A STUDY OF SOME ASPECTS OF SENESCENCE IN EMBRYOS OF *ZEA MAYS L.*

by

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ABSTRACT

The rate of ageing of a stock of one variety of maize seed of uniform genotype was accelerated by control of temperature and moisture conditions. The germinative capacity of seed samples subjected to increasing periods of the ageing treatment was found to have decreased from 98% to 0% over a period of 29 days.

Various ultrastructural, histochemical, autoradiographic and cytological studies were carried out on the root caps of unaged material and on this tissue for selected age stages of the seeds.

The presence of, and some of the characteristics of plant cell lysosomes have been established by the present writer (Berjak, 1968). It is suggested that senescence of the outermost root cap cells in unaged material is a genetically programmed, necessary event which involves hypersecretory dictyosomal activity and the release of lysosomal enzymes.

Results obtained indicated that the primary ultrastructural senescent change involves membrane deterioration, followed in some cases by respiratory failure and death of the embryos. Observations made suggested more than one pattern of senescence to occur, and ageing changes are postulated to be indicative of disturbances at the molecular control level.

C O N T E N T S

PART I - INTRODUCTION

PART II - MATERIALS AND METHODS

PART IV - DISCUSSION

ELECTRON MICROGRAPHS

The material for the ultrastructural surveys was fixed in glutaraldehyde, postfixed in potassium permanganate and post-stained with lead citrate according to Procedure 6c recorded in Part II, unless otherwise stated.

Key to Abbreviations used for Electron Micrographs

A	amyloplast
AER	agranular endoplasmic reticulum
BM	bounding membrane
CP	cell plate
cr	central region
CN	chromatin
CT	crista
CY	cytoplasm
DFL	developing first-phase lysosome
D	dictyosome
DC	dictyosomal cisterna
DV	dictyosomal vesicle
DS	dictyosomal secretion
ER	endoplasmic reticulum
FL	first-phase lysosome
IM	inner membrane
LD	lipid droplet
L	lysosome
LC	lysosomal content
LV	lysosomal vacuole
MC	micro-tubule
ML	middle lamella
M	mitochondrion
MM	mitochondrial matrix

MS	monosome
NOS	nucleolar organiser segment
NL	nucleolonema
NS	nucleolus
N	nucleus
NE	nuclear envelope
NP	nucleoplasm
OM	outer membrane
PA	pars amorpha
pe	peripheri
PL	plasmalemma (plasma membrane)
PD	plasmodesma
P	plastid
PI	plastid initial
PG	plastoglobuli
PS	polysome
PO	pore
PP	proplastid
R	ribosome
SL	second-phase lysosome
SG	starch grain
UM	unit membrane
V	vesicle
VA	vacuole
W	wall

PART I - INTRODUCTION

A. THE PROBLEM AT HAND - LOSS OF SEED VIABILITY

One of the major contributory factors to the qualitative and quantitative success of crop production is the utilization of seed having high germination capacity and vigour.

Successful seed storage is important in agriculture and forestry, as well as to the research worker investigating any problems associated with seeds because his material is continuously changing during ageing. Storage of the seeds of food crops is especially important, as the land which would be utilised for seed production is thus made available for other purposes.

Homozygous inbred lines of maize have been developed as a result of intensive research and breeding experiments, and the maintenance of these superior and vigorous lines depends largely on the successful storage of their seed-producing stocks, as well as seed for annual food crops.

Generally, signs of deterioration and decrease in the germination capacity are functions of the age of the seed (e.g. Haferkamp et al., 1953). Longevity of embryos within seeds depends basically on their genotype and there is considerable variability from species to species and between varieties. While the actual process of ageing of the embryo might be directly under genetic control, other inherited characteristics of the seed, especially the nature of its coat, are also extremely important factors in its longevity.

However, the environmental conditions under which a sample of seeds is stored have a marked effect upon its longevity, notwithstanding its genetic constitution. The investigations of Fleming (1966) illustrate this point. Using maize seed of the same age and of the same double cross hybrid, but from different producers, he showed that there were marked differences in the field germination and subsequent production of the plants. These differences reflect the treatment at source.

Environmental Conditions Affecting Seed Viability :

Although various environmental factors each contribute in some measure to loss of viability in stored seeds, it must be realised that the various factors are integrated in the overall production of deleterious effects.

In general, low moisture content and lower temperatures during storage extend the longevity of seed. This is true for cereals in general (Roberts, 1960). However, certain seeds prove exceptions to this generalisation (Owen, 1956).

1. Moisture

Barton (1961) has reviewed results of extensive experiments showing moisture content to be an extremely important factor in the retention of viability of stored seed.

(a) Humidity conditions and seed moisture uptake :

The amount of moisture absorbed was shown to vary with the seed type, but the pattern of moisture uptake proved to be similar for any one seed type under conditions of different relative humidity (Barton, 1941). Barton found that moisture uptake

was generally greater at 10°C than at higher temperatures, irrespective of the relative humidity. In addition, several investigators have shown that the state of viability of seeds has no effect on the amount of water which they will absorb (Barton, 1961). As seeds are hygroscopic, they will thus gain or lose moisture depending on the ambient humidity, until an equilibrium is reached (e.g. Sijbring, 1963).

No direct relationship was shown to exist between moisture content alone, and loss of viability, for any seed type under a given set of conditions. However, loss of viability in a variety of seeds was shown to progress at an accelerated rate under conditions of high relative humidity and high temperature, compared with lower relative humidity at the same elevated temperature and over the same time period (Barton, 1941).

Barton (1961) has reviewed literature showing that the 'critical' moisture content of seed (i.e. the maximum moisture content at which the seed may be stored without losing viability) varies from species to species, and is not always correlated with the capacity of the seed to absorb moisture. In addition the 'critical' moisture content of a given seed type decreases with increasing temperature.

(b) Effect of the fluctuation of relative humidity :

Barton (1961) reported results of experiments which demonstrated that the moisture content of seeds maintained under atmospheric conditions ('air-dry') differed with the locality and fluctuated with the season. She reported that the moisture content of

a variety of seeds was almost twice as much during the wet season than during the dry season.

This illustrates two important principles in the successful storage of seed; firstly, that the moisture content of seeds at harvest should be standardised to a safe level prior to storage, and secondly, that open storage (e.g. under the roof of an otherwise open shed) will contribute to deleterious changes in the seed.

In her review Barton (1961) has cited the results of certain experiments which illustrated that the viability loss was more rapid in seeds stored in cans which were repeatedly opened and resealed, than in those which were stored in sealed cans or sealed glass tubes, reopened only for the final germination test. Even storage in the unopened cans proved less efficient than storage in sealed glass tubes. Barton also pointed out that the initial moisture content of the seed was a determining factor in viability loss. Those seeds with a higher moisture content deteriorated far more rapidly (with all the storage methods used) than those in which the moisture content had been reduced. Barton concluded that, in general, stored seeds maintained their viability best if maintained at a constant high moisture content, than if fluctuations in the moisture content occurred, especially around the 'critical' level.

In a further investigation on the effects of storage conditions, it was found that if bean seeds were stored at sub-zero temperatures ($-2^{\circ}C$ and $-18^{\circ}C$) then sealing was without effect, and also that at $30^{\circ}C$ deterioration was rapid in both sealed and open storage.

However, at intermediate temperature sealing extended the longevity of the seeds (Barton, 1966).

2. Temperature

Low temperatures are superior to high in extending the longevity of seeds in storage. In general, however, unless the moisture content of the seeds is low, and the relative humidity of the store is maintained at a constant low level, seeds stored at low temperatures, but above $0^{\circ}C$, will deteriorate. However, the use of below-freezing storage temperatures has proved superior to storage temperatures above freezing, for certain seed types (Barton, 1961) and in general, conditions of high humidity have no effect on seeds stored at below-zero temperatures (e.g. Barton, 1966).

Although certain seeds appear to suffer no damage at very low temperatures e.g. $-40^{\circ}C$ for rye and hemp (Owen, 1956), Barton (1966a) reported that onion seed stored at $-18^{\circ}C$ showed some injury effect, compared with storage at $-2^{\circ}C$. Thus it appears that optimum storage temperature varies from species to species, as does e.g. the 'critical' moisture content.

Seeds which can be stored at below-zero temperatures must be able to withstand considerable drying, as freezing injury, causing death, occurs in seeds with higher moisture contents (e.g. Kantor and Webster, 1967).

However, there are certain seed types, mainly of tropical and sub-tropical origin, which cannot withstand extreme desiccation. If storage is necessary in such cases, the most favourable combinations of temperature and moisture content for viability retention, must be individually ascertained (Barton, 1961).

Roberts (1960) derived a mathematical relationship between viability of wheat seeds and the temperature and moisture content. He suggested that the same relationship holds for oat and barley seeds as well. Later, he illustrated that this equation also applied to rice seed.

The relationship is :

$$\text{Log } p = K_v - C_1 m - C_2 t \quad ,$$

where p = time taken for 50% of the seeds to be killed (half viability period)

m = moisture content (% wet basis)

t = temperature (^{o}C)

K_v, C_1, C_2 are constants.

The values of the constants for rice were found to differ from those for wheat.

Roberts et al. (1967) have cited the results of several independent investigations (e.g. Hukill, 1963; Burgess et al., 1963) showing that the viability period of a seed lot can be predetermined, provided the storage temperature and moisture content are known.

The Q_{10} for viability loss, derived from the above equation, is the same for all temperatures. However, Roberts and Abdulla (1968) have reviewed evidence suggesting that Q_{10} values increase with increasing temperatures, so that if the above equation is applied above the 25 - 45^{o}C temperature range it might overestimate the period of viability for a seed population.

3. Gaseous Environment

Although many of the earlier workers in the field of seed longevity reported the effects of gaseous environments on stored seeds, reviews of their work (Owen, 1956; Barton, 1961) show that the results presented were generally conflicting.

However, a few of these earlier investigators recognised that a decrease in environmental oxygen level was advantageous in extending the longevity of stored seed (or conversely, that an increase in environmental oxygen concentration was correlated with a decreased viability of stored seed).

Recently, however, more definite evidence has been obtained, showing that an extension of the viability of stored (wheat) seed appears to be associated with a decrease in environmental oxygen concentration during storage (e.g. Peterson et al., 1956; Glass et al., 1959). Roberts and Abdalla (1968), using barley, bean and pea seeds, confirmed that increased levels of environmental oxygen decreased the period of viability of the seeds. In addition, these investigators showed that the deleterious effects of oxygen were far more pronounced in seeds with a higher moisture content. They also demonstrated that the deleterious effects of oxygen were manifest at relatively low levels of this gas (0 - 21%).

Generally the deleterious effects of oxygen on seed viability were attributed to stimulation of respiration and/or to stimulation of activity of micro-organisms associated with the seed. Semenuik (1954) has reviewed the literature which relates increase in the activity of micro-organisms to decrease in viability of seed.

Roberts and Abdalla (1968), using a temperature (60°C) and moisture content (12%) at which micro-organisms would be unlikely to be active, showed that the rate of viability loss of seeds increased with increasing concentrations of oxygen. These authors concluded that the deleterious effects of this gas were thus due to a direct effect on the seed itself. However, micro-organisms may sometimes be the immediate cause of death, having established themselves on seed which has generally lost its resistance to attack, due to other factors.

In addition, Roberts and Abdalla (1968) demonstrated that the respiratory rate and respiratory quotient (0.63) of seeds stored in sealed ampoules at 25°C remained constant during an experiment, in which their environmental oxygen content fell from 21% to 1.4%, and their environmental carbon dioxide content rose from 0.03% to 12%. As the deleterious effect of oxygen was obtained with an increase from 0 - 21% (with little or no effect produced by a further increase to 100%) these authors postulated that the main deleterious effect of oxygen might be manifest at concentrations of this gas lower than 1.4% of the environmental atmosphere.

Other factors may also affect the viability of seeds, e.g. damage by insects, the effect of chemical pesticides, and mechanical damage to the seed. However, the effects of temperature, moisture and oxygen, and their interaction are the most likely to affect the metabolism of the embryo, causing viability loss.

B. PLANT PROTOPLASM - ULTRASTRUCTURAL AND FUNCTIONAL ASPECTS

Cellular Membranes

As a cell is subdivided both ultrastructurally and metabolically into discrete subcellular particles, it seems fitting that a discussion of the cell should begin with the structural components which effect this subdivision - the selectively-permeable, lipoprotein membranes. The biochemical systems which are necessary in the maintenance of a viable cell, are many and diverse, thus it is not surprising that for each system to operate at maximal efficiency, it should be isolated to some extent from other systems. The specific conditions required for a biochemical system to operate efficiently, form a micro-environment for that system, within the bounds of the selectively-permeable, lipoprotein membrane. In addition to the maintenance of the micro-environment, the bounding membrane must also facilitate the controlled two-way passage of certain substances between the surroundings and the interior of the organelle. Although the precise mechanism of the passage is as yet unknown, the bounding membranes must actively regulate metabolism by their ability to control transport. With the loss of this control, a cell could not remain viable.

Certain universal characteristics of membranes have become apparent from the evidence of numerous electron micrographs, illustrating cells fixed and stained by a variety of techniques (Thompson, 1965). Membranes appear to be tripartite: A band which is electron-transparent is bounded on either side by a 2 nm thick electron-dense layer. The average overall thickness of a membrane is 7.5 - 10 nm (Fig. I.B.1). These ultrastructural observations support


UM

the concept (first proposed by Danielli and Davson, in 1943) that cell membranes are lipoprotein in nature, with a bimolecular layer of lipid bounded on either side by a protein layer. This structure is termed a unit membrane. Suggestions as to the precise structural arrangement of the protein and lipid constituting the membrane, are conflicting. Covalent bonds are not operative in holding the lipid and protein membrane constituents together, and there is evidence that hydrogen bonding, London-van der Waals attractions and electrostatic attraction are responsible for lipid-protein interaction (Thompson, 1965).

The classes of lipid implicated in membrane structure are phospholipids, glycolipids, sulpholipids and isoprenoids. There is little information concerning the structure of membrane proteins (Thompson, 1965).

Perhaps the most important aspect of the membrane proteins is whether they are purely structural, or whether enzyme activity may also be attributed to them.

The Nucleus

The essential and controlling role of the nucleus of a cell has been illustrated by numerous experiments on enucleated cells, and by the fact that naturally-occurring anucleate cells have a limited period of viability.

Nuclear Envelope

The nucleus forms a discrete portion of the protoplasm and is bounded by the nuclear envelope, which consists of two unit membranes each about 7.5 nm thick (Fig. I.B.2). The two membranes enclose the perinuclear cisterna, which varies from 10 to 70 nm in width (Fig. I.B.3).

FIGURE I.B.2. Illustrates the nucleus bounded by the nuclear envelope. Note the prominent nucleolus.(x 45 050).

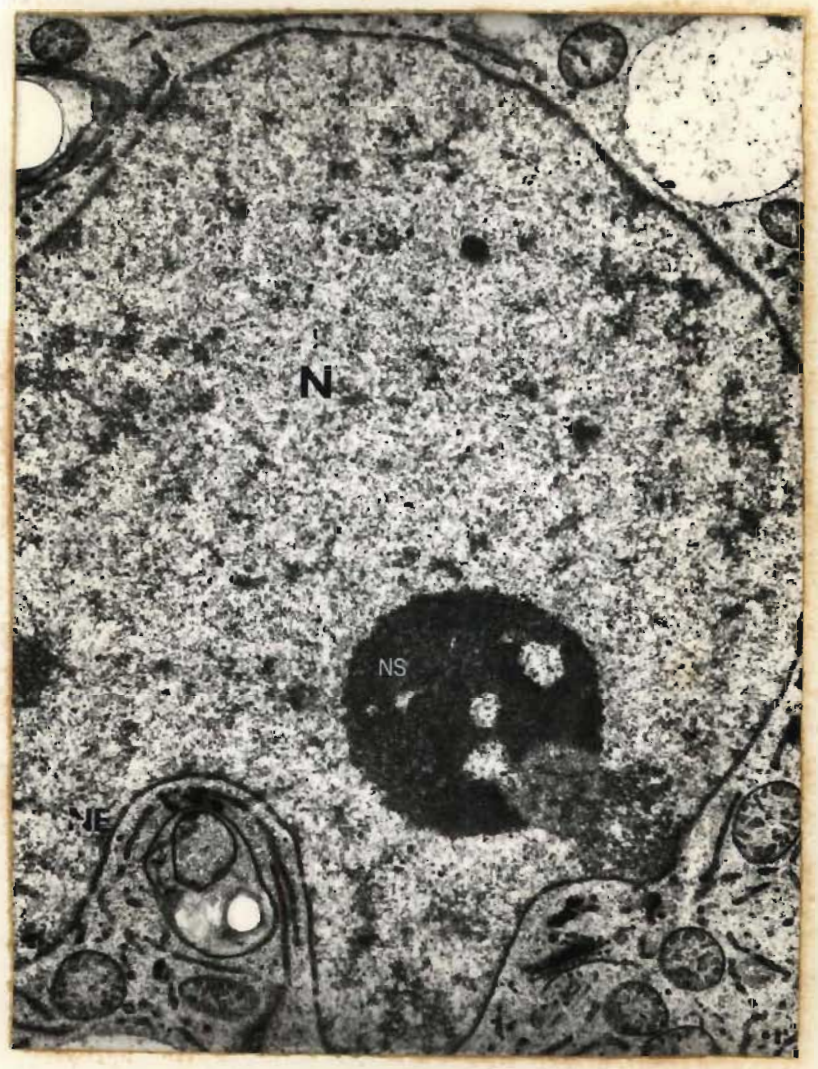

N
NS
IE

FIGURE I.B.3. Illustrates the nuclear envelope with the two membranes enclosing the perinuclear cisterna. Note the pores in the nuclear envelope. (x 29 250).


N
PO
NE

The outermost membrane of the nuclear envelope is commonly seen to be continuous with the membranes bounding the tubular profiles of the endoplasmic reticulum in the cytoplasm (Fig. I.B.4). Frey-Wyssling and Mühlethaler (1965) suggest that the perinuclear cisterna and the cisternae of the endoplasmic reticulum contain a low-density fluid, which they term enchylema.

Porter and Machado (1960) demonstrated that during cell division the nuclear envelope breaks into fragments which remain around the chromosome region, but are indistinguishable from elements of the ER. Towards the end of telophase the ER reconstitutes the nuclear envelope; thus the nuclear envelope is considered to be part of the ER.

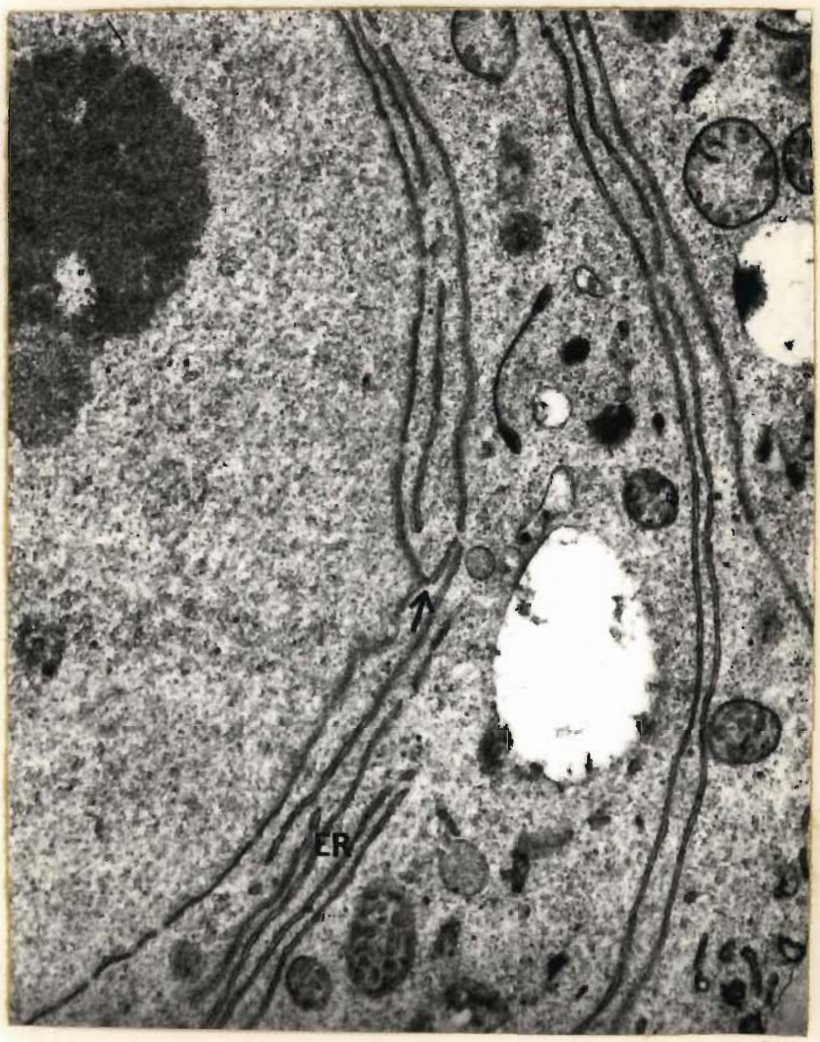
Pores

The nuclear membrane is frequently interrupted by pores, some 50 - 100 nm in diameter (Bonner, J. 1965).(Fig I.B.3). The nuclear pores are suggested to be more than mere holes. Yoo and Bayley (1967) have submitted evidence that the pores of pea nuclei are octagonal, with central granules or tubules, and surrounded by a complex annulus.

The pores do not appear to be static. Moor and Mülethaler (1963) demonstrated that the nuclear pores of yeast may be open or closed, according to certain circumstances.

Nuclear membrane resistance measurements have been carried out on a few cell types with nuclei 30 - 40 nm in diameter. The results are somewhat controversial, as the nuclear membrane of amphibian oocytes has a very low membrane resistance and no membrane potential (indicating little or no barrier between nucleoplasm and cytoplasm)(Fawcett, 1966), while the nuclear envelope in salivary glands of Drosophila shows a considerable electrical resistance, and sustains a resting potential with the nucleoplasm negative to the cytoplasm (Loewenstein and Kanno, 1962)

FIGURE I.B.4. Illustrates continuity between the outermost membrane of the nucleus envelope and the membranes bounding a tubular profile of the endoplasmic reticulum (at arrow). (x 22,750).


ER

Biochemical evidence exists, showing that the potential structural component molecules of chromatin pass into the nucleus, and that nuclear products such as ribosomes and RNA leave the nucleus, by way of the pores (Bonner, J., 1965). A controlled transport mechanism probably occurs at the pores, so that regulated transport between nucleus and cytoplasm occurs.

The nuclear membrane and permeases

Experimental evidence reviewed by Georgiev (1967) suggests the possibility that permeases attached to the nuclear membrane facilitate the inward transport of low molecular weight protein and nucleic acid precursors.

Subnuclear Components

The subnuclear components originally recognised by light microscopy are the chromatin, nucleolus, and karyolymph (nuclear sap). By physical rupture of the nuclear membrane, followed by density gradient centrifugation, the principal subnuclear fractions have been characterised as chromatin, nucleoli, nuclear ribosomes and supernatant (Bonner, J., 1965).

Chromatin

Chromatin consists of DNA, some RNA, and associated protein. The interaction, relationship and functioning of these constituents is discussed in Part C (Differentiation and Development), and only an outline will be given here.

DNA

DNA is a long-chain polymer which consists of four different repeating nucleotide units. The specific sequence of the nucleotides is interpreted as the basic genetic code. DNA exists as a double helix, with hydrogen bonding between complementary nucleotides of the two strands.

DNA Replication

DNA, the genetic material, has been shown to occur in constant amount in the nuclei of different specialised cells of a multicellular organism (e.g. Rasch and Woodward, 1959). The DNA of chromatin replicates during interphase, prior to division of a cell. Both resultant daughter cells thus receive the full complement of genetic information.

The two strands of the DNA helix are postulated to separate from one another, and DNA polymerase is then thought to function in catalysing the synthesis of the daughter long-chain nucleotide polymers, using both strands of the existing DNA as templates. Two double-stranded helices are postulated to result from this process, each reflecting the same base sequence as the original template DNA (e.g. Bonner, J., 1965).

RNA Synthesis

DNA serves as a template for the synthesis of RNA, catalysed by RNA polymerase. This process is termed transcription, and the RNA synthesized reflects the base sequence of the template DNA. RNA is a single-stranded nucleotide polymer which is produced utilising only one of the strands of DNA as template.

At least three major types of RNA are formed by DNA transcription:

(i) Messenger RNA (m-RNA), which interacts with ribosomes during protein synthesis.

(ii) Transfer (soluble) RNA (t-RNA or s-RNA), of low molecular weight, also involved in protein synthesis.

(iii) Ribosomal RNA, which forms an integral part of the ribosome.

Protein associated with chromatin

The chromatin-associated protein is of two main types.

(i) A basic (positively charged) histone type, which is postulated to be implicated in genetic repression.

(ii) The non-histone enzyme proteins.

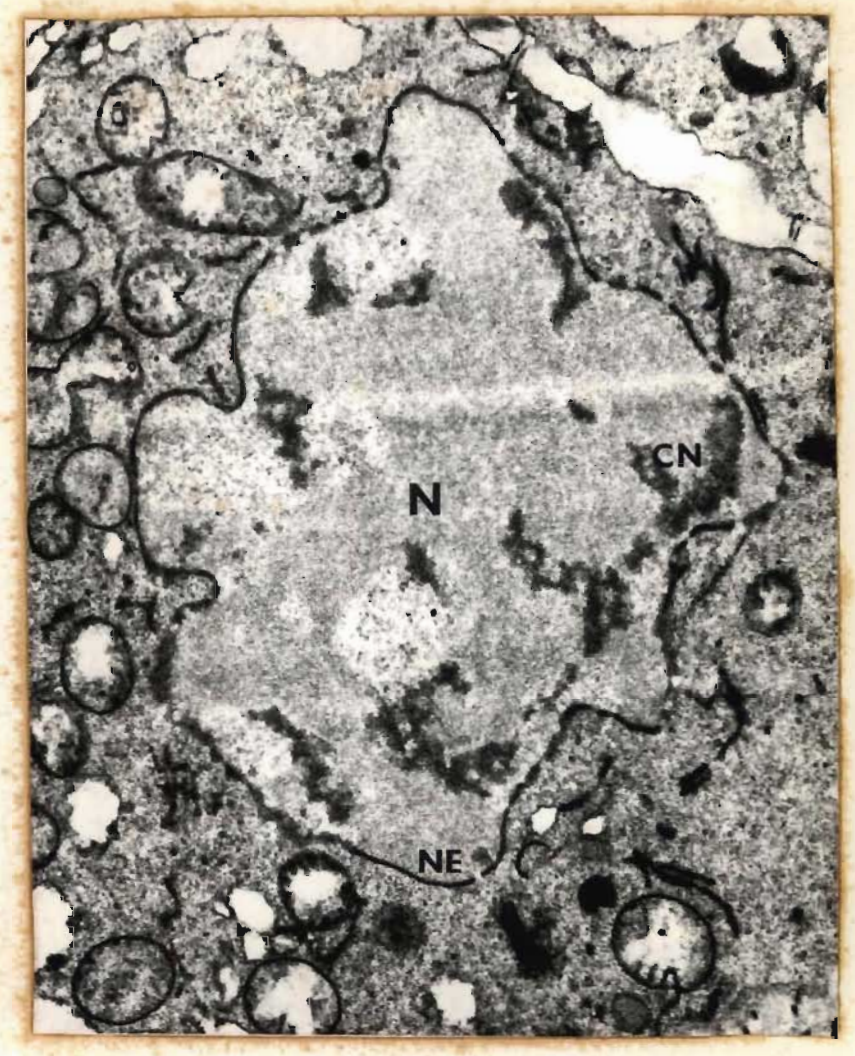
Nuclear Ultrastructure

Chromatin

The electron microscope has proved disappointing as a tool for investigating nuclear ultrastructure. The chromatin of an interphase nucleus appears as dense, granular masses, of ill-defined outline. The various phases of nuclear division are recognisable in electron micrographs, by the typical organisational aspects familiarised by light microscopy; however, the ultrastructure of the chromosomes remains somewhat of a mystery (Fig. I.B.5).

Ris (1956), working with chromosomes of a particularly loose structure showed that these basically consist of two microfibrils about 20 nm in diameter. This was later also demonstrated for the dense granular chromatin masses of the interphase nucleus of several plants and animals. Buvat (1963) showed that these two microfibrils are in turn each made up of two fibrils 10 nm in diameter.

Ultrastructural investigations on unsectioned interphase nuclei have confirmed and extended suggestions of the fibrillar structure of chromosomes (DuPraw, 1965). DuPraw (1966) has also proferred evidence for a folded-fibre organisation of chromosomes of honey-bee embryonic cells, and of human leucocytes and liver cells.


N
CN
NE

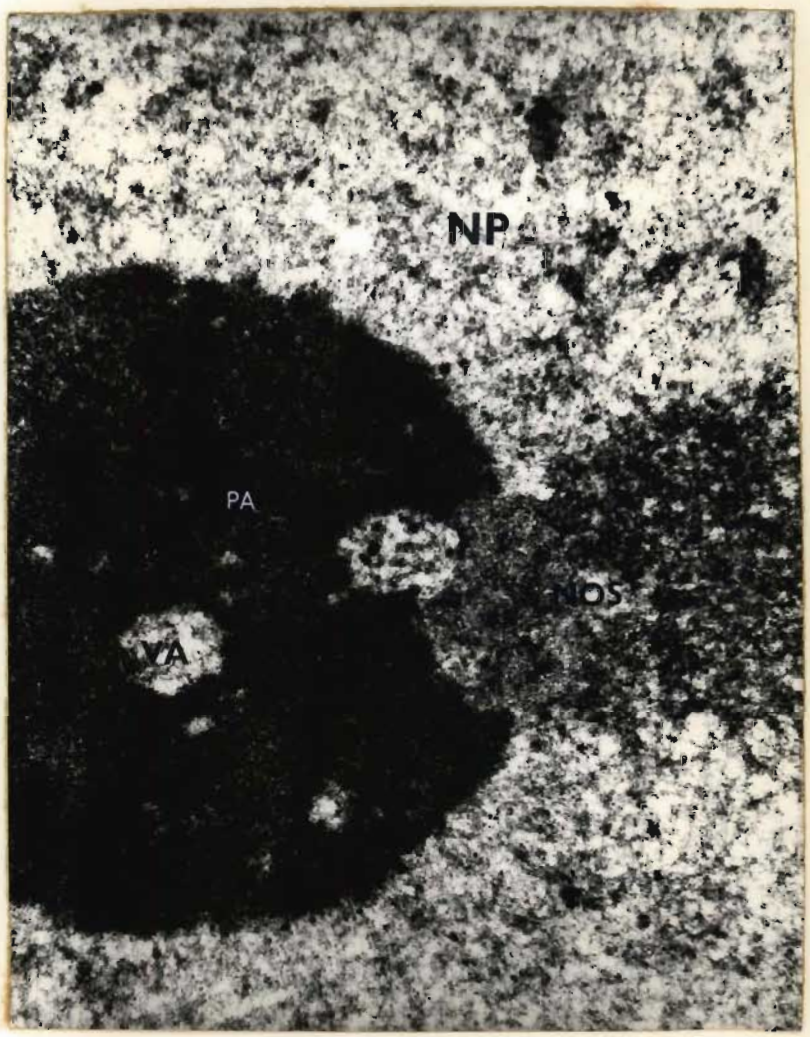
Nucleolus

The nucleolus is an eccentrically-placed, refractile body within the nucleus. Usually only one nucleolus is encountered per nucleus. This subnuclear particle is clearly demarcated from the nucleoplasm, but is not membrane-bound. (Fig. I.B.2.)

Estable in 1930 proposed that the nucleolus was not a homogeneous uni-constituent structure, and later investigations (Estable and Sotelo (1955); Bernard et al. (1955)) suggested a filamentous constituent, the nucleolonema which occurs sunk in the pars amorpha. Bopp-Hassenkamp (1959) has further suggested that the nucleolonemata contain helicoidal filaments, similar to chromosomal microfibrils.

Chouinard (1966) has shown that plant nucleoli present the appearance of two distinct zones, answering respectively to descriptions of the nucleonema and pars amorpha. The former presents an aspect of closely-packed thread-like structures associated with granules approximately 15 nm in diameter, while the latter consists of closely-packed thread-like fibrils 6 - 10 nm in diameter. In addition, Hyde (1967) has shown that nucleoli contain intrusions of heterochromatin corresponding to the attachment of a nucleolar-organising chromosome (Fig. I.B.6). This supports certain earlier observations of Buvat (1963). It has also been demonstrated that nucleoli contain one or more vacuoles (Chouinard et al. (1966); Hyde, (1967)) and that these may contain chromatin-like fibrils and what appear to be ribosome-like granules (Hyde, 1967). The granular particles, which average 15 nm in diameter, associated with the nucleolonema are judged to be nucleolar ribosomes, being rich in ribonucleoprotein.

FIGURE I.B.6. The nucleolus of a plant cell, illustrated. (x 29 250).


NP
PA
VA

Sirlin (1960) using labelled RNA precursors has shown that both the nucleolus and the chromatin contain RNA. The nucleolus was thought not to be a site of RNA synthesis. The use of autoradiography (Woods, 1959) and labelling experiments (Bonner, J., 1965a) showed that the newly-synthesized RNA in the chromatin first acquired the label; only after some time did labelled RNA appear in the nucleolus. However, the nucleolar organiser segment of the chromatin has been shown to be the template for ribosomal RNA synthesis (Ritossa and Spiegelman, 1965) thus associating this synthesis with the nucleolus.

The occurrence of ribosomes in the nucleolus was first demonstrated electron microscopically (Birnstiel and Hyde, 1963). Chipchase and Birnstiel (1963) subsequently characterised the nucleolar RNA. They found it to consist of the 28S and 18S components characteristic of ribosomal RNA.

The experiments of Birnstiel et al. (1963) which demonstrated that ribosomes could be prepared from isolated nucleoli completed the picture, and allowed for the conclusion that the nucleolus receives its RNA from the chromatin and incorporates this, with protein, into ribosomes.

Nucleolar ribosomes cannot support protein synthesis (Birnstiel et al., 1963), but some component system of the nucleolus is responsible for the production of the ribosomal protein (Birnstiel and Hyde, 1963). Bonner (1965) suggests that ribosomes leave the nucleus by way of the pores; ribosomes containing labelled RNA have been traced into the cytoplasm (Rho and Bonner, 1961).

Nuclear Sap (Supernatant fraction)

(i) Protein Synthesis Protein synthesis occurs in the nuclear sap (although apparently not in the nucleolus or chromatin). Allfrey et al. (1957) demonstrated the nuclear synthesis of proteins, and isolated peptides containing labelled amino acids from nuclei.

Particles identified as ribosomes (and found to be identical to cytoplasmic ribosomes) are found in the nuclear sap (Allfrey, 1963). The nuclear sap also contains all the other components necessary for protein synthesis (Georgiev, 1967).

(ii) Glycolytic Pathway McEwan et al. (1963) demonstrated the presence of the complete glycolytic sequence in nuclei, showing glucose to be converted to lactic acid. The enzymes of this pathway are located in the nuclear sap.

The enzymes of the hexose-monophosphate shunt have also been localised in the nuclear sap (McEwan et al., 1963) and the importance of this pathway is postulated to be in the formation of a pentose pool, pentoses being utilised in nucleic acid synthesis.

Note that the complete systems of glycolysis and the hexose-monophosphate shunt are also present in the cytoplasm.

(iii) Tricarboxylic Acid Cycle and Oxidative Phosphorylation :

Several tricarboxylic acid cycle (TCA) enzymes have been located in the nuclear sap (McEwan et al., 1963). The existence of a functional TCA cycle in

the nucleus has been postulated, but further work is necessary in order to establish this conclusively.

The electron transport system and oxidative phosphorylation, as described for mitochondria (see later), appear to be absent from the nucleus. However, oxidative phosphorylation does seem to occur in nuclei, and Georgiev (1967) suggests this nuclear process to differ essentially from the mitochondrial process.

(iv) Nucleotide Metabolism : The nucleus contains an appreciable amount of nicotinamide adenine diphosphate (NAD) (Stern and Mirsky, 1952). NAD pyrophosphorylase (NAD synthetase), an enzyme instrumental in the synthesis of NAD, has been purified from nuclei (Morton, 1961) and is thought to be localised in the nuclear sap.

Several other enzymes involved in nucleotide metabolism appear to occur in nuclei. Among these is glucose-1-phosphate uridylyl transferase which catalyses UDP-glucose formation (Smith et al., 1953). This is a key compound in several synthetic reactions in the cell, as are several other nucleotide compounds apparently synthesized in nuclei.

Nuclear Specialisation

Work on animal cells has shown certain specific enzymes to be present in nuclei from one type of tissue and not from another. Georgiev (1967) suggests that a degree of specialisation might exist in nuclei from various differentiated tissues.

RIBOSOMES

Robinson and Brown (1953) first reported ribosomes as round objects, approximately 20 - 30 nm in diameter, in electron micrographs of the cells of bean root. The connection of the ribosomes with protein synthesis was first demonstrated for animal tissue (Littlefield et al. 1955) and for plant tissue (Webster, 1955) at about the same time. Shortly thereafter Ts'o et al. (1956) isolated and characterised ribosomes from plant material.

The major components of the ribosomes of higher plants have been shown to be protein (50 - 60%) and RNA (40 - 50%) (Lyttleton, 1960; Wallace and Ts'o, 1961).

Magnesium occurs tightly bound to the ribosome. With the partial removal of magnesium the original cytoplasmic ribosome, of sedimentation coefficient 80S, is resolved into two subunits of sedimentation coefficient 60S and 40S respectively. These subunits have the same protein : RNA mass ratio as the original 80S particle. The dissociation is reversible, depending on the concentration of magnesium ions. The 60S particle can be resolved into a 40S and two 26S subunits by the further removal of magnesium (Bonner, J., 1961). Ts'o et al. (1958) reported ribosomes to have an open porous structure, one half of their volume being composed of water.

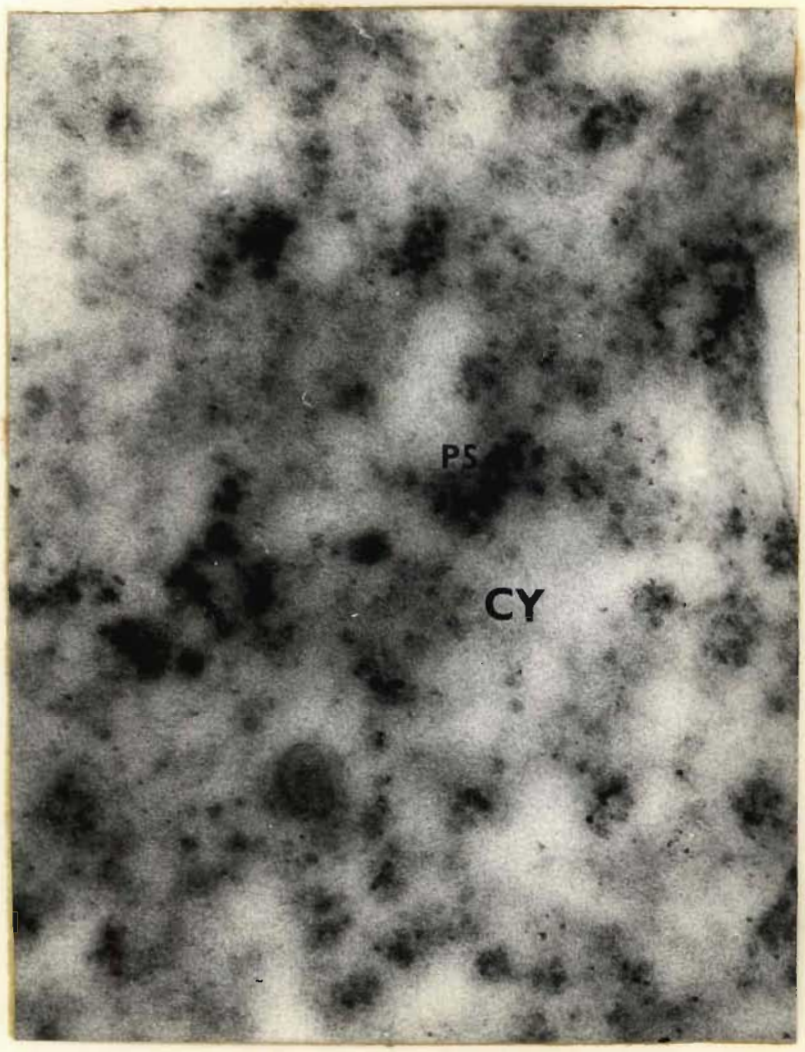
The function of the ribosomal RNA is not clearly understood. Chipchase and Bernstiel (1963) have shown this RNA to consist of two major components of sedimentation coefficient 28S and 18S respectively.

Structure

Bayley (1964) has shown that both the 60S and 40S particles consist of a disc of approximate diameter 20 nm. The 60S particle possesses in addition, two rod-shaped structures 25 x 9 nm, of sedimentation coefficient 26S.

Slayter et al. (1963) first reported that two or more ribosomes could occur bound together by a strand (approximately 1 nm in diameter) thus forming a polyribosome (Fig. I.B.7). The continuity of the strand is destroyed by treatment with ribonuclease, thus demonstrating it to consist of RNA. This structural arrangement, which is transitory, is explained as follows: A ribosome becomes attached to one end of a messenger RNA strand, at the start of the synthesis of a peptide molecule. The ribosome moves relative to the strand, being discharged on reaching the other end. Usually more than one ribosome is situated relative to the messenger RNA strand at any one time during peptide (protein) synthesis, thus accounting for the polyribosome, which leads to increased efficiency in energy utilisation. Hardesty et al. (1963; 1963a) have demonstrated the implication of ATP in the attachment reaction. Marcus and Feely (1966) have suggested that in the absence of amino-acyl formation, or without its transfer to the ribosome-messenger complex, polyribosome formation does not occur. Rich et al. (1963) demonstrated that 80S ribosomes not attached to messenger RNA are relatively inactive in peptide synthesis. In vitro studies on the progress of ribosomes relative to the messenger RNA strand have shown a decrease in polyribosomes, an increase in free 80S ribosomes, and the appearance of protein (of haemoglobin, in that case), with time (Hardesty et al., 1963; 1963a).

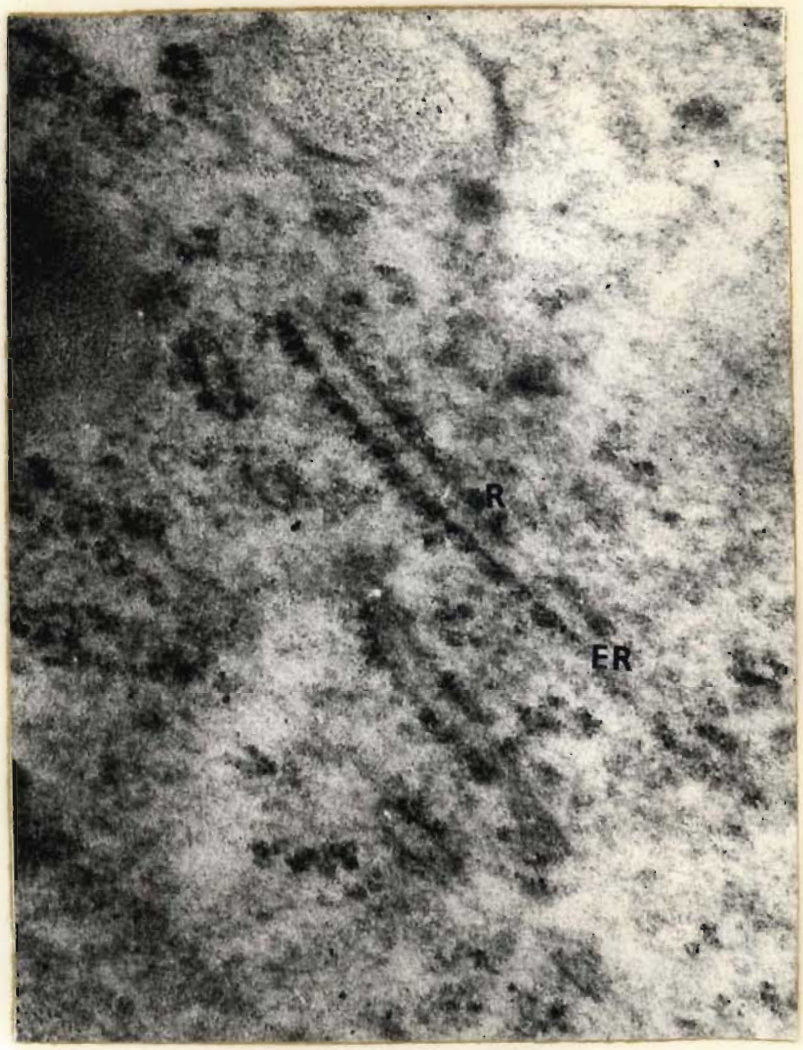
FIGURE I.3.7. Illustrates polyribosomes in the cytoplasm of a plant cell. The material was positioned in an osmium solution according to Procedure 6b. (x 55,250).


PS
CY

Marrè et al. (1965) have demonstrated that polyribosome formation also results from the linkage of monoribosomes to RNA, in plant material. These authors also demonstrated that the amino acid incorporation activity of polyribosomes far exceeds that of monoribosomes. Barker and Rieber (1967) have shown that the monoribosomes found in dry seeds form polyribosomes, following imbibition. Lin and Key (1967) have shown that under anaerobic conditions, polyribosomes are depleted, resulting in monoribosomes, with an accompanying loss of nascent polypeptide. Electron micrographs of unimbibed seeds have shown that the monoribosomes occur densely packed in those cells with a latent potential for protein synthesis (Chapman and Rieber, 1967).

A portion of the ribosomal complement of many cells occurs bound to the membranes of the endoplasmic reticulum (Fig. I.B.8). However, these ribosomes too, are formed in the nucleus, and their attachment to the ER is secondary (Porter and Machado, 1960). Mercer (1960) and Whaley et al. (1960) have demonstrated that ribosomes are initially free in the cytoplasm, in meristematic cells, becoming associated with the ER only during cell maturation. Meristematic and embryonic cells have a high rate of protein turnover, thus the correlation of ER-associated ribosomes with protein synthesis (for export) found in animal cells, appears not to be applicable in the case of these plant cells. Tissue culture experiments of Nicolson and Flamm (1965) have demonstrated that plant cells having a high proportion of ER-associated ribosomes in the exponential growth phase show an increase of free 80S ribosomes, apparently in a process of breakdown of these particles, as they senesce. Hallinan et al. (1968) have reported qualitative differences in protein biosynthesis by ER-bound ribosomes and polyribosomes, from liver cells. These authors

FIGURE I.B.8. Illustrates ribosomes bound to the membranes of the endoplasmic reticulum. This material was postfixed in an osmium solution according to Procedure 6b. (x 80 850)


R
ER

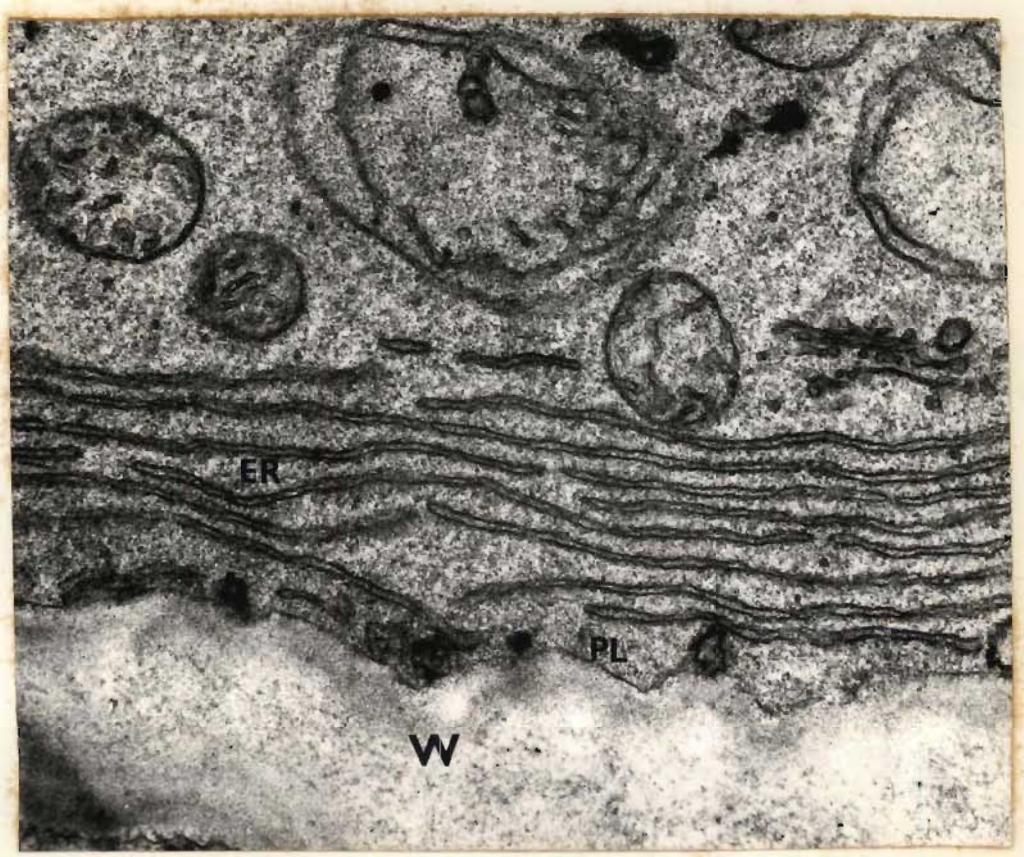
ascribe the specific function of the initiation of prosthetic group attachment to apopolypeptides, to the ER-bound liver cell ribosomes.

ENDOPLASMIC RETICULUM (ER)

In electron micrographs the ER is usually seen to consist of profiles of anastomosing tubules, 40 - 70 nm in diameter (Fig. I.B.9). Branton and Moor (1964), using techniques of serial sectioning and freeze-etching, have demonstrated that the ER actually forms a reticulate system of sheets of double membranes enclosing a variably-sized lumen, throughout the cytoplasm. Profiles of the ER seen in thin section show that the tubules sometimes dilate locally, forming vesicles.

The continuity of the nuclear membrane with profiles of the ER is commonly seen in thin sections (Fig. I.B.4). The ER and the nuclear envelope thus appear to belong to the same organelle system, and the origin of the ER from the nuclear envelope has been suggested as being more probable than its formation de novo, from the cytoplasm (Frey-Wyssling and Mühlethaler, 1965). Continuity between the plasma membrane and the ER, however, has rarely been reported (Frey-Wyssling and Mühlethaler, 1965). The tubular cisternae of the ER appear electron-transparent, and Frey-Wyssling and Mühlethaler (1965) suggest that these and the perinuclear cisterna contain a low-density fluid (enchylema).

Ribosomes often occur in close association with the outer surface of the membranes of the ER. However, as this is not always the case the ER is distinguished into two categories,


ER
PL
W

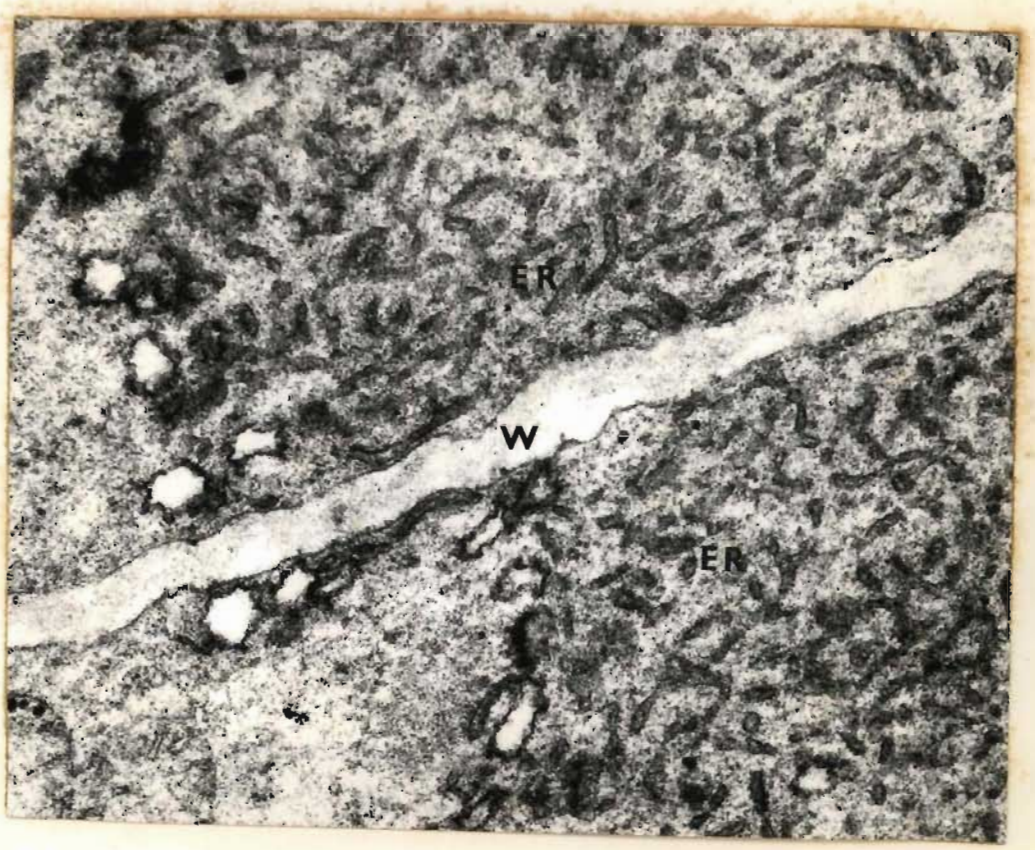
the granular or rough ribosome-associated ER, and the agranular (smooth) ER which lacks ribosomal association (Figs. I.B.8, 9 and 10). These correspond to the basophilic and acidophilic areas, respectively, of classical cytology. It has been previously mentioned that the association of ribosomes with the ER does not appear to be essential for protein synthesis, and therefore the role of this membrane system in the synthetic activities of the ribosomes is not yet clear.

Elements of the ER have often been reported to pass through the pores in the cell wall, communicating with the reticular structures of the adjacent cell (e.g. Whaley et al., 1960). However, no evidence has been reported either of intercellular traffic, or of an intracellular transport system within the tubular cisternae. Aggregates of ER are consistently observed at sites in the cell where an intensive consumption of matter occurs, e.g. the site of synthesis of a cell wall (Fig. I.B.11). Frey-Wyssling and Mühlethaler (1965) suggested that the ER is active in the distribution of substances within the cell, on the principle of the movement of these substances along the surface of the membranes, chemical forces being operative. Recent evidence from thin sections suggests that smooth ER is concerned with processes of transport to and from sites of aggregation of the microtubular subunits implicated in cell plate formation (Burgess and Northcote, 1968).

The ER is an extremely variable organelle. The degree of its development seems to vary with the cell type and also with the physiological activity of the same cell type (especially in animal tissue).

The ER in meristematic and embryonic cells appears sparse, with scattered, short cisternal profiles. However, in cells

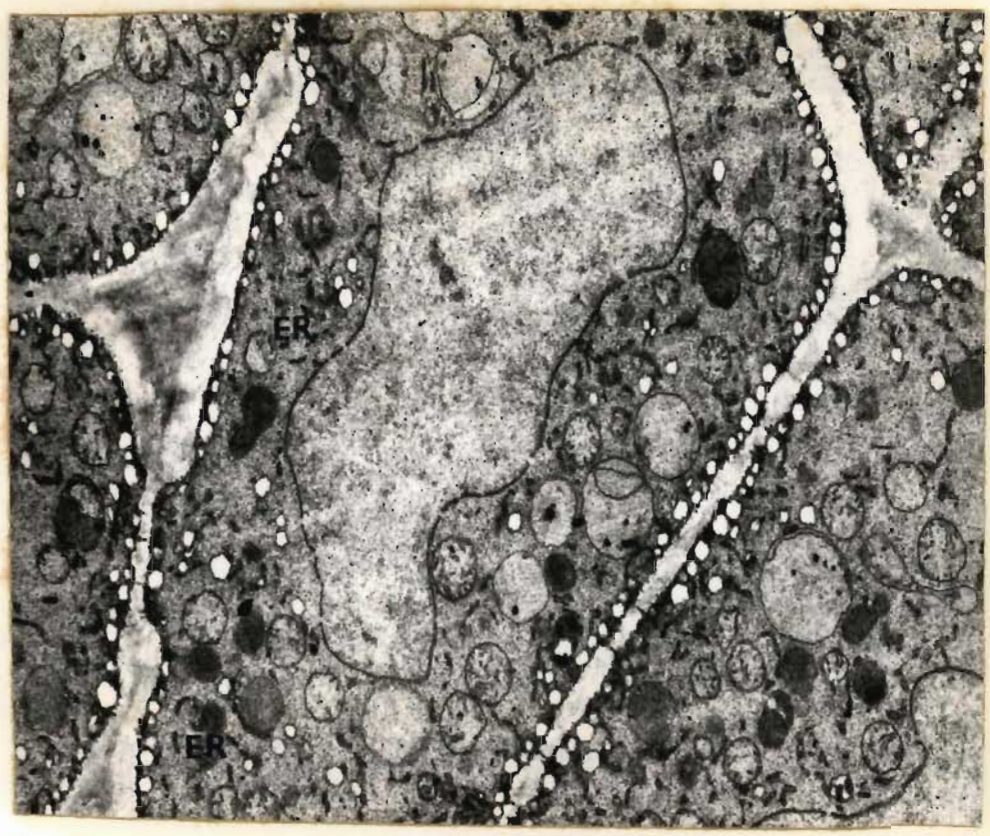

A.ER


ER
W
ER

which are growing and differentiating, there is a strong development of this organelle, evident in the parallel arrays of long cisternal profiles in thin section (Figs. I.B.12a & b). It has been reported that the ER is sparse in the mature plant cell without intensive metabolism. Porter and Bruni (1960) and also Yamada (1960) have suggested that extensive development of agranular ER in animal cells is linked to glycogenesis. Palay (1958) reported that the most extensive development of the agranular ER occurs consistently in cells which are engaged in lipid metabolism.

Enzymes associated with the ER.

The microsomal fraction obtained by the ultra-centrifugation of animal cells contains many enzymes, most of which are esterases or other hydrolases. In addition, cytochrome b_5 and its reductase are localised in this fraction consisting of fragmented ER (Dixon and Webb, 1964). The microsomal fraction obtained from plant cells is apparently homologous with that of animal cells, regarding its associated enzymes. However, cytochrome b_7 replaces the cytochrome b_5 of the animal microsomal fraction (Martin and Morton, 1957). Cytochrome b_7, which has protoporphyrin IX as its prosthetic group, is not reduced by the action of e.g. cyanide (Bonner, 1965). A type of cyanide-insensitive respiration has often been reported for plant tissue. All the electron transport components (see later) apparently become reduced, with the exception of some of the b cytochromes. W.D. Bonner (1961) proposed that all plant tissues have the capacity to exhibit cyanide- and carbon-monoxide-insensitive respiration by means of a pathway of electron transport to oxygen, which is alternative to cytochrome oxidase. The same author further postulated the existence of a mechanism for the control of the pathway of electron transport in the cell. It


ER
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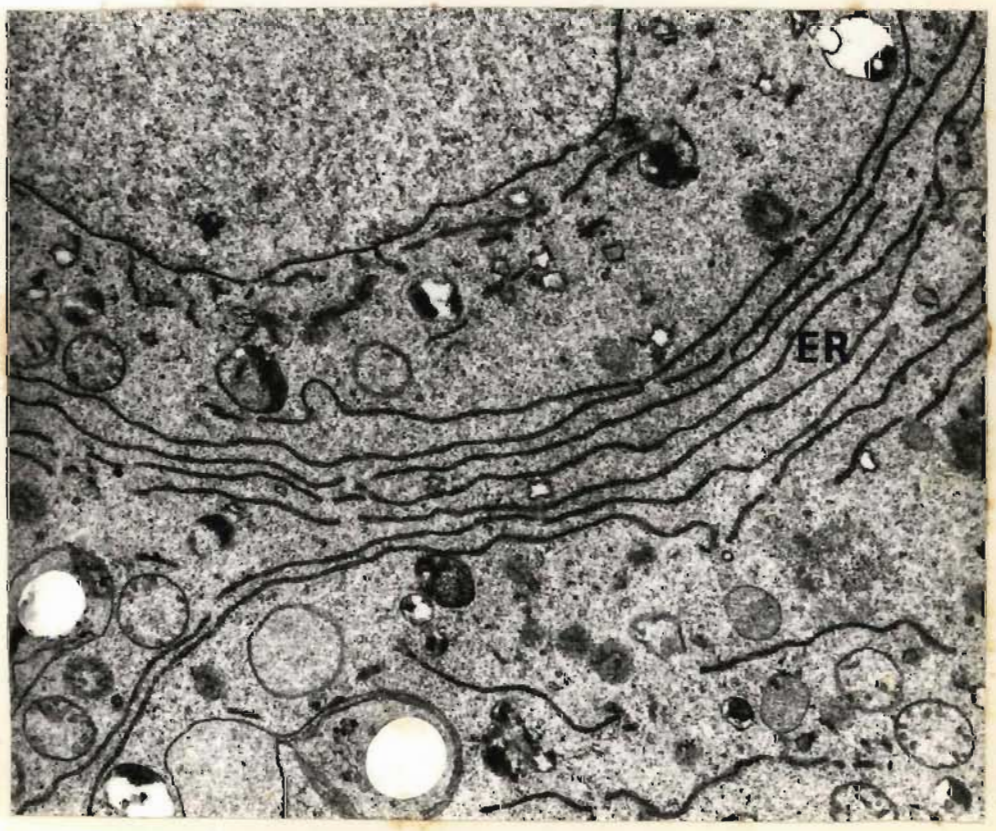

ER

is possible that an alternative pathway for the transport of electrons to oxygen, is linked up with the cytochrome b_7 associated with the ER.

An interesting observation in this respect is that the respiration of young carrot leaves was reported to be carbon monoxide- and cyanide-sensitive, whereas respiration in the old leaves was reported to proceed in the presence of these inhibitors (Marsh and Goddard, 1939).

The ER is reported to develop extensively under conditions of anaerobiosis (e.g. Linnane et al., 1962), thereby suggesting a transfer of metabolic function to this organelle, with the elimination of the mitochondrial system.

Many observations appear to support the concept that vacuoles arise as distentions of the ER (e.g. Buvat, 1963; Matile and Moor, 1968). Matile and Moor have demonstrated the homologous nature of the bounding membranes of vacuoles and the unit membranes of the ER. However, it must be mentioned in this respect, that vacuolar origin has been postulated by several authors to be dictyosomal, (e.g. Marinos, 1963; Ueda, 1966).

The results of freeze-etching (Matile and Moor, 1968) and ultra-thin sections (see later) suggest that the lysosome of the plant cell is derived from the ER. If the lysosomal apparatus can be equated, first with the pre-vacuolar body, and later with the vacuole (see later) then it might be concluded that the plant cell vacuole does, in fact, arise from the ER (Fig. I.B.16).

The close association of ER profiles with certain hepatic microbodies (Shnitka, 1966; Novikoff and Shin, 1964), with plastids (Wooding and Northcote, 1965), and with lysosomes

(see later) has led to several postulates on the functional significance of these relationships.

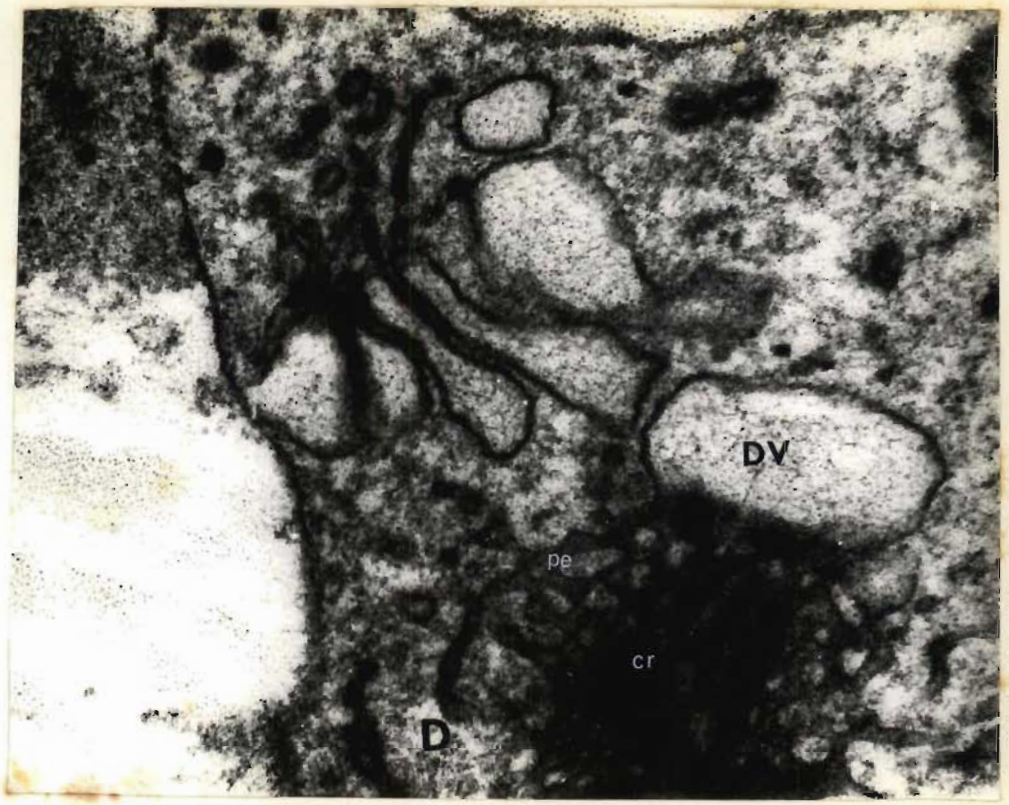
Frey-Wyssling and Mühlethaler (1965) have suggested that spherosomes are also derived from the ER, by constriction and subsequent cutting off of vesicles (see later).

DICTYOSOMES

Characteristically a dictyosome consists of a stack of flat cisternae in close parallel array. Each cisterna is disc-shaped, with a plate-like or fenestrated central region (about 500 nm in diameter), and reticulate, tubular peripheri (Fig. I.B.13) (Cunningham et al., 1966; Mollenhauer and Morre, 1966). Cisternal membranes are clearly distinguished from ER and other cell membranes (e.g. Matile and Moor, 1968), although also lipoprotein in nature, suggesting differences in constituent composition or organisation (Mollenhauer and Morre, 1966a).

Mollenhauer and Morre (1966a) have described two general types of vesicle which appear associated with the dictyosomal cisternae; one type, the so-called "shaggy vesicle" is found on all cisternae. It is approximately 50 nm in diameter, has a rough surface texture, and is located at the end of a single tubule. Secretory vesicles, on the other hand, are smooth-surfaced, vary in size (20 - 80 nm in diameter) and have definite tubular attachments (cisternal tubules) to the cisternal lumen (Mollenhauer and Morre, 1966).

Plant dictyosomes (Fig. I.B.14) appear to consist of a variable number of cisternae (e.g. Buvat, 1963; Voeller et al.,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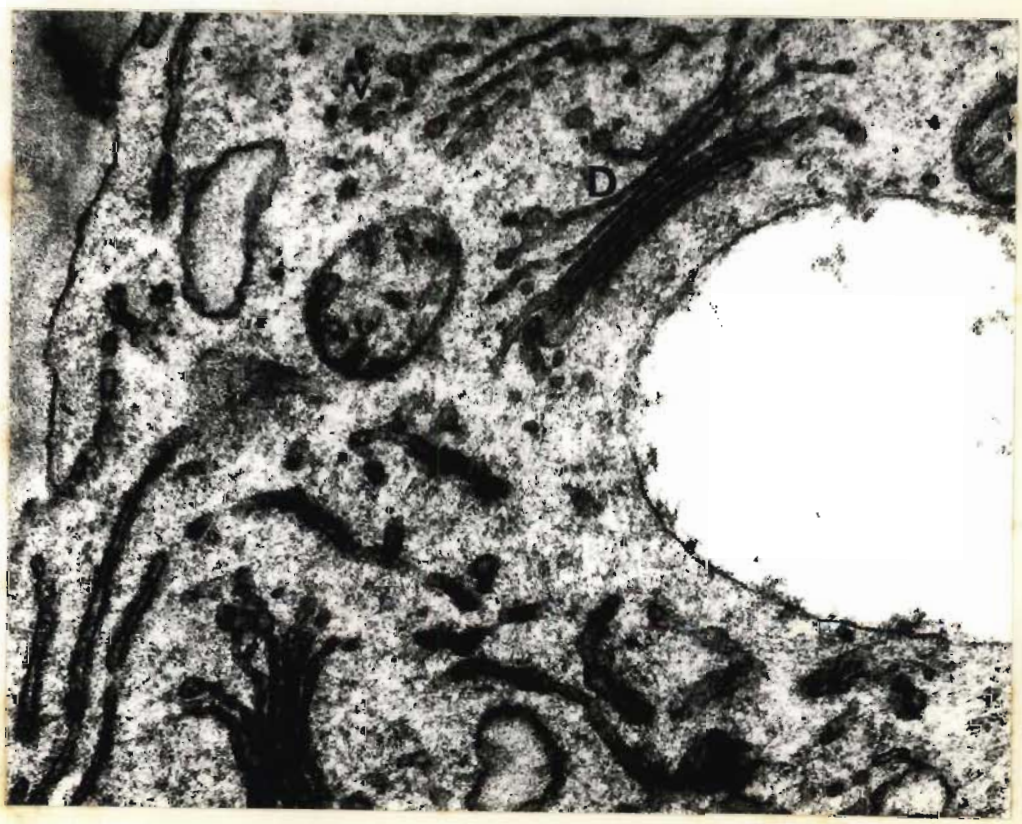
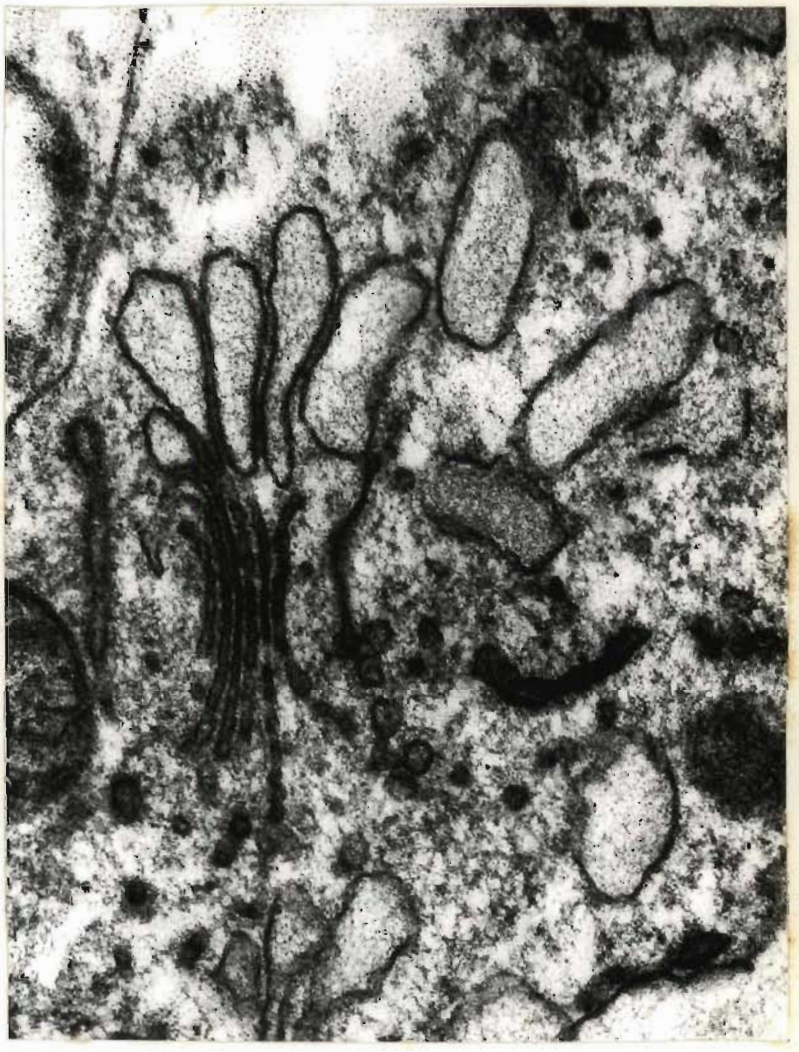

D

1964; Whaley et al., 1960). Mollenhauer and Morre (1966a) have suggested that the cisternae are joined by some bonding constituent, not synonymous with intercisternal elements. Turner and Whaley (1965) described intercisternal elements as being 7 - 8 nm in diameter, and centrally located between adjacent cisternae. These authors reported that the intercisternal elements were not seen in direct contact with the membranes of the cisternae, and suggested that these elements represented only one aspect of the intercisternal region. They also reported that the intercisternal elements varied in appearance with the dictyosome. Their function is unknown.

Dictyosomes of animal and plant cells are morphologically similar, but not all dictyosomes are alike (Mollenhauer et al., 1967). These authors describe the association of dictyosomes to form the Golgi apparatus as being definite in many types of animal cells, whereas in plant cells, if such an association exists, it is described as being loose. However, synchronous changes in the dictyosomes of a cell or tissue region are interpreted as being indicative of an informational connection.

The dictyosome is a variable organelle, the morphology of which is related to its functional status (e.g. Clowes and Juniper, 1964; Whaley et al., 1964; Mollenhauer et al., 1961). In quiescent cells, (undifferentiated non-meristematic cells in the root apex), Clowes and Juniper (1964) describe cisternae as few and loosely associated, while large secretory vesicles are associated with the well-developed cisternae of hypersecretory dictyosomes described in the outer root cap cells of maize (Fig. I.B.15), (Mollenhauer et al., 1961). Plant dictyosomes have also been reported to occur in a form which is different from either the quiescent or hypersecretory type, e.g. in the cortical cells of maize (Whaley et al., 1964).

FIGURE I.B.15. Illustrates a hypersecretory dictyosome in a mature root cap cell of maize. (x 61 600).



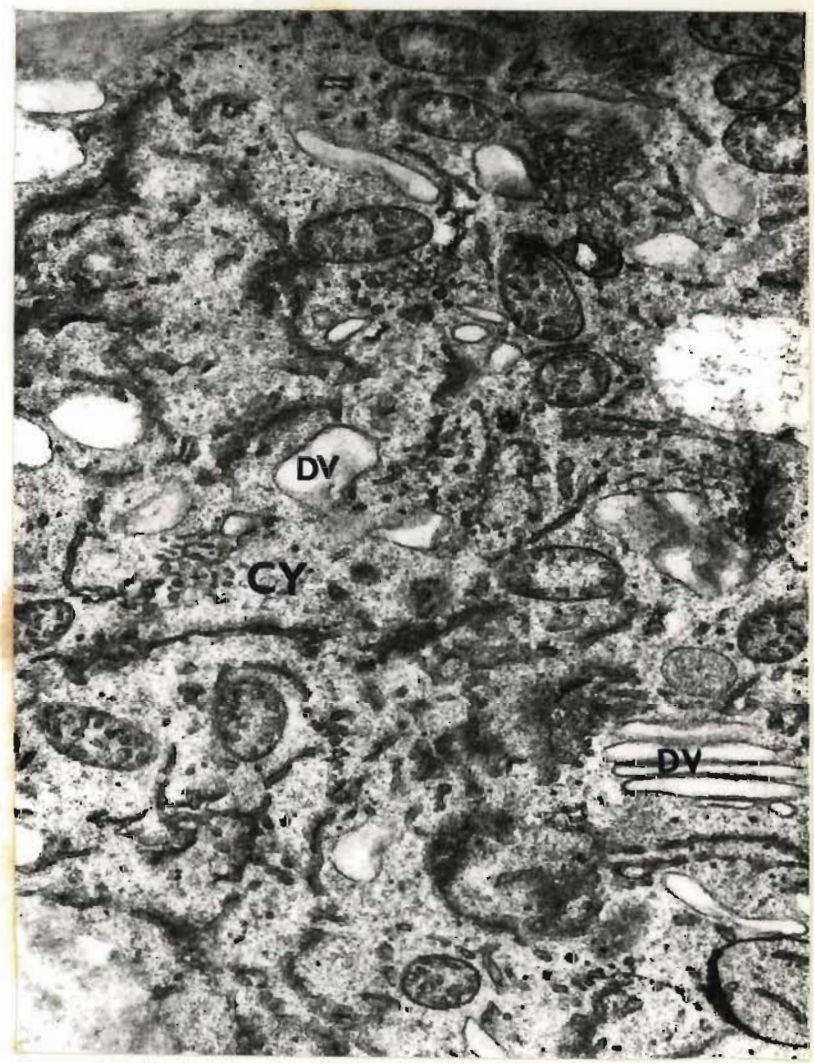
Functional Aspects

The function of the dictyosome was originally thought to be the synthesis of secretions. Palade et al. (1962) and Sjöstrand (1962) established, however, that this organelle is responsible for the segregation, concentration and transformation of secretions, rather than their elaboration. It is generally agreed in the case of proteins synthesized for export from the cell that these are elaborated by the ribosomal systems (associated with the granular ER) and transported across the membrane to appear segregated within the lumen of the ER (e.g. Palade et al., 1962; Sjöstrand, 1962). Caro (1961) and Caro and Palade (1964) using autoradiography combined with electron microscopy, have demonstrated the transfer of proteins from the ER to the dictyosomes, but the mode of this transfer has not been clarified.

As a dictyosomal cisterna matures, secretory vesicles are formed peripherally. These are continuous with the cisternal lumen, through the cisternal tubules during their formation (Mollenhauer and Morre, 1966). The secretory vesicles are ultimately released from the dictyosome becoming free in the cytoplasm (Fig. I.B.16). Changes in the secretory product have been reported both before and after the release of the vesicles from the dictyosome (e.g. Mollenhauer and Whaley, 1963).

The dictyosomes have been shown to play a role in cell plate formation, in which the content of distinct secretory vesicles contribute to this structure (e.g. Whaley and Mollenhauer, 1963) (see later). Dictyosomal secretion appears to play a part in the extension of the primary wall of an epidermal cell in root hair formation (Sievers, 1964), and the participation of secretions from this organelle in secondary wall formation has also been suggested (e.g. Wooding and Northcote, 1964).

FIGURE I.B.16. Illustrates the disposition of
vesicles derived from hypersecretory
dictyosomes in the cytoplasm of a
mature root cap cell of maize.
(x 17 100).


DV
CY
DV

Mollenhauer and Leech (1961) demonstrated that vesicles from hypersecretory dictyosomes migrate to, and fuse with, the plasma membrane in outer root cap cells of Zea mays, as a result of which a considerable accumulation of dictyosomal secretion builds up between the plasma membrane and cell wall (Fig. I.B.17). Morré et al. (1967) have shown that the secreted material is a highly hydrated polysaccharide, which moves through the cell wall under certain conditions to appear as a droplet adhering to the root tip. It appears that this secretion is involved in sloughing off the senescing outermost root cap cells (Fig. I.B.18) (see later).

In addition, some of these hypersecretory dictyosomal vesicles appear to be incorporated into the lysosomal vacuole (Matile and Moor, 1968). If the secondary lysosome is homologous with the plant cell vacuole, then dictyosomal secretion may also be bound up with the maintenance of osmotic activity (see later).

The secreted products of dictyosomes do not generally appear to be restricted to one class of compound. The most usual dictyosomal secretion in an animal cell appears to be proteinaceous, although lipid accumulation has also been associated with this organelle (Peterson and Leblond, 1964). Dictyosomal secretions in plants appear to be predominantly polysaccharide in nature (Sievers, 1964; Morré et al., 1967).

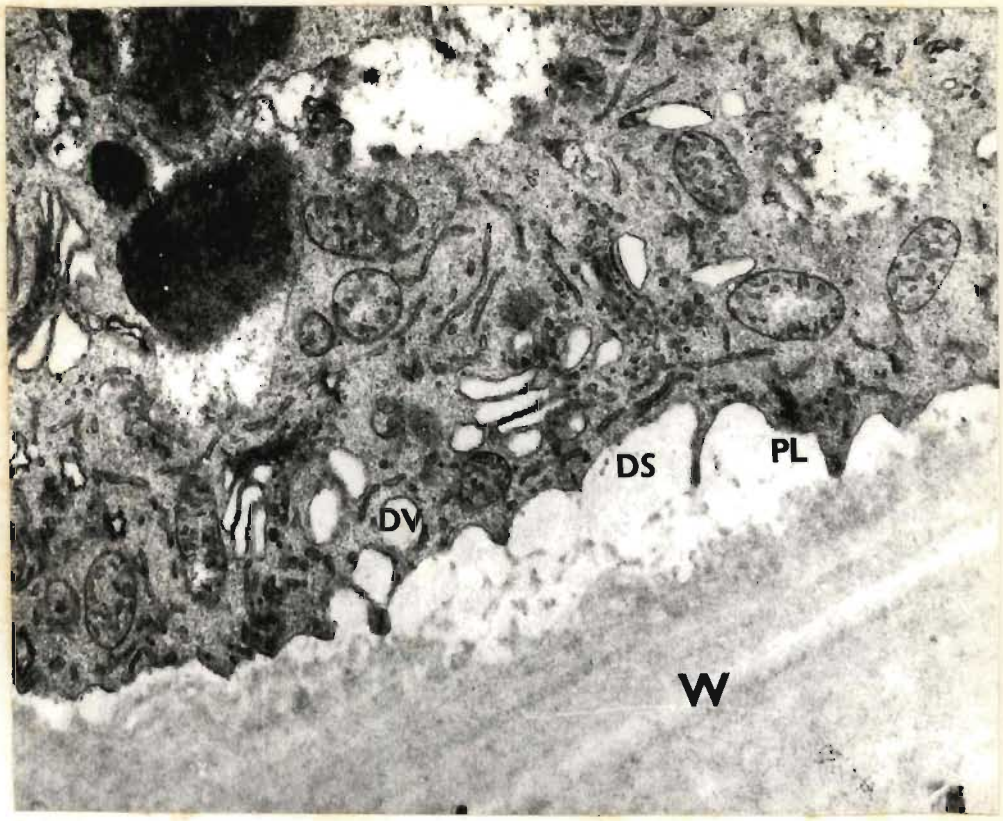
Falk (1962) and Schnepf (1963) demonstrated dictyosomal secretory activity to be dependent on respiratory energy.

Dictyosomal Enzymes

Dictyosomal enzymes have been demonstrated to contribute to the carbohydrate moiety of certain mucopolysaccharides (e.g. Petersen and Leblond, 1964).

FIGURE I.B.17. Illustrates the accumulation of dictyosomal secretion between plasma membrane and cell wall in a mature root cap cell of maize. (x 17 250).

