cannot be provided as the critical events lie beyond the resolution of the light microscope and electron microscope gives only static pictures". However, as emphasized by Wildman (1967), these changes in shape indicate that the surface of the chloroplast and the underlying stroma is dynamic and mobile. However, on the basis of our results it is

postulated that there is a possibility of the formation of mitochondria from the chloroplast through the formation of large protuberance of the chloroplast envelope called peripheral reticulum. But nothing can be said with certainty and this intriguing phenomenon merits further investigation.

Laetsch (1969) noted a similarity between the internal structure of the peripheral reticulum in chloroplasts of Amaranthus edulis and etioplasts of Saccharum officinarum and that of the large mitochondria, but it was later concluded (Goodchild, 1971) that the specialized mitochondria in C_4-plants are not extensions of the peripheral reticulum. Chapman et al. (1975) however, suggested strongly on the basis of their electron micrographs that some of the mitochondria could originate from peripheral reticulum of chloroplast in the bundle sheath cells of Amaranthus edulis and Atriplex spongiosa. The large vesiculated mitochondria-like bodies in the bundle sheath cells of these two plants have been shown to have mitochondrial activity by the cytochemical technique (Chapman et al., 1975). According to them the peripheral reticulum is a specialized mobile region of the chloroplast, capable of amoeboid-like movement; the ability of the peripheral reticulum to protrude, to partially envelop organelles like mitochondria and possibly to withdraw, can be reconciled with the apparent separation of particles observed by Wildman et al. (1962). Mitochondria could sit in what appeared to be a cup-shaped protrusion of the peripheral reticulum. Movement of these mitochondria away from the peripheral reticulum can give the impression that they are budded or segmented particles.

Membrane continuities have often been cited as evidence of the origin of one organelle from another (Crotty and Ledbetter, 1973). Although other supporting evidence for the possible origin of mitochondria-like structure from chloroplasts has been presented by Öpik (1963), but many other investigators have reservations (Maier and Maier, 1968). Later

investigators have pointed out the danger of making such interpretations based only on static electron micrographs. There is a possibility that such interconversion of organelles or some confluences between mitochondria and chloroplast have been induced by some unspecified variable in handling and preparation of the material. It is also possible that the phenomena are normal but are related to a peculiar physiological condition, or to a developmental situation atypical of more mature stages.

Since the prolamellar body is produced by the accumulation and contraction (or condensation) of perforated cisternae pinched off from the inner plastid membrane, it is irrelevant, for the process, whether the membrane material is freely dispersed in the stroma or aggregated in an organized structure. In both cases the greening is dependant upon the morphogenetic capabilities of the inner plastid membrane. In this respect the two membrnaes of the plastid envelope are rather different, since even in cases of anomalous lamellae formation in the space between the two limiting membranes, it is the inner membrane which is responsible for the initiation of such lamellae (Schötz and Diers, 1966). The developmental studies have shown that invaginations and vesicles formed form the inner membrane of the envelope are apparently involved in the development of the grana-fretwork system (reviewed by Kirk and Tilney-Basset, 1967).

Invaginations of the inner membrane of the envelope are observed very frequently in the plastids in the present study, mostly of small vesicular type and occasionally of flatter finger-like invaginations lying at the periphery of the plastids, some of them still connected to the inner membrane of the organelle, while others connected to the growing grana. These observations suggest that such vesicles could also contribute to the formation of grana by apposition to the growing structures irradiating from the prolamellar body. Often the invaginations are confluent with the inner membrane of the envelope at two or more places. Schötz and Diers (1967), from serial sections, have

reported that the invaginations are plate-like structures which enclose small enclaves and pockets of stroma.

In the present study, it becomes evident that a significant difference exists between the *in vivo* and *in vitro* studies of plastid development in the *Capsella bursa-pastoris* embryo. These are the early appearance of prolamellar body in the etioplasts of embryo on whole plants which are more frequent and more compact than in the excised embryos, the occurrence of a row of small circular tubules which are more frequent in the *in vitro* experiments and more developed grana formation in the *in vivo* plastids. *In vivo* studies demonstrate certain features in the plastids, which are not developed in the plastids of embryos on whole plants. These are the formation of regular vertical rows of small circular tubules in the stroma, alternating regularly with the flat lamellae of the plastids which may in some cases arise from the periphery of prolamellar body; the formation of large number of long parallel tubules arising from the inner membrane of plastid envelope and sometimes dominating the whole plastid stroma, and also the formation of mitochondria-like protrusions of plastid jacket.

These observations of the present investigation can be explained on the basis that plastid developmental sequences which occur in whole plants are fixed and possibly under the control of internal mechanisms. It seems clear, however, that the plastids of excised embryo have the capacity to follow different developmental pathways dependent on the environmental conditions and some of these changes are reversible.

Plate 45. Etioplast in 5-day dark cultured embryo (0.5% sucrose).

Fig. 84. Etioplast section from the cotyledon of embryo cultured in dark for 5 days on a growth medium containing 0.5% sucrose. Etioplast which is possibly dividing with crystalline prolamellar bodies hexagonal in outline, showing ordered hexagonals. A group of long tubules arranged parallel (→) arising from the inner membrane of etioplast envelope into the stroma is seen. x 47,500

Fig. 85. Etioplast section from the same embryo material as in Fig. 84. Etioplast with crystalline prolamellar body hexagonal in outline with several peripheral radiating lamellae alternating with one vertical row of small circular tubules (→). x 47,500

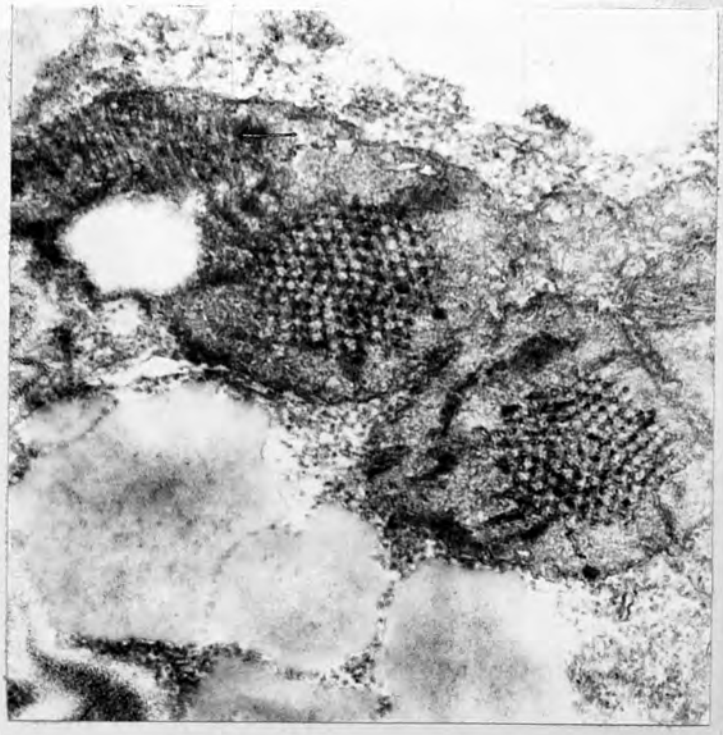
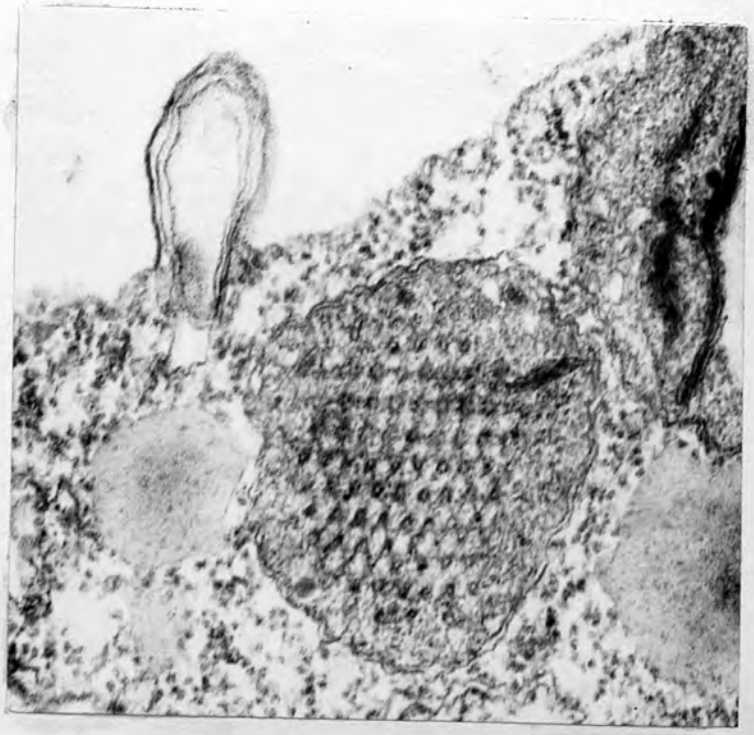


Plate 46. Structure of prolamellar body in 5-day dark cultured embryo.

Fig. 86. A thin etioplast section from the cotyledon of embryo cultured in dark for 5 days on a growth medium containing 0.5% sucrose. Prolamellar body appears as zigzag lines and dots in linear fashion as a result of breaking down of combination of hexagon due to thin sectioning. x 75,000

Fig. 87. Etioplast section from the same embryo material as in Fig. 86. Etioplast with a crystalline prolamellar body of more or less hexagonal outline showing elongated hexagon in one direction. x 52,000



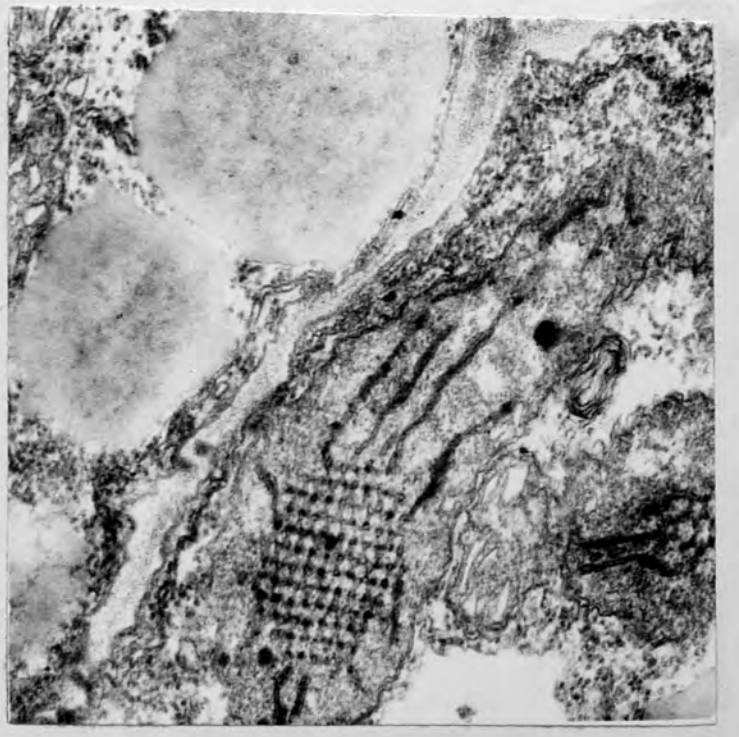
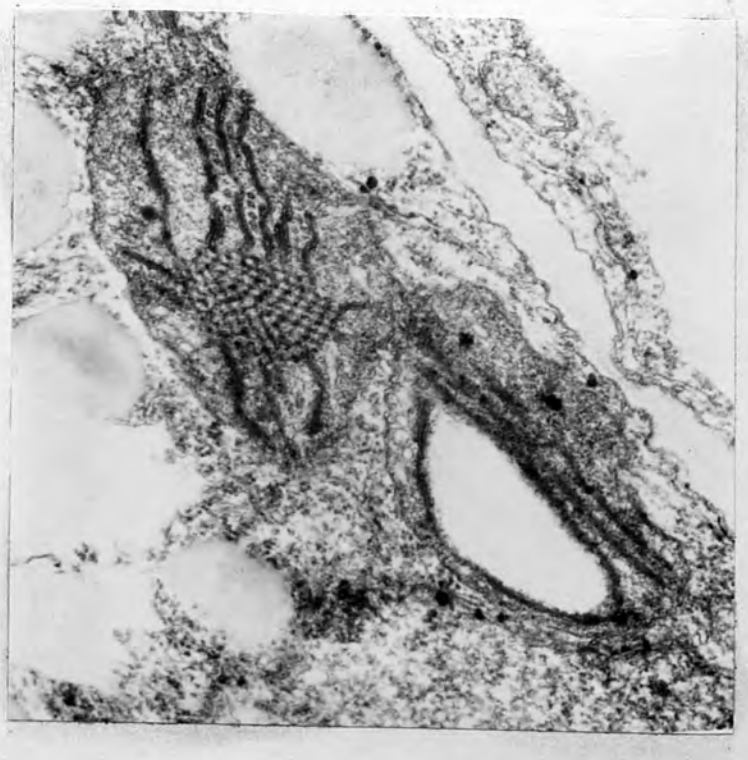


Plate 47. Dividing etioplasts in 5-day dark cultured embryo.