FIGURE I.B.18. Illustrates an accumulation of dictyosomally-derived secretion in the middle lamella region of the wall of an outermost root cap cell of maize. (x 17 100).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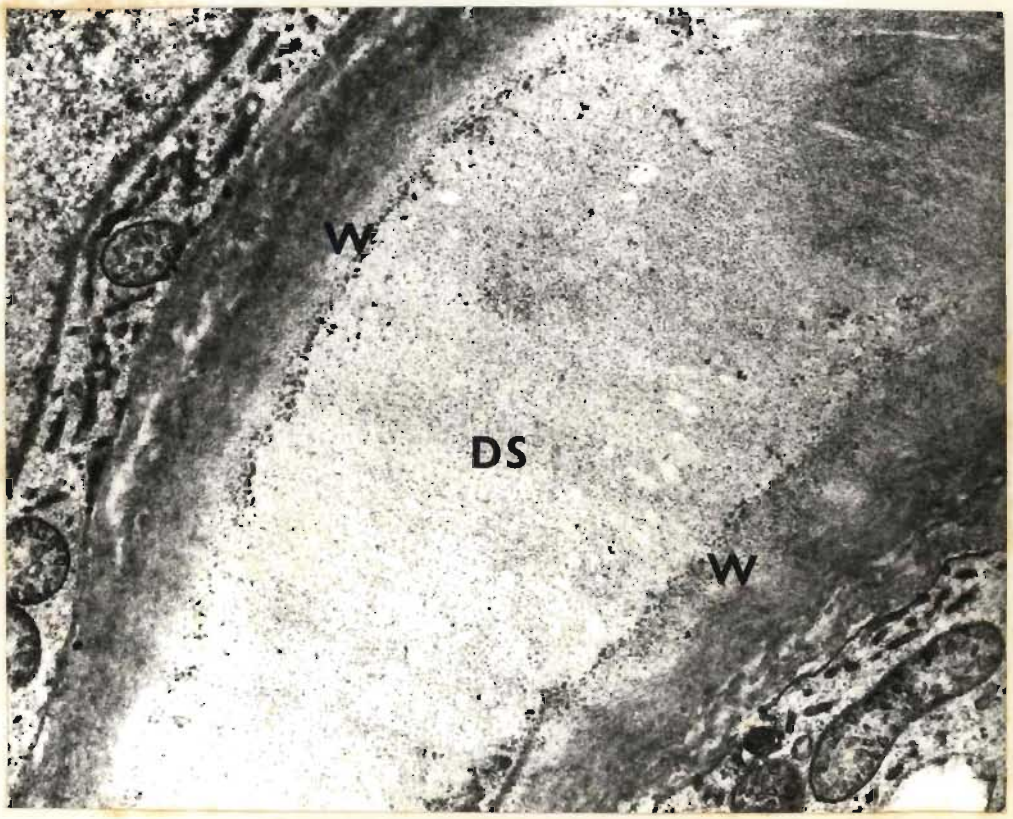

W
DS
W

Nucleotide diphosphatases have been shown to be associated with dictyosomal membranes for animal (Novikoff et al., 1962) as well as for plant tissue (Novikoff and Goldfischer, 1961). Axelrod (1956) suggested the activities of these enzymes to be part of a transferase or synthetase system; however, the significance of the dictyosomal association of these enzymes has not yet been ascertained.

Origin

Novikoff et al. (1962) suggested that a dynamic equilibrium existed between the membranes of the dictyosomes, the ER and the plasmalemma. However, the evidence concerning similarity between these and other cellular membranes is very contradictory (e.g. Yamamoto, 1963; Sjöstrand, 1963). Matile and Moor (1968) have presented evidence from freeze-etched material which suggests that the bounding membrane of the secretory vesicle is different from the membrane of the ER.

Whaley et al. (1960) reported that the number of dictyosomes per cell remains constant following cell division, and Clowes and Juniper (1964) demonstrated that the number of these organelles per unit volume of cytoplasm does not change during cell expansion. These observations suggest replication of this organelle. Mollenhauer and Morre (1966a) have suggested that fragmentation of existing dictyosomes into replicating units of one or more cisternae may be the basis of the reproduction of this organelle.

In general, individual dictyosomes, and those associated to form the Golgi complex, are suggested to be primarily concerned with segregation, concentration and transformation of a secreted product. The subsequent fate of this product generally appears to involve its removal from the dictyosome via secretory vesicles, its directional transport and finally its discharge.

MITOCHONDRIA

Mitochondria were first described in striated muscle by Köliker over a hundred years ago, and an intensive study of these organelles was under way early this century. The independent work of Palade (1953) and Sjöstrand (1953) on ultra-thin sections of mitochondria first enabled the ultrastructure of these organelles to be described.

1. Structural Aspects

A common organisational pattern exists, which characterises mitochondria in all cell types studied (Fig. I.B.19).

All mitochondria are bounded by membranes. The outer membrane (of average diameter 5.5 nm) has definite irregularly-spaced pits 2.5 - 3 nm in diameter (Parsons et al., 1965). A second unit membrane, the inner membrane, averaging 4.5 nm in diameter, bounds the mitochondrion, inside of the outer membrane. The organelle is thus bounded by a double unit membrane with a space, the outer compartment, between them.

The inner membrane exhibits invaginations in all cases, but these vary in number, dimension and complexity. The invaginations are termed cristae (the "cristae mitochondriales" of Palade, 1953), and their variations are associated with cell type and physiological and metabolic status.

Generally in plant mitochondria the cristae do not form the parallel arrays typical of these organelles in animal cells. Parsons et al. (1965) have found the cristae to be randomly orientated in a variety of plant mitochondria, sometimes connecting with each other, or forming closed loops. The lumen of the outer compartment appears to be continuous with the spaces within the cristae, but does not communicate with the lumen of the inner membrane (e.g. Lehninger, 1964).

FIGURE I.B.19. Illustrates a group of mitochondria in a mature plant cell. (x 25 500).


OM
IM
MM
M
CT

The lumen of the inner membrane system is filled with the finely granular matrix (Lehninger, 1964). Parsons et al. (1965) have shown that the matrix varies in density in mitochondria isolated by similar techniques. The matrix is known to contain protein and lipid (Lehninger, 1964). Parsons et al. (1965), working with plant material, reported that the mitochondrial matrix contained particulate aggregations of average diameter 10 nm, and 17 nm diameter particles resembling ribosomes, as well as mitochondrial granules of average diameter 30 - 40 nm.

Knob-like, stalked structures, the inner-membrane subunits (IMS), have been demonstrated in negatively-stained preparations of animal (e.g. Fernández-Morán, 1962) and plant mitochondria (Nadakavukaren, 1964; Parsons et al., 1965). These appear to cover the entire surface of the inner membrane, including the cristae and consist of a spherical head (averaging 10 nm in diameter) attached by a stalk (3.5 - 4 nm wide, and 4.5 nm long) to the inner mitochondrial membrane.

Nass and Nass (1963) demonstrated that fibrous inclusions in the matrix of mitochondria are, in fact, DNA fibrils. DNA is now considered to be a normal constituent of mitochondria, situated mainly in the matrix and possibly connected to the inner membrane by fine fibrils (Nass et al., 1965). Mitochondrial DNA is not identical with nuclear DNA (Roodyn, 1967).

2. Functional Aspects

(a) Respiratory activity

The oxidation of various substrates, accompanied by the formation of ATP (i.e. respiratory activity), is generally considered to be the main function of the mitochondrion. Mitochondria contain the enzymes

necessary for biological oxidation reactions. There is an underlying pattern common to most biological oxidations, starting with the removal of electrons and protons (in the form of hydrogen) from the substrate molecule coupled with their acceptance by a coenzyme molecule, usually NAD^+ or $NADP^+$. This type of reaction is catalysed by a specific dehydrogenase. The reduced coenzyme becomes reoxidised as electrons and protons are accepted by FAD, the next carrier. Thereafter the electrons and protons move through an assembly of carriers, the cytochromes, which are alternately reduced and oxidised. The final carrier in the assembly, cytochrome a_3 (cytochrome oxidase), releases the electrons which, together with the protons, reduce oxygen forming water.

This process, known as electron transport, is accompanied by phosphorylation of ADP to ATP, the process termed oxidative phosphorylation. NAD^+ and $NADP^+$ are pyridine nucleotides, FAD is a flavin coenzyme while each cytochrome consists basically of an iron-porphyrin prosthetic group bound to a protein (Conn and Stumpf, 1965).

Although the basic pattern of the respiratory chain is similar in both plants and animals, differences have been demonstrated in the constituent cytochromes. Pyridine nucleotide, flavoprotein, three cytochromes b, two cytochromes c (one of which is c_1-like and firmly membrane-bound), cytochromes a and a_3, have been characterised from a wide variety of higher plant tissues (Lance and Bonner, 1968). There is very little information on the sites of ATP formation accompanying the plant respiratory chain.

The components of the respiratory chain, viz. the carriers and their associated enzymes, are believed to be arranged in groups or complexes called respiratory assemblies (e.g. Lehninger, 1964; Roodyn, 1967). Lehninger (1964) described the respiratory assemblies to be firmly embedded in the inner membrane, and Roodyn (1967) has corroborated this idea.

The IMS were originally thought to contain cytochrome (Chance and Parsons, 1963) and were thus suggested to represent portions of the electron transport system. However, Chance et al. (1964) later demonstrated that the IMS contained few or no cytochromes, and Racker et al. (1964) suggested them to be related to the enzymes of oxidative phosphorylation.

Source of substrates for respiratory activity

The substrates which undergo oxidation (dehydrogen ation) as the starting point of mitochondrial respiratory activity have proved to be a variety of organic acids.

Carbohydrates are a major source of energy for all living organisms. The degredation of carbohydrates (e.g. starch, glycogen, glucose etc.) to form pyruvate, occurs in the cytoplasm of the cell (see later).

The resultant pyruvate is completely metabolised to carbon dioxide and water by a cyclic sequence of interconversions of organic acids. This sequence is termed the tricarboxylic acid (TCA) cycle (Krebs cycle, Citric acid cycle), and occurs in the mitochondrion. Certain of the interconversions involve dehydrogenation reactions, with subsequent electron transport accompanied by ATP formation.

The mitochondria also have the enzyme system for the β-oxidation of fatty acids (Lynen, 1955). The terminal product of this oxidation is acetyl coenzyme A (acetyl CoA) which may

be channelled directly into the TCA cycle.

TCA CYCLE

There is a considerable accumulation of evidence to show that pyruvate is oxidised via this cycle, in plants (e.g. Beevers, 1961). Although some of the TCA cycle enzymes have dual localization (i.e. in the mitochondria and elsewhere in the cell), certain enzymes of this cycle appear to occur uniquely in the mitochondria. Notable in this respect are the α-oxoglutarate dehydrogenase system and succinate dehydrogenase (Roodyn, 1967).

The localization of many of the TCA cycle enzymes in the inner-membrane-matrix fraction (following differential centrifugation and digitonin treatment of rat liver mitochondria) has recently been demonstrated (Schnaitman and Greenawalt, 1968). The authors also reported that this fraction has a high respiratory rate. These findings further substantiate the view of Lehninger (1964) and Roodyn (1967) that the functional TCA cycle may be ascribed to the mitochondrion.

Fatty Acid Oxidation

Mitochondria isolated in a relatively simple medium have the ability to oxidise fatty acids to completion. All the enzymes necessary for the β-oxidation of fatty acids (Lynen, 1955) are thus localised in the mitochondrion. β-oxidation of fatty acids results in the formation of acetyl coenzyme A, which is incorporated into the TCA cycle, by the action of the enzyme citrate synthetase.

(b) Some other mitochondrial reactions (enzyme systems).

Lipid Synthesis

Lipid synthesis occurs in mitochondria. Enzyme systems for lipid synthesis within mitochondria have been reported by

several workers (e.g. Webster et al., 1965).

RNA Synthesis

A DNA-dependent RNA polymerase in mitochondria has been suggested as a result of incorporation of certain radioisotopes into RNA. The incorporation was inhibited by actinomycin C (Neubert and Helge, 1965). RNA polymerase has been demonstrated in the mitochondria of a variety of animals, as well as in yeast by Wintersberger (1964).

Protein Synthesis

Mitochondria from a variety of sources have been shown to incorporate a wide range of amino acids (Roodyn, 1965). Incorporation is mainly into insoluble proteins associated with the mitochondrial membrane system (Roodyn, 1967).

Amino Acid Metabolism

Roodyn (1967) discusses the occurrence of several amino transferases in the mitochondrion. The action of these enzymes is not unique to the mitochondrion. Beaufay et al. (1959) demonstrated the action of the enzyme glutamate dehydrogenase to be unique to mitochondria.

Adenosine Triphosphatase (ATPase) Activity

ATPase generally occurs in a latent form in intact mitochondria, but considerable activity of this enzyme occurs in experimentally-damaged mitochondria or in mitochondria treated to uncouple oxidative phosphorylation (Roodyn, 1967).

Reversal of the terminal reactions of oxidative phosphorylation (Chance and Hollunger, 1961) may also result in net hydrolysis of ATP which enhances the effect of ATPase activity.

Ion Transport

The presence of a complex system for ion translocation appears to be a property of the mitochondrial membrane. Carafoli et al. (1964) demonstrated that the mitochondrial uptake of one ion-type is often accompanied by the release of another species of ion into the medium.

An interesting stoichiometric relationship between mitochondrial Ca^{++} and Sr^{++} uptake, and respiration (oxygen utilisation) has been reported for mitochondria (by Rossi and Lehninger, 1964 and Carafoli, 1965), in which the addition of low concentrations of the ions stimulates oxygen uptake.

Mitochondrial Swelling and Contraction

Frederic (1958) described changes in conformation of mitochondria in unfixed, living cells. He showed that changes in shape and volume were linked in some way to respiration and oxidative phosphorylation. Mitochondrial swelling and contraction have been subjects of considerable interest since that time.

Stoner and Hanson (1966) have carried out an intensive investigation on the swelling-contraction mechanism in corn (maize) mitochondria, and have pointed out certain differences between plant and animal mitochondria in this respect, for example, plant mitochondria appear to be more permeable than animal mitochondria.

The schematic representation of the swelling contraction mechanism in corn mitochondria according to Stoner and Hanson (1966), is shown in Fig. I.B.20.

Contraction of corn mitochondria is attributed to the non-phosphorylated high-energy intermediate of oxidative phosphorylation, and the degree of contraction at any one time is

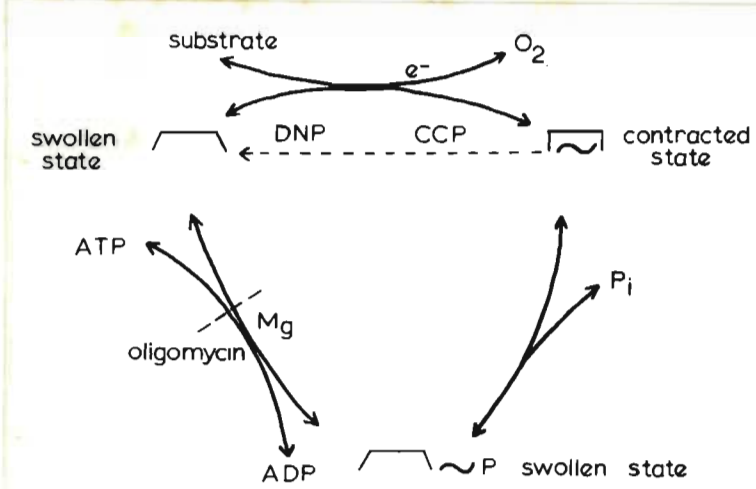


Fig. **I.B.20.** Scheme of the relationship between oxidative phosphorylation and mitochondrial swelling and contraction.

DNP = dinitrophenol CCP = carbonyl cyanide m - chlorophenyl hydrazone } uncoupling agents

Oligomycin is an inhibitor of ATP-powered contraction and the release of phosphate (after Stoner & Hanson, 1966)

thought to be related to the amount of this intermediate present. Under conditions which do not favour this intermediate (e.g. the absence of ATP, or of respiratory substrate), hydrolysis occurs, accompanied by mitochondrial swelling. The presence of excess phosphate is thought to effect phosphorylation of the intermediate, thus forming the phosphorylated high-energy intermediate of oxidative phosphorylation, not instrumental in the maintenance of contraction. Stoner and Hanson (1966) also suggest the possible implication of ions in the swelling-contraction mechanism.

Roodyn (1967) has suggested that ATPase activity may also be related to contraction of the mitochondrial membrane, in that a membrane-bound ATPase (similar in principle) to actinomyosin) might be operative.

The Implications of Mitochondrial DNA (Roodyn and Wilkie, 1968)

Mitochondria contain DNA, and appear to contain a DNA polymerase. In addition, a DNA-dependent RNA polymerase has also been established to be present. It seems probable that characteristic ribosomes (O'Brien and Kalf, 1967) and all the other necessities for protein synthesis are also present in mitochondria. In addition, labelled amino acids have been shown to be incorporated into peptide chains within mitochondria, and mitochondrial protein synthesis has been demonstrated to be an energy-requiring process.

Mitochondria thus appear to have some measure of an inherent self-replication system. But in addition, however, it is likely that systems outside the mitochondrion also influence the formation and replication of this organelle. The protein synthesized by the mitochondrial system appears to be insoluble structural protein, and there is no evidence of enzyme synthesis.

In addition, considerable cytological evidence implicates 'promitochondrial membranes' in mitochondrial biogenesis. Mühlethaler and Bell (1962) demonstrated that the nuclear membrane (of oöspheres of fern) formed evaginations, which subsequently became detached. This occurred after the disappearance of maternal mitochondria. The vesicles so formed were demonstrated to contain DNA and RNA, and were postulated to be mitochondrial (and plastid) initials (Bell and Mühlethaler, 1962; 1964). Thus the origin of these organelles still requires considerable investigation.

Dense Granules

The mitochondrial inclusions generally known as dense granules, have been shown to be composed of insoluble inorganic salts. Chappell and Crofts (1965), considering the granules to be stores of inorganic ions, have postulated them to be associated with ion-transport (exchange) and swelling-contraction phenomena.

MICROBODIES

Baudhuin et al. (1965) demonstrated microbodies from animal tissue to be bounded by a single membrane, and often to contain a characteristic central core. Shnitka (1966) grouped hepatic microbodies into 3 categories, based on the absence or presence (and nature) of the central core. de Duve and Baudhuin (1966) proposed the general term 'peroxisome' for all microbodies. They suggested that these particles (which contain the enzymes L-hydroxy-acid oxidase, catalase and urate oxidase) have a possible role in the extra-mitochondrial oxidation of NADH.

Microbodies from plant cells do not contain urate oxidase (Long, 1961). Frederick et al. (1968) suggested that non-crystal containing microbodies might either represent plant

lysosomes (see later), or be homologous with the microbodies of animal cells.

LYSOSOMES

Lysosomes are generally described as subcellular structures bounded by a single membrane, and having no definite internal structure. A number of acid hydrolases (with a common pH optimum of about 5) are associated with these organelles. Treatment of lysosomes with substances known to interfere with protein-lipid binding releases the hydrolases (Gahan, 1967). de Duve (1963) suggested enzymic differences between lysosomes in a tissue (but not to the extent of one lysosome-one enzyme).

Two main modes of lysosome functioning were postulated by de Duve (1963). (i) The action of the lysosome as an organ of intracellular digestion, and (ii) the liberation of lysosomal enzymes within a cell, resulting in destruction and cell death. Novikoff et al., (1964) suggested that lysosomes coalesce with pinocytic vesicles, the latter containing materials to be digested. This supports the role of lysosomes in intracellular digestion.

The lysosomal role in cellular autodigestion may result either in death of the entire cell, or may involve cytolysome formation (see later), resulting in the localised modification of certain cytoplasmic contents (Gahan, 1967).

The nature of the bounding membrane of an organelle which contains enzymes potentially injurious to the cell must be such as to retain these enzymes separate from their substrates. Therefore it is considered that an intact lysosome should exhibit a latency in the deposition of reaction products in histochemical tests for hydrolase activity (Gahan, 1967).

Lysosomes in plant cells

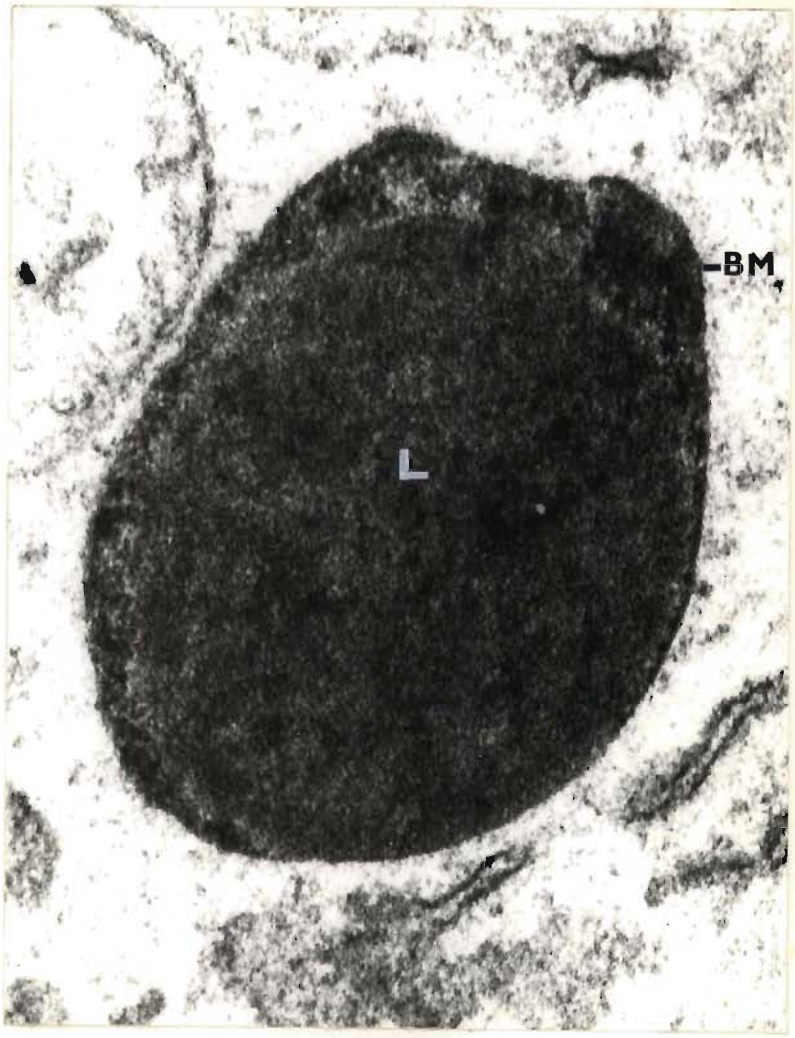
Establishment of the presence of lysosomes in plant cells has proved a difficult process. Several investigators have reported acid phosphatase-positive particles in plant cells as a result of light microscope histochemistry (e.g. Jensen (1956) in the root tips of Vicia faba, Allium cepa and Pisum sativum; Gahan (1965) in the root meristem of Vicia faba).

Gahan and Maple (1966) observed acid phosphatase activity in the root cap cells of Vicia faba, using the optical microscope. They showed that the acid phosphatase activity was confined to particulate sites in the meristematic cells, whereas the outermost (senescing) cells showed a diffuse reaction for the activity of this enzyme. This evidence suggests that senescence in the root cap cells is accompanied by the release of hydrolytic enzymes normally confined within an organelle.

However, until recently, little success was obtained in establishing the presence of lysosomes in ultra-thin sections of higher plant material. Poux (1963) demonstrated acid phosphatase activity in the vacuoles of shoot apex meristem cells in electron microscope studies, and Wardrop (1968) demonstrated structures with a positive reaction for acid phosphatase activity, in electron micrographs of Eryngium sp. collenchyma.

The present writer (Berjak, 1968) demonstrated organelles in ultra-thin sections of the root cap (and root apex) of Zea mays, which conform in appearance and enzyme activity, with lysosomes of animal tissue (Fig. I.B.21). A developmental sequence of these organelles (which will be discussed in detail later), was described in the root cap. In the meristematic cells these organelles appear as electron-dense bodies (of average diameter 900 nm) bounded by a single membrane, while the senescing cells

FIGURE I.B.21. Illustrates a lysosome in a mature root cap cell of maize. Note the electron-dense content and single bounding membrane. (x 58 500).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contain these organelles in various phases of their dissolution and show a diffuse cytoplasmic reaction for acid phosphatase. This author suggested that these organelles are plant lysosomes.

Matile (1968) showed the presence of organelles in freeze-etched preparations of corn root tip cells, which he suggested represented stages in the development of the lysosomal apparatus. Matile also used methods of cell fractionation to demonstrate the presence of three different types of lysosome, varying in density and hydrolase content.

Origin and subsequent fate of lysosomes.

Matile and Moor (1968), in a report on the morphology of vacuolation studied by freeze-etching, suggested that provacuoles derived from the ER, fused and subsequently expanded. The vacuoles were found to contain hydrolases and were suggested to be primary lysosomes. The present writer in ultrastructural studies on the development of lysosomes, has obtained evidence partly supporting these views. (See later). An expansion of ER-derived provacuoles, accompanied by a progressive increase in their dense content, appears to occur. This has been termed the first developmental phase (Figs. I.B.22 & 23). However, apparent fusion of first-phase lysosomes (in ultra-thin section) has only been observed when they appear fully expanded. (Fig. I.B.24). (See Part III).

An intimate association of the ER with first-phase lysosomes, immediately prior to their swelling (the second developmental phase) has been observed (Fig. I.B.25). Incorporation of large dictyosomal vesicles into second-phase lysosomes in root cap cells has also been observed in electron microscope studies of ultra-thin sections (Figs. I.B.26a,b,c & d) by the present writer, (See Part III), and autoradiographic results

FIGURES I.B.22 & 23. Illustrate the first developmental
phase of plant cell lysosomes.
Fig. 22 shows vesiculation of
the ER (x 38 250).
Fig. 23 illustrates several
ER-derived provacuoles in the
various stages of first-phase
lysosome development. (x 24 300).


ER
V

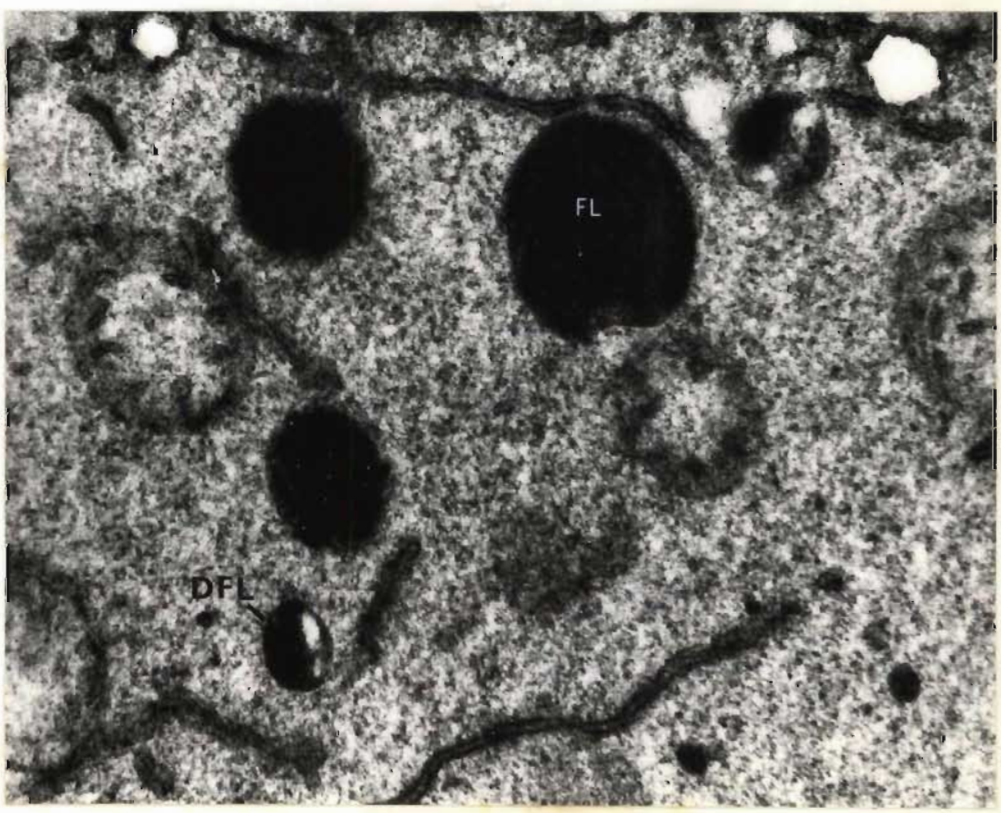
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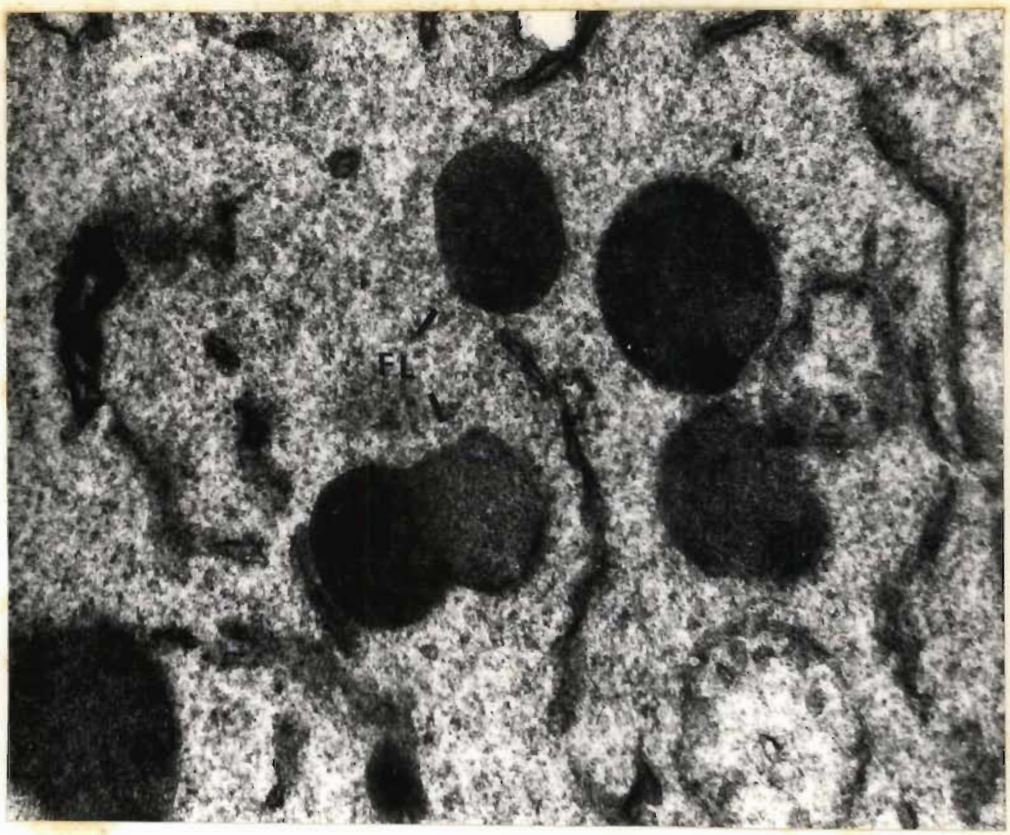
FIGURE I.B.24. Illustrates the apparent fusion of fully-formed first-phase lysosomes. (x 24 300).

FIGURE I.B.25. Illustrates the intimate ER-lysosome association observed immediately prior to the swelling of the latter under certain conditions. (x 13 050).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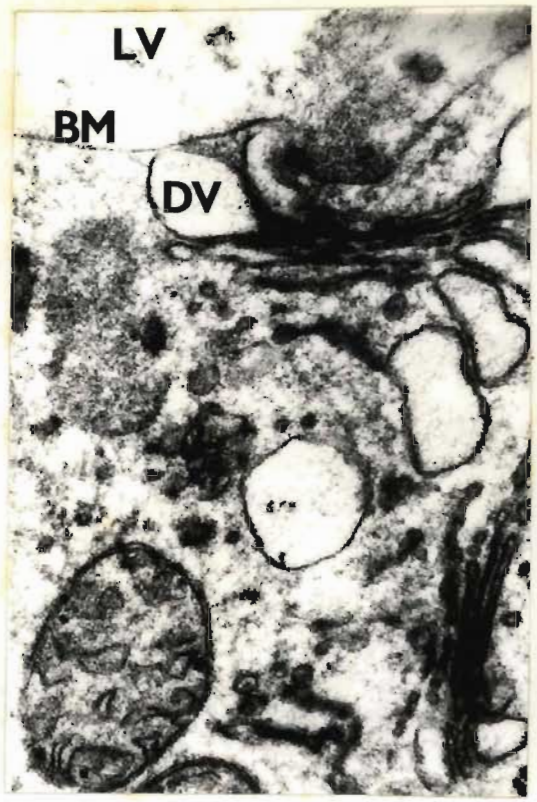
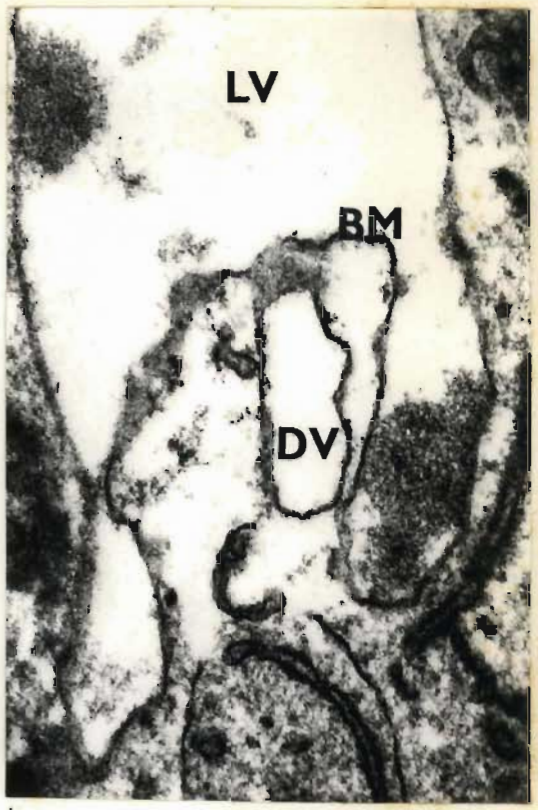
ER
FL

FIGURES I.B.26a-d. Illustrate stages in the incorporation of dictyosomal vesicles into second-phase lysosomes (lysosomal vacuoles).
(26a x 42 500; 26b x 50 400;
26c & d x 44 800).



a



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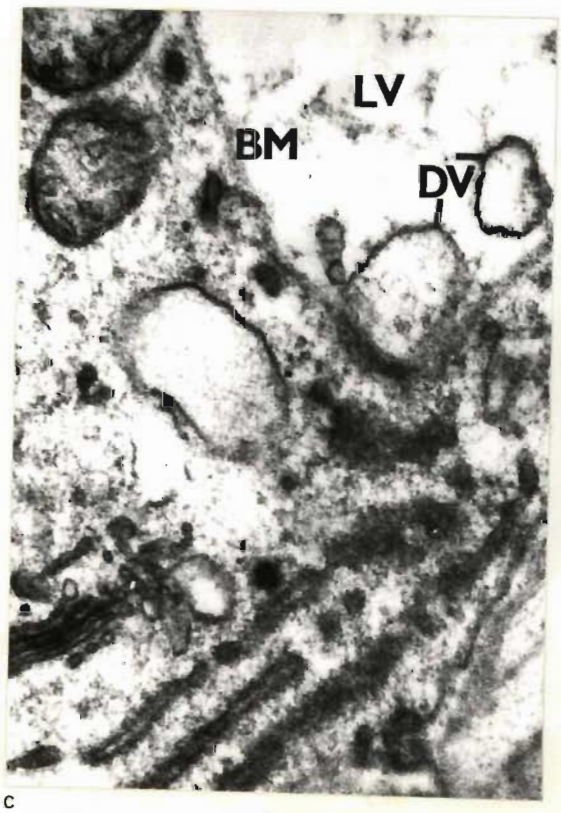
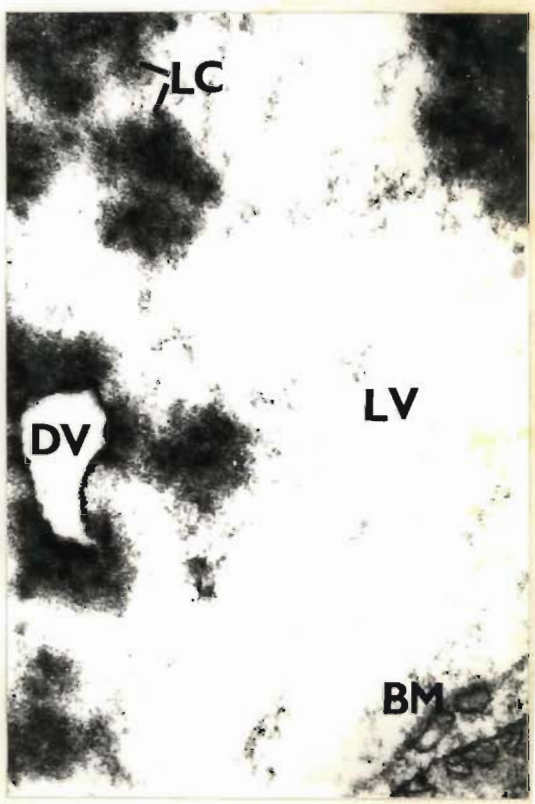


c



d

of Pickett-Heaps (1967) bear out this observation. Following this, the lysosomal vacuole may be termed a secondary lysosome (de Duve and Wattiaux, 1966).

Poux (1963a) and Brandes et al. (1965) have observed organelles normally encountered in the cytoplasm, in a process of digestion within the plant cell vacuole. In addition, Matile (1968) demonstrated the presence of hydrolases in the vacuoles of plant cells.

It seems likely that the plant cell vacuole is homologous with the secondary lysosome, and that it functions in two capacities: Firstly, as an organ of intracellular digestion, and secondly, in the maintenance of the turgor of the cell. The particulate concentration of the vacuole necessary for turgor maintenance may be partly dictyosomal in origin.

Lysosomes of root cap cells (and probably certain other plant cells as well) may act in yet another capacity - to bring about cell autolysis. The root cap cells are not typically vacuolated. This is probably a factor of the transient nature of a root cap cell, where only a short time elapses between formation and senescence. It has been demonstrated by the present writer (Berjak, 1968) that the lysosomal membrane undergoes dissolution in the outermost, senescing root cap cells. Accompanying this final phase of the lysosome is the autolysis of the cell.

The aleurone grains in the cotyledons of germinating pea seeds (Matile, 1968a) and cottonseed (Yatsu and Jacks, 1968) have been shown to contain hydrolytic enzymes. These authors suggest that the aleurone grains function as organs of intracellular digestion in connection with the mobilization of food reserves during germination.

CYTOLYSOMES

Intracellular membrane-bounded structures, containing other cytoplasmic organelles (in states of degradation) and having hydrolytic enzyme activity, are generally referred to as cytolysomes. However, the secondary lysosomes described above also answer to this description. Villiers (1967), working on embryos of Fraxinus excelsior, described cytolysomes which were bounded by a double membrane, and not the single membrane typical of a lysosome.

The term, cytolysome, might thus be better reserved for structures differing from secondary lysosomes, which also appear to function for intracellular digestion.

It must be mentioned that cytolysomes, while occurring in normal cells, are fare more conspicuous in cells which are under stress (Gahan, 1967).

SPHEROSOMES

Spherosomes are subcellular particles characterised by their high lipid content, but are differentiated from other lipid inclusions by the possession of a bounding membrane (Buvat, 1963). Spherosomes were suggested to originate by vesiculation of the ER, followed by detachment of the vesicle (Frey-Wyssling and Mühlethaler, 1965).

The precise origin and functional significance of the spherosomes is not yet clear. Frey-Wyssling and Mühlethaler (1965) attribute the final step in the synthesis of lipids to the spherosomes, and suggest that these are specialised organelles whose function is fat production. However, Sorokin, (1967)

reported that plants with a very low lipid content often contained a large number of spherosomes. In the opinion of this author the origin and function of the spherosomes, as explained by Frey-Wyssling and Mühlethaler (1965), are open to considerable question.

Jacks et al. (1967) suggested peanut cotyledon spherosomes to be sites of lipid storage, but not of lipid breakdown, and Ory et al. (1968) while agreeing that spherosomes are sites of fat storage, postulated that lipase activity is associated with these organelles in castor bean endosperm. Matile and Spichiger (1968) (in investigations of spherosomes of tobacco endosperm cells), also described these organelles as sites of lipid storage, having hydrolase activity, and interpreted them as a special type of lysosome.

Thus the general current opinion concerning the function of spherosomes appears to be that these organelles represent lipid stores, although their role in lipid synthesis is questionable. In addition, hydrolases associated with these organelles are thought to be responsible for the mobilization of lipid reserves.

ALEURONE GRAINS AND GLOBOIDS

Aleurone grains are protein-storing cytoplasmic particles. The cells of the outermost layer of the endosperm (in monocotyledons) contain many of these particles, thus this layer is termed the aleurone. Some aleurone grains consist of protein, with or without crystalline inclusions, while other aleurone grains contain spherical inclusions, called globoids, within the protein mass (Liu and Altschul, 1967). These authors found the globoids (from cottonseed aleurone grains) to contain 14.2% phosphorus and 10% metals and have suggested that this particle is the site of storage of phosphorus and certain metals.

PLASTIDS

A number of subcellular particles are generally classified as plastids. These are the plastid initials, the proplastids, the amyloplasts, chloroplasts and chromoplasts. The present discussion of plastids is confined to the plastid initials, proplastids and amyloplasts, as no aspects of chloroplasts or chromoplasts are pertinent to the work.

Origin.

In studies of oögenesis, Mühlethaler and Bell (1962) demonstrated that the chloroplasts and mitochondria of the oösphere of Pteridium aquilinum degenerated during maturation. These authors showed that, subsequent to the chloroplast and mitochondrial degeneration, the nuclear envelope evaginated to form vesicles. They also demonstrated that these vesicles, which presumably contained nucleoplasm, and were bounded by a double membrane derived from both components of the nuclear envelope, became detached from the nuclear envelope. Autoradiographic evidence showed further that the vesicles also contain DNA (Bell and Mühlethaler, 1964). The vesicles are postulated to be the initials of the plastids and the mitochondria (Bell and Mühlethaler, 1962; 1964).

However, Diers (1966), in studies of plastids and mitochondria during oögenesis in the bryophyte Sphaerocarpus donnellii Aust., found no indications of their degeneration or the development of new organelles from nuclear envelope evaginations. Diers suggested that the pre-existing plastids and mitochondria divide, thus accounting for the increase in numbers of these organelles during oögenesis.

The conflicting reports of these authors is indicative of the general controversy which is prevalent concerning the origin of these organelles.

Development

Growth of plastid initials to form undifferentiated oblate ellipsoids, is considered to be the next step in the developmental sequence of the plastids (Frey-Wyssling and Mühlethaler, 1965). These authors describe the subsequent developmental step to be represented by tangential invaginations of the inner membrane of these structures. Flat vesicles termed thylakoids (Menke, 1962) are then cut off from the invaginations. At this stage the plastids have a diameter greater than 0.5 μ, and are termed proplastids (Fig. I.B.27) (Frey-Wyssling and Mühlethaler, 1965).

These authors describe differences in the development of thylakoids under light or dark conditions. In the light, the thylakoids develop into the grana and stroma lamellae typical of the chloroplasts, whereas in the dark lamellation does not occur.

Menke (1961) showed that the vesicles arranged themselves to form an organised 3-dimensional lattice, which is now termed the prolamellar body, in proplastids maintained in the dark, and Hodge et al. (1956) showed that thylakoid lamellae grow from the organised lattice, if such proplastids are exposed to light.

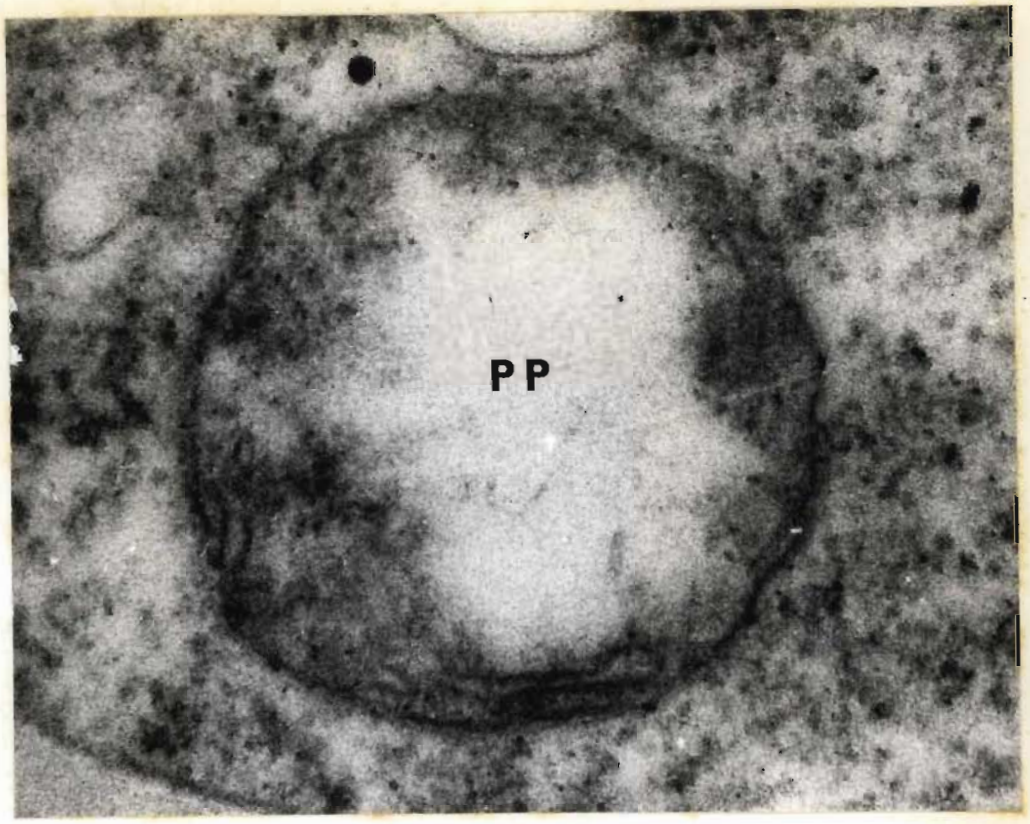
Amyloplasts

Amyloplasts are plastids which produce starch, and are generally devoid of pigment. (Fig. I.B.28). Badenhuizen (1964) discussed exceptions to this general description, citing amyloplasts which become green on exposure to light; amyloplasts which are always green; and amyloplasts which begin as chloroplasts.

Amyloplasts are differentiated from proplastids mainly by their size. These plastids contain only a few lamellae, but those which become green in the light are able to build up an

FIGURE I.B.27. Illustrates a proplastid in a root cap initial of maize. This material was postfixed in an osmium solution according to Procedure 6b. (x 67 200).

FIGURE I.B.28. Illustrates a starch-containing amyloplast. (x 42 500).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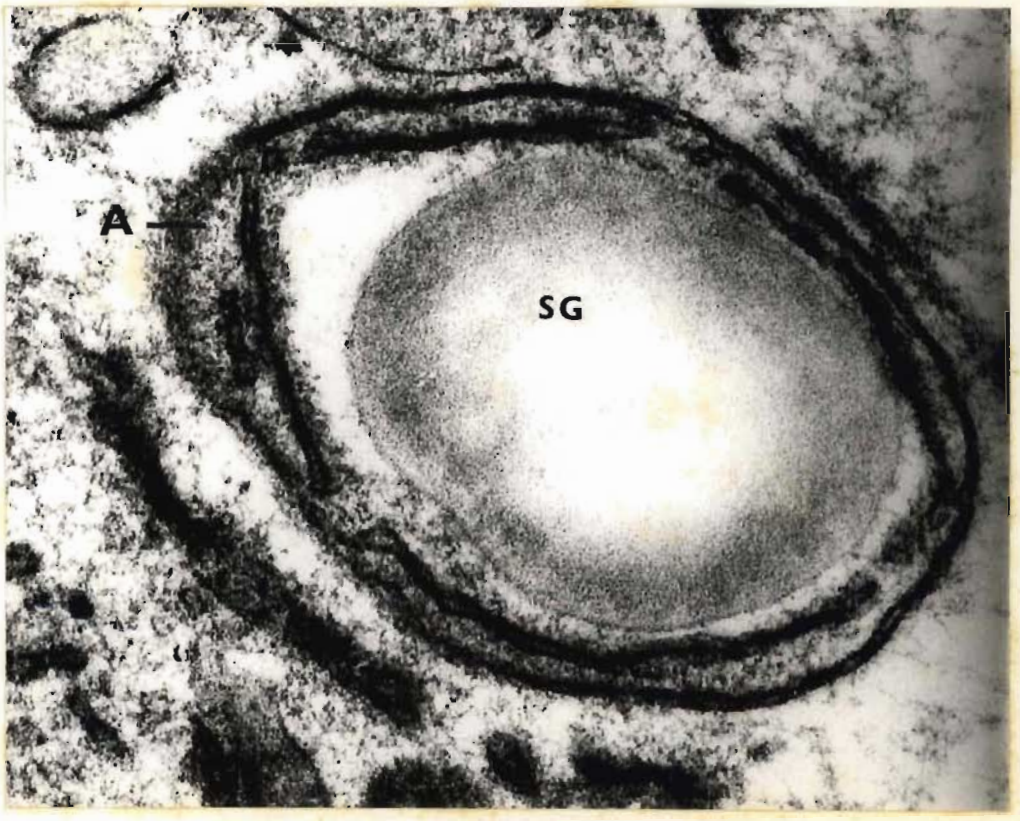
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organised thylakoid structure under these conditions. This type of amyloplast is described as storing ergastic material in the prolamellar body for thylakoid production. In addition, it stores starch in the form of granules in the stroma (Frey-Wyssling and Mühlethaler, 1965).

Certain amyloplasts do not become green on exposure to light. Bjorn (1963) has shown that, although protochlorophyll is present in corn root plastids, it is not particularly light-sensitive. Bjorn (1967) contrasts the 'active' (photo-convertible) protochlorophyll of leaf plastids, with the 'inactive' esterified protochlorophyll of corn roots.

Heitz (1957), in studies on amyliferous proplastids of corn roots, described these organelles as being devoid of a 'primary granum' (now defined as the prolamellar body). The amyloplasts of maize roots do not show a prolamellar body.

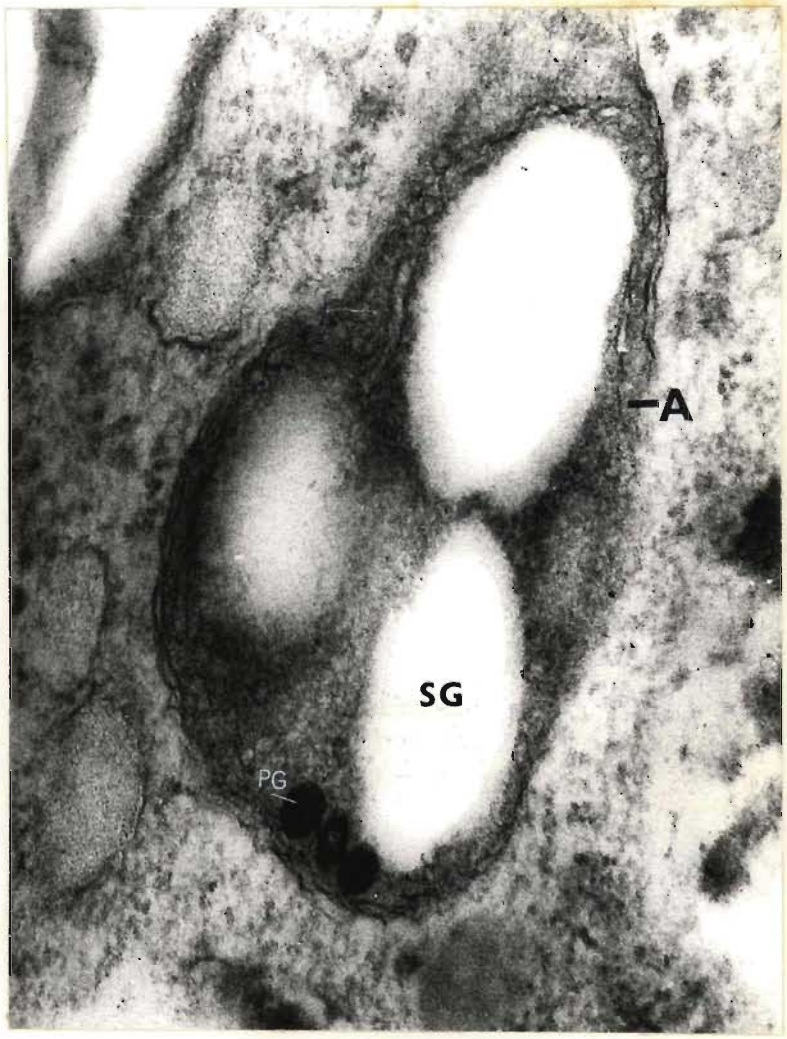
Plastid inclusions, other than starch.

Lichtenhaler (1966) described plastoglobuli as natural components of all types of plastids (Fig. I.B.29). This author suggested that the ontogeny and interrelationships of the various plastid types could be based on the size and number of plastoglobuli which they contain. Heslop-Harrison (1966) suggested that these osmiophilic plastoglobuli might serve as a pigment reservoir within the plastid, while Lichtenhaler and Peveling (1967) related lipoquinone and plastoglobuli content of onion plastids.

RNA and plastid ribosomes

RNA has been identified within particles which occur in the stroma of plastids (e.g. Jacobson et al., 1963), and Eisenstadt and Brawerman (1963) demonstrated that these particles could support protein synthesis. These particles are now considered to be plastid ribosomes.

FIGURE I.B.29. Illustrates plastoglobuli in an amyloplast. This material was postfixed in an osmium solution according to Procedure 6b. (x 44 800).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Dyer and Leech (1968) have demonstrated a low molecular weight RNA as a component of chloroplast ribosomes, as well as an apparently unique soluble RNA within chloroplasts.

Gunning (1965) has suggested that the plastid ribosomes might be partly responsible for prolamellar body formation. O'Brien and Thimann (1967) suggest that this might explain the absence of prolamellar bodies in plastids which do not apparently contain ribosomes. Maize root cap amyloplasts do not appear to contain either ribosomes or prolamellar bodies.

DNA

DNA was first characterised as a plastid inclusion by its guanine-adenine ratio, which differs from that of nuclear DNA (Chun et al., 1963; Kirk, 1963).

Kislev et al. (1965) demonstrated ^{3}H-thymidine incorporation into chloroplasts *in vivo*, and also showed a fibrillar DNAse-sensitive component within young plastids. It has been suggested that the plastid DNA may contribute to a measure of genetic autonomy of these organelles (Heslop-Harrison, 1966).

Phytoferritin.

Several investigators have reported the presence of phytoferritin in plant tissues (e.g. Hyde et al., 1963; Robards and Humpherson, 1967).

Robards and Humpherson (1967) reported 'crystalline' and 'paracrystalline' arrays of granules within the plastids of *Salix fragilis* L., which they suggested to be phytoferritin. These authors further suggested that phytoferritin occurs as an iron-protein complex, thereby effecting iron storage without toxic effects.

Starch production and storage

Starch stored within the amyloplasts is termed reserve starch, and is thus differentiated from the assimilation starch found within photosynthetically-active chloroplasts.

In general, the starch encountered within plastids is a mixture of the linear polymer, amylose (10 - 30%), and the branched-chain amylopectin (70 - 90%)(Akazawa, 1965). The amylose and amylopectin content of the starch is determined by the action of certain genes and their modifiers.

Starch is deposited in the form of granules within the plastids. Badenhuizen (1961) demonstrated that crystalline pattern and overall shape and size of a starch grain are genetically determined, but that there are environmental effects. The use of $^{14}CO_2$ has demonstrated that the layered structure of a starch grain results from apposition growth of the grain (e.g. Badenhuizen and Dutton, 1956).

As a result of their X-ray diffraction patterns, starch grains can be grouped into one of three general categories, viz. cereal starch, tuber or bulb starch and an intermediate type. Hizukuri et al. (1961) showed that the pattern of the starch grain is temperature dependant.

Carbohydrate is transported in the form of sucrose in cereal plants (Akazawa, 1965) and the conversion of sucrose to starch is considered to occur as follows :

$$\text{sucrose} \xrightarrow[\text{ADP or UDP}]{\text{sucrose synthetase}} \underset{\text{UDPG}}{\overset{\text{ADPG or}}{}} \xrightarrow{\text{starch synthetase}} \text{starch}$$

The production of a starch granule in corn endosperm plastids is reported to be preceded by the accumulation of material in the stroma. The crystallisation of such a granule has been demonstrated to be accompanied by the disappearance of the

accumulated material, which is therefore assumed to consist of starch precursors (Salema and Badenhuizen, 1967). These authors suggested that the chain length and concentration of the starch molecules increase, until these molecules assume a paracrystalline pattern. These authors reported further that the accumulation of starch in corn plastids, although occurring in any part of the stroma, is promoted by enclosing pocket-like lipid membranes. These pockets apparently form prior to the starch accumulation which occurs within them.

WALL

The wall is a structure characteristic of plant cells which bounds the cell entirely, and is in close contact with the plasma membrane of the cytoplasm. (Fig. I.B.30). The wall affords protection to the cell, but is not a selectively-permeable barrier, this function being attributed to the plasma membrane. The wall of higher plant cells consists basically of a cellulose framework, which may be impregnated with other macromolecules, e.g. pectin, hemicellulose and sometimes with lignin. Proteins are also present.

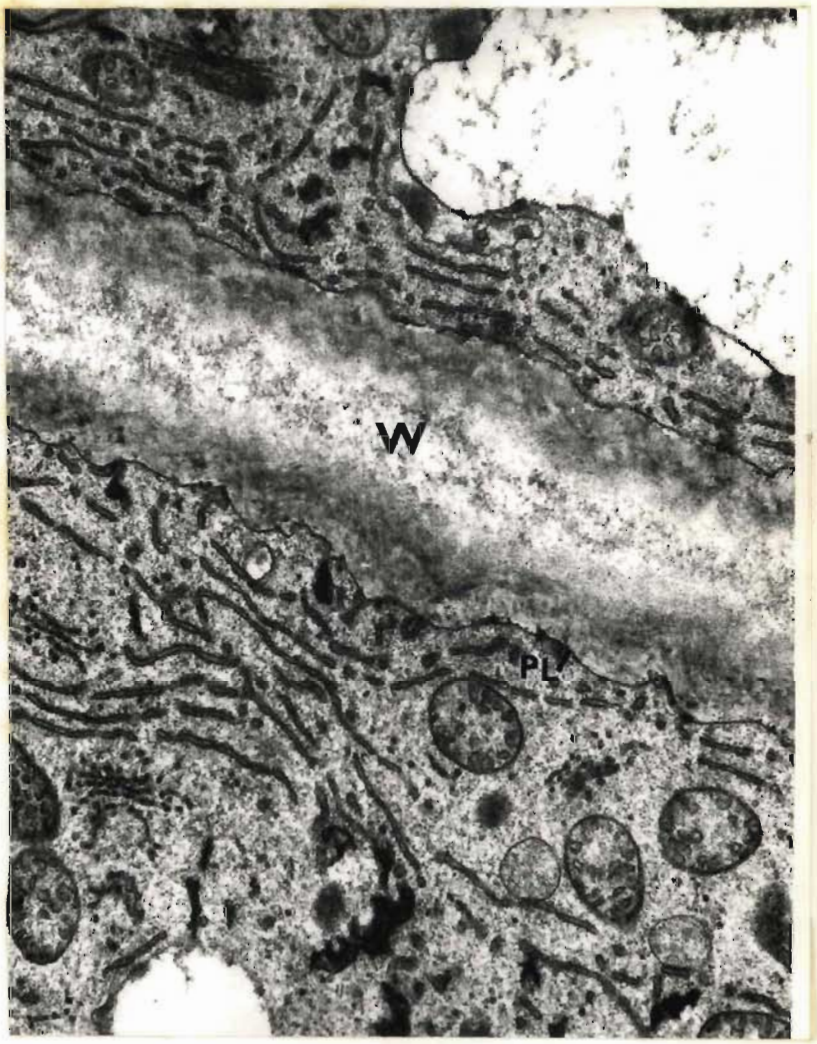
In a dividing cell, the new wall is initiated as a structure termed the cell plate, and a number of sub-cellular structures appear to be connected with its formation, viz. microtubules, dictyosomes and ER.

Cell wall synthesis.

Ontogeny of the cell plate.

Ledbetter and Porter (1963) observed the presence of microtubules (23 - 27 nm in diameter) within plant protoplasm, and suggested that these elements were involved in wall formation. These authors suggested a relationship between microtubules and protoplasmic streaming.

FIGURE I.B.30. Illustrates the wall between two mature cells of the root cap of Zea mays L. (x 45 500).


W
PL

Several workers have observed a morpholical relationship between microtubules and developing cell wall thickenings, and suggested that the microtubules might function in wall organisation (e.g. Wooding and Northcote, 1964). However, Newcombe and Bonnett (1965) showed a lack of coincidence between the orientation of the microtubules and the cellulose microfibrils.

Recently Hepler and Newcombe (1967) observed that microtubules aggregate during the early stage of cell plate formation, and later disappear. These authors described this early stage to be further characterised by an aggregation of dictyosomally-derived vesicles in the cell plate region, and suggested that the microtubules function to determine the pathway taken by the migrating vesicles.

Role of the dictyosomes.

During anaphase and telophase, the dictyosomes, especially those in the equatorial region of the cell, produce a great number of small vesicles (e.g. Whaley and Mollenhauer, 1963). These vesicles move towards the equator of the cell and fuse to some extent, in or near the zone of cell plate formation (Fig. I.B.31) (e.g. Hepler and Newcombe, 1967).

Using histochemical methods, Wooding and Northcote (1964) demonstrated similarities between dictyosomal vesicles and the plate matrix. Dashek and Rosen (1966) concluded that these dictyosomal vesicles are the site of pectin synthesis, while subsequent methylation and polymerization of the pectin occurs within the coalesced vesicles of the forming plate.

Frey-Wyssling et al. (1964) suggested that the bounding membranes of the coalesced vesicles constitute the plasmalemma lining the new cell plate. This concept appears to have been substantiated by subsequent investigations (e.g. Hepler and Newcombe, 1967).

FIGURE 11B, PL. Illustrates the formation of a new cell plate in a dividing cell. Note the dictyosomes in the region of the plate. (x 17 400).


D
N
CB
N
D

Profiles of the ER are often seen in association with cell plate formation. Frey-Wyssling et al. (1964) suggested that profiles of the ER bridge the forming wall, becoming components of the plasmodesmata, fine passages which traverse cell walls.

Robards (1968) demonstrated that plasmodesmata are lined by the plasmalemma. This author also demonstrated that a microtubule, probably originating from the spindle, traverses the plasmodesma. This tubule (for which Robards (1968) suggests the name 'desmotubule'), is closely bounded at its ends by the plasma membrane. He further suggested that spindle elements and ER become trapped during cell plate formation, but that there is no continuity either between the cytoplasm from cell to cell, or through the lumen of the ER, between cells.

Olszewska et al. (1966) demonstrated the presence of certain hydrolases in the newly formed plate, and suggested that these enzymes act to hydrolyse cytoplasmic and spindle elements in this region.

Subsequent wall formation

Once the entire cell plate, lined by the plasmalemma, is laid down, subsequent growth of the wall depends on the penetration of substances through the membrane.

Frey-Wyssling and Mühlethaler (1965) cited the results of several investigators who demonstrated the fusion of dictyosomal vesicles with the plasmalemma, and the contribution of the vesicular contents to primary wall formation. Mühlethaler (1967) suggested that dictyosomal vesicles initially fuse with the plasmalemma, releasing non-cellulosic matrix substances into the wall.

Mühlethaler (1967) has described results of freeze-etching experiments which demonstrate the presence of plasmalemma particles

associated with the outer surface of this membrane. These particles, of obscure origin, are believed to be multi-enzyme systems and are thought to be responsible (i.a.) for cellulose production.

King and Bayley (1965) demonstrated that the primary wall contains protein. The amino acid, hydroxyproline, predominates in the composition of the primary wall protein, which is thought to be significant in the growth of the wall and possibly implicated in auxin action (Mühlethaler, 1967). This reviewer cites the observations of several investigators concerning the concept that proteins, probably associated with the plasmalemma particles, are responsible for the regulation of cell wall properties.

Vesicles, other than those derived from dictyosomes, may be implicated in cell wall formation. Walker and Bisalputra (1967) described vesicular structures associated with the surfaces of cells (of Helianthus shoot). These structures were reported to arise deep within the cytoplasm, but their precise origin was not known. These authors regarded these tubule-containing vesicles as differing essentially from any dictyosomally-derived vesicle described in the literature. Walker and Bisalputra (1967) suggested that the vesicles fused with the plasmalemma, with subsequent integration of their content with the cell wall (Fig. I.B.32).

However, the origin and function of such vesicles is not really clear. There is neither definite evidence for their origin deep within the cytoplasm, nor that they actually fuse with the plasmalemma. There is also the possibility that they are pinocytotic.

Primary and Secondary Wall.

The cellulose microfibril is the basic structural unit of both the primary and secondary wall, which differ primarily

FIGURE I.B.32. Illustrates a vesicular structure associated with the plasmalemma in a root cap cell of maize. This structure appears to contain micro-tubules. (x 45 500).


V
PL
MC

in the amount of cellulose deposited. Secondary walls are characterised by a greatly increased cellulose deposition, with the microfibrils deposited in layers of approximately parallel bundles, each layer varying by a certain angle, from the adjacent layer. Thus the secondary wall has an extremely ordered structure compared with the primary wall, in which the cellulose microfibrils tend to be randomly arranged. In addition, the secondary wall may contain lignin, which is thought to be deposited on the microfibrils (Albersheim, 1965).

The cell walls of corn roots have been partially chemically characterised by Dever et al. (1968), who obtained mainly glucose, xylose and galactose, as well as a little galacturonic acid, by wall hydrolysis.

CYTOPLASM

The cytoplasm is considered in this section to be the ground substance surrounding the organelles and particles visible with the electron microscope. (Fig. I.B.33). As work which has been done on the cytoplasm has mainly been concerned with its biochemical characterisation, the term 'soluble phase' of the cell, is also used.

Enzyme Systems.

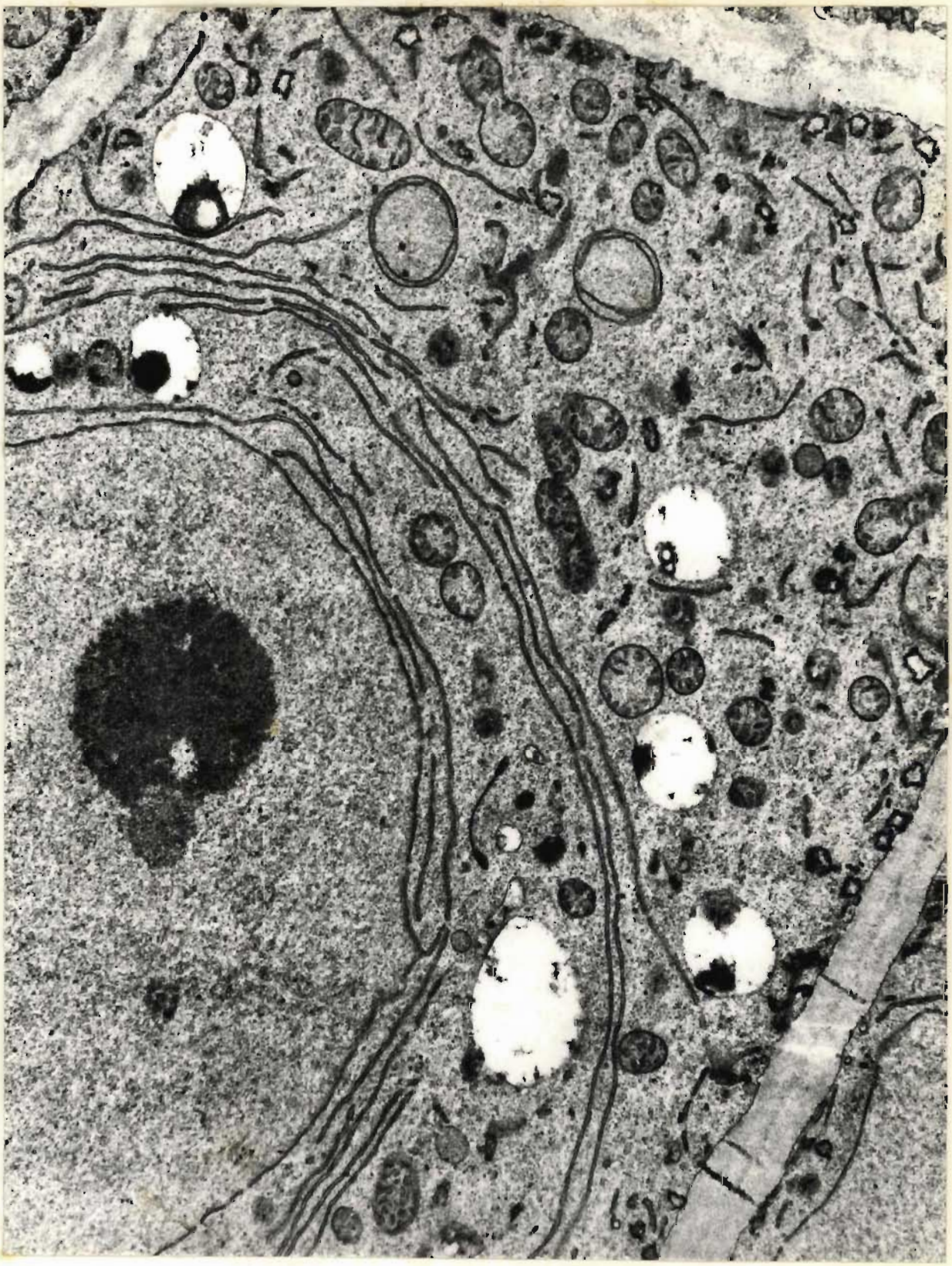
Several enzyme systems are believed to be located in the cytoplasm.

(i) Glycolysis

Glycolysis generally described the conversion of hexose to pyruvic acid.

Stumpf (1950) demonstrated that plant tissue extracts could catalyse the transformation of fructose 1, 6-diphosphate to pyruvic acid, and Axelrod and Bandurski (1953)

FIGURE I.B.33. Shows a general view of portion of a root cap cell of Zea mays L. (x 16 100).



demonstrated that ATP production accompanied this process. In addition, all the enzymes of this pathway have been purified from plant material (Bonner and Varner, 1965). Under aerobic conditions, pyruvic acid is produced in the cytoplasm, transported into the mitochondria and there converted to acetyl coenzyme A and carbon dioxide. The acetyl Co A enters the TCA cycle (q.v.).

(ii) Hexose monophosphate shunt.

Axelrod and Bandurski (1953), (i.a.), first established the details of this alternative pathway for the degradation of hexoses. The enzymes concerned are thought to be located in the cytoplasm.

(iii) Amino acid activation

Amino acid activation, a necessary step in protein synthesis, involves the reaction of an amino acid with ATP, forming an aminoacyl adenylate. The activating enzymes are highly specific (each for a single amino acid) and the activity of most of them has been demonstrated in higher plants (Holley, 1965). These enzymes are thought to have cytoplasmic localization.

(iv) Fatty acid biosynthesis

Stumpf (1965) has described experimental work demonstrating the presence (in the supernatant fraction obtained by centrifugation of plant tissue homogenate) of most of the enzymes involved in fatty acid biosynthesis. This author has pointed out, however, that the enzyme system does not seem to be complete in the soluble cell phase.

However, in equating the cytoplasm with the soluble phase of the cell following differential centrifugation, one must consider whether the *in vivo* system is, in fact, equivalent to the representation *in vitro*. In this respect, Zalokar (1960) has pointed out

that most of the 'soluble' proteins encountered *in vitro*, may not exist as such, *in vivo*, and Kempner and Miller (1968) have found the cytoplasm of cells of *Euglena gracilis* to apparently be free of macromolecules. Kempner and Miller (1968a) have suggested that, at least in *Euglena*, the entire array of macromolecules (normally associated with the soluble phase of a cell) is associated with larger particles.

PLASMA MEMBRANE

The plasma membrane (plasmalemma, cytoplasmic membrane), the unit membrane bounding the protolasm of a cell, is primarily responsible for control of substances entering and leaving the cell. (Ref. Fig. I.B.33).

Ionic transport.

Ions move across the plasma membrane by a process of active transport, which has been shown to be energy-requiring (Thompson, 1965). The process of active transport is not yet clearly understood, and it is not known whether the ionic transport is mediated by enzymes which are an integral part of the membrane, or whether the enzymes are bound to the membrane surface.

Pinocytosis.

This term describes the invagination of the plasma membrane, with the subsequent pinching off of the vesicle so formed. Fluid and particulate inclusions have often been demonstrated to be taken into the cell by pinocytosis. The pinocytotic vesicles have been reported to remain, as such, in the cytoplasm, or to become dissolved (e.g. Wohlfarth-Bottermann, 1960). The reverse of pinocytosis has also been suggested to occur, and the extrusion of cell products by this process may be of considerable importance (e.g. in cell wall formation, Walker and Bisalputra, 1967).

Certain small molecules, depending on their charge and lipid solubility, can diffuse through the 'pores' of the plasma membrane (Frey-Wyssling and Mühlethaler, 1965).

It is unlikely that a cell can remain viable, with loss of the transport control mechanisms maintained by the plasma membrane.

C. DIFFERENTIATION AND DEVELOPMENT

Chromatin

Chromatin consists of DNA, some RNA and associated protein of two main types, viz. histone (basic protein containing the positively-charged groups of arginine and lysine in a high proportion), and the non-histone enzyme proteins (Bonner, J., 1965a).

DNA, the genetic material, has a double-stranded, helical structure, each strand consisting of an unbranched, long-chain nucleotide polymer. Hydrogen bonding occurs between the bases of the complementary nucleotides, adenine and thymine, and cytosine and guanine, between the two strands (Watson and Crick, 1953).

Genetic code.

The nucleotide sequence of the DNA determines the nature of the basic genetic information, in providing the 'code' for specific protein structure. Evidence has accumulated indicating that one or more unique triplets of nucleotides of DNA is responsible (via the transcribed complementary nucleotide sequence of messenger RNA) for the relative positioning of each amino acid of the protein (Jukes, 1963).

Messenger RNA (m-RNA)

The genetic information implicit in the DNA molecule is transcribed into RNA. The earliest views on this subject (Volkin and Astrachan, 1956) were confirmed by Chamberlin and Berg (1962) and by Hurwitz et al. (1962), showing also that the RNA synthesized mirrors the nucleotide composition of the template DNA. The enzyme, RNA-polymerase, is postulated to separate the two strands of DNA, concomitantly synthesizing an RNA molecule which is complementary to one of the DNA strands (Bonner, J., 1965a).

Experiments with labelled precursors of RNA have shown that RNA moves from the nucleus, and appears in association with the ribosomes (Brenner et al., 1961; Gros et al., 1961). This is messenger RNA.

Transfer (soluble) RNA (t-RNA; s-RNA)

Another species of RNA, transfer (soluble) RNA is also formed in the nucleus, from where it moves into the cytoplasm. All or part of this molecule is postulated to be formed on a DNA template, but it is thought that the molecule is somehow subsequently modified (Fleissner and Borek, 1962).

A specific transfer RNA exists for each amino acid species. An amino acid becomes activated, and subsequently bound to its specific transfer RNA molecule. Specificity is postulated to be conferred on a transfer RNA molecule by means of a particular coding triplet of nucleotides.

Transfer RNA contains 70 - 80 nucleotides. Fresco et al. (1960) postulated that the secondary structure of this molecular species, is largely helical, with hydrogen bonding between complementary bases. As t-RNA is a single-stranded nucleic acid, the molecule must loop back on itself, to have this secondary structure. A loop is thought to be formed in a region of the chain where base sequences are not favourable for complementary pairing. The coding triplet of unpaired nucleotides is thought to be situated at the loop. The helical part of the molecule appears to consist of a long run of guanine-cytosine pairs, ending with the unpaired sequence cytosine-cytosine-adenine, to which the amino acid becomes attached.

The various species of t-RNA, each with its specific amino acid attached, interact by means of their coding triplet with that region of the ribosome-associated m-RNA which bears the complementary nucleotide triplet. Thus the amino acids are aligned in specific positions relative to one another.

Peptide bonds are established between the amino acids, starting from the N-terminal end of the potential protein (or polypeptide). This has been demonstrated for both plant and animal proteins (Webster, 1961).

The complete protein is then detached from the ribosome-m-RNA-t-RNA complex, ATP being necessary for this process.

MOLECULAR BASIS OF DIFFERENTIATION

Genetic repression and derepression

Within the nuclei of cells (of multicellular organisms with specialised tissues), there exists a mechanism of control of genetic activity, which allows for specialisation of cells in definite directions. A controlled sequence of repression and derepression of genes is basically responsible for cellular differentiation.

The positively-charged histone proteins appear to be implicated in repression of genes. Histone bound to DNA (as nucleohistone) has been demonstrated for animal as well as plant cells (Bonner and Huang, 1963). The histone-complexed DNA will not support RNA synthesis, these proteins thus appearing to be the physical basis of control of genetic activity (Bonner et al., 1963).

However, it has been shown that there are few variations in histone structure, and furthermore that the histones isolated from different organisms (which have different genes), are similar. Thus an extension of the 'repression by histone alone' mechanism suggests itself, to account for the specificity shown in the repression of particular genes in differing cell types.

It is suggested that species of RNA exist (chromosomal RNA), which anneal with DNA at specific genetic sites, thus repressing particular genes, and that histones are bonded to these complexes. This has been demonstrated for plants by Huang and Bonner (1965), and for animals (Benjamin et al , 1966).

Bonner et al. (1968) demonstrated that chromosomal RNA is a short-chain molecule with extremely heterogeneous base sequences, and hybridises specifically only with DNA from the same organism. These authors suggest that this nuclear RNA is probably responsible for base-sequence recognition, and that it binds to the complementary portion of the DNA. They suggest further that the histone is ionically bonded to the complex.

Schwimmer and Bonner (1965) showed that nucleohistone as well as the non-annealed DNA, is active in DNA replication, i.e. that the entire genome is replicated during interphase preceding nuclear division.

Kaufman (1967) has suggested, since the molecules which effect repression are not themselves replicated during genomic replication, that a newly-synthesized gene will initially be de-repressed. This author has further postulated that the pattern of repression which develops in the newly-synthesized genes is not necessarily the same as the repression pattern in the original set, which has persisted during replication. This would account for differences in the genetic activity of two sister chromatids, which, through mitosis, could play a role in cellular differentiation.

Regulator - Operator - Effector concept.

The existence of certain genes (regulators) which control the activity of other genes, has been established for micro-organisms (e.g. Jacob and Monod, 1961) and also for higher plants, e.g. for maize by McClintock (1956).

A genetic unit, the operon, which consists of an operator gene adjacent to and controlling a set of structural genes has been described. The structural gene (or genes) of an operon are responsible for the manufacture of a specific protein, implicated in the general metabolism of the cell, while the operator gene controls the activity of the structural gene.

A regulator gene is postulated to control the activity of an operon by the production of a substance (the repressor) which acts upon the operator gene (Bonner, 1965a).

By effector, is meant a substance which will counteract a repressor, thereby bringing about derepression of an operon.

Bonner (1965a) has also described a possible extension of this control mechanism, in which a 'master' regulator gene acts upon operators of a series of regulator genes (sub-regulators) which, in turn, produce repressors thereby limiting the activity of specific operators. Effector substances may act upon the repressors produced by the sub-regulators (thereby derepressing the operons concerned) as a consequence of the action of an effector on the 'master' regulator.

Effectors

Recent studies on hormone action in both animals and plants have shown that these small molecules can induce derepression of genes, resulting in de novo synthesis of enzymes, in specific target tissues (Bonner et al., 1968).

Tuan and Bonner (1964) demonstrated that in dormant potato buds, which have a very limited capacity for DNA-dependent RNA synthesis, template activity is greatly increased after treatment with ethylene chlorohydrin, which mimics the action of gibberellic acid. These authors concluded that the action of ethylene chlorohydrin was to effect derepression of the genetic material.

Several investigators have demonstrated that gibberellic acid stimulates RNA synthesis, with subsequent de novo enzyme synthesis during germination in a variety of seeds, especially of the cereal plants (e.g. Varner and Chandra, 1964; Chandra and Varner, 1965; Jacobsen and Varner, 1967). Their work will be discussed later, in the consideration of germination. However, it is pertinent that these authors consider that gibberellic acid functions to derepress the genetic material.

However, addition of an appropriate hormone to isolated chromatin does not enhance template activity, as it does in vivo (Bonner et al., 1968). Johri and Varner (1968) demonstrated that nuclei isolated from dwarf pea shoots show an enhanced rate of DNA-dependent RNA synthesis when treated with gibberellic acid, compared with untreated nuclei. Apparently, therefore, the effect of the hormone depends on some substance, or substances, present in the nucleus.

Bonner et al. (1968) have reviewed the work of several investigators on the nature of this nuclear component in endometrial cells. A soluble protein has been demonstrated in endometrial nuclei, which binds only with oestrogenically-active substances, and Maurer and Chalkley (1967) showed that this substance, once it is bound to the hormone, becomes bound to some somponent of the chromatin.

However, the molecular basis of the interaction between the hormone-protein complex, and the chromatin, thus effecting derepression, remains to be elucidated.

Cytokinins

The cytokinins are a group of hormones encountered in plant tissues. In plants, cytokinins stimulate cell division and are implicated in the control of fruit development.

Osborne (1967) demonstrated that senescence is retarded in kinetin-treated leaf-discs, kept in the dark. Use of this hormone caused chlorophyll retention, as well as enhanced RNA and protein turnover. This author suggested that the action of kinetin is either at the DNA level (possibly derepression) or at the transcription level, and Woolhouse (1967) suggested that kinetin effects depression by becoming incorporated into the chromosomal RNA.

Several investigators have demonstrated that cytokinins are actually incorporated into transfer (soluble) RNA in the cells of a variety of plants, e.g. tobacco and soybean (Fox, 1966), maize (Letham and Ralph, 1967) and spinach and peas (Hall et al., 1967). Therefore it seems possible that, in addition (or as an alternative) to their postulated effect of depression, cytokinins may function to facilitate synthesis of certain species of transfer RNA.

The role of the cytoplasm in differentiation.

Experiments involving the transplantation of nuclei from one cell type to another, have demonstrated that the cytoplasm exerts considerable influence on nuclear function (e.g. in frog ova, Gurdon, 1966). Gurdon (1966) suggested that cytoplasmic components, which may play a part in genetic control, enter the nuclei.

Heslop-Harrison (1967) has described the cytoplasm as containing extra-chromosomal components of the control circuits which regulate gene repression and derepression. He suggested that these cytoplasmic components are sub-divided into those adapted to sense changing cellular environment and those which form feed-back loops to 'actuate timed transcription sequences'.

D. GERMINATION

Seeds can generally persist in a fully viable, but quiescent state for prolonged periods, the actual time varying from species to species. Germination is that process whereby, under appropriate conditions, intensive metabolic activity occurs in the seed leading to morphological changes and the establishment of a normal, growing plant.

1. Seed structure

A seed basically consists of the following component parts (e.g. Lang, 1965) :

(i) The embryo, derived from the zygote, which resulted from the fusion of the female and a male gamete. The embryonic tissue is usually diploid.

(ii) The endosperm, a polyploid (usually triploid) tissue, derived from the fusion of the second male gamete with the (usually) diploid central nucleus of the embryo sac.

(iii) The testa (seed coat) which develops from the integuments of the ovule, following fertilization.

A cereal grain is not a simple seed, but a caryopsis, i.e. an achene with pericarp and testa united (Willis, 1955). As the external covering of a cereal grain is the fruit wall, there is no micropyle establishing communication between the interior of the seed and the external environment (Brown, 1965). However, according to that author, the enveloping layers of a cereal grain are similar to the testa of e.g. the pea seed, in that the cellular layers of the seed coat are contracted and non-living, and several of the layers are separated by fatty, cuticular material.

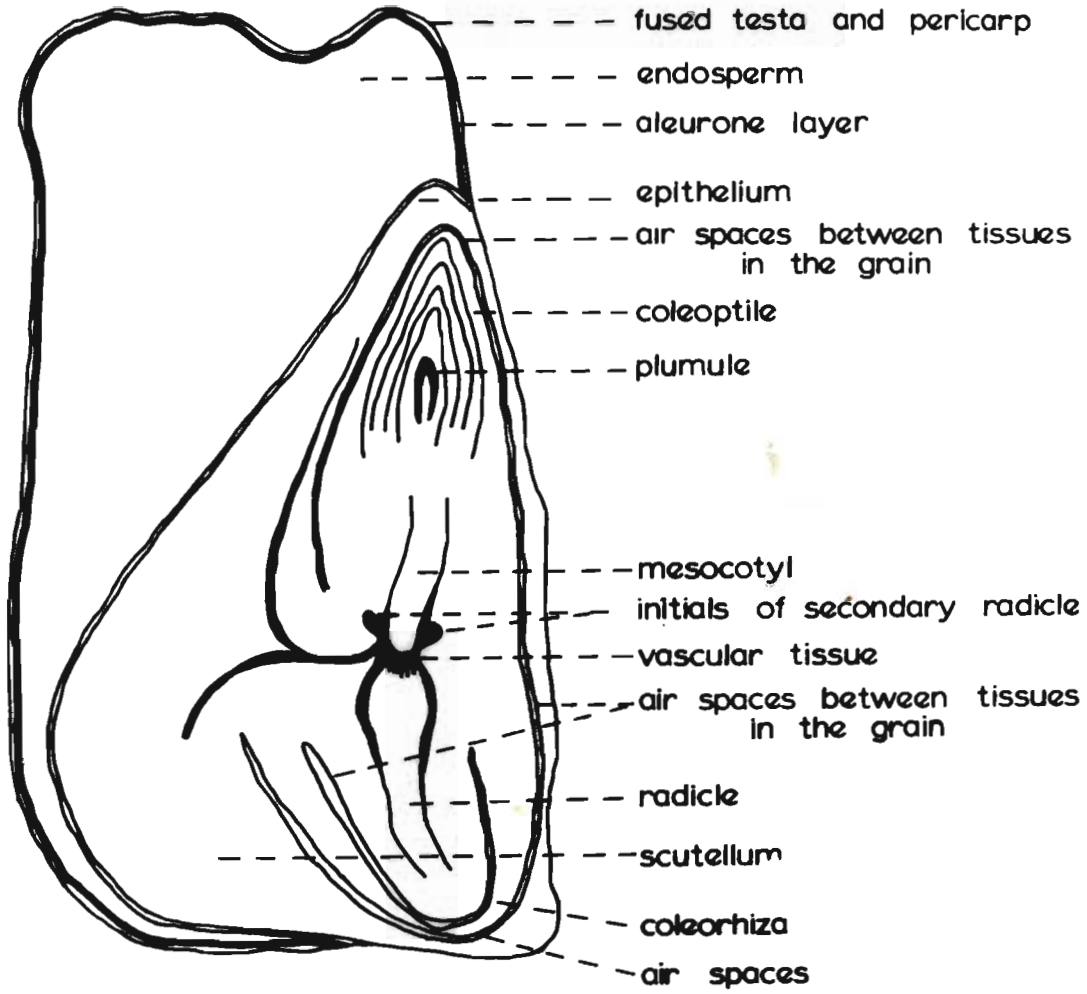


FIG. I.D.1.
LONGITUDINAL SECTION OF THE MAIZE CARYOPSIS (after Crocker & Barton, 1957)

The embryo contained within a cereal grain consists basically of plumule, radicle and one cotyledon, termed the scutellum. Apical meristems occur in both radicle and plumule, leaf primordia being a feature of the latter meristem. The lowest leaf primordium is developed to form the coleoptile, a sheathing structure around the plumule.

The food reserve of a cereal grain is contained within the cells of the endosperm. The endosperm comprises a central mass of starch-containing cells, and a peripheral aleurone layer, usually one cell wide. The cells of the aleurone layer do not accumulate starch (Varner, 1965) but may contain protein in the form of aleurone grains (Brown, 1965). They have a specific active metabolic function during germination (see later), unlike the starchy endosperm cells which Brown (1965) described as 'unquestionably inert'.

Mature caryopsis of maize (Fig. I.D.1.)

Endosperm

The endosperm tissue of the maize caryopsis is triploid, with the outermost layer differentiated as the aleurone (Kisselbach, 1949). Crocker and Barton (1957) have described the aleurone layer to be specialised for the production of enzymes, and the starch endosperm as an inert, parenchymatous food-storing tissue.

Embryo

The diploid maize embryo consists of the embryonic stem bearing five primordial leaves, which is sheathed by the coleoptile; the radicle, which is sheathed by the coleorhiza; and the scutellum (Kiesselbach, 1949). According to Maheshwari (1950), that side of the scutellum which is in contact with the endosperm develops finger-like processes penetrating the endosperm and presumably facilitating nutrient absorption.

Other features of the mature caryopsis.

According to Kiesselbach (1949), there is a 1,400-fold volume increase during the 50-day interval from fertilization to maturity of the caryopsis.

The ovary wall develops into the thickened and somewhat lignified pericarp, while the integuments largely disintegrate. During development of the maize caryopsis, the outer cell walls of the nucellar epidermis become thickened and suberised. This structure, representing the remains of the nucellus, persists to form the nucellar membrane between the aleurone layer and the pericarp in the mature caryopsis.

The interior of the mature caryopsis is occupied by embryo and endosperm, constituting respectively 10% and ± 85% of the dry weight of the kernel. The remaining ± 5% is constituted by the pericarp, nucellar membrane, integument remains and pedicel (Kiesselbach, 1949).

2. Factors necessary for germination

Quiescent seeds have a very low metabolic rate. However, the activities of such seeds increase (providing they are not dormant) in moist conditions, with sufficient oxygen and at a suitable temperature. These conditions are prerequisites for the ensuing process of germination.

Water

The moisture content of mature seeds is low, in general representing only some 5 - 20% of the total weight (Lang, 1965), although it is influenced by the relative humidity of the environment (Barton, 1961). Although it is not known how this low moisture content is established, its decrease with increasing maturity of the seed has been shown

to be essential to the establishment of germinability of certain seeds, e.g. maize (Sprague, 1936).

The metabolic rate of a mature seed is limited by its low moisture content, and this is postulated to be the basis of the capacity of seeds to remain quiescent for prolonged periods (Brown, 1965).

Increase in metabolic rate of a seed can only be initiated once the dry seed has an adequate water supply, which is therefore a prerequisite for germination.

Oxygen

The onset of germination is an energy-requiring process and Toole et al. (1956) described this energy requirement to be met by an increased respiratory rate. Although anaerobic respiration (via the glycolytic pathway) has been cited as the major energy-liberating mechanism in the germination of certain seeds (notably, rice), this is exceptional, and is now subject to some doubt (Mayer and Poljakoff-Mayber, 1963; Brown, 1965). Typically, oxygen is a requirement for the onset of germination, and Brown (1965) has reported that an increased oxygen uptake accompanies water uptake from the start, in barley at least.

Temperature

Mayer and Poljakoff-Mayber (1963) reported that the optimum temperature for germination varies from species to species, between varieties of a species, and also with the age of the seed. The particular temperature range within which different seeds germinate also differs with species, variety and seed age. Mayer and Poljakoff-Mayber (1963) reported that maize seed germinates within the range 8 - 44°C, the optimum being between 32 and 35°C.

Light

Light is not an essential factor for the germination of all seed types. The germination of certain seeds is promoted by light, and the germination of other seeds is inhibited in the presence of light, while the germination of many seeds is described as being indifferent to light (Toole et al., 1956; Mayer and Poljakoff-Mayber, 1963).

A photoreversible pigment, phytochrome, is implicated as a "trigger-mechanism" in the germination of light-sensitive seeds (e.g. Amen, 1968).

Dormancy

Lang (1965) described a completely dormant (but viable) seed as one which is unable to germinate under any set of environmental conditions.

Amen (1968) used the concepts of constitutive and exogenous dormancy, introduced by Sussman and Halvorson (1966). Constitutive dormancy implies that the dormant state is maintained by endogenous control and may be prevented by pre-treatment, or be relieved by specific environmental factors. Exogenous dormancy, on the other hand, is generally equated with quiescence.

Mature maize seed does not exhibit constitutive dormancy, and may be described as quiescent or resting.

3. Course of Germination

(a) General morphological and cytological changes.

When a fully viable, non-dormant seed is exposed to favourable conditions of moisture, air and temperature, germination follows.

The cells of dry seeds have been described as being shrunken, with small vacuoles and a plasmolysed appearance

(e.g. Toole et al., 1956). The first phase of germination is rapid water imbibition, which establishes turgidity. Generally this phase lasts about 10 - 12 hours, and no morphological changes accompany it (e.g. Toole et al., 1956; Brown, 1965).

During the following 12 - 24 hours the first morphological changes are evident in extension of the radicle (Brown, 1965) or of the coleorhiza (in cereals). Toole et al. (1956) reported that for maize extension of the coleorhiza is evident about 20 hours after the beginning of imbibition. The coleorhiza (of maize) then breaks through the pericarp. The following morphological change is extension of the radicle, which then breaks through the coleorhiza, the latter not developing any further. Cell division is first noticed in the radicle at about this stage (Toole et al., 1956).

The emergence of the plumule generally shortly follows that of the radicle. In certain seeds (e.g. pea), the cotyledons remain within the seed coat, functioning for food storage alone (hypogeal germination) and emergence of the plumule is achieved by elongation of the epicotyl. In other seeds (e.g. sunflower), the hypocotyl extends, and both the plumule and cotyledons emerge from the testa. In such cases the cotyledons expand and function as foliage leaves (epigeal germination). In maize, under optimum temperature conditions, the coleoptile sheathing the plumule emerges about 24 hours after the coleorhiza and radicle (Brown, 1965).

During this period too, Toole et al. (1956) report that the cells of maize scutellum enlarge greatly, their nuclei becoming very prominent, but do not divide. In general, the early morphological changes in germination result from cell expansion and not cell division (Brown, 1965).

Key (1964) reported that both RNA and protein synthesis occur in the elongating soybean hypocotyl, and showed that their synthesis is essential for the elongation process. Key and Shannon (1964) also showed that auxin enhances nucleotide incorporation into RNA and suggested the effect of auxin to be more closely associated with RNA synthesis than with subsequent protein synthesis. Auxin is known to increase cell wall plasticity and stimulate cell elongation; however, it appears that these effects of this hormone are indirect, and that its primary action might be at the control level.

The work of Nooden (1968) on artichoke discs, supports the concept that auxin acts to induce RNA synthesis (presumably m-RNA), resulting in the synthesis of new enzymes, which may modify the cell wall, thus allowing for cell expansion. This RNA is not instrumental in the actual process of cell enlargement, at least in artichoke discs.

Nitsan and Lang (1966) showed that DNA synthesis occurs in elongating, non-dividing cells of lentil epicotyl. They suggested that this DNA synthesis (which is enhanced by gibberellin) is necessary for ribosomal RNA synthesis, which is required for cell elongation in certain plant tissues.

(b) Water absorption.

When dry seeds are immersed in water, the rate of absorption is at a maximum immediately after immersion, and decreases with time (Brown, 1965). Stiles (1948), working with maize and cotton, showed that the amount of water absorbed by the embryo is relatively much greater than by the rest of the seed. This implies that while water uptake of the entire seed is by imbibition, that of the embryo is determined by metabolic processes as well (Brown, 1965).

Hunter and Erickson (1952) showed that the minimal amount of water absorbed, which facilitates germination, varies with the seed type. For maize it is 30.5% of the fresh weight of the seed.

The state of viability of seed apparently has no effect on their early water absorption (e.g. Barton, 1961), and Brown (1965) suggested that in these stages the process is a physical one of imbibition, water being adsorbed onto the hydrophilic seed colloids.

Generally the rate of water absorption by seeds is enhanced with rise in temperature. Water absorption is accompanied by swelling of the seed, and evolution of heat. The pressure developed in this process is instrumental in seed coat rupture (Brown, 1965).

Effect of the seed coat

According to Brown (1965) seeds generally take up water and germinate faster when the micropylar end is in contact with the moist surface than when the seeds are otherwise positioned. Even cereal caryopses are reported to germinate most rapidly when water is available to the micropylar end, although there is no direct communication, via a pore, in these grains.

However, seeds will germinate, although more slowly, when the micropylar end is not in contact with the moist surface, indicating that water absorption does occur across the seed coat.

The rate of water absorption has been shown to be increased by removal of the seed coat, indicating some measure of resistance to this process across the coat (e.g. Brown, 1965). However, that author reported that germination proper may not be enhanced by this process. Larson (1968) has shown that the rapid imbibition following seed coat removal led to seed injury. He demonstrated that mono-saccharides, disaccharides, amino acids and other solutes were lost from the de-coated seeds, and suggested that in addition, rapid imbibition caused cell membrane damage, thereby enhancing the solute loss.

(c) Molecular Aspects of Germination.

Marcus and Feeley (1964) showed that ribosomes which were isolated from resting seeds (peanut) are inactive in supporting protein synthesis. However, these investigators demonstrated amino acid incorporation on the addition of exogenous RNA (e.g. polyuridylic acid) to the isolated ribosomes.

Marcus and Feeley (1965) and Barker and Rieber (1967) showed, on the other hand, that ribosomes isolated from germinating seeds are active in protein synthesis, and are associated to form polysomes, requiring the presence of m-RNA.

Marcus and Feeley (1964) and Barker and Rieber (1967) thus suggested that m-RNA is either synthesized or activated when a seed is imbibed with water. Dure and Waters (1965) demonstrated that actinomycin D does not inhibit protein formation in imbibing cotton seeds, nor does it interfere with polysome formation. These authors suggested that this early protein synthesis is supported by a stable m-RNA which is present in the mature seed. Waters and Dure (1966) obtained additional evidence supporting this concept, from subsequent work.

Marcus and Feeley (1966) demonstrated that amino acid incorporation by activated ribosomes (polysomes) requires ATP and magnesium to be present. However, Jachymczyk and Cherry (1968) suggested that the actual process of attachment of monosomes to m-RNA is non-enzymic, and requires the presence of magnesium, but not ATP, the energy requirement being for peptide formation.

Vold and Sypherd (1968) have shown that the soluble (transfer) RNA complement of resting wheat embryos changes during the first 24 hours of germination. They presented evidence showing an initial decrease in t-RNA level, followed by a subsequent synthesis of t-RNA. These authors suggested that either the RNAse present in the resting seed is responsible for degradation of t-RNA at the start of germination, or that the t-RNA becomes annealed with part

of the long-lived m-RNA, in a manner analogous to DNA repression.

Following the initial germination stage, embryonic development depends upon protein synthesis which is at least partly supported by newly-synthesized RNA (Waters and Dure, 1966). In this respect, Fujisawa (1966) demonstrated that following the phase of water absorption (by the embryonic axis of Raphanus) is a period characterised by increasing incorporation of ^{14}C-uracil (RNA synthesis) and protein synthesis. This author suggested continued RNA synthesis to be essential for normal growth.

The RNA content of cotyledons (where these represent a site of food reserve) has been shown to increase markedly during the earlier part of germination, and to decline as the seedling becomes established (Cherry, 1963; Cherry et al., 1965). The increase is thought to be related to activation and mobilization of reserves, and the subsequent decrease to senescence of the cotyledons. The pattern of enzyme activity was found to resemble closely that of RNA level, during germination (Cherry, 1963).

Marrè et al. (1965) have shown that the RNA content of castor bean endosperm also increases markedly during the earlier stages of germination. These authors suggested that enzyme synthesis and subsequent metabolic activation depends on this RNA synthesis, (m-RNA) which is DNA-dependent.

Hormonal Control

Gibberellic acid is implicated in the control of nucleic acid synthesis and also therefore of subsequent protein synthesis. This has been demonstrated e.g. in the germination of barley (Chandra and Varner, 1965) and of maize (Ingle and Hageman, 1965) as well as for metabolic processes other than germination.

During germination, the embryonic tissues themselves produce gibberellin or gibberellin-like substances, and the role of gibberellins in germination has been established to be the mobilization of endosperm reserves (Cohen and Paleg, 1967).

There appears to be some controversy as to the primary site of gibberellin production. Radley (1967) suggested that the scutellum was the primary site of its production. However, MacLeod and Palmer (1968) state that gibberellin is produced in the embryonic axis and translocated via the scutellum, to the endosperm. Whatever the case, excision of the embryo prevents endosperm mobilization, and gibberellins have been shown to substitute for the embryo in this function (Paleg, 1960).

In cereal grains, gibberellins are effective in controlling RNA and protein (enzyme) production in the aleurone cells. Isolated aleurone layer has been shown to react to gibberellins as it does in the intact grain (e.g. Paleg and Hyde, 1964; Varner and Chandra, 1964)

Varner and Chandra (1964) showed that the development of α-amylase activity by isolated aleurone layer of barley resulted from a de novo synthesis of the enzyme, and was completely dependent on added gibberellic acid. These authors postulated that gibberellic acid, by effecting the production of specific RNA, controls synthesis of α-amylase and other proteins in the aleurone cells.

Chandra and Varner (1965) demonstrated that RNA synthesis occurs in the aleurone cells of barley, in response to gibberellic acid, and this response has also been demonstrated in systems other than those from germinating seeds (e.g. dwarf pea shoot nuclei, by Johri and Varner, 1968).

Although synthesis of a messenger RNA specific for α-amylase production has not yet been demonstrated, Varner (1967) suggested that this is, in fact, the case, and in addition that gibberellic acid controls the production of several other hydrolases in the cereal aleurone layer in the same way. Gibberellic acid induced synthesis of protease in isolated barley aleurone has recently been demonstrated (Jacobsen and Varner, 1967).

The response to gibberellin has been demonstrated for other cereals e.g. for maize (Ingle and Hageman, 1965), rice (Ogawa,1966),

wheat and oat (e.g. Paleg et al., 1962), as well as for barley.

Although only the gibberellins are capable of eliciting the response in barley (e.g. Coombe et al., 1966), it seems probable that other hormones may be active in certain other seeds. Sprent (1968) has reported the inability of gibberellic acid to stimulate amylase activity in pea cotyledons.

(d) Respiration.

Oxygen uptake by an imbibed seed increases from the start of the phase of water absorption (Brown, 1965), and the evolution of carbon dioxide shows a similar increase. Brown (1965) reported that at least 90% of the carbon dioxide evolved during cereal germination is due to the embryo, and the other 10%, attributed to the endosperm, is largely produced by the aleurone layer.

According to Varner (1965), once the preformed respiratory mechanism in the embryo is fully activated during the early phase of germination, further increases in respiratory capacity are presumed to be parallel with cell division and growth of the embryo.

R.Q.

R.Q. values decrease from about 2,5 to 1.0 at 12 hours and 48 hours respectively after the start of imbibition in germinating barley (Brown, 1965). Although the nature of the respiratory substrate largely determines the R.Q. value, Brown (1965) suggested that the availability of substrate type is not a significant factor in the observed drop in R.Q. value. This author suggested that the seed coat is largely responsible for the change in R.Q., being initially freely permeable to carbon dioxide, and permeable to oxygen only to a limited extent, but later becoming more permeable to this gas. The fact that the permeability of the seed coat increases with soaking, indicates that gases move along continuous water channels, into the seed, and as carbon dioxide is far more soluble in water than is oxygen, the seed coat is more permeable to the former than to the latter gas (Lang, 1965). It is possible that, while the testa

restricts oxygen uptake, fermentative respiration might prevail, giving way to aerobic respiration as oxygen becomes more freely available to the seed (Mayer and Poljakoff-Mayber, 1963).

An increase in the bulk activity of mitochondrial fractions during germination has been reported for various seed components, e.g. castor bean endosperm (Akazawa and Beevers, 1957) corn scutellum (Cherry and Hageman, 1961), peanut cotyledons (Cherry, 1963) and bean cotyledons (Öpik, 1965). Breidenbach et al. (1967) demonstrated that biogenesis of mitochondria occurs in peanut cotyledons during germination. These authors showed that respiratory enzymes and mitochondrial DNA increased as a function of germination time, and Lado and Schwendimann (1967) demontrated an early synthesis of RNA in mitochondria of castor bean endosperm, which they suggested supported at least part of mitochondrial protein synthesis during germination.

(e) Other aspects of metabolism during germination.

During germination, food reserves in the storage tissues of a seed are mobilized and translocated to the embryo.

Although some nutrient absorption from the endosperm does occur during the 24-48 hour phase of germination, this is limited, and the metabolic activity of the embryo in the early germination phase is thought to be at the expense of its own reserves (Brown,1965).

(i) Carbohydrate metabolism.

β-amylase is present, but in a latent form, in quiescent seeds (e.g. oats, Simpson and Naylor, 1962; wheat, Varner,1965). The activity of this enzyme is stimulated during germination, by the activation of the latent form. On the other hand, α-amylase is produced by de novo synthesis, triggered by gibberellin (or possibly other hormones in certain genera) as described above.

Endosperm starch reserves are hydrolysed by α- and β-amylase, producing mainly maltose. However, maltose is not

translocated from endosperm to embryo, and Simpson and Naylor (1962) showed that in oats, maltase is necessary for glucose formation. These authors demonstrated that maltase is also formed by **de novo** synthesis, triggered by gibberellic acid. The resultant glucose is absorbed by the scutellum (converted to sucrose) and translocated to the embryonic axis (Edelman et al., 1959; Varner, 1965)

Apparently both the aleurone layer and the scutellum produce the hydrolytic enzymes α-amylase and maltase during germination of cereal grains (Brown, 1965; Varner, 1965).

The level of other carbohydrases also increases during germination. Swain and Dekker (1966) have proposed a pathway for starch degradation during germination, which is alternative to hydrolysis by α- and β-amylases and maltase. The authors were working with germinating peas, and the alternative pathway involves phosphorylytic enzymes, and (i.a.) the nucleotide-glycosyl intermediate UDP-glucose. This pathway also ultimately yields sucrose for transport in the embryonic axis. Several investigators have identified nucleotide-glycosyl compounds, produced during germination e.g. UDP-fructose (Brown and Mangat, 1967), also in germinating pea seeds.

Degradation of polysaccharides other than starch also occurs during germination. The enzymes responsible have been studied in germinating barley. These include specific endo-β-glucosanases, exo-β-glucosanases and cellobiase (Varner, 1965).

(ii) Protein and amino acid metabolism.

Reserve protein of the endosperm or cotyledons is hydrolysed to peptides and amino acids, and translocated to the embryonic axis during germination. The maximum growth rate of the embryo has been found to coincide with the maximum rate of protein hydrolysis (Varner, 1965).

Varner and Chandra (1964) demonstrated that the activity of protease in the barley aleurone layer increases in response to gibberellic acid, and Jacobsen and Varner (1967) showed that the synthesis of protease in the aleurone layer is induced by this growth hormone.

However, Ingle and Hageman (1965) demonstrated that the initiation of amino acid production (i.e. protein degradation) in isolated maize endosperm is only partially dependent on exogenous gibberellic acid. Protease has been purified from mature, dry pea seeds (Soedigo and Gruber, 1960). Thus, apparently, the active enzyme complement responsible for protein breakdown during germination is partly preformed and arises partly by de novo synthesis.

Stewart and Beevers (1967), in studies on germinating castor bean, have shown that certain amino acids (which can, on deamination, give rise to intermediates in the lipid ⟶ sucrose pathway) are largely converted to sucrose, the nitrogen being incorporated into, and transported as glutamine. This gluconeogenesis occurs at the same time as a major production of sucrose from lipid, in germinating castor bean endosperm. However, these authors suggested that this phenomenon may be largely confined to seedlings whose main storage product is lipid.

(iii) Lipid metabolism.

In oil-storing seeds there is an increase of neutral lipase activity during germination, and rapid conversion of lipid to carbohydrate. Ory et al. (1967) reported the isolation of a protein activator for castor bean lipase, which restored the reduced activity of the isolated apoenzyme. Fatty acids resulting from lipase activity do not accumulate, and Stumpf and Barber (1956) demonstrated their conversion (via β-oxidation) to acetyl coenzyme A(acetyl Co.A).

Acetyl Co.A so formed is ultimately converted to sucrose

via the glyoxalate cycle and thence via glycolysis (the reversed pathway). Two key enzymes of the glyoxalate cycle (malate synthetase and isocitrate lyase) are absent, or occur in very low concentrations, at the start of germination. Varner (1965) suggested these enzymes to be synthesized during the course of germination. Pinfield (1968) has shown that exogenous gibberellin promotes the specific activity of isocitrate lyase in hazel cotyledons.

The glyoxalate cycle enzymes do not occur in those seeds which contain starch as their predominant reserve material (Conn and Stumpf, 1965).

(iv) Nucleic acid metabolism.

At least part of the RNA present in reserve-storing cells of the seed (i.e. endosperm, cotyledons) appears to be degraded and translocated to the embryo during germination. An increase in ribonuclease of cereal endosperm has been reported to occur during germination (e.g. in barley by LeDoux et al., 1962) and it is suggested that this enzyme is produced by the aleurone cells and released into the endosperm where it degrades RNA. Beevers and Guernsey (1966) demonstrated that the initially high RNA content in germinating pea cotyledons declines, with no accumulation of nucleotides. Concommitantly the RNA content of the axis increases. In this respect, Barker and Douglas (1960) have suggested that ribonuclease (which catalyses a reversible RNA degradation reaction), functions to synthesize RNA in the embryonic axis, and Ingle and Hageman (1965a) reported an increase in total ribonuclease and RNA in the embryonic axis of corn, during germination.

However, Varner (1965) considers it more likely that the ribonuclease in the embryonic axis functions by limiting the lifetime of the m-RNA in the growing cells.

Another suggestion is that the undegraded acids are translocated to the growing embryo. LeDoux et al. (1962)

have shown that exogenous DNA and RNA are taken up by germinating barley embryos; however, this phenomenon appears to occur only during the early germination stages. Thus Olsson and Boulter (1968) suggested that the embryos of certain seeds might be partially dependent upon their reserve tissues for information which might be connected with utilization of the food reserves.

(v) Phosphorous Metabolism

Phosphates play an extremely important role in a variety of reactions, including the all-important formation of nucleic acids and ATP.

Some aleurone grains contain spherical inclusions, called globoids, within the protein mass (Lui and Altschul, 1967). These authors reported that the globoids (from cotton seed aleurone grains) contain 14.2 per cent phosphorous and 10 per cent metals. They have cited this particle to be the site of storage for phosphorous and certain metals.

In many seeds phosphorous is principally in the form of phytin (calcium, magnesium and potassium salts of inositolhexa-phosphoric acid) which may constitute up to 80 per cent of the total phosphorous content of the seed (Mayer and Poljakoff-Mayber, 1963). The level of inorganic orthophosphate is very low and may be the limiting factor in metabolic reactions (Varner, 1965). Phytin may be regarded as a store of inorganic phosphate which is released as germination proceeds. In germinating cotton seeds the phytin level drops so that after six days most of it has disappeared. Concurrently inorganic phosphate accumulates in the seeds (Ergle and Guinn, 1959).

Phosphate is liberated from phytin by the enzyme phytase. Mayer (1958) has demonstrated a good correlation between phytin breakdown and phytase activity. In cereal grains phytase activity

is highest in the scutellum and aleurone layer (Varner, 1965). Much of the metabolism of the seed may be dependant on the hydrolysis of phytin and the concomitant release of magnesium and potassium ions.

(f) Ultrastructural changes during germination.

(i) Cotyledons

Treffry et al (1967) reported that the only ultrastructural changes in the cotyledons (of germinating soybean) during imbition is a general swelling. Shortly thereafter differentiation of the vascular elements occurs from the procambium (Smith and Flinn, 1967). Mitochondria and ER develop completely, 24 to 48 hours after the start of imbibition (Bain and Mercer, 1966). Mollenhauer (1967) reported that lipid bodies (present in most of the cells of the mature bean cotyledon) are converted into an extensive smooth membrane system during the initial stages of germination. That author suggested that the resultant system resembles the smooth ER and postulated that this feature, which is consistent in the cells of germinating peas, might represent a mechanism for rapid membrane elaboration, when required. These developmental changes in the cotyledons are dependent on some factor which passes from the embryonic axis (Varner et al., 1963).

Certain cells which differ somewhat in appearance, are scattered throughout the storage tissue with increasing frequency towards the periphery of the cotyledons of pea (Smith and Flinn, 1967). Flinn and Smith (1967) suggested that these are specialised as sites of enzyme synthesis. During the later stages of germination the food reserves are progressively digested away (e.g. Öpik, 1966).

Once the food reserves have been depleted progressive degenerative changes occur in the cells as the cotyledons senesce.

Plastid thylakoids gradually disappear (Butler, 1967) and plastid membranes become disrupted (Treffry et al., 1967). Butler (1967) reported that mitochondria (in senescing cucumber cotyledons) become smaller and ultimately contain very few cristae, but that they remain morphologically intact until a very late stage. Öpik (1965) in ultrastructural studies on the mitcohondria of senescing Phaseolus cotyledons, reported darkening of the mitochondrial matrix, and swelling of the cristae with senescence.

Öpik (1966) observed that the ER showed a tendency to become orientated into parallel lamellae with age and Butler (1967) reported that subsequent vesiculation of the ER occurred. According to Öpik (1966) a binding of ribosomes (which were free during the initial germination stages) to membranes of the ER occurs as ageing of the cotyledons progresses. Fragments of ER with ribosomes attached are reported to be among the last organelles remaining in the final stages of senescence (Öpik, 1966).

Breakdown of the tonoplast apparently precedes hydrolysis and the final destruction of all the subcellular particles (Butler, 1967; Treffry et al., 1967). This is in keeping with the concept of the cell vacuole as the lysosomal apparatus (see "Lysosomes").

(ii) Aleurone Cells.

Van der Eb and Nieuwdorp (1967) reported that during germination of barley, the protein matrix of the aleurone grains disappears and the globoids are decomposed. These authors also reported extensive development of the ER during

the first eight days following imbibition. Elaboration of the ER at the expense of the globoids has been suggested by Perner (1966) and Mollenhauer (1967a) (see below). The ER has been observed to make contact with the spherosomes, the contents of which gradually diminish during the course of germination. Perner (1966) has also discussed the participation of the ER in food reserve mobilisation.

Mitochondria of the aleurone cells, which have been described as very underdeveloped at the start of germination, are reported to show an increase in the number and length of cristae, and van der Eb and Nieuwdorp (1967) have related these ultrastructural changes to the established physiological activity of the aleurone cells during germination.

(iii) Embryo

Mollenhauer (1967a) reported that membrane synthesis and development begin about 8 to 10 hours after the start of imbibition, in corn embryonic cells. Perner (1966) suggested that membranous components of myelin-like bodies give rise to the elements of the ER, and the observations of Mollenhauer (1967a) support this view. The myelin-like bodies have been suggested to arise by some modification of the globoids within certain protein bodies (Perner, 1966; Mollenhauer, 1967a). Mollenhauer (1967a) suggested that the ER also develops by extension of its pre-existing components.

E. ASPECTS OF SENESCENCE

Maynard-Smith (1966) defined an ageing process as '... any process occurring in an individual which renders that individual more likely to die in a given time interval, as it grows older ..'

It is highly unlikely that any one factor can be classified as the cause of ageing. The possible underlying causes of senescence are grouped into two main types, for the purposes of this discussion.

1. Genetic Processes

This includes those processes bringing about senescence which are under control of specific parts of the genome (programmed senescence).

2. Environmental Processes.

(a) Both internal and external environmental factors may bring about changes at the control level within cells. If such changes are operative at the genome (DNA) level, then subsequent senescent change becomes genetically controlled, although not programmed in the genome prior to the change. Environmental processes effecting changes in RNA species are also included in this group, as are any changes brought about at the translation level (i.e. in the process of protein synthesis.)

(b) Certain internal or external environmental events which effect senescence may do so by affecting the molecular components of an organism, other than those functioning at the control level.

1. Genetic Processes.

Although the mechanisms underlying senescence might be similar in organisms generally, each species nevertheless has a characteristic life-span under normal environmental conditions. Calloway (1966) suggested that the onset of senescence accompanies the origin of the individual, at the time of zygote formation, and that members of a species have a predetermined time of death, even in a perfect environment. This suggests that ageing might be at least a part-consequence of a genetically programmed complex of factors. A process of programmed senescence, under genetic control, has a direct advantage in the survival of a species at the expense of individuals. A process of programmed senescence could similarly be postulated to maintain the efficiency of a population of cells (e.g. comprising an organ), at the expense of aged cells.

Strehler (1967), in summing up ideas on the origins of the types of failure associated with the ageing process, reported that genes controlling senescence have either been the object of natural selection, or have been indirectly selected as an accidental byproduct of some other, advantageous, developmental process.

Programmed Senescence as an Extension of Differentiation.

The postulate that differentiation of cells within multicellular organisms results from ordered sequences of repression and derepression of the genes has been discussed (see 'Differentiation and Development'). Differentiation (within a multicellular organism) results in a variety of tissues, each of which shows a particular structural and functional pattern. However, from the start of the differentiation of a cell type, there is interaction between

it and other cell types, and each interaction may have modifying effects upon the general pattern of differentiation in the organism as a whole.

A consideration of the aspects presented by a fully differentiated plant or animal cell does not usually suggest any abrupt discontinuity from which the onset of senescence can be dated. However, with the consideration of an organism as a whole, certain changes which are apparent early in its development, are later associated with the 'ageing syndrome' (e.g. fibre to ground-substance ratio in tissue matrices). Thus some events associated with development may be described as part of the ageing process (Weiss, 1966).

If senescence is an extension of differentiation, then it is possible that a group of genes exists, controlling the former process and which remains repressed during the sequence of ordered repression and derepression that culminates in the fully differentiated cell. Derepression of such a group of genes would lead to processes ultimately effecting death of the cell, and it is suggested that such genes would somehow be derepressed at a predetermined time (Woolhouse, 1967). Weiss (1966) attributes ageing (as a corollary of development) to disturbed mutual relationships between activities of component parts of an organism. Such relational disturbances could be involved with the trigger-mechanisms which activate the genetic system described above, culminating in cell, organ or organism death.

Strehler (1967a) suggested that whereas differentiation involves the systematic repression of syntheses not required within a specialised cell type, senescence may involve repression of certain syntheses necessary for cell maintenance. This suggests senescence to result from a progressive, probably ordered, decrease in the availability of information. Strehler (1967a) proposed two general models of senescence. The first involves selective repression or activation of m-RNA synthesis, and the second, selective inhibition of messenger translation. In the light of results obtained, mainly

by J. Bonner's group (see 'Differentiation and Development'), the first model appears the more likely.

Several authors have shown that leaf senescence may be retarded by hormones, e.g. kinetin, (Osborne, 1967; Woolhouse, 1967). The fact that foliar senescence may be retarded suggests that the process is one of gene repression rather than derepression, at least in leaves. Woolhouse (1967) suggested that kinetin might act to effect derepression by becoming incorporated into the chromosomal (histone) RNA. As a result, the structure of the RNA portion of the repressor would become altered, with loss of its complementarity to the corresponding portion of the DNA, resulting in depression.

Error-Inducing Systems.

In connection with genetically-programmed senescence, Medvedev (1967) suggested that certain genes exist, whose specific activity is to induce errors at the control level (e.g. slow synthesis of mutagenic analogues of nucleotides which are subsequently incorporated into both DNA and RNA). This author suggested that such genes act to determine the ageing rate and characteristic lifespan of a species. In this respect, it is interesting to note that McClintock (1956, 1961) has demonstrated the presence of genes controlling mutation frequency (in the production of somatic mosaicism), in maize.

Precipitous Cellular Senescence.

Degeneration and death of cells is not confined to chronologically aged organisms. In this respect, cellular senescence followed by death of specified cells and/or tissues is a widespread phenomenon during embryogenesis. Saunders (1966, 1966a), concluded that the death of cells, and also the destruction of tissues, organs and organ systems, occur as genetically-programmed events

in the development of multicellular organisms. This author pointed out that selective processes of this kind are not only useful, but necessary, especially during embryogenesis. However, Saunders (1966, 1966a) reported that no visible senescent changes occur in such embryonic cells, even shortly before their death. In fact, by grafting experiments, this author demonstrated that such cells could follow developmental pathways in embryonic systems, other than those culminating in their death. This suggests the immediate cellular environment to be instrumental in determining the fate of these cells. Saunders (1966, 1966a) concluded that morphogenetic death during embryogenesis results from 'programmed suicide', triggered by some undertermined factors in the micro-environment, and not from the accelerated accumulation of deleterious (ageing) changes normally associated with chronological ageing.

It is possible that if genes exist which control precipitous cell senescence, such as that observed in embryonic systems, then these act to release hydrolytic enzymes, normally confined within lysosomes, thereby effecting autolysis of the cells concerned. However, this view is open to question. Several investigators have reported that regression of the tadpole tail is accompanied by an increase in hydrolytic enzymes, but that these are elaborated by macrophages (e.g. Weber, 1964; Tata, 1966).

Precipitous senescence in outermost root cap cells has been shown to be accompanied by the release of hydrolytic enzymes (Gahan and Maple, 1966; Berjak, 1968), and the cells do not show changes associated with chronological ageing, immediately prior to their senescence (Berjak, 1968). The probability of macrophages is eliminated in a plant system, and it seems probably that autolysis which accompanied normal cell death in root caps, may be effected by the genetically release of lysosomal enzymes.

However, whether cell death results from changes associated with chronological ageing, or whether it is a precipitous process, there seems little doubt that the genome must normally play a major role in its determination.

2. Environmental Processes.

Environmental factors, both internal and external, may play a part in bringing about changes associated with the ageing process. These changes are reflections of molecular variations.

Environmental factors causing molecular variation may be one or more of several types.

(i) Physical factors, e.g. temperature extremes (especially associated with moisture content in seeds), and indirectly, threshing damage and damage by insects (in seeds).

(ii) Chemical factors, e.g. endogenously-formed radicles and nucleotide analogues (formed by errors in biosyntheses of nucleotides (Medvedev, 1967); chemical toxins and nutrient deficiencies (Woolhouse, 1967); oxygen tension associated with stored seeds (Roberts and Abdalla, 1968; Abdalla and Roberts, 1968); auto-immune effects (Walford and Troup, 1966; Ram, 1967)

(iii) Radiation. Natural radiation may cause ageing changes, if the damage is accumulated, i.e. in organisms with long life-spans (Medvedev, 1967). Radiation is not considered to be a significant factor in seed senescence under normal storage conditions; however, administration of X-rays may produce ageing damage, the severity of which varies with the other environmental factors (Roberts et al., 1967)

The relative significance of the various environmental factors in producing deleterious ageing changes, has not been generally clarified. However, the effects are manifest in changes at the control level (DNA, RNA and protein synthesis) and in changes in other components of an organism, e.g. enzymes and structural components.

2a. Changes at the Control Level.

DNA functions not only in transmitting information from generation to generation, but also in generally controlling differentiation and maintenance of somatic cells. Thus any error which occurs in this informational macromolecule may have a far-reaching effect. Such errors are the basis of mutation,

both in gametes and in somatic cells. A certain instability of the genetic material (DNA) is a prerequisite for evolutionary change. This factor, which partly determines biological success, is also the basis for deleterious changes.

Somatic Mutations.

Genome mutations, especially those which occur in post-mitotic cells, are widely considered to contribute to the ageing process (e.g. Curtis, 1966, 1967). The accumulation of microscopically-visible chromosome damage with increasing age has been reported for several organisms {e.g. mice, Curtis,(1963); seeds, Barton, (1961); Roberts et al., (1967); Abdalla and Roberts, (1968)}. Such observations are especially pertinent in the case of seeds, as there is no mitotic activity, and thus no selection against aberrent cells, during the quiescent period prior to the start of germination.

Chromosome damage has generally been found to be a reliable index of ageing, and probably results from damage to the constituent macromolecules i.e. to DNA (e.g. alteration in nucleotide sequences, thymine dimer formation, breakages and loss of sections of the molecule, or formation of abnormal cross-linkages between various DNA double helices).

The theory that ageing is caused by an accumulation of somatic mutations has wide support (Comfort, 1964). However, although somatic mutations are probably the basis of a certain proportion of ageing changes, it is unlikely that they are the only factors involved. Szilard, (1959), suggested that all, or most of the genes on a chromosome may be rendered ineffective, thus unmasking the action of any recessive lethal gene on the homologous chromosome. On this basis Szilard, (1959) suggested that such a cell would die and death of the organism would occur when a critical number of cells had become non-functional. However, evidence has accumulated to show that such a recessive-mutation theory is not generally applicable (e.g. Maynard-Smith,1966).

Repair Systems.

Errors in DNA may be retained. However, Setlow and Carrier (1964) described specific enzyme systems which operate to eliminate damage at the DNA level (e.g. the elimination of thymine dimers), and processes of repair are known to occur at various organisational levels of the individual. However, any repair system which operates, notwithstanding the organisational level, is itself controlled by specific genes. Damage to the DNA of such genes would result in an inability to repair (or a lowered maintenance efficiency of) the target of the system, and thus the accumulation of deleterious changes. Such effects could be at the control level (non-elimination of errors in DNA), with far-reaching consequences, or at the cellular structural level (e.g. non-repair or maintenance of membrane systems), or at the levels of tissues and organs.

In this respect, Alexander (1967) suggested that some differentiated, non-dividing cells might not have DNA-repair systems. Thus damage to DNA in such cells would accumulate and might eventually cause death of the cells concerned.

Active Genes.

Mutation of an active gene would affect the process which it controlled (via the enzyme produced), and to a greater or lesser extent; e.g. on the one hand, resultant changes in the enzyme may not involve its active sites, or, on the other hand, error at the DNA level might be of an extent to block m-RNA and thus enzyme synthesis.

Repressed Genes.

Somatic mutations may lead to incompatibility between a gene and its repressor. Most of the genome is thought to be repressed in a differentiated cell. Thus, if mutation is equally likely in repressed and derepressed portions of the genome, then most mutations would occur in the repressed portion

of the genome (e.g. Medvedev, 1967; Roberts et al., 1967). If, as Bonner et al. (1968) suggested, chromosomal RNA is bound to complementary cistrons in the process of repression, then it follows that alterations in the structure of the DNA would interfere with this bonding. Consequently derepression would probably occur, resulting in 'nonsense information' and thus cellular imbalance. Such mutation might, for example, bring about synthesis of inappropriate repressors, thereby blocking vital pathways and resulting in cell death.

DNA Replication (in cells which normally retain the capacity to divide).

It is possible that DNA replication, a necessary prerequisite of normal cell division, becomes impaired with age. Deterioration in the ability for DNA replication may result from mutation in that portion of the genome controlling this function. Loss of the potential for DNA replication may also be explained on the basis of repression (ordered or otherwise) of that part of the genome controlling the process. According to Medvedev (1967), there has been but a single report relating changed properties of DNA-polymerase (suggested to be due to accumulation of a specific inhibitor), to the ageing process (Mukundan et al., 1963).

Hayflick (1966) has shown that cell strains in culture (which have the karyotype of the tissue of origin) have a limited capacity for division (as opposed to cell lines, which do not have the karyotype of the tissue of origin).

It is possible that such cells (e.g. meristems in plants) may lose their capacity to divide, thus limiting the life of the organism.

Regulatory Genes.

Mutations of regulatory genes would have multiple consequences, and are thus suggested to make the greatest contribution to ageing changes caused by disturbances at the

DNA level (Medvedev, 1967).

The precise role and contribution of mutation, as a cause of ageing, requires quantitative and qualitative investigation. Thus the occurrence of mutations, and the rate of their accumulation, must be evaluated. Ideally, complete elucidation of normally-occurring nucleotide sequences, and variations caused by mutation, would be necessary. The extremely heterogeneous nature of DNA, the number of nucleotides involved, and the random nature of mutational change make this a formidable task. However, hybridisation experiments, involving 'young' (unmutated) DNA, and aged DNA are promising tools in this investigation (von Hahn, 1966).

Irreversible Repression

von Hahn, (1966a) suggested that age-related loss of the ability to synthesize RNA and protein might be a consequence of permanent repression of an operator or structural gene. He proposed that 'irreversible' bonds are formed, as the result of random processes, thus effecting permanent repression of genes.

RNA

RNA, whether of the messenger, transfer or ribosomal type, is generally short-lived. It is possible that chromosomal RNA, having functioned for a definite time, may also be degraded. Degraded RNA is replaced by DNA-dependent synthesis of new RNA, and the process is catalysed by RNA polymerase.

Medvedev (1967) suggested that, in addition to transcription errors which may be inherent in DNA as a result of mutation, other factors may function to produce variation in any of the RNA types. He suggested that malfunction of RNA polymerase might occur, or that ribonucleotide analogues might be incorporated, so that the RNA produced could contain many more errors than the template DNA.

It is possible that the longevity of RNA molecules increases with age. If this is so, then RNA might be subject to molecular accidents, as described for DNA.

Determinations of RNA content have demonstrated that the level increases in certain animal cell types, and decreases in others (Wulff, 1966; Medvedev, 1967). A gradual decline in the RNA content of leaves, with increasing age, has been demonstrated by a number of investigators (e.g. Shaw et al., 1965; Woolhouse, 1967). However, increased RNA turnover (evidenced by incorporation experiments) has been reported to occur in plant cells (e.g. Cherry, 1967; Srivastava and Atkin, 1968) and animal cells (e.g. Wulff, 1966), during ageing. Wulff (1966) suggested that there is enhanced m-RNA synthesis in old cells, which might compensate for mutational errors. Cherry,(1967) and Srivastava and Atkin (1968) who used ^{32}P in their incorporation experiments, suggested that a decline in the phosphorus pool resulted in less dilution of the absorbed ^{32}P, causing an increased labelling of RNA. In addition, Cherry (1967) suggested that increased degradation of RNA could be coupled with near-constant rate of its production, or an increase in RNA polymerase activity.

Medvedev (1967) suggested that the observed increase in RNA production might be a consequence of increased availability of template DNA, as a result of derepression caused by mutation. That is, that the RNA produced, in fact, represents 'nonsense information'.

Strehler (1967a) has suggested (but without any evidence) that failure in the activity of specific soluble-RNA-activating enzymes could be an important factor in the mechanism of ageing.

Enzyme (Globular) Proteins.

A change produced in even a single, vital enzyme, might cause breakdown of an essential pathway, resulting in

death of a cell, a tissue or organ, or of an organism. In general, the turnover of enzyme proteins apparently eliminates those molecules which are abnormal (either by faulty transcription or translation, or by molecular accident to the enzyme itself), as well as normal, functional molecules.

However, Orgel (1963) has pointed out that theoretically, such defective molecules could accumulate with possible deleterious side-effects, in the absence of a specific system for their removal. Anychanges in template DNA or in RNA, irrespective of their causes, would be reflected in synthesis of abnormal enzyme molecules.

2b. Ageing Changes, other than those at the Control Level

Considerable attention has been focussed on the physico-chemical changes in structural (fibrous) proteins, which accompany ageing. Fibrous proteins (e.g. collagen, elastin) function for a relatively long time, the molecules showing only a very low rate of turnover. Cross-linkages between the molecules and microfibrils of collagen have been shown to occur with time, accompanying loss of elasticity, the general ageing change associated with connective tissues. However, little is known about the relative effects of environmental factors and of programmed phenomena, in the ageing changes of this tissue (e.g. Hall, 1966; 1967).

In general, little attention has apparently been focussed on changes in subcellular organelles, accompanying the senescence process. Street (1967) described cells of aged root apices in culture as apparently viable, judging from their structure.

Various investigators have studied changes associated with cotyledon senescence in the later germination stages of several plants. Butler (1967) and Treffry et al. (1967)reported degenerative changes in plastid membranes. Öpik (1965) investigating ultrastructural changes in Phaseolus cotyledons, reported that the mitochondrial matrix darkened, and the cristae became swollen, with senescence,

while Butler (1967) observed size reduction with apparent degeneration of cristae in mitochondria of senescing cucumber cotyledons. Butler (1967) and Treffry et al. (1967) described breakdown of the tonoplast to precede hydrolysis and disorganisation of the cells.

As the selectively-permeable lipoprotein membranes are the main structural components of both plant and animal cells, it is possible that both genetically-programmed changes, and environmental factors might be functional at this level of organisation, during the ageing process. In this respect, changes in membrane properties (notably, permeability) have been demonstrated to accompany senescence (ripening) in fruit tissue (e.g. Sacher,1967).

PART II : MATERIALS AND METHODS

A. MATERIALS

Preliminary investigations were carried out on Zea mays L. var. Hickory King. However, this white variety of maize (which is produced principally for human consumption) is produced under open pollination. There is little selection in its production and thus it is genetically variable and is not uniform in its reactions to various conditions. In addition, it proved difficult to obtain suitable samples of these seeds in bulk.

As it is generally held that the rate of ageing in seeds can be altered by specific regimes of temperature, moisture content and oxygen tension (e.g. Roberts et al., 1967), it was decided to purchase a stock of one variety of seed, of uniform genotype and place of origin (and thus of handling and exposure to infection), and to subject this material to a particular treatment which would accelerate the ageing process.

Subsequent investigations, which constitute the bulk of this report, were therefore carried out on SA 4, a yellow double-cross hybrid variety of Zea mays L. The seeds were purchased (shortly after harvest) from the Pioneer Seed Company, Greytown, Natal, South Africa, who are specialist producers of hybrid maize.

SA 4 is regarded to be a medium-maturity hybrid, which normally takes about 135 days to reach maturity from the date of planting. It is a vigorous germinator, of uniform character and is judged to be the highest-yielding form of yellow maize in the areas of the country where this crop is cultivated. This hybrid is susceptible to most of the common diseases of maize in South Africa, but exhibits considerable drought-resistance. SA 4 is predominantly produced as a feed crop in South Africa.

B. METHODS

1. ACCELERATED AGEING OF SEEDS (Grabe, 1968)

The moisture content of thoroughly-mixed samples of the seed was determined (see below), and was found to be 14%. This is slightly above the level recommended (12.7%, Grabe, 1968), and it was decided to retain the seeds at this moisture content. The seeds were then immediately divided into 35 lots, and treated as follows:

(i) The seeds were sealed into glass jars.

(ii) The jars, with the exception of one (subsequently used as unaged material) were placed in an incubator which was maintained at 40°C.

(iii) A sample of seeds from the jar containing the unaged material was removed to test for germination capacity, and the jar resealed and placed in cold storage at 4°C.

(iv) One jar was removed from the incubator each day, for 34 days.

(v) Samples of seeds for germination tests were removed from each jar after cooling to room temperature, on removal from the incubator.

(vi) The jars were then placed in cold storage at 4°C.

2. DETERMINATION OF MOISTURE CONTENT

The kernels were minced, the sample weighed and then dried in a ventilated oven at 105°C to constant weight. Moisture content determinations were carried out on 10 samples, and expressed as a percentage of the air-dry weight.

3. GERMINATION TESTS

Samples of 100 seeds were used in each test for each stage (day) of the ageing treatment.

The seeds were:

(i) Imbibed with water at 25° ± 2°C for 12 hours.

(ii) Set out with the embryo side in direct contact with moist

cellulose wadding, in loosely closed plastic containers.

(iii) Maintained at 25° ± 2°C, under normal laboratory lighting for a period of 60 hours.

Seeds were deemed viable if the radicle protruded at least 5mm. from the ruptured coleorhiza, and the results were expressed as percentage viability.

4. TETRAZOLIUM TEST

Samples of 50 seeds were used in each test for selected stages (days) of the ageing treatment.

The seeds were:

(i) Imbibed with water at 25° ± 2°C for 12 hours.

(ii) Bisected longitudinally.

(iii) Immersed in a 1% aqueous solution of 2, 3, 5-triphenyltetrazolium chloride for two hours.

Seeds were classified as viable if the plumule, radicle, scutellar node and central scutellar area were completely stained (after Lakon, 1949).

5. DETERMINATION OF CHROMOSOME ABERRATIONS

A preliminary investigation showed that after a total germination period of 48 hours in the younger seeds and 60 hours in the aged seeds, the first peak of mitosis in radicle apices of apparently viable embryos occurred at about 2 o'clock in the afternoon.

The seeds were:

(i) Imbibed with water at 25° ± 2°C for 12 hours.

(ii) Placed with the embryo side in direct contact with moist cellulose wadding, in a loosely-closed plastic container.

(iii) Maintained at 25° ± 2°C for 48 or 60 hours, depending on the age of the seed.

Shortly after the emergence of the radicle from the coleorhiza, the extreme tip of the radicle was cut off and treated as follows:

(i) Fixed in Clarke's Fluid (see below) for at least 12 hours.
(ii) Washed in water for 10 minutes.
(iii) Hydrolysed in 1.0 N hydrochloric acid at 60°C for 10 minutes.
(iv) Stained in Schiff's reagent (see below) for 150 minutes.
(v) Rinsed briefly in water.
(vi) Bleached in three washes of 2% sodium bisulphite, 2 minutes each.
(vii) Placed on a microscope slide in a drop of acetic orcein (see below), and crushed with the handle of a scalpel.

A cover slip was then put onto the preparation, which was then further squashed. The preparations were viewed and photographed, using a Zeiss photomicroscope. A total of 300 anaphase figures from 10 root tips was examined for selected stages (days) of the agening treatment. The fraction of aberrent anaphase configurations were expressed as a percentage of the total anaphase figures observed in each case.

Clarke's Fluid (Peacock, 1966)

Glacial acetic acid	- 25ml.
Ethanol (100%)	- 75ml.

Schiff's Reagent (after Peacock, 1966)

(i) 1g. of basic fuchsin was dissolved in 200ml. of boiling distilled water.
(ii) The solution was well shaken (5 minutes), then cooled to 50°C and filtered
(iii) 20ml. 1.0 N hydrochloric acid was added to the filtrate.
(iv) 1g. sodium metabisulphite was added to the solution, which had been cooled to 25°C.
(v) The solution was placed in a stoppered bottle, in the dark, for 24 hours.
(vi) 2g. activated charcoal were added to the solution, which was then shaken for 1 minute, and then filtered.
(vii) The resultant filtrate (leuco-fuchsin) was stored at 4°C in the dark, stoppered bottle, and allowed to reach room temperature before use.

Acetic Orcein (Peacock, 1966)

Stock solution.

Glacial acetic acid	- 100ml.
Orcein	- 2.2g.

The stock solution is diluted as follows, for use:

Stock solution	- 10ml.
Distilled water	- 12ml.

6. METHODS USED IN THE PREPARATION OF MATERIAL FOR ELECTRON MICROSCOPY

Imbibed Material

The seeds were imbibed with water for 12 hours at 25° ± 2°C after which the embryos were excised. The extreme tip of the radicle was cut off and subjected to one of the procedures described below.

24-and 48-hour material

The seeds were imbibed with water for 12 hours at 25° ± 2°C and then maintained at this temperature with the embryo side in contact with moist cellulose wadding within a loosely-closed plastic container. After a further 12 or 36 hours (24- and 48-hour material respectively) the extreme tip of the radicle was cut off and subjected to one of the procedures described below.

Preparative Procedures

6(a) Postfixation with Osmium Tetroxide

The root tips were:

(i) Fixed at 22°C for 2-12 hours in 6% purified glutaraldehyde (see below), buffered with phosphate at pH 7.15

(ii) Washed in phosphate buffer (see below) for 3 hours with 6 changes.

(iii) Placed in Palade's fixative (see below) at 0°C for 1 hour.

(iv) Washed in 10% ethyl alcohol for 30 minutes with three changes.

(v) Dehydrated in an alcohol series (75%, 100% ethyl alcohol 30 minutes each, with 2 changes).

(vi) Finally dehydrated in propylene oxide for 30 minutes with 2 changes.

(vii) Embedded in Araldite (see below) which was set to polymerise in an oven at $90^{\circ} \pm 3^{\circ}C$ for 60 hours.

Purified Glutaraldehyde (Smith and Farquhar, 1966)

(i) The glutaraldehyde was twice-distilled and made up to 6% with phosphate buffer (see below) at pH7.2

Phosphate Buffer {(Sørensen)- Hale, 1958}

Solutions:

A. disodium hydrogen orthophosphate ($Na_2HPO_4 . 12H_2O$) - 26.91g./l.

B. potassium dihydrogen phosphate (KH_2PO_4) - 9.078g./l.

Buffer, pH7.15:

698ml. of solution A
302ml. of solution B

The buffer solution was stored at $4^{\circ}C$.

Osmium Solution {(Palade's Fixative) - Mercer and Birbeck, 1961}

Solutions:

A. sodium barbitone	- 14.7g.
sodium acetate	- 9.7g.
distilled water	- 500ml.
B. 0.1N hydrochloric acid	

Palade's Fixative:

solution A	- 20ml.
solution B	- 22ml.
solid osmium tetroxide	- 1g.
distilled water	- 60ml.

Araldite

'CIBA' Araldite CY 205	- 9.5ml.
'CIBA' Hardener HY 905	- 9.5ml.
dibutyl phthalate	- 0.8ml.
'CIBA' accelerator DY 064	- 0.2ml.

The solution was rotated on a wheel until the components were thoroughly mixed.

The above procedure was unsuccessful, as most of the organelles were found to be destroyed when the material was viewed in the electron miscroscope. Thus the procedure was modified as follows:

6(b) Staining with Osmium Tetroxide - Modified Procedure.

The root tips were:

(i) Fixed in glutaraldehyde and washed in phosphate buffer as described in procedure 6a, above.

(ii) Postfixed in an osmium solution, buffered with phosphate (at pH7.15), and containing certain salts (see below).

(iii) Washed, dehydrated and embedded as described in procedure 6a above.

Salt Solution (Zetterqvist, 1956)

sodium chloride	- 40g.
potassium chloride	- 2g.
calcium chloride	- 1g.
distilled water to make 500ml.	

Fixative (modified after Zetterqvist, 1956).

buffer solution (Phosphate, as in procedure 6a above)	- 10ml
salt solution	- 3.4ml.
0.1N hydrochloric acid	- 11.0ml
distilled water to make 50ml.	
solid osmium tetroxide	- 0.5g.

This procedure gave better results than that described in 6a, above. Most of the organelles were encountered in a good state of repair. However, the endoplasmic reticulum was not well preserved, and only fragments of this organelle could be seen. However, this modified procedure of postfixation with osmium had to be used in order to view the ribosomes, as these particles are destroyed by potassium permanganate, the eventual fixative of choice for most of the work.

6(c) Postfixation with Potassium Permanganate {(Luft's Permanganate Fixative) - modified after Mercer and Birbeck, 1961}

The root tips were:

(i) Fixed at 22°C for 2 - 12 hours in 6% purified glutaraldehyde and washed in phosphate buffer as described in procedure 6a above.

(ii) Postfixed at 0°C in Luft's Permanganate for 45 minutes.

(iii) Washed, dehydrated and embedded as described in procedure 6a above.

This method, followed by post-staining of the ultra-thin sections with lead citrate (see below) gave very good preservation and resolution. Therefore this method was used preferentially, whenever possible.

6(d) Acid Phosphatase Localisation (modified after Gomori, 1952).

The root tips were:

(i) Fixed at 22°C for 2 - 12 hours in 6% purified glutaraldehyde, buffered with acetate at pH7.2 (see below).

(ii) Washed in acetate buffer (pH7.2) for 3 hours with 6 changes.

(iii) Incubated in Gomori's medium (see below) at 37°C for 30 minutes.

(iv) Washed as follows:

Acetate buffer, pH5.0 (see below)	- 10 minutes;
2% acetic acid	- 1 minute;
acetate buffer, pH7.2	- 5 minutes

(v) Postfixed, dehydrated and embedded as described in procedure 6c above.

The control incubation medium consisted of the Gomori medium containing 0.42% sodium fluoride as an enzyme inhibitor (Gahan, 1967a).

Acetate Buffer pH7.2 {(modified Walpole), after Hale, 1958}

Solutions:

A. 0.1N sodium acetate.

B. 0.1N acetic acid

Solution B was added dropwise to solution A, until a pH of 7.2 was obtained.

Acetate Buffer, Ph5.0 {(Walpole), Hale, 1958}

Solutions:

A. 0.1N sodium acetate

B. 0.1N acetic acid

59ml. of solution B were added to 141ml. of solution A.

Gomori's Medium

(i) 3.4g. sodium acetate were made up to 500ml. with distilled water.

(ii) The pH was adjusted to 5.0, using acetic acid.

(iii) 0.6g. lead nitrate were dissolved in the above solution.

(iv) 1.5g. sodium glycerophosphate were dissolved in 50ml. distilled water and this solution was added to the above solution.

(v) The medium was incubated at 37°C for 24 hours.

(vi) The control medium consisted of the above, with the addition of 0.42% sodium fluoride.

6(e) Esterase Location (modified after Wachstein et al., 1961)

The root tips were:

(i) Fixed in glutaraldehyde as described in procedure 6d above.

(ii) Washed in a series of acetate buffers as follows:

pH7.2 (see procedure 6d, above)	- 90 minutes, 3 changes;
pH6.5 (see below)	- 30 minutes;
pH6.0 (see below)	- 30 minutes;
pH5.5 (see below)	- 30 minutes.

(iii) Incubated in an acetate-buffered medium which contained thioacetic acid as substrate (see below), for 90 minutes at 22°C.

(iv) Washed as described in procedure 6d (iv), above.

(v) Postfixed, dehydrated and embedded as described in procedure 6c, above.

The control incubation was carried out in the thioacetic acid incubation medium, containing 1mM sodium fluoride.

Acetate Buffers (modified Walpole, after Hale, 1958).

Solutions:

A. 0.1N sodium acetate.

B. 0.1N acetic acid.

Solution B was added dropwise to solution A, until the requisite pH (6.5; 6.0; 5.5) was obtained.

Incubation Medium

(i) 0.15ml. thioacetic acid were pipetted into 5ml. distilled water.

(ii) The pH was adjusted to 5.5, using 0.1N sodium hydroxide.

(iii) The volume was made up to 100ml., using pH5.5 acetate buffer.

(iv) The control medium consisted of the above, containing 1mM sodium fluoride.

The solutions were made up immediately before use.

6(f) Ultramicrotomy

Ultrathin sections, 50 $\pm$ 10nm thick, were cut on a Cambridge (Huxley) ultramicrotome, using glass knives. The sections were picked up on copper grids.

6(g) Post-staining.

All the ultrathin sections obtained, irrespective of the preparative method used, were post-stained with lead citrate (Reynolds, 1963).

Lead Citrate Solution (Reynolds, 1963).

(i) 1.33g. lead nitrate and 1.76g. sodium citrate were placed in a 50ml. volumetric flask.

(ii) 30ml. distilled deionised water were added.

(iii) The solution was shaken vigorously for 1 minute, and thereafter intermittently for 30 minutes.

(iv) 8ml. of 1N sodium hydroxide (which was freshly prepared, using distilled, deionised water) were added to the solution.

(v) The volume was made up to 50ml. with distilled, deionised water.

Staining Procedure

(i) A piece of filter paper was fitted into the base of a Petri dish, and saturated with 1N sodium hydroxide.

(ii) Drops of the lead citrate solution were deposited onto a piece of dental wax, which had been placed on the filter paper in the Petri dish.

(iii) The grids were floated, section-side down, on the drops of staining solution for 45 minutes, in the closed Petri dish.

(iv) After staining, the grids were thoroughly washed with 0.1N sodium hydroxide solution, followed by distilled, deionised water, and then allowed to dry on a piece of filter paper.

6(h) Electron Microscopy

The ultrathin sections were viewed in a Philips EM 200 electron microscope. All electron micrographs were taken on 35mm. film.

7. ORGANELLE COUNTS AND DIMENSIONS

Counts.

Organelle counts were carried out per 100 cm^2 cytoplasm, at a magnification of 10,350 times.

Dimensions

Organelle dimensions were measured for at least 20 of each organelle type, unless otherwise stated.

8. AUTORADIOGRAPHIC PROCEDURES FOR LIGHT MICROSCOPY
(Modified after Caro et al., 1962.)

For all the autoradiographic studies, the seeds were imbibed with water for 12 hours at $25^{\circ} \pm 2^{\circ}C$, and then maintained at this temperature with the embryo side in contact with moist cellulose wadding in a loosely-closed plastic container for a further 36 hours. The embryos were then excised.

The isotopes used were thymidine (methyl-T) (17.8c/mM), uridine-5-T (25.5c/mM) and DL-leucine -4, 5, T (14.7c/mM) and were all obtained from the Radiochemical Centre, Amersham, U.K.

8(a) Incorporation of Tritiated Thymidine (^{3}H-thymidine)

The embryos were:

(i) Incubated in a small volume of solution containing 10µc/ml. of ^{3}H-thymidine, on a slowly-rotating shaker for 4 hours.

(ii) Washed twice with distilled water.

(iii) The extreme tips of the radicles were cut off and treated as follows:

(iv) Fixed in F.A.A. (1:8:1 glacial acetic acid/70% ethyl alcohol/40% formaldehyde - Peacock, 1966)

(v) Dehydrated through the following alcohol series:

Ethyl alcohol	- 10%	-	30 minutes
	- 25%	-	30 minutes
	- 50%	-	30 minutes
Tertiary-butyl alcohol (TBA) in S.V.R.	- 50%	-	2 hours
	- 70%	-	2 hours
	- 85%	-	2 hours
	- 95%	-	3 hours
	-100%	-	8 hours with 2 changes, and then 12 hours.

(vi) Placed in a 1:1 mixture of TBA/paraffin wax for 3 hours.

(vii) Placed in pure paraffin wax, in an oven at 80°C, for 12 hours.

(viii) Cast in fresh paraffin wax.

8(b) Incorporation of Tritiated Uridine (^{3}H-uridine)

The embryos were:

(i) Incubated in a small volume of solution containing 10µc/ml. ^{3}H-uridine, on a slowly-rotating shaker, for 2 hours.

(ii) Washed twice with distilled water.

(iii) The extreme tips of the radicles were cut off and treated as described in procedure 8a, above.

8(c) Incorporation of Tritiated Leucine (^{3}H-leucine)

The embryos were:

(i) Incubated in a small volume of solution containing 10μc/ml. ^{3}H-leucine, on a slowly-rotating shaker, for 1½ hours.

(ii) Washed twice with distilled water.

(iii) The extreme tips of the radicles were cut off and treated as described in procedure 8a, above.

8(d) Microtomy

Sections, approximately 12μ thick were cut on a Cambridge rotary microtome, and mounted on microscope slides, using chrome-alum adhesive.

8(e) Processing

The slides upon which the wax-embedded sections had adhered were treated as follows:

(i) Immersed in 100% ethanol for 10 minutes with 2 changes.

(ii) Immersed in 1:1 ethanol/xylene.

(iii) Immersed in pure xylene for 10 minutes with 2 changes.

(iv) Coated by dipping into Ilford L4 nuclear emulsion diluted 2:1 with water.

(v) Exposed for 4 days at 4°C in a sealed wooden case containing dry silica gel.

8(f) Processing of the Coated Slides

The slides were:

(i) Developed for 5 minutes in a stock solution of either Phen-X (Ilford) or Microdol-X (M & B), at 20°C.

(ii) Rinsed in 1% acetic acid for 1 minute.

(iii) Fixed in Amfix (M & B) for 5 minutes.

(iv) Washed in running water for 20 minutes.

8(g) Dehydration and Mounting.

The slides were:

(i) Taken through the following ethyl alcohol series (5 minutes each): 10%, 25%, 50%, 75%.

(ii) Immersed in absolute ethanol for 20 minutes with 2 changes.

(iii) Immersed in 1:1 ethanol/xylene for 10 minutes.

(iv) Immersed in pure xylene for 20 minutes with 2 changes.

The sections were mounted in Canada Balsam.

8(h) Microscopy

The sections were viewed and photographed by phase contrast, using a Zeiss photomicroscope.

9. AUTORADIOGRAPHIC PROCEDURES FOR ELECTRON MICROSCOPY (modified after Caro et al., 1962)

The seeds were imbibed, set to germinate and the embryos excised as described for procedure 8 above.

The isotopes used were described in procedure 8, above.

9(a) Incorporation of Tritiated Uridine (^{3}H-uridine)

(i) The embryos were incubated in the isotope solution, washed and the radicle tips cut off, as described in procedure 8b, above.

(ii) The radicle tips were fixed, postfixed, dehydrated, and embedded as described in procedure 6b, above.

9(b) Incorporation of Tritiated Leucine (^{3}H-leucine)

(i) The embryos were incubated in the isotope solution, washed and the radicle tips cut off, as described in procedure 8c, above.

(ii) The radicle tips were fixed in freshly-made formaldehyde buffered with phosphate at pH7.2 (Peters and Ashley, 1967 - see below) for 2 hours.

(iii) The radicle tips were washed, postfixed, dehydrated and embedded as described in procedure 6c, above.

Formaldehyde Fixative (modified after Peters and Ashley, 1967)

(i) 4g. paraformaldehyde were suspended in ± 90ml. phosphate buffer at pH7.2 (ref. procedure 6a, above)

(ii) The suspension was heated to 70°C, with constant stirring.

(iii) The suspension was cooled, filtered and the volume made up to 100ml. with distilled water.

9(c) Ultramicrotomy

Sections ± 80nm. thick were cut on a Cambridge (Huxley) ultramicrotome, using glass knives. The sections were picked up on formvar-coated copper grids.

9(d) Coating Procedure

(i) The grids were mounted by means of double-sided adhesive on the tips of wooden pegs, inserted vertically into bases.

(ii) Ilford L4 nuclear emulsion was diluted 1:2 with water.

(iii) The grids were individually coated by the loop method.

(iv) Exposure took place in a sealed wooden case, containing dry silica gel, which was renewed weekly.

(v) Exposure time was 6 weeks, in the case of the ^{3}H-uridine incorporation, and 10 weeks in the case of the ^{3}H-leucine incorporation.

9(e) Processing of the Coated Grids

The grids were:

(i) Developed for 2 minutes in a stock solution of D 19 B (Kodak) at 20°C.

(ii) Washed in distilled water for 20 seconds.

(iii) Fixed in Amfix for 5 minutes.

(iv) Washed in running water for 10 minutes.

9(f) Poststaining

Poststaining of the grids, after developing, was carried out as described in procedure 6g, above.

9(g) Electron Microscopy

The ultrathin sections were viewed in a Philips EM 200 electron microscope, and the electron micrographs taken on 35mm. film.

PART III - RESULTS.

A. ULTRASTRUCTURE OF UNAGED MATERIAL.

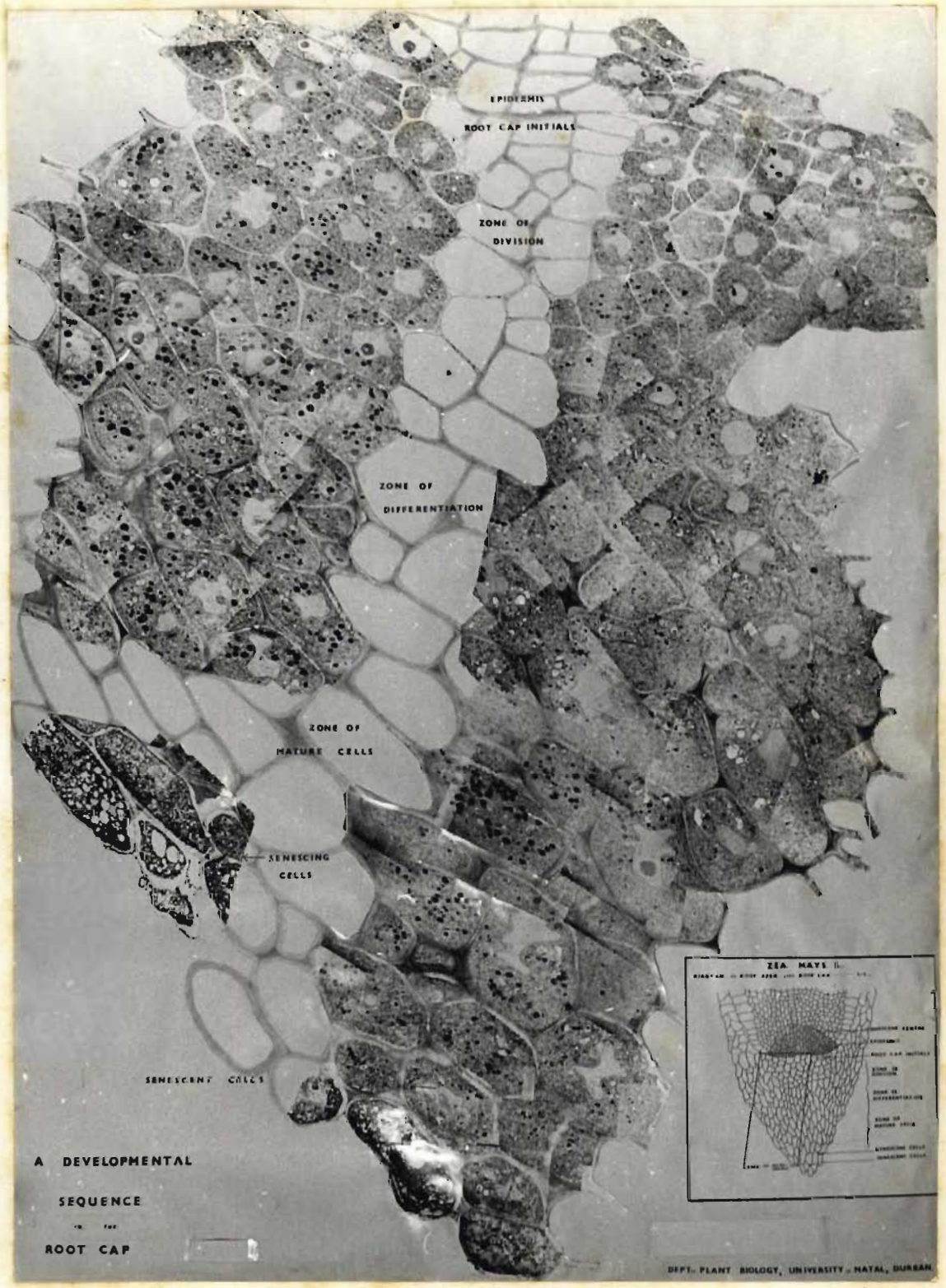
All the ultrastructural observations on the unaged (and subsequently, on the ageing material) were carried out in the cells of the root cap. This tissue was chosen for three reasons. Firstly, the cells of the root cap do not become typically vacuolated. Thus the observations were not confined to a narrow, peripheral band of cytoplasm within the cells. Secondly, this organ shows a rapid developmental sequence, starting with a meristematic zone (the root cap initials) which is followed by a zone of cell division. Distal to this is a zone where cells are growing and differentiating to form mature cells and, finally, the outermost layer of root cap cells which are senescing. Thus, within a tissue of limited extent an entire developmental sequence is encountered from formation to destruction of the cells (Fig. III.A.I). Thirdly, the fact that the outermost cells of the root cap do senesce makes possible a study of a type of senescence within an organ where this is a regular and necessary feature.

The developmental sequence in the root cap zones of unaged material has been arrested in the quiescent seed, and is reactivated in each of these zones by imbibition. The ensuing development is presumably a continuation of the developmental sequence prior to quiescence.

A.1. ULTRASTRUCTURAL OBSERVATIONS IN THE ROOT CAP CELLS OF IMBIBED, UNAGED EMBRYOS.

The embryos at this stage had only just been rehydrated. Thus the intracellular situation reflects a relatively quiescent state, prior to the start of intensive metabolism. Counts of organelles were carried out per unit area of cytoplasm (100 cm^2), at a magnification of 10,350 times.

FIGURE III.A.1. Illustrates the cellular zonation of the root cap. (x 733).


EPIDERMIS
ROOT CAP INITIALS
ZONE OF DIVISION
ZONE OF DIFFERENTIATION
ZONE OF MATURE CELLS
SENESCING CELLS
SENESCENT CELLS
ZEA MAYS L.
A DEVELOPMENTAL SEQUENCE IN THE ROOT CAP
DEPT. PLANT BIOLOGY, UNIVERSITY OF NATAL, DURBAN

Organelle dimensions were measured for at least 20 of each organelle type (unless otherwise stated), per cell zone.

Nucleus

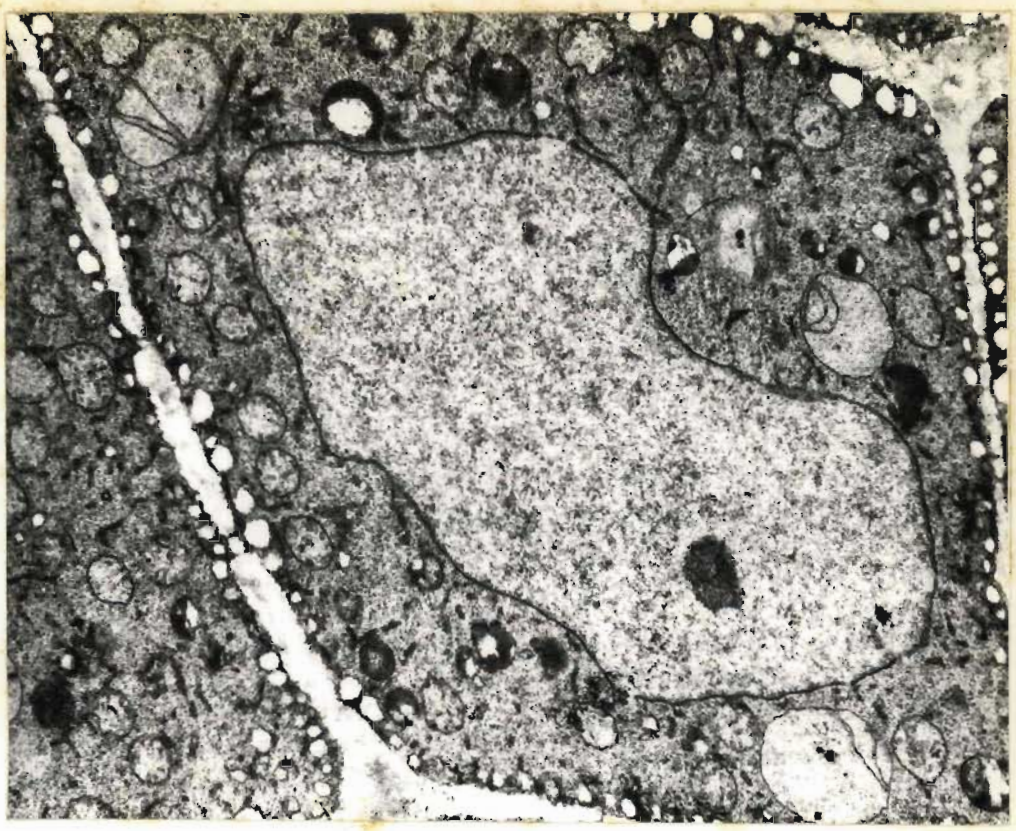
The nucleus was very prominent in the root cap initials (Fig III.A.2a) and cells of the zone of division (Fig. III.A.2b) where it was roughly spherical to oval. Nuclear dimensions did not vary much as the cells enlarged, thus nuclei in cells of the differentiating and mature zones were relatively less conspicuous. This is a factor of the increase in cell size, as there is an overall 15-fold volume increase from the cells of the cap initials to those of the mature zone (Juniper and Clowes, 1965). Nuclei of mature cells showed pronounced lobing compared with those of the younger cap cells (Fig. III.A.2c). Chromatin did not stain with potassium permanganate at this stage of germination.

Mitochondria

The mitochondria in the cap cells in general showed little differentiation. They were circular in section, and profiles differing in shape were seldom encountered. This was probably an indication that these organelles were roughly spherical at this stage, their elongation accompanying subsequent development. Cristae were very short and sparse, although they appeared slightly more developed in the mitochondria of the mature cells, compared with those in the initials (Figs. III.A.3a & 3b). However, some of the mitochondria in the mature cells had somewhat distorted profiles. This may be interpreted as a membrane phenomenon and is probably a function of the age of these cells in the root cap (Fig. III.A.3c).

There was an overall increase in size of mitochondria with increasing maturity of the cells. Their average

FIGURES III.A.2a & 2b. Illustrate the nucleus in a cap initial and a cell of the zone of division respectively, in embryos which had been imbibed for 12 hours.
(2a x 13 050; 2b x 10 350).



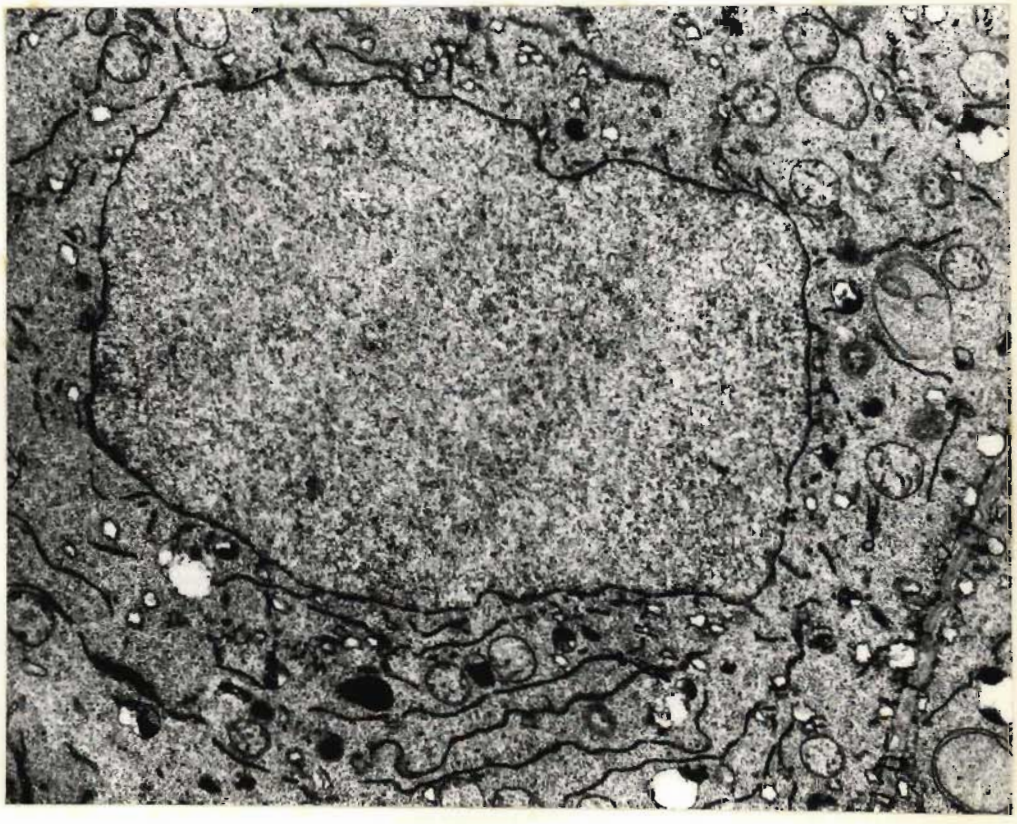
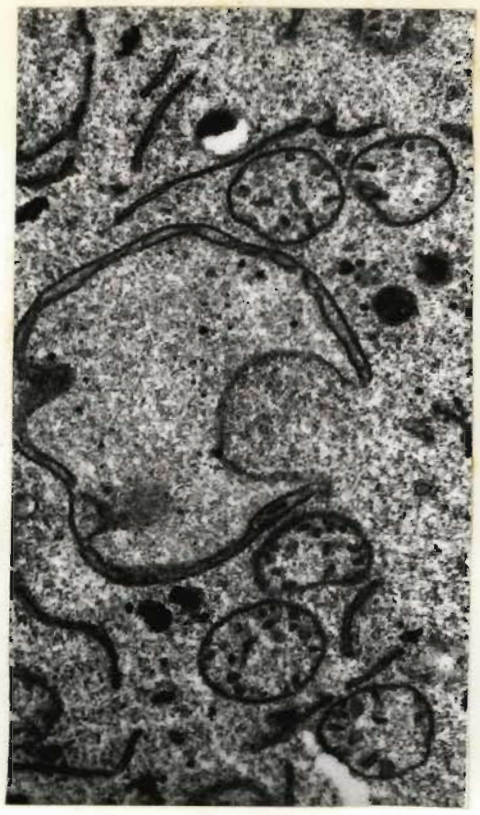


FIGURE III.A.2c. Shows the lobed nuclear profile which is characteristic of cells of the mature zone at the 12-hour germination stage. (x 10 350).