Fig. 88. Etioplast section from the cotyledon of embryo cultured in dark for 5 days on a growth medium containing 0.5% sucrose. In a dividing etioplast an invagination has been formed midway along the etioplast axis. The two resultant etioplasts show dissimilar lamellar organization. x 39,000

Fig. 89. Etioplast section from the same embryo material as in Fig. 88. A dividing etioplast in which the two resultant etioplasts show similar lamellar organization. x 47,500



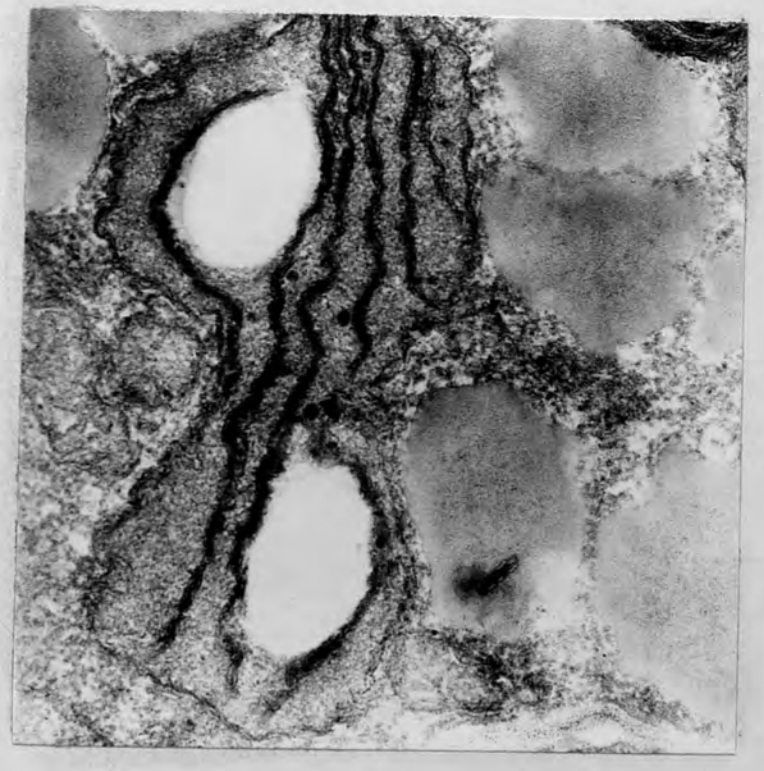
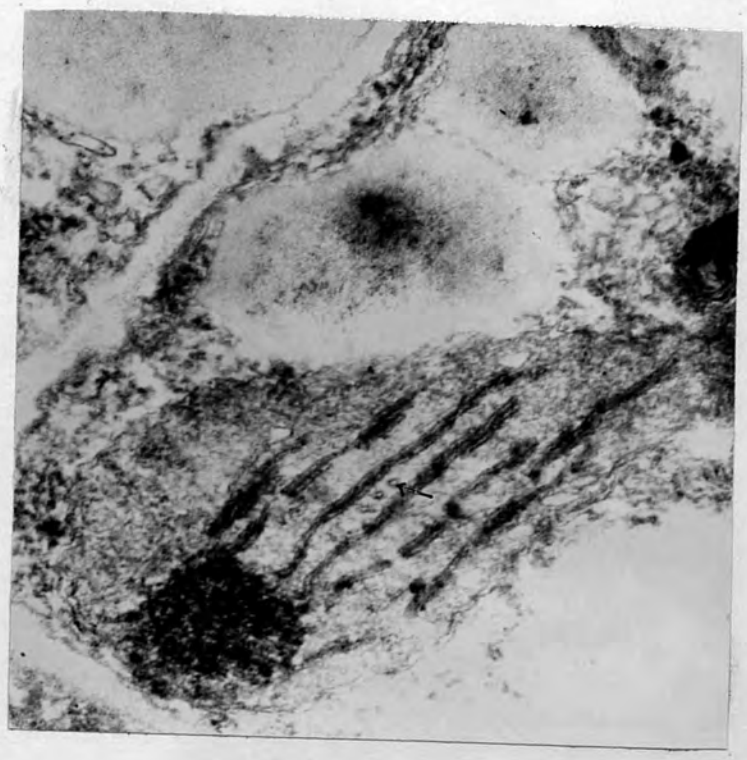


Plate 48. Small circular tubules in 5-day dark cultured embryo.

Fig. 90. Etioplast section from the cotyledon of embryo cultured in dark for 5 days on a growth medium containing 0.5% sucrose. An etioplast with amorphous prolamellar body with peripheral radiating lamellae. One row of small circular tubules (→) alternates with the peripheral radiating flat lamellae.
x 64,000

Fig. 91. Etioplast section from the same embryo material as in Fig. 90. Etioplast with two vertical rows of about 15 small circular tubules alternating with single layered flat lamellae. No prolamellar body is seen in this etioplast.
x 72,000



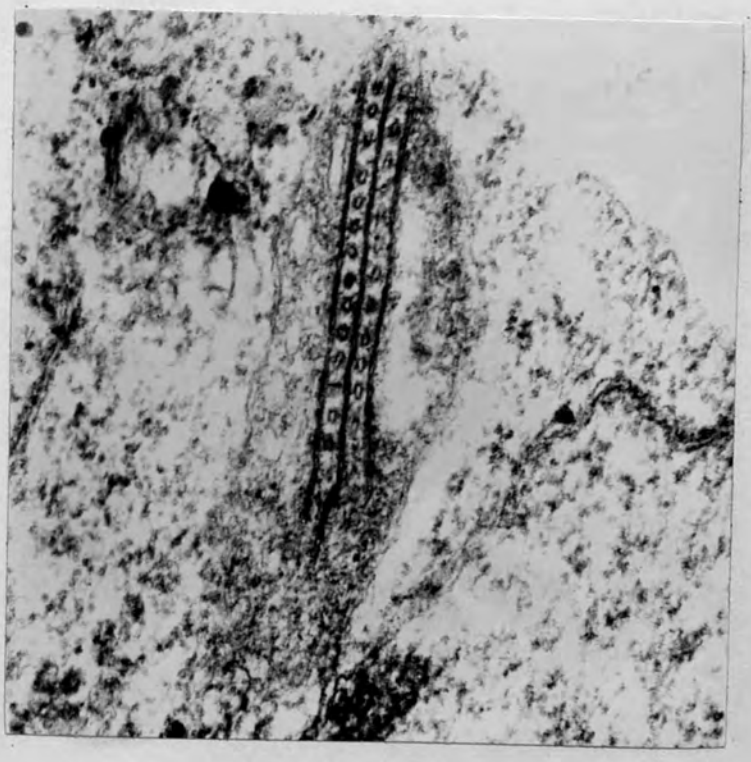
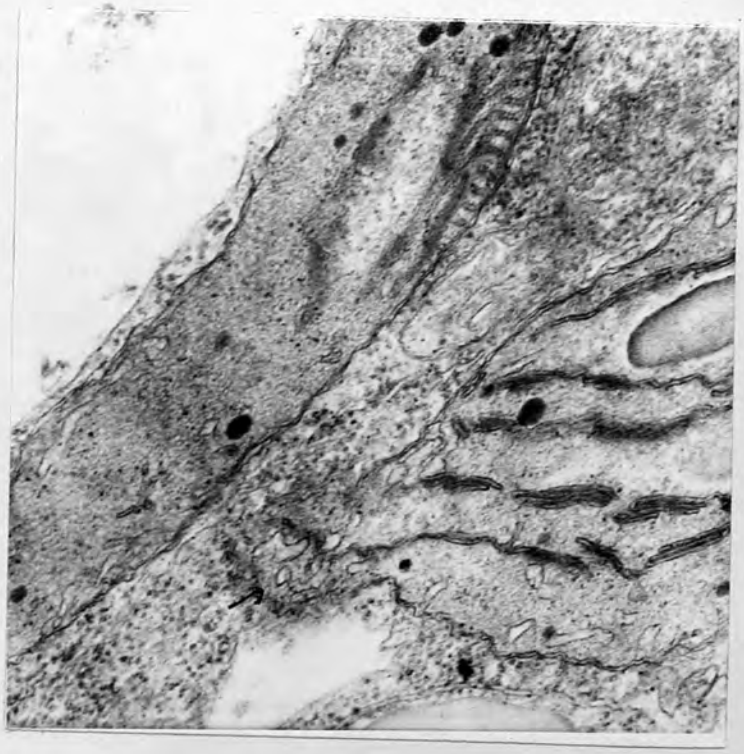


Plate 49. Etioplasts in 6-day dark cultured embryo (0.5% sucrose).

Fig. 92. Etioplast section from the cotyledon of embryo cultured in dark for 6 days on a growth medium containing 0.5% sucrose. Large number of infoldings of small vesicles and finger-shaped from inner membrane of etioplast envelope are seen. Some of these infoldings are contiguous with grana and others fill the protruberance of the plastid envelope- the peripheral reticulum (→) Abnormally long adjacent etioplast shows only a vertical row of small circular tubules cut obliquely near to inner membrane of etioplast envelope. x 52,000

Fig. 93. Etioplast section from the same embryo material as in Fig. 92. Etioplasts with crystalline prolamellar body and a vertical row of 5 small circular tubules (→). x 39,000



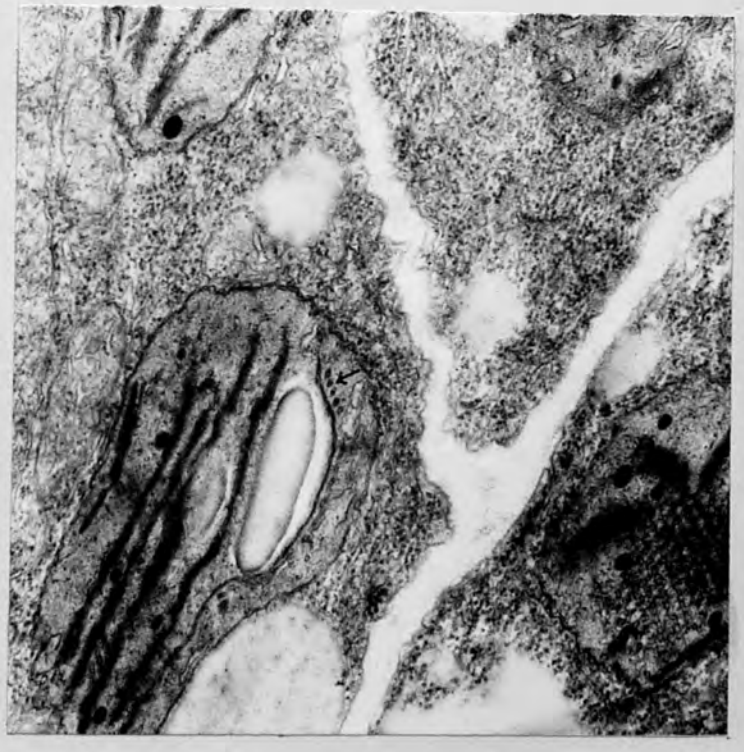
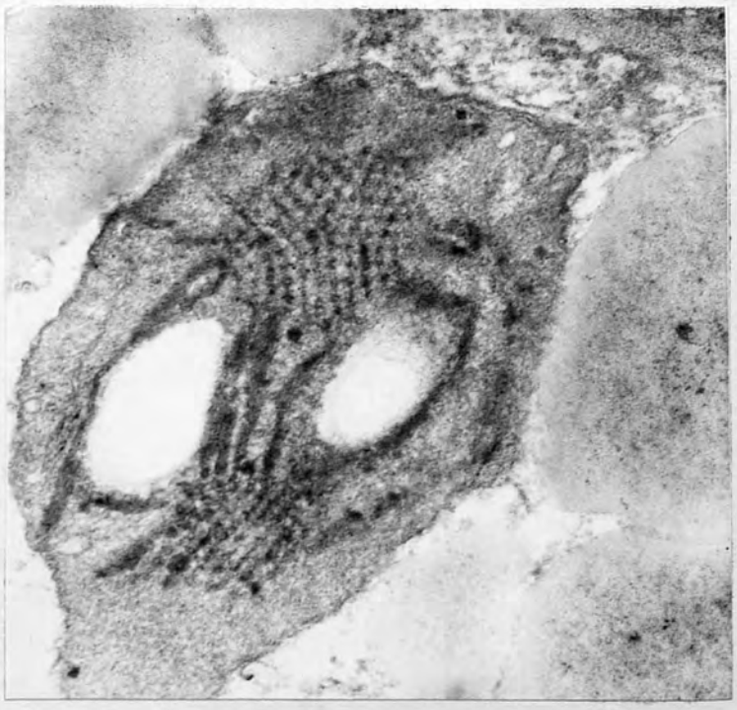


Plate 50. Effect of brief illumination on the prolamellar body of dark cultured embryo.

Fig. 94. Etioplast section from the cotyledon of embryo cultured in dark for 5 days and then exposed to white light for 1 minute. The etioplast shows early stage of disruption of two crystalline prolamellar bodies. x 52,000

Fig. 95. Etioplast section from the cotyledon of embryo cultured in dark for 5 days and then exposed to white light for 15 minutes. The tubules in the prolamellar body have changed from the crystalline to the transformed configuration. x 48,000



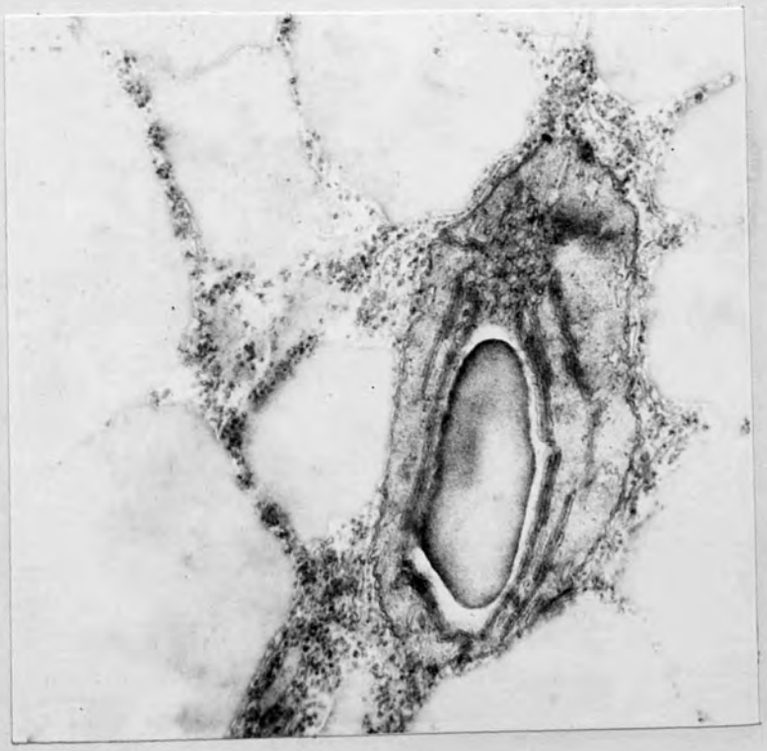
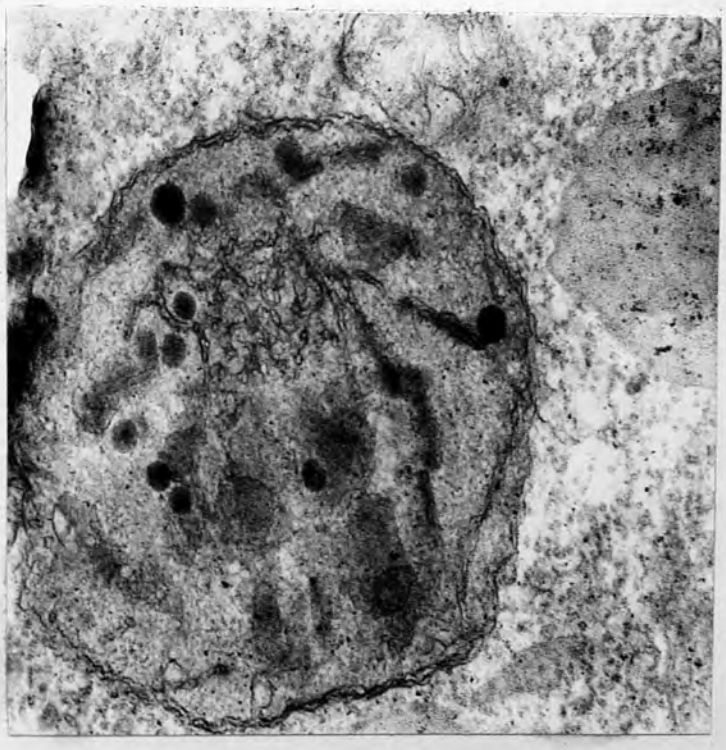


Plate 51. Effect of 30-minutes exposure on the prolamellar body of dark cultured embryo.