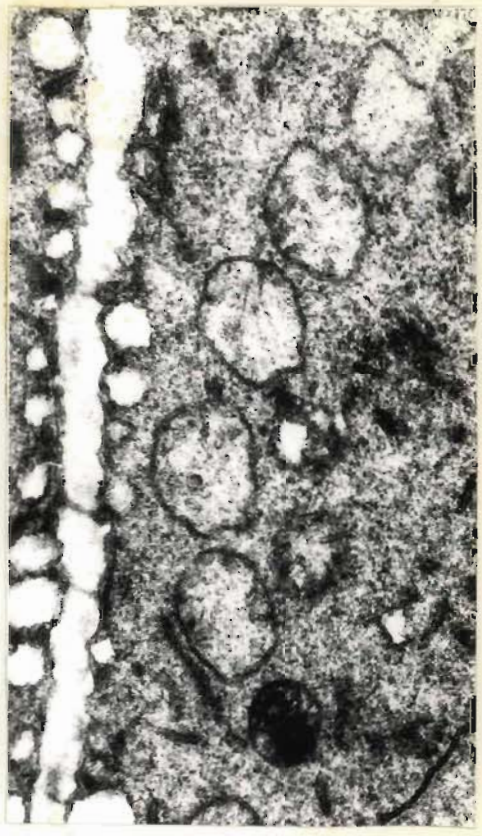
FIGURES III.A.3a & 3b. Illustrate the relative development of mitochondria in a mature cell and a cap initial respectively, 12 hours after the start of imbibition. (3a x 18 900; 3b x 32 400).

FIGURE III.A.3c. Shows a distorted mitochondrion (at arrow) in a mature cap cell of material at the 12-hour germination stage. (x 18 400).

FIGURE III.A.3d. Illustrates degenerating mitochondria in an outermost cap cell at the 12-hour germination stage. (x 18 400).



a



b

c

d

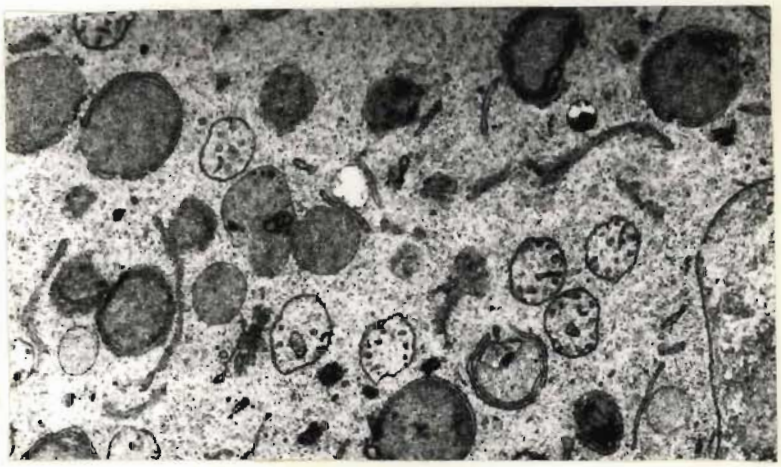
diameter in the cap initials was 410 nm., while in the mature cells it was 660 nm. The number of these organelles per unit area of cytoplasm fell from 47 in the intitials, through 29 in the cells of the zone of division, to 17 in cells of the zones of differentiation and maturity. This apparent drop in mitochondrial count probably does not reflect a drop in the total number of these organelles per cell, as there is a 15-fold volume increase between initials and mature cells (Juniper & Clowes, 1965). The outermost cells, which although in a state of senescence were not in an advanced phase of disorder in imbibed material, had a similar number of mitochondria to the mature cells. However, in these cells the mitochondria appeared somewhat swollen (average diameter was 725 nm.) with a less dense matrix than in the cells of the mature zone. The swelling tended to eliminate the distorted profiles seen for some of these organelles in mature cells. This swelling is interpreted as a degenerative change in these organelles, in the senescing cells (Fig. III.A.3d).

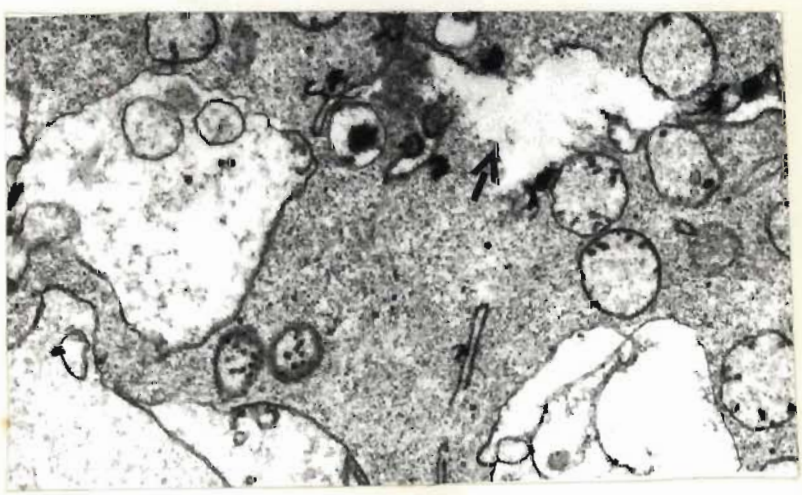
Lysosomes

Lysosomes are thought to originate from dilation of cisternae of the ER in cells of the zones of initials, division and differentiation and these organelles occurred in various stages of their formation (ref. Fig. III.A.2b). However, they only occurred as fully-formed, first-phase lysosomes, of average diameter 550 nm., in the mature cells. In these cells the fully-formed lysosomes were all characteristically in close association with a profile of the ER (Fig. III.A.4a). In the outermost cell layer, the bounding membrane of each lysosome had lifted (Fig. III.A.4b). There was an overall increase in size of the lysosome (now in the second developmental phase), which was interpreted as a swelling of this organelle.

FIGURE III.A.4a. Shows the intimate ER-lysosome association which is encountered in mature cap cells of 12-hour embryos. (x 13 800).

FIGURE III.A.4b. Illustrates second-phase lysosomes in an outermost cap cell of an embryo at the 12-hour germination stage. Note the regions where apparent dissolution of the lysosomal membrane has occurred (at arrow). (x 16 000).





The intimate association of ER with the lysosomes, which preceded their swelling might be significant in this process. There appeared to be some measure of dissolution of the bounding memberanes of some of the lysosomes in the outermost cells.

Counts of lysosomes per unit area of cytoplasm showed that the number fell with the development of the root cap. The average counts were 27 in the initials, 18 in (cells of) the zone of division, and 13 in (cells of) the zones of differentiation, maturity and senescence. However, this drop in lysosome count probably does not reflect a drop in the total number per cell, as increase in cell volume is reported to be 15-fold between the initials and mature cells.

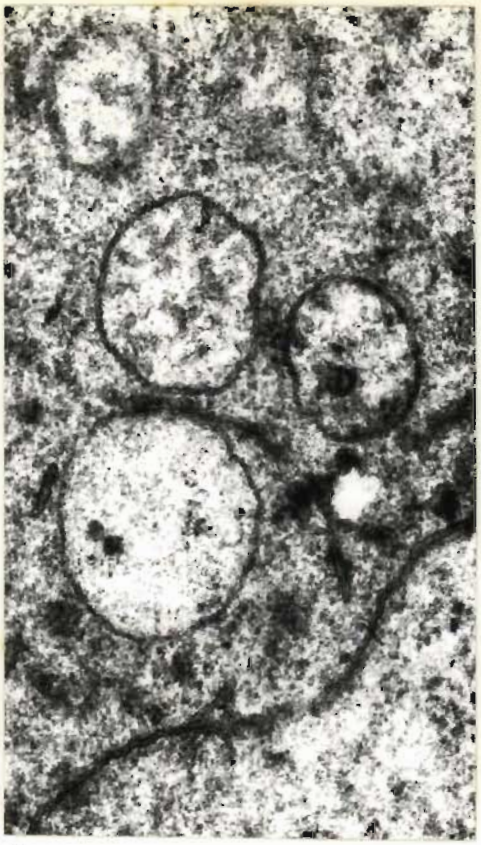
Plastids

Plastids in root cap initials of the imbibed material fell into two general classes. Some of them were undifferentiated organelles having a circular profile in cross-section (Fig. III.A.5a). Their average diameter is 510 nm. in these cells, and they are probably best described as plastid initials. Other plastids in the cap initials showed invaginations of the inner membrane, and were larger than the plastid initials, and had an average diameter of 740 nm. (Fig. III.A.5b). These are considered to be proplastids. There were few starch-storing amyloplasts in the root cap cells of imbibed maize embryos, and the cells of the zones of division and differentiation contained these organelles virtually only in the form of proplastids. However, ultrastructural observations on root cap cells after only 4 hours imbibition, showed that some of the plastids contained starch at that stage. It appears, therefore that plastids of these cells contain some starch in the mature seed, and that this reserve is utilised during the imbibition phase.

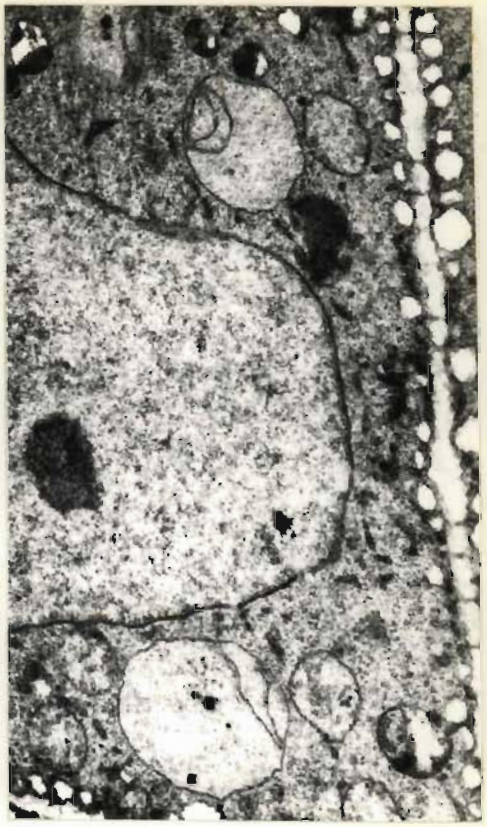
FIGURES III.A.5a & 5b. Illustrate a plastid initial and proplastids respectively. Both these micrographs are of cap initials at the 12-hour germination stage. (5a x 43 200; 5b x 17 400).

FIGURE III.A.5c. Shows a relatively large proplastid typical of cells of the mature zone at the 12-hour germination stage. (x 32 400).

FIGURE III.A.5d. Illustrates a swollen, disorganised plastid in a senescing outermost root cap cell in material which had been imbibed for 12 hours. (x 16 000).

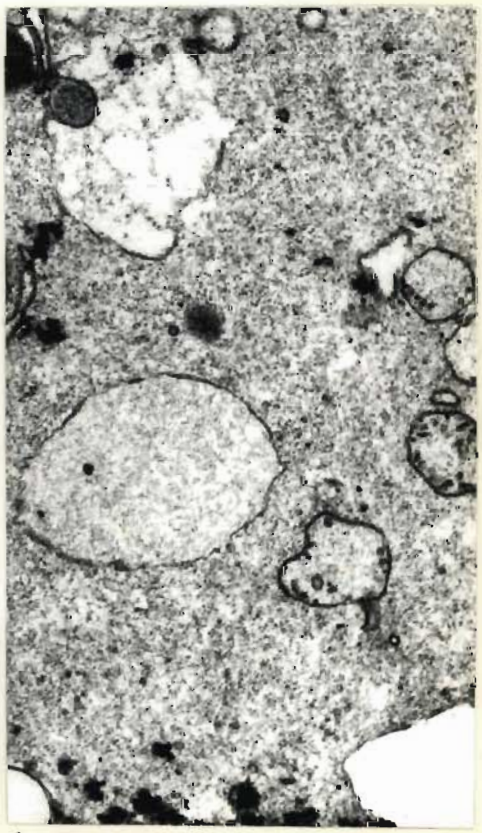


a



b

c



d

The proplastids increased in size with increasing development of the cells, reaching an average diameter of 1,100 nm. in the mature zone (Fig. III.A.5c). In the outermost cells, the plastids were interpreted as being swollen, with a marked decrease in their density, and appeared completely disorganised (Fig. III.A.5d). These outermost cells were senescing, and this change in the plastids is interpreted as being degenerative.

There was a slight decrease in the counts of plastids per unit area with increasing maturity of the cells, which does not reflect a drop in the total per cell, as there is a 15-fold volume increase between initials and mature cells. The counts were 7 in initials, 5, 3 and 4 in the zones of division, differentiation and maturity respectively, and 4 in senescing cells.

Dictyosomes

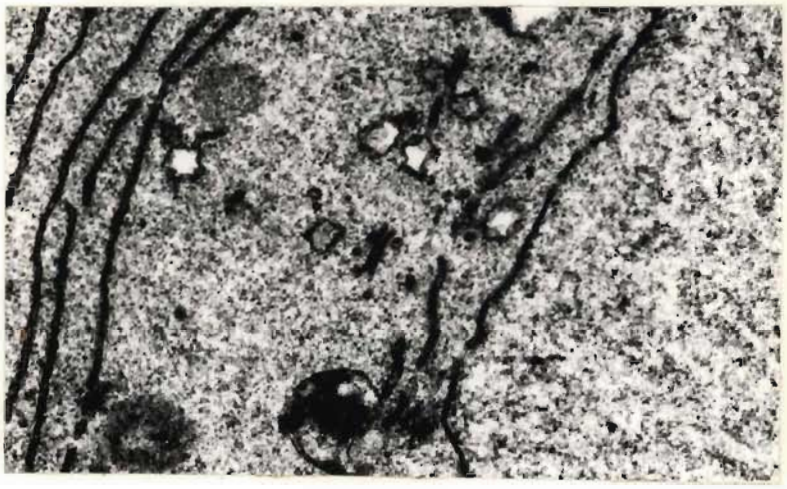
Dictyosomes were perhaps the least conspicuous, both in size and developmental status, of all the organelles in the root cap cells of imbibed, unaged embryos. Generally, in these cells, the dictyosomes consisted of two or three loose-associated cisternae and small associated vesicles. These organelles reflect a similar developmental status throughout the cap (Fig. III.A.6a).

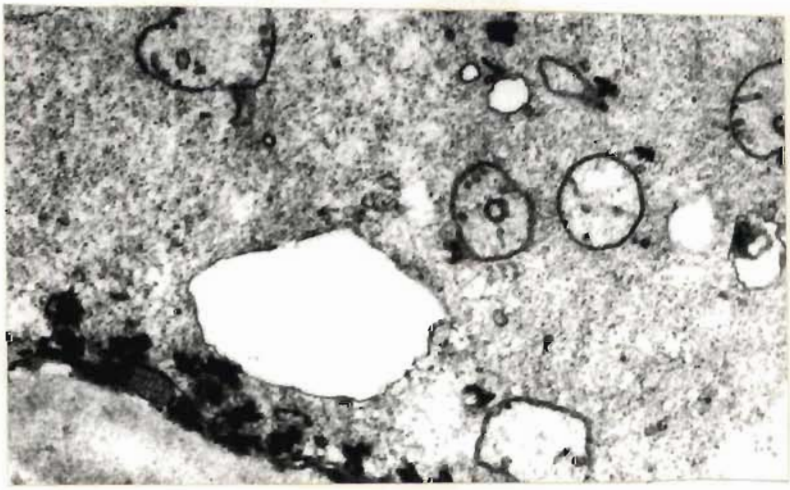
Dictyosome counts per unit area of cytoplasm were low (4 in initials, 2 in cells of the zone of division and 1 in the zones of differentiation and maturity).

No dictyosomal cisternae were apparent in the outermost, senescing cells. These are the only organelles of which there is no trace in the outermost cells. However, vesicles which are presumably dictyosomal in origin still persisted in these cells (Fig. III.A.6b).

FIGURE III.A.6a. Illustrates a dictyosome which consists of two cisternae, in a cell of the zone of division at the 12-hour germination stage. (x 48 600).

FIGURE III.A.6b. Shows portion of the cytoplasm of an outermost cap cell, with a few dictyosomal vesicles still apparent at the 12-hour germination stage. (x 19 950).





Endoplasmic Reticulum

It is difficult to asses the ER quantitatively, thus the relative length of the profiles, and their degree and type of orientation are used as criteria of the development of this organelle.

The ER was sparse, with only short profiles appearing in some of the initials (Fig. III.A.7a). In cells of the zone of division, the ER profiles were longer, but not orientated (Fig. III.A.7b). Both development and orientation of the ER were apparent in the arrangement of long profiles parallel with each other and with the nuclear envelope, in cells of the zone of differentiation (Fig. III.A.7c). This development is probably indicative of the physiological status of differentiating cells, as the ER is known to be an organelle which varies according to the cell type and physiological activity. In cells of the mature zone, the ER appeared relatively sparse. Many of the profiles occurred in intimate association with the lysosomes, while the remaining profiles were short and scattered through the cytoplasm (Fig. III.A.7d).

The senescing cells of the outermost layer showed only short, disorganised profiles of the ER, many of which were loosely associated with the (second phase) lysosomes (ref. Fig. III.A.4b).

Ribosomes

Ribosomes were encountered as disaggregated monosomes for up to 4 hours after the start of imbibition at $25^{\circ} \pm 2^{\circ}C$. (Fig.III.A.8a). However, by 6 hours after the start of the imbibition phase, the ribosomes were aggregated, forming polysomes in all but the outermost, senescing cells. This probably indicates the presence of long-lived m-RNA within the embryos, and is in keeping with the results of Dure and Waters (1965). In material which has been imbibed for the

FIGURE III.A.7a. Illustrates the sparse, relatively short ER profiles typical of the cap initials 12 hours after the start of imbibition. (x 13 050).

FIGURE III.A.7b. Shows development of the ER in a cell of the zone of division in material which had been imbibed for 12 hours. (x 10 350).

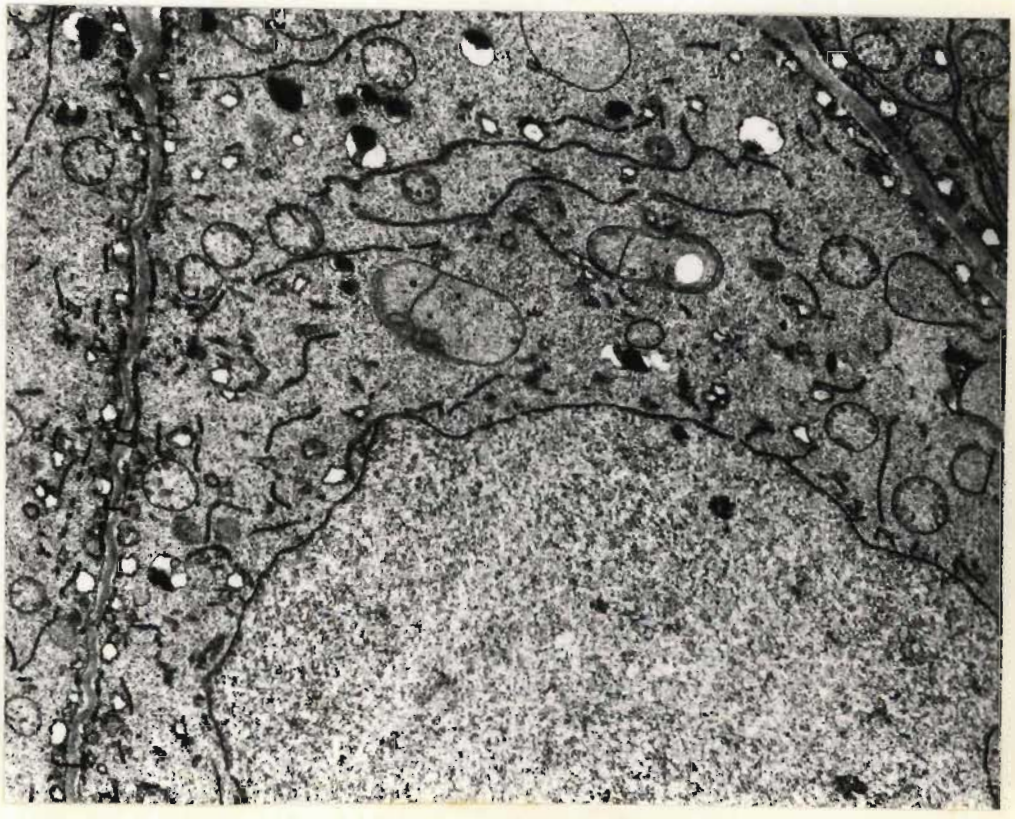
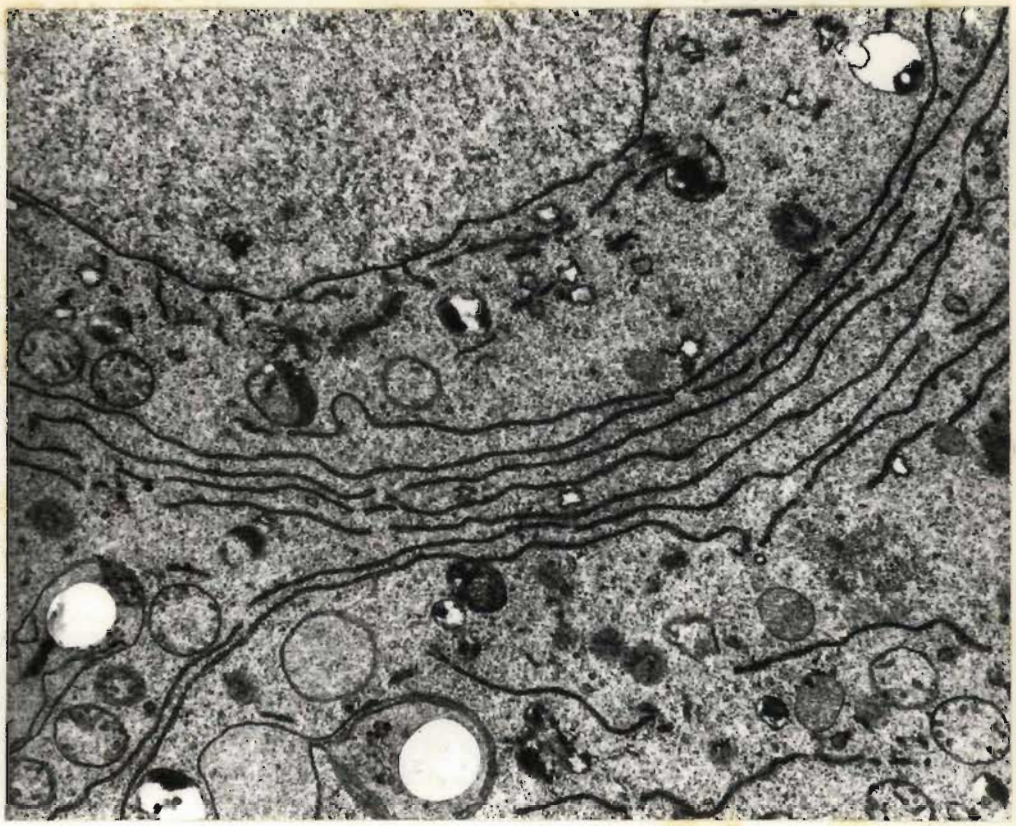
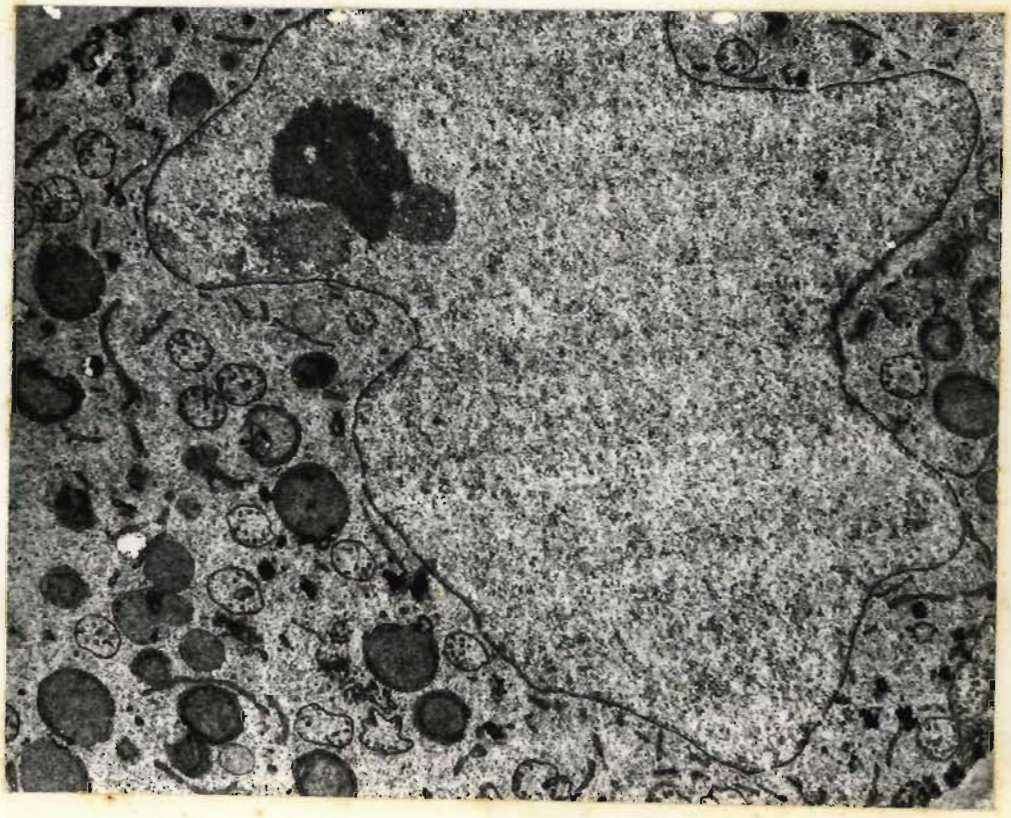


FIGURE III.A.7c. Illustrates the orientation of long ER profiles parallel with each other and with the nuclear envelope, in a cell of the zone of differentiation at the 12-hour germination stage. (x 13 050).

FIGURE III.A.7d. Shows the disposition of ER profiles typical of cells of the mature zone, 12 hours after the start of imbibition. (x 10 350).





full 12 hour period this same situation existed, viz. polysomes occurred in all the cells excepting those of the outermost layer (Fig. III.A.8b).

Lipid Droplets

These aggregations of reserve lipid generally were situated at peripheri of the cells in all the zones of the root cap in the imbibed material (ref. Fig. III.A.7a). However, in cells of the zones of division, differentiation and maturity, there was evidence that lipid droplets had migrated more deeply into the cytoplasm. This is interpreted as being indicative of the metabolic and physiological status of these cells.

Wall

The root cap in maize is separated by a very thick, somewhat irregular wall, from the root apex proper. This was apparent both in the imbibed state and in the later germination stages of the embryo (ref. Fig. III.A.1). However, this thick wall, in common with cell walls in general, was also found to be traversed by plasmodesmata.

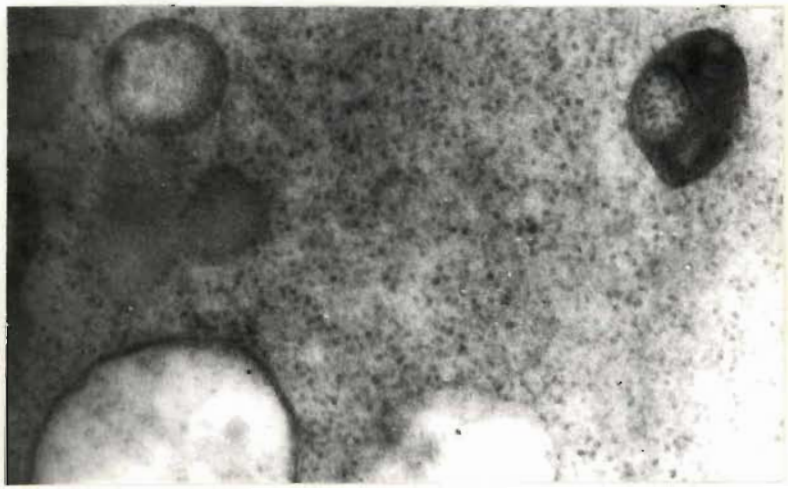
These walls of the cap initials and the zone of division which were parallel to the axis of the root, were consistently thicker than the transverse walls. This is indicative of the periclinal plane of cell division (Fig. III.A.9a). The cell walls in this region appeared relatively electron-transparent. The relative electron density, and the dimensions of the cell walls increased with increasing development of the cells. Walls of mature cells were relatively electron-dense, and thicker than those of younger cells (Fig. III.A.9b).

The outermost layer of the cap cells was complete in the imbibed material. There was no evidence of the sloughing-off process (involving changes in the middle lamella) which occurs in later developmental stages of the embryo.

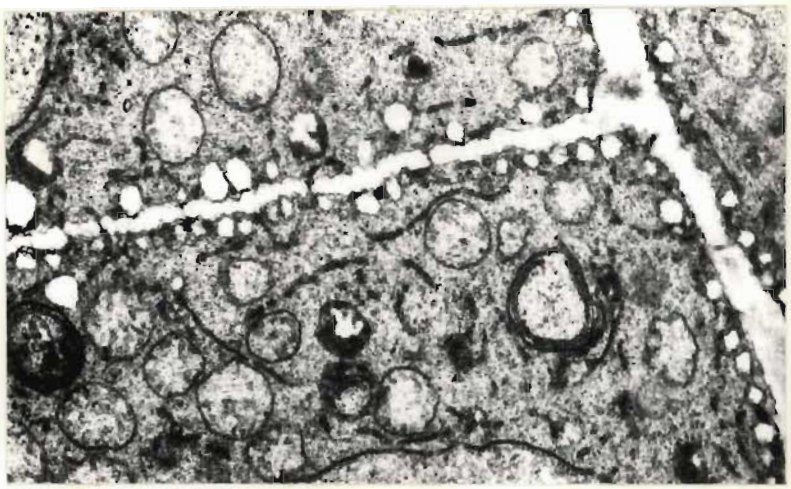
FIGURE III.A.8a. Shows monosomes in a cap cell after 4 hours after imbibition. (x 39 200).

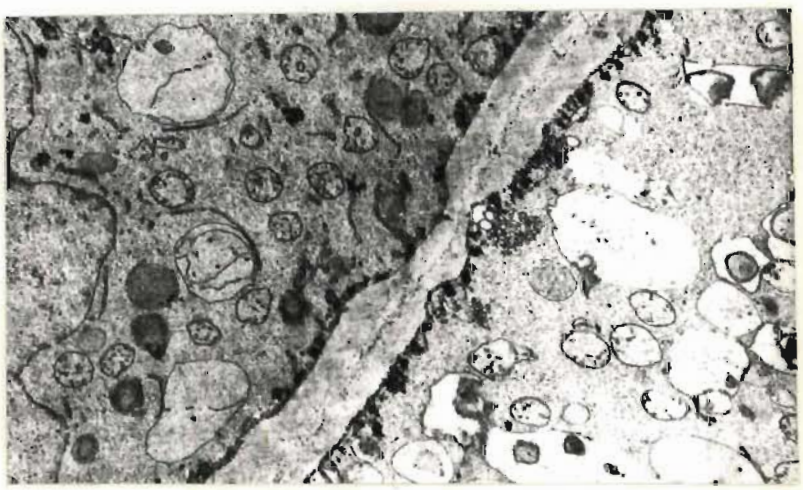
FIGURE III.A.8b. Illustrates polysomes in a cap cell of an embryo 12 hours after the start of imbibition. (x 44 800).

The material represented in both these micrographs was postfixed in an osmium solution according to Procedure 6b.



FIGURES III.A.9a & 9b. Illustrate the argon ion
pregarded by cap fall while
in the zonus of Zaleia and
mature cells respectively.
in material which had been
imbibed for 12 hours.
(9a x 17 400; 9b x 8 050).





A.2. ULTRASTRUCTURAL OBSERVATIONS IN THE ROOT CAP CELLS OF UNAGED EMBRYOS - 24 HOURS AFTER THE START OF IMBIBITION

The material described as '24-hour material' was imbibed with water for 12 hours at 25°C ± 2°C followed by a further 12 hours at this temperature with the embryo side in contact with moist cellulose wadding. While coleorhiza and radicle elongation had occurred 24 hours after the start of imbibition in no case had the radicle yet broken through the coleorhiza.

The organelle counts are presented per unit area of cytoplasm (100 cm^2 at a magnification of 10,350) and organelle dimensions represent the average for at least 20 of each organelle type, unless otherwise stated.

Nucleus

The aspect of the ultrastructure of the nucleus in the 24-hour material embryonic root cap was similar to that in this organ in the imbibed material. There was no sign of cell division (as evidenced by nuclear division) having started, at this stage. Thus the radicle extension which had occurred was presumably as a result of cell enlargement. In addition, the chromatin does not stain with potassium permanganate in any of the zones of the root cap at this germination stage.

Mitochondria

There was no significant change in the average cross-sectional diameter (460 nm.) of the mitochondria in the cap initials at this stage compared with the imbibed state. However, their average cross-sectional diameter in mature cap cells was somewhat lower (540 nm.) than in mature cells of the imbibed material. These organelles were sometimes seen in longitudinal section, and their elongation was apparent in all the cap cell types (excepting the initials).

The decrease in diameter might be interpreted as elongation having occurred, without a significant volume increase.

Cristae are generally more numerous and more developed in cap cells of the 24-hour material than in comparable cells of imbibed material (Fig. III.A.10a & 10b). The development of the mitochondria was in keeping with the general increase in activity known to accompany the early stages of germination.

The average number of mitochondria per unit area of cytoplasm dropped from 34 in the initials, to 22 in cells of the zone of division, but thereafter remains constant (21 and 22 in cells of the zones of differentiation and maturity respectively). Thus, replication of these organelles must occur, as cell enlargement accompanies increasing maturity of the cells. Profiles of mitochondria, showing what might be interpreted as division, were encountered in this material (Fig. III.A.10c). In the senescing, outermost cells, the mitochondria appeared somewhat disorganised, with the matrix less dense than in mitochondria of non-senescing cells and some appeared to be in a state of degradation. (Fig. III.A.10d).

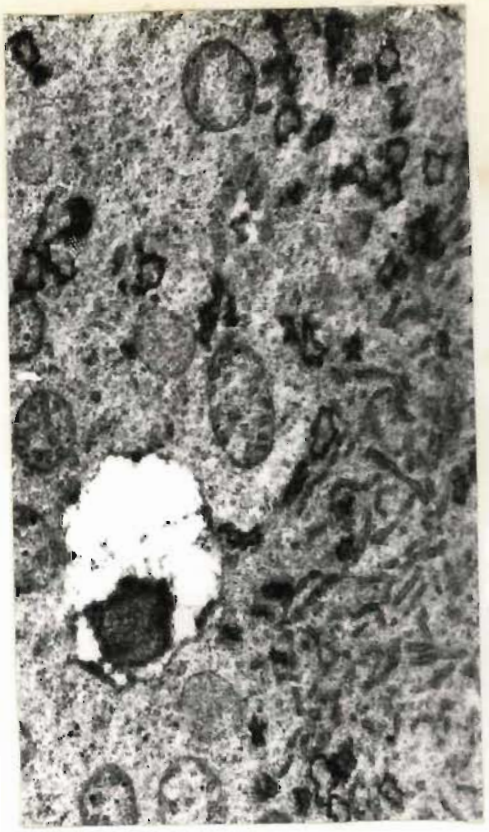
Lysosomes

Lysosomes were encountered mainly in their various stages of formation, in the cap initials 24 hours after the start of imbibition. However, in cells of the zone of division these organelles appeared as fully formed first-phase lysosomes, and as second-phase lysosomes. In cells of the zone of differentiation the second developmental phase of these organelles predominated. The second-phase lysosomes formed small vacuoles in these cells (Fig.III.A.11a). The presence of many relatively large (average cross-sectional diameter 1072 nm), first phase lysosomes (as well as second phase lysosomes) in mature cells suggested that

FIGURES III.A.10a & 10b. Illustrate mitochondria in cap cells of the zones of division and maturity respectively, 24 hours after the start of imbibition. (10a x 22 800; 10b x 14 500).

FIGURE III.A.10c. Shows a mitochondrion in an apparent state of division in a cell of the zone of division at the 24-hour germination stage. (x 18 400).

FIGURE III.A.10d. Illustrates the disorganised mitochondria typical of the senescing outermost cap cells, 24 hours after the start of imbibition. (x 18 400).



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formation of more of these organelles had occurred (Fig.III.A.11b) Counts bear out this impression being 27 in initials, 13 and 6 in the zones of division and differentiation respectively and reverting to 13 in mature cells. In the outermost root cap cells of 24-hour embryos the bounding membranes of the lysosomal vacuoles appeared to be discontinuous (Fig. III.A.11c). This was coupled with (and may have been responsible for) general degradative changes in these cells.

Plastids

In the cap initials of the 24-hour material, the plastids occurred mainly as proplastids and young amyloplasts (Fig. III.A.12a). Thus these organelles had developed further in the period 12-24 hours following the start of imbibition, and starch reserves had begun to accumulate within some of them. Their average cross-sectional diameter in these cells was 800 nm.

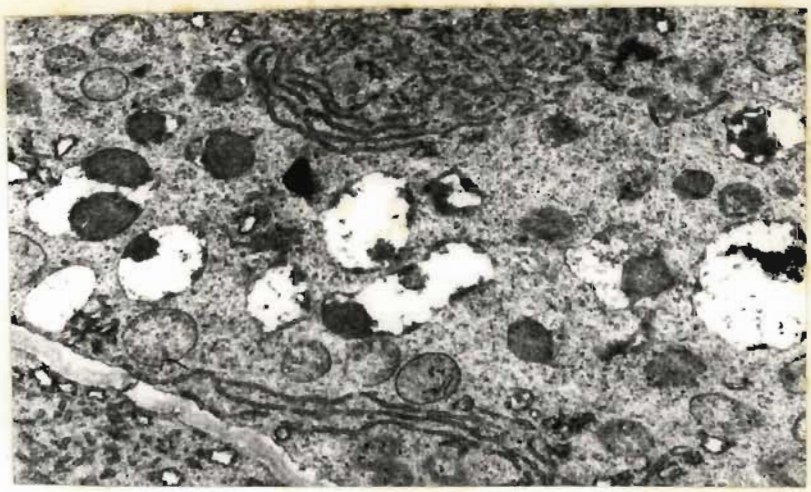
Some of the plastids in the cells of the zone of division appeared themselves to be in a state of division. Plastids in these cells appeared to contain little or no starch (Fig. III.A.12b). Plastids in the cells of the zone of division were somewhat smaller (average cross-sectional diameter 600 nm) than those in the initials. This difference is probably a factor of the reserve starch in plastids of the initials, and of the apparent division of these organelles in cells of the zone of div. sion.

Plastids in the cells comprising the zone of differentiation showed a marked increase in size (average cross-sectional diameter 1,900 nm). There is evidence of intensive starch accumlation in these plastids which may account for at least part of the increase in size of these organelles (Fig. III.A.12c).

FIGURE III.A.11a. Shows the small vacuoles formed by the second-phase lysosomes in cells of the zone of division, at the 24-hour germination stage. (x 11 500).

FIGURE III.A.11b. Illustrates the relatively large first-phase lysosomes, and the second-phase lysosomes which are typical of mature cells 24 hours after the start of imbibition. (x 13 050).

FIGURE III.A.11c. Shows the apparent lack of continuity in bounding membranes of lysosomes in an outermost cap cell at the 24-hour germination stage. (x 16 100).



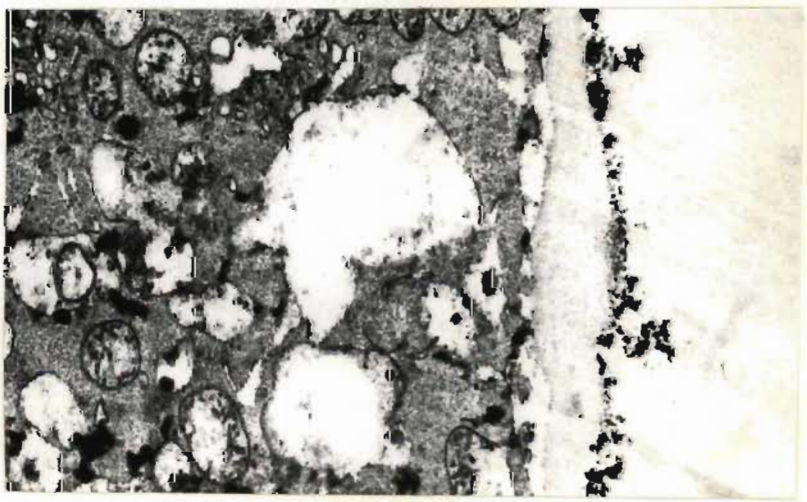
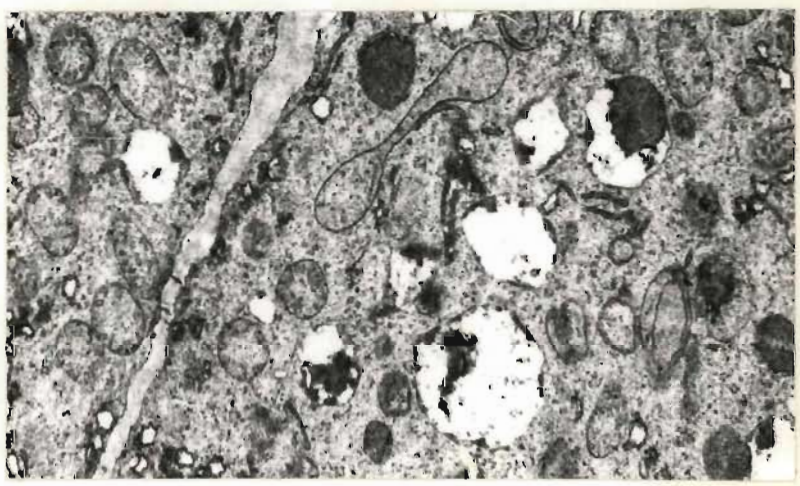
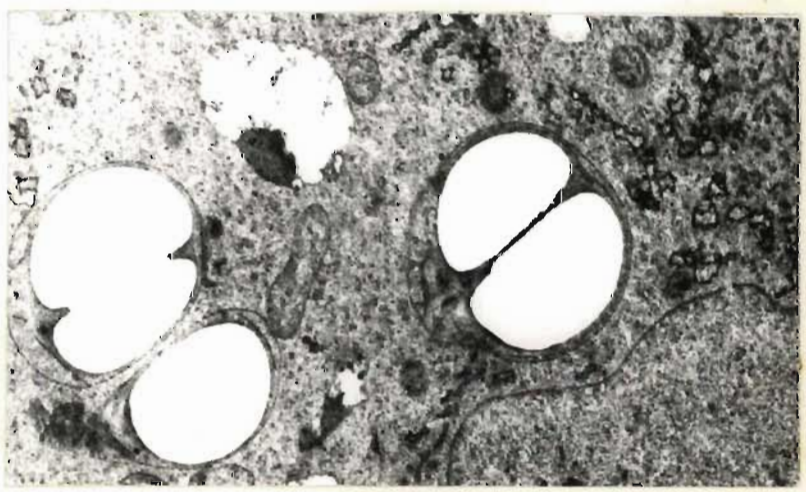


FIGURE III.A.12a. Shows proplastids and young amyloplasts in a root cap initial at the 24-hour germination stage. (x 18 400).

FIGURE III.A.12b. Illustrates a proplastid in an apparent state of division in a cell of the zone of division, 24 hours after the start of imbibition. (x 11 500).

FIGURE III.A.12c. Shows plastids in a cell of the zone of differentiation, at the 24-hour germination stage. There is evidence of intensive starch accumulation. (x 10 800).





Plastids in the mature cap cells of 24-hour embryos were similar in size and appearance to those in the cells of the zone of differentiation. These plastids also contained substantial starch deposits.

Plastid counts per unit area of cytoplasm reflected a decrease from the zone of initials (11) to the zone of division (4) after which the count remained virtually constant (3 and 4 in cells of the zones of differentiation and maturity respectively). Thus division of plastids must have occurred, as indicated by their numerical constancy per unit area of cytoplasm despite the overall increase in cell size.

The intact plastids in the cells of the outermost root cap layer showed a lessening in density and their internal structure was disorganised, while some of these organelles were in an apparent state of breakdown.

Dictyosomes

The dictyosomes in the root cap cells generally appeared to have become active at the 24-hour germination stage compared with their appearance in the imbibed material. Dictyosome counts per unit area of cytoplasm were 4 in the initials, 2 and 1 in the zones of division and differentiation respectively, and 6 in mature cells, indicating re-association or replication of these organelles, as there is a 15-fold volume increase from the initials to the mature cells.

In the initials and cells of the zone of division, although the dictyosomal cisternae still appeared to be somewhat loosely associated, the number of cisternae (4 on the average) per dictyosome had increased compared with these organelles in comparable zones of the imbibed embryos (Fig. III.A.13a). These organelles also appeared to be

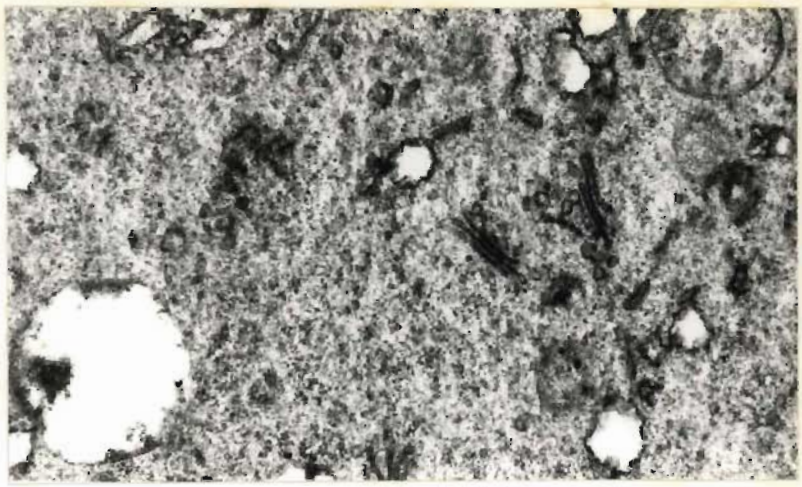
active in vesicle formation in these cells in the 24-hour embryos. While the exact functional significance of the dictyosomes at this stage is not known, it seems possible that their activity might be preparatory to cell division (e.g. to new cell plate formation) which is known to follow shortly after this germination stage. The aspect presented by the dictyosomes in the zone of differentiation was essentially similar to that in the preceding tissue zones. However, in mature cells dictyosomal count per unit area of cytoplasm has increased, and these organelles presented an appearance of greatly enhanced activity. While the average number of cisternae per dictyosome was unchanged, compared with the less mature cells of the cap at this stage, there was evidence of very active vesicle production in the mature cells (Fig. III.A.13b). There was an increase in the length of dictyosomal cisternae in section with increasing maturity of the cells in the cap. (The average of the length of the longest cistena per dictyosome was taken, and these measurements were made on 10 dictyosomes in each case). In cells of the zone of differentiation the average of the longest cisternae was 500 nm. compared with 800 nm. in mature cells. The dictyosomal vesicles in the mature cells (average cross-sectional diameter 123 nm.) were larger than those of any other zone (average cross-sectional diameter 100 nm.), at the 24-hour germination stage. This development of the dictyosomes is interpreted as being preparatory to their hypersecretory activity in the mature cells at subsequent germination stages (see below).

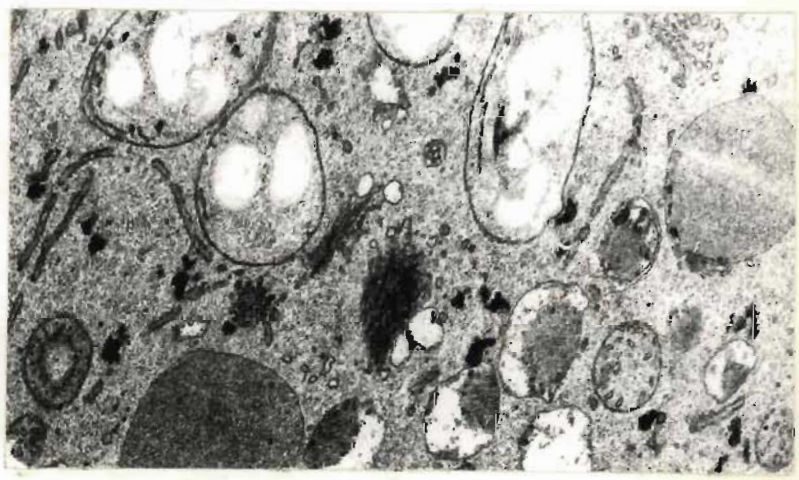
Localised groups of dictyosomal vesicles were encountered in the outermost cells, but cisternae were not in evidence. These organelles appeared to be most sensitive to general conditions prevailing in the cytoplasm of senescing cells (Fig. III.A.13c).

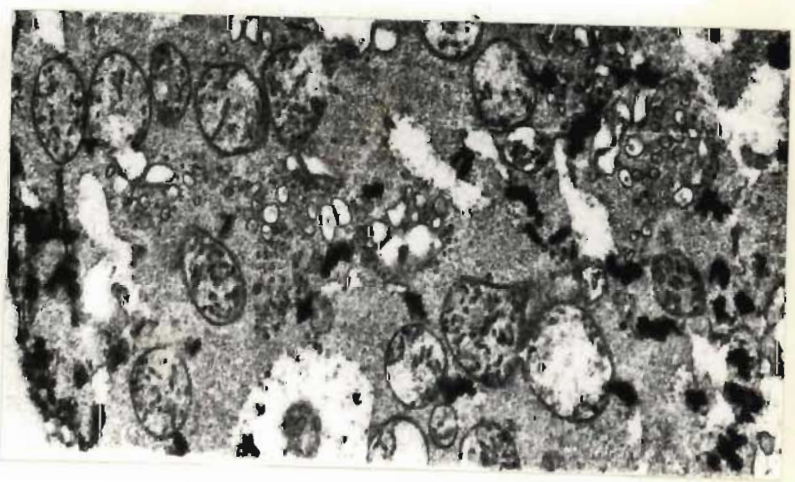
FIGURE III.A.13a. Illustrates dictyosomes in a cap cell of the zone of division, 24 hours after the start of imbibition. (x 24 050).

FIGURE III.A.13b. Shows dictyosomes in a mature cap cell. Their apparently enhanced activity at this stage (24 hours after the start of imbibition) is evident. (x 15 950).

FIGURE III.A.13c. Shows localised groups of vesicles which occur in the outermost cap cells at the 24-hour germination stage. (x 18 400).







Endoplasmic Reticulation

While the ER in the cap initials of the 24-hour embryos was very sparse, this organelle showed development in cells of the zone of division. Although the ER profiles were not relatively long, they appeared in profusion, generally localised in the midregion of the cell (Fig. III.A.14a). It is difficult to suggest a functional significance for this aggregation of the ER; however, localisation was in the peri-nuclear region and it might be related to the subsequent nuclear (and cell) division which occurred in this zone. The remainder of the cytoplasm showed very few ER profiles.

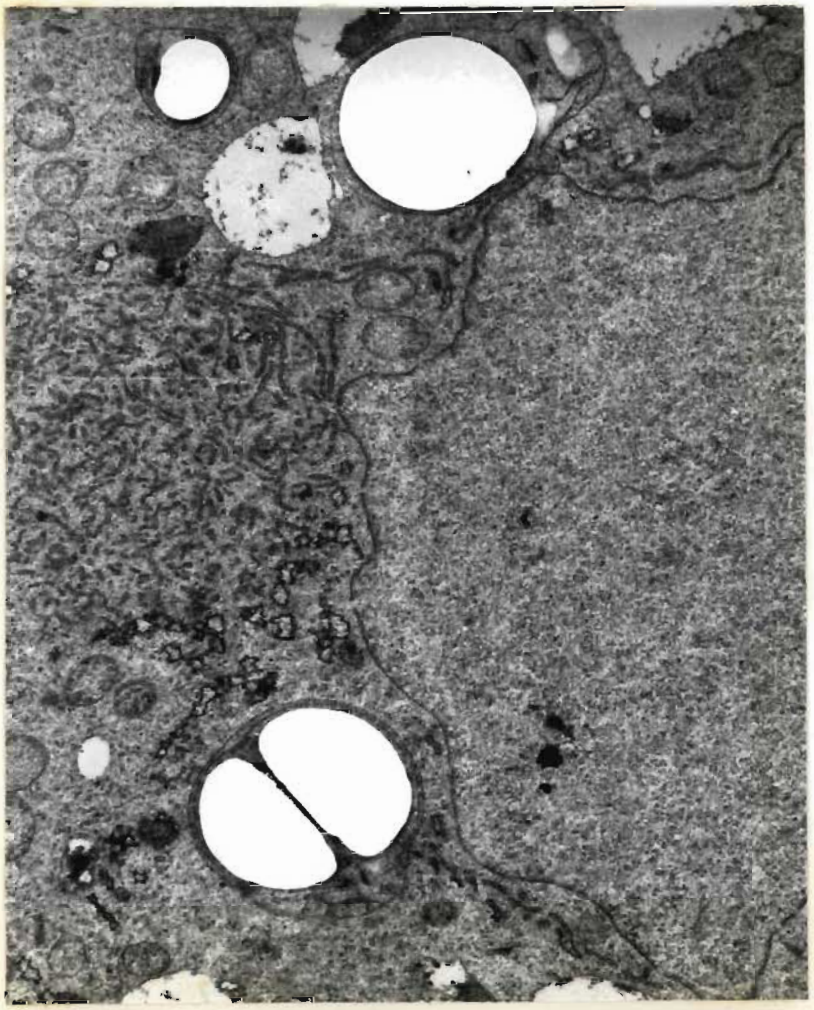
Cells of the zone of differentiation showed a similar situation with respect to development and aggregation of the ER, as described for cells of the zone of division (Fig. III.A.14b). However, in addition there was development and aggregation of short profiles of the ER near the cell peripheri (Fig. III.A.14c). In mature cells, the ER was orientated in short parallel profiles throughout the cytoplasm (Fig. III.A.14d).

ER profiles were absent from the cytoplasm of the outermost, senescing cells. Destruction of the short, disorganised ER fragments (encountered in the outermost cap cells of imbibed embryos) presumably accompanied the degradative changes which occur in the senescing cells at this germination stage.

Ribosomes

The ribosomes, which were aggregated to form polysomes in the imbibed material, persisted in this form in cap cells of embryos 24 hours after the start of imbibition (Fig. III.A.15). Only in the outermost, senescing cap cells did the ribosomes occur as monosomes.

FIGURES III.A.14a & 14b. Illustrate the localisation of relatively short ER profiles in cells of the zones of division and differentiation respectively, at the 24-hour germination stage. (14a x 10 350; 14b x 16 200).



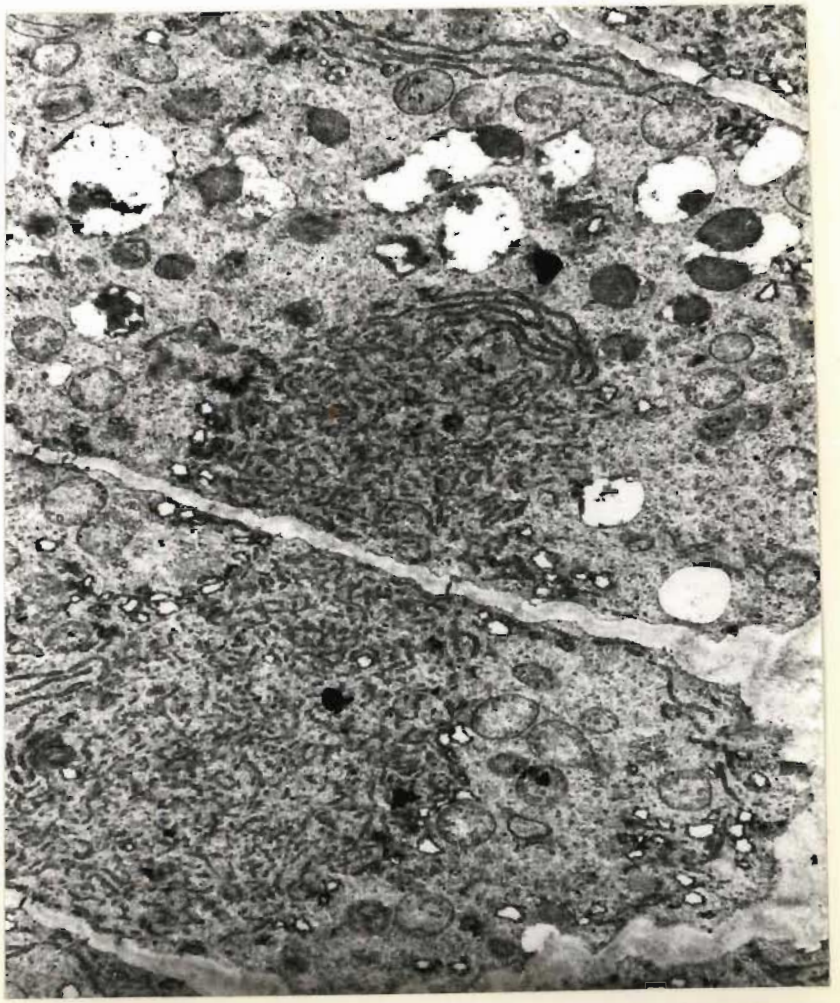
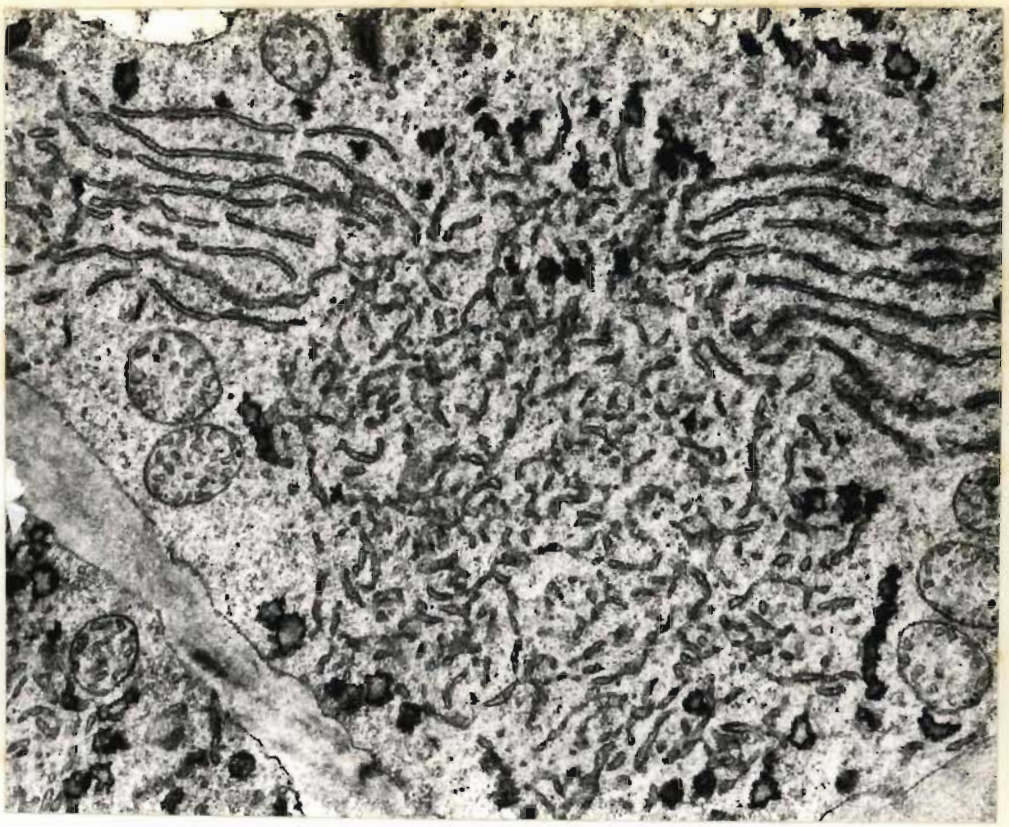


FIGURE III.A.14c. Shows an aggregation of the ER near the cell peripheri in the zone of differentiation, 24 hours after the start of imbibition. (x 17 100).

FIGURE III.A.14d. Illustrates the disposition of the ER in the cytoplasm of a mature cap cell at the 24-hour germination stage. (x 13 050).



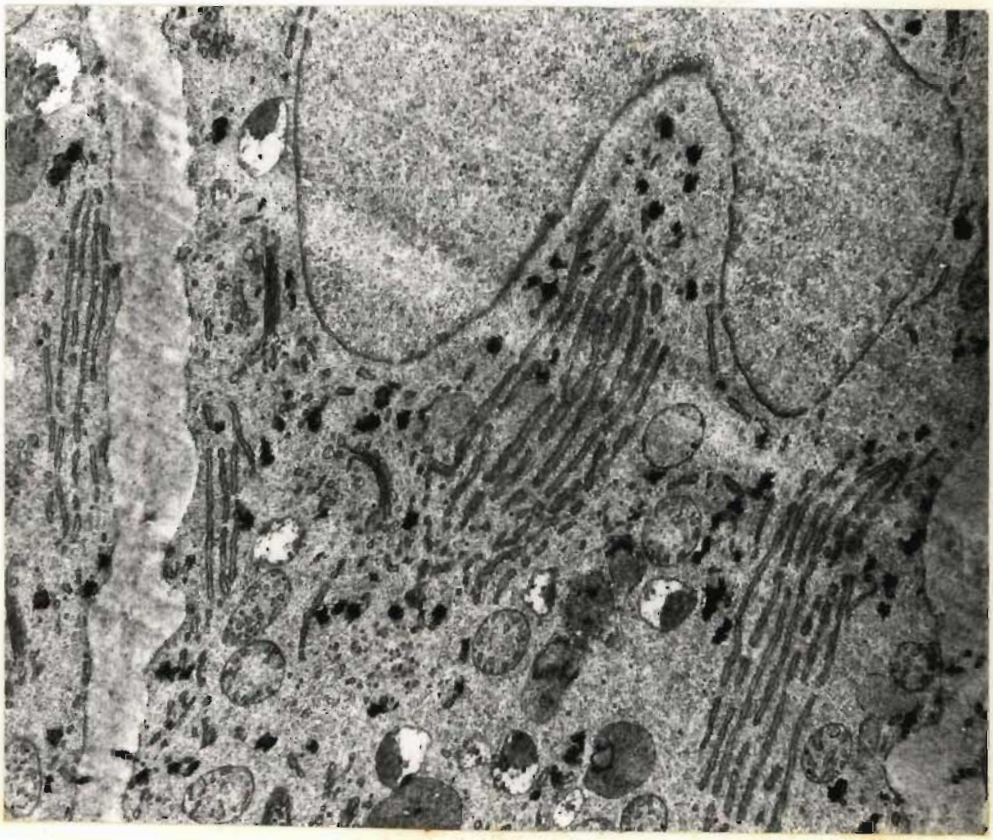


FIGURE III.A.15. Illustrates polysomes in a root cap cell, 24 hours after the start of imbibition. The material was postfixed in an osmium solution according to Procedure 6b. (x 50 400).

Lipid Droplets

Mobilization of reserve lipid was indicated by the fact that the lipid droplets, which were largely localised at the cell peripheri in cap cells of the imbibed material, had migrated inwards to the deeper parts of the cytoplasm. This was evident in all the zones of the root cap at the 24-hour germination stage. In cells of the zone of division, association of lipid droplets with ER aggregates was apparent (ref. Fig. III.A.14a), and occurrence of lipid droplets in the perinuclear area and associated with the more peripheral ER aggregates, was also seen in the zone of differentiation (ref. Figs. III.A.14c and 16a). In addition, there was a change in the lipid droplets, which decreased in size, and changed in staining reaction towards potassium permanganate with increasing maturity of the cells (Fig. III.A.16b). The change in disposition and appearance of the lipid can be interpreted as progressive utilization of this reserve, probably associated with proliferation of membranes as germination progresses.

Wall.

The cell wall pattern in the root cap of the 24-hour embryos was essentially similar to that described for the imbibed material. However, there was a tendency for the outermost, senescing cells to become separated from the cap at this stage (Fig. III.A.17). The separation occurred in the middle lamella region, between the outermost cells and the adjacent layer, and a senescing cell became dissociated from the root cap, completely enclosed by its wall.

FIGURE III.A.16a. Shows the disposition of lipid droplets in the perinuclear cytoplasm in a cap cell of the zone of division, at the 24-hour germination stage. (x 13 050).

FIGURE III.A.16b. Illustrates the changed appearance of the lipid droplets in a mature cap cell, 24 hours after the start of imbibition. (x 14 500).

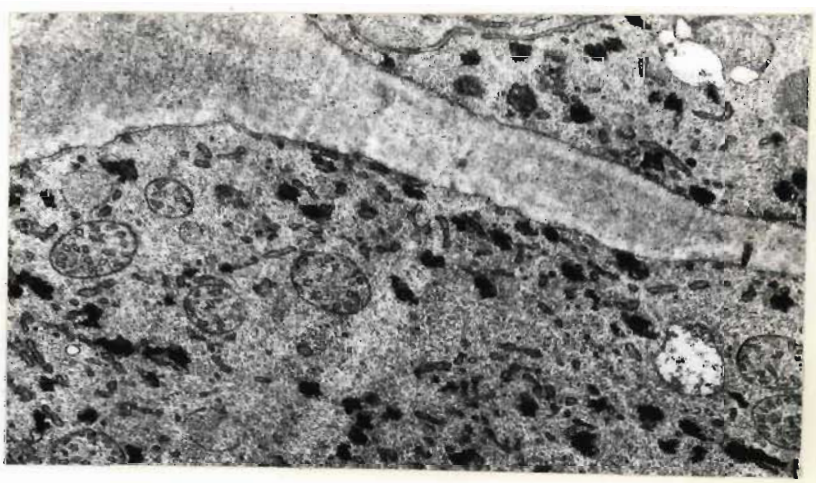
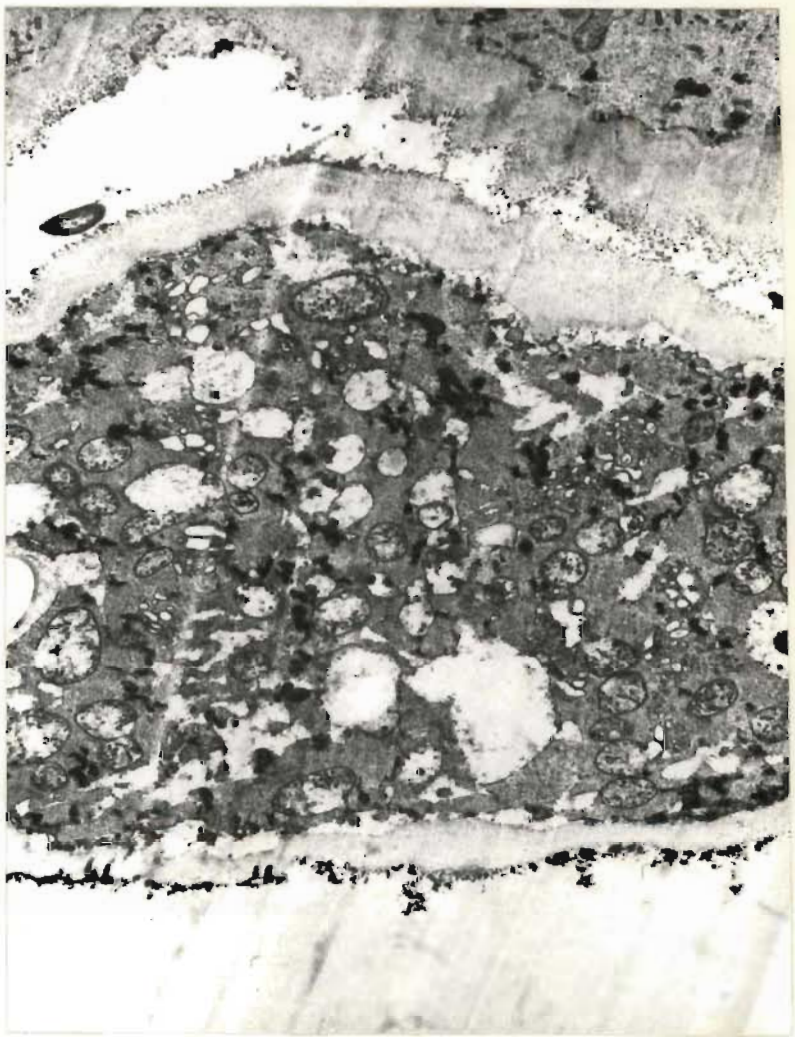


FIGURE III.A.17. Illustrates dissociation in the middle lamella region of the wall between a cell of the most distal part of the mature zone, and an outermost cell, 24 hours after the start of imbibition. (x 10 350).



A.3 ULTRASTRUCTURAL OBSERVATIONS IN THE ROOT CAP OF UNAGED EMBRYOS - 48 HOURS AFTER THE START OF IMBIBITION.

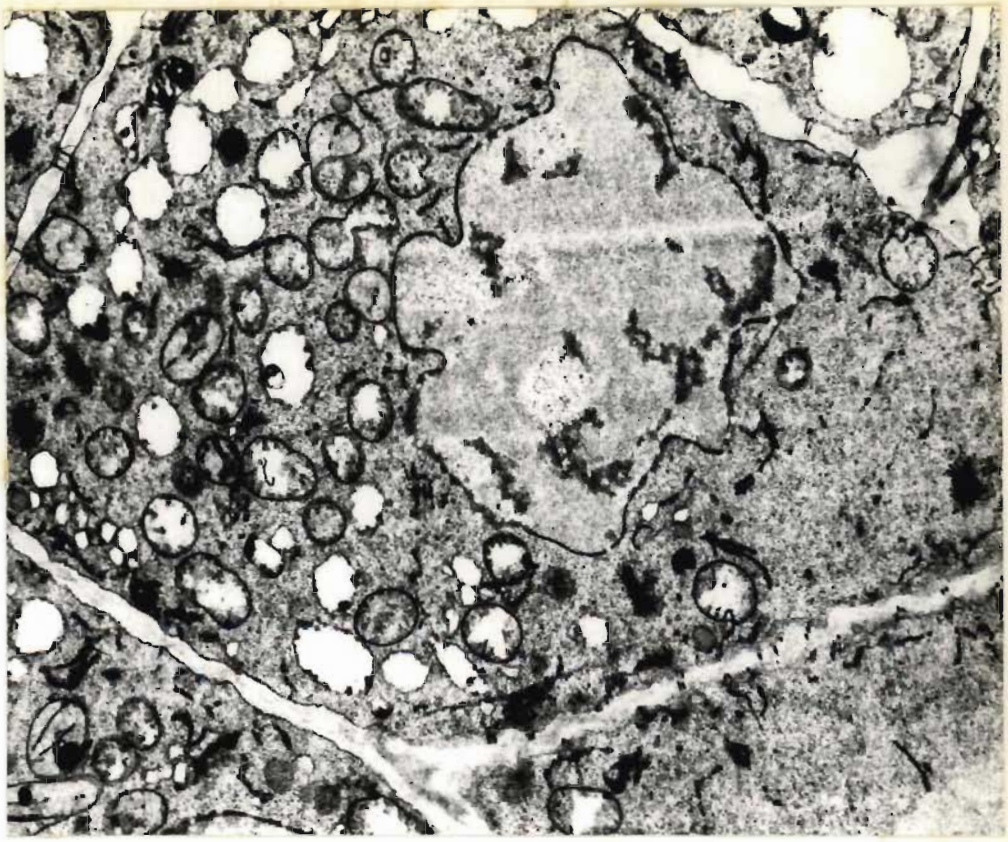
By 48 hours after the start of imbibition (12 hours imbibition followed by 36 hours on moist cellulose wadding), the radicle had broken through the coleorhiza, and cell division had started.

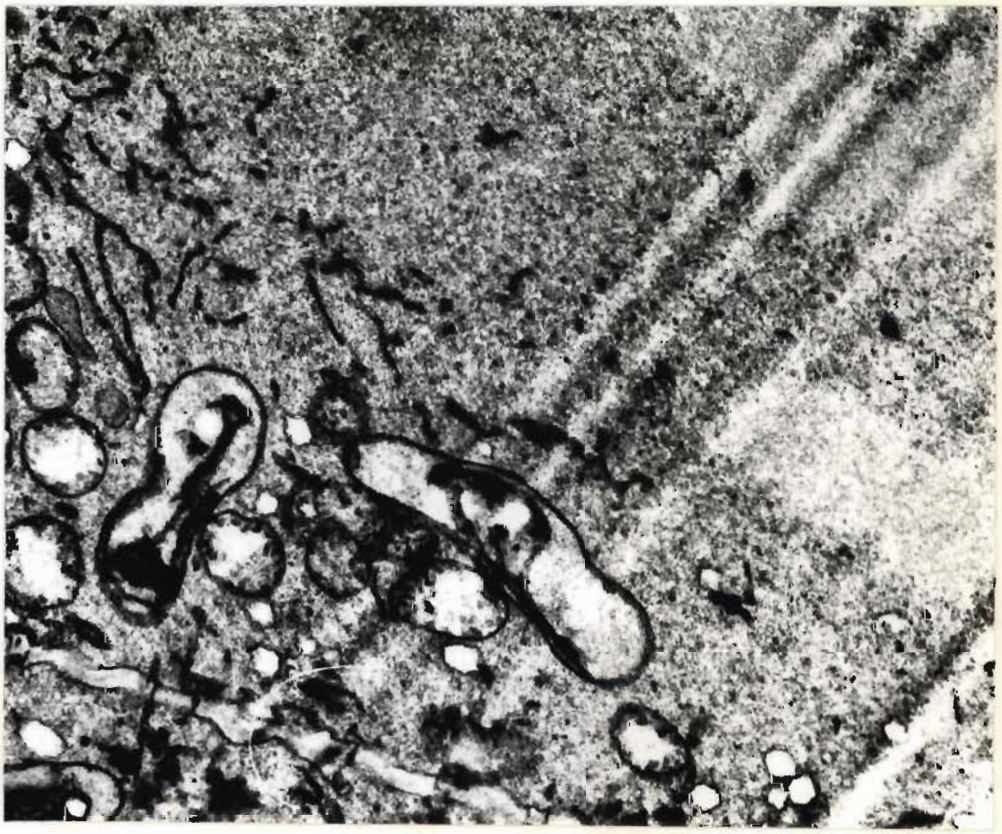
Preliminary investigations on the developmental sequence in the root cap of maize were carried out on the variety Hickory King. It was found that, for this variety, the outermost root cap cells were in a state of disorder, indicating a late stage of senescence. In fact, these cells appeared non-functional and were deemed senescent (Berjak, 1968). Subsequent investigations on maize were made on the hybrid SA 4 (for the reasons outlined in Part II). In this variety the outermost root cap cells 48 hours after the start of germination were found to be in an earlier stage of senescence than those of Hickory King. It is important to bear in mind that the apparently senescent outermost cap cells described for the 24-hour material (SA 4) have been lost and these cells have been replaced by distal cells of the mature zone, by the 48-hour stage. These cells in SA 4 were sloughed off in a senescing state, and the senescent cells were thus detatched and only infrequently encountered, as they were largely lost in the preparation of these root tips for ultramicrotomy and electron microscopy. Thus cells in late stages of senescence have been described from Hickory King, as the senescent cells occasionally encountered in ultra-thin sections of SA 4 at the 48-hour stage reflect an apparently identical condition.

Nucleus

Meristematic activity of the cap initials was evident, as cells in stages of division were encountered. Figures III.A.18a and 18b show two stages of mitosis in cap initials.

FIGURES III.A.18a & 18b. Illustrate stages of mitosis in cap initials, 48 hours after the start of imbibition. (18a x 10 350; 18b x 16 100).





In the zone of dividing cells too, there was evidence of mitotic activity.

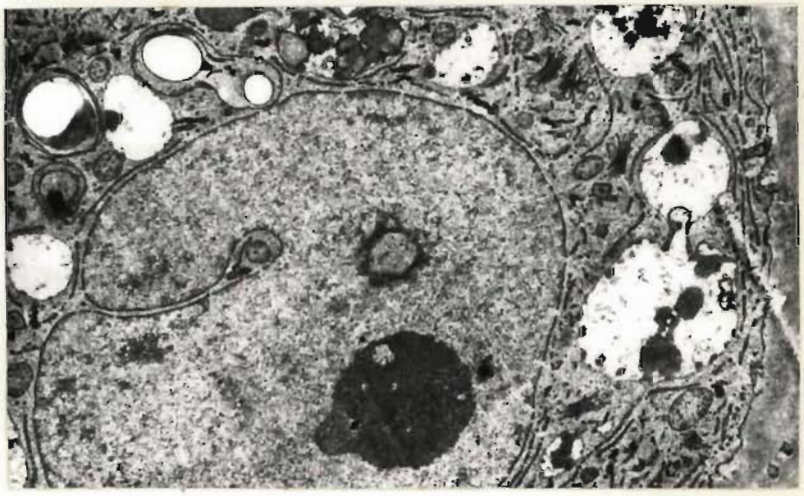
The same trend of nuclear size relative to cell size described for imbibed and 24-hour material occurred in the various zones of the root cap of 48-hour embryos. That is, as the nuclear size did not vary much, and cell size increased with increasing maturity, the nuclei occupied a decreasing volume in the protoplasm. Nuclei of the mature cells had a somewhat lobed profile (Fig. III.A.18c), which is interpreted as an expression of the age of these cells within the cap.

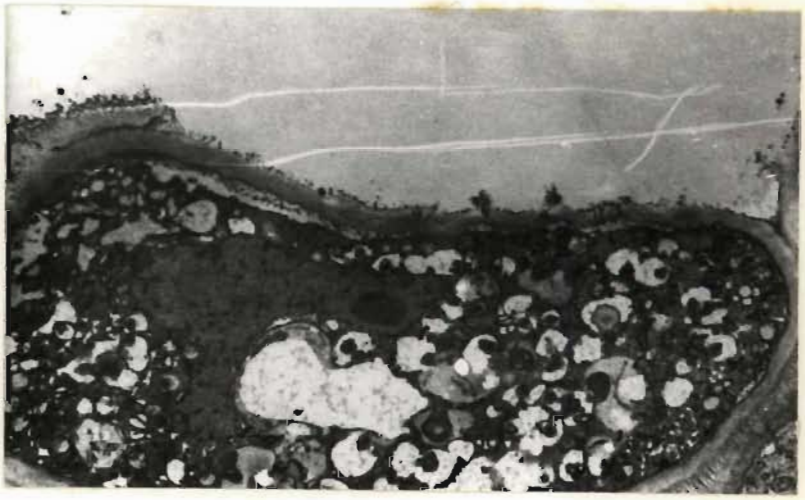
There was a change in the staining reaction of nuclei to potassium permanganate in all regions of the 48-hour root cap. Whereas there was no staining of chromatin by potassium permanganate in nuclei at either the imbibed or the 24-hour stage, staining of chromatin is general in the nuclei in 48-hour material. This is interpreted as being indicative of the physiological status of the cap cells in the actively-growing embryo, where not only cell division occurred, but intensive nuclear-directed metabolism must accompany differentiation and the establishment of mature cells, and possibly also the process of senescence in the outermost cell layer.

In cells in the earlier stages of degradative changes associated with their senescence, the nuclei had a similar appearance to those in mature cells. However, the staining properties of nuclei changed in cells where degradative processes were advanced (evidence from Hickory King). In these cells the nucleoplasm stains relatively darkly as compared with nuclei in other zones of the root cap (Fig. III.A.18d).

FIGURE III.A.18c. Shows the somewhat lobed nuclear profile in a mature cap cell, at the 48-hour germination stage. (x 6 300).

FIGURE III.A.18d. Illustrates the darkly-stained nucleoplasm in a cap cell showing advanced degradative change (Hickory King). (x 3 500).





Mitochondria

The number of mitochondria per unit area of cytoplasm was approximately double (29 in the actively-dividing initials compared with the count (14) in non-dividing cap initials. Thus replication of these organelles (or their derivation from the nuclear envelope) must occur either immediately preceding, or accompanying nuclear division. As mitochondria in an apparent state of division were encountered (Fig. III.A.19a) and no evidence has been obtained for their derivation from the nuclear envelope, it is thought that replication of these organelles occurs, at least in the root cap cells of maize.

Mitochondrial development accompanied cell maturation, and these organelles in differentiating and mature cells showed a progressive increase in cristae which were more developed, compared with those in the dividing cells and the initials (Fig. III.A.19b).

Some of the mitochondria in senescing cells (of SA 4) had a darkened matrix. This is interpreted as a degenerative change and is in keeping with the results of Öpik (1965).

Mitochondrial counts per unit area of cytoplasm initial rose from the non-dividing initials (14) to the cells of the zone of division (19). The count was 12 in differentiating cells, increasing to 17 in cells of the mature zone. In a comparison of the counts in initials and mature cells, it is evident that mitochondrial replication had occurred with increasing size and maturity of the cap cells. In senescing cells of the variety SA 4 the number of mitochondria per unit area of cytoplasm had dropped to 8. This suggests that the degradation of these organelles accompanied the general changes in senescing cells. In senescent outermost cap cells (Hickory King) the remaining mitochondria had a swollen, disorganised

appearance, with a lessening in density of the matrix. This is interpreted as a sign of advanced mitochondrial degeneration and is in keeping with the appearance of these organelles in outermost cap cells at the 24-hour stage.

The average cross-sectional diameter of mitochondria is 575 nm. in those initials not in an active state of division. In cells of the zone of division the average cross-sectional diameter of the mitochondria had increased to 650 nm. and remained fairly constant in differentiating cells (660 nm.). Mitochondria in the mature cells had a somewhat lower cross-sectional diameter (610 nm.). These measurements indicate that there is an initial increase in mitochondrial diameter at the time of their most active replication, probably without much increase in length, longitudinal sections of these organelles not being prevalent. This is followed by an increase in the length of these organelles (Fig. III.A.19b) probably without much volume change.

Lysosomes

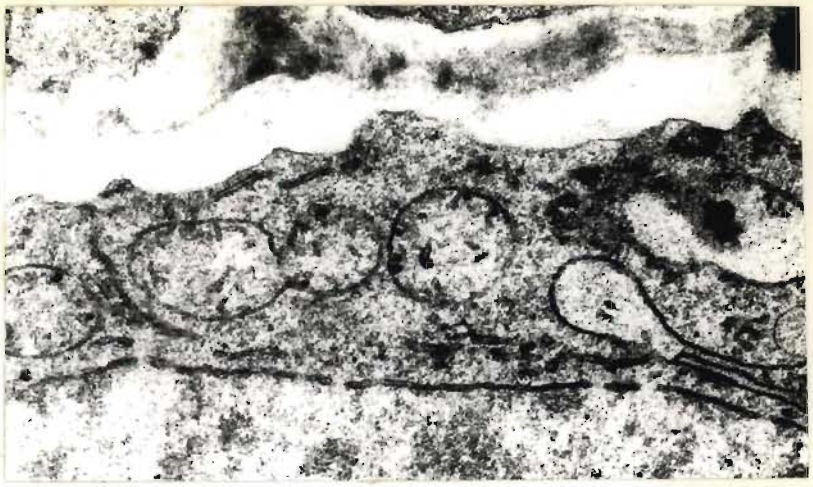
Lysosomes in the cap initials were generally both of the fully-formed first-phase variety (i.e. compact, electron-dense organelles in which the bounding membrane was closely applied to the surface of the dense content) and of the second-phase variety (in which lifting of the membrane from the dense content had occurred). However, in actively-dividing initials, second-phase lysosomes predominated. The second-phase lysosome might play some role, for example destruction of the spindle fibres, related to cell division.