Fig. 96. Etioplast section from the cotyledon of embryo cultured in dark for 5 days and then exposed to white light for 30 minutes. Spherical etioplast with a reacted prolamellar body in which tubules have changed from the crystalline to the transformed configuration thus consisting of loose complex tangle of tubules. Some tubules are forming lamellae. Top and side views of lamellae are seen. Densely stained osmiophilic plastoglobuli most of which are contiguous with lamellae are also present. Double membrane of the etioplast is resolved and inner membrane shows some infoldings of small vesicles. x 76,000

Fig. 97. Etioplast section from the same embryo material as in Fig. 96. The tubules in the prolamellar body show complete transformed configuration and a more loose complex tangle of tubules as compared to Fig. 96 is seen. The formation of lamellae from tubules and their occasional overlapping and stacking is clearly seen. Two membranes of etioplast envelope are resolved and large number of infoldings of small vesicles from the inner membrane are seen, some of which are joining the lamellae and primary grana. Osmiophilic plastoglobuli are also seen some of which are contiguous with lamellae and primary grana. x 47,500



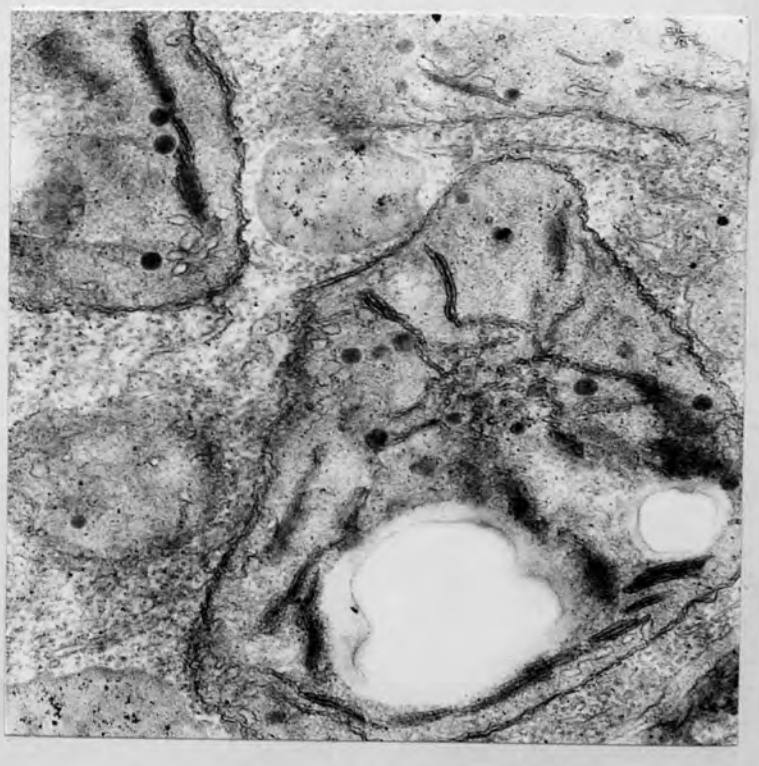
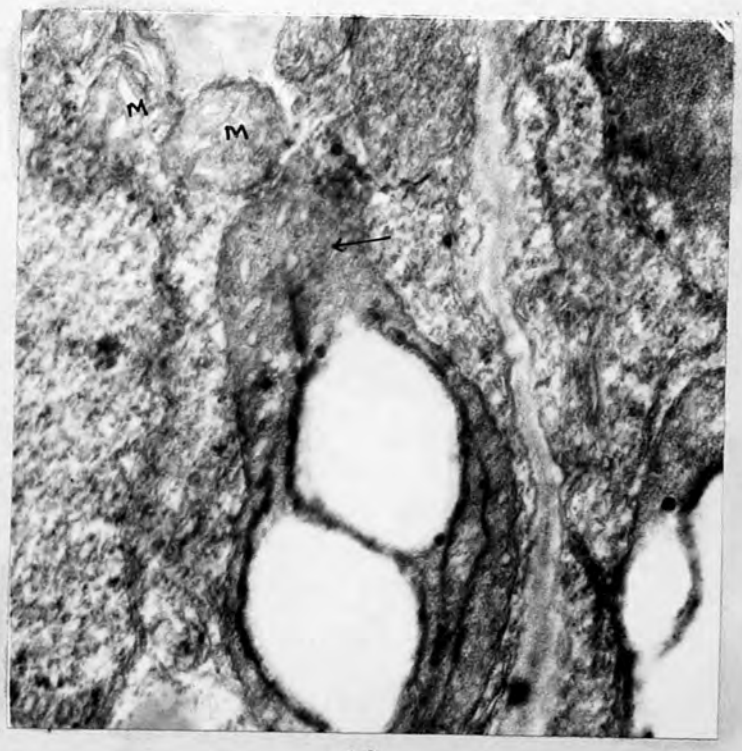


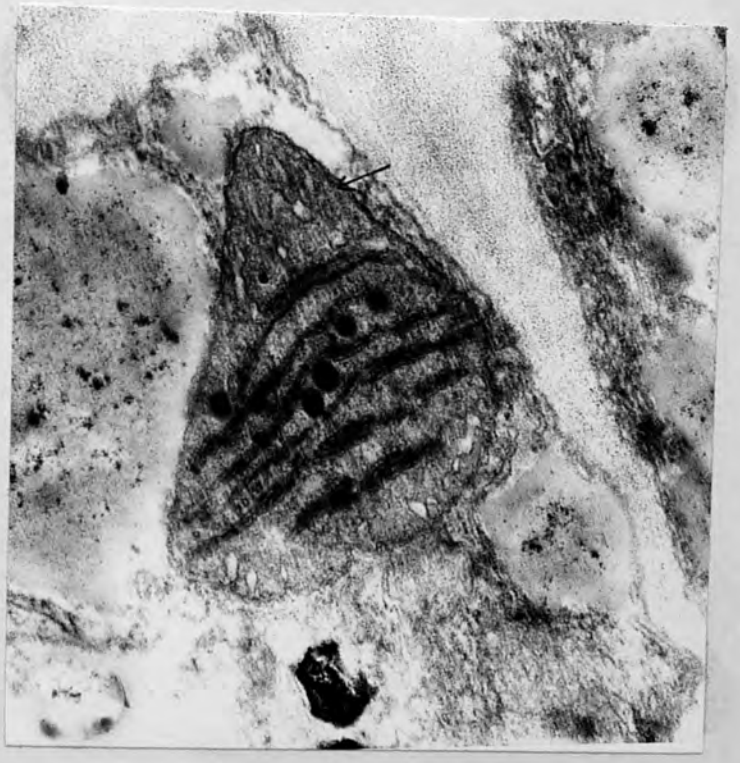
Plate 52. Effect of 60-minutes exposure in the etioplast of dark cultured embryo.

Fig. 98. Etioplast section from the cotyledon of embryo cultured in dark for 5 days and then exposed to white light for 60 minutes. Etioplast shows a conspicuous protuberance (→) with great development of internal vesiculation, and such protuberances is regarded as peripheral reticulum. x 47,500

Fig. 99. Etioplast section from the same embryo material as in Fig. 98. Two circular mitochondria (M) like structures appear as if being constricted off from the peripheral reticulum (→) of the etioplast. x 39,000



99

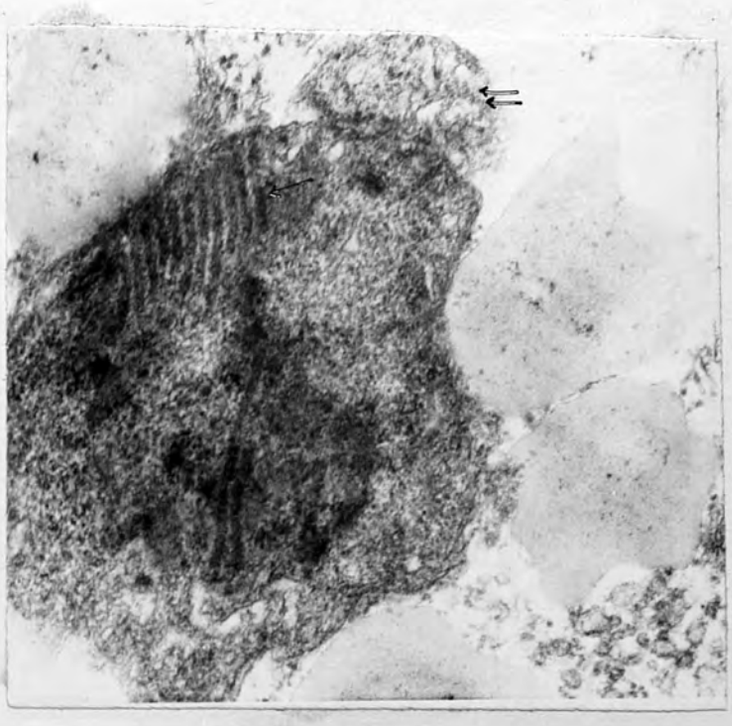


98

Plate 53. Long parallel tubules ~~containing~~ in etioplasts of dark cultured embryos exposed to light for 60 minutes.

Fig. 100. Etioplast section from the cotyledon of embryo cultured in dark for 5 days and then exposed to white light for 60 minutes. Etioplast shows large number of long tubules (→) arising parallel from the inner membrane of etioplast envelope running into the stroma. Large number of infoldings of small vesicles from the inner membrane of envelope are seen only in that part of the etioplast where long tubules are not being produced. A small circular mitochondria like structure with great development of internal vesiculation is seen contiguous with the etioplast envelope (→) x 52,000

Fig. 101. Etioplast section from the same embryo material as in Fig. 100. Etioplast with large number of long tubules (→) arranged parallel as in Fig. 100 shows a conspicuous protuberance (→) with no development of internal vesiculations. x 78,000



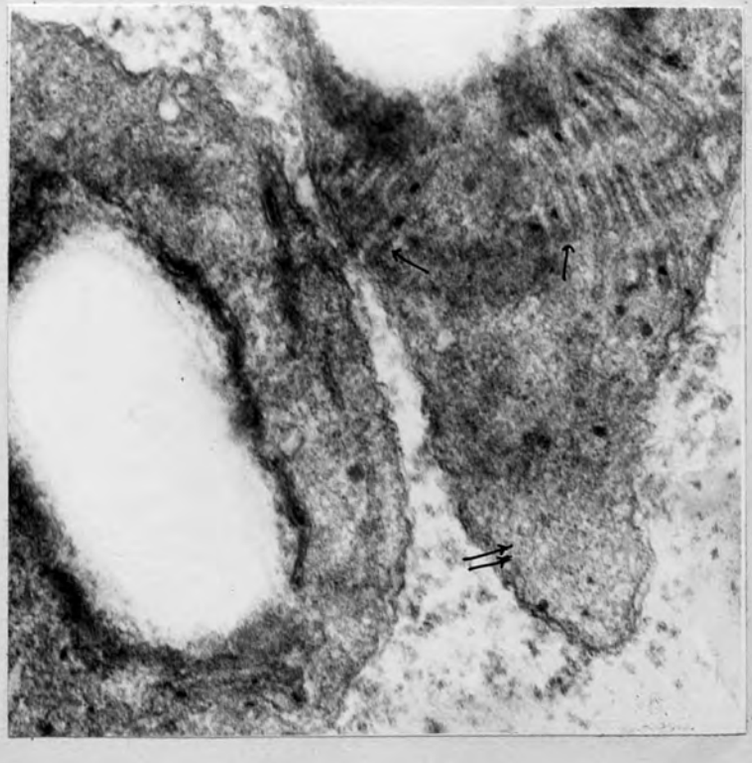
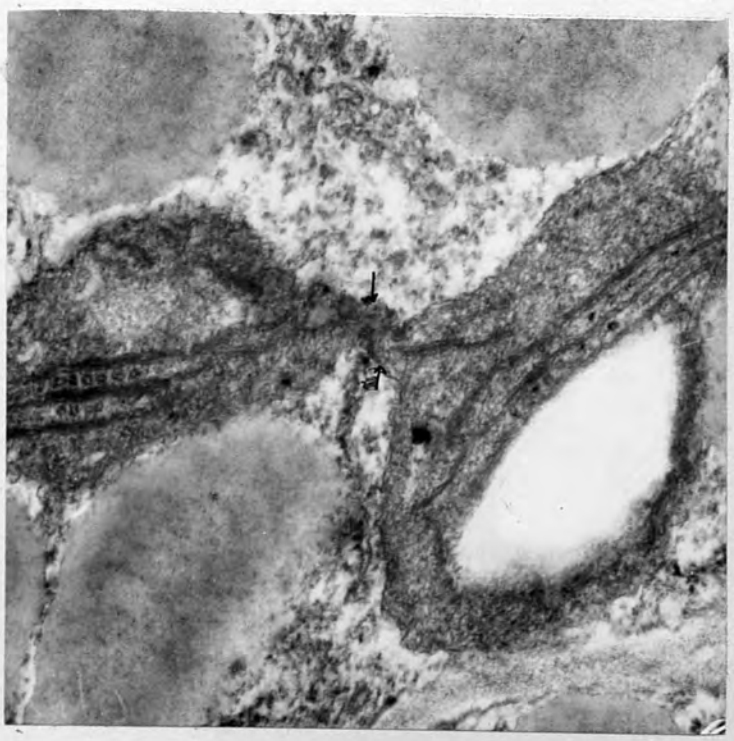


Plate 54. Small circular tubules ~~containing~~ in etioplasts of dark cultured embryo exposed to light for 60 minutes.

Fig. 102. Etioplast section from the cotyledon of embryo cultured in dark for 5 days and then exposed to white light for 60 minutes. Two etioplasts showing a peculiar fusion by the fusion of beaks like protrusion (→). One of these etioplast shows 2 rows of small circular tubules alternating with flat lamellae and in the other etioplast these small circular tubules are probably cut obliquely. x 52,000

Fig. 103. Etioplast section from the same embryo material as in Fig. 102. Three vertical rows of about 15 small circular tubules (→) alternating with flat lamellae are seen in this etioplast. x 52,000



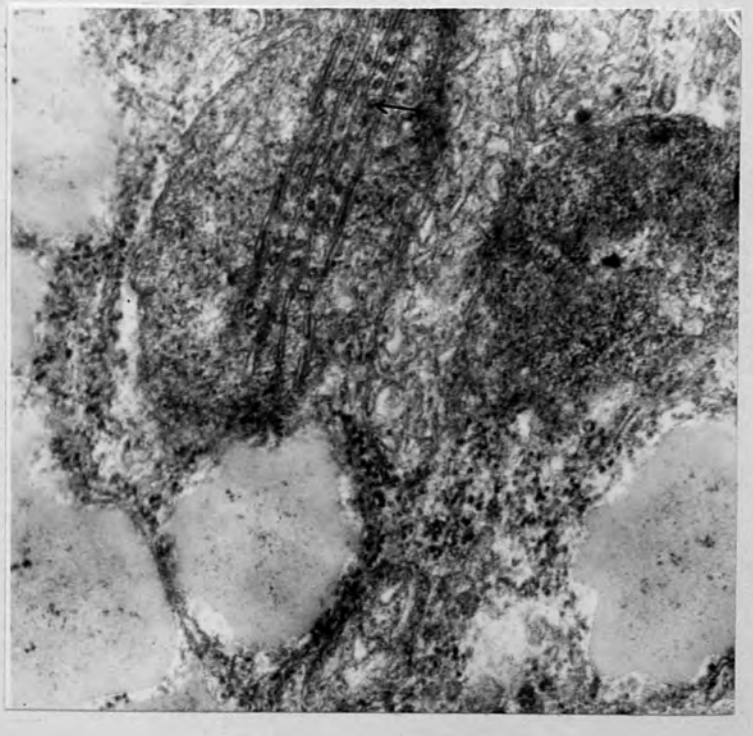
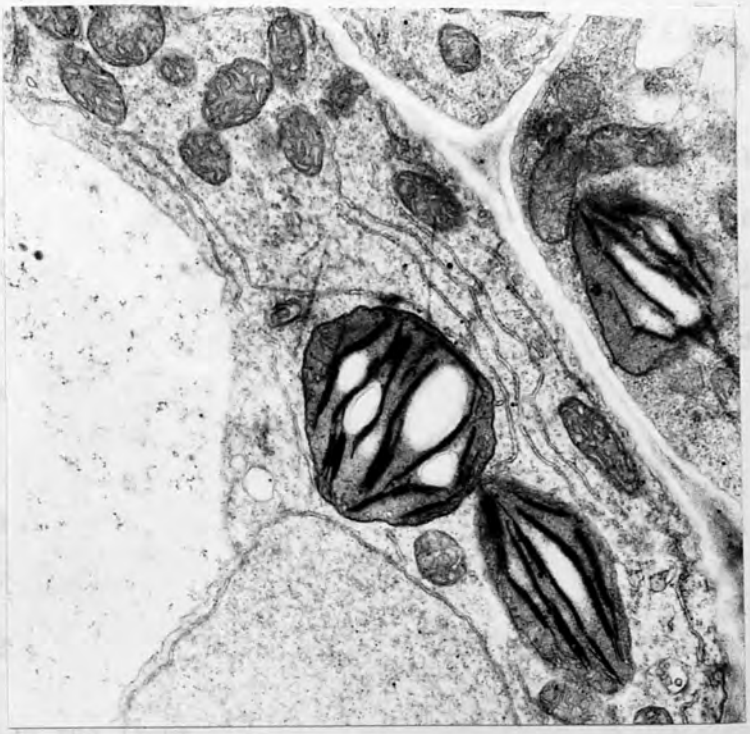
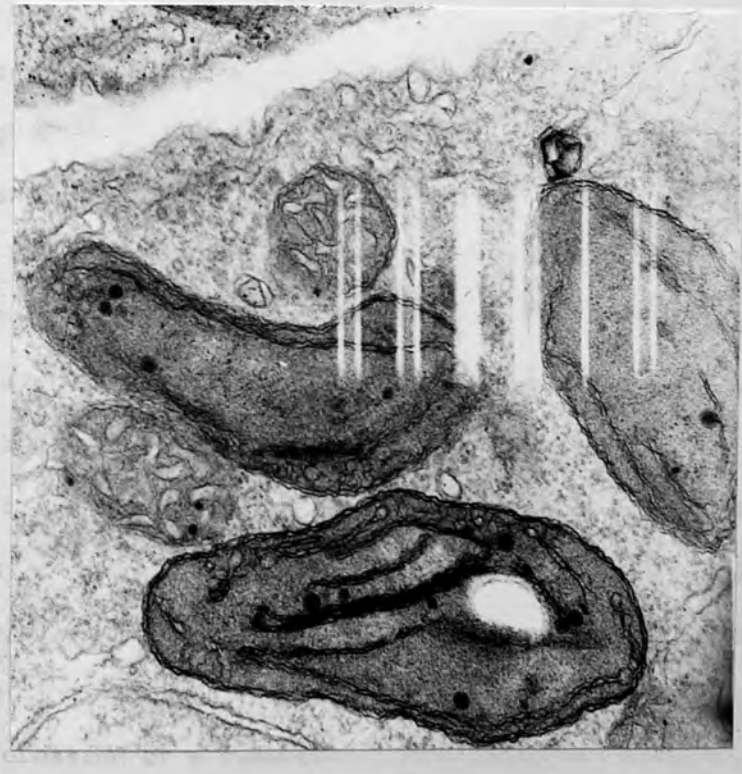


Plate 55. Effect of long illumination on etioplast development of dark cultured embryo.

Fig. 104. Chloroplast section from the cotyledon of embryo cultured in dark for 5 days and then exposed to white light for 3 hours. Fully developed chloroplasts with well developed grana and intergrana lamellae and some starch grains are seen. x 19,000

Fig. 105. Chloroplast section from the same embryo material as in Fig. 104. Chloroplasts are in early stages of development. Two membranes of chloroplast envelope are resolved. The production of parallel sheets of lamellae from the inner membrane of envelope are seen. The involvement of the infoldings of small vesicles from inner membrane of envelope in lamellae formation is also seen. x 47,500





4. The effect of light of different wavelengths on the plastid development in the cultured embryo.

We have determined on the basis of our results of earlier culture experiments that 5-day dark period and 0.5% sucrose in the growth medium give the best results for the formation of prolamellar body in the etioplast of the young embryo. This experiment is performed on the basis of these findings.

(A) Etioplast development under illumination with red light.

(i) Disruption of the crystalline prolamellar body.

(a) Two-minutes red light.

The etioplasts of 5-day dark cultured embryos when given 2-minutes exposure to red light, show prolamellar bodies which are either crystalline or more or less crystalline and are in a stage of losing their regularity to become non-crystalline. Thus the immediate effect of a brief red light illumination is the disruption of the crystalline prolamellar body, such that the tubules first become loosely and irregularly connected. This effect is similar to that as has been observed in the etioplasts of 5-day dark cultured embryos exposed to 1-minute white light. One etioplast shows one half of the prolamellar body where tubules have retained crystalline configuration (crystalline prolamellar body), while in the other part of the prolamellar body the crystalline configuration is lost (reacted or open prolamellar body). It is seen in most etioplasts that the crystalline lattice of the prolamellar body becomes loose as a result of disruption and this is the first step towards the formation of a non-crystalline (reacted) prolamellar body

from a crystalline prolamellar body. The crystalline prolamellar bodies are extensively developed occupying larger part of the etioplast, and have few peripheral radiating lamellae with occasional stack to form primary granum. An occasional etioplast contains a comparatively smaller but almost circular densely stained amorphous type of prolamellar body with greater number of peripheral radiating lamellae forming grana and alternating with a row of small circular tubules. Increase in number of lamellae in such etioplasts can be explained as a result of transformation of some of the tubules into the lamellae. Occasional etioplast with no prolamellar body show a lamellar system with few primary grana in different stages of formation showing at places short overlaps which at places appear as simple, short lamellae appressed to longer lamellae, or a single lamella may appear to double back on itself forming grana.

Etioplasts demonstrate the formation of tubules. Some etioplasts with extensively developed crystalline prolamellar body also contain either a group of long tubules arranged parallel arising from the inner membrane of etioplast envelope or 2-3 vertical rows of small circular tubules alternating with flat lamellae. But the etioplasts with a large crystalline prolamellar body and a group of long parallel tubules are more frequent. Vertical rows of small circular tubules alternating with flat lamellae are usually present in etioplasts containing no prolamellar body. Certain etioplasts have become exceptionally long maximum up to 3.2 μm long (2.5 μm long in 5-day dark cultures) showing a crystalline prolamellar body and alternating rows of small circular tubules. Long etioplasts show various stages of division by the formation of constriction in their middle. Etioplast elongation and division is possibly stimulated with short exposure to red light.

(b) 15-minutes red light.

The etioplasts of 5-day dark cultured embryo when exposed to red light for 15-minutes show prolamellar bodies which are either in a stage of losing their regularity or have almost lost their regularity through transformation from crystalline to non-crystalline form. Occasional etioplast shows small, circular densely stained amorphous form of prolamellar body with well developed peripheral radiating lamellae and starch grains. Vertical rows of small circular tubules alternating with flat lamellae, and long tubules arranged parallel and some dividing etioplasts are seen but not as frequently as in earlier treatments. Moreover these two types of tubules are found only in those etioplasts which do not contain prolamellar body.

(c) 30-minutes red light.

Rapid disruption of prolamellar body in the etioplasts of 5-day etiolated embryos which starts just after 2-minutes exposure to red light appears to be complete with 30-minutes exposure to red light. Remnant of transformed prolamellar body is seen in occasional etioplast which also shows two rows of alternating small tubules connecting transversly with flat lamellae with which they alternate. Such transversly connected tubules with flat lamellae or small circular alternating tubules are also seen frequently in etioplasts. Long parallel tubules arising from the inner membrane of etioplast envelope into the stroma are also seen in occasional etioplasts. Occasionally an etioplast has transformed into a chloroplast which has almost normal lamellar organization and a great number of peripheral vesicles formed by the invagination of the inner etioplast envelope membrane. One set of etioplasts show a group of densely stained broken particles of various shapes (Fig. 106) which

presumably are formed by the breaking up of the plastid lamellae including starch. The nature and significance of such structure is not clear at present. This cannot be an artifact as other organelle structures are normal in the same electron micrograph. However, these appear somewhat similar in structure to the phloem plastids containing fibrils and stringy starch in granular matrix of Pinus resinosa (Neuberger and Evert, 1974).

(ii) Reformation of crystalline prolamellar body.

Etioplasts of 5-day dark cultured embryos given 2-minutes exposure to red light, and then returned to dark for 2 hours, show a return to a crystalline prolamellar body condition (Fig. 107). A hexagonal crystalline prolamellar body is found in most etioplasts following the treatment. The etioplasts appear exceptionally long (up to 3.8 μm long) and assuming various shapes (Fig. 108). Such elongated etioplasts also show an excessive formation of long tubules arranged parallel from the inner membrane of etioplast envelope into the stroma and some of these tubules join the tubules of prolamellar body. Such long tubules are more frequently seen following the present dark treatment than in earlier treatments described above. These long tubules are very conspicuously developed in this treatment as these increase in number, thickness (Fig. 109), and frequency, and are seen arising from the different parts of the inner membrane of etioplast envelope. This excessive production of parallel elongated tubular ingrowths of the inner membrane of etioplast envelope has initially been stimulated possibly by the brief red light exposure. The formation of alternating rows of small circular tubules is similar to as found in the etioplasts after 2-minutes red light treated cotyledons (Fig. 107). Some etioplasts

still retain reacted prolamellar body.

One of the electron micrograph presents a rare phenomenon of fusion of plastids in higher plants in which 3 etioplasts are fusing together lying around the nucleus (Fig. 110). This is one of the ways in which the plastid number in a cell is regulated, besides the division of plastids. The number of chloroplasts per cell falls in at least some leaves in autumn (Stearns and Wagenaar, 1971) and fusion of plastids has earlier been reported in higher plants (Esau, 1972). Dividing etioplasts are also seen in occasional electron micrographs (Fig. 111).

We have seen complete disruption of prolamellar body in the etiolated cotyledons by 30-minutes exposure to red light. When these embryos are returned to dark for 2 hours prolamellar body reformation occurs, which appears to be almost complete within 2 hours, as etioplasts show the formation of a crystalline prolamellar body hexagonal in outline with few peripheral radiating lamellae. Nevertheless alternate rows of small circular tubules are still seen in some of the etioplasts similar to those found in the etiolated cotyledons exposed for 30 minutes to red light.

(B) Etioplast development under illumination with blue light.

The 5-day dark cultured embryos were given 30-minutes, 60-minutes and 90-minutes exposure to blue light to study the etioplast development into the chloroplast. Brief exposures similar to red were not given as it is known that the blue light effects on the development of etioplast are slow (Rosinki and Rosen, 1972).

(a) 30-minutes blue light.

When given 30-minutes exposure to blue light the etioplasts of the

cotyledon either show transformed (reacted) prolamellar body with peripheral radiating lamellae extending into the stroma up to the inner membrane of etioplast envelope and stacked at places to form grana; or fully formed chloroplast with well developed lamellar system. Such plastids also contain smaller or bigger transparent starch grains lying in the interlamellar spaces in the stroma. Most etioplasts contain several very big starch grains. One of such etioplast, which is also probably dividing, contains four starch grains in each of the resulting etioplast, a transformed prolamellar body in the middle with few peripheral radiating lamellae and a large number of densely stained plastoglobuli. Plastoglobuli are also seen in other plastids which have fully developed lamellar system with grana and no prolamellar body, but their number is more in those plastids where prolamellar body is transformed and lamellae are not yet formed. An occasional etioplast shows one to two rows of small circular tubules alternating with flat membranous lamellar sheets in the etioplasts which contain a large starch grain and lesser lamellae. Long tubules arranged parallel arising from the inner membrane of plastid envelope are seen very occasionally.

(b) 60-minutes blue light.

The etioplasts of 5-day dark cultured embryo when given 60-minutes exposure to blue light, contain either a normal lamellar system or prolamellar body in the transformation stages. Most of the etioplasts contain 3 regular vertical rows of small circular tubules (maximum 20 in one row) alternating regularly with flat membranous lamellar sheets either radiating out from the periphery of the transforming prolamellar body (Fig. 112) or lying free in the etioplast alternating with similar rows of tubules (Figs. 114, 115). Other etioplasts show these tubules cut in different plane so that they do not appear as circular but flat

more or less similar to normal flat lamellae which are darkly stained and radiating out from the transforming prolamellar body (Fig. 113). Thus such etioplasts show flat densely stained lamellae radiating out from prolamellar body which alternate with 3 faintly stained flat lamellae like structures, nearer to inner membrane of plastid envelope. Occasional etioplast shows long tubules arranged parallel arising from the inner membrane of etioplast envelope and group of such elongated tubules is seen in one of the dividing etioplast lying nearer to a crystalline prolamellar body.

Dividing etioplasts are not uncommon. Osmiophilic plastoglobuli are not very conspicuous and starch grains are very small in the etioplasts of the embryo of this treatment as compared to etioplasts of embryo exposed to 30-minutes of blue light.

(c) 90-minutes blue light.

When 5-day dark cultured embryos are given 90-minutes exposure to blue light, most of the plastids in the cotyledon show normal development of lamellar system forming longer grana of 4-5 stacks interconnected with intergrana lamellae (Fig. 116) and each may contain big and small starch grains (Fig. 117) filling the plastid or may not contain any starch grain (Fig. 116). Plastids contain few osmiophilic plastoglobuli.

Many plastids still retain transforming prolamellar body with well developed peripheral radiating lamellae, and other plastids demonstrate 3-4 rows of small circular tubules alternating with flat normal lamellae (Fig. 116). Others have large numbers of long tubules arranged parallely to the inner membrane of plastid envelope running into the stroma of plastid which does not show any other lamellar structure or just a few loose tubules of prolamellar body.

Dividing etioplasts are not uncommon and one of the dividing etioplast has transforming prolamellar body with peripheral radiating lamellae in one of the resultant etioplasts and 2 rows of small circular tubules alternating with flat lamellae in the other. This dividing etioplast also contains several small vesicles formed by the invagination of inner etioplast envelope membrane.

Comments.