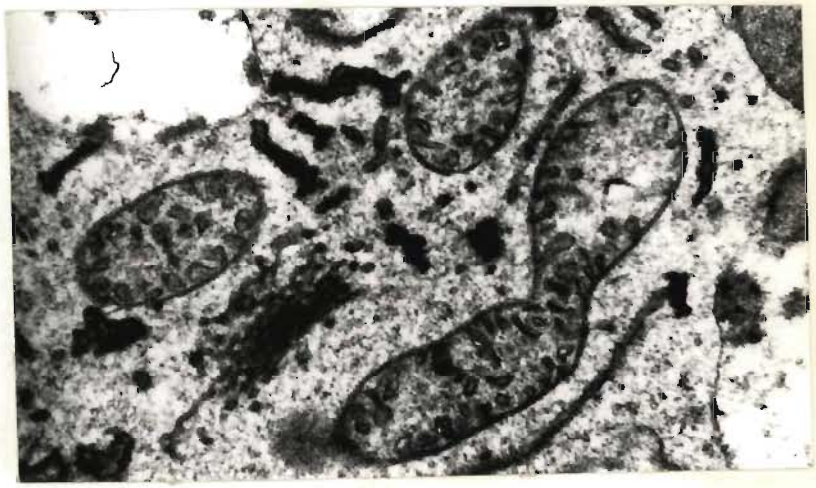
In cells of the zone of division virtually all the lysosomes were in the second developmental phase, and this was also the case in the differentiating cells (Fig. III.A.20a). In mature cells, once again second-phase lysosomes predominated.

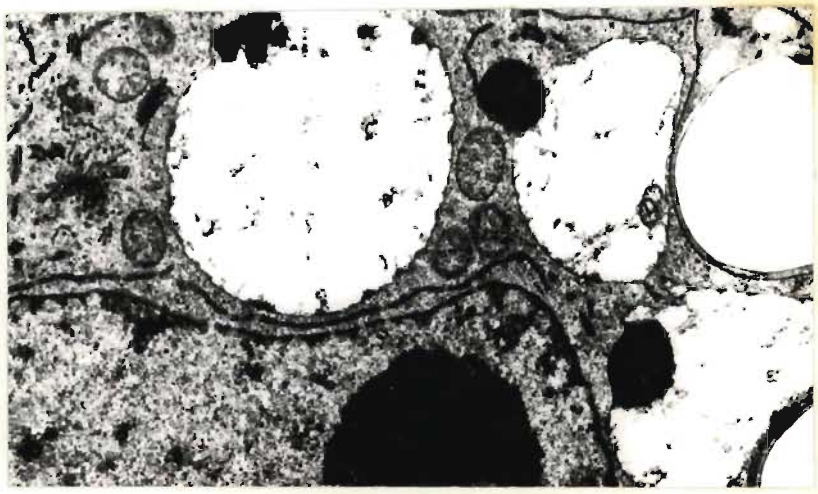
FIGURE III.A.19a. Illustrates a mitochondrion in an apparent state of division in a cap initial, 48 hours after the start of imbibition. (x 24 700).

FIGURE III.A.19b. Shows developed mitochondria in a mature cap cell, at the 48-hour germination stage. (x 24 050).

FIGURE III.A.20a. Illustrates second-phase lysosomes in a cell of the zone of division, 48 hours after the start of imbibition. (x 11 700).







The counts of lysosomes per unit area of cytoplasm (9, 10, 7 and 9 in initials, dividing, differentiating and mature cells, respectively) showed that these organelles were produced with increasing maturity of the cells.

Some degree of coalescence of lysosomes appeared to occur, especially in mature cells. The lysosomal vacuoles are thought to coalesce, but more than one core of dense content was encountered within them (Fig. III.A.20b).

In the mature cells the presence of secondary lysosomes containing closely-packed membranes resembling myelin figures suggests that these organelles act as organs of intracellular digestion (Fig. III.A.20c).

There is evidence for the incorporation of large dictyosomally-derived vesicles into second-phase lysosomes in mature cells (Fig. III.A.20d). Incorporated vesicles appeared surrounded by lysosomal content (Fig. III.A.20e), and were subsequently presumed to be broken down. Following this the interior of the lysosomal vacuole appeared to contain aggregations of very finely granular substance but no intact vesicles, as well as the typical darkly-staining lysosomal content which is somewhat dispersed (Fig. III.A.20f).

β-amylase and α-glucosidase occur within plant lysosomal vacuoles (Matile, 1968) and it is possible that hydrolysis of the polysaccharide dictyosomal secretion (Morré et al., 1967) occurs within these organelles. Whether or not the polysaccharide is hydrolysed, the concentration of particles within the vacuole must increase. Subsequent uptake of water could cccount for the very marked lysosomal swelling which occurs in root cap cells in the early stages of senescence (Fig. III.A.20g). Subsequent to this the lysosomal membrane appeared to undergo dissolution (Fig.III.A.20h, SA 4), and this is followed by general

FIGURE III.A.20b. Shows two cores of dense content within a lysosomal vacuole in a mature cap cell 48 hours after the start of imbibition. (x 22 750).

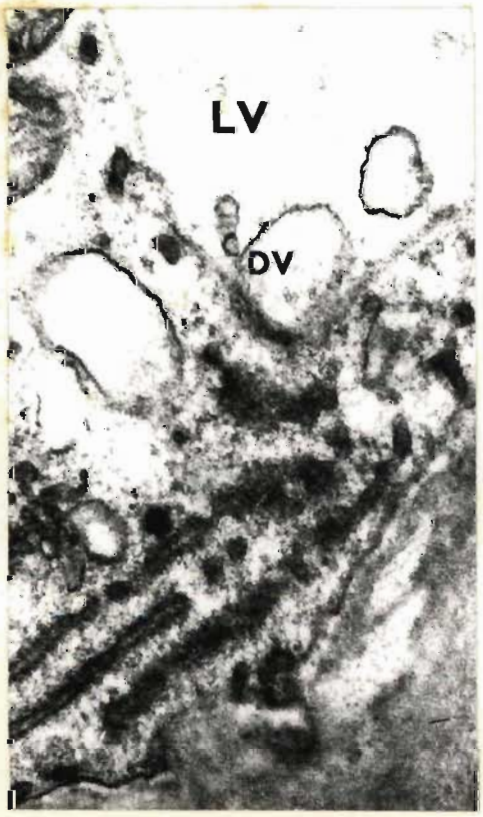
FIGURE III.A.20c. Shows close-packed membranes within a secondary lysosome in a mature cap cell at the 48-hour germination stage. (x 22 750).

FIGURE III.A.20d. Illustrates the incorporation of dictyosomally-derived vesicles into a lysosomal vacuole in a mature cap cell 48 hours after the start of imbibition. (x 44 800).

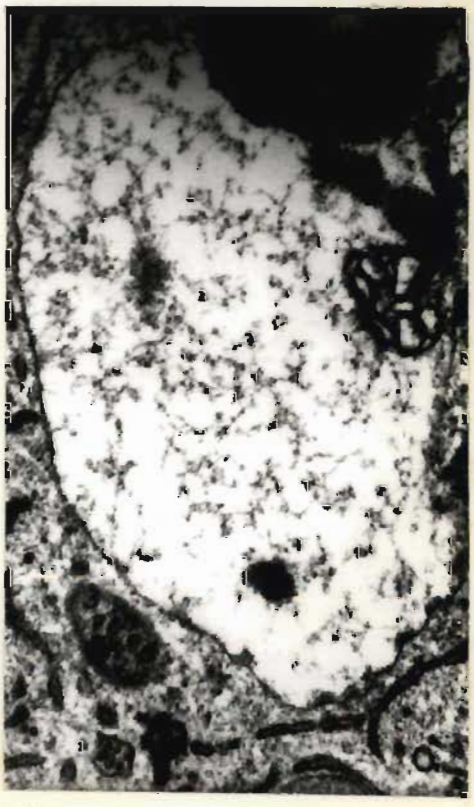
FIGURE III.A.20e. Shows vesicles, presumed to be dictyosomally-derived, within a second-phase lysosome in a mature cap cell at the 48-hour germination stage. (x 32 500).

b

c



d



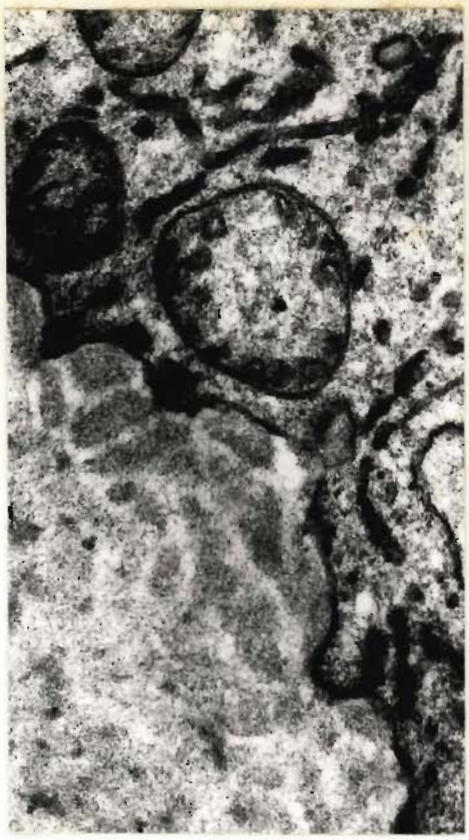
e

FIGURE III.A.20f. Illustrates the finely-granular inclusion material, thought to be of dictyosomal origin, within a lysosomal vacuole in a mature cap cell, 48 hours after the start of imbibition. (x 32 500).

FIGURE III.A.20g. Shows the markedly swollen lysosomal vacuoles in a cap cell which is in the early stages of senescence, 48 hours after the start of imbibition. (x 8 050).

FIGURE III.A.20h. Illustrates dissolution of the bounding membrane of a lysosome in an outermost cap cell which is in the early stages of senescence, at the 48-hour germination stage. (x 22 750).

FIGURE III.A.20i. Illustrates general cytoplasmic degradation in an outermost cap cell, at the 48-hour germination stage (Hickory King). (x 7 000).



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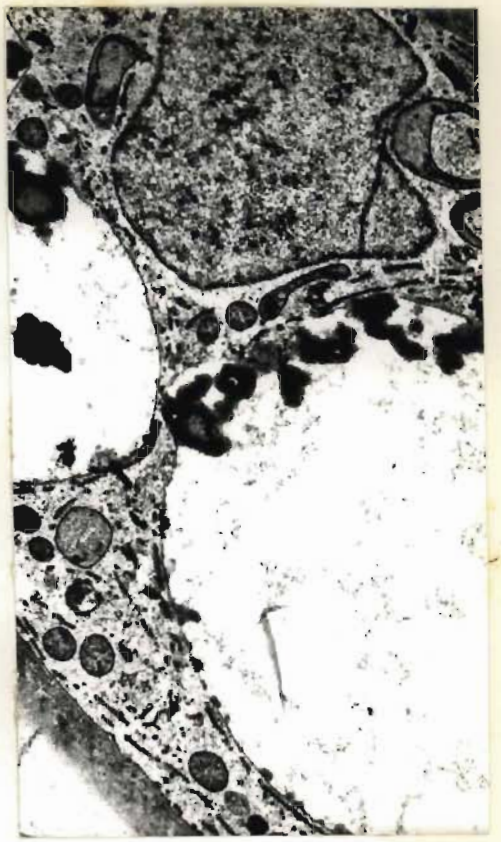

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i

degradative changes in the cytoplasm (Fig. III.A.20i - Hickory King).

Degeneration within these cells is ascribed to the action of hydrolases normally confined within the lysosome by the intact membrane (see below).

Because of the wide variation in size due to lysosomal swelling, and to coalescence of some of these organelles, measurement of actual dimensions are considered to serve very little purpose.

Plastids

The plastids in the cap initials contained little reserve starch, and were often encountered in an apparent state of division (Fig. III.A.21a). These organelles varied widely within the initials, ranging from relatively small proplastids to relatively large young amyloplasts (Fig. III.A.21b). There was evidence of increasing accumulation of reserve starch within plastids in dividing and differentiating cells in which cross-sectional diameter of the grains was 920 nm. and 1,300 nm. respectively (Fig. III.A.21c). The average cross-sectional diameter of the starch grains decreased to 670 nm. in plastids of mature cells, probably indicating utilisation of these reserves (Fig. III.A.21d).

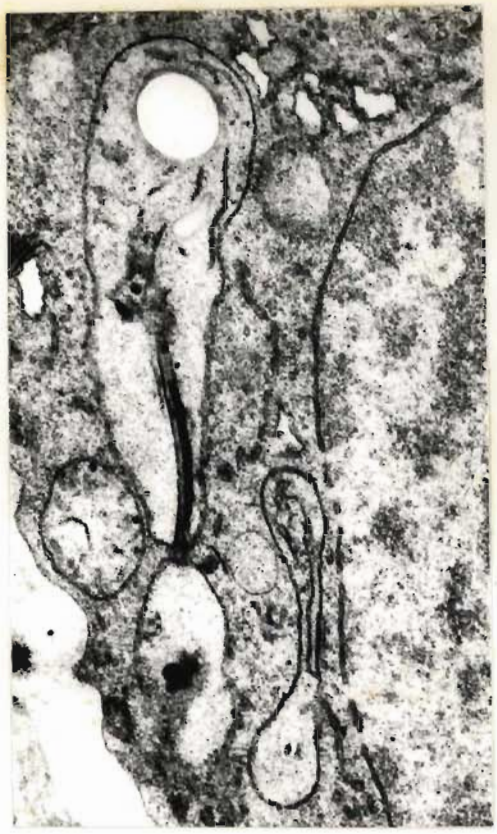
The number of plastids per unit area of cytoplasm fell from 7 in the initials, through 5 in both dividing and differentiating cells, to two in mature cells. These counts probably indicate that plastid division is largely restricted to the young cells of the root cap, and occurs at a far lower level in the older cells. This interpretation is in keeping with the results of Juniper and Clowes (1965) who concluded that most, if not all plastid division occurs in the dividing root cap cells of maize.

FIGURE III.A.21a. Illustrates apparent division of a plastid in a cap initial, at the 48-hour germination stage. (x 13 300).

FIGURE III.A.21b. Shows several plastids in a dividing cell, 48 hours after the start of imbibition. (x 15 200).

FIGURE III.A.21c. Illustrates accumulations of starch within plastids of a differentiating cap cell, 48 hours after the start of imbibition. (Postfixed in an osmium solution according to Procedure 6b). (x 10 350).

FIGURE III.A.21d. Shows plastids in a mature cap cell, at the 48-hour germination stage. (x 12 650).



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In those cells in the early stages of senescence (SA 4), the plastid count per unit area of cytoplasm remained at 2. In cells where senescence was more advanced (Hickory King) these organelles were swollen and disorganised.

Dictyosomes

Dictyosome counts per unit area of cytoplasm (6 in initials; 6 in dividing cells; 5 in differentiating cells and 9 in mature cells) had increased markedly when compared with counts in the corresponding cell zones in the earlier germination stages. In addition, the counts in the 48-hour material indicated active formation of dictyosomes, with increasing size and maturity of the cells.

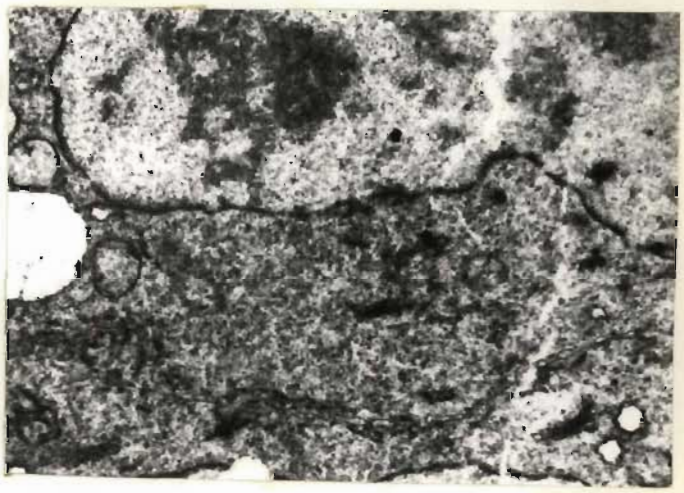
In the initials and the dividing cells, dictyosomes consisted of 4 cisternae on the average, were small (average longest cisternal length is 370 nm.) and had many small vesicles of average cross-sectional diameter 40 nm. associated with them (Fig. III.A.22a). The content of these vesicles was relatively electron-transparent at this stage. These dictyosomes were presumably concerned with synthesis of the new cell plate following division, and were situated in this region during plate formation (Fig. III.A.22b). In non-dividing initials the dictyosomes were mainly encountered near the peripheri of the cell.

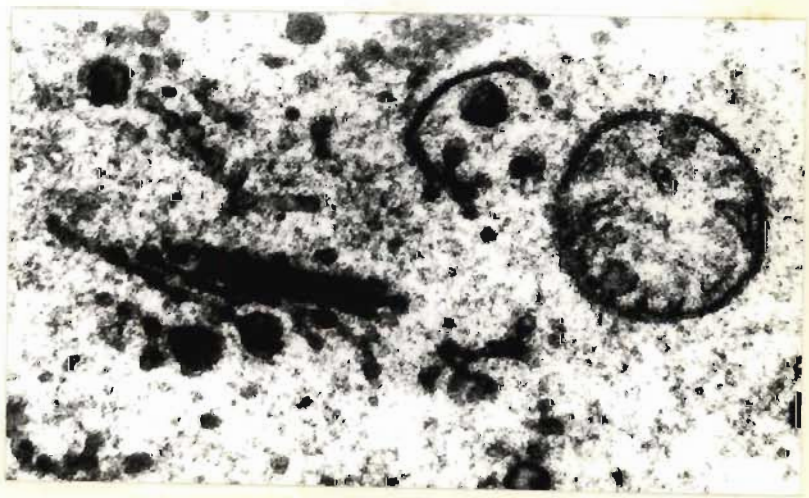
Dictyosomes in differentiating cells had increased in overal size, having an average of 6 cisternae and the average longest cistera was 650 nm. There was evidence of activity of the dictyosomes in these cells, with usual vesicle production. The vesicular content had altered in staining property, being electron-dense and the vesicles had an average cross-sectional diameter of 88 nm. (Fig. III.A.22c). The exact function of the dictyosomes in these cells is not known, but presumably the vesicular secretion plays some

FIGURE III.A.22a. Illustrates a dictyosome with associated small vesicles in a cap initial, at the 48-hour germination stage. (x 34 200).

FIGURE III.A.22b. Shows dictyosomes in the region of formation of a new cell plate in a dividing cell, 48 hours after the start of imbibition. (x 17 400).

FIGURE III.A.22c. Illustrates the nature of the dictyosomes and associated vesicles in a differentiating cap cell, 48 hours after the start of imbibition. (x 42 500).





active role in the differentiation process. It is noteworthy that the dictyosomes often occurred localised in regions of the cytoplasm where many mitochondria and lipid droplets were aggregated (Fig. III.A.22d).

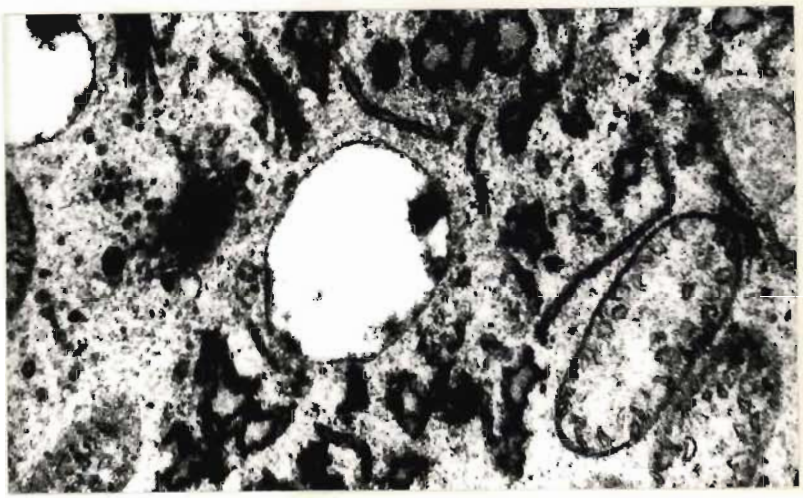
Dictyosomes in mature cells showed a striking degree of development. While the average number of cisternae per dictyosome remained at 6, the average cisternal length (of the longest cisterna per dictyosome) had increased to 850 nm. These organelles were in an apparently highly-active state within the mature cells, and proliferation of many relatively very large, electron-transparent vesicles occurred. (It was difficult to estimate the size of these vesicles, which were irregularly shaped) (Fig. III.A.22e). These are the hypersecretory dictyosomes of Mollenhauer et al. (1961). Figure III.A.22f illustrates the typical disposition of hypersecretory dictyosomes and vesicles in the cytoplasm of a mature cell.

The function of dictyosomal secretion in the mature cap cells appears to be at least two-fold. Firstly, some of these vesicles are incorporated into lysosomal vacuoles, where their secretion may play a part in osmotic activity (see above). Secondly, dictyosomal vesicles migrate to the plasma membrane discharging their content between the plasma membrane and cell wall. This results in a considerable accumulation of dictyosomal secretion between the plasma membrane and cell wall (Fig. III.A.22g).

It appears that this secretion eventually moves through the wall (of the cell in which it was produced) to accumulate in the middle lamella region (Fig. III.A.22h). This intramural accumulation only occurs between the cells of the most distal part of the mature zone, and those of the outermost cap layer. It is suggested that the secretion is instrumental in accelerating the separation of senescing cap cells. It

FIGURE III.A.22d. Shows the disposition of dictyosomes, lipid droplets and mitochondria in a differentiating cap cell, at the 48-hour germination stage. (x 22 750).

FIGURE III.A.22e. Illustrates a hypersecretory dictyosome in a mature cap cell, 48 hours after the start of imbibition. (x 44 800).



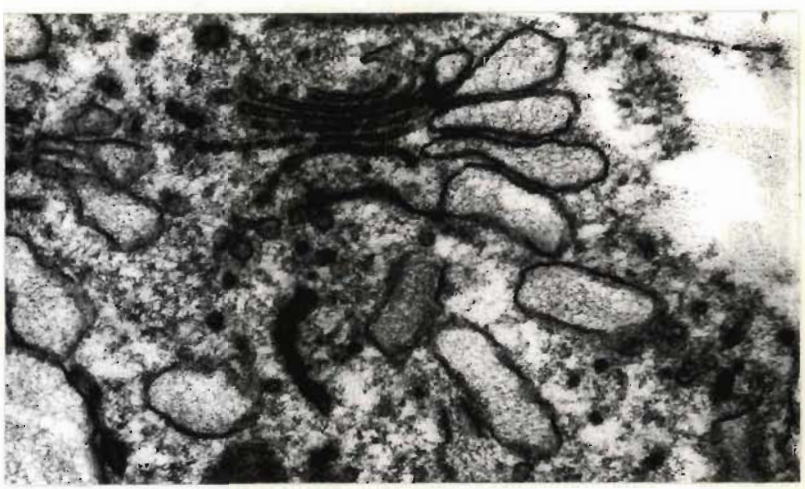
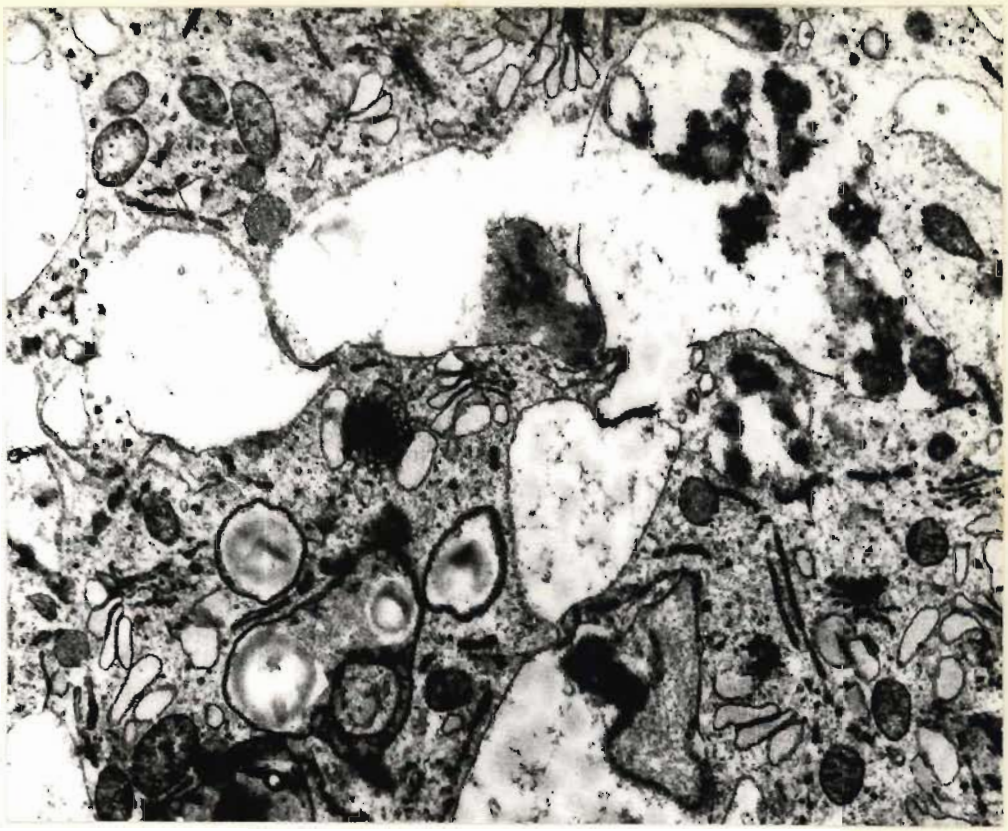


FIGURE III.A.22f. Shows the disposition of hyper-secretory dictyosomes and vesicles in the cytoplasm of a mature cell, 48 hours after the start of imbibition. (x 13 800).

FIGURE III.A.22g. Illustrates the accumulation of dictyosomal secretion between the plasma membrane and the cell wall. (x 7 000).

FIGURE III.A.22h. Shows an accumulation of dictyosomal secretion in the middle lamella region between a cell of the most distal layer of the mature zone, and an outermost cell, at the 48-hour germination stage. (x 13 300).



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is probably not the primary factor in the separation process, as partial loosening of the outermost cap cells occurred in the 24-hour material, where the secretion was not in evidence.

The dictyosomal count per unit area of cytoplasm decreased from 9 in mature cells, to 2 in cells in early stages of senescence suggesting that dissolution of these organelles accompanies the degradative changes marking the early stages of senescence. Hypersecretory dictyosomal vesicles were not in evidence in these cells. They were probably not being produced in these cells, and all those vesicles observed in mature cells had either been incorporated (as described above) or destroyed as part of the general senescent change.

There are no dictyosomal cisternae in senescent cells (Hickory King).

Endoplasmic Reticulum

The ER was very sparse, with short, scattered profiles in the cap initials (Fig. III.A.23a), and thus the developmental status of this organelle had not really altered when compared with the initials in imbibed and 24-hour embryos.

In cells of the zones of division and differentiation the ER was far more developed than in the initials and was orientated approximately parallel with the nucleus and cell peripheri (Figs. III.A.23b and 23c). However, in comparable cap cells of the 24-hour material, the ER appeared far more profuse and differently orientated. It is possible that the earlier development of this organelle was somehow involved with the reactivation of these formerly quiescent cells preparatory to their activity both in division and differentiation. Another possible explanation is that as the localised ER in these cells in the 24-hour material consists predominantly of short profiles, this could represent a site of formation of this organelle which is followed in

FIGURE III.A.23a. Illustrates the sparse, short ER profiles in a cap initial, 48 hours after the start of imbibition. (x 10 350).

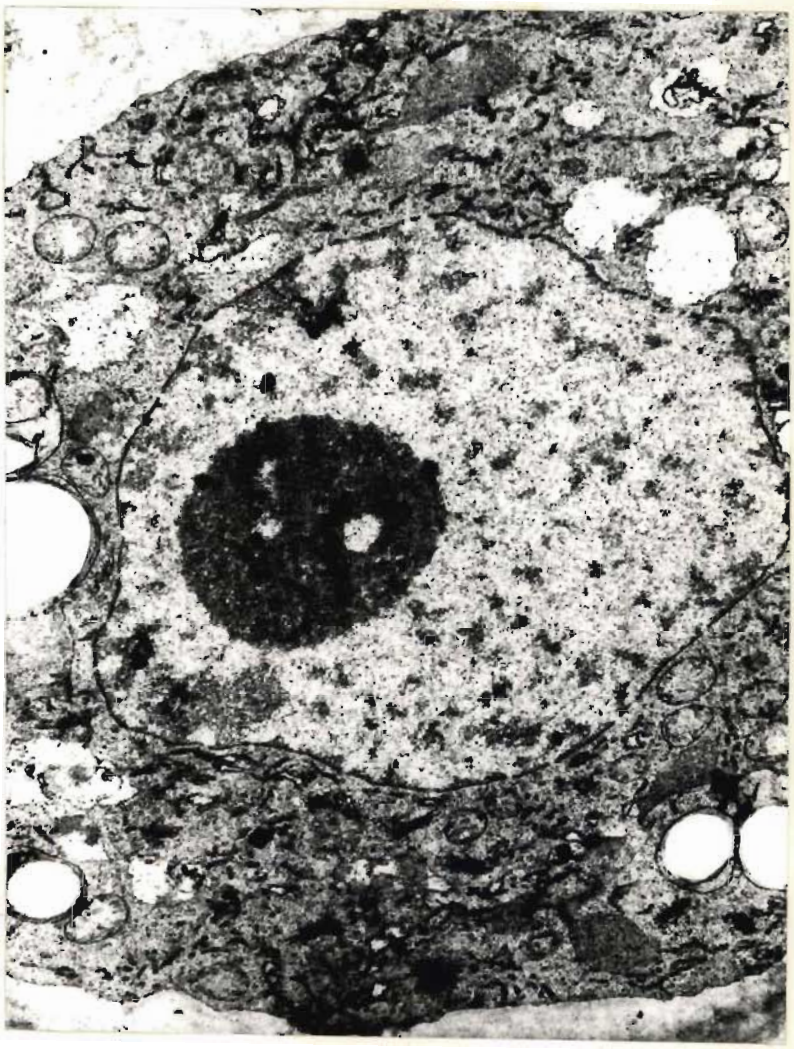
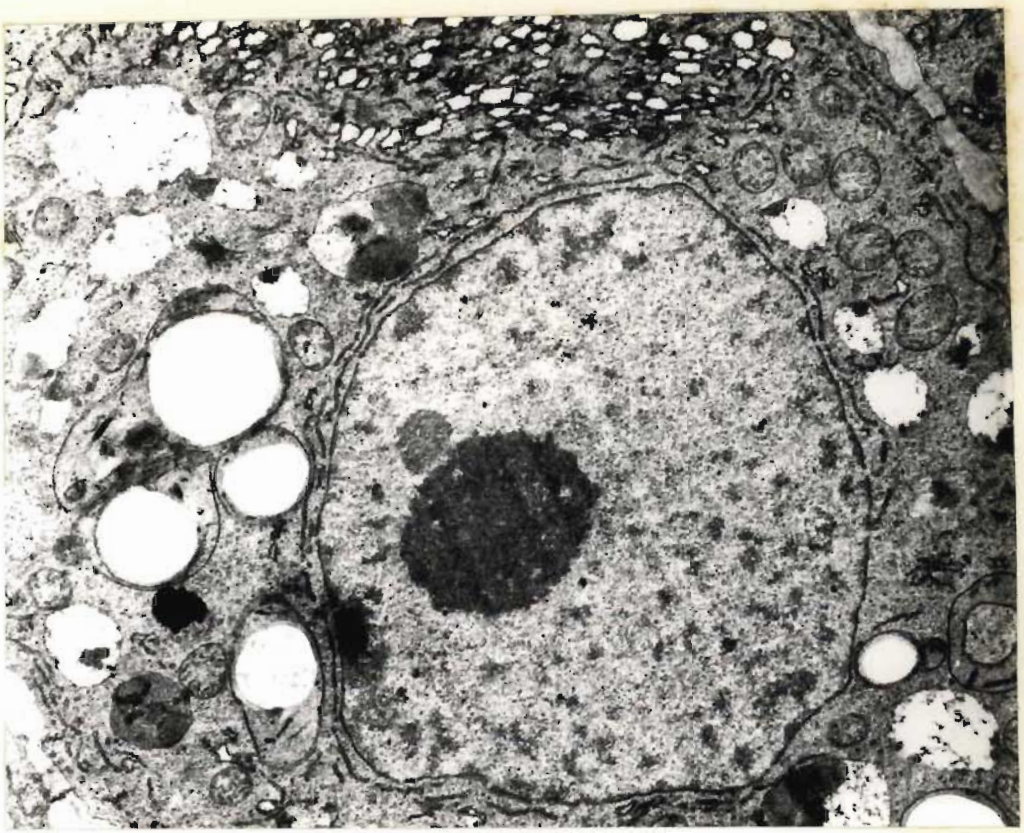
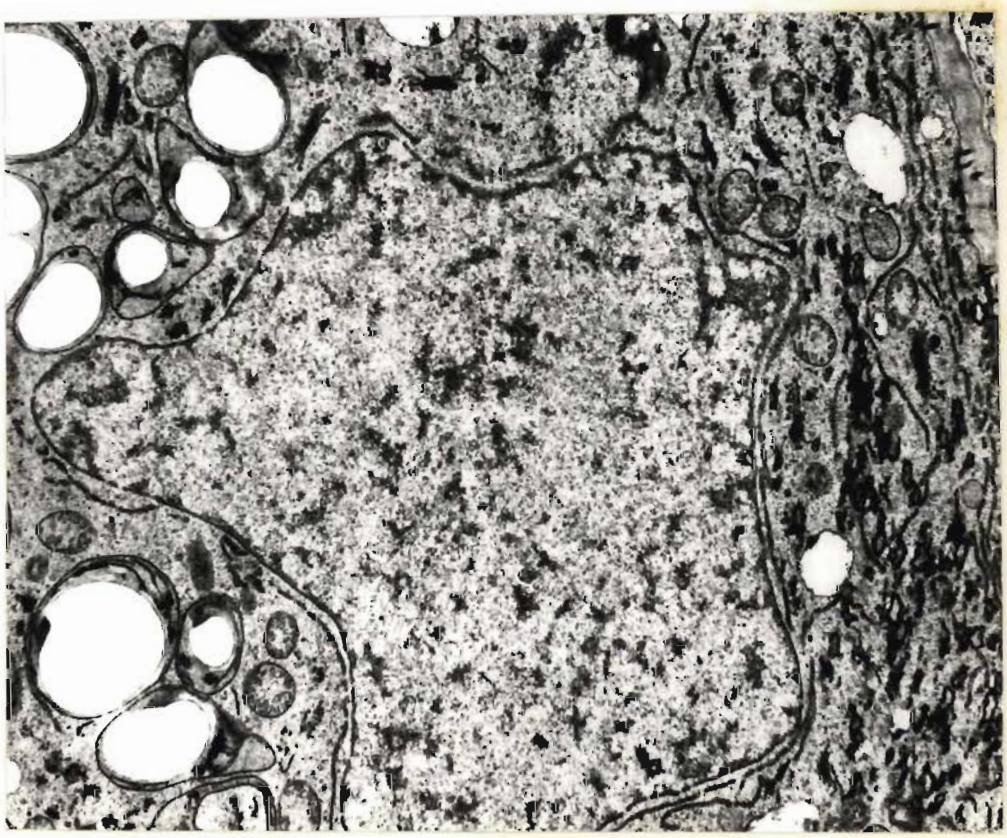


FIGURE III.A.23b. Illustrates the orientation of the ER profiles approximately parallel with the nuclear membrane and cell peripheri in a dividing cap cell at the 48-hour germination stage. (x 10 350).

FIGURE III.A.23c. Illustrates the disposition of the ER in a cell of the zone of differentiation, 48 hours after the start of imbibition. (x 9 000).





later germination stages, by elongation of the cisternae and their re-orientation in the cytoplasm.

The ER in mature cells occurred orientated parallel with and between the cell boundaries and the nuclear envelope. This organelle appeared to be more developed in these cells than in those of the zone of differentiation. In mature cells there were more profiles which were orientated parallel with each other and with the cell peripheri (Fig. III.A.23d).

While fragments of the ER still occurred in cells in the early stages of senescence (SA 4), this organelle appeared to have disappeared from the cytoplasm of senescing cells, once the degradative changes were more advanced (Hickory King) (Fig. III.A.23e).

Ribosomes

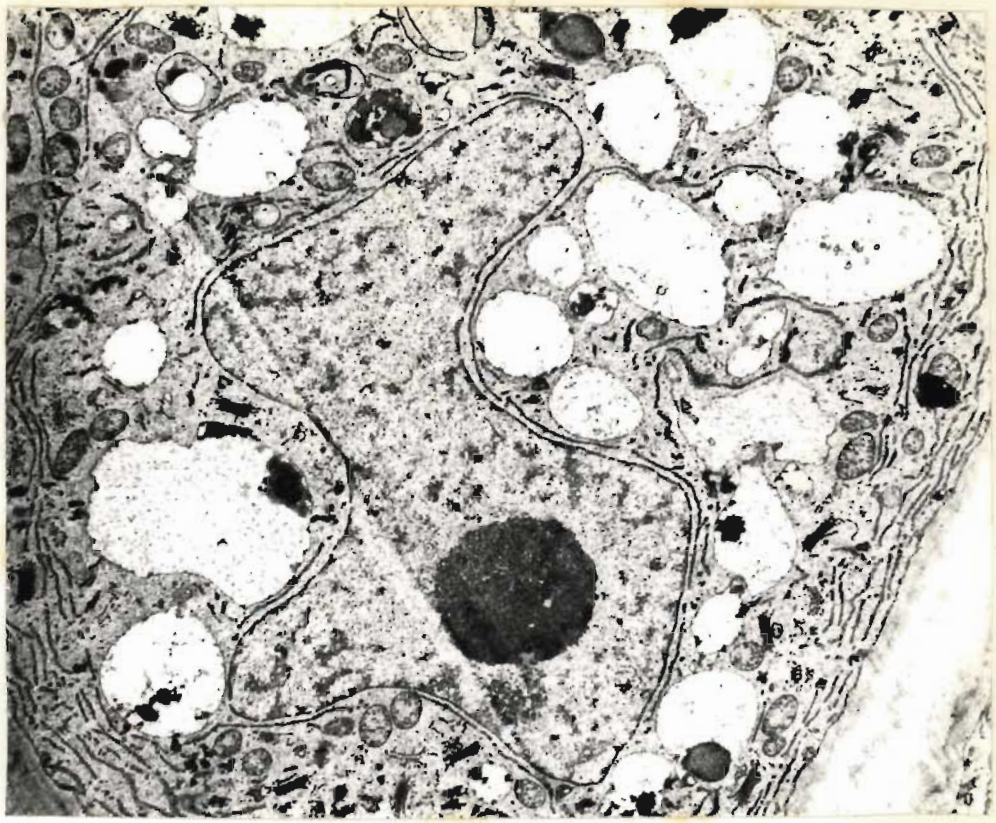
Ribosomes occurred aggregated as polysomes in the cell types of the root cap of unaged material 48 hours after the start of imbibition (Fig. III.A.24a). Polysomes also occurred in the cells which were in the early stages of senescence. However, only monosomes occurred in cells which were in an advanced state of senescent change (Hickory King) possibly indicating that the m-RNA responsible for polysome formation had been degraded (Fig. III.A.24b).

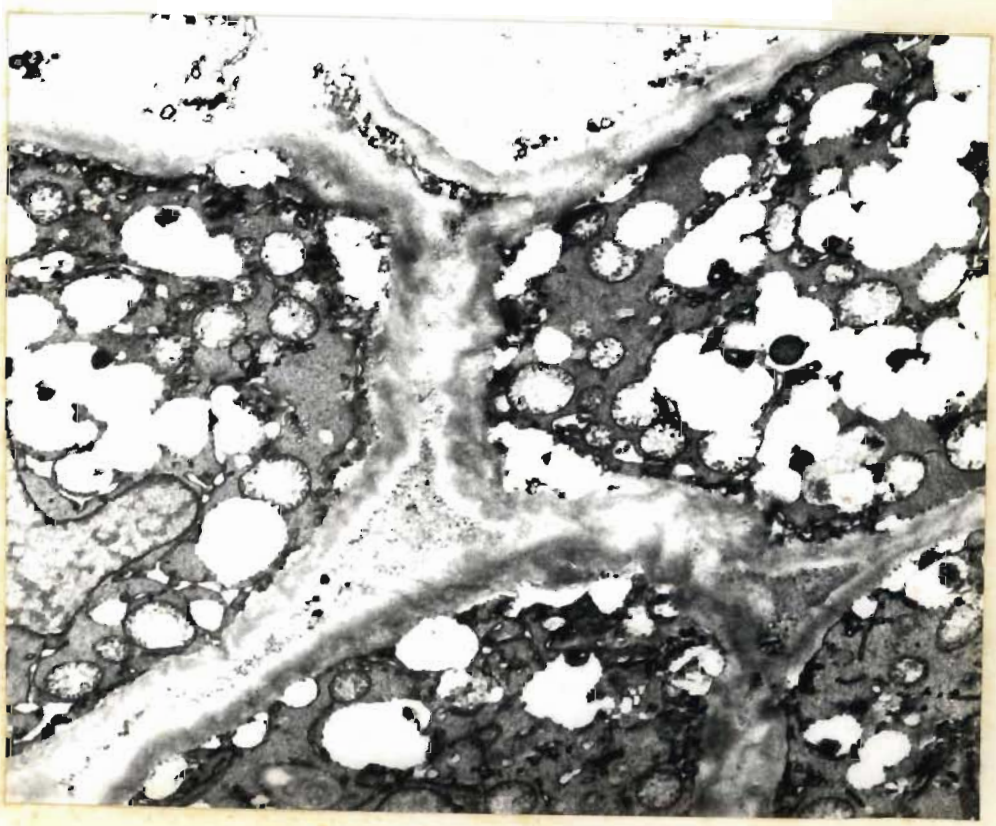
Lipid Droplets

There was evidence of utilisation of lipid (presumably continued from the earlier germination stages), in initials and cells of the zone of division. In these cells, the lipid droplets occurred aggregated in the cytoplasm, and the reserves were presumably being utilised for the elaboration of the membranes in the formation and replication of organelles (Fig. III.A.25a). The lipid droplets changed in staining properties and size, assuming a dark and

FIGURE III.A.23d. Illustrates the disposition of ER profiles in an ultra-thin section of a mature root cap cell, at the 48-hour germination stage. (x 6 300).

FIGURE III.A.23e. Illustrates that the ER has virtually disappeared from the cytoplasm of a senescing cap cell (Hickory King), 48 hours after the start of imbibition. (x 12 650).





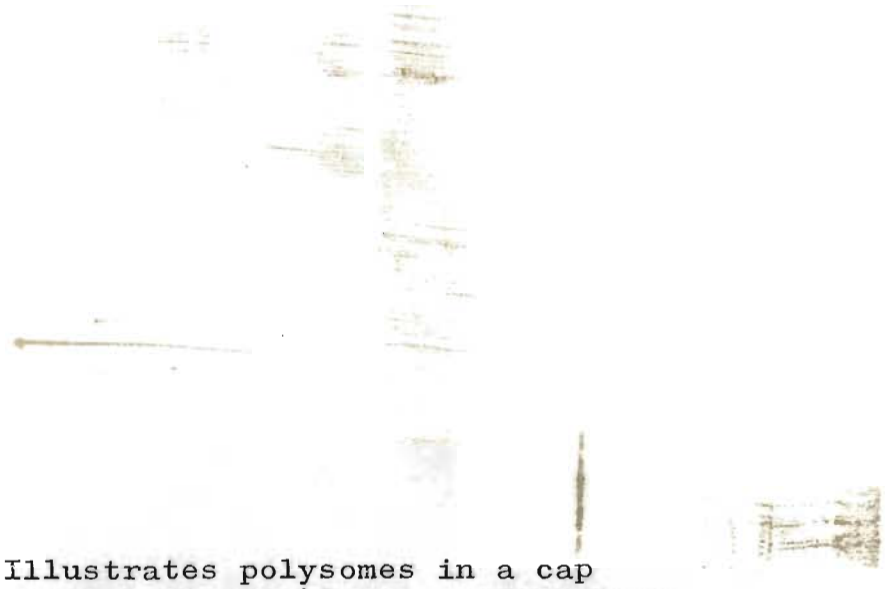
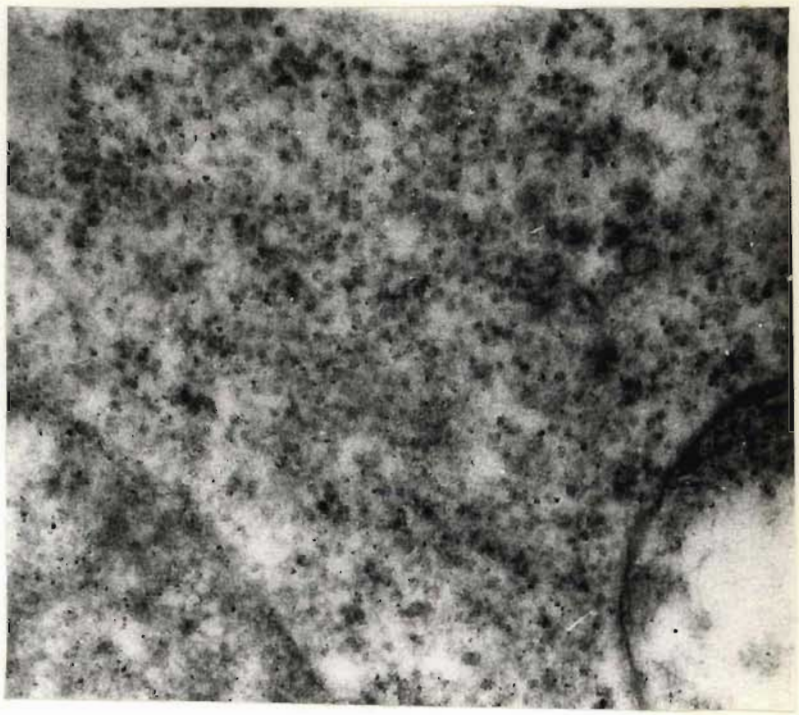


FIGURE III.A.24a. Illustrates polysomes in a cap initial at the 48-hour germination stage. The material was postfixed in an osmium solution according to Procedure 6b. (x 66 150).

FIGURE III.A.24b. Shows monosomes in an outermost cap cell which is in an advanced stage of senescence (Hickory King). The material was postfixed in an osmium solution according to Procedure 6a. (x 46 750).



somewhat shrunken appearance, with increasing maturity of the cells (Fig. III.A.25b). This is interpreted as depletion of the lipid reserves. The fact that these droplets became electron-dense indicates some biochemical change within them, suggesting a non-lipid product, as lipid is normally completely oxidised by potassium permanganate when utilised as a stain.

Wall

The wall separating the root proper from the cap was typically thick, somewhat irregular and was more electron-dense than the relatively young walls of the initials and dividing cells (Fig. III.A.26a).

Electron-density and relative thickness of the walls increased with increasing maturity of the cap cells until, in the zone of mature cells, this became a thick and relatively electron-dense structure (Fig. III.A.26b).

The walls of the cells of the distal part of the mature zone showed a change, the middle lamella region becoming relatively electron-transparent (Fig. III.A.26c). This is interpreted as being preparatory to the separation process, in which apparent disintegration of the middle lamella region occurs. The separation process is probably accelerated by accumulation of dictyosomal secretion in this area (see above).

FIGURE III.A.25a. Illustrates the disposition of lipid droplets in the cytoplasm of a cell of the zone of division, 48 hours after the start of imbibition. (x 10 150).

FIGURE III.A.25b. Shows the changed appearance and staining reaction to potassium permanganate of lipid droplets in a mature cap cell, at the 48-hour germination stage. (x 29 250).

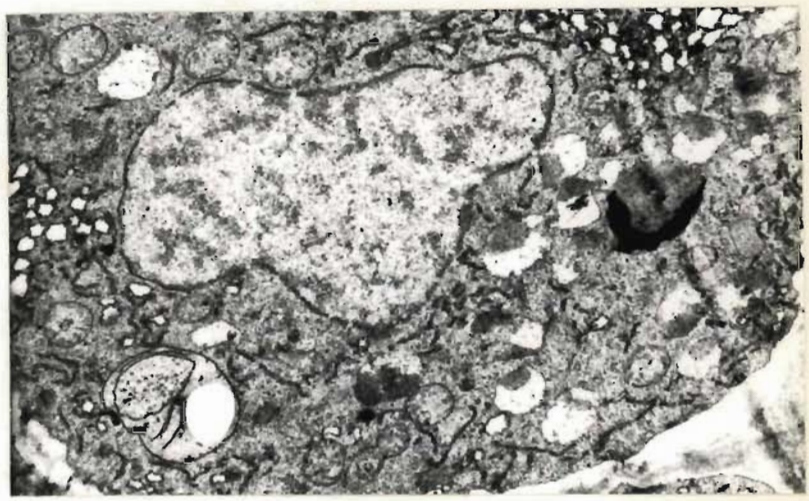
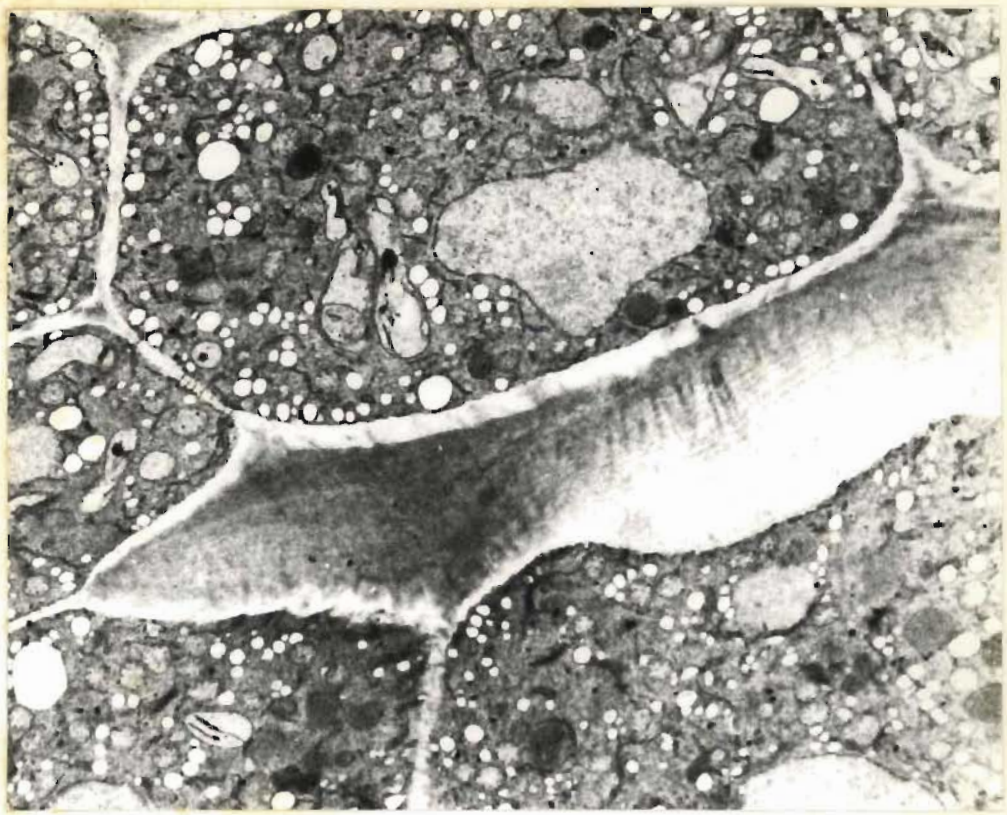


FIGURE III.A.26a. Illustrates the wall separating the cap from the root apex, 48 hours after the start of imbibition (x 7 000).

FIGURE III.A.26b. Shows the relatively thick, electron-dense wall between two cells in the mid-region of the mature zone, 48 hours after the start of imbibition. (x 32 500).

FIGURE III.A.26c. Illustrates a change in the nature of the middle lamella region of the wall, typical of the distal portion of the mature zone, 48 hours after the start of imbibition. (x 22 750).



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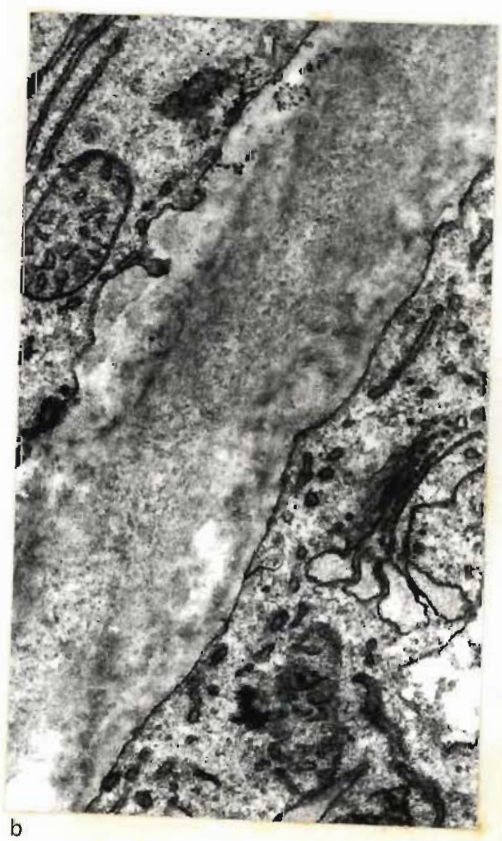

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B. HISTOCHEMISTRY OF UNAGED MATERIAL

Lysosomes are best defined as subcellular organelles, bounded by a single membrane and having hydrolases with a common pH of about 5, associated with them (Gahan, 1967). Thus it was decided to test for the activity of acid phosphatase (a hydrolase normally associated with lysosomes) in these organelles, and in the cytoplasm of the cap cells.

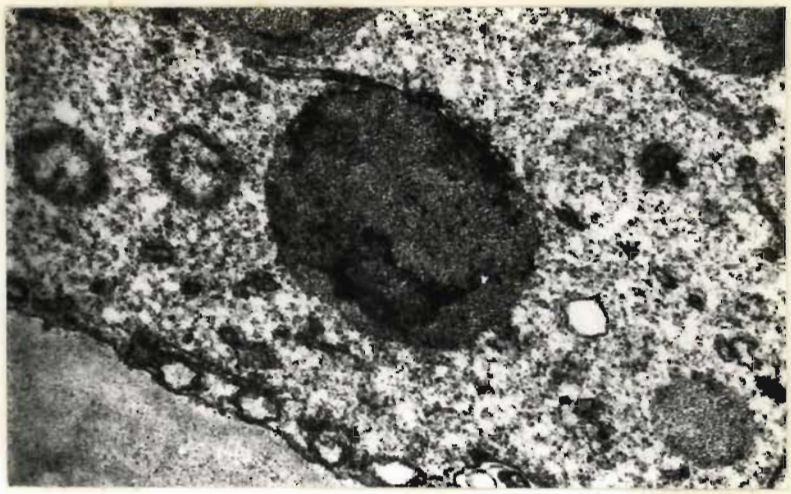
Tests for acid phosphatase activity were carried out on unaged material at the imbibed, 24-hour (12 hours imbibition followed by 12 hours on moist cellulose wadding) and 48-hour (12 hours imbibition followed by 36 hours on moist cellulose wadding) germination stages.

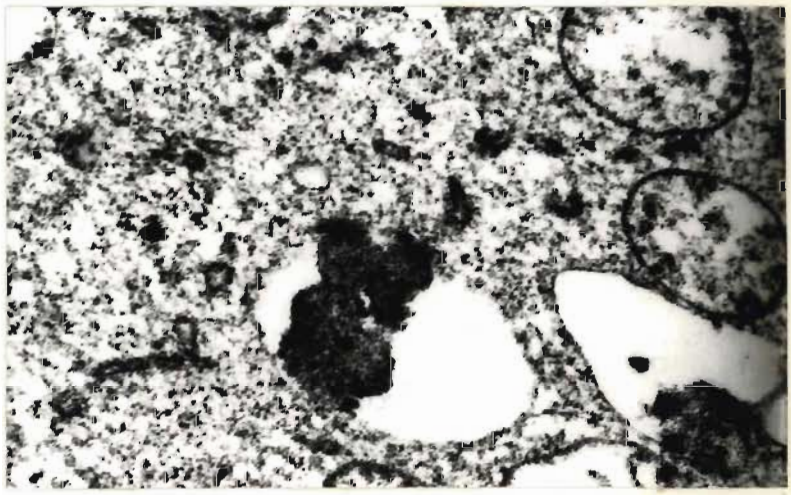
B.1. IMBIBED MATERIAL

Use of the Gomori method for acid phosphatase localisation illustrated that there is no activity of the enzyme within the lysosomes until these organelles are fully formed at the end of their first developmental phase. At this stage, which occurred only in the cells of the mature zone, there is an indication that this enzyme first becomes active (Fig. III.B.1a). This activity, which is localised at points in the lysosomes, also occurred in the second-phase lysosomes encountered in the outermost cells (Fig. III.B.1b). In addition, there is a small amount of acid phosphatase activity in the cytoplasm of the outermost cells (Fig. III.B.1c). These cells were in an early stage of senescence, and the activity of this (and presumably other) hydrolase(s) is suggested to be instrumental in the further degradation which is evident in these cells in the 24-hour material. No acid phosphatase activity was seen in the control material, where sodium fluoride was used as an enzyme inhibitor (Fig. III.B.1d and 1e).

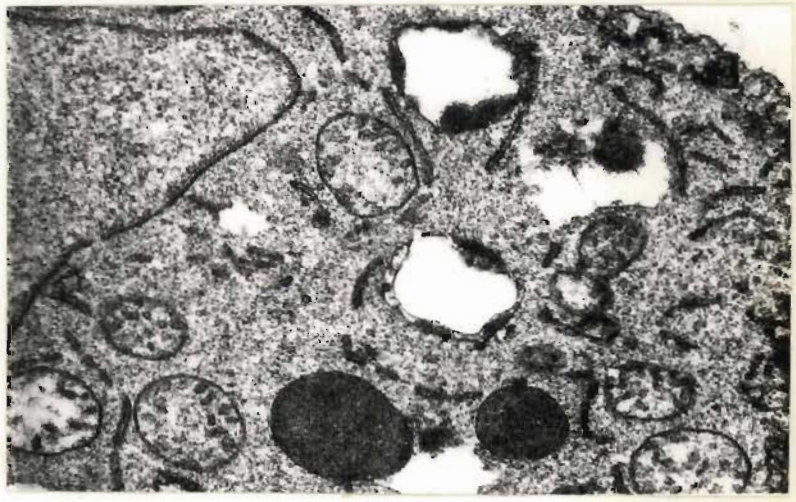
FIGURES III.B.1a & 1b. Illustrate that there is a small amount of acid phosphatase activity in first- and second-phase lysosomes respectively, 12 hours after the start of imbibition. (x 22 750).

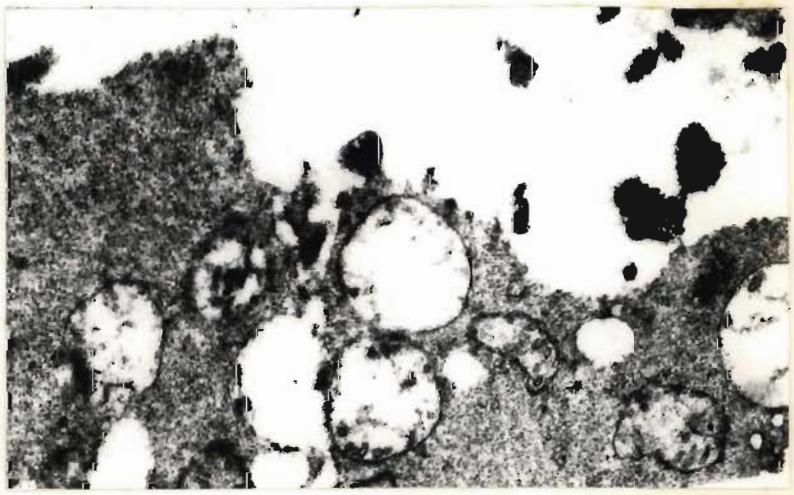
FIGURE III.B.1c. Illustrates acid phosphatase activity in the cytoplasm of an outermost cap cell, at the 12-hour germination stage. (x 13 300).





FIGURES III.B.1d & 1e. Illustrate that no positive reaction for acid phosphatase activity occurs either within the lysosomes in mature cap cells, or in the cytoplasm of outermost cells, when sodium fluoride was used as an enzyme inhibitor. The material illustrated in these micrographs was fixed a 12-hour period of imbibition. (1d x 17 000; 1e x 24 700).





It is suggested that hydrolases may occur in an inactive form within the fully-formed first-phase and second-phase lysosomes of mature embryos in quiescent seeds, becoming active during germination as acid phosphatase actively occurs in fully-formed first-phase and second-phase lysosomes in cap cells of all the zones at later germination stages.

B.2. 24-HOUR MATERIAL

Lysosomes in their first developmental phase (prior to the fully formed first-phase lysosomes) did not show acid phosphatase activity. However, there was evidence of greatly-increased activity of this enzyme in the fully formed first-phase lysosomes, and in these organelles in their second developmental phase, in the root cap cells of 24-hour embryos (Fig. III.B.2a). The outermost root cap cells were in a relatively advanced state of degeneration in the 24-hour material, and acid phosphatase activity was found to occur throughout the cytoplasm (Fig. III.B.2b).

The control material in which sodium fluoride was used as an enzyme inhibitor did not show a positive reaction for the activity of this enzyme in any of the lysosomes (Fig. III.B.2c) or in the cytoplasm of the outermost cells.

B.3. 48-HOUR MATERIAL

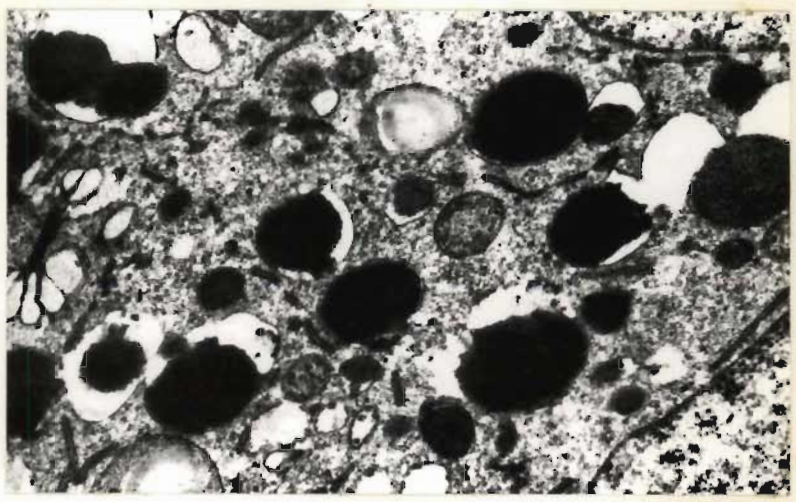
Using the Gomori method, there was a strong positive reaction for acid phosphatase activity in fully formed first-phase and second-phase lysosomes in the root cap cells of the 48-hour embryos (Figs. III.B.3a and 3b). No activity of this enzyme occurred in the control material in which sodium fluoride was used as an enzyme inhibitor (Fig. III.B.3c).

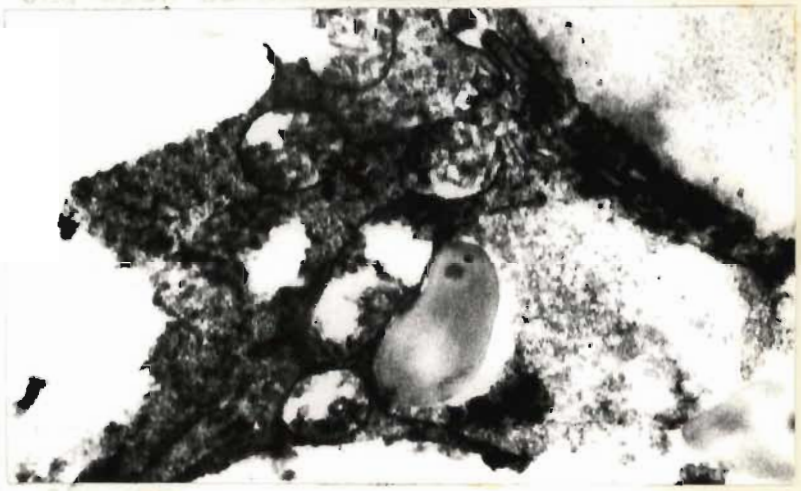
Cells in the early stages of senescence (encountered

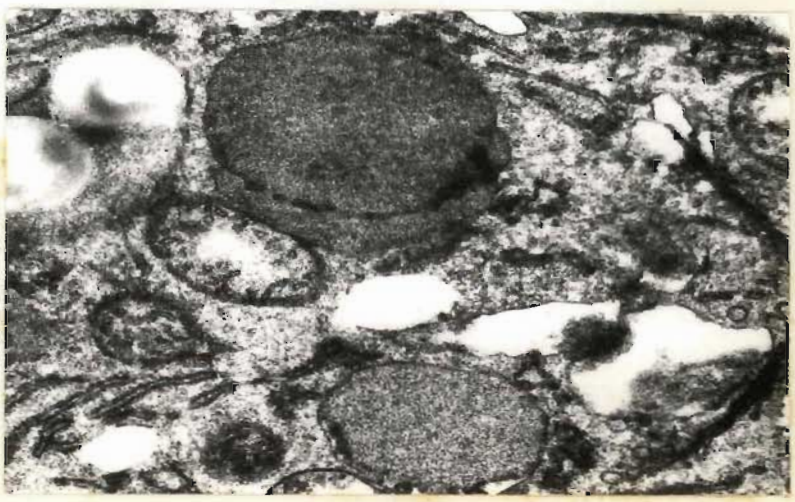
FIGURE III.B.2a. Illustrates acid phosphatase activity within intact first-phase and second-phase lysosomes, 24 hours after the start of imbibition. (x 13 300).

FIGURE III.B.2b. Illustrates that acid phosphatase activity occurs in the cytoplasm of outermost cap cells at the 24-hour germination stage. (x 32 500).

FIGURE III.B.2c. Shows that no acid phosphatase activity occurs in the control material where sodium fluoride was used as an enzyme inhibitor. (x 22 750).

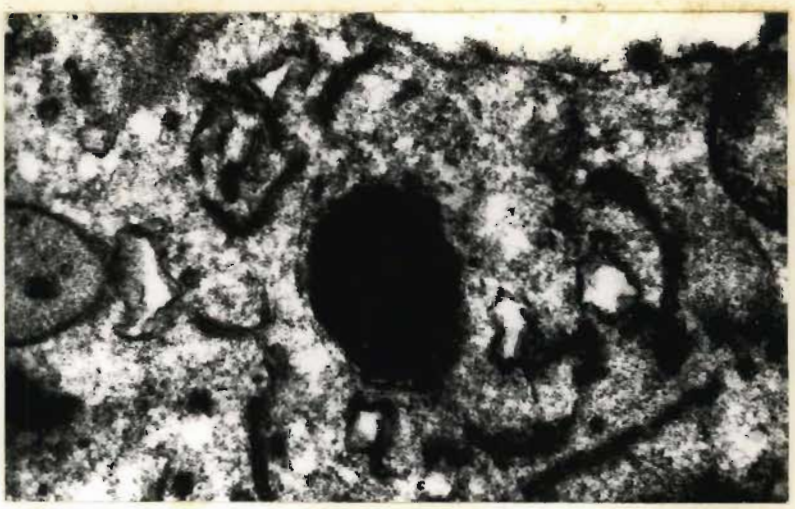


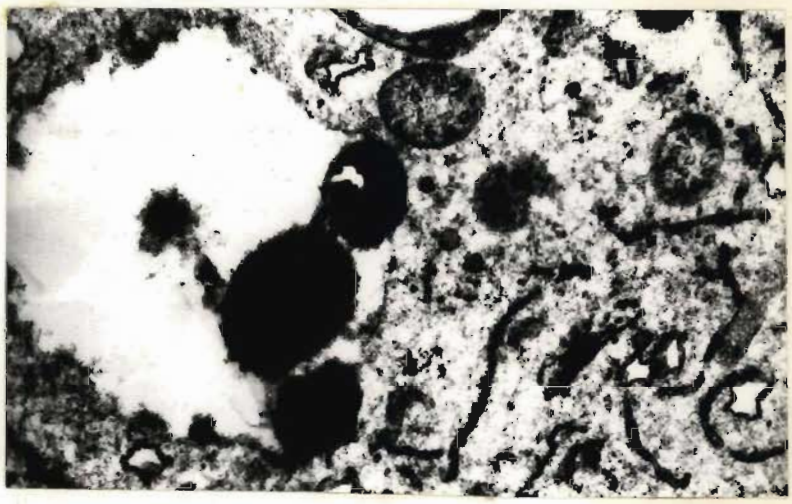


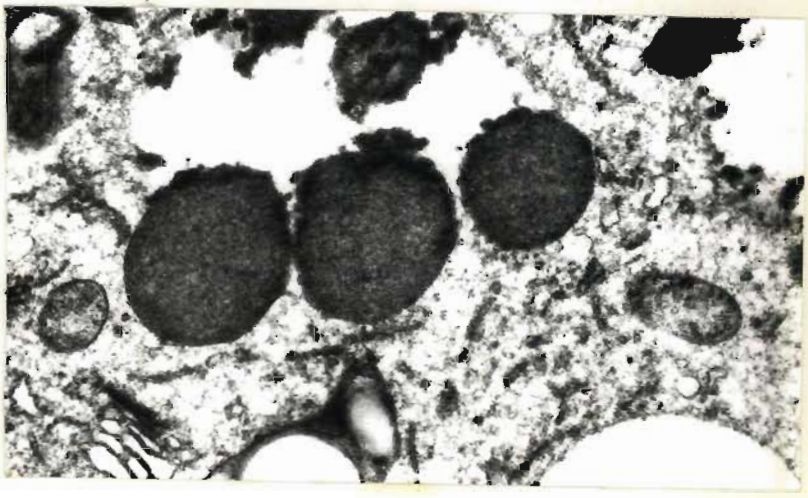


FIGURES III.B.3a & 3b. Illustrate that acid phosphatase activity occurs within first- and second-phase lysosomes respectively, at the 48-hour germination stage.
(3a x 29 750: 3b x 18 900).

FIGURE III.B.3c. Shows that no reaction for the activity of acid phosphatase occurs within lysosomes in the control material where sodium fluoride was used as an enzyme inhibitor. The material was fixed at the 48-hour germination stage.
(x 17 000).





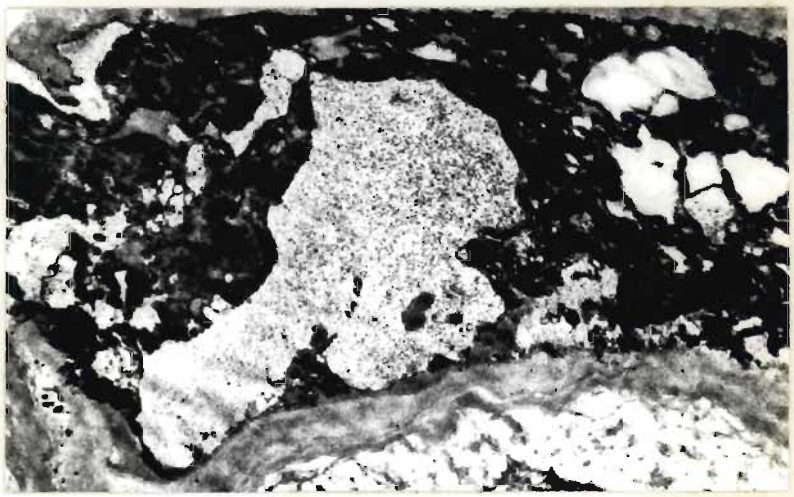


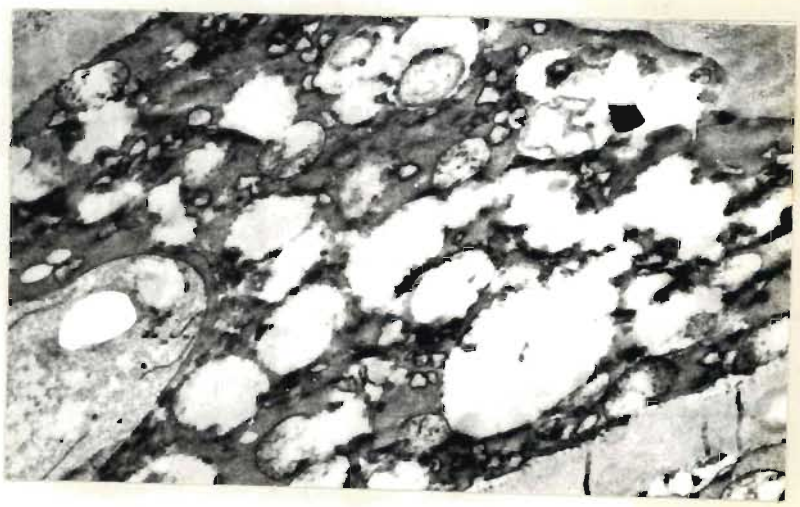
in the outermost cap layer of the hybrid, SA 4) showed localised reaction for acid phosphatase in the cytoplasm. However, in the outermost cap cells where the degradative processes were advanced a diffuse positive reaction for the activity of this enzyme occurred throughout the cytoplasm (Hickory King) (Fig. III.B.3d). The control material, in which enzyme activity had been inhibited, gave a negative result (Fig. III.B.3e).

Note that during preliminary investigations on Zea Mays L. var. Hickory King, the present writer demonstrated that esterase first became active in the lysosomes at the beginning of their second developmental phase, in the cap cells of the 48-hour germination stage. A progressive increase in the activity of this enzyme was demonstrated, which reached a maximum in the outermost cap cells (Berjak, 1968).

FIGURE III.B.3d. Illustrates a diffuse reaction for the activity of acid phosphatase in the cytoplasm of an outermost cap cell (Hickory King), at the 48-hour germination stage. (x 9 000).

FIGURE III.B.3e. Illustrates an outermost cap cell of the control material where sodium fluoride was used as an enzyme inhibitor in the Gomori incubation medium, at the 48-hour germination stage (Hickory King). (x 12 650).





C. AUTORADIOGRAPHY OF UNAGED MATERIAL.

Autoradiographic investigations were undertaken in order to follow the synthesis of nucleic acids and proteins. All the autoradiographic studies at both the light and electron microscope levels were carried out on the hybrid SA 4 at the 48-hour germination stage.

C.1. ^{3}H-Thymidine Incorporation (light microscopy)

^{3}H-thymidine was incorporated into nuclei of the initials and dividing cells only during a 4-hour incubation period showing DNA replication to be confined to these cap zones (Fig. III.C.1). Cells showing labelled nuclei are expressed as a percentage of the total number of cells in the root cap. The mean percentage of labelled nuclei per root cap was found to be 6.0 for unaged material 48 hours after the start of imbibition.

C.2. ^{3}H-Uridine Incoporation

Light Microscopy

^{3}H-uridine was incorporated into the nuclei and cytoplasm of all the zones of the root cap, during a 2-hour incubation period in the isotope solution (Fig. III.C.2a). Silver grains were counted over 10 cells in each of the cap zones. Incorporation per cell was least in the initials (an average of 61 grains per cell), and progressively increased to the to the zone of mature cells (164 grains per cell). Senescing cells of the outermost cap layer showed a lower incorporation of ^{3}H-uridine (74 grains per cell) than those of the mature zone. Figure III.C.2b illustrates the comparative incorporation of ^{3}H- uridine throughout the root cap of unaged material at the 48-hour germination stage.

FIGURE III.C.1. Illustrates that the incorporation of ^{3}H-thymidine is limited to nuclei in the meristematic cap zones in unaged material. (x 252).

FIGURE III.C.2a. Illustrates silver grains over nuclei and cytoplasm, showing incorporation of ^{3}H-uridine in cap cells of the zone of division, in unaged material. (x 2 520).

1

2. a

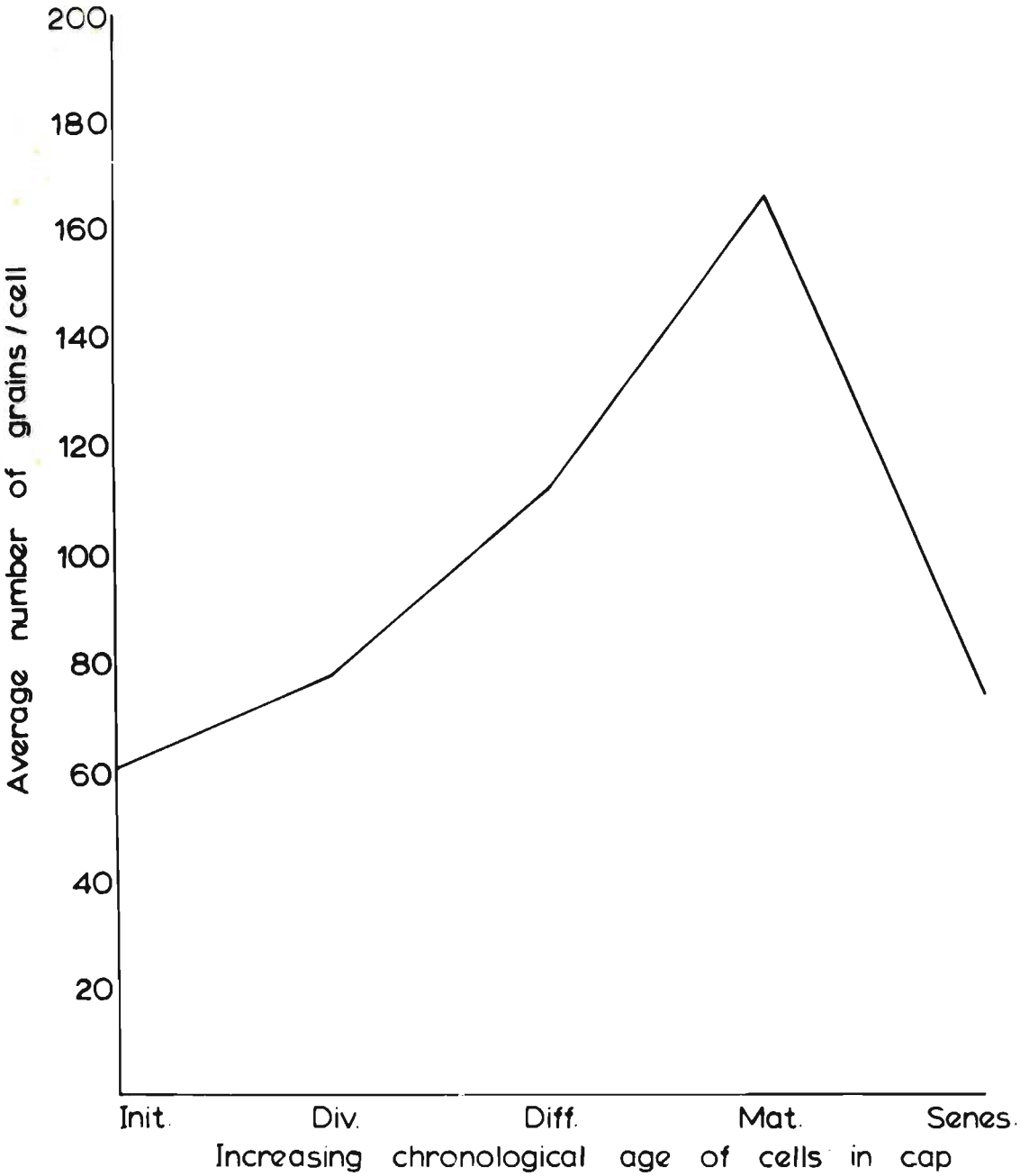


Fig. III. C. 2b Illustrating the incorporation of ^{3}H-uridine in the root cap of unaged material, at the 48-hour germination stage.

Silver grains were also counted over the nucleoli, nucleoplasm and cytoplasm in each of the cap cell zones (Fig. III.C.2c). It was found that there was little variation in the counts over the nucleoli from zone to zone in the cap, with the exception of the outermost, senescing cells where the count was lower. Counts of silver grains over the nucleoplasm and cytoplasm increased, the latter greater than the former, with increasing maturity of the cells. However, counts of silver grains over both nucleoplasm and cytoplasm decreased markedly for the outermost, senescing cap cells.

Electron Microscopy

Electron microscopic observations illustrate that the cytoplasmic incorporation of ^{3}H-uridine was not confined to any one organelle, but was general in the cytoplasm (Fig. III.C.2d).

C.3. ^{3}H-Leucine Incorporation

Light Microscopy.

^{3}H-leucine was incorporated into cells of all the zones of the root cap in unaged material during a 1½-hour incubation period, 48 hours after the start of imbibition. Figure III.C.3a shows that the incorporation was least in the initials, and increased with increasing maturity of the cap cells, to reach a maximum in the outermost, senescing cells. Figures III.C.3b and 3c illustrate comparative incorporation of the label in meristematic (intitials and dividing) and mature cells, repsectively.

Counts of silver grains over 10 cells in each of the cap regions bear out the above impression. An average of 37 grains was counted over the initials, and the count over the outermost, senescing cells was 296. Figure III.C.3d

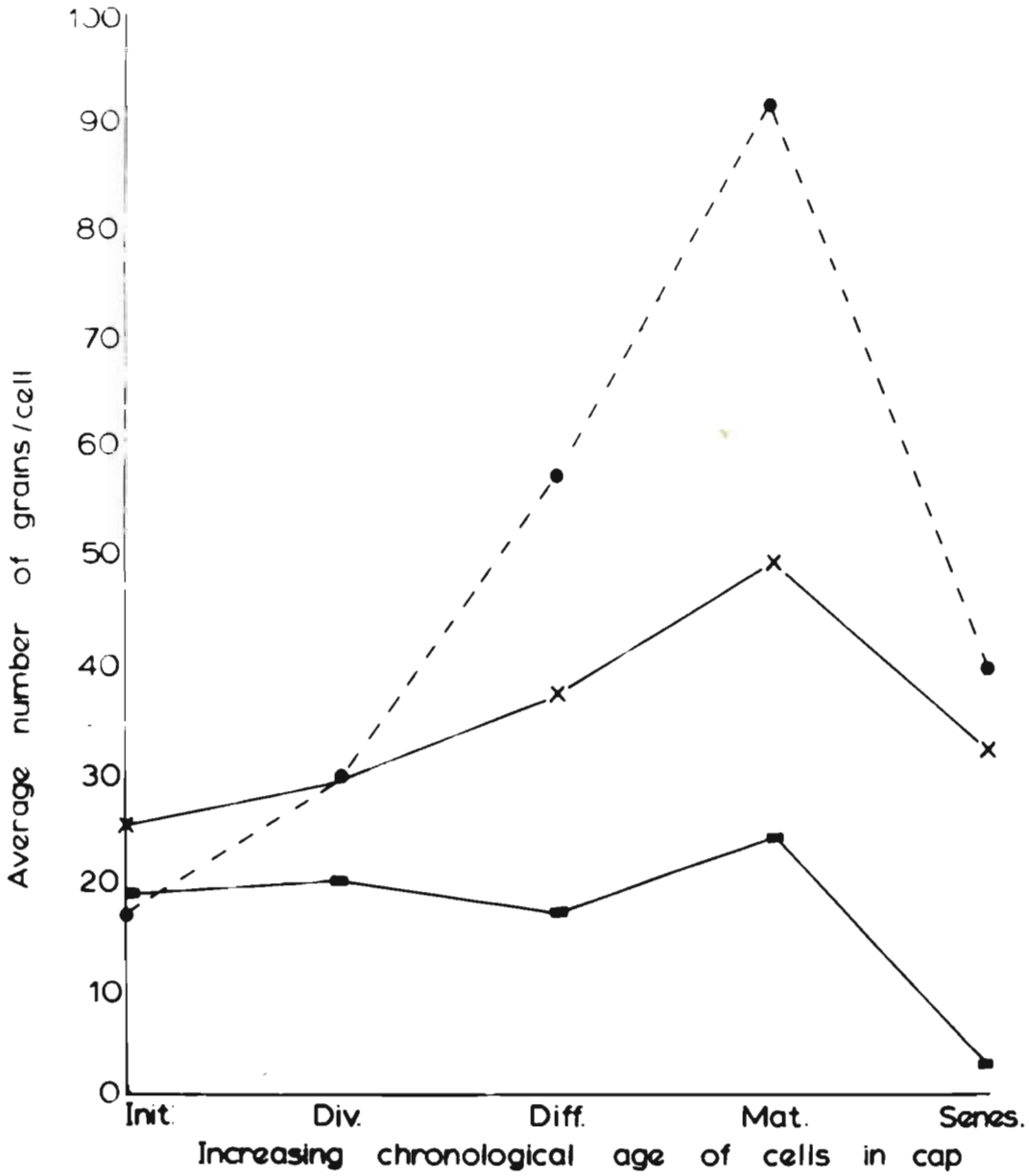


Fig. III C. 2c. Illustrating the incorporation of ^{3}H-uridine into nucleolus, nuclear sap and cytoplasm in the root cap of unaged material at the 48-hour germination stage.