Our results support the view that chloroplasts are autonomous self-duplicating bodies (Frey-Wyssling and Mühlethaler, 1965; Kirk and Tilney-Basset, 1967). The plastids increase in number by the division of proplastids, by the transverse constriction of chloroplast, by the growth of an invagination of the inner envelope membrane right across the chloroplasts, and also by budding the chloroplasts.

Most plastids divide by the formation of a constriction in the mid-region. However, this division appear to be stimulated by brief red light exposure especially when such treated embryos are returned to dark. Such plastids become exceptionally long before the constriction appears in their mid-region. In general dividing plastids are more numerous in embryos exposed to red and white light.

The effect of red light appears analogous to white light as both result in the disruption of the prolamellar body and both stimulate the extensive formation of long tubules in the etioplasts. Effect of blue light is however, analogous to that of dark which stimulate the extensive formation of small circular tubules in 2-4 vertical rows alternating with flat membranous lamellar sheets. It appears that morphogenetic capabilities of the inner membrane of plastid envelope is exceptionally enhanced in the cultured embryos resulting in the excessive production of tubules. The inner membrane of plastid envelope may form THREE types of tubules:

1. Large number of small circular tubules arranged regularly in vertical rows which alternate with the flattened lamellae extending mostly from the periphery of the prolamellar body. These tubules are not inter-connected.

2. Excessive production of long tubules arising from the inner membrane

running into the stroma in a parallel order, sometimes completely filling the plastids. These tubules are also not interconnected.
3. Other type of tubules which gets pinched off from the inner membrane accumulate and contract to form a regular arrangement- the prolamellar body. The prolamellar body is formed by the development of interconnections between these tubules. This type of tubules are formed in all plastids, and can be called as normal type tubules.

The plastids usually contain one type of tubules, or less frequently two types of tubules lying separately in the plastid and rarely a plastid is seen to have all three types of tubules.

The closest approach for such tubular structures is the tubular extensions from the prolamellar bodies of isolated etioplasts reported in the recent years (Wellburn and Wellburn, 1971, 1973; Wrischer, 1973; Kohn and Klein, 1976; Wellburn et al., 1977). The formation of long tubules have also been reported in intact etiolated leaves mainly under conditions unfavourable to plastid development (Wettstein and Kahn, 1960; Eriksson et al., 1961).

Wellburn and Wellburn (1971, 1973) reported that in isolated Avena etioplasts light induced the transformation of the crystalline prolamellar body into 'long' tubules. Short illumination results in the emergence of the long straight tubules from the prolamellar body into the stroma, but instead the expected immediate conversion into perforated lamellae, the tubules persist. In longitudinal section they are seen to emerge from the dispersing prolamellar body in regular rows. In transverse section the tubules are seen to be alternate and the spacing between the individual tubules corresponds to the tubule spacing in the quasi-crystalline prolamellar body. With continuous illumination the tubules become less distinct and are not observed in plastids illuminated for 3 hours. Wrischer (1973) reported that after exposure to light for

one hour all prolamellar bodies in isolated maize etioplasts contained long, straight tubules which disappeared after 2-3 hours illumination with strong light. Light may favour their formation but under certain conditions they also occur in the dark (Wrischer, 1973; Kohn and Klein, 1976). But the tubules observed in the present investigations show different structure and arrangement and are developed both in the darkness as well as in light, although these become less distinct and eventually disappear in plastids illuminated for 2-3 hours.

The tubular structures cannot be regarded as part of normal plastid ultrastructural development. This phenomenon appears to be of an abnormal nature probably as a reaction to a changed environment (Wrischer, 1973) and is not likely to be inherent to normal plastid development.

The formation of various types of tubules in the plastids of cultured embryos of Capsella bursa-pastoris might be the result of a reaction to the changed environmental conditions as the excised embryos experience an artificial medium rather than a natural medium of intact plants. During the present investigations, however, various types of plastid tubules could never be observed in the control material, ie. in intact etiolated leaves and embryos either in darkness or in light, similar to the findings of those working with isolated plastids. The various types of tubules are observed only under two conditions, i) when the excised embryos (containing plastids) are cultured on an artificial culture medium as observed in the present investigations, and, ii) in isolated plastids which come in direct contact with isolation medium as reported by others. It is very probable that the appearance of tubules in the plastids is dependent on the composition of either the culture medium or the isolation medium. The fact that the inner membrane of plastid envelope of cultured embryo demonstrate an excessive production of tubules, while the embryo plastids from the intact plants

only have one type of tubule may be a result of differences in the nutritional status due to growth on the artificial medium.

Certain unknown chemicals in the culture medium might stimulate such abnormal growth of plastid tubules in the plastids of cultured embryos of Capsella bursa-pastoris. Under the influence of such unknown factor (possibly-sucrose) the membranes of the plastids cannot reorganize quickly and efficiently resulting in the formation of several types of tubules in the plastids. The present investigations, with media containing high and low concentrations of sucrose have shown that the extensive formation of several types of plastid tubules can be decreased by increased concentration of sucrose in the medium. In the experiments performed with medium containing 2% sucrose, only occasional plastids show only one type of tubule formation- small circular tubules only in one row. In other experiments performed with medium containing 0.5% sucrose, majority of plastids show the extensive formation of tubules of one or more than one type in each plastid. Wrischer (1973) also reported that the percentage of isolated etioplasts with long tubules was decreased when a media containing higher concentration of sucrose was used.

Gunning (May 1977, personal communication), believes that the formation of such tubular structure observed in the present investigation is likely to be a sign of degeneration rather than a normal stage of development. Kohn and Klein (1976) also believe the formation of long tubules as a result of the deterioration of the isolated prolamellar body. Since these tubules are also formed in darkness, Wrischer (1973) suggests this formation is an abnormal way of differentiation of the prolamellar body.

Brief red light treatments lead to rapid disruption of all prolamellar bodies followed by their reformation in the subsequent dark period

regaining its crystallinity. Disruption of prolamellar body which starts with 2-minutes exposure to red light is completed by 30-minutes red light exposure. Brief light treatments might bring about an immediate change in the chemical constitution of the membranes making up the prolamellar body (Berry and Smith, 1971).

Our results show the reformation of crystalline prolamellar body which appear slightly larger than dark control prolamellar body, when brief red light treated embryos are returned to darkness. Berry and Smith (1971) found that short red light treatment induced the crystallization of prolamellar body in the dark grown leaves of Hordeum vulgare. They found that brief red-light treatments lead to rapid disruption of all prolamellar body followed by a slow reformation in the subsequent dark period. They also found a marked increase in the proportion of crystalline prolamellar body when red light treatments were given with intervening 3 hours dark period. Gunning and Jagoe (1967) also found the reattainment of 100% crystallinity when the prolameller body reformation occur in the etioplasts of Avena sativa when given red light treatment and then returned to darkness. A considerable reformation of prolamellar body is also reported in the etioplasts of leaflets of Pisum sativum (Treffrey, 1973) when given similar treatments with brief red treatments.

Bradbeer et al. (1974b) reported a similar increase in prolamellar body volume per plastid but with much greater increase in the number of prolamellar bodies per plastid section, than in the dark control. It seems possible that after complete disappearance of the prolamellar bodies, recrystallization of prolamellar body material during subsequent darkness may occur at a greater number of loci than the original number of prolamellar bodies in the plastids.

Brief exposure (30 minutes) to red light resulted in our experiments

to the fragmentation of the lamellae. Similar structures have been reported in the sieve cell plastids of Pinus. During the normal course of development of the sieve cell in Pinus strobus (Srivastava and O'Brien, 1966) and Pinus resinosa (Neuberger and Evert, 1974) the plastids present various stages of breakdown resulting into collapse or distortion of the internal lamellae. Neuberger and Evert,(1974) have reported most of the plastids occupied by fibrils and a stringy to amorphous substance which is interpreted by them as starch. Even Parameswaran (1971) reported the presence of both intact an ruptured plastids in mature sieve cells of Pinus silvestris needles. Eventually the 'filaments' set free from ruptured plastids formed a net-like structure in the cell interior. Parameswaran considered this condition to be normal and suggested that the free plastid filaments could be considered 'Plasmafilamenten'. Harris (1972) has also reported that, as the sieve cells in the leaves of Pinus strobus and Picea abies become mature, the plastid envelope ruptures and the fibrils and starch grains become free in the lumen of the cell.

In the blue light treatment the majority of the prolamellar bodies in the etioplasts retained the transformed configuration. Prolamellar bodies almost get transformed even after the 30 minutes of blue light treatment. The number of grana in the plastids under blue light is higher than under red. Similar effect of blue light on formation of lamellar structures of the chloroplasts in greening plants has been reported in greening pea seedlings (Vlasova and Drozdova, 1971) and in Phaseolus vulgaris (Bradbeer et al., 1974b).

Plate 56. Effect of 30 minutes red light exposure on the etioplast of dark cultured embryo.

Fig. 106. Plastid section from the cotyledon of embryo cultured in dark for 5 days and then exposed to red light for 30 minutes. An etioplast with a group of densely stained broken particles of various shapes formed possibly by the breaking up of the lamellae. Other plastid with normal lamellae and big starch grain is seen. x 19,500

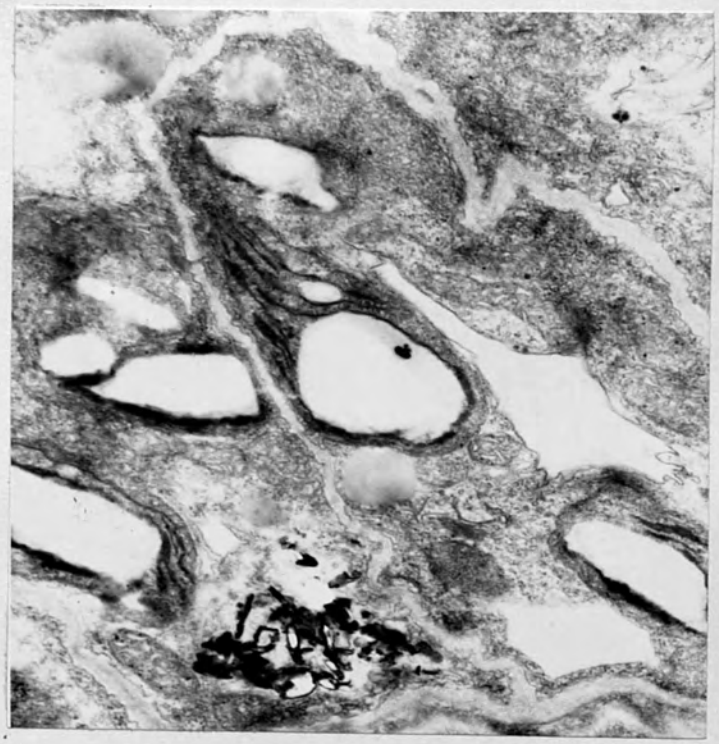
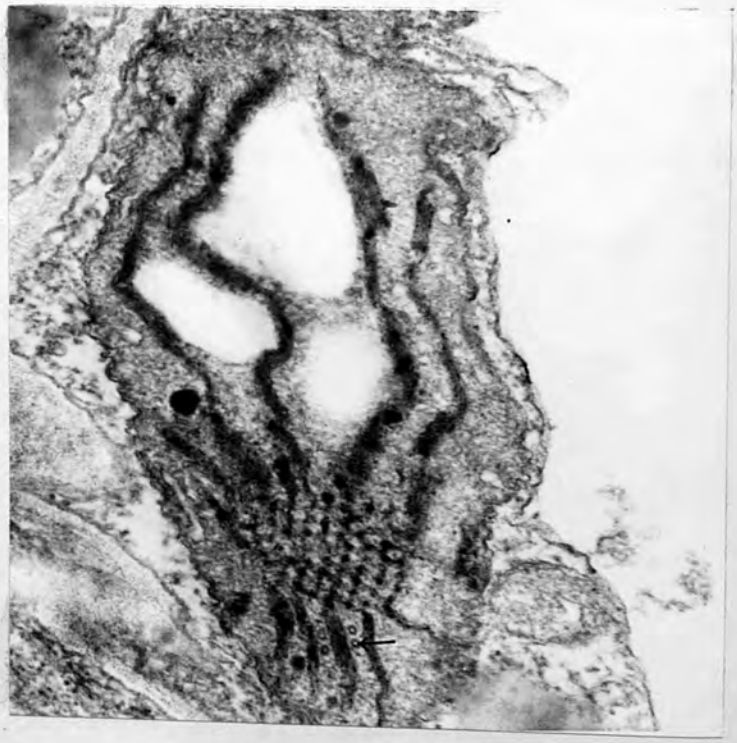


Plate 57. Effect of red light on the formation of small circular tubules in the etioplasts of dark cultured embryo.

Fig. 107a. Etioplast section from the cotyledon of embryo cultured in dark for 5 days, exposed to red light for 2 minutes and then returned to dark for 2 hours prior to fixation. Etioplasts with reformed crystalline prolamellar body and vertical rows of few small circular tubules (→) alternating with flat lamellae radiating out from the prolamellar body. x 52,000

Fig. 107b. Etioplast section from the same embryo material as in Fig. 107a. Some peripheral lamellae stack to form a thick granum on the periphery of crystalline prolamellar body. Two vertical rows of small circular tubules (→) alternating with peripheral radiating lamellae are also seen. x 52,000



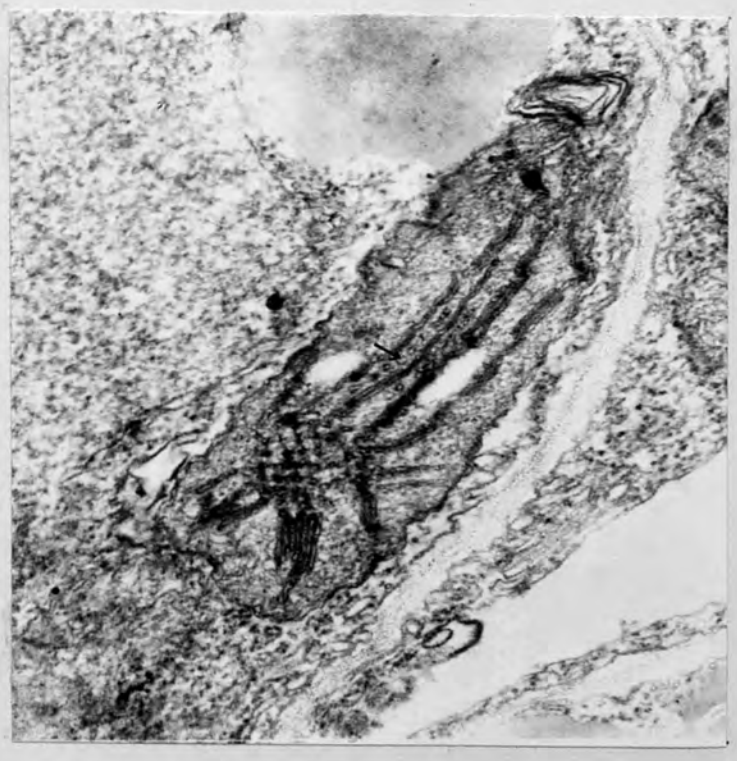


Plate 58. Effect of red light on the formation of long parallel tubules in the etioplast of dark cultured embryo.