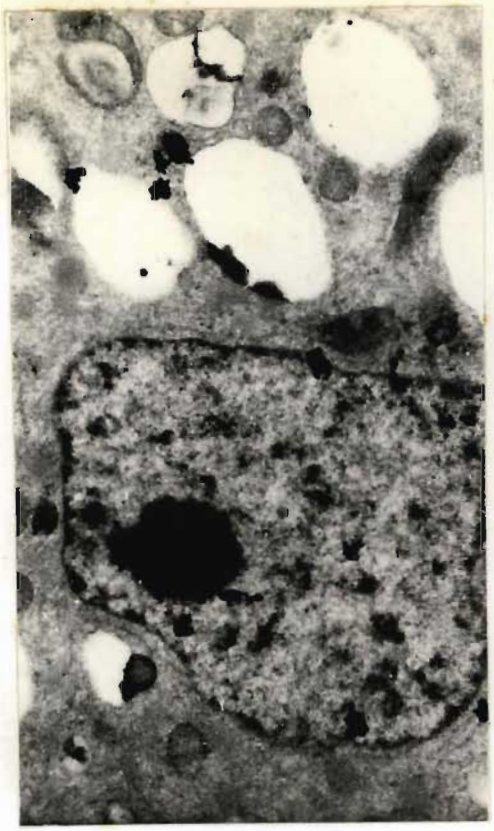
●– – –● cytoplasm x——x nuclear sap

■——■ nucleolus

FIGURE III.C.2d. Illustrates that the incorporation of ^{3}H-uridine is general in the cytoplasm, rather than localised in any one organelle. (x 8 100).

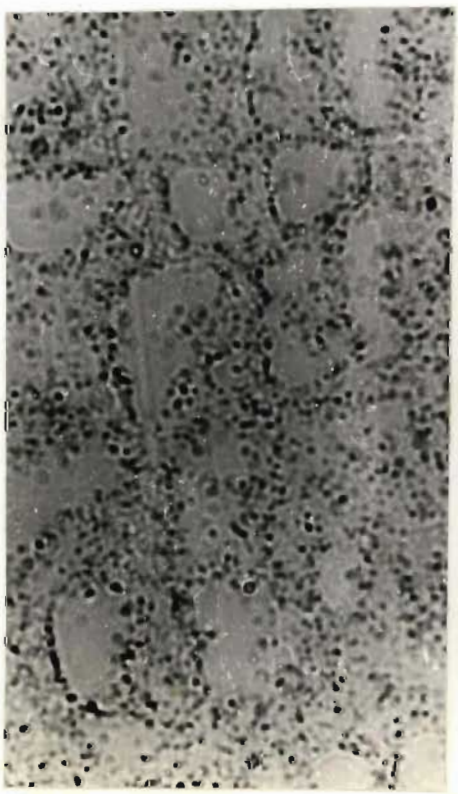
FIGURE III.C.3a. Shows the pattern of ^{3}H-leucine uptake in the root cap of an unaged embryo. (x 500).

FIGURES III.C.3b & 3c. Illustrate the comparitive incorporation of ^{3}H-leucine in unaged material, evidenced by silver grains over meristematic and mature cap cells, respectively. (x 1 600).



2d

3a



3b

3c

illustrates the progressive increase in ^{3}H-leucine incorporation with increasing age of the cells within the root cap.

Electron Microscopy

Figures III.C.3e and 3f illustrate that ^{3}H-leucine was incorprated into the nucleus and generally the cytoplasm. Nuclear incorporation of ^{3}H-leucine occurred both in the nucleoplasm and nucleolus.

While generally an increase in ^{3}H-leucine incorporation followed that of ^{3}H-uridine, there is a lack of correspondence for the relative incorporation of these two labelled molecules in the outermost, senescing root cap cells.

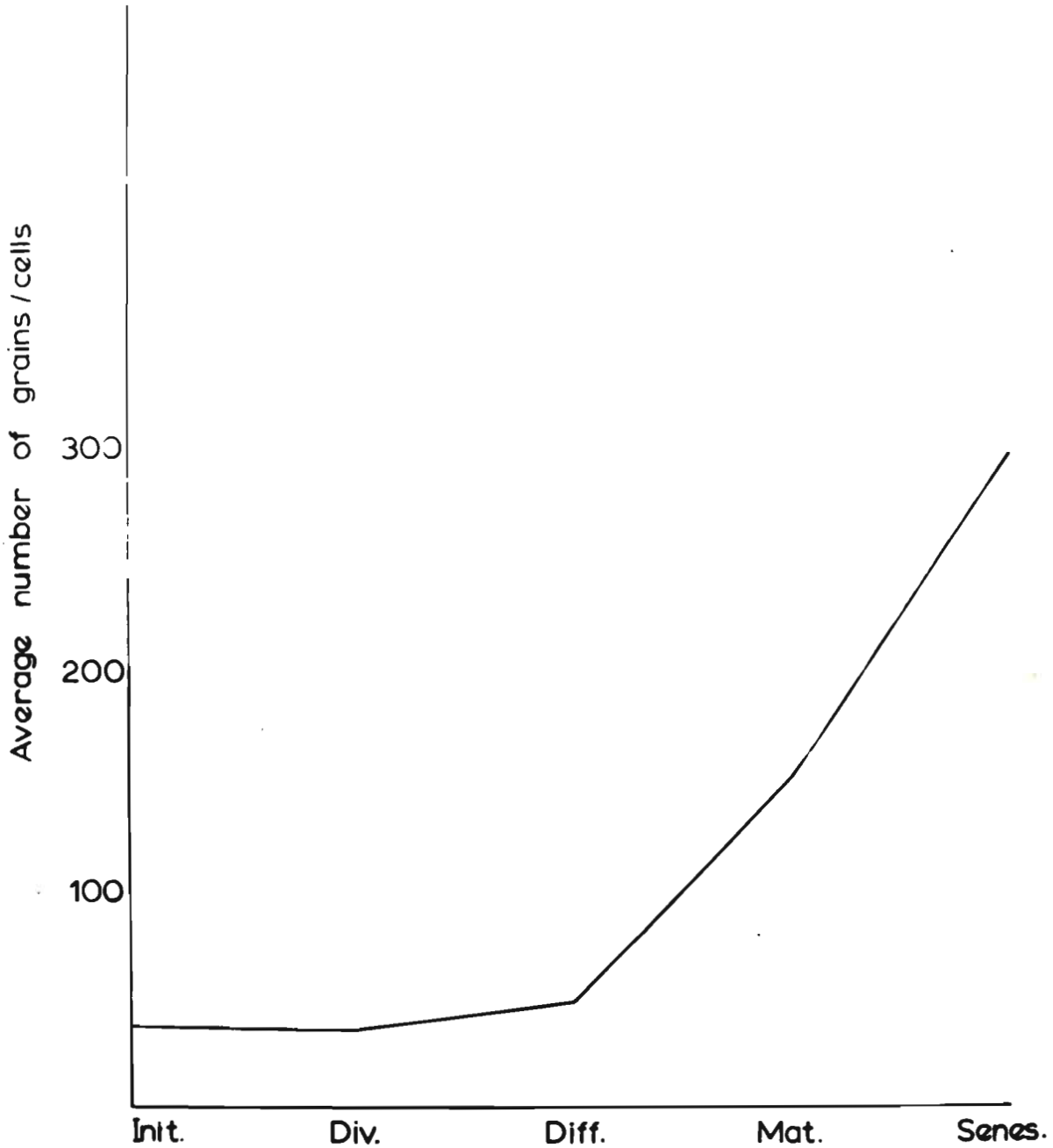
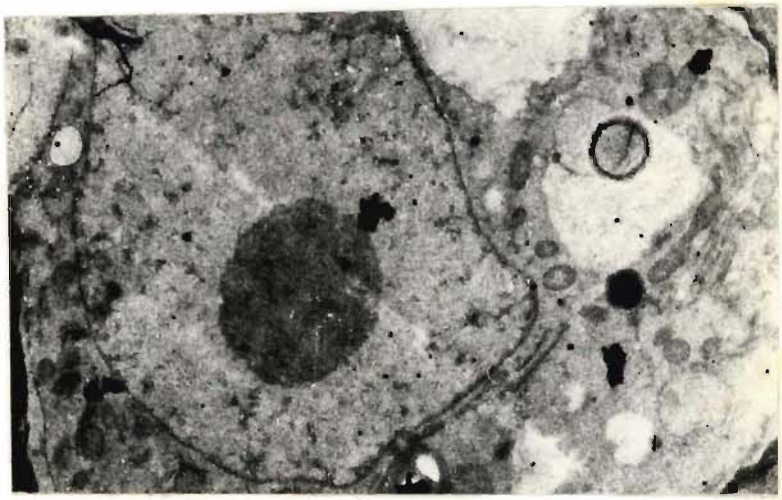
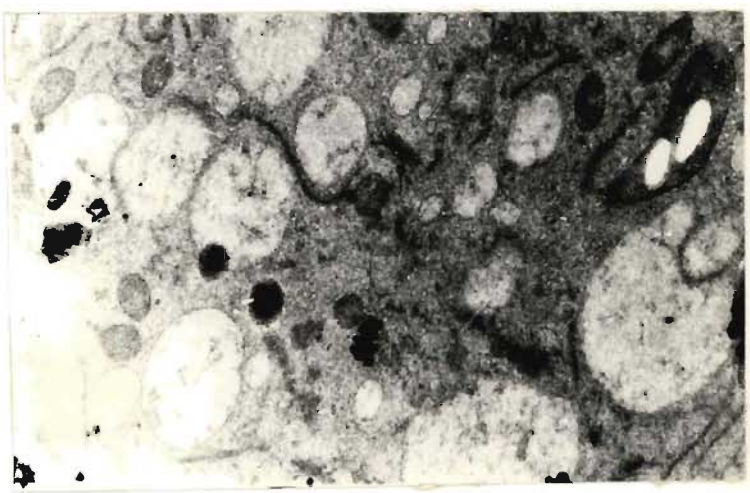


Fig. III. C. 3d. Uptake of ^{3}H-leucine in the cap cells of unaged material, 48 hours after the start of germination.

FIGURE III.C.3e. Illustrates ^{3}H-leucine incorporation in the nucleus of a cap cell of an unaged embryo. (x 8 100).

FIGURE III.C.3f. Shows that incorporation of ^{3}H-leucine occurs generally in cap cell cytoplasm in unaged embryos. (x 10 350).





D. VIABILITY IN RELATION TO THE AGEING SEQUENCE

1. Germination behaviour

Figure III.D.1 shows the survival curve for the population of maize grain maintained at 40°C and 14% moisture content. The values for percent viability were obtained from the germination behaviour in samples of 100 seeds. The results show that the percentage germination falls with increasing storage time. This is in keeping with the results of other investigators (e.g. Roberts et al., 1967; Roberts and Abdalla, 1968).

Seeds are known to lose their viability with time, when stored in an air-dry condition and at ambient temperatures, and increasing temperature and moisture accelerate the viability loss (e.g. Owen, 1956; Barton, 1961).

Oxygen, even in relatively low concentrations, has been demonstrated to have a deleterious effect on stored seed (Roberts and Abdalla, 1968). The samples of maize grain in the present investigation were sealed in glass jars prior to being placed in an incubator. Thus the oxygen content of their gaseous environment was that of the atmosphere at the start, falling somewhat as a result of respiratory exchange during the incubation. The oxygen level was, no doubt, a contributory factor to the viability loss of these seeds.

2. Tetrazolium test.

Figure III.D.2 shows the survival curve for the population of maize grain stored at 40°C and 14% moisture content, determined by the tetrazolium test. Seeds were classified as viable if the plumule, radicle, scutellar node and central scutellar area were completely stained

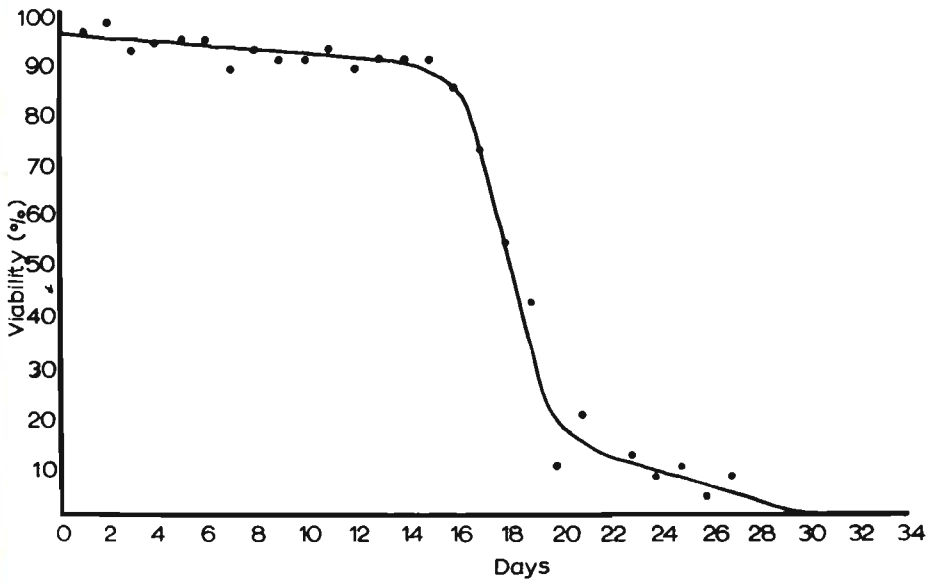


Fig. **III.D.1.** Survival curve for the population of maize grain stored at 40°C and 14% moisture content as determined by germination behaviour

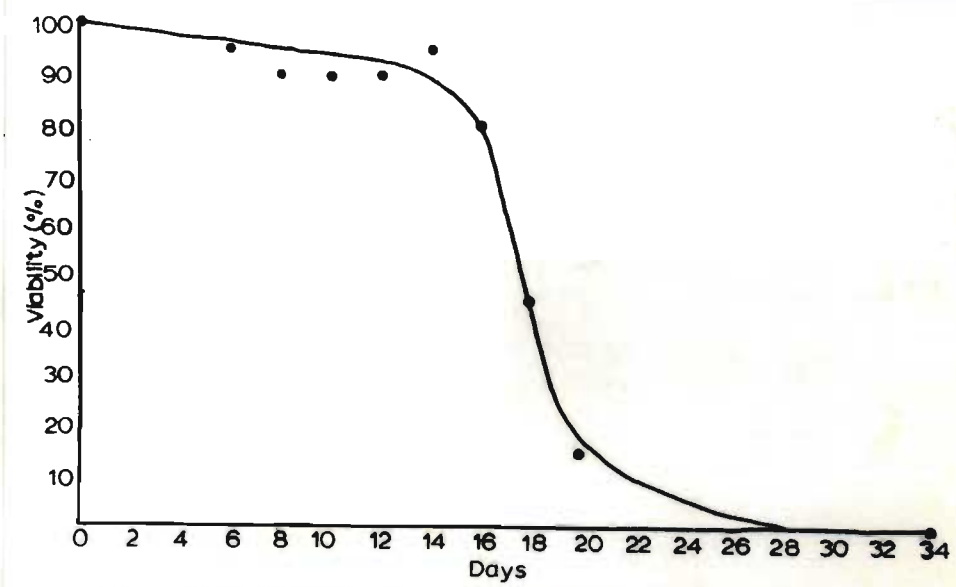


Fig. **III.D.2.** Survival curve for the population of maize grain stored at 40°C and 14% moisture content as determined by the tetrazolium test

(after Lakon, 1949). Reaction with 2,3,5,triphenyltetrazolium chloride is a very sensitive test, being an index of respiratory enzyme activity, and these results bear out the observations obtained by the germination behaviour.

3. Chromosome damage.

Accumulation of chromosome damage with increasing age of the seed has been demonstrated by many investigators. Barton (1961) has reviewed this subject, and it has been established that chromosomal aberrations are produced in the non-dividing cells of the embryo, as the seed ages.

Figure III.D.3 shows the relationship between the mean frequency of chromosomal aberrations (in the surviving population of maize seeds maintained at 40°C and 14% moisture content) and increasing age (storage time). Chromosome damage was best observed at anaphase, and a total of 300 anaphase figures from 10 root tips was examined for each estimation. The fraction of aberrent anaphase configurations is expressed as a percentage of the total anaphase cells, observed, in each case. It is apparent that chromosome damage in embryonic root tips of maize increases with increasing age (storage time) of the seeds. Abdalla and Roberts (1968), working with pea, bean and barley seeds under various conditions of temperature, moisture and oxygen levels, demonstrated similar increases in aberrent cells, with increasing time. These authors showed that any combination of the above factors which led to loss of seed viability also led to an accumulation of aberrent cells, as indicated by chromosome damage, in the embryo. They also reported that an upper limit of the frequency of aberrent cells exists, the value of which is peculiar to a species.

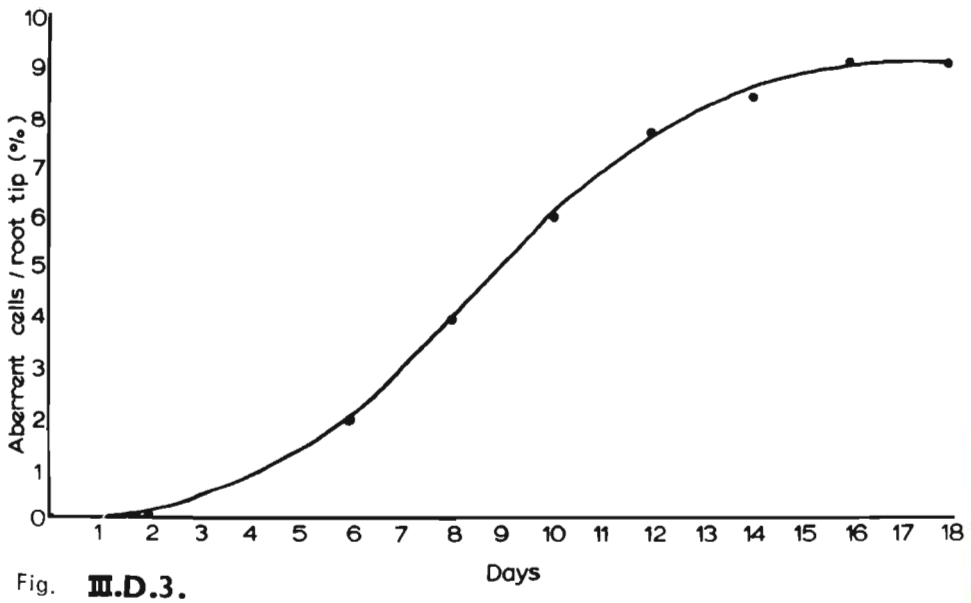


Fig. **III.D.3.**

Relationship between the mean frequency of aberrant cells / root tip, and seed age

Roberts et al. (1967) suggested that the embryonic cells are all in early interphase in the quiescent seed during storage, and it is at this time that the chromosome damage occurs. However, the damage is only demonstrable during the first embryonic cell divisions.

Maize seeds which had been stored for longer than 18 days (at 40°C and 14% moisture content) were also examined for aberrent embryonic cells. However, although some of these seeds appeared to start to germinate, coleorhiza and radicle elongation having occurred, no cell division followed, and thus chromosome aberrations could not be estimated. These seeds were presumably non-viable, and this is borne out by the results of the germination and tetrazolium tests. In addition, there was no significant indication of DNA replication in the root tip cells of these seeds, when autoradiographic methods were used (see later).

The types of chromosome aberration which were encountered in maize root tip cells were generally of three types, viz. lagging chromosomes, chromosome deletions and bridge formation (Figs. III.D.4 (a) - (e)).

4. Chromosome aberration and viability loss.

Figure III.D.5 illustrates that a correlation exists, up to a point, between percentage viability and increase in frequency of chromosome aberrations. It must be noted that the samples examined for chromosome aberrations become more biased towards the longest-lived members of the population, with increasing time, as a consequence of increasing viability loss.

The storage conditions used (40°C and 14% moisture content) were relatively severe, and the loss of viability occurred suddenly and rapidly (ref. Figs. III.D. 1 and 2) .

FIGURE III.D.4a - 4e. Illustrate the types of chromosome aberration encountered in maize root tip cells.

(4a, 4b, 4d & 4e x 1 500; 4c x 2 400).

a

b

c

d

e

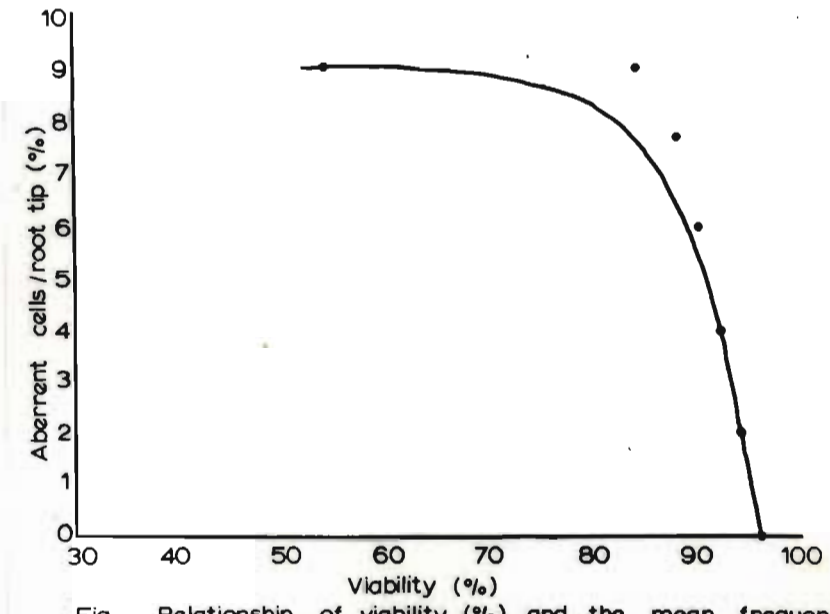


Fig. E.D.5. Relationship of viability (%) and the mean frequency of aberrent cells / root tip in the surviving population of maize grain (stored at 40°C and 14% moisture content)

Cell division was not apparent to a significant extent in those seeds which had been stored for more than 18 days, and the incidence of chromosome aberration occurring at 18 days, might have been limiting.

Roberts et al. (1967) and Abdalla and Roberts (1968) suggested that a certain number of key cells may exist in the embryo, and when a critical non-functional level is reached in these cells, then germination becomes impossible. These authors extended this suggestion by postulating that under constant storage conditions the probability of any of these cells (at random) becoming non-functional (because of molecular accident) is fixed, per unit time, and that the probability is increased by external factors, e.g. increased temperature, moisture content and oxygen tension.

It is possible that the frequency of chromosome damage occurring after 18 days of storage under the conditions used, becomes a limiting factor in the germination capacity of the seed. However, chromosome damage, which has been established as a reliable index of ageing (Roberts et al., 1967), is probably a gross manifestation of other damage at the sub-cellular and ultimately at the molecular control level. Thus the investigation of ultrastructural changes which might have occurred with increasing age (storage time) was undertaken.

E. ULTRASTRUCTURAL CHANGES IN EMBRYOS OF ZEA MAYS L. WHICH OCCUR WITH INCREASING AGE OF THE MATERIAL.

There is a progressive increase in the time taken to germinate under the experimental conditions, with increasing age of the seeds. In unaged material the radicles extend 1cm or more within 48 hours of the start of imbibition. In aged material all the apparently viable embryos took up to 84 hours to reach this stage. Seeds which had been subjected to an intermediate period of the ageing treatment (12 days) had reached this stage of development by 60 hours.

Generally, three patterns or types of intracellular deterioration were seen at the ultrastructural level in aged material. These will be elaborated below. It is pertinent that whatever the pattern of degenerative change it is always progressive in that the chronologically oldest cells of the cap (outermost and mature) show deterioration first, and the initials last.

Embryos of aged material (18 to 20 days) can be divided into three types, based on the degree and type of cellular degenerative change. The first type (type 1 aged embryos) is characterised by cells in which general deterioration of the protoplast is in an advanced state. These embryos appear non-viable, extreme degenerative changes having occurred during ageing. The second type (type 2 aged embryos) is characterised by cells in which a disorientation of organelles has occurred. The organelles show a measure of degenerative change, and occur crowded around the nucleus leaving large areas of cytoplasm virtually devoid of organelles. Both the first and second type of aged embryo will be discussed in detail below (III.F) except that certain aspects of both types are included in the present section (III.E). The third type of aged embryo (type 3 aged embryos) is discussed in detail in this section, and is

characterised by cells in which, although the organelles reflect a measure of degenerative change, the protoplast is still relatively well-organised.

Note that although all the results of this work are presented for the root cap, for the reasons outlined above (III.A) general surveys of the root apices in all cases have shown that the pattern of degenerative change occurring in the cap and described here (III.E), also occurs in the cells of the apex.

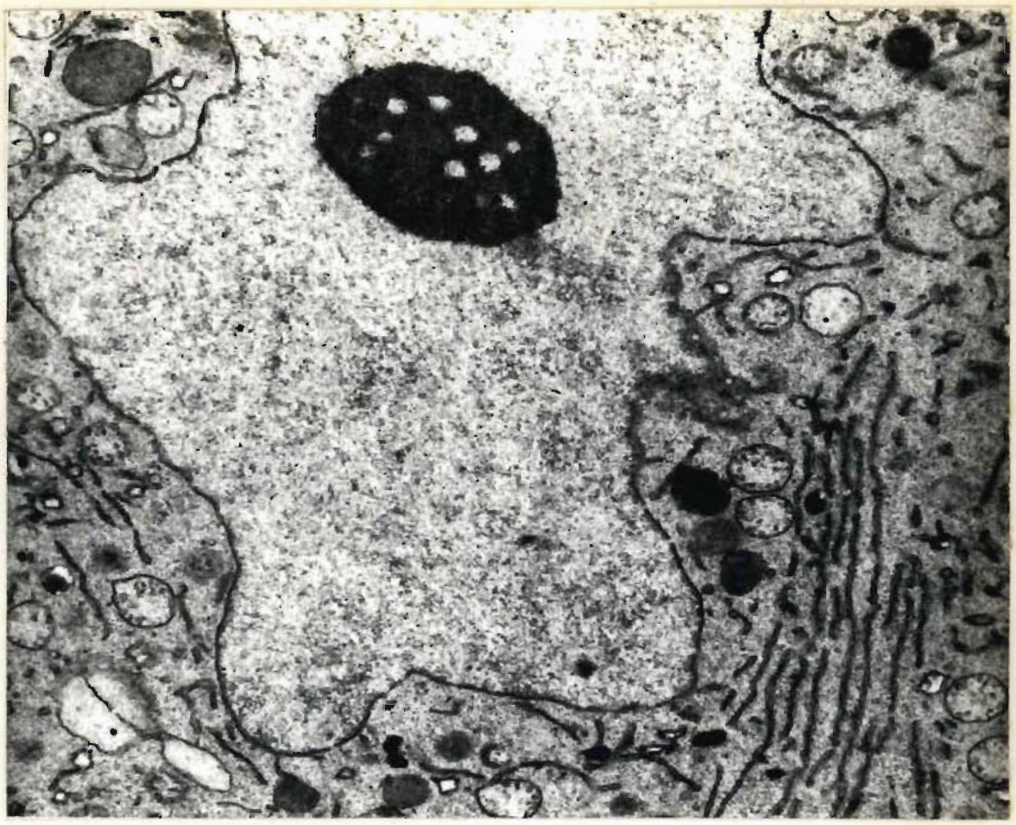
E.1. IMBIBED MATERIAL

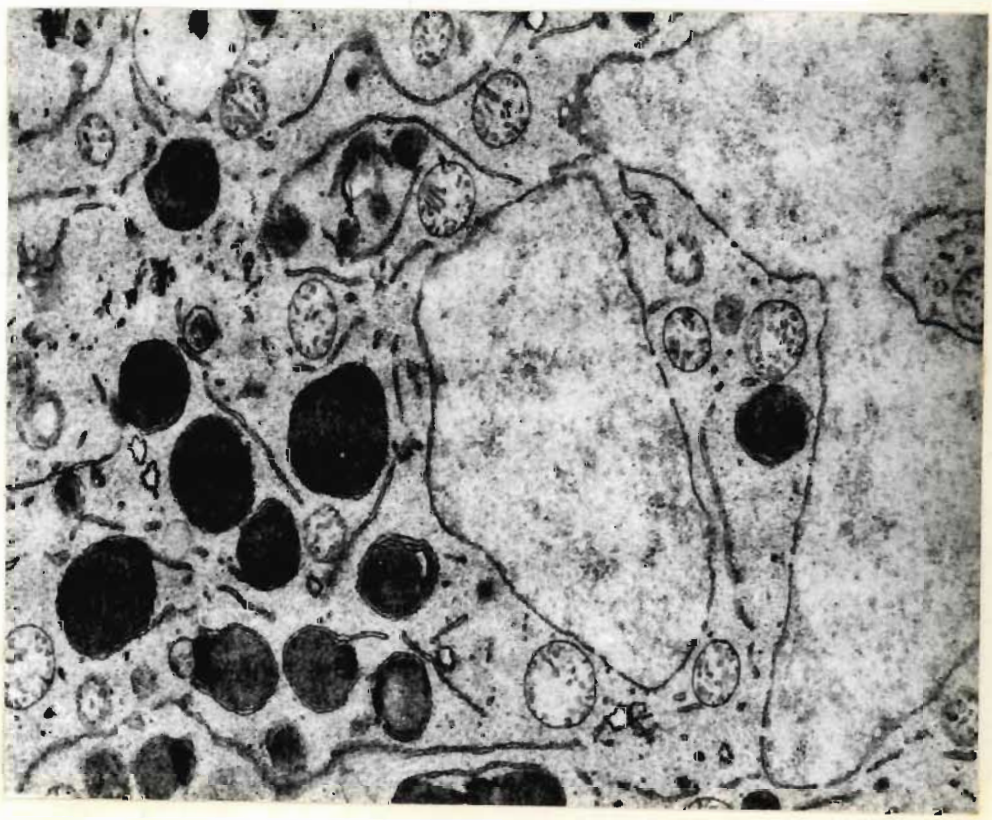
Embryonic tissue which has been obtained from maize seed following a 12-hour imbibition period at $25^{\circ} \pm 2^{\circ}C$ will be referred to as 'imbibed material' or '12-hour material'.

Nucleus

There is a marked tendency for the nucleus to become lobed with increasing age of the embryo. In early stages of the ageing sequence (6 days), nuclear lobing was evident only in the cells of the mature zone. This was also found to be the case in this zone of the unaged embryos; however, after 6 days of ageing the lobing of these nuclei appears to be more pronounced (Fig.III.E.1a). In embryos after 12 days of the ageing treatment nuclear lobing is more severe, and was evident in cells of the zone of differentiation as well as in the zone of mature cells (Fig. III.E.1b). In aged embryos (after 18 to 20 days of the ageing treatment) nuclear lobing was encountered in all the cap cells of imbibed embryos from initials to mature. Figure III.E.1c illustrates the type of lobing encountered in nuclei of the zones of initials and division in an aged embryo.

FIGURES III.E.1a & 1b. Illustrate nuclear lobing in mature cap cells of embryos which received 6 and 12 days of ageing treatment, respectively.
(1a x 10 350; 1b x 9 200).





Nuclear lobing is interpreted as resulting from fundamental changes in the basic nature of the membrane. The average cross-sectional diameter of the nuclear envelope in unaged material was 74 nm. as compared with 48 nm. in aged material. In addition, the perinuclear cisterna had a smaller diameter in aged material (Figs. III.E.1d and 1e). The lobing is suggested to result from membrane weakness and bulging of the nucleoplasm causes stretching of the envelope.

The staining reaction of the nucleus to potassium permanganate changed with increasing age of the material. While chromatin was not found to stain in any cap nuclei of the unaged, imbibed embryos, chromatin staining occurred in the aged condition (ref. Fig. III.E.1c) in cells which appeared to be alive and in those which appeared to be dead. This is interpreted as a change in the chromatin, or in its reactivation during imbibition, which occurred during the ageing process.

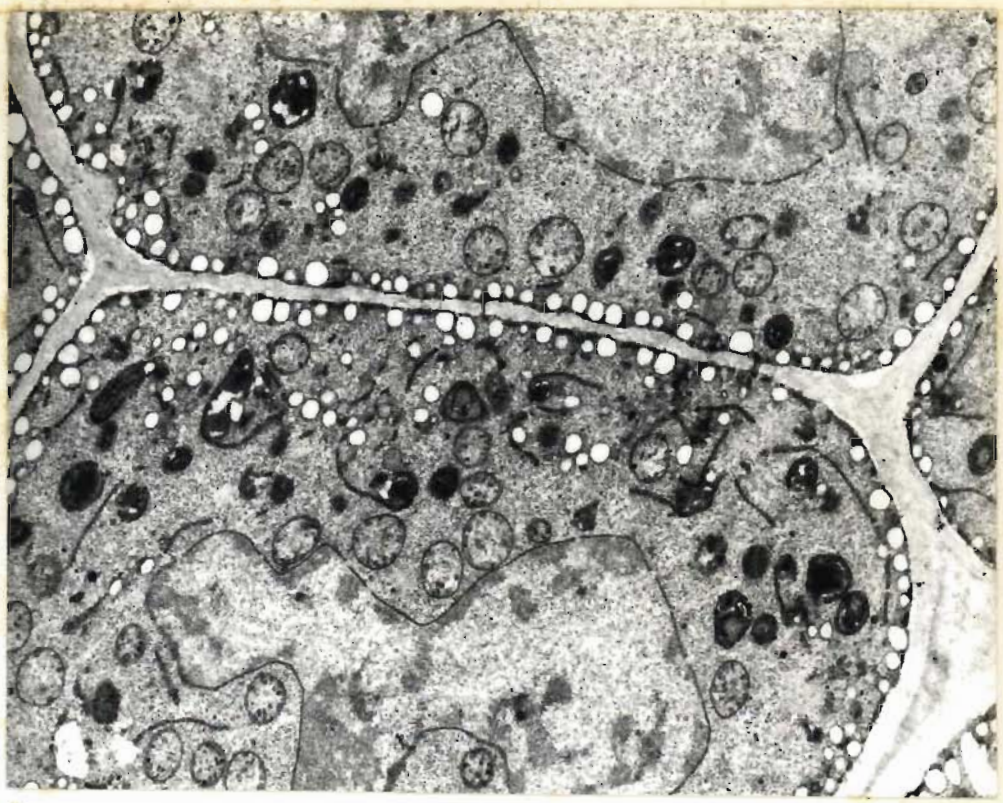
These nuclear changes were indicative of damage which occurred in the quiescent seed (stored at 40°C and 14% moisture content), as these seeds had only been imbibed for 12 hours and the embryos had not as yet undergone any visible signs of elongation indicative of germination.

Mitochondria

Changes in the fine structure of some of the mitochondria were encountered in the cap cells beginning after only 6 days of the ageing treatment, when the percentage viability had not yet dropped. Figure III.E.2a illustrates that some of these organelles had somewhat irregular profiles in thin section, even in the meristematic zones. Mitochondria with distorted profiles were encounteredin all the cap cell

FIGURE III.E.1c. Illustrates lobed nuclei in the zone of initials in an aged embryo. (x 10 350).

FIGURES III.E.1d & 1e. Illustrate the nuclear envelope in cap initials of unaged and aged embryos, respectively. (x 16 100).



c

d

e

types of the 6-day imbibed material. However, there was an increasing frequency of distorted mitochondria with increasing maturity of cells in the root cap, and cells of the mature zone had few apparently normal mitochondria. This distortion of mitochondrial profiles is interpreted as a degenerative ageing change, and is suggested to result from fundamental changes in the membrane structure.

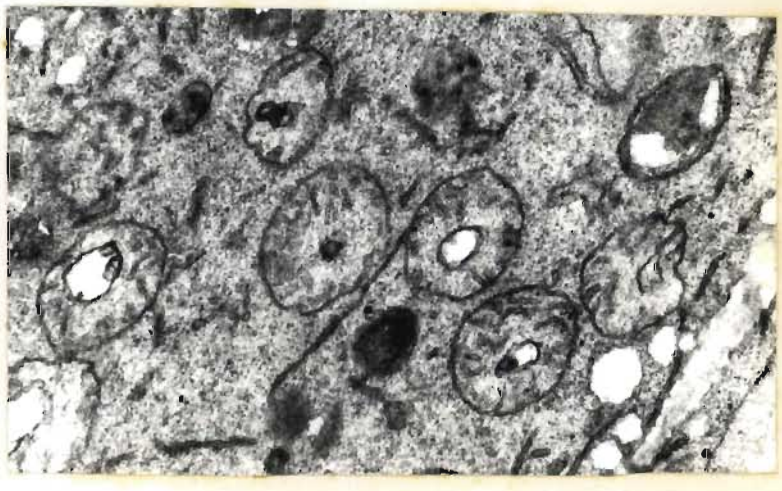
In addition to the distorted profiles, mitochondria in cells of the mature zone showed an unusual intra-mitochondrial structure. In cross-section this appeared as a membrane-bounded space (Fig. III.E.2b). This is in keeping with reports of Villiers (1968) who has observed this type of intramitochondrial structure in aged embryos of Fraxinus excelsior which were kept in an imbibed, dormant condition in the laboratory for three years. If this structure represents a vesicle, then the morphology of this organelle has undergone considerable change to a hollow sphere (if the vesicle is localised) or to a somewhat cylindrical structure (should the vesicle persist throughout the length or diameter). Alternatively, and what is more probable, this structure could represent a degree of disorganisation of the inner membrane of the mitochondrion. Virtually every mitochondrion in all the cap cell zones of type 3 aged embryos showed these signs of degeneration (Fig. III.E.2c)

The pattern of development of the cristae (very short and sparse in initials and a little more developed in mature cells) was similar to that in comparable cells of the unaged material.

There was no significant change in the counts of mitochondria (per unit area of cytoplasm) in any of the cap cells of embryos in the early (6 days) and intermediate

FIGURES III.E.2a & 2b. Illustrate abnormal mitochondria in initials and mature cells in root caps of embryos which had received 6 days of ageing treatment. (x 14 950).

FIGURE III.E.2c. Illustrated mitochondrial damage in a cap cell of a type 3 aged embryo. (x 21 600).



(12 days) ageing stages. However, there was a general drop in the counts of these organelles in cap cells of aged (18 to 20 days) type 3 embryos (initials, 20; zones of division, differentiation and maturity, 15, 18 and 10 respectively). This may reflect breakdown of some of these organelles or decrease in their replication, should this normally occur during the imbibition phase.

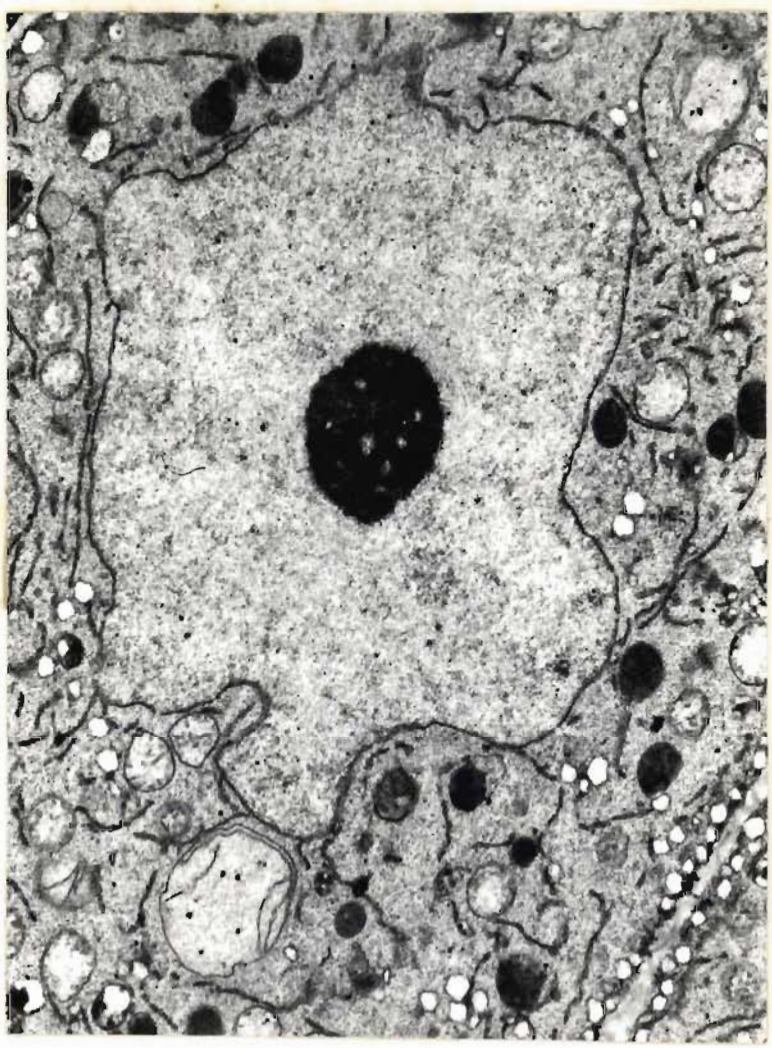
Mitochondria in cap cells of embryos which had received short (6 days) and intermediate (12 days) periods of ageing treatment reflected a sequence of increasing diameter with increasing maturity of the cells, which was similar to that described for unaged, imbibed material; This pattern was not much changed for cap cells of aged type 3 embryos.

Lysosomes.

Lysosomes showed what is interpreted as a precocious development with increasing age of the embryos. In the root cap of the unaged, imbibed embryos, fully-formed first-phase lysosomes were only found in the mature cells. However, this form of lysosome was encountered with increasing frequency in the chronologically younger cap cells with increasing age of the material.

In material which had received 6 days of the ageing treatment, although the initials and cells of the zones of division and differentiation contained lysosomes in their various stages of development, a fair proportion of these organelles were fully-formed at the end of the first developmental phase (Fig. III.E.3a). Their average diameter was 600 nm. in initials and cells of the zone of division, and 720 nm. in the zones of differentiation and maturity. The intimate ER-association with the lysosomes

FIGURE III.E.3a. Illustrates lysosomes in their first developmental phase in a cap cell of the zone of division, 12 hours after the start of imbibition. The material received 6 days of ageing treatment. (x 10 350).



was only encountered in mature cells, as was the case for unaged material.

Lysosomes in the root cap cells of embryos which had received 12 days of the ageing treatment, showed a similar developmental sequence, and in addition, the ER-association was encountered in cells of the zone of differentiation as well as in those of the mature zone (Fig. III.E.3b).

In aged material (18 to 20 days) in the relatively organised cells of type 3 embryos, fully-formed first-phase lysosomes were seen in association with ER profiles in thin sections of the zones of division and initials (Fig. III.E.3c). In addition, some of the lysosomes had swollen and second-phase lysosomes were encountered in all the cellular zones of the cap (Fig. III.E.3d).

However, in type 2 aged embryos, most of the lysosomes were crowded in the perinuclear region with the other organelles and were of the ER-associated first-phase type (Fig. III.E.3e). Some of these organelles appeared to have swelled, but only to a limited extent.

Lysosomes showing membrane rupture occurred in cells of type 1 aged embryos, which appeared non-viable. The possible significance of this will be discussed below (III.F)

Counts of lysosomes per unit area of cytoplasm did not vary significantly with increasing age of the material, from those obtained for unaged, imbibed cap cells. Counts of these organelles in cap cells of aged (type 3) embryos were 29 in initials and 15, 11 and 13 in the zones of division, differentiation and maturity, respectively.

FIGURE III.E.3b. Shows the intimate ER-lysosome association in a cap cell of the zone of differentiation, at the 12-hour germination stage. This material received 12 days of the ageing treatment. (x 9 200).

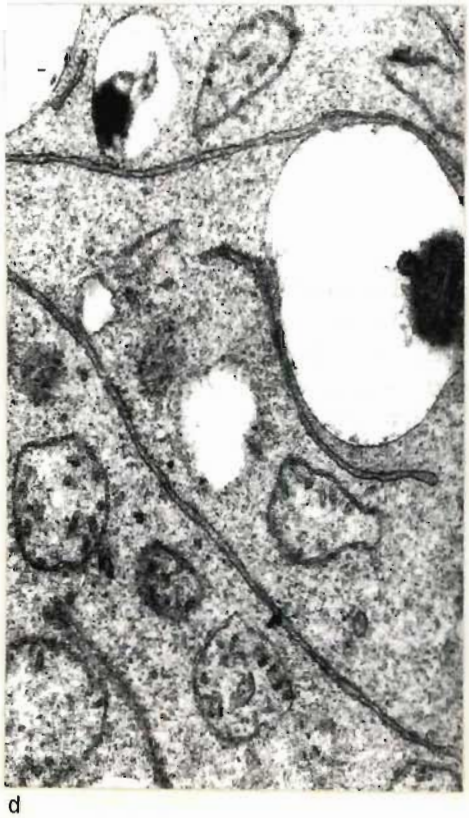
FIGURE III.E.3c. Illustrates ER-associated lysosomes in a cap initial of a type 3 aged embryo, 12 hours after the start of imbibition. (x 13 800).

FIGURE III.E.3d. Shows second-phase lysosomes which occur in all the cap cell zones of type 3 aged embryos, 12 hours after the start of imbibition. (x 21 600).

FIGURE III.E.3e. Illustrates lysosomes (among other organelles) in the perinuclear area in a cap cell of a type 2 aged embryo, at the 12-hour germination stage. (x 11 500).

b

c



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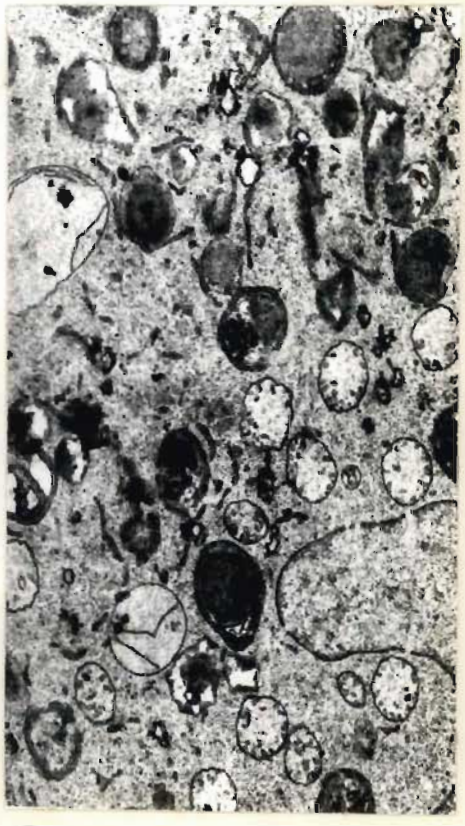


e

Plastids

A change in the appearance of most of the plastids in thin sections of imbibed root caps occurred at an early stage of the ageing treatment. After only 6 days of the ageing treatment the plastids in all the cell zones had distorted profiles. In addition, the internal membranes of the proplastids reflected a change. In the unaged, imbibed material these membranes had smooth profiles compared with the distorted profiles seen in early (and subsequent) ageing stages (Fig. III.E.4a).

This type of distorted plastid was encountered throughout the ageing sequence without much sign of further degeneration. Figure III.E.4b shows the proplastids encountered in a cell of the zone of differentiation of a type 3 aged embryo.

In cap cells of type 2 aged embryos, all the plastids had a distorted appearance, while in cells of type 1 aged embryos, there was a tendency for the reversal of the distorted profiles. In addition, the plastids in type 1 aged embryos showed a marked lessening in their density. (Fig. III.E.4c).

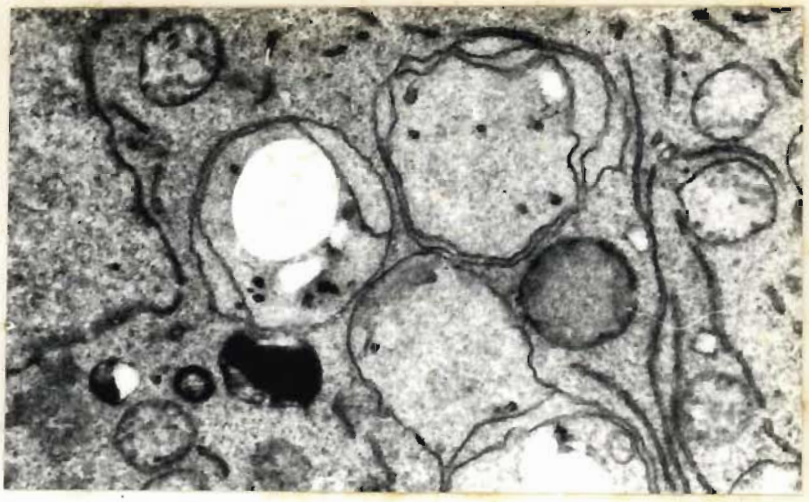
The ageing changes apparent in the plastids are attributed to some basic change in the nature of the membranes. Their appearance in the cells of type 1 aged embryos is thought to be the result of loss of transport control, together with a measure of swelling of these organelles (see below, III.F.).

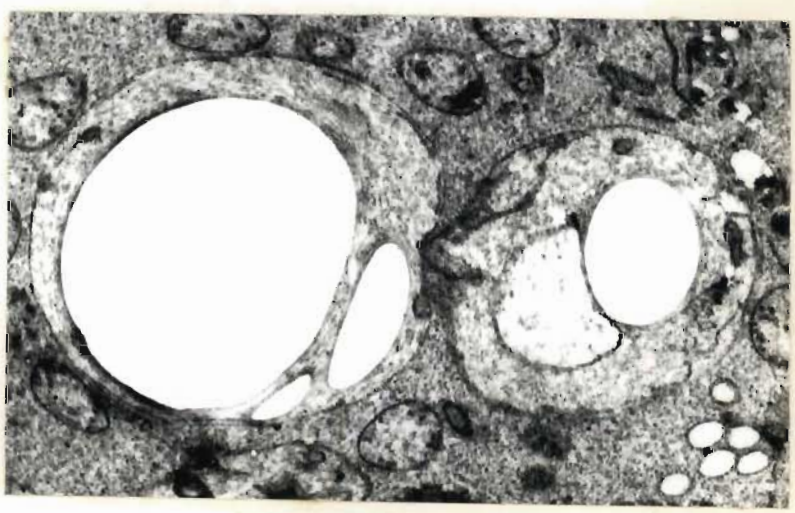
The number of plastids per unit area of cytoplasm did not vary significantly in aged material compared with unaged, suggesting that complete breakdown of the organelles had not occurred during the ageing sequence.

FIGURE III.E.4a. Illustrates visible damage to plastids in a cap cell of material subjected to 6 days of the ageing treatment, at the 12-hour germination stage. (x 14 950).

FIGURE III.E.4b. Shows plastids in a cap cell of the zone of differentiation in a type 3 aged embryo, 12 hours after the start of imbibition. (x 21 600).

FIGURE III.E.4c. Illustrates swollen plastids in a cap cell of a type 1 aged embryo, 12 hours after the start of imbibition. Note the lessening in density of the content compared with these organelles in unaged material. (x 13 800).





The pattern of average diameter of the plastids was similar at the early (6 days) and intermediate (12 days) ageing stages to that encountered in unaged material. However, in type 1 aged embryos the average plastid diameter for the cap cells irrespective of zone, was 1,900 nm. This is interpreted as a consequence of swelling.

Dictyosomes

Dictyosomes, which were judged to be the least conspicuous of the organelles in the cap cells of unaged material, were even less in evidence with the increasing period of ageing treatment of the material. Counts of dictyosomes in root cap cells of embryos having received short (6 days) and intermediate (12 days) ageing treatments (1 dictyosome per unit area of cytoplasm in each of the cell types) reflected a probable loss of these organelles.

When dictyosomes were encountered in the cytoplasm of any of the cap cells (irrespective of the length of the ageing treatment) they appeared disorganised. Figure III.E.5 illustrates an 'unstacked' dictyosome, typical of ageing material.

It is suggested that these organelles become disorganised with the ageing treatment, and that the disorganisation takes the form of 'unstacking' and probable loss of individual cisternae. It is also possible that inactive individual cisternae might persist, but are not recognised as such because of their isolation.

Because of the sparseness of these organelles, estimations of their average dimensions are not valid.

FIGURE III.E.5. Illustrates an 'unstacked' dictyosome, typical of ageing material, 12 hours after the start of imbibition. (x 37 800).

Endoplasmic Reticulum

The development of the ER in the various cell zones of root caps which had received a short ageing treatment (6 days) was not changed from its development in the comparable cap zones of unaged material. That is, the profiles were short and sparse in the initials (Fig. III.E.6a); longer, but largely disorientated in cells of the zone of division (Fig. III.E.6b); in cells of the zone of differentiation the profiles were longer and orientated parallel with one another and with other organelles (Fig. III.E.6c) while in mature cells the ER appeared largely associated with the lysosomes (Fig. III.E.6d).

There was, however, a marked change in this organelle and its degree of development in cap cells of embryos after 12 days of the ageing treatment. The short, scattered profiles typical of the initials in younger material, persisted in cells of the zones of division and differentiation (Fig. III.E.6e). In the mature cells, although there was an increase in length of some of the ER profiles, these were clearly distorted, showing an increase in diameter of the cisternal lumen (Fig. III.E.6f). The average diameter of the cisternal lumen is 48 nm. in mature cells of material having received 12 days of the ageing treatment.

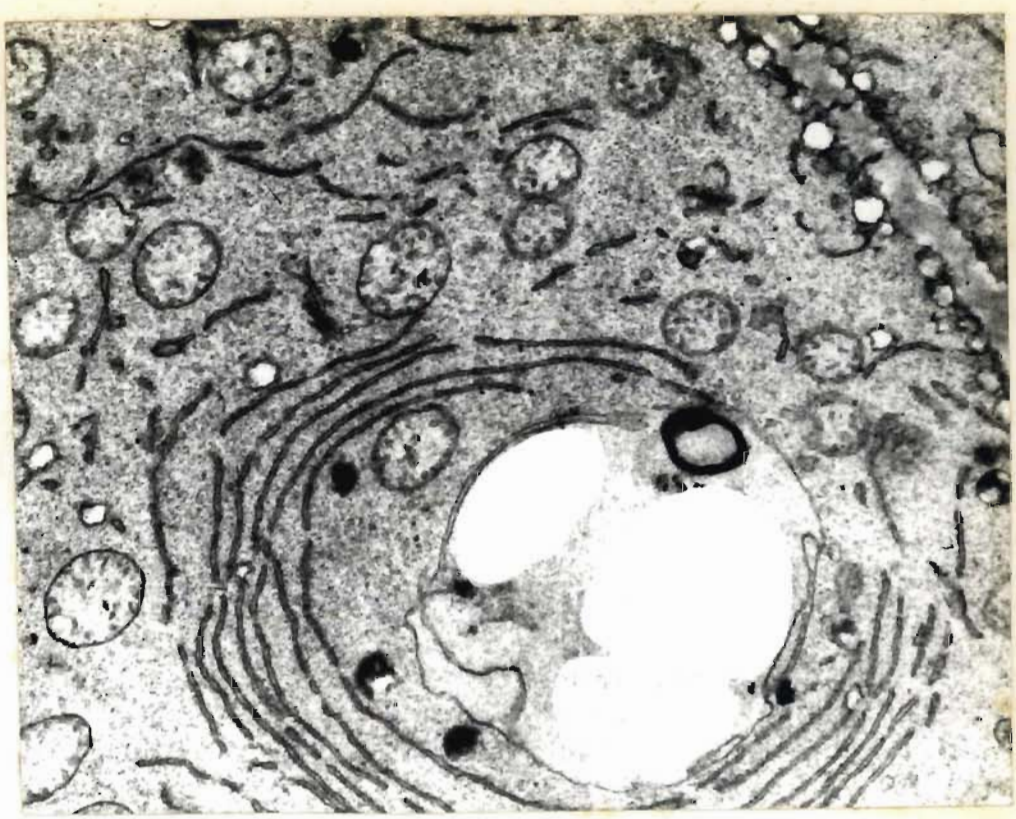
In cells of type 3 aged embryos ER distortion appeared to be reversed. Here the profiles were longer and thinner in all the cell zones (Fig. III.E.6g) when compared with unaged material. This was especially marked in cells of the mature zone. The average diameter of profiles in mature cells of this aged material was 32 nm. as compared with 48 nm. in these cells of unaged material.

Distortion of the ER with increasing age of the material is suggested to result from changes in the membranes.

FIGURES III.E.6a & 6b. Illustrate the disposition of relatively short ER profiles in cells of the zones of initials and division respectively, at the 12-hour germination stage in material which had received 6 days of the ageing treatment.
(6a x 12 650; 6b x 10 350).

FIGURE III.E.6c. Shows orientation of ER profiles in a cap cell of the zone of differentiation in material which had received 6 days of the ageing treatment, at the 12-hour germination stage. (x 13 800).

FIGURE III.E.6d. Illustrates the intimate ER-lysosome association in a mature cap cell of material which received 6 days of the ageing treatment, 12 hours after the start of imbibition. (x 10 350).



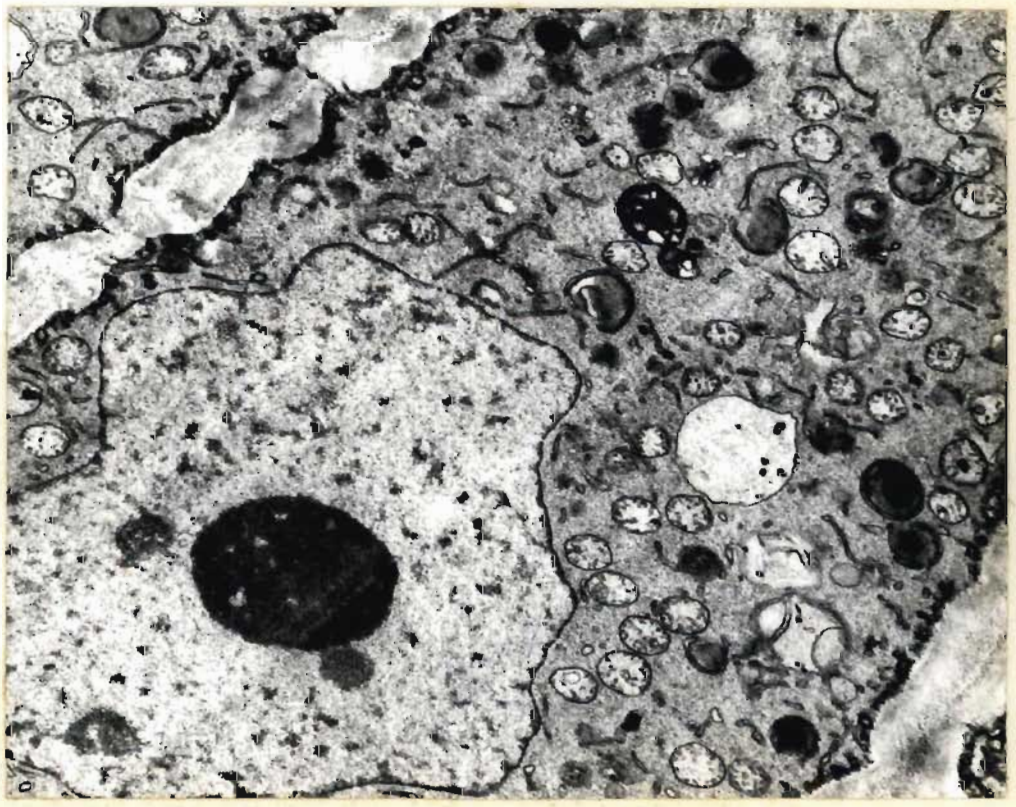
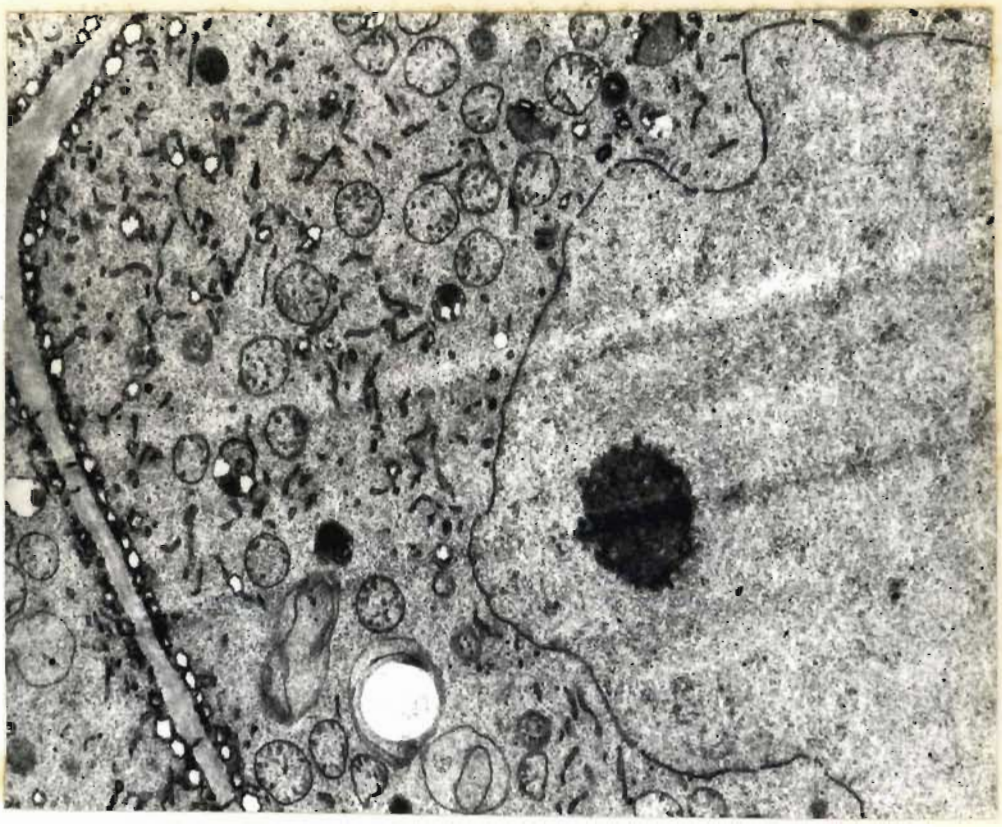


FIGURE III.E.6e. Shows the short, scattered ER profiles which persist in cells of the zones of division and differentiation in material which receives 12 days of the ageing treatment, 12 hours after the start of imbibition. (x 10 350).

FIGURE III.E.6f. Illustrates the distorted ER profiles which are visible in mature cap cells of material which received 12 days of the ageing treatment, at the 12-hour germination stage. (x 13 800).



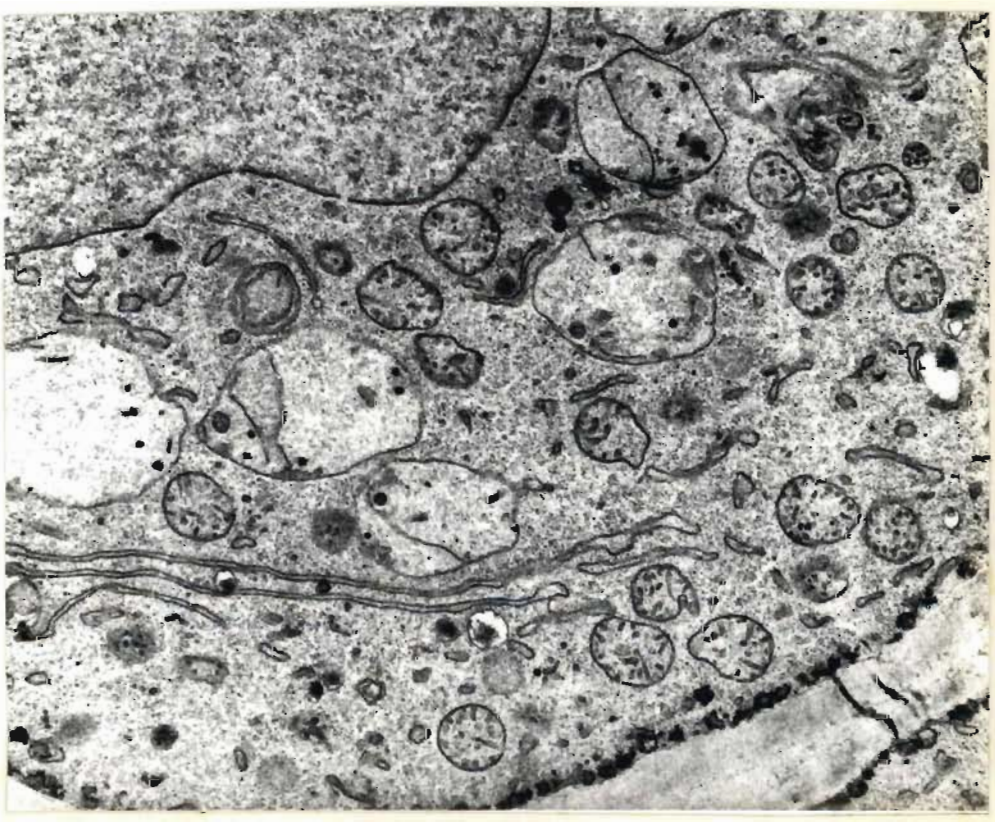


FIGURE III.E.6g. Illustrates the relatively long, thin ER profiles which are seen in cap cells of type 3 aged embryos at the 12-hour germination stage. The average diameter of these profiles is less than in comparable cells of unaged material. (x 17 250).

The final extension in length, with accompanying loss in diameter is interpreted to result from loss of the normal properties of these membranes so that the pressure of the enchylema may cause profile distortion.

ER profiles were virtually absent from type 1 aged embryos, while in the cells of type 2 aged embryos, the profiles were short, sparse and without orientation (see below III.F).

Ribosomes

Ribosomes were seen to be aggregated to form polysomes after 12 hours of imbibition in the cap cells of the unaged material and also in cells of material which had received short (6 days) and intermediate (12 days) periods of ageing treatment (Fig. III.E.7a). However, in cap cells of aged material (18 to 20 days), ribosomes were still largely disaggregated as monosomes, following a 12-hour period of imbibition (Fig. III.E.7b). This was the situation in all three types of aged embryos.

This change, which occurs with age, is thought to result from a general slowing down of various cellular processes which occur during germination, and possibly not from the destruction of the long-lived m-RNA, at least in types 2 and 3 aged embryos (see below, III.F).

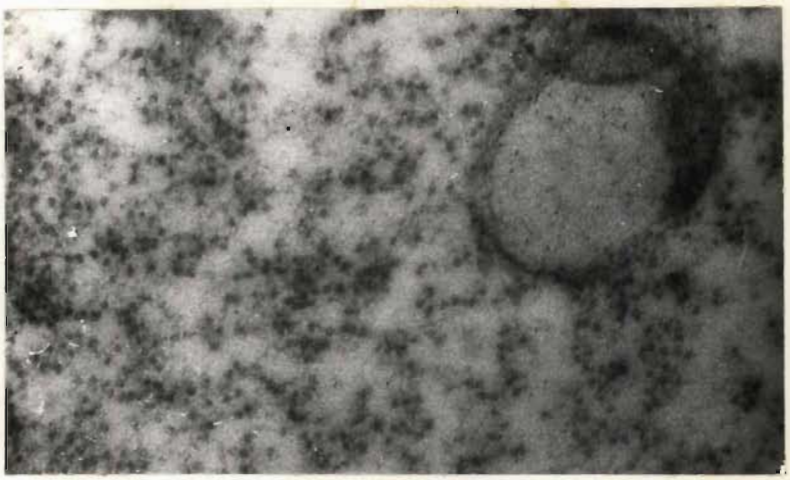
Lipid Droplets

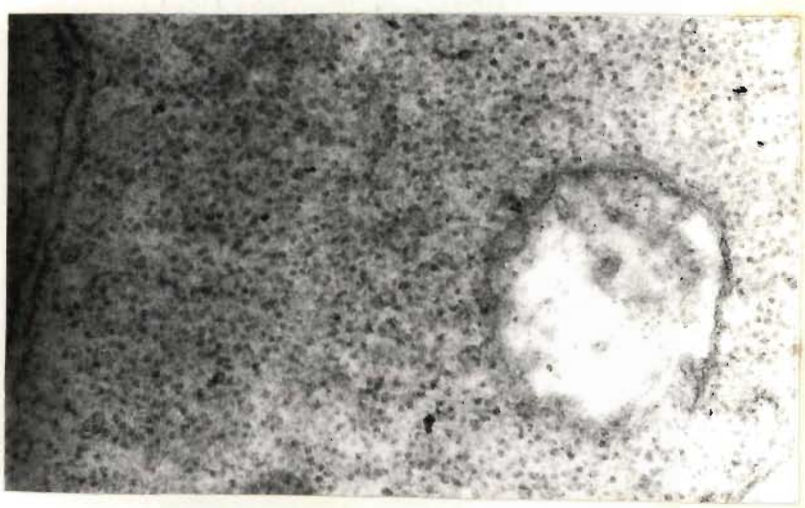
The disposition of lipid droplets in the cytoplasm of the cap cells was not basically altered in the aged imbibed material compared with the unaged. It is thought that the disposition of lipid within the various cap cell types in imbibed material, might represent the situation as it was prior to the drying of the seed.

FIGURE III.E.7a. Illustrates that polysome formation has occurred by the 12-hour germination stage in material which received 12 days of the ageing treatment. (x 50 400).

FIGURE III.E.7b. Shows that monosomes predominate in cap cells of type 3 aged embryos at the 12-hour germination stage. (x 44 800).

The material illustrated in both these micrographs was postfixed in an osmium solution according to Procedure 6b.





Walls

There was no apparent change in cell walls of the aged material when compared with walls of unaged material. However, occasionally cell wall collapse appeared to occur in cells of type 1 aged embryos (see below, III.F).

As seeds increase in age, not only does the germination percentage drop, but also those seeds which do germinate take a longer time for the process and may develop into somewhat stunted seedlings.

The ultrastructural changes which are apparent with increasing age, appear long before there is any substantial drop in the germination percentage. However, the time taken for seeds to germinate increases after about 8 days of the ageing treatment. This is probably a result of impaired efficiency of organelles, especially perhaps that of the mitochondria.

E.2 24- AND 48-HOUR MATERIAL

The seeds from which the material was obtained for the 24- and 48-hour observations were imbibed for 12 hours at 25° ± 2°C and set to germinate at this temperature with the embryo side of the seed in contact with moist cellulose wadding for 12 and 36 hours respectively.

Nucleus

Nuclear lobing with progressive age, which was described for imbibed material, persisted in the cap cells of the 24- and 48-hour material.

In material which had received relatively short ageing treatment (6 days at 40°C and 14% moisture content) nuclear lobing was encountered only in mature cells, and these nuclei were similar in appearance to those of mature cap cells of unaged material (i.e. the lobing was moderate and the nucleus retained a basic, central mass). Figure III.E.8a shows part of the nuclear profile typical of a mature cap cell which had undergone 6 days of the ageing treatment.

Nuclear lobing was more pronounced in mature cap cells of material which had been subjected to 12 days of the ageing treatment, both 24 and 48 hours after the start of imbibition. The lobes of the nucleus are of greater magnitude, leaving a somewhat smaller central mass (Fig. III.E.8b). In addition, nuclear lobing is encountered in cells of the zone of differentiation (Fig.III.E.8c).

In the aged material (18 to 20 days) at both the 24- and 48-hour germination stage, nuclear lobing is seen throughout the root cap. In the initials and cells of the zone of division the lobing may be described as moderate. Differentiating and mature cap cells of type 3.

FIGURES III.E.8a & 8b. Illustrate lobed nuclear profiles in mature cap cells of material which received 6 and 12 days of the ageing treatment respectively, at the 24-hour germination stage.
(8a x 10 350; 8b x 13 800).

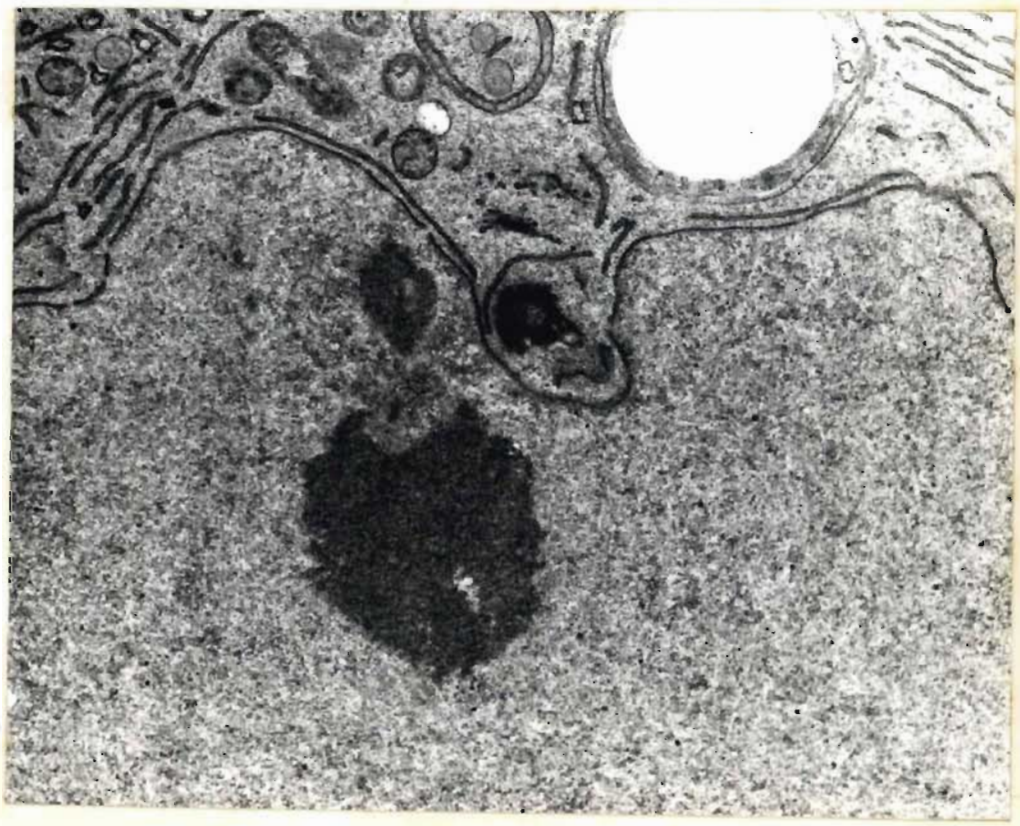
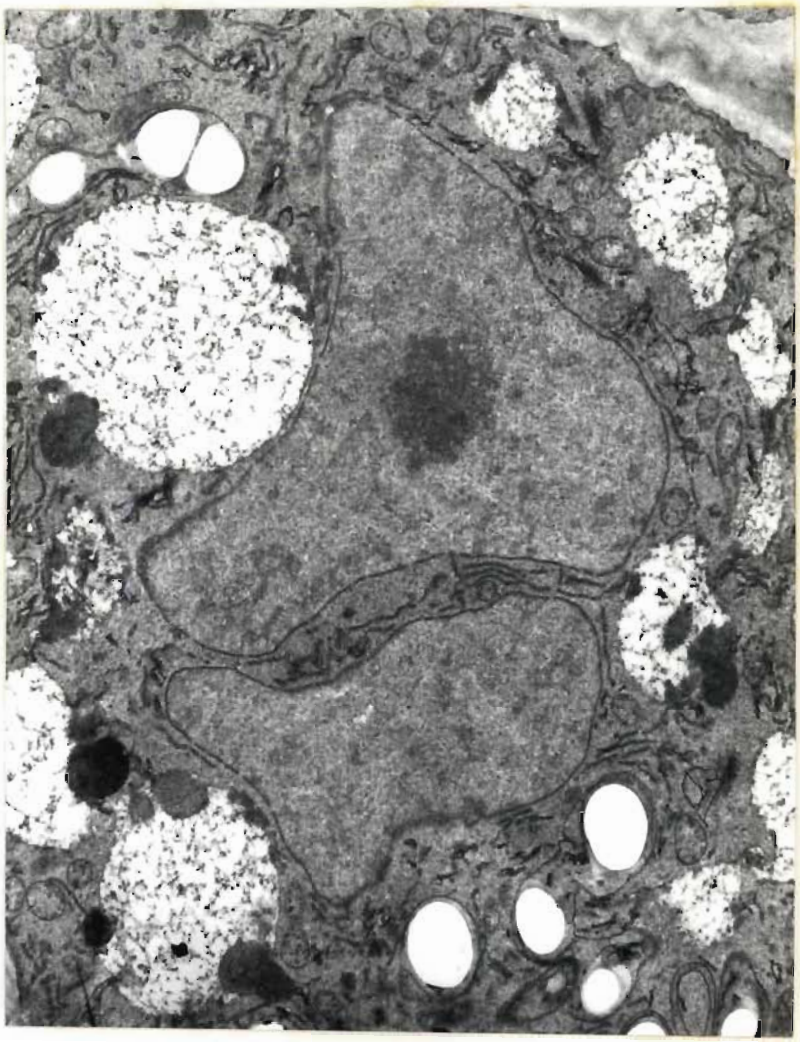


FIGURE III.E.8c. Illustrates a section cut through two nuclear lobes in a cap cell of the zone of differentiation in material which received 12 days of the ageing treatment, at the 48-hour germination stage. (x 8 100).



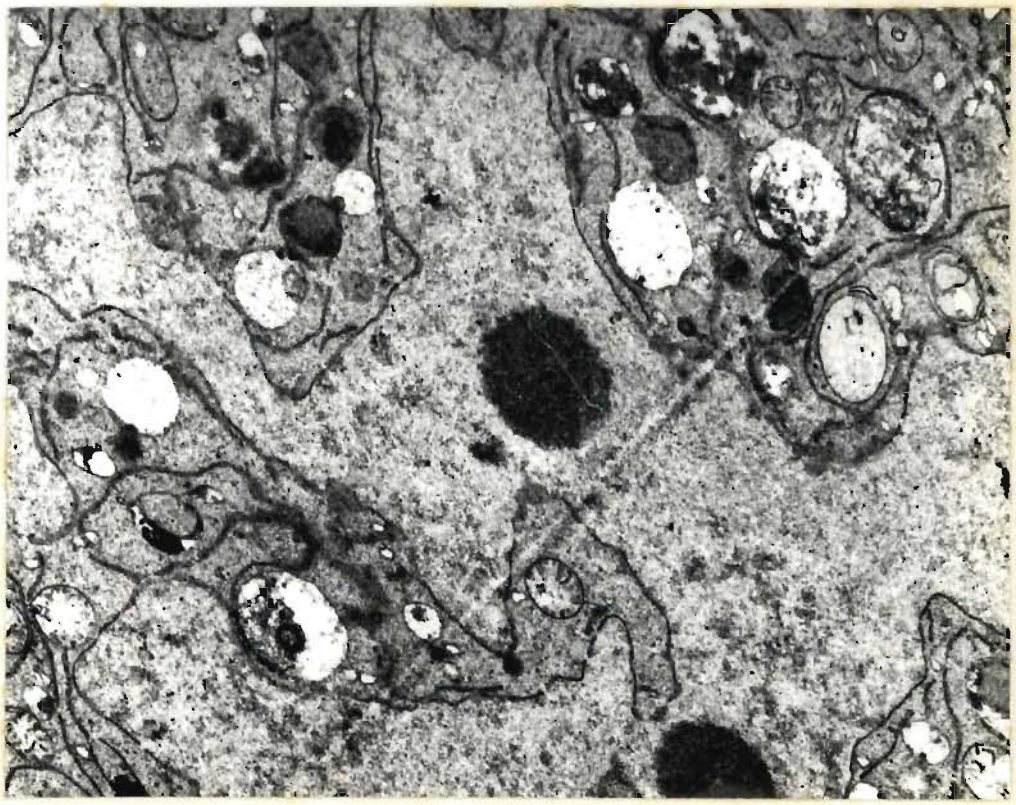
aged embryos have more pronounced nuclear lobing at both of the germination stages under discussion (Fig. III.E.8d). However, cells of type 2 aged embryos show nuclear lobing which can only be described as grotesque. These nuclei have no central mass and are represented by a mass of processes extending through the cytoplasm (Fig. III.E.8e). The average diameter of the envelope of such nuclei is 37 nm. compared with 64 nm. in nuclei of unaged material at these germination stages.

Thus the nuclear lobing encountered with progressive age of the embryos, 12 hours after the start of imbibition is not reversed by the 24 and 48 hours germination stages. However, repair of the nuclear membrane may subsequently occur, as this type of embryo from which samples are described, are able to develop into viable seedlings. Nuclear lobing is suggested to result from basic alterations in the configuration of the membrane. It is possible that some measure of membrane repair has been (or may subsequently be) effected in those type 3 aged embryos which would develop into viable plants. However, in the cells of type 2 aged embryos marked nuclear deterioration was evident with progressive time after imbibition. Two suggestions are possible in this respect. Firstly, if a measure of genetic control does exist in such cells, then that part of the genome controlling nuclear membrane synthesis and repair is not operative, being either altered or repressed. Secondly, that irrespective of the state of the genes in question, there may be partial or complete loss of transport control across the nuclear envelope.

Nuclear division was evident 48 hours after the start of imbibition in the meristematic cap cells of embryos which had been subjected to a short (6 day) period of

FIGURE III.E.8d. Illustrates the relatively pronounced nuclear lobing which is visible in mature cap cells of material which received 12 days of the ageing treatment, at the 24-hour (and 48-hour) germination stage. (x 13 800).

FIGURE III.E.8e. Shows the marked degree of nuclear lobing visible in cap cells of type 2 aged embryos. This micrograph is of a mature cap cell, 48 hours after the start of imbibition. (x 10 800).



ageing treatment and to a far lesser extent in those subjected to a 12 day ageing treatment. However, there are no signs of nuclear division at the 48-hour germination stage in older embryos. This is probably bound up with the slower germination rate shown by apparently viable seeds with progressive age. However, it is probable that cells of type 2 aged embryos have lost the capacity to divide. Cells of this type of embryo are probably non-viable.

Mitochondria

Mitochondrial abnormalities which were first noticed in cap cells of imbibed material after 6 days of the ageing treatment were apparently reversed in this material by the 24-hour stage of germination. The mitochondria in all the cap cell zones were comparable in size, development and appearance with those of the unaged material both 24 and 48 hours after the start of imbibition (Fig. III.E.9a). Counts of these organelles per unit area of cytoplasm were also similar to those obtained for unaged material.

There are two possible explanations of the apparent reversal of abnormal mitochondria. Firstly, the damage which has occurred in these organelles during the relatively short period of ageing treatment may not be severe i.e. the changes in the mitochondrial membrane seen in imbibed (6 day) material may not be indicative of fundamental damage at this stage. The second possibility is the operation of a membrane repair system, under genetic control, which could be operative 24 hours after the start of imbibition, and could effect membrane repair and the reversal of the earlier damage.

Mitochondrial damage observed in the cap cells of imbibed embryos after 12 days of the ageing treatment also appeared to be reversed by the 24-hour germination stage. The mitochondria throughout the cap cells of this material appeared normal and were often encountered in an apparent state of division (Fig. III.E.9b). There was no apparent structural abnormality in these organelles in cap cells of embryos 48 hours after the start of imbibition. These observations suggest the operation of a controlled membrane repair system.

However, a consideration of type 3 aged embryos shows that at the 24-hour germination stage the mitochondria in the cap cells generally have distorted profiles, and the membrane-bound intra-mitochondrial vesicle described earlier is still present (Fig. III.E.9c). Some mitochondria of normal appearance are encountered in the cap initials, but the majority of mitochondria in these cells show signs of damage. It is possible that the apparently normal mitochondria may have been produced in the initials during the period 12 - 24 hours after the start of imbibition (see below).

By the 48-hour germination stage, mitochondrial damage appears to be largely reversed in all the cap cells of type 3 aged embryos, suggesting the operation of a repair system. As all the visible signs of germination occur more slowly with increasing age of the seed, it is possible that the action of such a repair system could also be delayed, accounting for the degree of mitochondrial damage which persists at the 24-hour germination stage. Alternatively, the slower rate of germination could be a consequence of the damaged mitochondria.

FIGURE III.E.9a. Illustrates mitochondria in a mature cap cell of an embryo which received 6 days of the ageing treatment, 48 hours after the start of imbibition. (x 12 650).

FIGURE III.E.9b. Illustrates mitochondrial division in a cap cell of the zone of division in material which received 12 days of the ageing treatment, at the 24-hour germination stage. (x 9 900).

FIGURE III.E.9c. Shows that visible mitochondrial damage persists in cap cells of type 3 aged embryos at the 24-hour germination stage. (x 17 250).

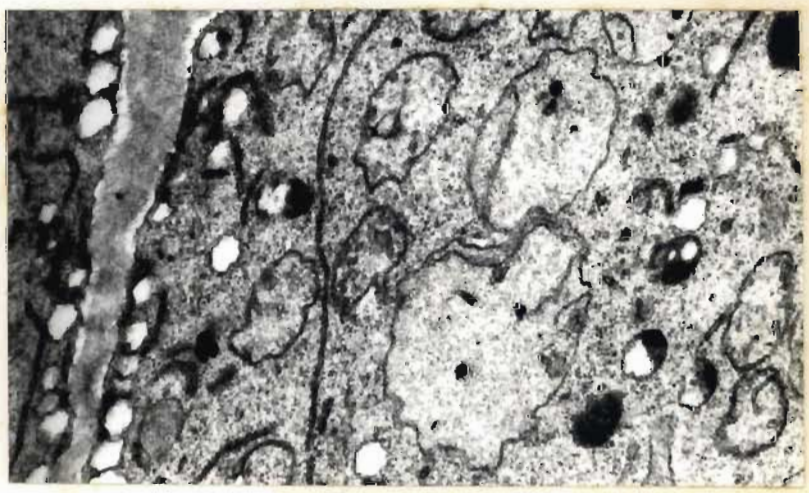


TABLE III.E.1.

Counts of mitochondria/100 cm^2 of cytoplasm, (at a magnification of 10 350) 24 hours after the start of imbibition

Type of cap cell	days of ageing treatment			
	unaged	6	12	18/20 *
non-dividing initials	34	36	51	60
zone of division	22	25	23	15
zone of differentiation	21	23	18	19
Zone of mature cells	22	23	27	20

Table III.E.2.

Counts of mitochondria/100 cm^2 of cytoplasm, (at a magnification of 10 350) 48 hours after the start of imbibition

Type of Cap cell	days of ageing treatment			
	unaged	6	12	18/20 *
non-dividing initials	14	16	31	33
zone of sivision	19	20	18	35
zone of differentiation	12	14	20	22
zone of mature cells	17	18	19	24

* type 3 aged embryos.

A different type of mitochondrial damage is also evident in some of these organelles at the 24-hour germination stage in type 3 aged embryos. Some of the cristae have elongated in an atypical manner (Fig.III.E.9d). This type of abnormality which is not encountered in the mitochondria of any of the aged cap cells at the imbibed stage, occurs at the same time as general development of the cristae. It is possible that this abnormal membrane development might be a type of 'compensatory mechanism', allowing for a greater surface area for respiratory enzyme attachment, to meet the energy requirements for germination. Alternatively, this development of the cristae might be 'nonsense', resulting from slight changes in the genes (and/or in the DNA localised within individual mitochondria). This type of mitochondrial abnormality is more marked in some of these organelles (of type 3 aged embryos) 48 hours after the start of imbibition, where cristae sometimes extend right across the mitochondrion (Fig. III.E.9e).

Table III.E.1. shows that mitochondrial counts per unit area of cytoplasm increased markedly for the cap initials which had received an intermediate ageing treatment (12 days) and for cap initials of aged type 3 embryos, 24 hours after the start of imbibition. A similar trend in mitochondrial count per unit area of cytoplasm occurs in cap initials of the same material (intermediate and type 3 aged embryos) 48 hours after the start of imbibition. In addition, there was a marked increase in the mitochondrial count in the zone of division, of type 3 aged embryos at this germination stage (Table III.E.2).

These results indicate an increase in the rate of mitochondrial replication in chronologically younger cells within the root caps. This might be classed as a

FIGURE III.E.9d. Illustrates mitochondria in which atypical elongation of some of the cristae has occurred. This micrograph is of a cell of the zone of division in a type 3 aged embryo, 24 hours after the start of imbibition. (x 18 400).

FIGURE III.E.9e. Illustrates mitochondria in a mature cap cell of a type 3 aged embryo 48 hours after the start of imbibition. Note the atypical development of the cristae. (x 29 700).

'compensatory mechanism' by which more efficient mitochondria are produced to meet the energy requirements necessary for germination. Mitochondrial counts per unit area of cytoplasm are comparable with those obtained for unaged material, in the chronologically older regions of the root cap. Thus there is apparently no enchancement in the rate of production of these organelles in the more mature cap cells of the intermediate (12 days) and type 3 aged embryos. Note that as cell division does not appear to have started to any great extent in these embryos at the 48-hour germination stage, the more mature cells could not have been replaced by cells with increased mitochondrial content, derived from the meristematic zone.

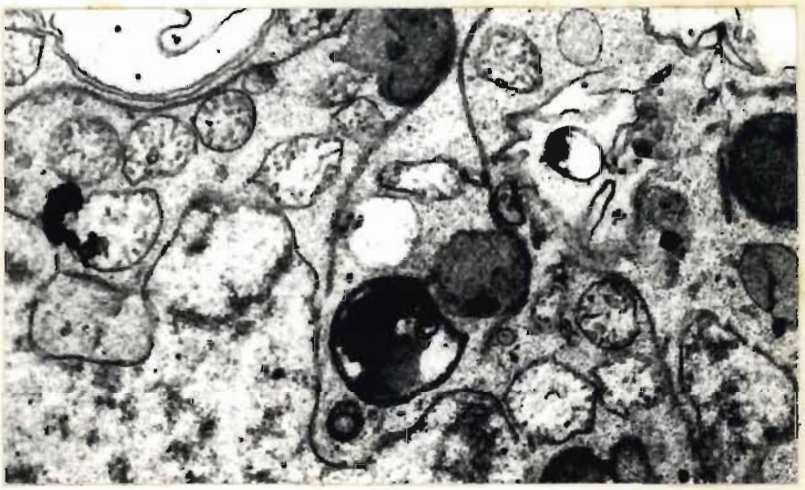
Mitochondria in cap cells of type 2 aged embryos show all the abnormalities described for similar imbibed material both 24 and 48 hours after the start of imbibition (Figs. III.E.9f and 9g). It is possible that a genetically-controlled repair system (perhaps operative in other embryos) has broken down. There is also little development of cristae in the disorientated, damaged mitochondria of type 2 aged embryos. In a few cells of such embryos mitochondria with a measure of development of cristae are encountered. However, this development is often of the atypical type described above (Fig. III.E.9h), and is suggested to result from slight changes in the nuclear DNA involved and/or in the DNA localised within the individual mitochondria.

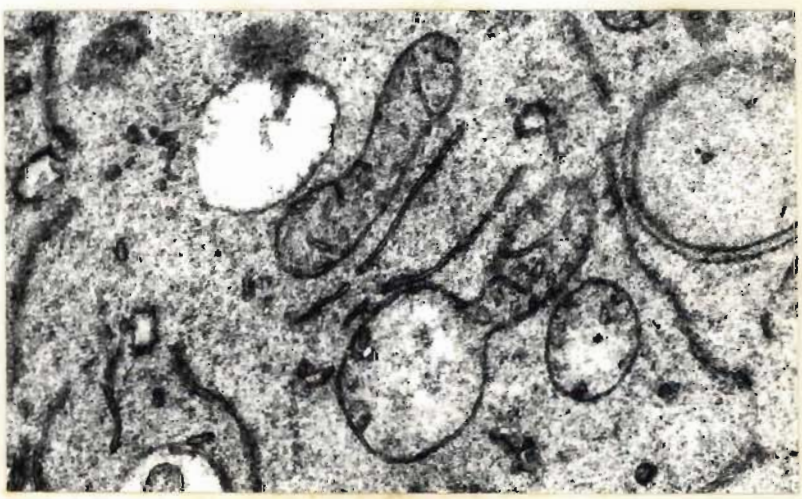
Lysosomes

In the cap cells of embryos which had received a short period (6 days) of ageing treatment, the disposition of lysosomes in their various developmental phases was comparable to that in unaged material, at both the 24- and 48-hour germination stages. That is, for 24-hour

FIGURES III.E.9f & 9g. Show damaged mitochondria in mature cap cells of type 2 aged embryos 24 and 48 hours after the start of imbibition, respectively. (9f x 17 250; 9g x 12 600).

FIGURE III.E.9h. Illustrates mitochondria in which atypical development of the cristae is apparent, in a mature cap cell of a type 2 aged embryo at the 48-hour germination stage. (x 29 700).





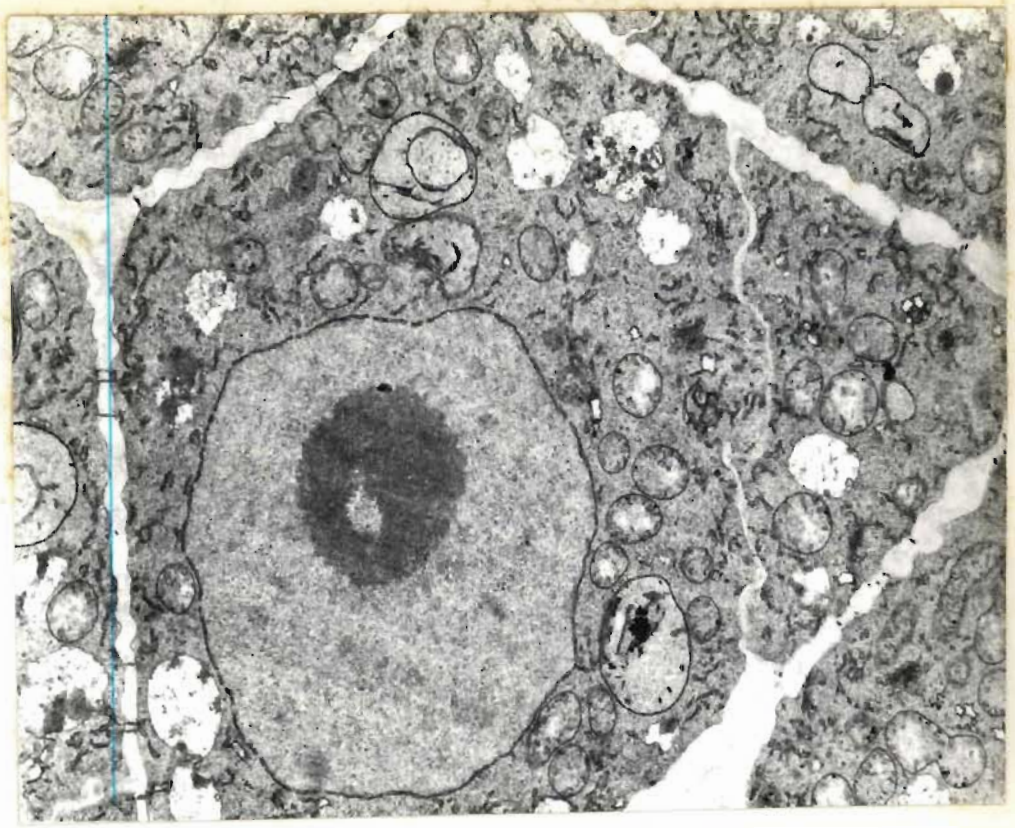
material lysosomes were encountered in their various developmental stages prior to the fully-formed first phase, in the cap initials. In dividing differentiating and mature cells these organelles occurred both in the fully-formed first phase and as second-phase lysosomes. In addition, there was apparent formation of additional lysosomes in mature cells, while in outermost cells there is evidence of lysosomal membrane dissolution. In the 48-hour material, fully-formed first-phase lysosomes predominate in the initials only, and these organelles occur as lysosomal vacuoles (second developmental phase) in all other cell types.

Counts of lysosomes per unit area of cytoplasm in all the cap cell types of 6-day material are similar to those obtained in comparable cells of unaged material, both 24 and 48 hours after the start of imbibition.

In cap cells of material which had received intermediate ageing treatment (12 days), the disposition of lysosomes throughout the cap cells at the 24-hour germination stage is similar to that encountered in younger material. However, in this material 48 hours after the start of imbibition, second-phase lysosomes are very prevalent in the initials. In addition, lysosomal vacuoles in cells of the zones of division, differentiation and maturity appear far more extensive in this material than in comparable cells of younger embryos (Figs. III.E.10a and 10b). It is possible that these organelles function as organs of intracellular digestion in cells other than those of the mature zone in this material. Lysosomal vacuoles containing, for example, mitochondria and ER in various stages of degeneration are sometimes encountered in dividing and differentiating cells, both 24 and 48 hours after the start of imbibition (Figs. III.E.10c and 10d). This is an unusual feature for

FIGURE III.E.10a. Illustrates second-phase lysosomes in a dividing cell of material which received 6 days of the ageing treatment, at the 48-hour germination stage. (x 10 350).

FIGURE III.E.10b. Illustrates a cell of the zone of division in material which received 12 days of the ageing treatment, at the 48-hour germination stage. Note the occurrence of second-phase lysosomes. (x 10 350).



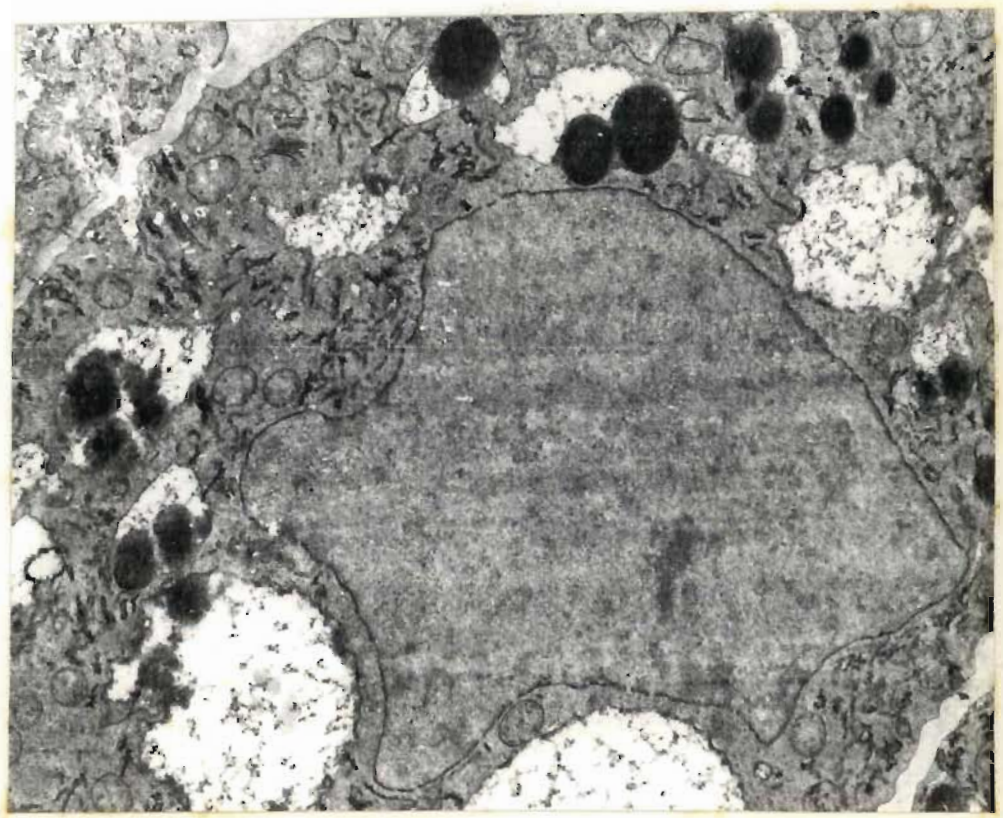
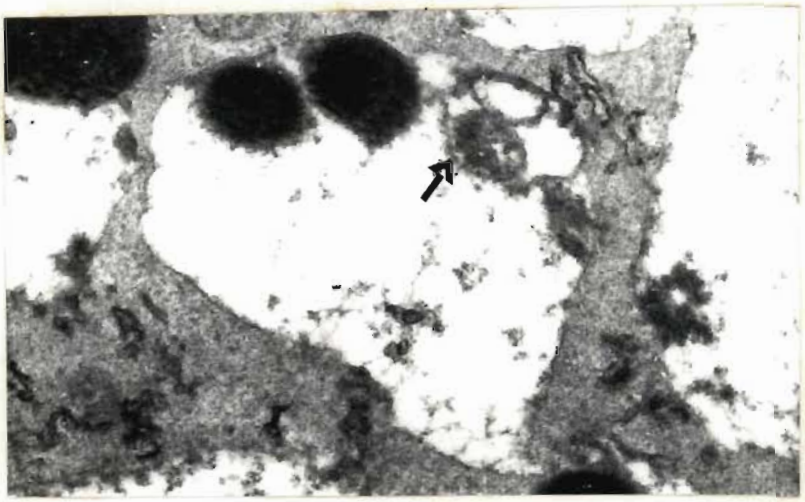
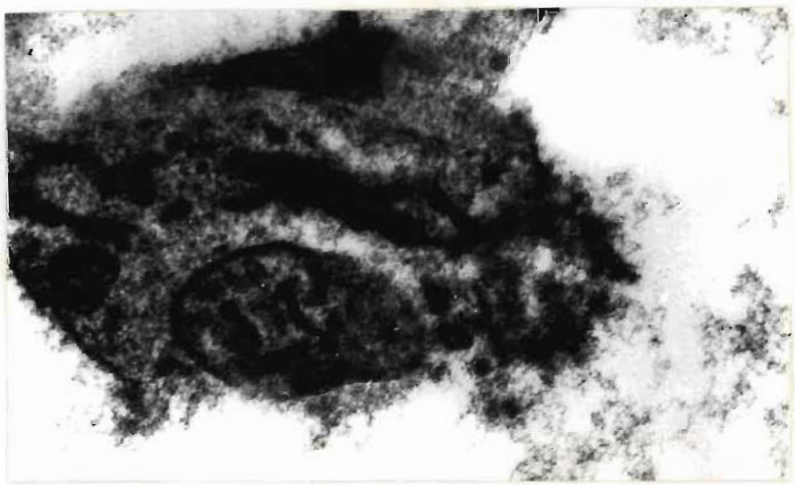


FIGURE III.E.10c. Illustrates a cell of the zone of division in material which received 12 days of the ageing treatment, at the 48-hour germination stage. Mitochondrial remains are visible within a lysosomal vacuole (at arrow). (x 18 400).

FIGURE III.E.10d. Illustrates partially degraded mitochondria within a lysosomal vacuole in a cell of the zone of differentiation. The material received 12 days of the ageing treatment and was fixed at the 48-hour germination stage. (x 61 600).





cap cells other than those of the mature zone. This suggests activation (derepression) of a genetic control system normally repressed in non-mature cap cells, and it is possible that organelles which have become inefficient or non-functional are eliminated from the cell by this means.

Counts of lysosomes per unit area of cytoplasm in cap cells of 12-day material do not differ significantly from those obtained for younger material.

Lysosomes showed a similar disposition and development in cap cells of type 3 aged embryos as described for embryos having received 12 days of the ageing treatment (Fig. III.E.10e). In addition, counts of these organelles per unit area of cytoplasm are similar.

However, in cap cells of type 2 aged embryos 24 and 48 hours after the start of imbibition, the lysosomes mainly occurred in the ER-associated first-phase form (Fig.III.E.10f). Second-phase lysosomes occurred only in cells which show marked deterioration of other organelles. Some of these lysosomal vacuoles contained what appeared to be remnants of other cytoplasmic organelles (Fig. III.E.10g).

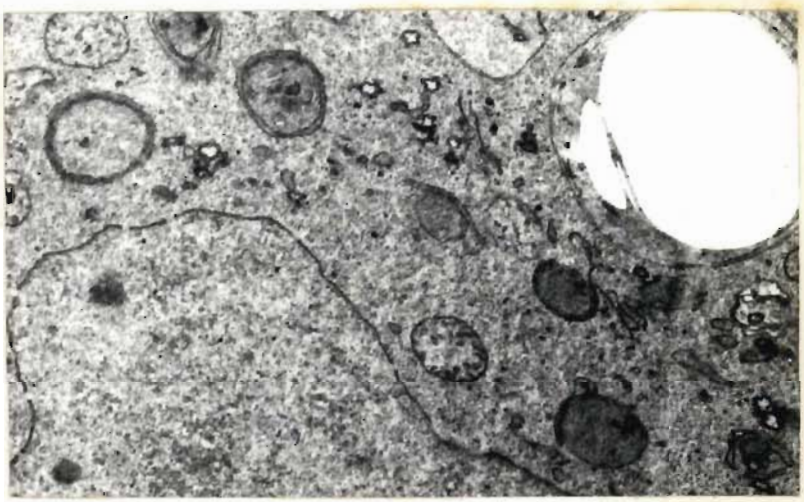
Plastids

Plastid distortion was evident in all the cap cell types of embryos which had received a short ageing treatment (6 days), 12 hours after the start of imbibition. However, by the 24-hour germination stage this apparent damage appears to be reversed. It is possible that the membrane distortion seen at the earlier germination phase did not represent fundamental change in the molecular components. Alternatively, early activation of a genetically-controlled membrane-repair system might occur, accounting for the apparent reversal of the earlier damage.

FIGURE III.E.10e. Shows closely-packed membranes, which resemble myelin figures, within lysosomal vacuoles in a cell of the zone of differentiation of a type 3 aged embryo 24 hours after the start of imbibition. (x 16 100).

FIGURE III.E.10f. Shows ER-associated first-phase lysosomes in a cap cell of a type 2 aged embryo, 24 hours after the start of imbibition. (x 16 100).

FIGURE III.E.10g. Illustrates relatively small second-phase lysosomes, which appear to contain remnants of other organelles, in a cap cell of a type 2 aged embryo at the 24-hour germination stage. Note that the ER-lysosome association persists in this material, although the lysosomes are in the second developmental phase. (x 16 100).



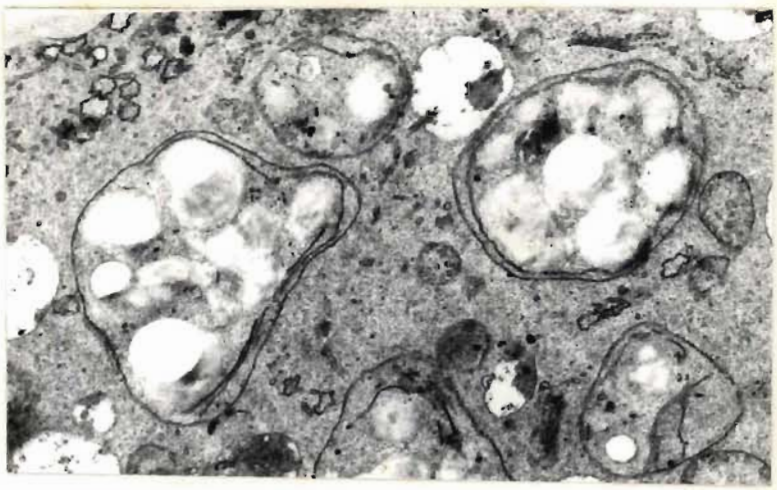
The plastids in cap cells of the 6-day material are similar in all respects to those in comparable cells of unaged material both 24 and 48 hours after the start of imbibition. Also there is no significant variation in the counts of these organelles per unit area of cytoplasm in cap cells of embryos which had received 6 days of the ageing treatment, when compared with unaged material.

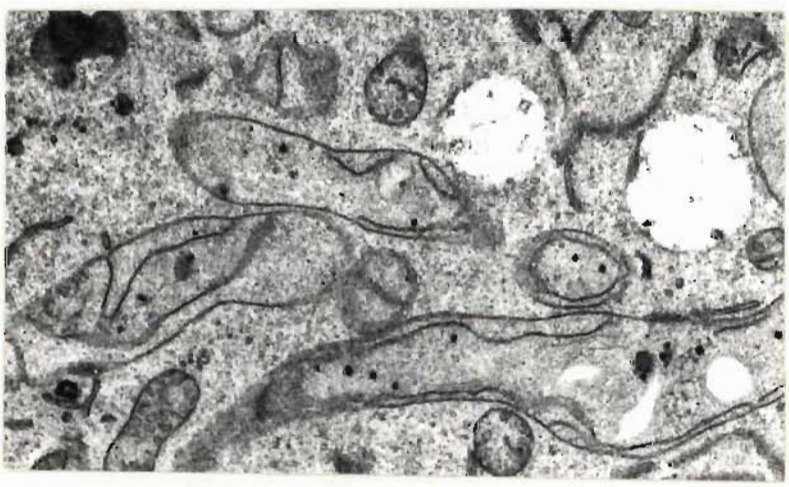
Plastid damage appears to be reversed in all cap cell types of embryos which have been subjected to 12 days of the ageing treatment by the 24-hour germination stage. This might indicate the activity of a genetically-controlled membrane repair system. However, there is a change in the degree of development of these plastids. While starch deposition was seen in plastids in all the cell types of the unaged material at this germination stage, very little starch accumulation had occurred in these organelles in cap cells of material which had been subjected to 12 days of the ageing treatment (Figs. III.E.11a and 11b). This is probably a factor of the decreased rate of the germination process encountered with increasing age of the seed. It is probable that mobilization of the endosperm reserves with subsequent establishment of reserves within the embryo is retarded with increasing age of the seed.

Starch deposition within the plastids in all the cap cells had occurred by the 48-hour germination stage. However, the starch grains were smaller in plastids within some of the cap cell types of embryos which had undergone 12 days of the ageing treatment, than in comparable cells of unaged material. In the 12-day material the average diameters of the starch grains were 750 nm. and 1,050 nm. in cells of the zones of division and differentiation respectively, compared with 920 nm. and 1,300 nm. for the cells in unaged material.

FIGURE III.E.11a. Illustrates plastids in a mature cap cell of material which received 6 days of the ageing treatment, at the 24-hour germination stage. (x 12 650).

FIGURE III.E.11b. Illustrates plastids in which there is little visible starch accumulation in a mature cap cell of material subjected to 12 days of the ageing treatment, at the 24-hour germination stage. (x 16 100).





The starch grains within amyloplasts in mature cells of the 12-day material had an average cross-sectional diameter of 855 nm. which is greater than their average diameter (670 nm.) in comparable cells of unaged material.

These differences may be explained in terms of the lowered germination rate. That is, starch deposition within the embryo is slowed, and in addition, utilization of the reserves in the mature cap cells also proceeds at a lower rate.

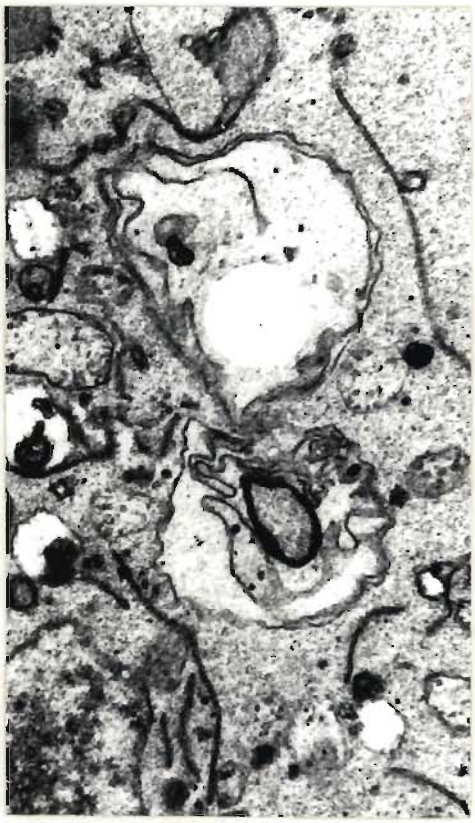
The counts of plastids per unit area of cytoplasm in all the cap cell zones are similar to those obtained for unaged material at both germination stages under consideration.

At both the 24- and 48-hour germination stages, plastids in the cap cells of type 3 aged embryos were very similar in appearance, development and number to those described for 12-day material.

However, in cells of type 2 aged embryos the plastids showed a marked degree of degeneration (Fig. III.E.11c). Membrane damage was not reversed either 24 or 48 hours after the start of imbibition. The outer memberanes of these plastids were markedly distorted. A lack of internal organisation was apparent, and inner membrane damage seemed to be more marked than in this material in the imbibed state. Very little starch occurred in any of the capcell plastids of aged type 2 embryos.

The persistent nature of the plastid damage in cells of type 2 aged embryos again suggests failure of a membrane repair mechanism. In addition the ability either for endosperm mobilization or for starch accumulation within the embryonic cells (or for both) has apparently been seriously impaired, or has been lost. These changes are suggested to

FIGURE III.E.11c. Illustrates plastids in a mature cap cell of a type 2 aged embryo at the 48-hour germination stage. Note that there is visible membrane damage in these organelles. (x 13 500).



be indicative of breakdown in control at the molecular level.

Dictyosomes

Counts of dictyosomes per unit area of cytoplasm indicated that there had been replication of these organelles or re-association of 'unstacked' cisternae during the period 12 - 24 hours after the start of imbibition, in cap cells of embryos which had received 6 days of ageing treatment. The counts were as follows: initials - 2, zones of division, differentiation and maturity - 2, 3 and 4 respectively, compared with the count of 1 dictyosome per unit area of cytoplasm for all comparable cells of 6-day material 12 hours after the start of imbibition.

Dictyosomes in this material were structurally similar to those encountered in unaged material. However, the number of associated cisternae per dictyosome was variable (up to 5) and single cisternae were also encountered (Fig. III.E.12a). Although dictyosomes in cap cells of the 6-day material (at the 24-hour germination stage) showed signs of activity, the vesicles were all relatively very small, having an average cross-sectional diameter of 48 nm. in all the cell types. There was no sign of the relatively large dictyosomal vesicles which were produced in mature cap cells of unaged material at this germination stage.

Dictyosomes are apparently very sensitive to the conditions used to accelerate ageing of the seeds, and the damage caused by only 6 days of the ageing treatment is not totally reversed in the early stages of germination. The damage apparently involves disruption of the intercisternal region, possibly affecting both the intercisternal elements and the bonding constituent.

Most of the dictyosomal damage present 24 hours after the start of imbibition appears to be reversed by the 48-hour

germination stage, in cap cells of embryos which have been subjected to 6 days of the ageing treatment. However, the cisternae still appear to be somewhat loosely associated (Fig. III.E.12b). Dictyosomal counts and dimensions are similar to those for unaged material in all the cap cells of the 6-day material. In addition, the apparent activity of these organelles appears to be normal by the 48-hour germination stage.

Although dictyosomal damage was evident after a short period of the ageing treatment, the control systems for replication and maintenance of these organelles were apparently unaffected, at least at this stage.

Dictyosomes in cap cells of embryos which had received an intermediate period of ageing treatment (12 days) developed in the manner described for the 6-day material at both the 24- and 48-hour germination stages.

However, in type 3 aged embryos at the 24-hour germination stage dictyosomal counts per unit area of cytoplasm were lower for all the cap cell types, than in younger material. Dictyosomes appeared to be absent from the initials, while the counts for the zones of division, differentiation and maturity were 1, 1 and 3 respectively. In addition, dictyosomes consisting of only one, or two loosely-associated cisternae were quite often encountered. Such dictyosomes appeared to be active as vesicles are associated with them (Fig. III.E.12c).

Damage to the dictyosomes is apparently reversed by the 48-hour germination stage in type 3 aged embryos, and counts of dictyosomes in all the cap cell types at this stage are comparable with those obtained for unaged material. The secretory activity, judged by vesicle production, also appeared to be unaffected at the 48-hour germination stage (Fig.III.E.12d).

FIGURE III.E.12a. Illustrates single dictyosomal cisternae in a cap cell of material which received 6 days of the ageing treatment, at the 24-hour germination stage. (x 18 400).

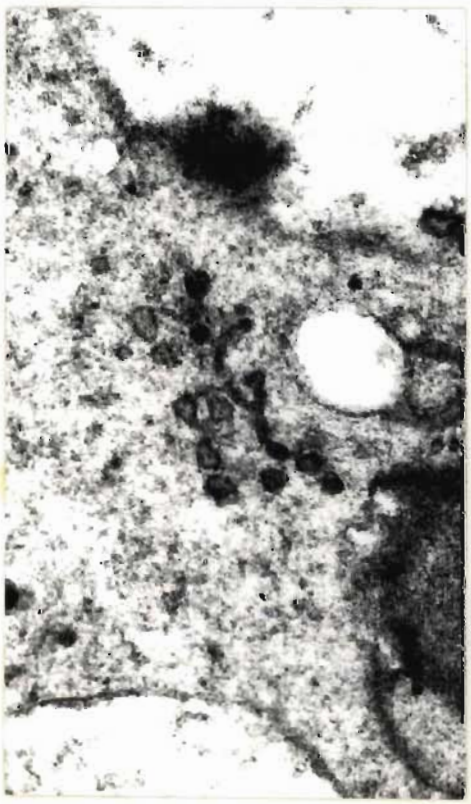
FIGURE III.E.12b. Illustrates a mature cap cell of an embryo which received 6 days of the ageing treatment, at the 48-hour germination stage. Note the apparently loose association of the dictyosomal cisternae. (x 18 850).

FIGURE III.E.12c. Illustrates loosely-associated dictyosomal cisternae which are apparently active. These are illustrated for a cap cell of the zone of differentiation in a type 3 aged embryo, 24 hours after the start of imbibition. (x 43 200).

FIGURE III.E.12d. Active hypersecretory dictyosomes are illustrated in a mature cap cell of a type 3 aged embryo at the 48-hour germination stage. (x 11 500).

a

b



c

d

However, the average cisternal length (of the longest cisterna per dictyosome) is less than that obtained for the unaged material at the 48-hour germination stage, irrespective of the cell type. For example, the average cisternal lengths in cells of the zones of differentiation and maturity were 362 nm. and 580 nm. respectively, compared with 650 nm. and 850 nm. in these cells in unaged material.

It appears that the control mechanisms for dictyosome elaboration and activity are functional in type 3 aged embryos.

In the cap cells of type 2 aged embryos both at the 24- and 48-hour germination stages, no recognisable dictyosomes were present. This suggests that those dictyosomes which were originally present have become disorganised, and that the control mechanisms for elaboration and activity of these organelles have become seriously impaired or non-functional.

Endoplasmic Reticulum

Development of the ER in cells of the various root cap zones in material which had received a short ageing treatment (6 days) was not changed from the development of this organelle in unaged material. In the 6-day material at the 24-hour germination stage the ER was very sparse in the initials. A profusion of short ER profiles occurred mainly in the perinuclear region of cells of the zones of division and differentiation, while in mature cells short ER profiles were orientated parallel with one another throughout the cytoplasm. At the 48-hour germination stage the ER was sparse in the initials while in cells of the zones of division and differentiation the profiles were more developed and orientated. In mature cells of the 6-day material at the 48-hour germination stage the ER profiles were orientated mainly parallel with the cell peripheri and the nucleus.

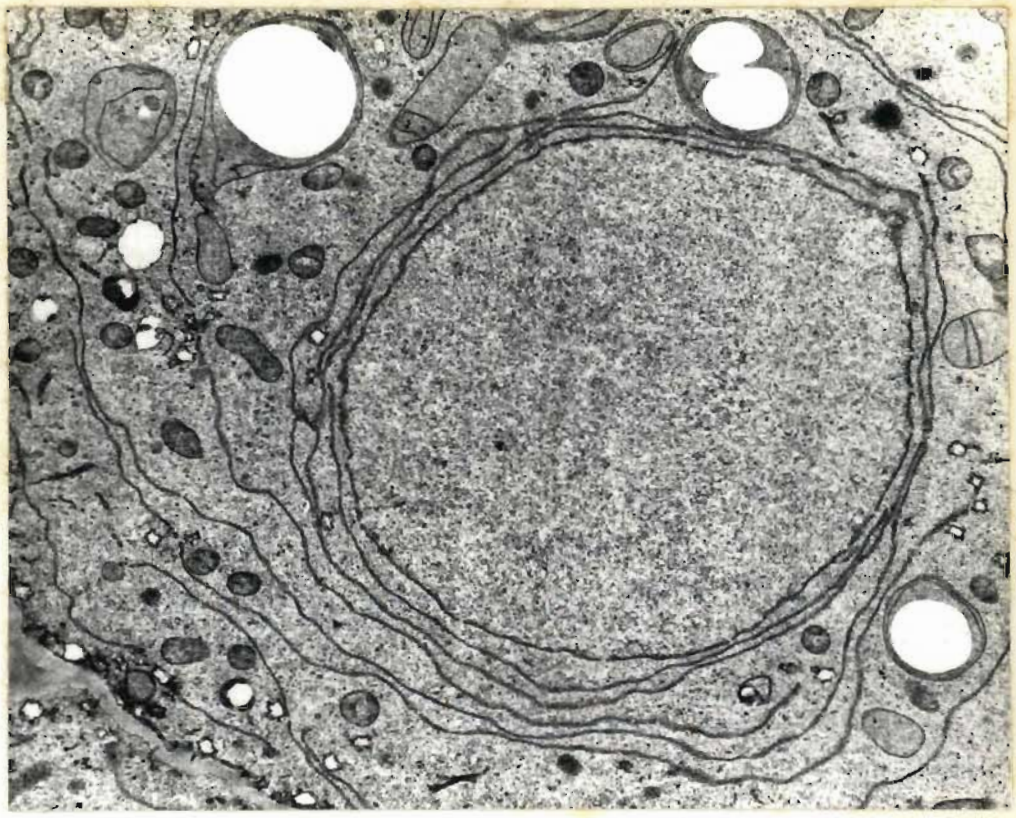
However, changes occurred in ER development with increasing age of the embryos. In material which had received an intermediate ageing treatment (12 days) the ER profiles in the cap initials were typically short and scattered at the 24-hour germination stage. However, ER profiles were atypically long in the chronologically older cells within the cap of this material. Figure III.E.13a illustrates the long ER profiles which occurred loosely parallel with one another and with the nuclear membrane, and also scattered in the cytoplasm in cells of the zone of differentiation, 24 hours after the start of imbibition. The profusion of short ER profiles which occurred in the perinuclear region of these cells in unaged material at this germination stage was not seen in any of the cap cells of of the 12-day material. Mature cap cells of the 12-day material at the 24-hour germination stage also showed ER development which was atypical for comparable cells of unaged material. The profiles were relatively long, some of them being orientated loosely parallel with the nucleus, but most of them occurred scattered in the cytoplasm (Fig. III.E.13b).

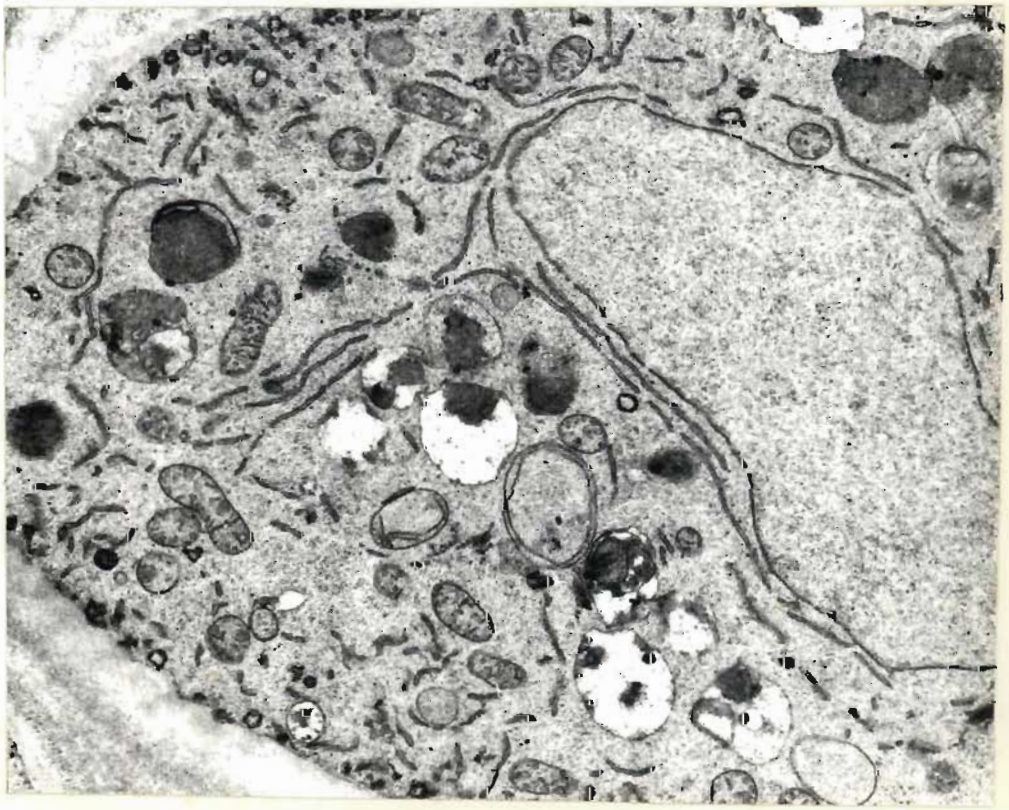
In cap cells of imbibed 12-day material, ER profiles were often distorted, especially in the mature cells. Such distortion appears to be reversed by the 24-hour germination stage. It is possible that the atypical length of ER profiles in the cap cells of this material at the 24-hour germination stage might result from membrane weakness, with distortion occurring in the lengthwise direction. However, and what is more likely, this development of the ER might represent a type of 'compensatory' mechanism by which respiratory function is partly transferred to this organelle as a result of decreased efficiency of the mitochondrial system.

Development of the ER in the cap cells of the 12-day material at the 48-hour germination stage was comparable with

FIGURE III.E.13a. Illustrates the long ER profiles which occur loosely parallel with each other and with the nuclear membrane, in a cell of the zone of differentiation in material which received 12 days of the ageing treatment, at the 24-hour germination stage. (x 10 350).

FIGURE III.E.13b. Shows ER development in a mature cap cell of material which was subjected to 12 days of the ageing treatment, 24 hours after the start of imbibition. (x 13·050).





that in unaged material. Figure III.E.13c shows the development and orientation of the ER in a mature cap cell (the nucleus of which is markedly lobed) at the 48-hour germination stage. Apparently the control mechanisms regulating ER development and its repair and maintenance are functional and unaltered in embryos which have received 12 days of the ageing treatment, as the earlier distortion and atypical development of this organelle had been reversed by the 48-hour germination stage.

Root cap cells of type 3 aged embryos had a pattern of ER development which was similar to that described for the 12-day material at both the 24- and 48-hour germination stages. However, orientation and development of the ER in cells of type 2 aged embryos differed strikingly when compared with any other material whether aged or not.

At the 24-hour germination stage ER profiles were sparse and relatively short, and occurred scattered in the cytoplasm especially in the perinuclear region (Fig. III.E.13d) in all the cap cell types. No aggregations of ER profiles occurred in this material.

By the 48-hour germination stage, striking proliferation and atypical orientation of the ER had occurred. Banks of long parallel ER profiles occurred, encircling the perinuclear region and the ends of the profiles were often distended (Fig. III.E.13e).

The proliferation of the ER in these cells is suggested to result from uncontrolled derepression of the genes responsible for the elaboration of this organelle, implying that the cells are not 'technically dead' at this stage. It is considered unlikely that this represents a 'compensatory mechanism' in these highly disorganised cells.

FIGURE III.E.13c. Shows development and orientation of the ER in a mature cap cell of material which received 12 days of the ageing treatment, at the 48-hour germination stage. Note the marked nuclear lobing. (x 11 500).

FIGURE III.E.13d. Illustrates short, scattered ER profiles in the perinuclear region, in a cap cell of the zone of differentiation of a type 2 aged embryo at the 24-hour germination stage. (x 11 900).

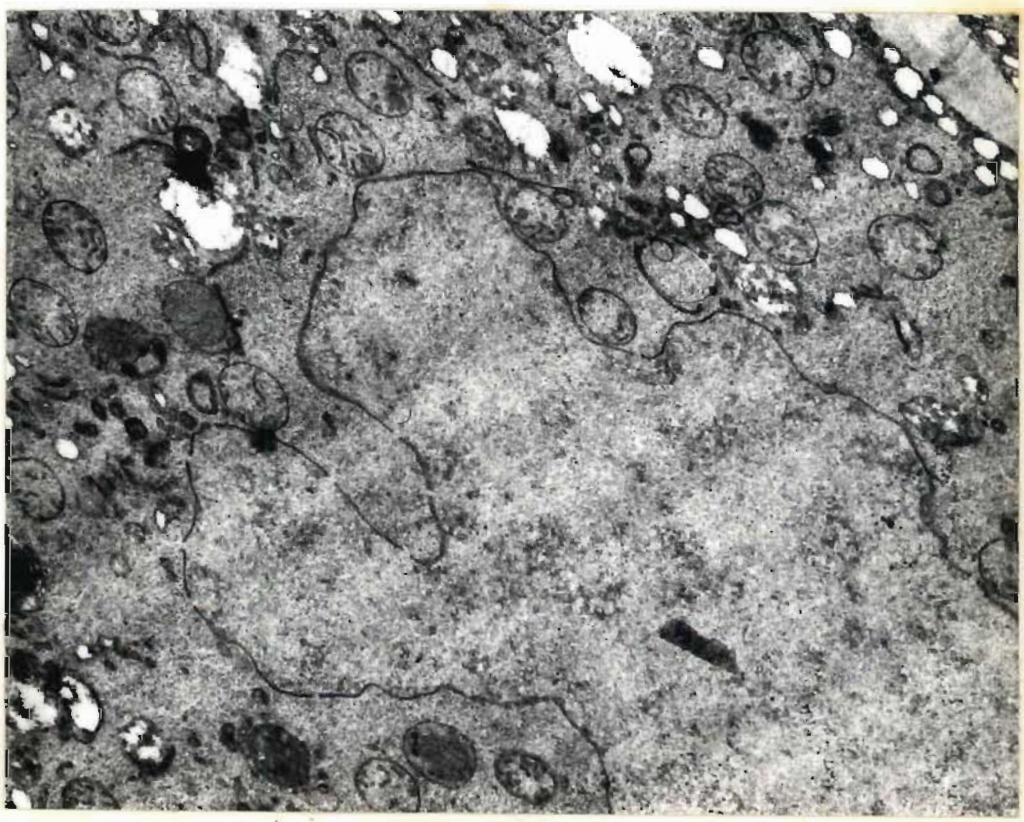
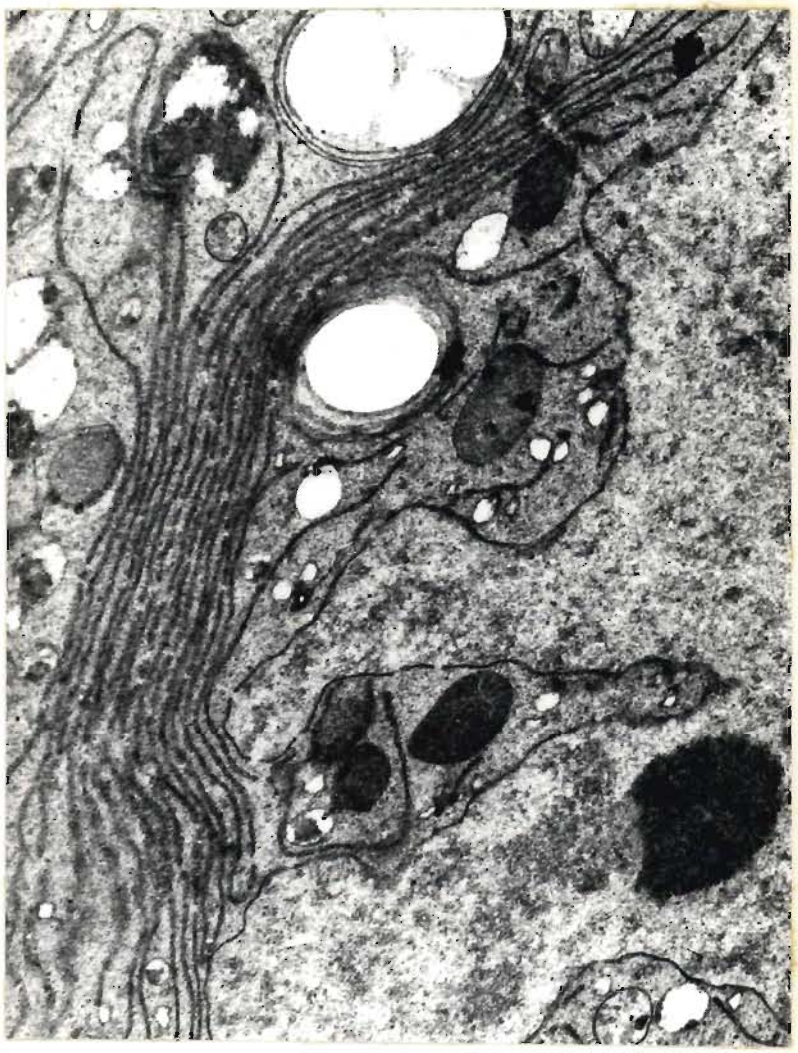


FIGURE III.E.13e. Illustrates the striking proliferation of the ER in the perinuclear region of a cap cell in a type 2 aged embryo, 48 hours after the start of imbibition. (x 10 800).



Ribosomes

Ribosomes were mainly aggregated, forming polysomes in all cap cell types (excepting the outermost cells as before stated in the case of unaged material) of embryos which had received short (6 days) and intermediate (12 days) periods of the ageing treatment, at the 24-hour germination stage. This was also the case for these embryos 48 hours after the start of imbibition (Fig. III.E.14a). In addition, at the 48-hour germination stage, polysomes also occurred in the outermost cap cells. This is probably not due to polysome formation in the cells which comprised the outermost layer at the earlier germination stages, those cells having been largely sloughed off. The cells which comprise the outermost cap layer by the 48-hour germination stage have been derived from the mature zone during the period 24-48 hours after the start of imbibition, and are in early stages of senescence. Cytoplasmic disorganisation had not occurred to any great extent within these cells.

Polysome formation had occurred in cap cells of type 3 aged embryos (again, with the exception of the outermost cells) by the 24-hour germination stage, although only monosomes were present in these cells in the fully-imbibed condition (Fig. III.E.14b). At the 48-hour germination stage polysome formation occurred in all the cap cells of type 3 aged embryos (Fig. III.E.14c). Cap cells of type 2 aged embryos also contain polysomes both 24 and 48 hours after the start of imbibition.

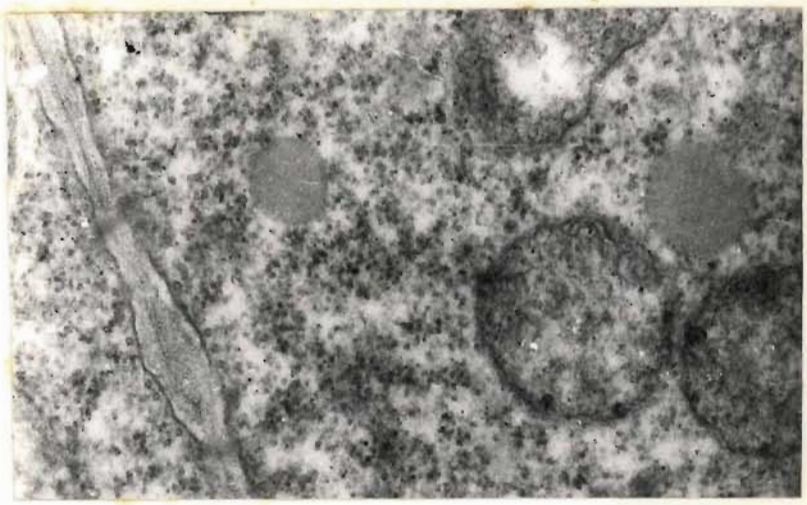
It is thought that initial formation of polysomes (which is evident after only 6 hours of imbibition in unaged material) is dependent on long-lived m-RNA present in the quiescent seed (e.g. Dure and Waters, 1965). However, it is probable that synthesis of new RNA must also be effected relatively early

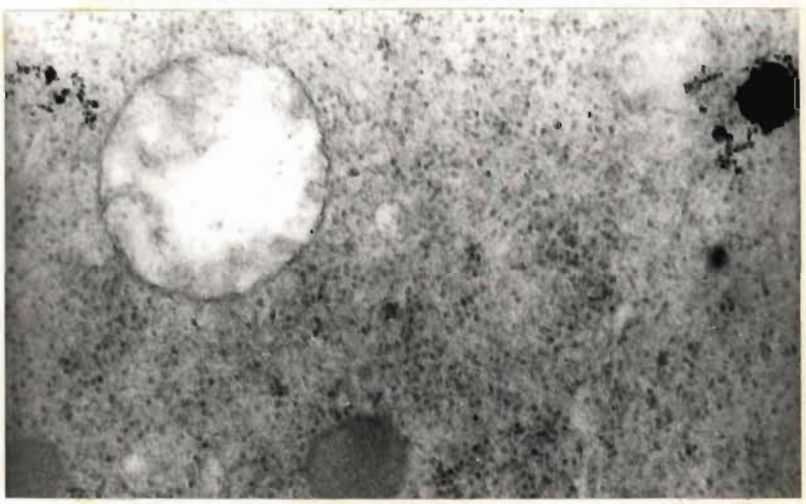
FIGURES III.E.14a - 14c. Illustrate polysomes in cap cells of embryos at selected stages in the ageing sequence. The material used for the ribosome survey was postfixed in an osmium solution according to Procedure 6b.

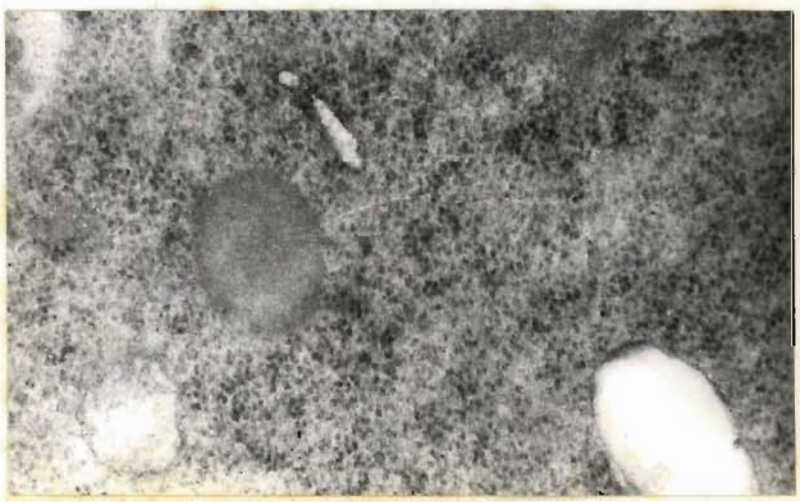
14a. Illustrates polysomes in a mature cap cell of material which received 6 days of the ageing treatment, at the 48-hour germination stage. (x 48 200).

14b. Illustrates polysomes in a cap cell of the zone of division in a type 3 aged embryo, at the 24-hour germination stage. (x 44 800).

14c. Illustrates polysomes in a cap cell of the zone of differentiation in a type 3 aged embryo, 48 hours after the start of imbibition. (x 44 800).







in the germination process (e.g. Key, 1964). The fact that polysome formation is somewhat delayed in type 3 aged embryos may be attributed to the generally lowered germination efficiency which occurs with increasing age of the seed. It seems probable that at least some of the polysomes in cells of type 3 aged embryos 24 and even 48 hours after the start of imbibition are dependent on the long-lived stored m-RNA present in the quiescent seed. It is possible that the lowered rate of germination shown by these seeds might be at least partly due to delayed initiation in the synthesis of new m-RNA (or its synthesis at a lower rate) suggested to be necessary even for the early stages of cell elongation (e.g. Key, 1964).

Formation of polysomes in cells of type 2 aged embryos (by the 24-hour germination stage) probably indicates that long-lived m-RNA had not been degraded. In addition, the manifestation of what might be 'nonsense information' in the proliferation of ER at the 48-hour germination stage, indicates that new m-RNA was probably being formed, but was a result of uncontrolled synthesis.

Lipid Droplets

The disposition of lipid droplets in the cytoplasm of cap cells of 6-day material is similar to that described for unaged material both at the 24- and 48-hour germination stage (Fig. III.E.15a).

However, in the cap cells of material which had received 12 days of the ageing treatment, the disposition of lipid droplets in the cytoplasm had not changed much in the period 12 - 24 hours after the start of imbibition (Fig. III.E.15b). By the 48-hour germination stage, however, there was little sign of reserve lipid in any of the cap cells of the 12-day material (Fig. III.E.15c). This probably indicates a very

rapid mobilization and utilisation of these reserves. If this is considered in conjunction with the apparently increased rate of mitochondrial replication in the cap initials and the elaboration of the ER in the cap cells generally in this material it seems possible that these reserves have been utilised in the elaboration of membranes. In addition, if general membrane repair is occurring in the cap cells at this stage, then reserve lipid might well be utilised.

The same general situation as described for 12-day material exists, regarding disposition and apparent utilisation of lipid reserves in cap cells of aged type 3 embryos at both the 24- and 48-hour germination stages.

However, in cells of type 2 aged embryos there is a change in the pattern of lipid disposition in the cytoplasm. The droplets were not situated peripherally at the 24-hour germination stage, but occurred interspersed among the disorientated organelles in the perinuclear region (Fig. III.E.15d). There was evidence of the partial depletion of lipid reserves in this material 48 hours after the start of imbibition. It is probable that these reserves had been partially utilised in the elaboration of the ER which occurs in these cells.

Wall

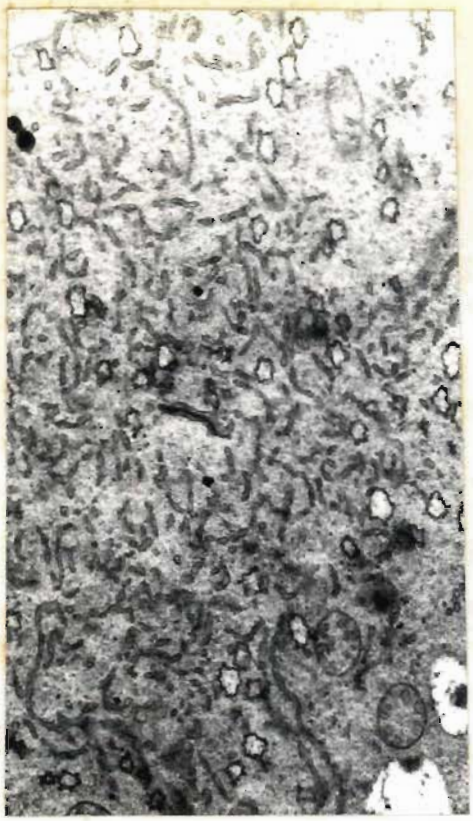
There is no apparent degenerative change in the walls of any of the cap cells, with progressive age of the material. However, in type 1 aged embryos there is some evidence of wall breakdown (see below).

FIGURE III.E.15a. Illustrates the disposition of lipid in the region of an aggregation of short ER profiles in a cell of the zone of differentiation in material which received 6 days of the ageing treatment, at the 24-hour germination stage. (x 13 800).

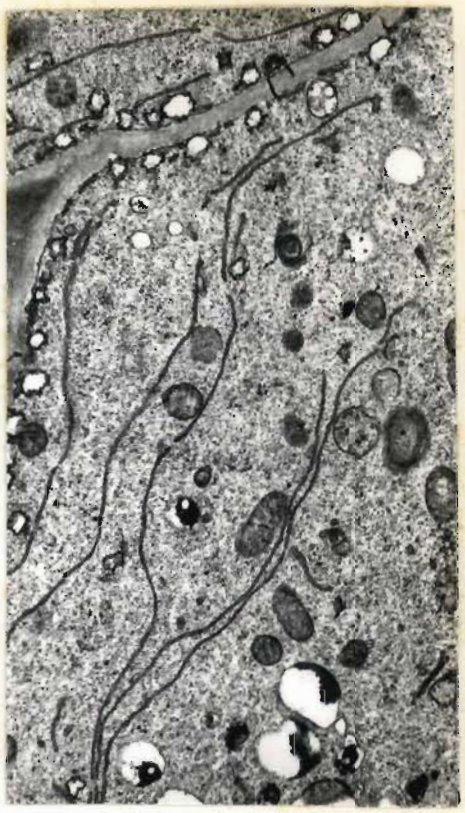
FIGURE III.E.15b. Shows that the lipid is orientated in the peripheral cytoplasm in cap cells of material which received a 12-day ageing treatment, at the 24-hour germination stage. (x 9 200).

FIGURE III.E.15c. Illustrates that lipid droplets are not in evidence in cap cells of material which received a 12-day ageing treatment, at the 48-hour germination stage. A cell of the zone of division is illustrated. (x 12 650).

FIGURE III.E.15d. Shows the lipid droplets to be interspersed among the disorientated organelles in the perinuclear area of a cap cell of a type 2 aged embryo, at the 24-hour germination stage. (x 10 350).



a



b

c

d

F. PATTERNS OF SENESCENCE

Ultrastructural studies of aged embryos which had been stored for 18 to 20 days at 40°C and 14% moisture content revealed that extreme degenerative changes in cells, which are interpreted as being irreversible, did not follow one pattern only. This suggests that death in stored seeds (as in other organisms) may not be attributable to any single cause.

Three basic patterns of death in embryos of stored seed were encountered. These are apparently elaborations of the three types of degenerative changes initially observed in imbibed aged material.

F.1. TYPE 1 AGED EMBRYOS

Certain aged embryos (18 and 20 days of treatment) appeared non-viable when examined 12, 24 and 48 hours after the start of imbibition. Generally all the root cap cells of such embryos appeared to have undergone extreme degenerative changes. Cellular degenerative changes which were encountered after 12 hours of imbibition can be followed through 24 and 48 hours after the start of imbibition.

Nucleus

Nuclei of cells of type 1 aged embryos showed extreme lobing, with the chromatin staining in dark patches (with potassium permanganate) (Fig. III.F.1.), 12 hours after the start of imbibition. Both nuclear lobing and the staining properties of the chromatin are interpreted as degenerative changes, and have been observed in nuclei of viable, but aged, embryos. The nucleoli are not visible in the nuclei of these cells. This senescent change is not encountered in ageing material generally, nor in type 2 or type 3 aged embryos.

FIGURE III.F.1. Illustrates portion of the nucleus in a cap cell of a type 1 aged embryo, 12 hours after the start of imbibition. Note the darkly-stained clumps of chromatin. (x 11 500).

Mitochondria

Mitochondria in the cap cells of type 1 aged embryos showed signs of extreme degeneration at the 12-hour germination stage. These organelles were swollen (average cross-sectional diameter 702 nm) and there was a marked reduction in the density of the matrix (Fig. III.F.2.). Distortion of the mitochondrial profile, which was the first degenerative change encountered in these organelles, was completely reversed in these cells. However, reversal of the mitochondrial distortion was presumably caused by their swelling. It is suggested that the early mitochondrial damage, which results in membrane distortion, represents fundamental changes in the membranes. In the cells of type 1 aged embryos, which are suggested to be non-viable, there is presumably no genetically-controlled maintenance of mitochondrial or other membranes. Apparently, control of transport across the damaged membranes is lost, resulting in the swollen mitochondria and their "diluted" appearance.

Lysosomes.

Lysosomes encountered in the cap cells of type 1 aged embryos after 12 hours of imbibition were not the compact organelles observed in comparable unaged material. The membrane bounding the lysosomes in this material was not closely applied to the surface of the dense contents, and these organelles generally gave the impression of being in their second developmental phase (Fig. III.F.3.) However, it is not thought that the lysosomes have followed an ordered developmental sequence. Lysosomal membranes, although presumed to have properties differing somewhat from other cell membranes, are also suggested to have undergone basic changes during the ageing treatment. The swelling of this organelle in type 1 material is attributed to these changes which have resulted in the loss of the properties normally attributed to the lysosomal membrane. Some of the lysosomes in this

FIGURE III.F.2. Shows mitochondria in a cap cell of a type 1 aged embryo, at the 12-hour germination stage. These organelles are swollen and there is a marked reduction in the density of the matrix. (x 11 500).

FIGURE III.F.3. Illustrates lysosomes in a cap cell of a type 1 aged embryo, at the 12 hour germination stage. Note the swollen appearance of these organelles, and the apparent dissolution of the bounding membrane (at arrows). (x 16 800).

material showed a degree of dissolution of their bounding membranes.

Plastids

Striking degenerative changes were observed in the plastids of cap cells of type 1 aged embryos, 12 hours after the start of imbibition. These organelles were extremely swollen and showed little indication of an internal membrane system. In addition, there was a marked lessening in the density of their contents (Fig.III.F.4).

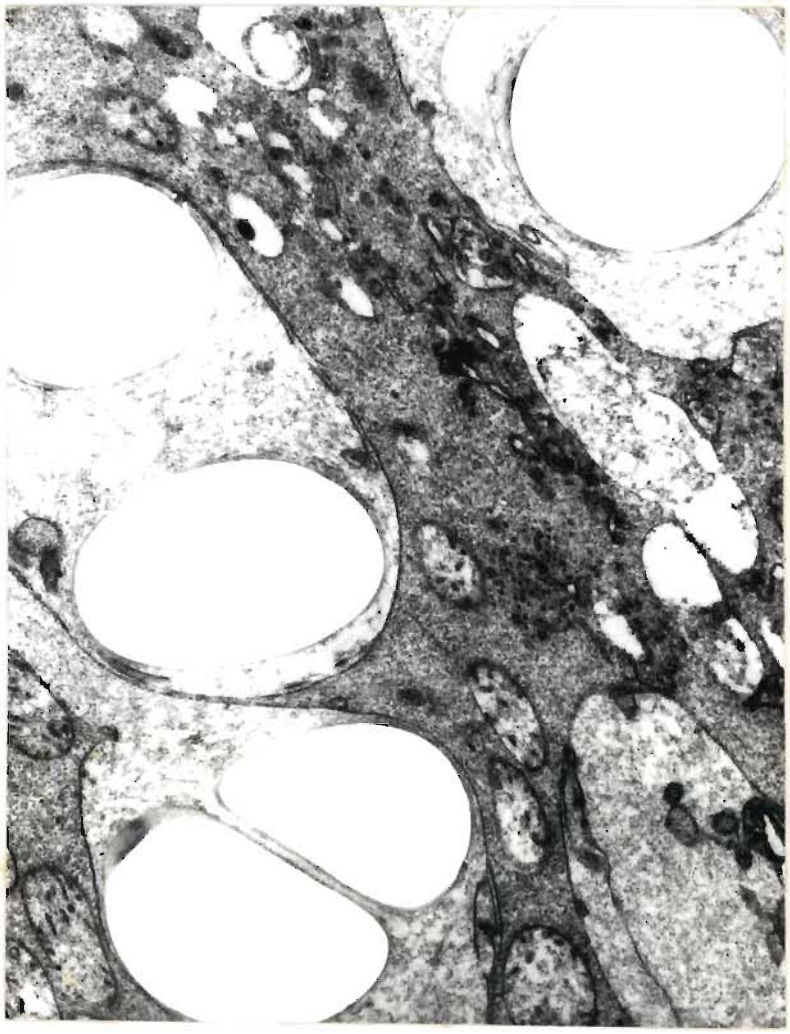
Plastids were among the earliest of the organelles to show degenerative change (after only 6 days of the ageing treatment), and the change was manifest as membrane aberration. It is suggested that the marked degree of swelling observed in the plastids is a consequence of the earlier membrane abnormality which has become irreversible during storage. It would appear that transport control across the membranes of the plastids has been completely lost, resulting in their extreme swelling.

These plastids apparently contain starch reserves, which is atypical for cap cells of imbibed material. It is suggested that plastids which occur in the cap cells of mature seed do contain some starch, but that this reserve is utilised during the imbibition phase. Thus in normal embryos these organelles are largely in the form of (secondary) proplastids, and not amyloplasts. Plastids in unaged material after 2 and 4 hours of imbibition have been observed to contain starch grains. The cells of type 1 aged embryos are suggested to have lost their viability during the storage period. Thus their carbohydrate reserve is not utilised and persists in the plastids.

Dictyosomes

No subcellular structures that can be identified as dictyosomes were present in the cells of imbibed type 1 aged embryos. This is in accordance with the apparent disorganisation of these organelles encountered early in the ageing sequence.

FIGURE III.F.4. Shows the extremely swollen plastids typical of cap cells of type 1 aged embryos, 12 hours after the start of imbibition. (x 17 100).



Endoplasmic Reticulum.

Short, distended profiles, presumed to represent sections of the degenerated ER, were encountered in the cytoplasm of cells of type 1 aged embryos 12 hours after the start of imbibition (Fig. III.F.5). However, as these profiles were only seldom encountered in any of the cap cells of this material, it is suggested that this organelle has virtually completely degenerated during storage of the seed.

Degeneration of the ER is suggested to be basically due to irreversible changes in the membranes. However, there is a possibility of action of hydrolases. These enzymes are normally confined within the lysosomes but these organelles are sometimes encountered with their bounding membranes incomplete in this material.

Ribosomes

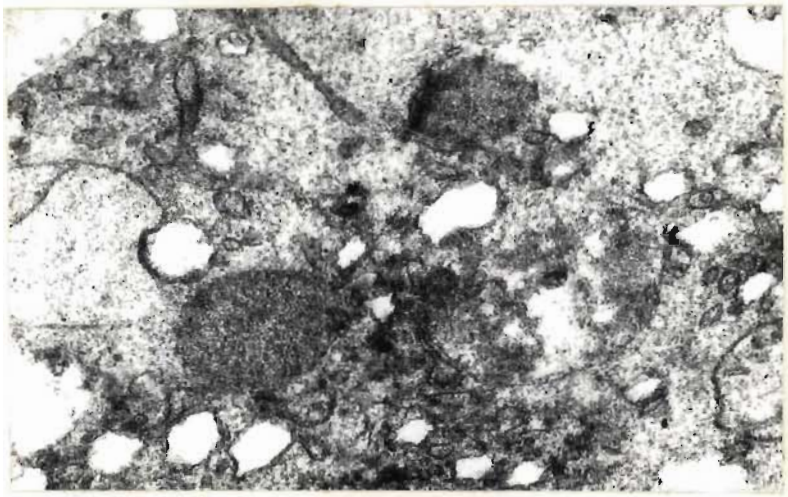
Monosomes occurred throughout the cytoplasm of cap cells of type 1 aged embryos 12 hours after the start of imbibition (Fig.III.F.6). This was expected in view of the fact that polysomes had not formed by the equivalent stage in viable (type 3) aged embryos. It is notable that only monosomes occurred 24 and 48 hours after the start of imbibition in the cap cells of type 1 aged embryos.

Lipid Droplets

Lipid droplets occurred scattered randomly throughout the cytoplasm in cap cells of type 1 ages embryos which had been imbibed for 12 hours. (Fig. III.F.7.). However, this is not taken to indicate any ordered initiation of reserve utilisation, but rather random disorientation accompanying viability loss which occurred during storage.

FIGURE III.F.5. Illustrates the short, distended ER profiles which are the only trace of this organelle in cap cells of type 1 aged embryos at the 12-hour germination stage. (x 17 100).

FIGURE III.F.6. Shows monosomes in the cytoplasm of a cap cell of a type 1 aged embryo, 12 hours after the start of imbibition. The material was postfixed in an osmium solution according to Procedure 6b. (x 39 200).



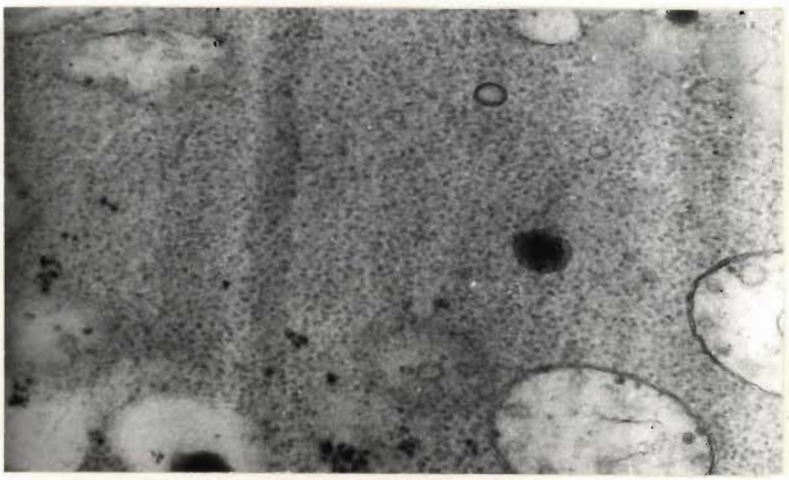
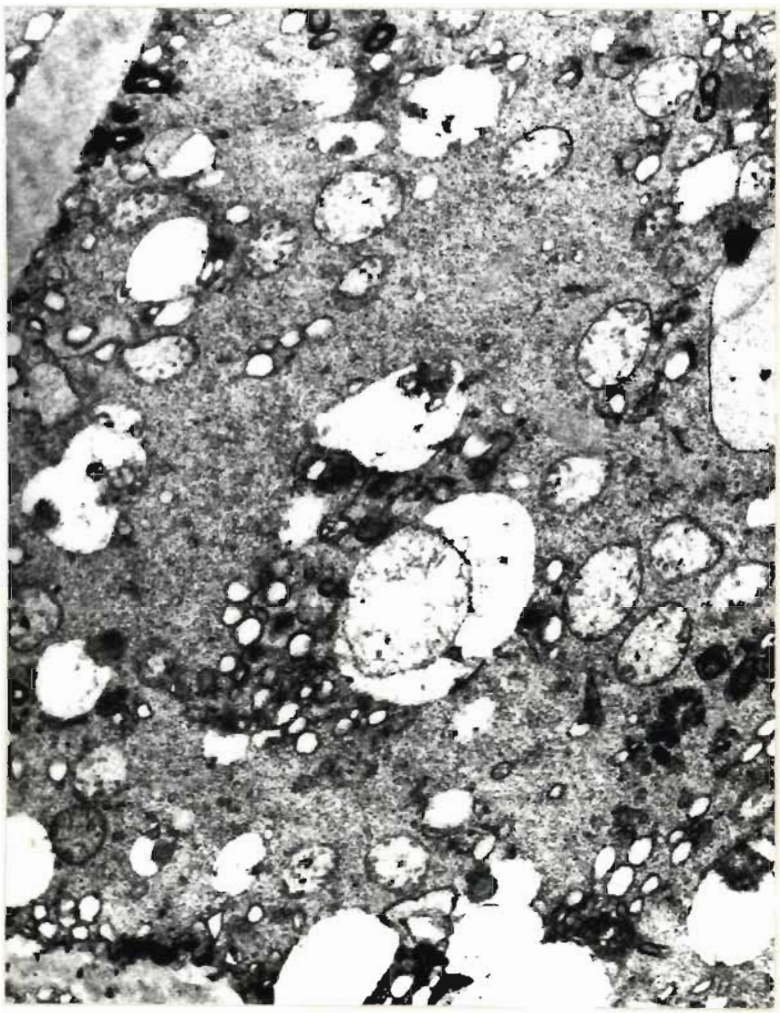


FIGURE III.F.7. Illustrates the random disposition of lipid droplets in the cytoplasm of a cap cell of a type 1 aged embryo, 12 hours after the start of imbibition. (x 16 100).



Wall and Plasma Membrane

Complete cell wall breakdown was occasionally encountered in type 1 aged embryos 12 hours after the start of imbibition (Fig. III.F.8a). This degenerative change, which accompanied viability loss during storage, was not encountered before in embryos during the ageing sequence.

Wall breakdown was generally accompanied by breakdown of the plasma membrane in that region (Fig. III.F.8b). In certain cells of the presumably non-viable, type 1 aged embryos, 12 hours after the start of imbibition, the plasma membrane had moved inwards, away from the wall, giving the entire cell a somewhat plasmolysed appearance. The resultant open "space" between the plasma membrane and the cell wall appeared to be filled with a finely granular deposit. Such cells showed a positive reaction for acid phosphatase in the cytoplasm (see below), and it suggested that hydrolytic reaction products might accumulate between the wall and plasma membrane (Fig. III.F.8c).

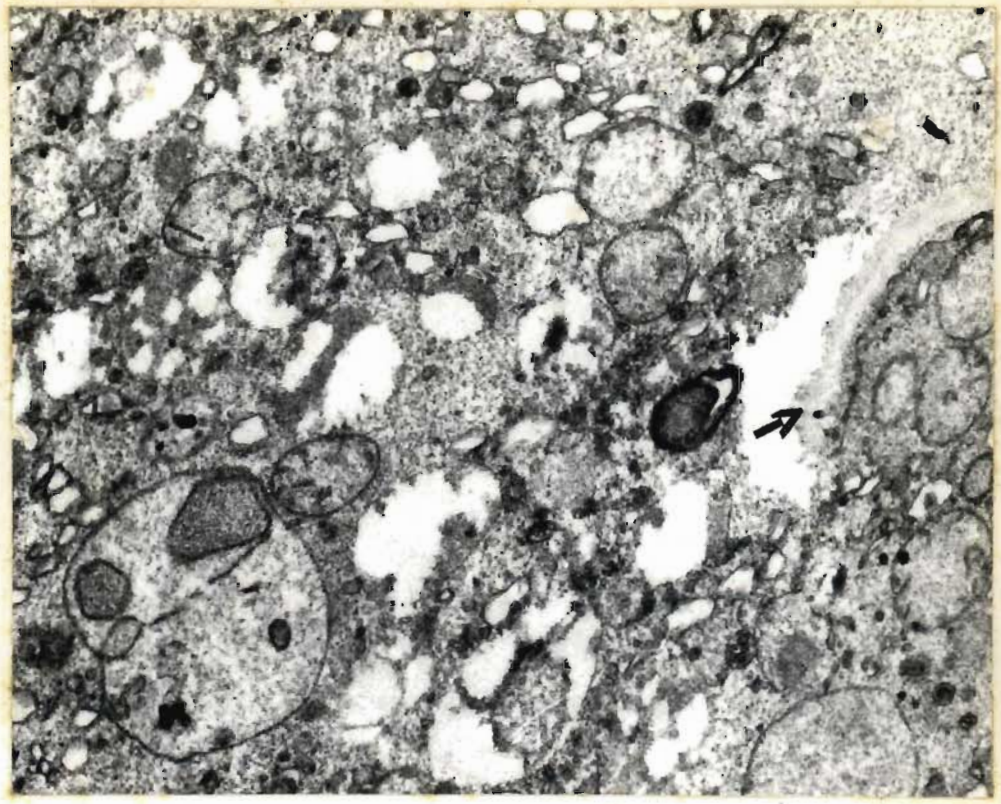
Histochemistry of Type 1 Aged Embryos

Activity of acid phosphatase occurred generally scattered in the cytoplasm of cap cells of type 1 aged embryos. In addition, there was marked activity of this enzyme within intact lysosomes (Fig. III.F.9a) (which was not the case in lysosomes of comparable unaged material). Sodium fluoride was used as an enzyme inhibitor in the control material, which showed no reaction for the activity of this enzyme (Fig. III.F.9b). It is suggested that the enzyme is present mostly in an inactive (bound) form within fully-formed first phase lysosomes in unaged material, but that it is somehow stimulated by the ageing treatment to appear in the active form 12 hours after the start of imbibition.

However, the general degradative changes encountered in cap cells of type 1 aged embryos are probably not brought about

FIGURE III.F.8a. Shows a region in the root cap of a type 1 aged embryo at the 12-hour germination stage, where cell wall breakdown has occurred (at arrow). (x 17 250).

FIGURE III.F.8b. Illustrates wall breakdown, accompanied by dissolution of the plasma membrane in that region, in the root cap of a type 1 aged embryo at the 12-hour germination stage. (x 11 500).



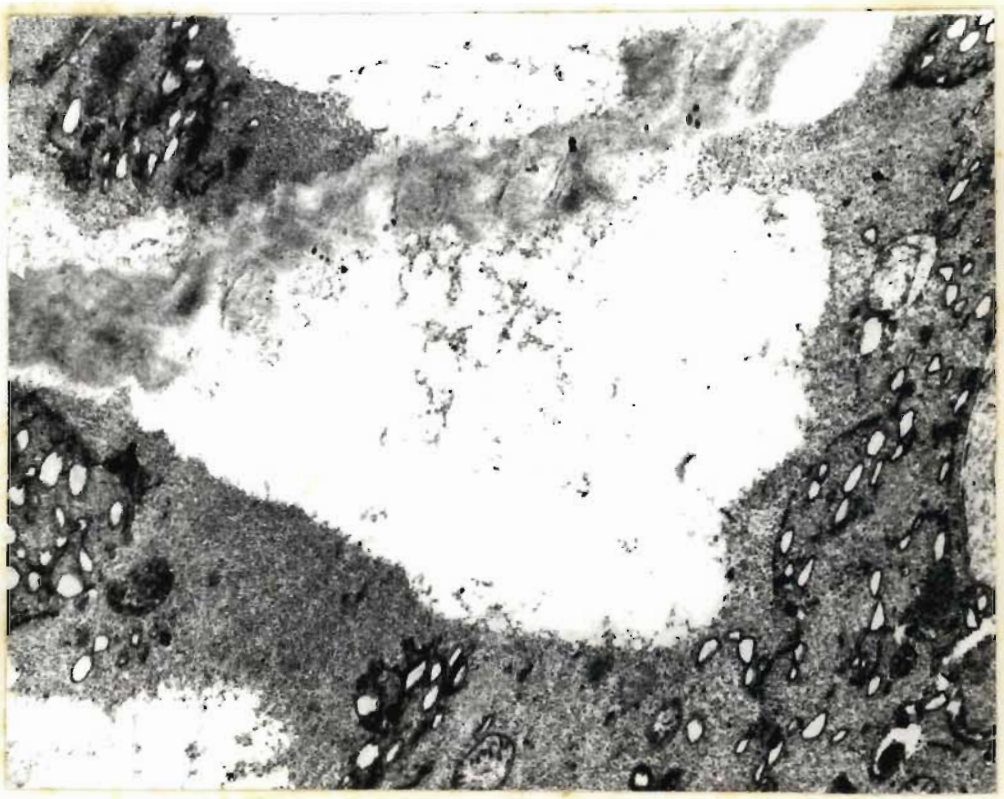


FIGURE III.F.8c. Shows activity of acid phosphatase in the cytoplasm and the accumulation of a finely-granular substance suggested to be hydrolytic reaction product, between cell wall and plasma membrane. A cap cell of a type 1 aged embryo at the 12-hour germination stage is illustrated. (x 10 350).