Fig. 108. Etioplast section from the cotyledon of embryo cultured in dark for 5 days, exposed to red light for 2 minutes and then returned to dark for 2 hours prior to fixation. Etioplast with a grpup of long tubules arising parallel from the inner membrane of the envelope, and some getting contiguous with the periphery of crystalline prolamellar body. This etioplast is exceptionally long, developing a constriction in the mid-region. Other etioplasts show either a crystalline prolamellar body or a group of long tubules. x 32,500

Fig. 109. Etioplast section from the same embryo material as in Fig. 108. Etioplasts with a group of long tubules arising parallel from inner membrane of envelope and some of which are contiguous with the tubules of prolamellar body. x 52,000

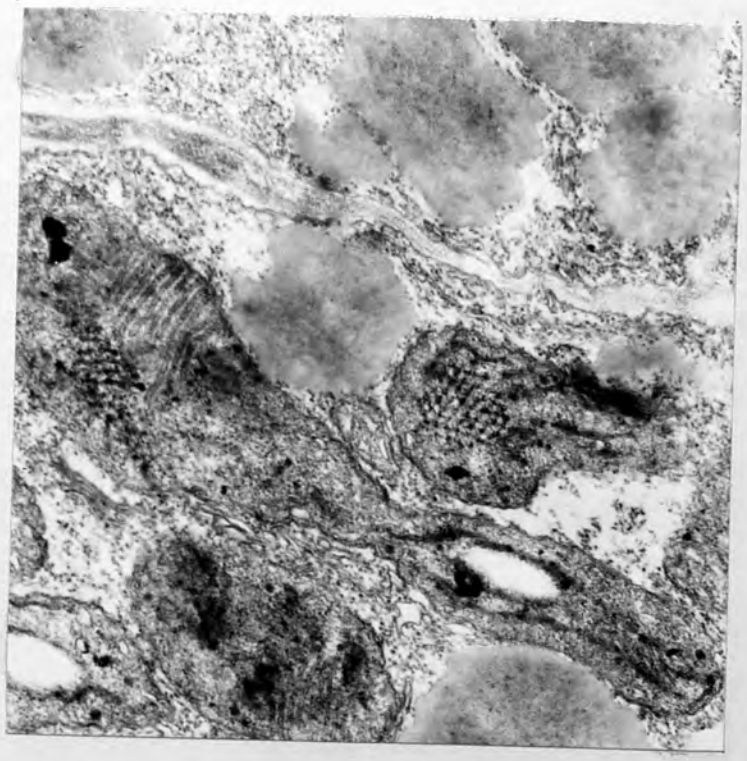


Plate 59. Effect of red light on the fusion and division of etioplasts of dark cultured embryo.

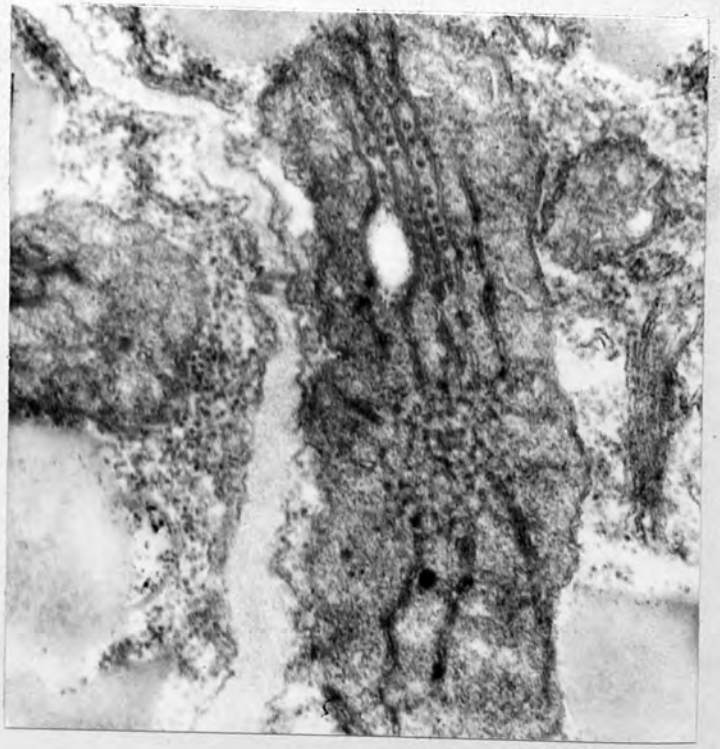
Fig. 110. Etioplast section from the cotyledon of embryo cultured in dark for 5 days, exposed to red light for 2 minutes and then returned to dark for 2 hours prior to fixation. Three etioplasts are fusing together through their envelopes. A rare phenomenon in higher plants. x 32,500

Fig. 111. Etioplast section from the same embryo material as in Fig. 110. An etioplast with crystalline prolamellar body is developing a constriction in its mid-region. x 39,000

Plate 60. Effect of blue light on the formation of small circular tubules in the etioplasts with prolamellar bodies of dark cultured embryo.

Fig. 112. Etioplast section from the cotyledon of embryo cultured in dark for 5 days and then exposed to blue light for 60 minutes. An etioplast with 3 vertical rows of small circular tubules alternating with flat lamellae radiating out from a transforming prolamellar body. Flat lamellae alternating with tubules do not stack to form grana while such lamellae may stack at other places in the etioplast. x 52,000

Fig. 113. Etioplast section from the same embryo material as in Fig. 112. An etioplast showing 3 vertical rows of tubules possibly cut obliquely alternating with darkely stained flat lamellae radiating out from a completely transformed prolamellar body. x 52,000



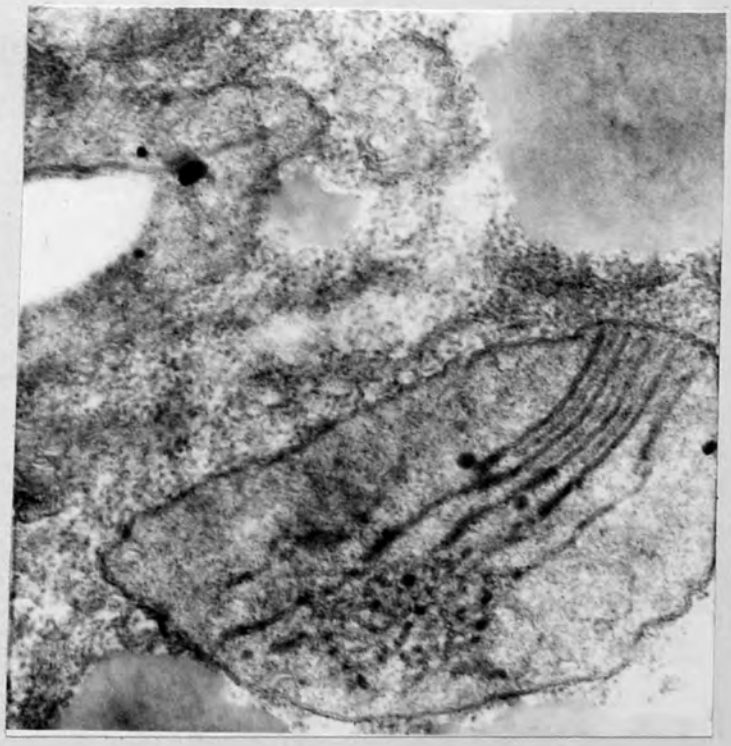
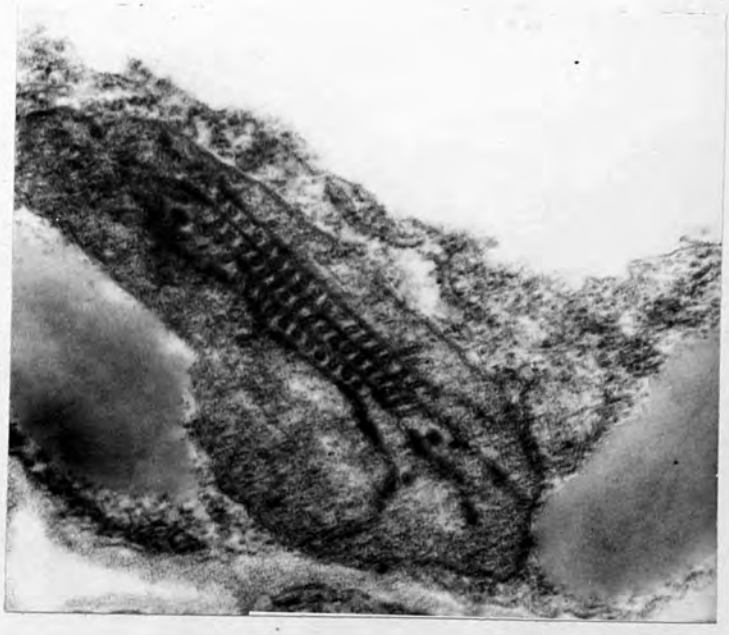


Plate 61. Effect of blue light on the formation of small circular tubules in etioplast with no prolamellar body of dark cultured embryo.

Fig. 114. Etioplast section from the cotyledon of embryo cultured in dark for 5 days and then exposed to blue light for 60 minutes. An etioplast showing 3 vertical rows of small circular tubules cut in a plane that they appear connected with the flat lamellae with which they alternate. This etioplast does not contain a prolamellar body and flat lamellae in it do not stack to form grana. x 32,500

Fig. 115. Etioplast section from the same embryo material as in Fig. 114. Two etioplasts showing 3 vertical rows of small circular tubules alternating with flat lamellae. These lamellae do not stack at all and no prolamellar body is seen in this etioplast. x 26,000



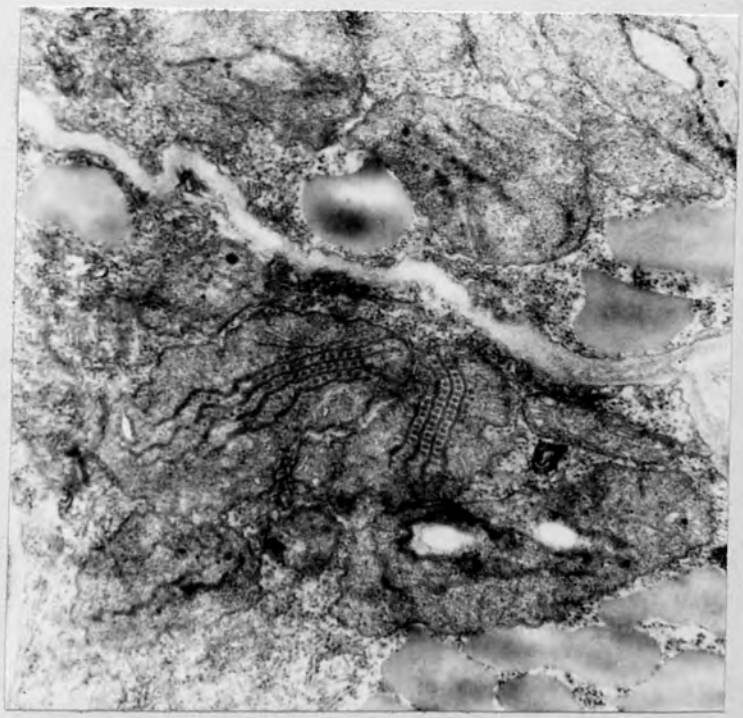
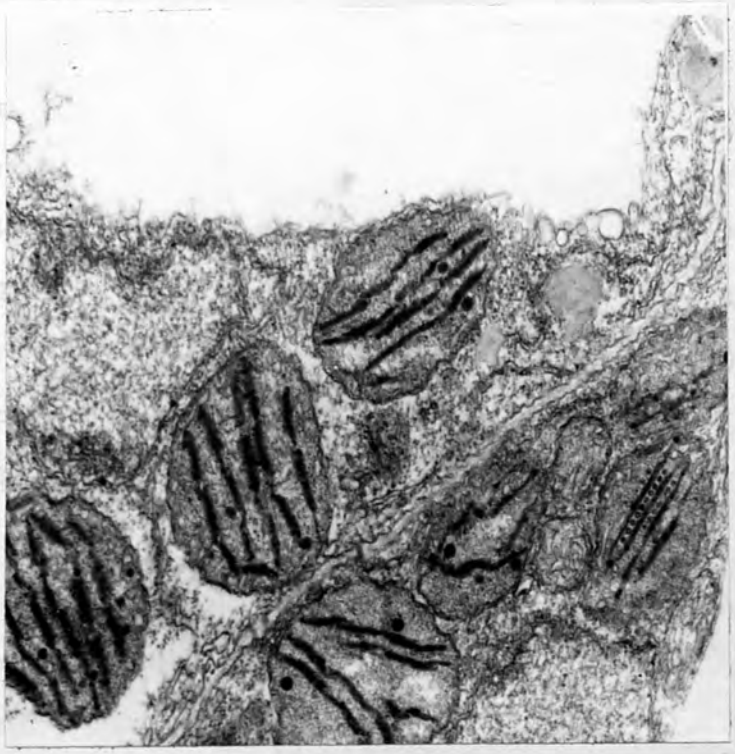
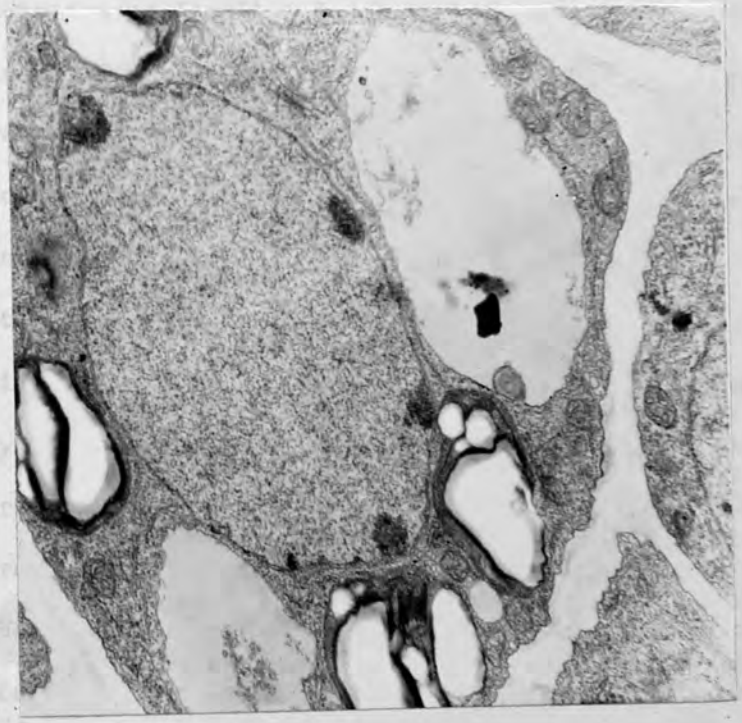


Plate 62. Effect of blue light on the chloroplast development of dark cultured embryo.

Fig. 116. Chloroplast section from the cotyledon of embryo cultured in dark for 5 days and then exposed to blue light for 90 minutes. A group of chloroplasts with normal development of lamellar system. One of the chloroplast shows 2 vertical rows of small circular tubules alternating with flat lamellae. No starch grain is seen in any of the chloroplasts. x 26,000

Fig. 117. Chloroplast section from the same embryo material as in Fig. 116. Chloroplasts completely filled with bigger and smaller starch grains. x 13,000





5. Abnormal development and arrangement of lamellae in etioplasts.

In one of the repeats of the experiments described above a very peculiar type of arrangement of lamellae is seen in the etioplast of the cotyledon of 5-day dark cultured embryo. Instead of the formation of a crystalline prolamellar body, a network of intermingled lamellae along with few densely stained osmiophilic plastoglobuli is seen (Fig. 118). This arrangement of lamellae probably represents an intermediate stage between the completely formed prolamellar body (in the dark) formed by the condensation (Bradbeer et al., 1974c) or contraction (Weier et al., 1970) of lamellae, and fully formed chloroplast (when etioplast is illuminated). A part of the etioplast envelope is incomplete in one of such etioplasts (Fig. 118) and is possibly used up in the formation of abnormal arrangement of lamellae.

Since the fully developed lamellae of a chloroplast have not been condensed completely to form a prolamellar body in the dark, we propose the term semi-condensed lamellae for such lamellae.

Interestingly enough, when the etioplasts of these 5-day dark cultured embryos are given 2 minutes illumination in red light, they show a typical well organized crystalline prolamellar body with many peripheral radiating lamellae (Fig. 119). Such crystalline prolamellar bodies gradually lost their crystalline form when the embryos were given 5-minutes and 15-minutes exposure to red light. Exposure of 5-day dark cultured embryos to 30-minutes red light resulted in complete disruption of prolamellar body in the etioplast similar to that seen in the normal experimental results.

When the 5-day dark cultured embryos were given 2-minutes red light and returned to dark for 2-hours the etioplasts again showed the formation of the network of semi-condensed lamellae similar to those in the

etioplasts of 5-day dark cultured embryos before red light treatment (Figs. 120, 121), but with some increase in semi-condensed lamellae. The etioplasts with such lamellae usually contain starch grains, although a few are without it. One of the etioplasts shows the network of semi-condensed lamellae along with a prolamellar body which appears almost reacted.

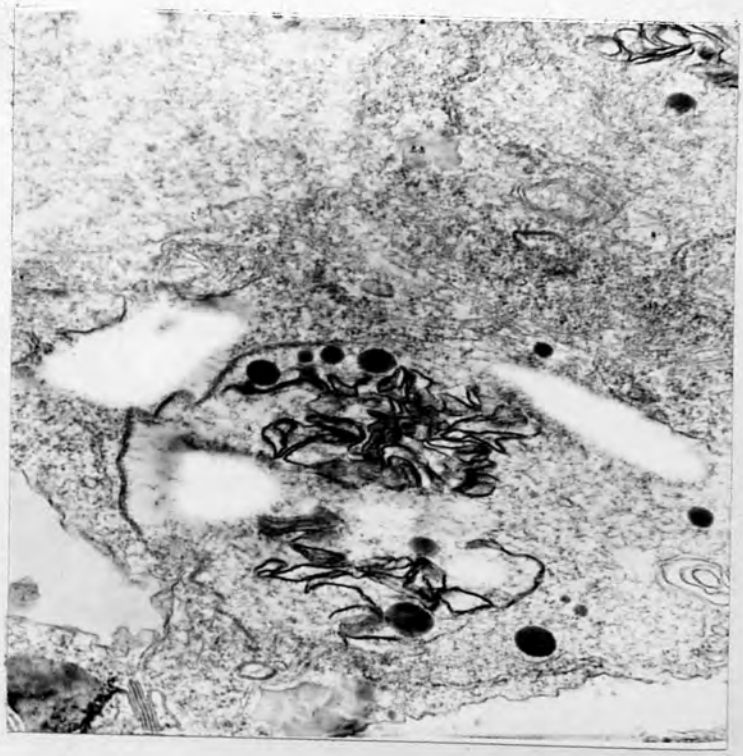
This abnormal type of network formed by the intermingling of semi-condensed lamellae is still present in the occasional etioplasts after exposure to 30-minutes blue light. These etioplasts also contain big densely stained osmiophilic plastoglobuli and some starch grains along with the network (Fig. 122). These abnormal lamellae show the loosening of network and stack at places to form structure almost similar to grana (Fig. 122). This opening of network and stacking into the grana like structure is possibly due to the effect of blue light.

The factors responsible for this type of abnormal arrangement of semi-condensed lamellae is not known at present and needs further investigation to find out the cause of their formation.

Plate 63. Etioplasts with abnormal lamellar system.

Fig. 118a. Etioplast section from the cotyledon of embryo cultured in dark for 5 days. An etioplast showing an abnormal lamellar system consisting of a network of semi-condensed lamellae and few plastoglobuli. x 32,500

Fig. 118b. Etioplast section from the cotyledon of same embryo material as in Fig. 118a showing the abnormal lamellar system as in Fig. 118a. x 39,000



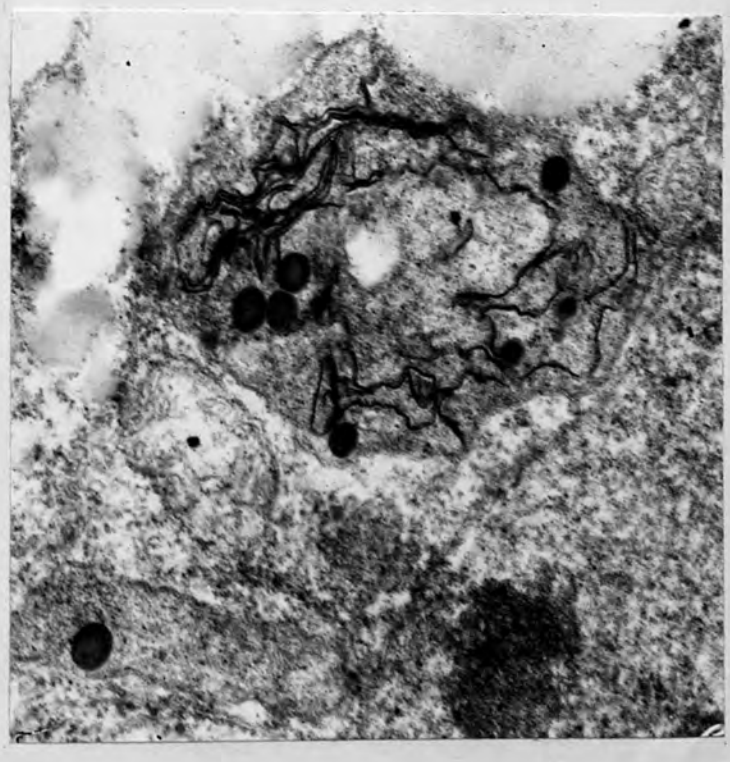
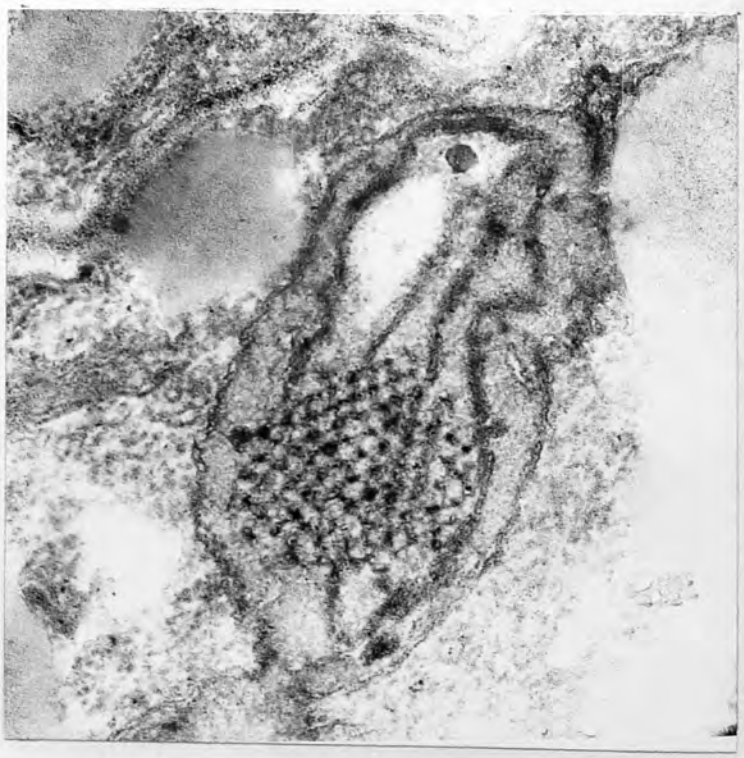


Plate 64. Effect of brief exposure of red light on the formation of crystalline prolamellar body in etioplasts with abnormal lamellae.

Fig. 119a. Etioplast section from the cotyledon of embryo cultured in dark for 5 days and then exposed to red light for 2 minutes. An etioplast with normal crystalline prolamellar body. The abnormal lamellar system is changed to crystalline form of prolamellar body. x 76,000

Fig. 119b. Etioplast section from the cotyledon of same embryo material as in Fig. 119a. Two etioplasts with normal crystalline prolamellar body are seen. x 47,500



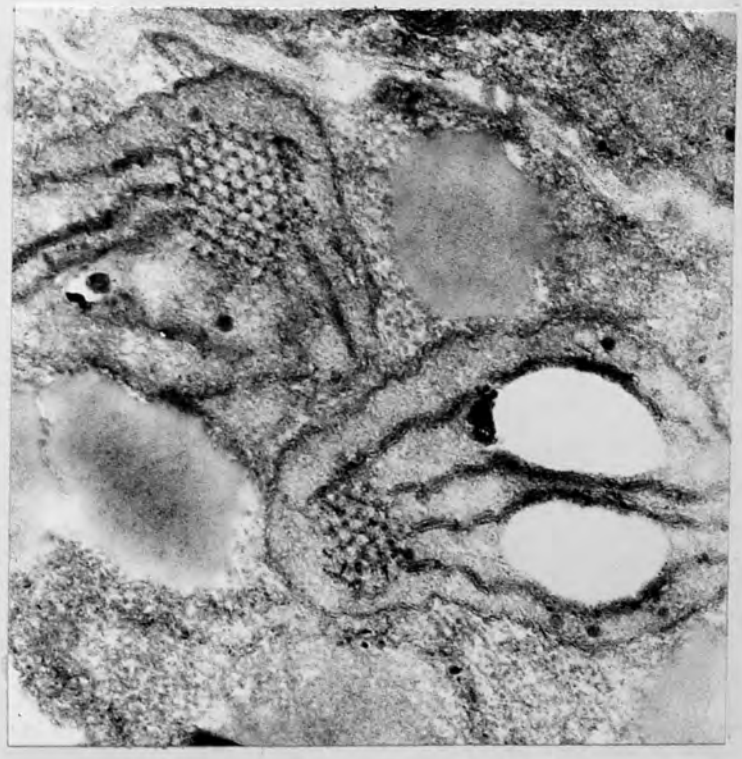
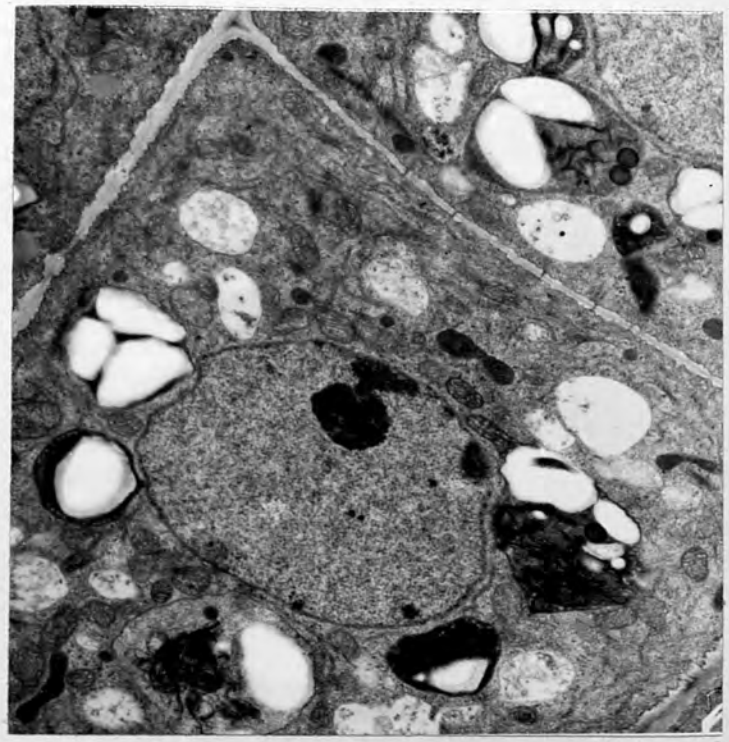


Plate 65. Effect of red light on the etioplast with abnormal lamellae of 5-day dark cultured embryo.

Fig. 120. Section through the cotyledon of embryo cultured in dark for 5 days, exposed to red light for 2 minutes and then transferred to dark for 2 hours prior to fixation. Most etioplasts in a cell show abnormal lamellar system. x 9,750

Fig. 121. Section through the same embryo material as in Fig.120. One of the etioplast shows a peculiar arrangement of abnormal lamellae while the other etioplast is full with large starch grains. x 19,500



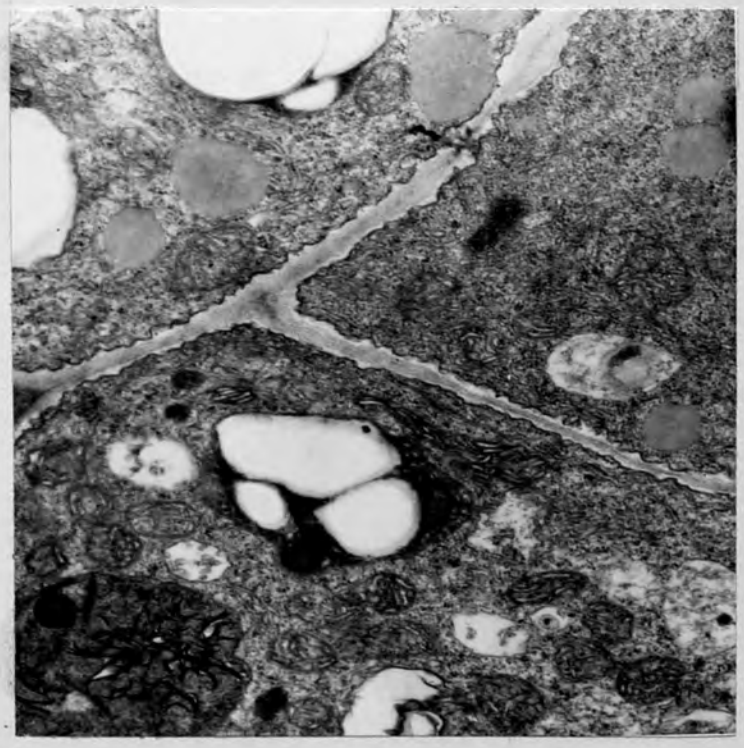
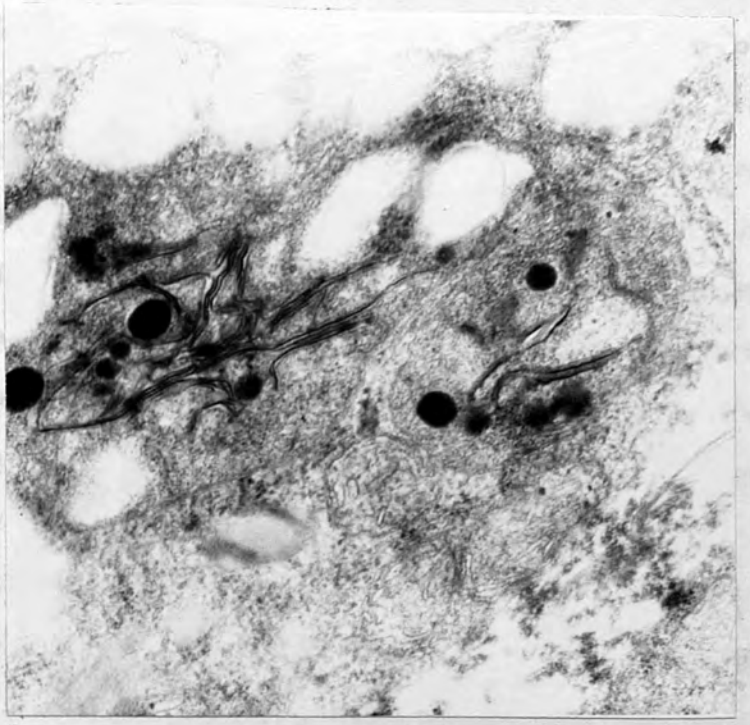
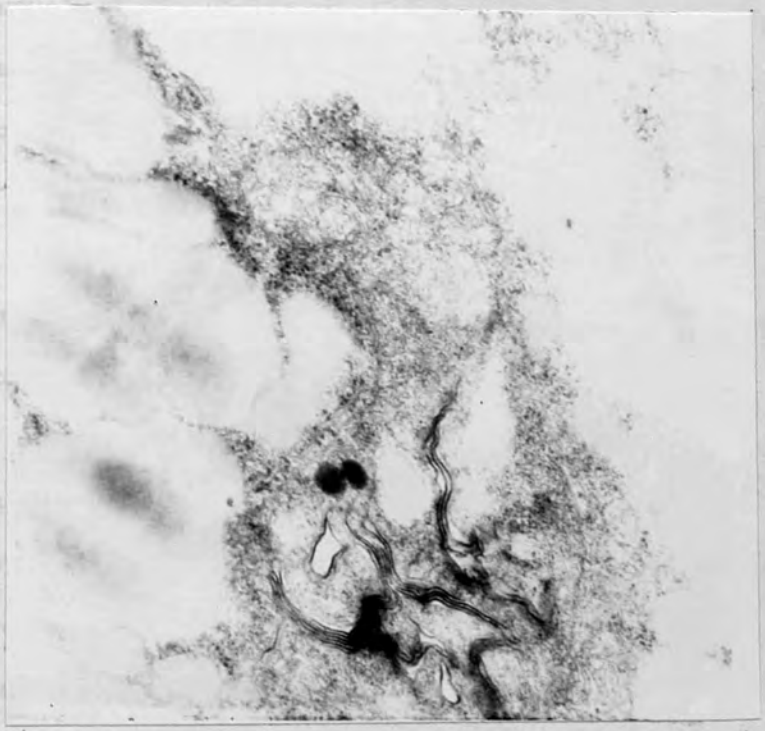


Plate 66. Effect of blue light on the etioplast with abnormal lamellae of 5-day dark cultured embryo.

Fig. 122a. Etioplast section from the cotyledon of embryo cultured in dark for 5 days and then exposed to blue light for 60 minutes. Etioplast still shows the abnormal lamellar system with some stacking to form grana like structure.
x 39,000

Fig. 122b. Etioplast section from the cotyledon of the same embryo material as in Fig. 122a. Etioplast with abnormal lamellar system with some stacking to form grana like structure.
x 39,000





SUMMARY

1. Changes in the ultrastructural cytology in the cotyledon during the development of the embryo from the torpedo - stage of embryogenesis to the mature dry embryo - stage are described. A comprehensive review is presented of the literature relating to ultrastructural studies on embryos. The new observations fit well into the general sequence of events that has emerged from other studies. During the early stages of seed development, some of the organelles appear to have a low degree of organization as reflected by the complexity of the membrane system and low efficiency in performing their specific type of function. The organelles then follow a sequence of structural and chemical changes which lead to a more complex organelle of higher efficiency. After a certain time they start to lose their organized structure and become inactive. The frequency of occurrence of a certain type of organelle and duration of its function vary from cell to cell and seem to be related to the type of function served by a particular cell. Although physical and chemical changes occur continuously during the lifetime of a particular organelle, the changes seem to be most rapid during seed development and maturation - a period characterised by high metabolic activity in the seed. Subsequent changes which take place in the dry seed are slow and very hard to follow.

2. Certain organelles of the dry seed of Capsella appear disorganised as in the case of external membranes of mitochondria and plastids. At maturity the cotyledons of most angiosperm seeds contain no chloroplast although the disorganised granal plastids may still be recognised in some cases (Klein and Pollock, 1968) similar to those observed in the mature ivory - white cotyledons of Capsella. The reduction of mitochondrial cristae is regarded as visible indication of decreased respiratory activity. Other organelles like Golgi bodies and endoplasmic reticulum are

usually reported missing from most dry seeds studied so far. But we were able to find Golgi bodies and the formation of concentric whorls of endoplasmic reticulum in dry Capsella cotyledons. It has been suggested in the present investigation that by modifying the fixation techniques the integrity of some organelles can be preserved, such as that of Golgi bodies which other investigators have not been able to envisage in dry tissue and other organelles which usually appear less organised than those in developing tissue.

3. The dry embryo of Capsella shows a general lack of membrane clarity, absence of some organelles normally present in metabolically active cells of early stage of embryogenesis and the presence of large amounts of reserve food materials. Oil and protein are the two major food reserves present in oil bodies and protein bodies. Certain questions such as the origin of oil bodies, relationship between spherosomes and oil bodies, and nature of membrane around each oil body still remain unsettled. In recent years new evidence has been presented concerning the site of the origin of oil bodies. Spherosomes and oil bodies have been reported to be separate entities, with different sites of origin and function within the cell. (Smith, 1974). As there is little change in the size of oil bodies during later developmental stages and the increase in the amount of oil may be entirely by the synthesis of further oil bodies suggests the de novo origin of oil bodies in the plant cell. The fact that oil bodies do not appear to coalesce with adjacent oil bodies in the cell indicates that a limiting membrane of some nature is present around each oil body. The nature of this membrane is still unsettled question although it has been proposed in recent years as a monomolecular layer of phospholipid (Rest and Vaughan, 1970). Only future biochemical and ultrastructural research will solve these problems.

4. It was established in the last century that scattered cells

filled with myrosin (an enzyme, a thioglucosidase) called myrosin cells are present in various organs of all cruciferous plants which were studied (Werker and Vaugham, 1974). Our results are contradictory to this as in the Capsella embryos we could not trace the presence of such protein grains which are without inclusion (i.e. myrosin grains). All cells contain only one type of protein grains with inclusions (globoid and crystalloid) called aleurone grain which present a vacuolar origin. The problems regarding the origin of protein bodies still remains unsolved as so far it is not understood that how the protein enters the vacuole.

5. A major controversy is the difference between cytological and biochemical results as to the fate of polysomes in the seeds during development, maturation, quiescence and germination. Cytological data, derived from electron micrographs of tissues fixed in situ indicate that polysomes are common in young developing embryos, rare in maturing embryos, missing in the dry embryos and common in the embryo of germinating seeds. The predominance of polysomes over the monosomes up to the heart - shaped embryo stage and the complete absence of polysomes beyond the heart - shaped embryo stage of Capsella embryo is a notable feature as the loss of polysomes has never been observed before in seeds at such an early stage as observed in the present investigation. This loss of polysomes from very early stages of embryogenesis which continues to grow up to the maturity of seed demands further investigation and explanations. This early return to a random distribution of the ribosome may be related to a change in nutritional and metabolic relationships between the embryo and its environment at this time as it is at this stage that the initiation of endosperm breakdown and resorption starts, oil bodies begin to appear and starch accumulation starts in plastids.

6. The present investigation deals with the studies on the morphogenesis of embryo chloroplast, on which no information is available in

the literature. To provide the basis for comparison the normal chloroplast structure in the light grown leaf of Capsella was studied along with the formation of etioplast and prolamellar body and their dispersal on subsequent illumination was attempted. The plastids of leaves subjected to darkness do not form prolamellar bodies but instead an extensive development of very variable, irregularly - shaped, stroma lamellae with characteristic ring or cup - shaped or looped lamellae connecting together forming bridge - like connections in the plastid stroma. This extensive development of an irregularly - shaped lamellar system showing peculiarly interesting structures are hitherto unknown in the plastids of normal higher plants. One observation shows that the lamellar synthesis is probably stopped in the chloroplasts as soon as they are transferred to the dark and thus resulting in the formation of irregularly - shaped lamellar systems rather than tho formation of prolamellar bodies. It is concluded that such exceptional lamellar system might have developed in the normal dark growing seedlings due to some disharmony resulting from an incipient senescence.

7. Prolamellar body formation in the etioplasts of young embryos of intact plants occurs after 3 days in the dark while in the etioplasts of cultured embryos these are formed after 5 days in the dark. Treatment with 3 hours of continuous illumination with white light of low intensity completes the transformation of the etioplast into chloroplast. Transformation of the prolamellar body takes place via a direct conversion of the prolamellar body to thylakoids, without a intervening stage of vesicle formation. The conversion occurs through the extension of membranes from the prolamellar body, not through the alignment of discrete vesicles, which have broken off from the prolamellar body. The development of the etioplast and formation of the prolamellar body have been shown by present investigation to involve growth of continuous membranes which may be att-

ached to the inner membrane of the chloroplast envelope as well as the prolamellar body. Thus considerable evidence from the present investigation has accumulated which shows that plastid membranes maintain their continuity to a great exent - in the mature chloroplast, during formation of prolamellar bodies in the dark, and during light - induced thylakoid formation.

8. That the inner plastid membrane does have a role in thylakoid synthesis has been shown in the present investigation as the vesicular and flat finger - like invagination of inner membrane of the plastid envelope have been observed very frequently throughout the present study. It has been shown that these invaginations are contiguous with the stroma lamellae and that the stroma lamellae are contiguous with tubules of prolamellar body. The role of such vesicles in the formation of grana by opposition to the growing structure irradiating from the prolamellar body is clearly presented in our results. But the question arises about the production of such cisternae and lamellae that have different staining characteristics than the existing stroma and grana lamellae. The present investigation has also provided further evidence for the existence of extensive, if discontinuous, lamellar sheets in etioplasts. These lamellae increase in number after illumination, but do not appear to differ from those in the etioplasts.

9. The present investigation has greatly expanded our information about the prolamellar body. But several questions still remain unanswered: What is the role of prolamellar bodies, mechanism of prolamellar body formation, what causes it to stop growing and to grow. This structure persists in the chloroplasts under normal light conditions. It may serve as a storage body for material that will be used in the formation of lamellae. Its configuration would allow a maximal storage surface for membranes until used in this manner. The development of etioplast stops when the

etioplast and its prolamellar body/bodies reach a critical size, containing much less membrane material than the mature chloroplasts. It is not yet known what determines the critical size, but it is presumed that the supply of key enzymes or precursors run out or become limited, and that illumination is a pre-requisite for their replenishment.

10. Also observed in cultured embryos are mitochondrion-like protuberances and lobes of the plastid jacket with a great deal of internal vesiculation which we regard as peripheral reticulum. The peripheral reticulum is mostly considered as the only unique structure of the C_4 plants but now is also reported from many C_3 plants. We have postulated that there is a possibility of the formation of mitochondria from the chloroplast through the formation of large protuberance of the chloroplast envelope called peripheral reticulum. Peripheral reticulum is therefore, regarded as a specialized mobile region of the chloroplast, capable of amoeboid - like movement; the ability of the peripheral reticulum to protrude, to partially envelope organelles like mitochondria and possibly to withdraw. There is a possibility that such interconversions of organelles or some confluences between mitochondria and chloroplasts have been induced by some unspecified variable in handling and preparation of material. It is also possible that the phenomenon is normal but are related to a peculiar physiological condition, or to a developmental situation atypical of more mature stages.

11. The plastids of cultured embryos show 2 novel structures, that is, the formation of two types of tubules distinct from the tubules forming prolamellar body. It appears that morphogenetic capabilities of the inner membrane of plastid envelope is exceptionally enhanced in the cultured embryos resulting the excessive production of tubules. The final type of tubules appears as vertical rows of small circular tubules alternating with the flattened lamellae extending mostly from the periphery

of the prolamellar body. The second type of tubules appears as a group of long parallel tubules arising from the inner plastid membrane running into the stroma. These tubules cannot be regarded as part of normal plastid ultrastructural development - The phenomenon appears to be an abnormal type possibly as a reaction to a changed environmental condition as the excised embryo experience and artificial medium rather than a natural medium of intact plants. The sucrose in the culture medium possibly stimulate such abnormal growth of plastid tubules as the membranes of the plastids cannot reorganize quickly and efficiently resulting in the formation of several types of tubules. The formation of such tubular structures are regarded as sign of degeneration rather than a normal stage of development.

12. It is difficult to devise 1 general scheme for chloroplast differentiation in any detail as structural changes during plastid development are highly flexible - they can be drastically altered by the number of environmental factors. The nutritional state of plants as well as changes in light intensity and temperature have very marked effects on chloroplast structure and chlorophyll content. The future research should be involved in determining how these and other environmental factors exert their control on plastids development.

13. At this time we are at the beginning of a new phase of embryological study in which the ultrastructural techniques are applied to cultured embryos and much more remains to be achieved. For example, very few attempts have as yet been made to use a defined medium which can be considered to contain the minimal nutritional requirements in respect of particular tissue or organ or an organelle (say plastids). Moreover, we know very little about the utilization and metabolic fate of various chemicals present in the complex media. This should not be very difficult to follow with the use of labelled chemicals. Plant tissue culture should

not be an end in itself, but be a creative medium in which all kinds of problems can be better studied than *in vivo*.

14. We need much more basic data from the observations made by experimental embryologists looking at more stages of development of more species of angiosperms. We also need more data that combines dynamic and analytical studies with the ultrastructural work. Histochemical and cytochemical studies at both the light and electron microscope level show promise in extending the morphological observations and tying them in with the physiological data which are available from culture experiments. This is just the beginning of modern study of plant embryogenesis. The available results suggest that ultrastructural procedures offer a way of obtaining data that will help in to understand fundamental problems in cell and organism development. The next few years should be exciting ones indeed in the field of embryogenesis

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* Original not seen.