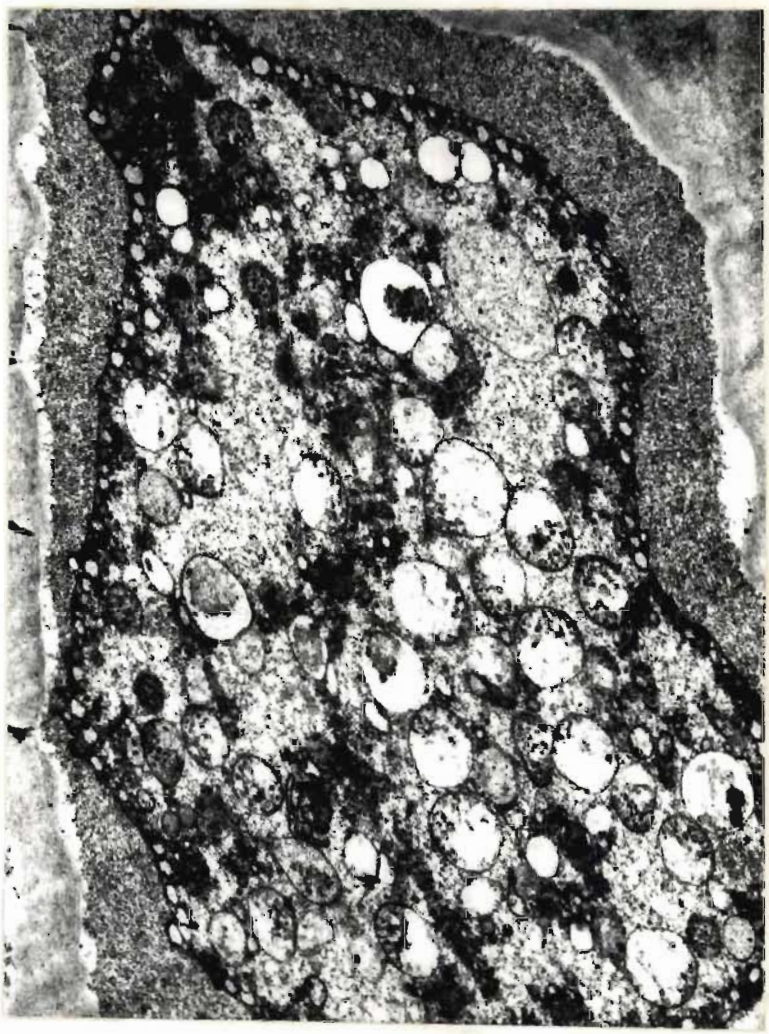
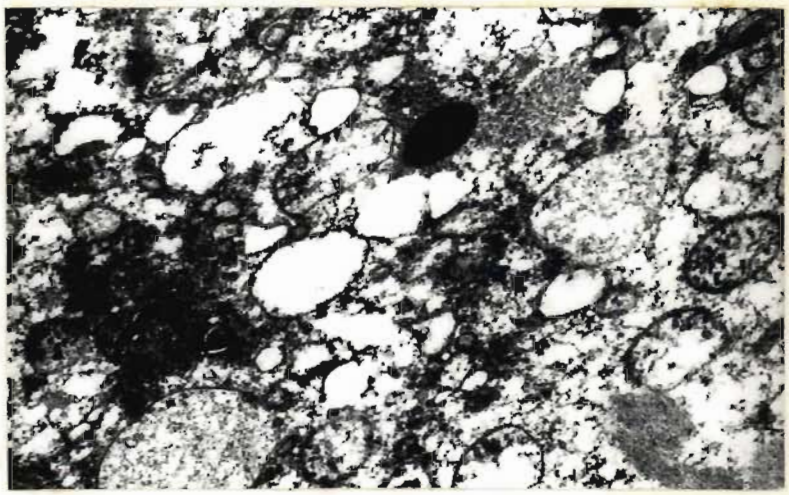
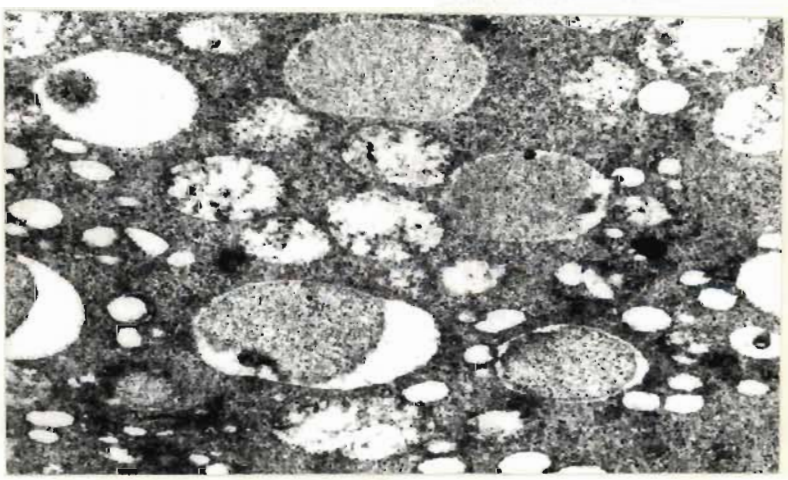


FIGURE III.F.9a. Illustrates acid phosphatase activity both within an intact lysosome and in the cytoplasm in a cap cell of a type 1 aged embryo 12 hours after the start of imbibition. (x 13 300).

FIGURE III.F.9b. No acid phosphatase activity is seen in the lysosomes or in the cytoplasm in cap cells of type 1 aged embryos at the 12-hour germination stage, when sodium fluoride was used as an enzyme inhibitor. (x 13 300).





by hydrolases, as most of the organelles are intact, although distorted. In addition it is unlikely that hydrolytic activity could occur to any marked extent, or that hydrolases could diffuse through the cytoplasm, in the relatively dehydrated conditions which prevail within the cells of mature stored seeds.

All the degenerative changes which occurred in the organelles within the stored seed appear to result from alterations in the membranes. In the cells of type 1 aged embryos these membrane alterations appear generally to have reached the point of being irreversible.

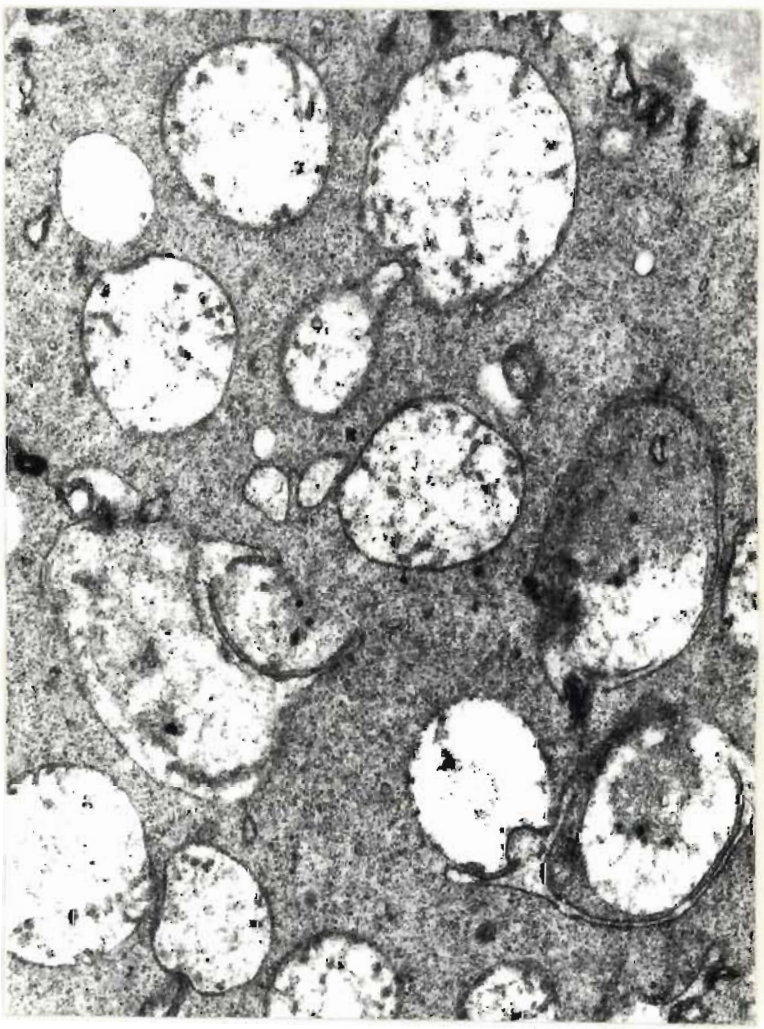
Type 1 aged embryos 24 and 48 hours after the start of imbibition.

Intact mitochondria in cap cells (irrespective of cell zone) continued to swell during the 12 to 24 hours period after the start of imbibition, and their average cross-sectional diameter was 860 nm (Fig. III.F.10).

The general state of deterioration encountered in this material was more advanced than at 12 hours after the start of imbibition and there was evidence of organelles in early stages of breakdown. This is attributed to release of active hydrolytic enzymes normally encountered within the lysosomes. Few intact lysosomes remained in the cells of this material, and acid phosphatase activity occurred scattered throughout the cytoplasm and in association with partially-degraded organelles (Figs. III.F.11a and 11b). No acid phosphatase activity occurred in the control material, where sodium fluoride was used as an enzyme inhibitor (Fig. III.F.11c).

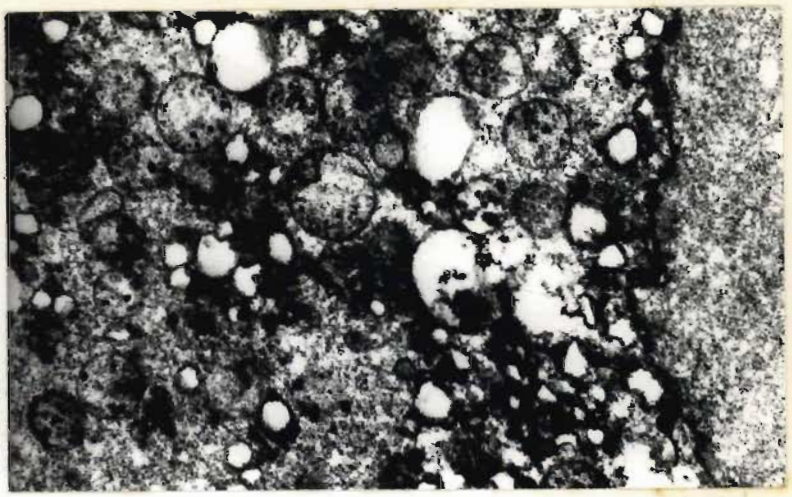
By 48 hours after the start of imbibition all stages of intracellular destruction were encountered in cap cells of type 1 aged embryos. Figures III.F.12a to 12e illustrate the sequence

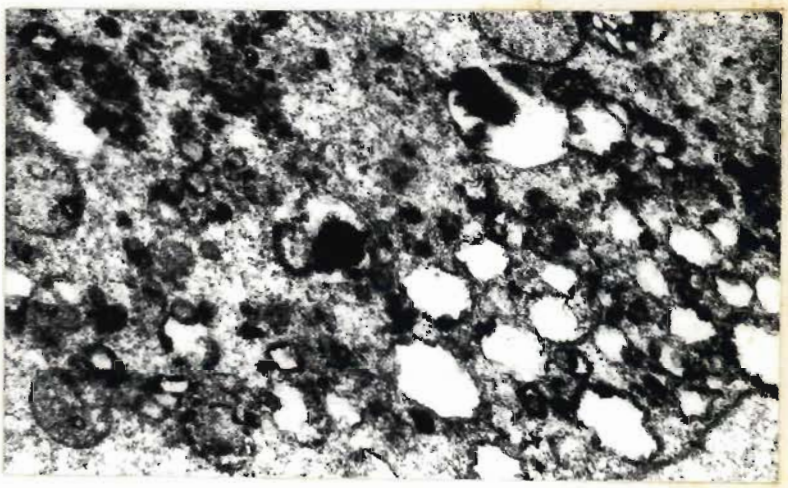
FIGURE III.F.10. Illustrates swollen, disorganised mitochondria in a cap cell of a type 1 aged embryo 24 hours after the start of imbibition.
(x 32 400).

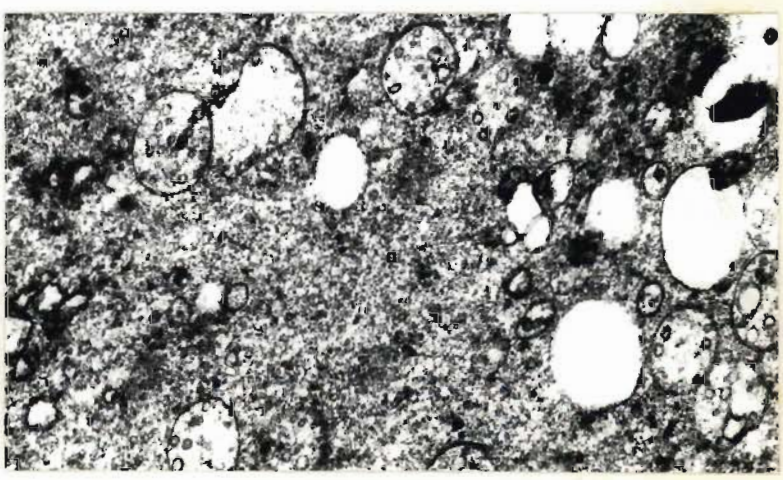


FIGURES III.F.11a & 11b. Illustrate that acid phosphatase activity is located in the cytoplasm and in the lysosomes in cap cells of type 1 aged embryos, 24 hours after the start of imbibition. (11a x 13 300; 11b x 22 750).

FIGURE III.F.11c. Illustrates that no activity of acid phosphatase occurs in cap cells of type 1 aged embryos at the 24-hour germination stage, with the use of sodium fluoride as an enzyme inhibitor. (x 18 900).







of destruction encountered. There was progressive disappearance of virtually all the organelles and progressive accumulation of a granular deposit between the cell wall and the plasma membrane in most cases. In a few cells, however, the granular deposit occurred within the bounds of the plasma membrane. The granular deposit is taken to represent cytoplasmic remains, containing hydrolytic reaction products. In the penultimate stages of cellular destruction a few swollen mitochondria remained and sometimes the nucleus remained in an otherwise blank cell (Fig. III.F.12e). The final stage in cellular destruction was represented by remnants of the plasma membrane, a few lipid droplets and the occasional mitochondrion somewhere within the mass of homogeneous granular deposit which probably represents the hydrolysed remains of the protoplast. Acid phosphatase activity was associated with the (recognisable) cellular remains within the granular deposit (Figs. III.F.12f and 12g), and was encountered within the protoplast at all the stages preceding this final cellular destruction (Fig. III.F.12h). The control material showed no reaction for the activity of this hydrolase (Fig. III.F.12i).

F.2. TYPE 2 AGED EMBRYOS

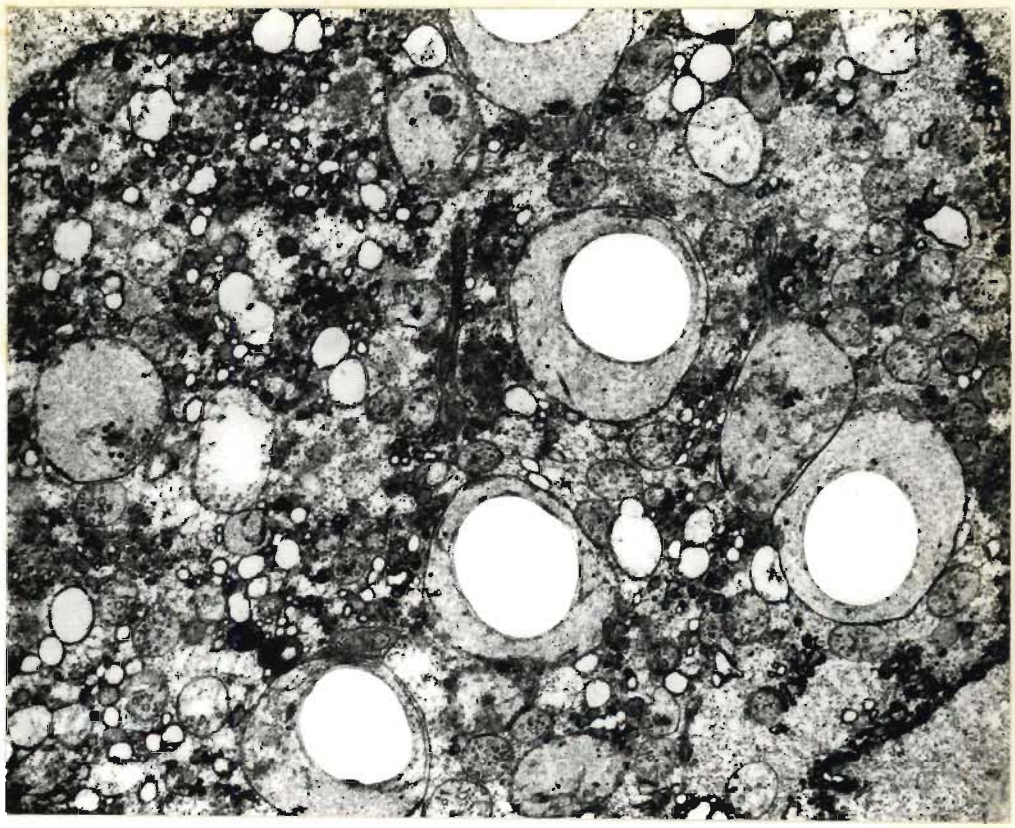
Type 2 aged embryos are those in which cells show general organelle disorientation. The patterns of ageing changes with respect to the various organelles in this material have been described above (III.E.) Thus a general synopsis of the situation within these cells will be given here.

Deviation from the normal disposition of organelles throughout the cytoplasm in cells of type 2 aged embryos was evident 12, 24 and 48 hours after the start of germination. Disorientated organelles occurred crowded in the perinuclear area, leaving a relatively sparse scattering of organelles in the peripheral cytoplasm (Fig. III.F.13).

FIGURES III.F.12 a - 12e. Illustrate the sequence of intracellular destruction encountered in cap cells of type 1 aged embryos 48 hours after the start of imbibition.

FIGURE III.F.12a. x 11 500.

FIGURE III.F.12b. x 10 350.



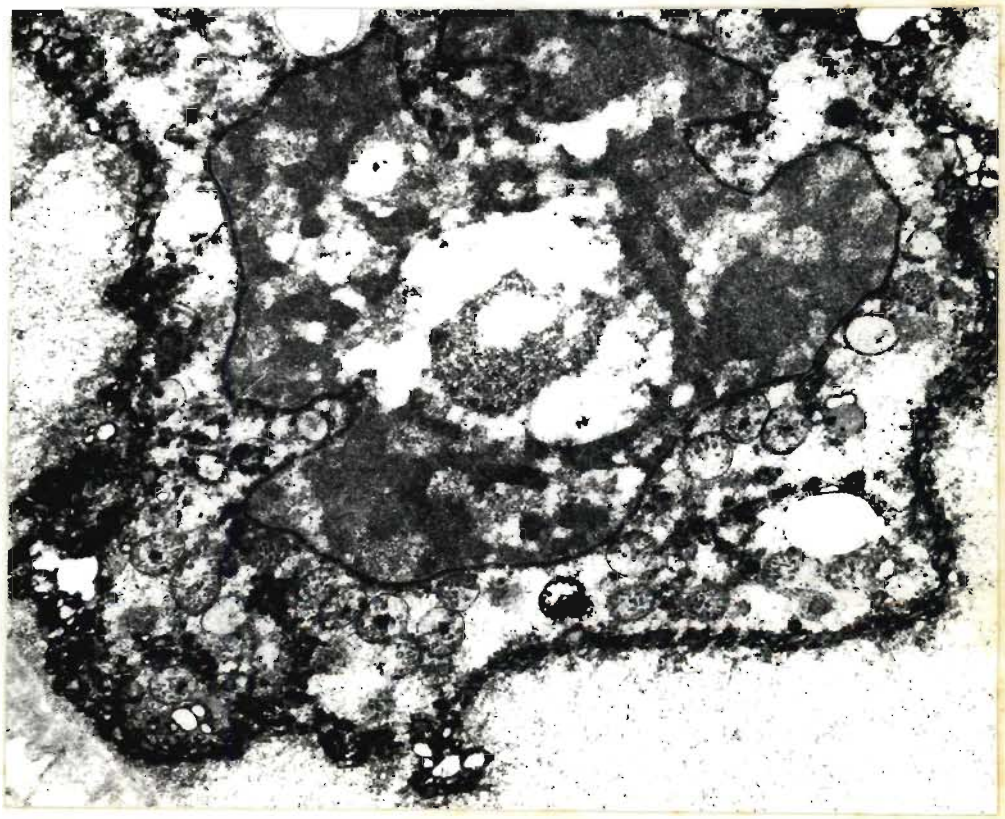
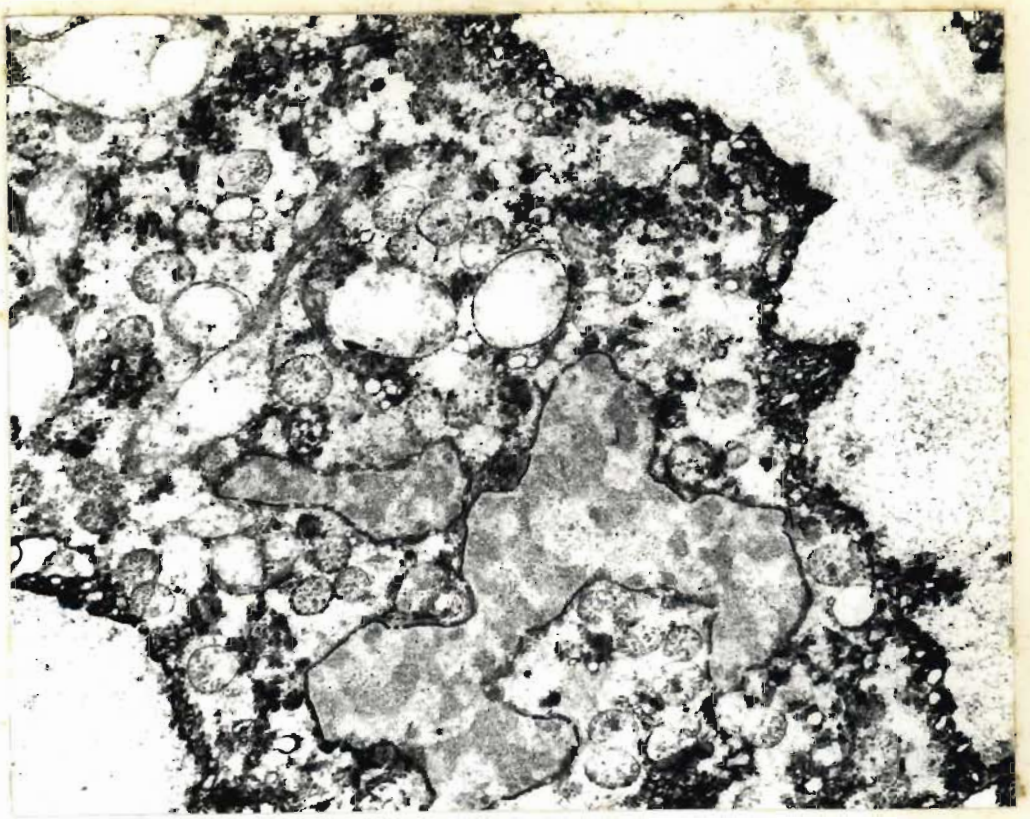


FIGURE III.F.12c. x 10 350.

FIGURE III.F.12d. x 10 350.



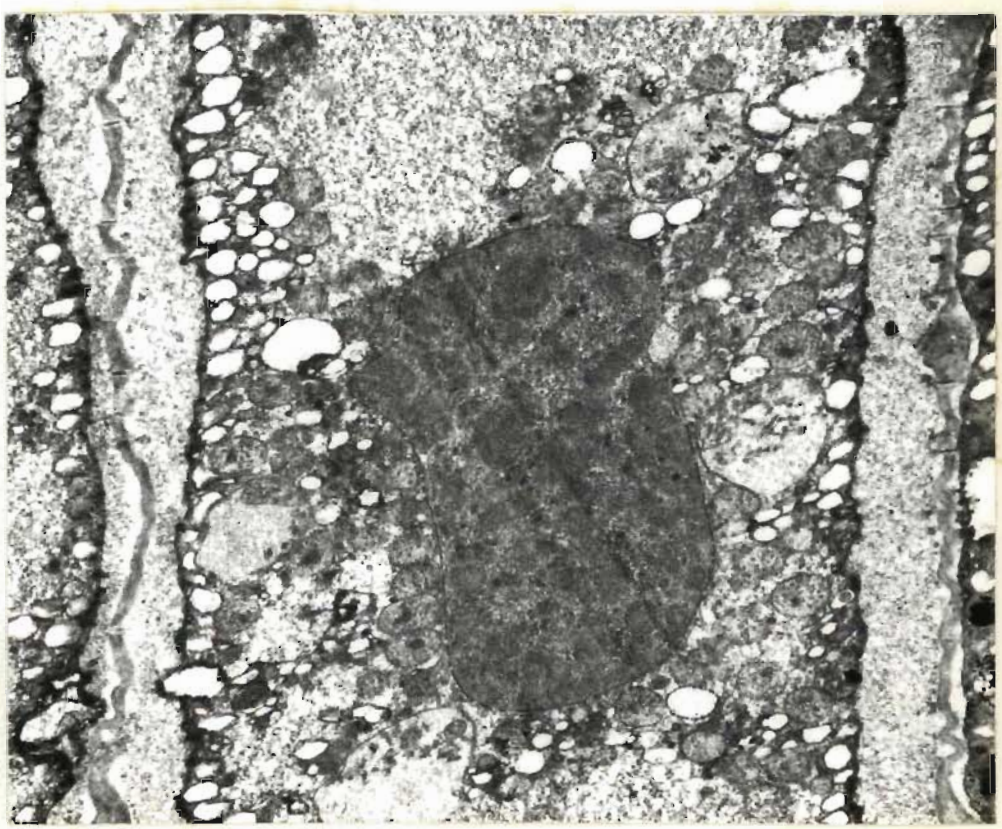
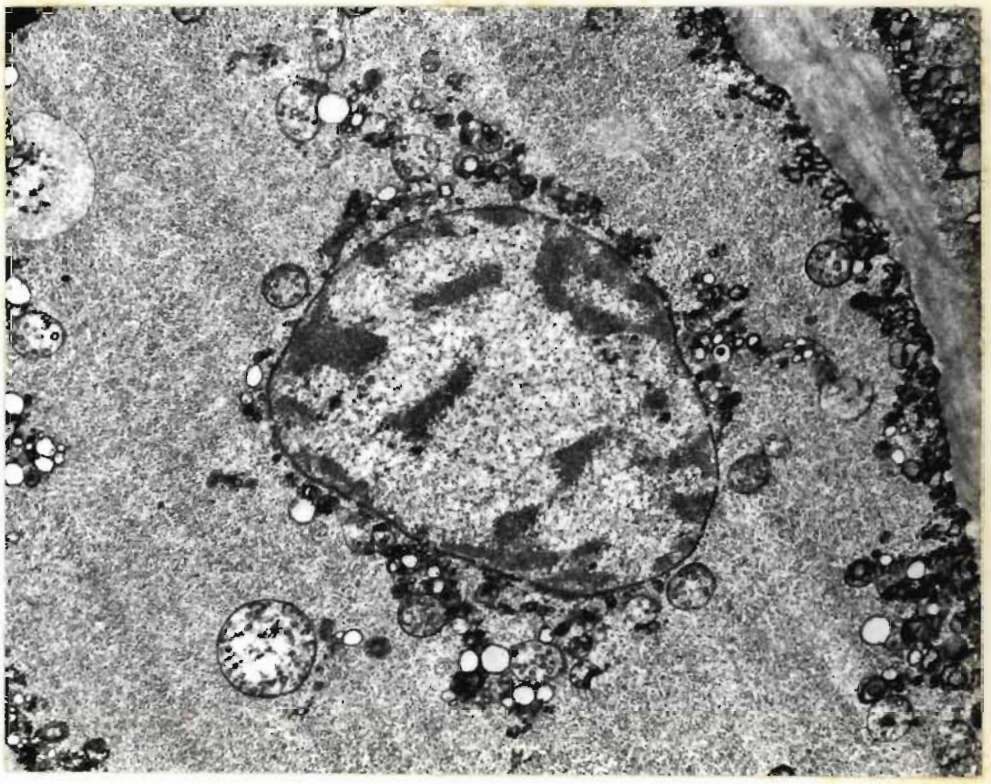


FIGURE III.F.12e. x 10 350.

FIGURE III.F.12f. Illustrates that acid phosphatase activity is associated with the remains of the protoplast in the final stages of cellular destruction. (x 11 500).



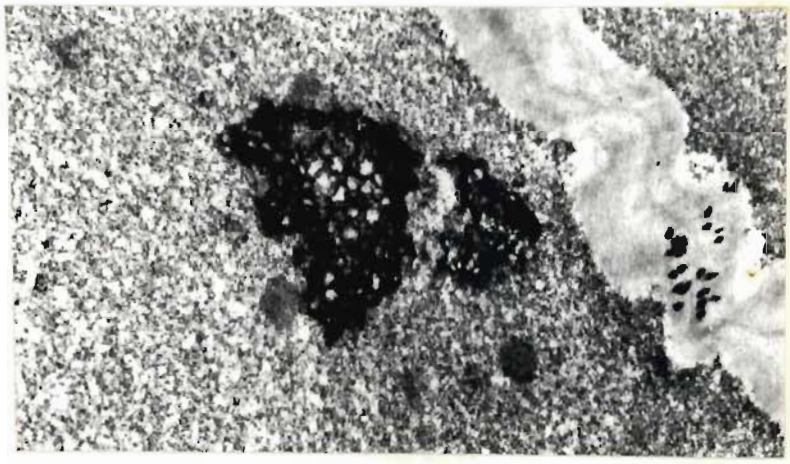


FIGURE III.F.12g. Illustrates the association of acid phosphatase activity with the remains of the protoplast in the final stages of destruction. (x 11 500).

FIGURE III.F.12h. Illustrates a diffuse reaction for acid phosphatase activity within the protoplast of a cap cell of a type 1 aged embryo 48 hours after the start of imbibition. (x 17 100).

It is thought that the homogeneous, granular deposit represents the products of hydrolytic reactions.

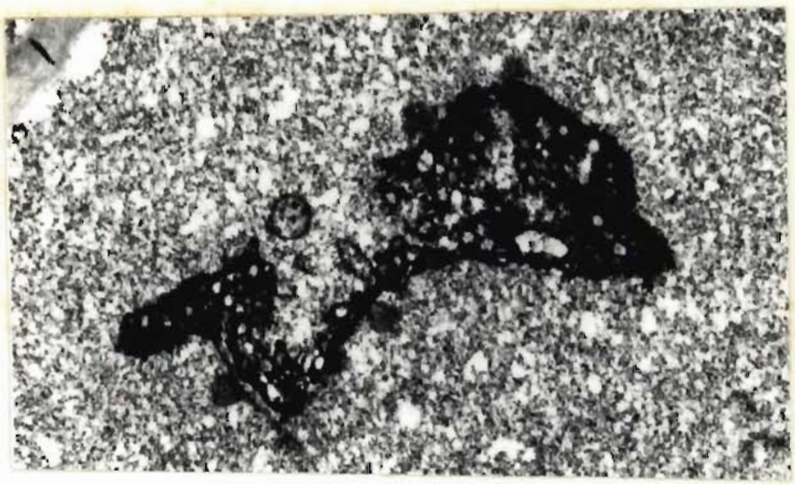


FIGURE III.F.12i. The control material which was incubated in the Gomori medium containing sodium fluoride as an enzyme inhibitor, is illustrated. No reaction for acid phosphatase occurs. (x 10 350).

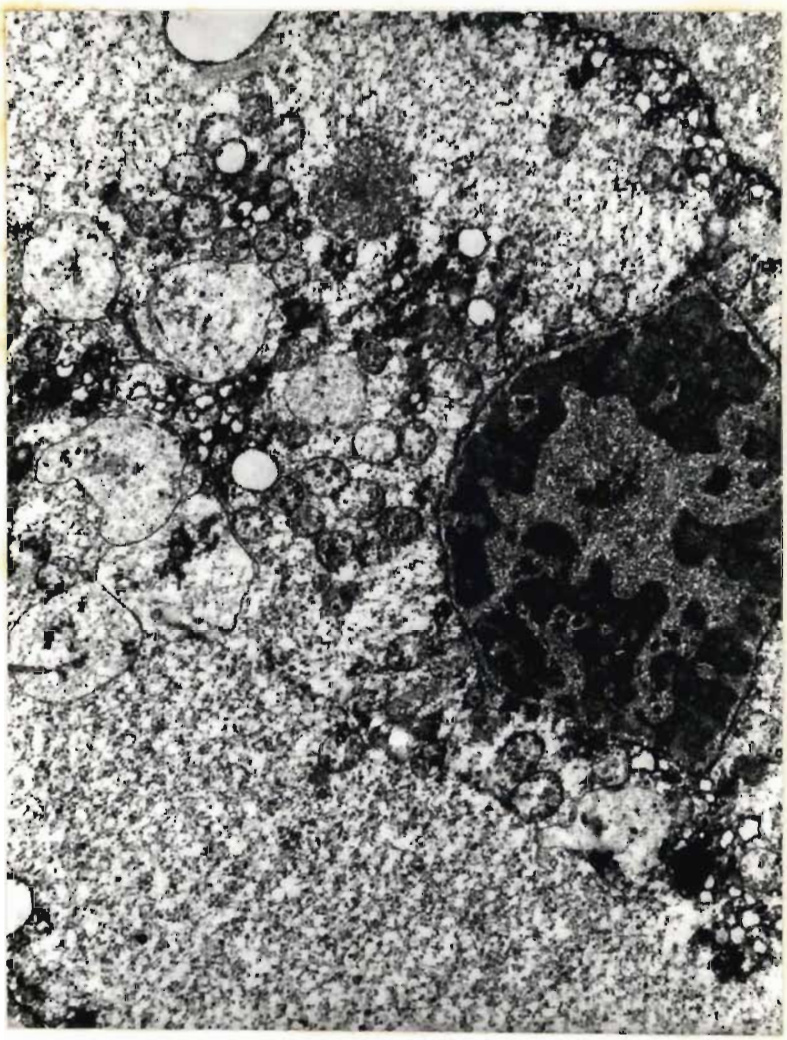
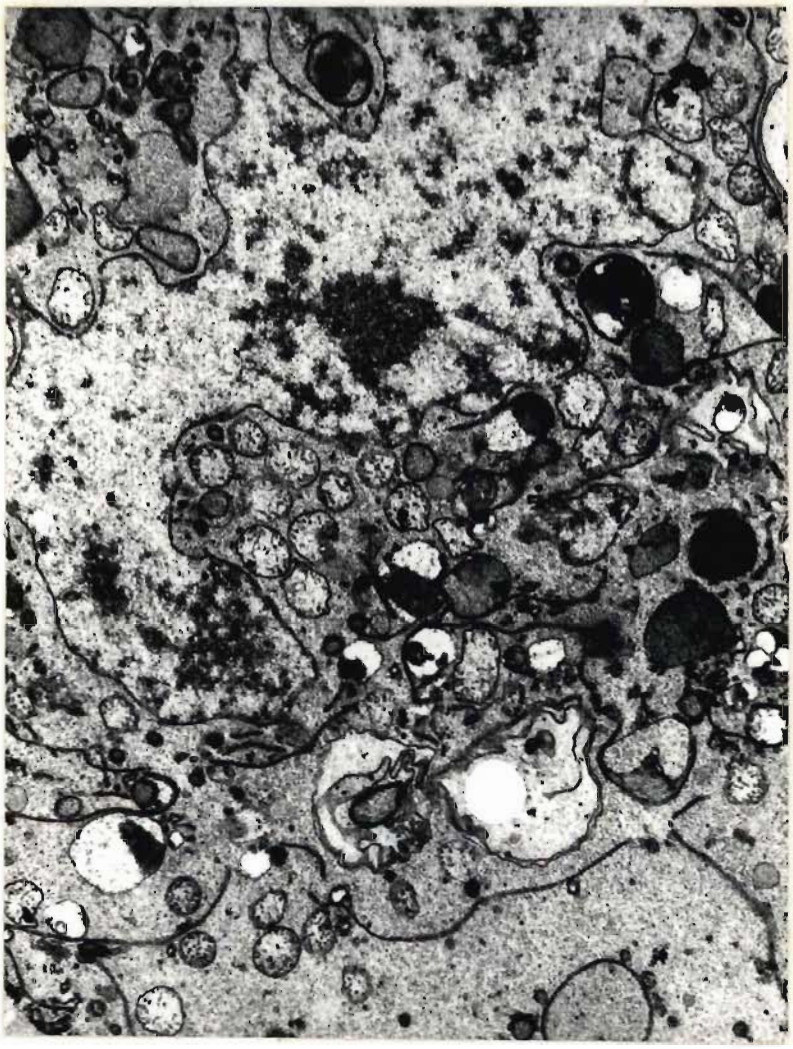


FIGURE III.F.13. Shows the aggregation of organelles in the perinuclear cytoplasm in a cap cell of a type 2 aged embryo, at the 48-hour germination stage. (x 8 100).



Chromatin stained within these nuclei, even after the imbibition phase, and marked nuclear lobing was apparent 12 and 24 hours after the start of imbibition. By the 48-hour germination stage, lobing had reached a point where no central nuclear mass was encountered. The nucleus consisted of several distorted processes which extended throughout the cytoplasm. Disorientated organelles were encountered between and around these processes (Fig. III.F.14).

Mitochondria and plastids encountered in cap cells of type 2 aged embryos showed the damage typical of these organelles in aged material 12, 24 and 48 hours after the start of imbibition (ref. Fig. III.F.13).

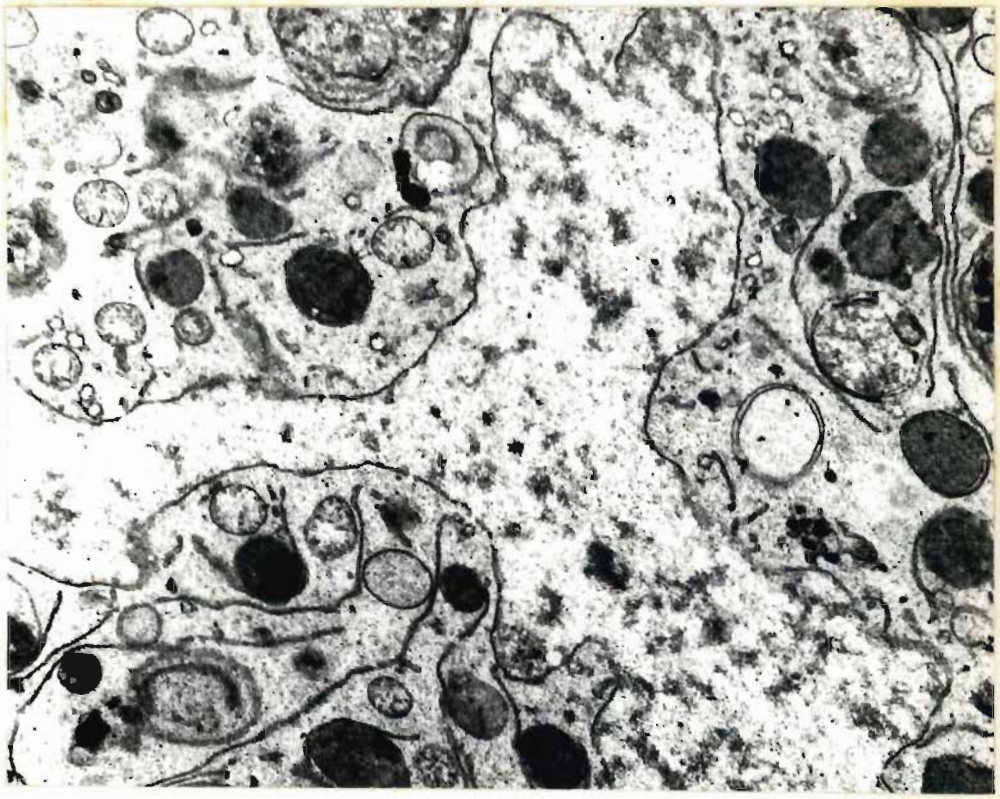
Genetically-controlled membrane maintenance systems are suggested to be active in normal cells, in addition to systems which control replication of the various organelles. Failure to repair damaged mitochondria and plastids is apparent in cells of type 2 aged embryos, and is suggested to result from breakdown of a genetically controlled pathway (or pathways).

Lysosomes did not follow a typical developmental sequence in the cap cells of type 2 aged embryos. Most of the lysosomes were crowded in the perinuclear area. These organelles appeared mainly as ER-associated first-phase lysosomes in the imbibed material, and generally persisted in this form 24 hours after the start of imbibition. In some cells of this material (in which degenerative changes are generally very marked) lysosomal vacuoles occurred, containing what appear to be the remnants of other organelles normally encountered in the cytoplasm.

First and second-phase lysosomes occur in the cap cells of type 2 aged embryos 48 hours after the start of imbibition. The intimate ER association of first-phase lysosomes (which was noticed in this material in the earlier phases of germination) has been lost to a large extent (Fig. III.F.15.)

FIGURE III.F.14. Illustrates portion of the nucleus which consists of a mass of distorted processes, in a cap cell of a type 2 aged embryo, 48 hours after the start of imbibition. (x 9 900).

FIGURE III.F.15. Illustrates lysosomes in a cap cell of a type 2 aged embryo at the 48-hour germination stage. Note that the intimate ER-lysosome association present in this type of aged embryo in the earlier germination stages, is largely lost. (x 14 400).



There was no sign of lysosomal membrane dissolution having occurred in any of the disorganised cells of type 2 aged embryos investigated.

No structures recognisable as dictyosomes were found in the cap cells of this material, either in the imbibed stage or subsequently. Apparently those dictyosomes which were present in the cells during seed storage became disorganised. In addition, molecular control mechanisms for the elaboration and activity of these organelles appear to be completely non-functional in the cap cells of type 2 aged embryos.

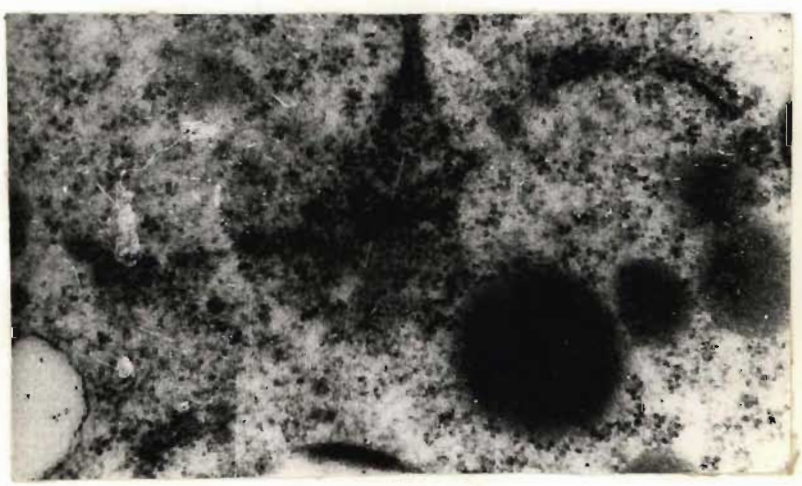
The ER profiles were short and sparse and occur without orientation in the cytoplasm, especially in the perinuclear region, 12 and 24 hours after the start of imbibition. Development of this organelle is a striking feature of cap cells of type 2 aged embryos by the 48 hour germination stage. In this material long ER profiles, the ends of which are often distended, were orientated in parallel banks in the perinuclear region (Fig. III.F.16). The proliferation of this organelle suggests that molecular control (of a sort) still exists.

Although only monosomes occurred 12 hours after the start of imbibition in the cap cells of type 2 aged embryos, some aggregation to form polysomes was evident 24 hours after the start of imbibition and persisted in this material at the 48-hour germination stage. In addition, an apparently atypical association of ribosomes with membranes of the ER was sometimes encountered in these cells 48 hours after the start of imbibition (Fig. III.F.17).

Polysome formation which occurred at the 24 hour germination stage implies the presence of long lived m-RNA which has persisted in the mature seed. It is possible that this m-RNA has been subjected to molecular accidents during the ageing treatment. Alterations produced in the long-lived m-RNA could partly account for some of the abnormalities encountered in the cap cells of type 2 aged embryos.

FIGURE III.F.16. Illustrates the long, parallel ER profiles which encircle the perinuclear area in cells of type 2 aged embryos at the 48-hour germination stage. (x 9 900).

FIGURE III.F.17. Shows polysomes in a cap cell of a type 2 aged embryo at the 48-hour germination stage. Note the atypical association of ribosomes with ER profiles. This material was postfixed with osmium according to Procedure 6b. (x 39 200).



However, a measure of DNA-dependent RNA synthesis probably occurred in these cells 24 to 48 hours after the start of imbibition. Some of this RNA may represent 'nonsense information' resulting from uncontrolled DNA derepression, and it seems most likely that this type of failure at the molecular control level would account for the ER proliferation described above.

Lipid droplets, which occurred peripherally in the cytoplasm of cap cells of imbibed type 2 aged embryos, were interspersed between the disorientated organelles 24 and 48 hours after the start of imbibition. There was some evidence of depletion of these in the 48 hour material, which had probably been partially utilised in elaboration of membranes of the ER.

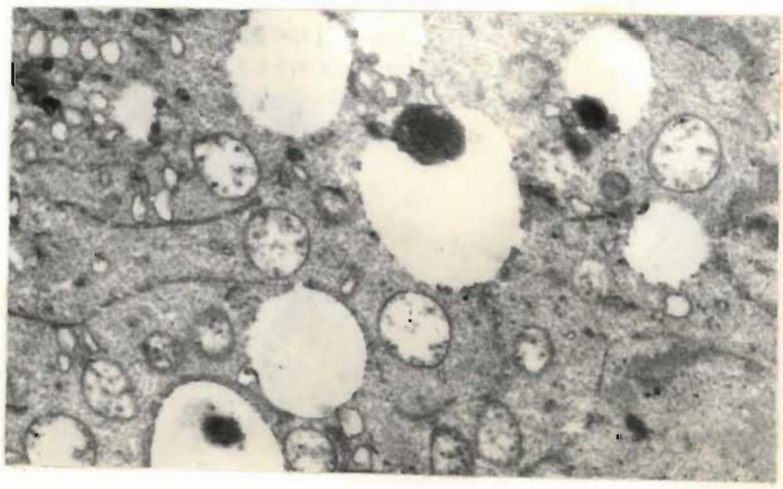
There was no apparent change in the cap cell walls in type 2 aged embryos in any of the material studied.

Use of the Gomori method for acid phosphatase localisation has shown the activity of this hydrolase to be confined within the lysosomes during all the germination stages studied (Fig.III.F.18a). However, there was no reaction for the activity of this enzyme when sodium fluoride was used in the control incubation (Fig.III.F.18b).

It is suggested that the pattern of degenerative changes which occurred in type 2 aged embryos are a consequence both of damage to cell membranes and of accidents at the molecular level. Some aspects of the disorganisation suggest some failures in the genetic pathways, while others suggest untimely depression of genes. The overall impression gained from these cells is that initially membrane aberration occurred, followed by general breakdown at the molecular control level.

FIGURE III.F.18a. Illustrates that acid phosphatase activity is localised within the lysosomes in cap cells of type 2 aged embryos at the 48-hour germination stage. (x 13 300).

FIGURE III.F.18b. The control material in which sodium fluoride was used as an enzyme inhibitor, shows no reaction for the activity of acid phosphatase. (x 16 100).



F.3. TYPE 3 AGED EMBRYOS.

Type 3 aged embryos are those in which the cells have retained basic cytoplasmic organisation, despite the duration (18 and 20 days) of the ageing treatment.

Generally, in cells of type 3 aged embryos, there is apparent repair and/or replacement of damaged organelles (which are evident in imbibed material) so that by 48 hours after the start of germination the cells appear to be normal and active.

However, it is possible that not all the type 3 aged embryos are viable, although this is not evident 12 and 24 hours after the start of imbibition. In some of the 48-hour embryos a pattern of development and differentiation occurs which culminates in cell death. This process, which occurs in cap cells, will be termed "precocious senescence". It must be noted in this respect that examination of the root apices of ageing embryos show that the various ultrastructural changes in organelles, and the patterns of senescence described for types 1 and 2 aged embryos, occur in cells of the root proper as well as in cap cells. However, precocious senescence as such is apparently confined to the root cap.

Cell death associated with this pattern is also progressive, from the chronologically oldest cap cells to the youngest. Precocious senescence is interpreted as being an acceleration of the usual cap developmental sequence which terminates in senescence.

Root caps of type 3 aged embryos which exhibited precocious senescence had a senescent zone which varies in extent. That is, the mature zone may be partly or entirely senescent, or the mature zone and part of the zone of differentiation may be composed of senescent cells, etc. However, no

viable cells occurred distal to those which were senescent. In addition, there were no intermediate zone between apparently highly-organised viable cells, and those which are senescent, i.e. the senescent cells occurred immediately adjacent to cells which appeared to be highly organised. Figure III.F.19 illustrates precocious senescence within the zone of differentiation.

Viable cells which occurred in root caps which exhibited precocious senescence were characterised by a marked degree of differentiation (Fig. III.F.20). In this case all the cells of the mature zone and the distal part of the zone of differentiation were non-viable (ref. Fig. III.F.19).

Nuclei in the viable cells were lobed, but this is interpreted as a function of the ageing treatment in general, and not especially characteristic of precocious senescence. The mitochondria and plastids showed an apparent reversal of the damage which was evident after 12 hours of imbibition, and were typical in form and disposition for this cell zone in unaged material. These organelles were also encountered in an apparent state of division within the cells of the zone of differentiation, including those cells immediately adjacent to senescent cells. Dictyosomes were regularly encountered in these cells, and were apparently active as typical vesicles were associated with them. ER profiles were long and orientated parallel to the nucleus and cell peripheri. The ribosomes were largely aggregated as polysomes.

Lysosomes encountered within these cells were large, and in their second developmental phase. However, there was evidence of lysosomal membrane dissolution in those viable cells immediately adjacent to the senescent cells (Fig. III.F.21).

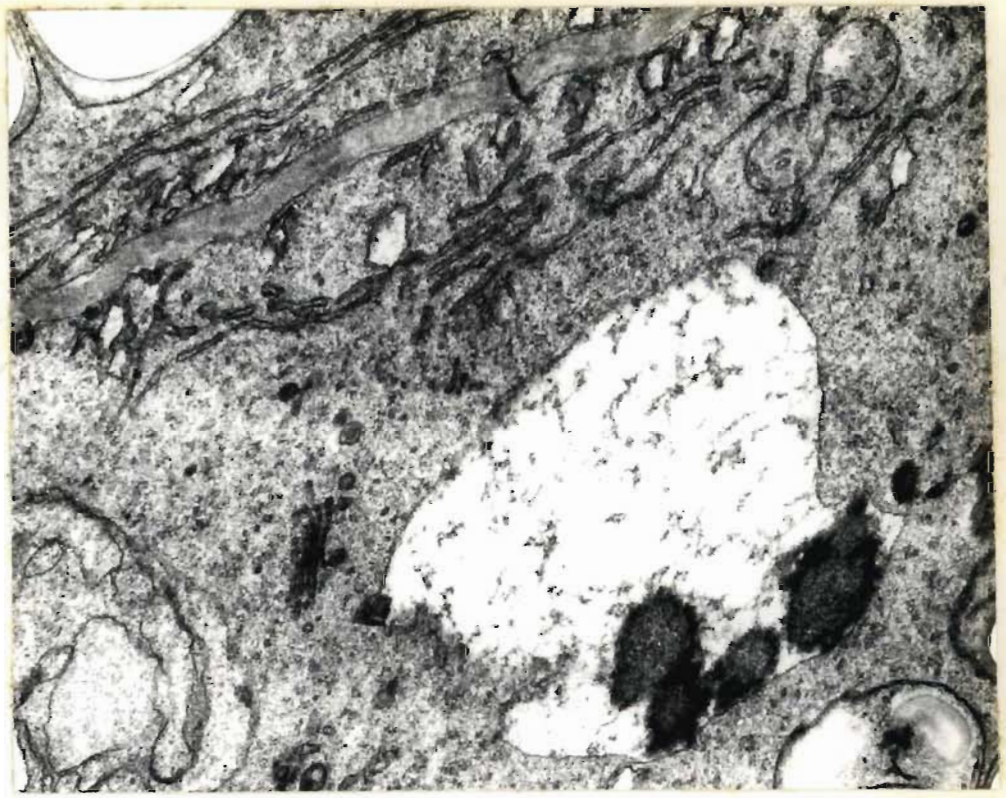
The senescent cells, including those immediately adjacent to the outermost rank of viable, apparently active cells, showed complete breakdown of the protoplast. Figure III.F.22 illustrates a senescent cell immediately adjacent to a viable cell. Organelles

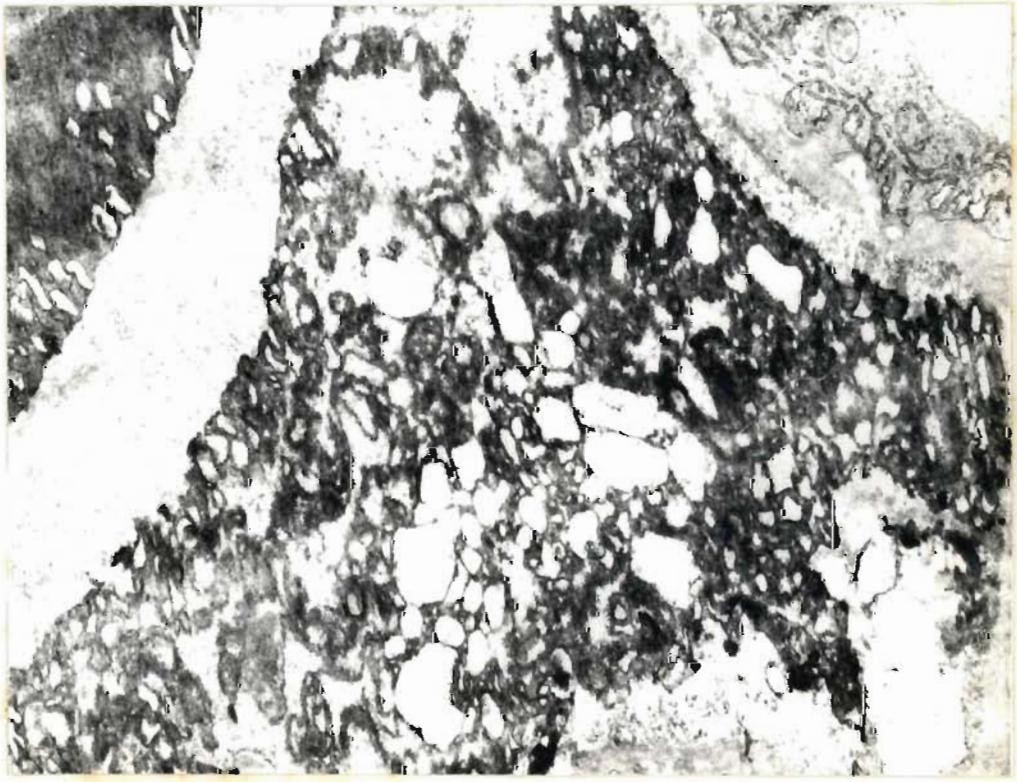
FIGURE III.F.19. Illustrates precocious senescence within the zone of differentiation at the 48-hour germination stage. (x 3 150).

FIGURE III.F.20. Illustrates an apparently viable cell which is immediately adjacent to the innermost rank of senescent cells in the zone of differentiation of a type 3 aged embryo which shows precocious senescence in the root cap. (x 10 350).

FIGURE III.F.21. Illustrates a second-phase lysosome in an apparently viable, highly organised cap cell, which is immediately adjacent to the innermost rank of senescent cells. Note the apparent dissolution of the lysosomal membrane. (x 24 300).

FIGURE III.F.22. Illustrates a senescent cell which is immediately adjacent to an apparently organised cell (top right) within the zone of differentiation of a type 3 aged embryo showing precocious senescence of the root cap. (x 10 350).





were virtually unrecognisable in these cells. However, monosomes persisted, and Figure III.F.23 illustrates a senescent cell containing only monosomes immediately adjacent to a viable cell containing polysomes. Thus the m-RNA associating the ribosomes appears to be completely hydrolysed with senescence, or possibly the reaction mechanism or the ribosomes themselves are damaged.

The appearance of the senescent cells, in conjunction with the fact that senescence is apparently precipitous, is suggestive of hydrolytic enzyme action. In this respect senescence occurring in this material is comparable with that described for the outermost cap cells of unaged material at the 48-hour germination stage ('Hickory King'). However, the notable difference lies in the fact that precipitous senescence described for unaged material is confined to the outermost cap cells, whereas in this aged material it moves progressively inwards.

Histochemical localisation of acid phosphatase showed that the activity of this enzyme was confined within the lysosomes in all the viable cells (Fig. III.F.24a), while in the senescent cells it was scattered throughout the protoplast (Fig. III.F.24b). The control material showed no activity for this enzyme (Fig. III. F.24c).

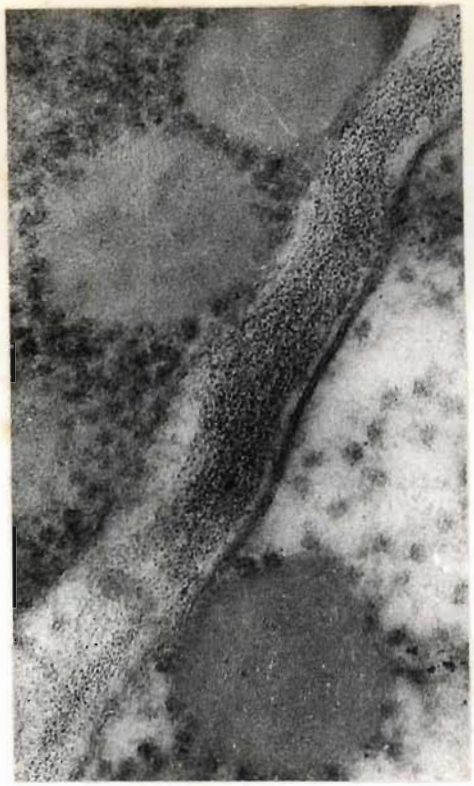
Thus the precocious senescence of cap cells which occurred in certain type 3 aged embryos was accompanied by dissolution of the lysosomal membranes and release of hydrolytic enzymes.

Cell death is suggested to be a consequence of the release of hydrolases normally confined within the bounds of the lysosomal membrane. The histochemical results in a zone showing precocious senescence are in keeping with those obtained for the normal senescence encountered only in the outermost cap cells of unaged material.

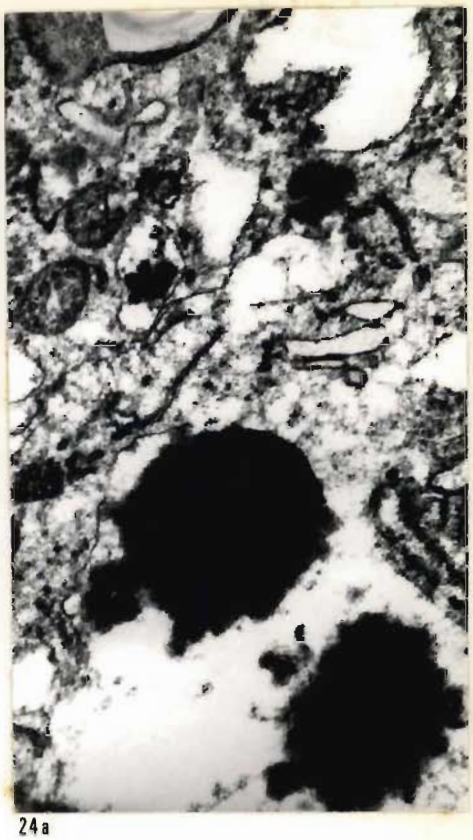
FIGURE III.F.23. Illustrates polysomes in a viable cell (lower right) which is immediately adjacent to a senescent cell in which only monosomes are present, in a type 3 aged embryo which shows precocious senescence of the root cap. This material was postfixed in osmium according to Procedure 6b, in order to preserve the ribosomes. (x 116 200).

FIGURES III.F.24a & 24b. Illustrate that acid phosphatase activity is confined within the lysosomes in viable cap cells, and occurs dispersed in the cytoplasm of senescent cells in type 3 aged embryos which show precocious senescence of the root cap at the 48-hour germination stage. (x 22 750).

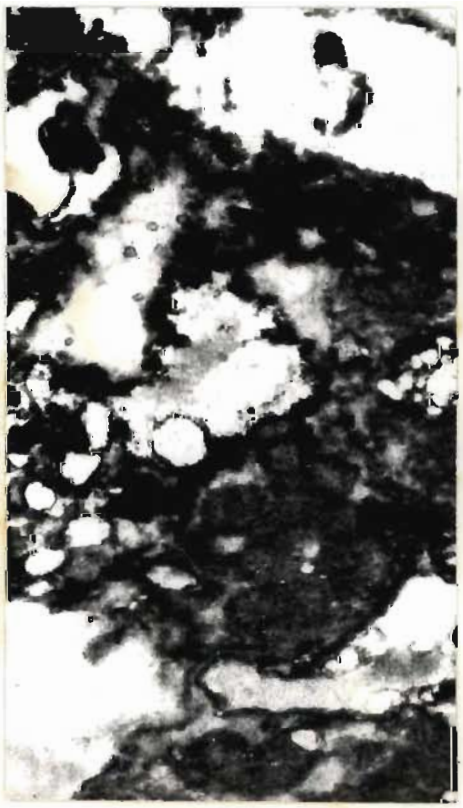
FIGURE III.F.24c. Illustrates that there is no reaction for acid phosphatase activity in a type 3 aged embryo showing precocious senescence of the root cap, with the use of sodium fluoride as an enzyme inhibitor. (x 16 100).



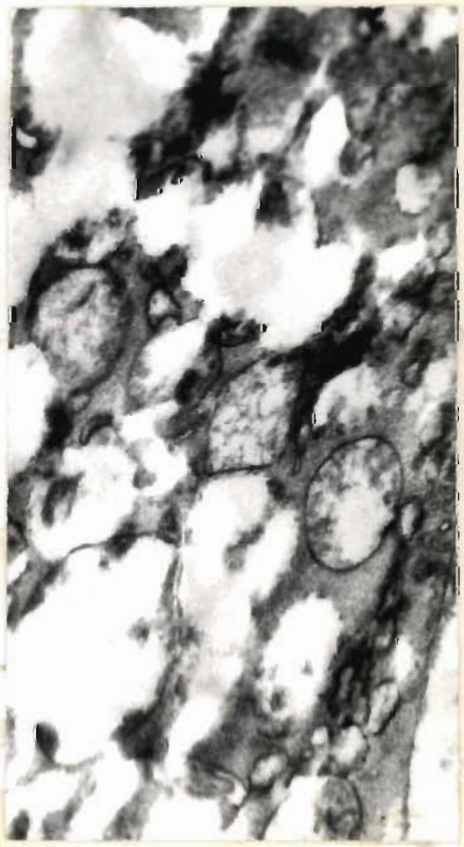
23



24a



24b



24c

G. AUTORADIOGRAPHY AND THE AGEING SEQUENCE

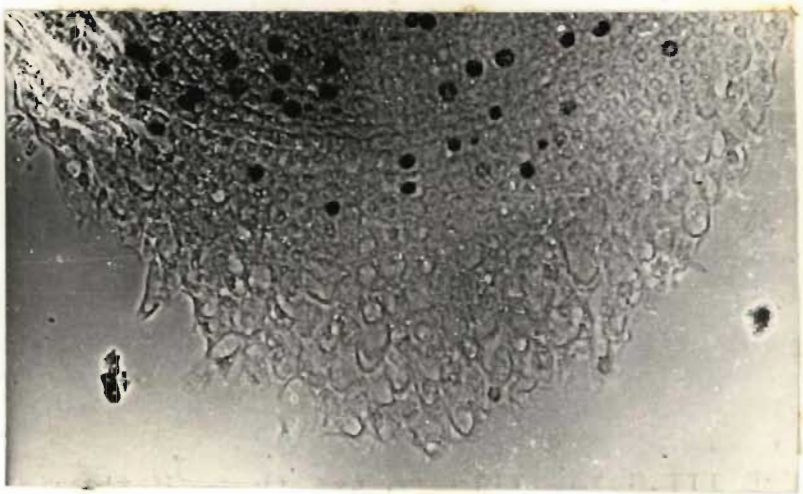
G.1. ^{3}H-Thymidine Incorporation

In cap cells of embryos which had been subjected to a relatively short period (6 days) of the ageing treatment, the pattern of ^{3}H-thymidine incorporation was found to be similar to that described for unaged material. Figure III.G.1a shows that labelled nuclei were confined to the meristematic zones of initials and dividing cells in 6-day material at the 48-hour germination stage. The proportion of cap cells with labelled nuclei (expressed as a percentage of the total number of cap cells) was 5.8%, which is similar to the value obtained for unaged material.

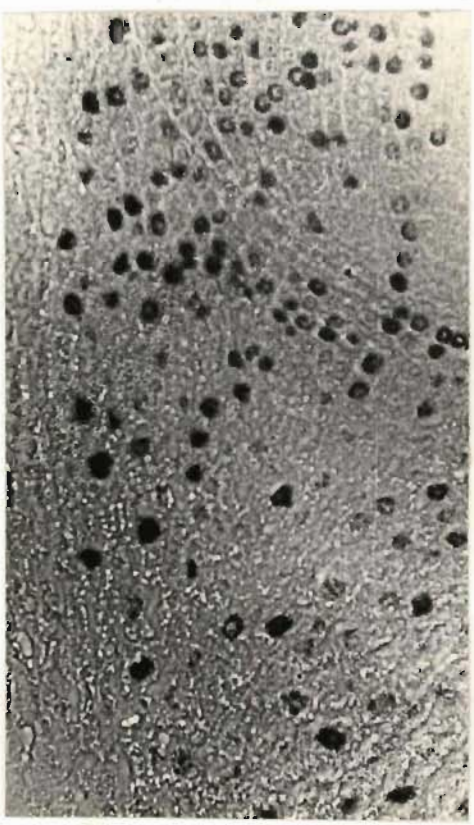
However, the percentage of cap cells which had incorporated ^{3}H-thymidine increased in those embryos which had been subjected to intermediate periods of the ageing treatment. Embryos which had been subjected to 10, 12 and 14 days of the ageing treatment had values of 9.1, 8.3 and 8.6 respectively, for the percentage of labelled nuclei per root cap. However, while ^{3}H-thymidine incorporation occurred in the nuclei of the initials and of the zone of division, there is evidence that the increase in the proportion of labelled nuclei per root cap was at least partly due to incorporation into zones which are normally non-meristematic. Figures III.G.1b and 1c show the pattern of ^{3}H-thymidine incorporation in root caps of embryos which had received 12 days of the ageing treatment. The proportion of cap cells with labelled nuclei dropped markedly to an average of 0.67% in some of the aged (18 to 20 days) embryos. This incorporation was random, and seldom occurred in the zones of initials or of division (Fig. III.G.1d). There was no evidence of ^{3}H-thymidine incorporation within cap cells of most of the

FIGURE III.G.1a. Illustrates that ^{3}H-thymidine incorporation is limited to nuclei of meristematic root cap cells in material which received 6 days of the ageing treatment. (x 252).

FIGURES III.G.1b & 1c. Illustrate the pattern of ^{3}H-thymidine incorporation into nuclei of root caps which received intermediate periods of ageing treatment. Note that incorporation of ^{3}H-thymidine occurs in non-meristematic as well as meristematic zones of the cap.
(1b x 500; 1c x 640).



a



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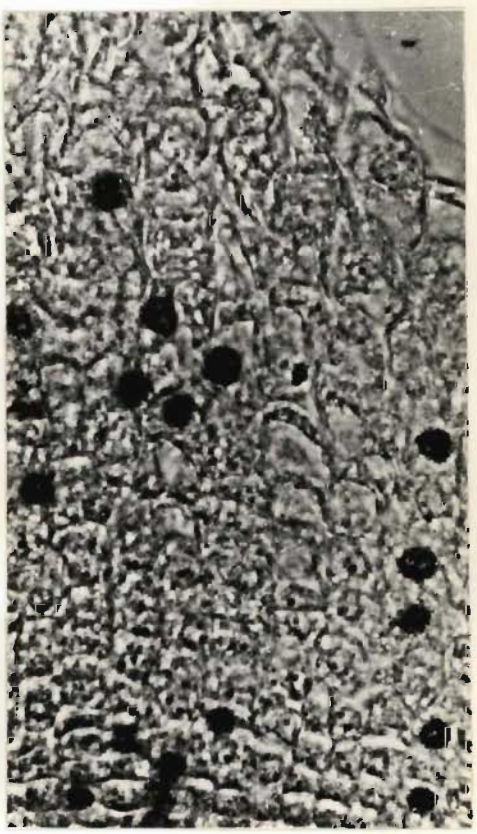


c

aged embryos. It is possible that those aged embryos which showed a measure of ^{3}H-thymidine incorporation corresponded to type 3 aged embryos which are apparently viable, or to type 2 aged embryos. The fact that little ^{3}H-thymidine incorporation occurred in the meristematic region of such material may be a function of the lowered germination rate which occurs with increasing age. Figures III.G.1e and 1f illustrate root caps of a type 1 aged embryo and a type 3 aged embryo showing accelerated precocious senescence respectively. No evidence of ^{3}H-thymidine incorporation can be seen in either of these types of aged embryos.

G.2. ^{3}H-Uridine Incorporation.

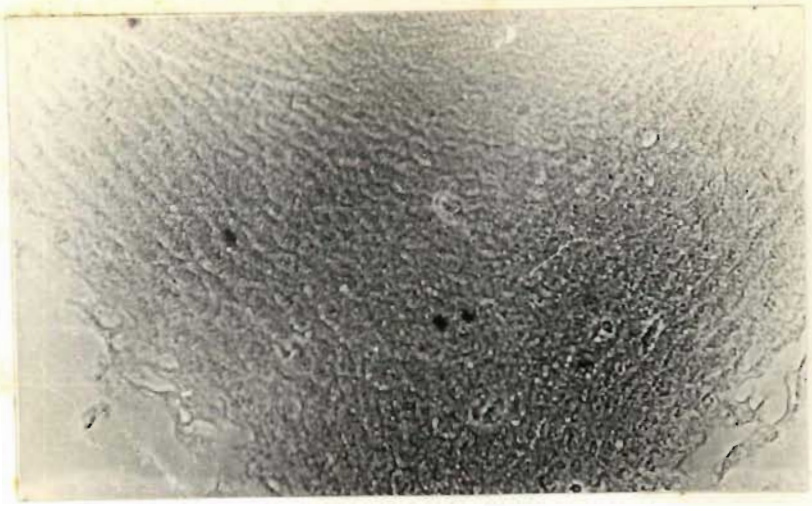
Light Microscopy

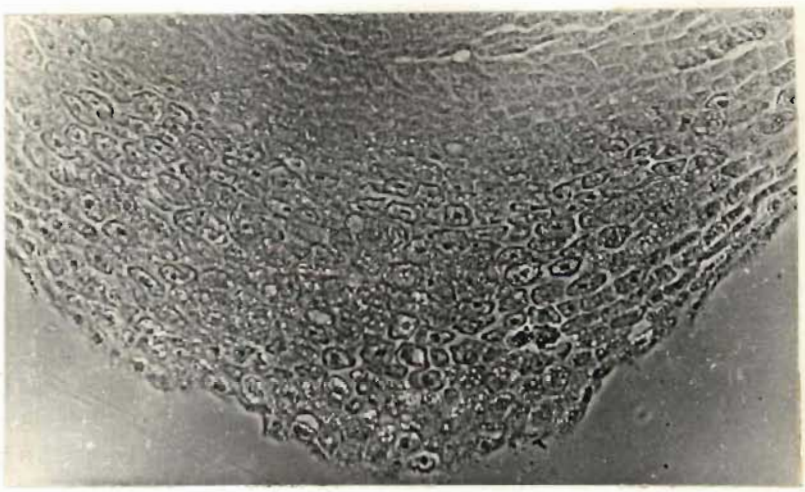
Silver grains were counted over 10 cells in each of the cap zones for selected stages of the ageing sequence. There was no difference between these counts for material which received a short period (6 days) of the ageing treatment, and the unaged material. However, the silver grain count (which is an index of ^{3}H-uridine incorporation) per cap cell type increased for all the cap cell zones, reaching a maximum in material which had been subjected to 14 days of the ageing treatment. Thereafter there was a drop in ^{3}H-uridine incorporation to a minimum in the aged material. (Fig. III.G.2a). The pattern of ^{3}H-uridine incorporation was similar for comparable zones of the root cap, reaching a maximum in mature cells, irrespective of the length of the ageing treatment. Figures III.G.2b and 2c are light micrographs showing silver grains over mature cap cells of material which had received 6 days and 14 days of the ageing treatment respectively.

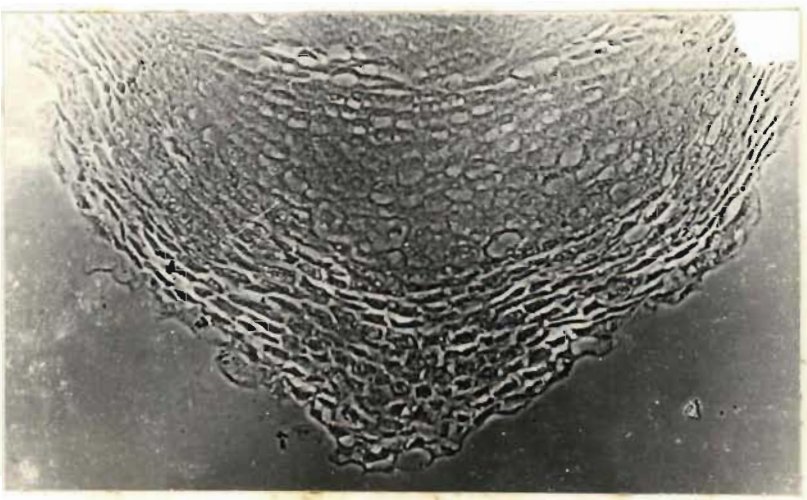
There are three different patterns of ^{3}H-uridine

FIGURE III.G.1d. Illustrates random incorporation of ^{3}H-thymidine into nuclei of non-meristematic cap cells of an aged embryo. (x 320).

FIGURES III.G.1e & 1f. Light micrographs illustrating root caps of a type 1 and a type 3 aged embryo (which shows precocious senescence), respectively. The material was incubated in a solution of ^{3}H-thymidine according to Procedure 8a. (x 252).







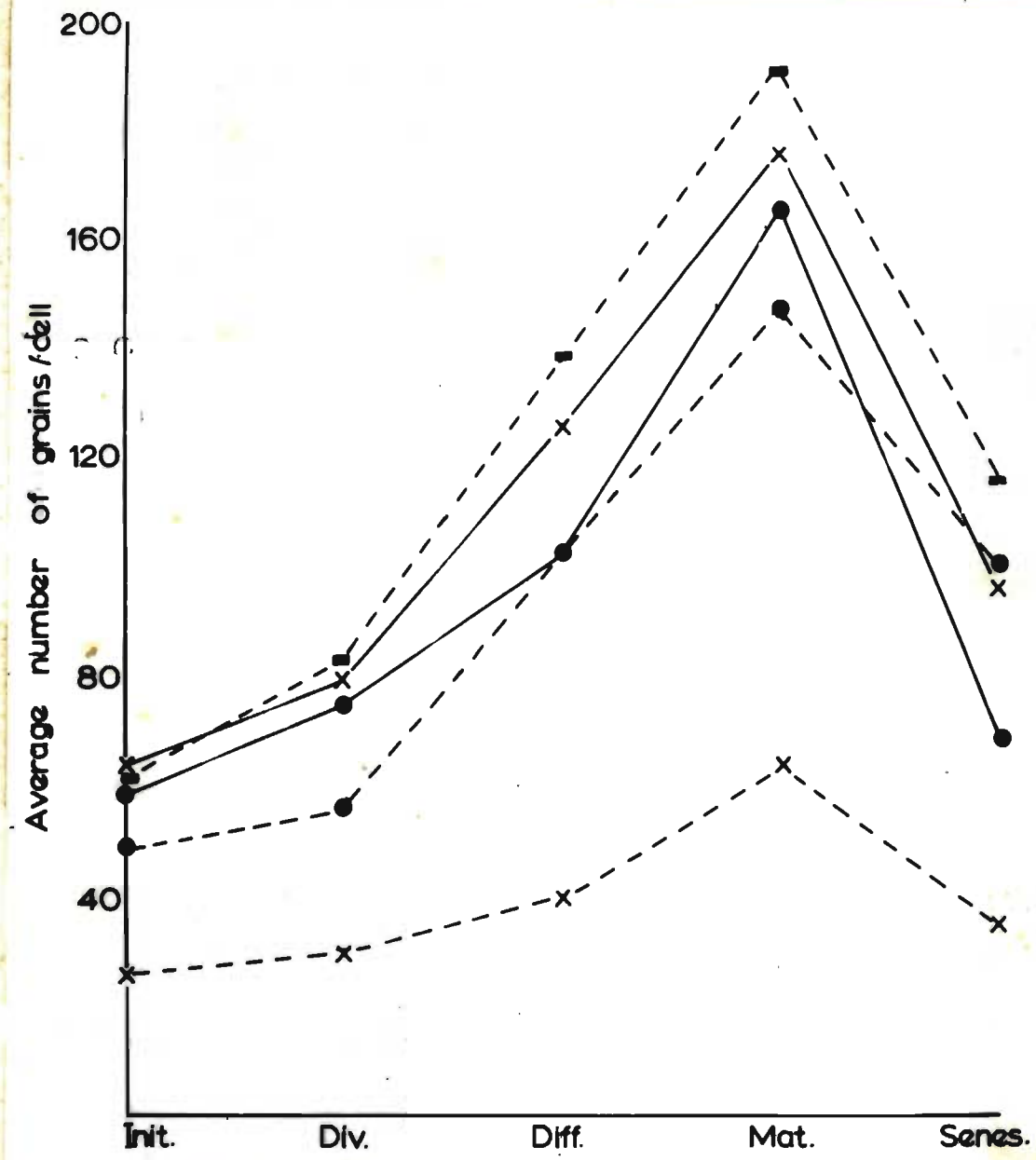


Fig. III. G. 2a. Illustrating ^{3}H-uridine incorporation in the various cap cell zones for selected stages in the ageing sequence.

o——o 6 days x——x 12 days ----- 14 days
o- - -o 16 days x- - -x 18/20 days type 3 aged embryos

FIGURES III.G.2b & 2c. Illustrate silver grains which indicate incorporation of ^{3}H-uridine over mature cap cells of embryos which received 6 and 14 days of the ageing treatment, respectively. (x 2 520).

incorporation in material which had received 18 to 20 days of the ageing treatment (Fig. III.G.2d). Curve (a) is taken to be representative of type 3 aged embryos as the basic pattern of incorporation is similar to that in younger material (ref. Fig. III.G.2a). Curve (b) might represent type 2 aged embryos where a lower incorporation of ^{3}H-uridine occurred and the pattern of the incorporation is somewhat changed. Curve (c) represents the pattern of incorporation which occurred in type 3 aged embryos showing precocious senescence of the cap distal to the zone of differentiation. It is interesting to note that a peak in ^{3}H-uridine incorporation occurs in the zone of differentiation which is adjacent to the senescent cells. Figure III.G.2e illustrates silver grains over a cell of the zone of differentiation, which is immediately adjacent to the senescent cells.

Certain of the aged embryos (18 to 20 days) showed no incorporation of ^{3}H-uridine. These are representative of type 1 aged embryos which have lost their viability during storage.

Figure III.G.2f illustrates the intracellular distribution of the label in the various cap zones for selected stages in the ageing sequence. It is seen that in general the bulk of the label occurred in the cytoplasm and least in the nucleolus, irrespective of the age of the material. In addition, the decline of ^{3}H-uridine incorporation which occurs in embryos at the later ageing stages was reflected in nucleolus, nucleoplasm and cytoplasm. Thus it appears that a decline in RNA synthesis occurs rather than a transport barrier between its origin in the nucleus and destination in the cytoplasm.

Figure III.G.2g illustrates that a peak of incorporation of ^{3}H-uridine accurred in all the cap cell types after about 14 days of the ageing treatment and that incorporation

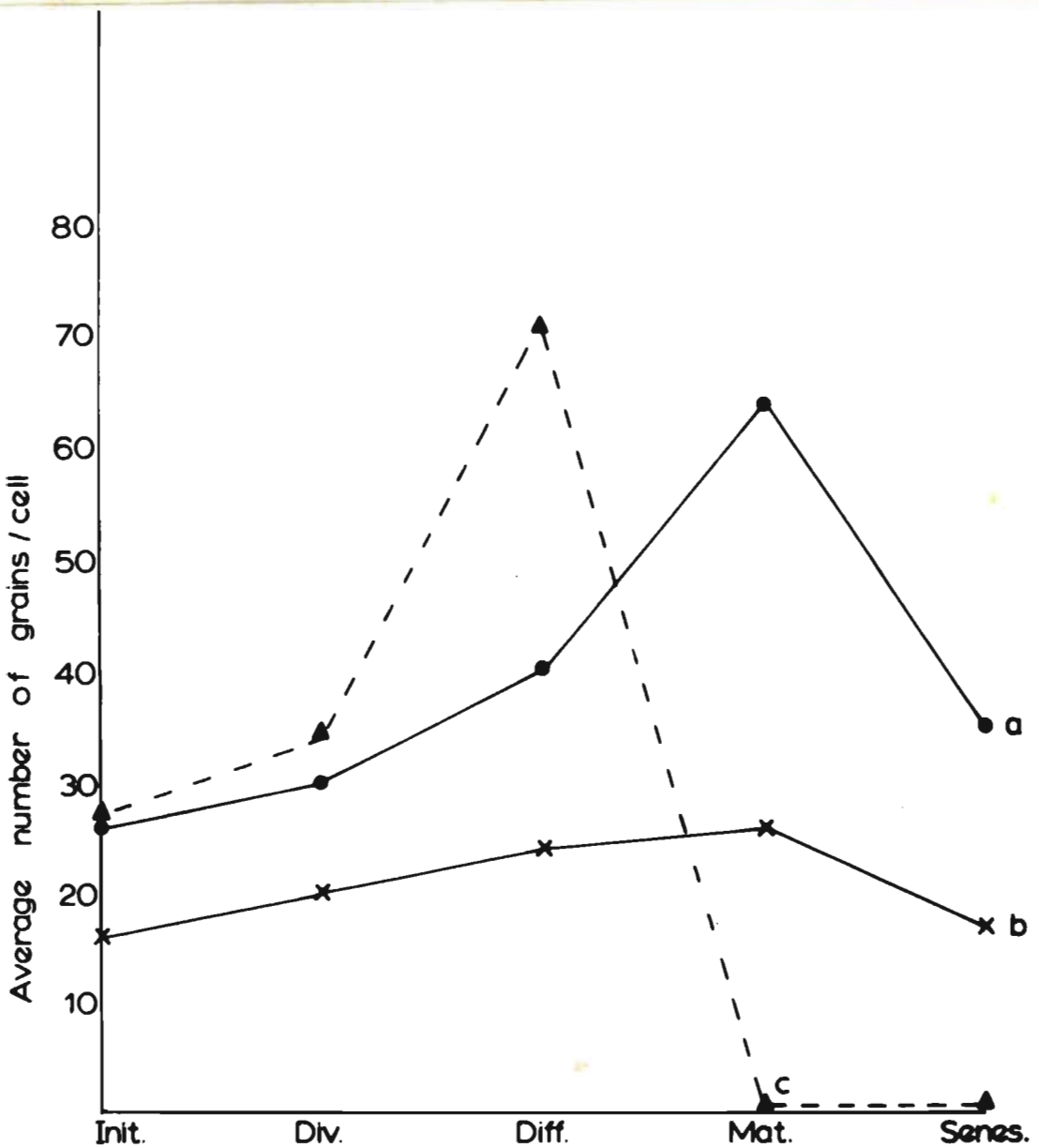


Fig. III. G. 2d. Illustrating the various patterns of ^{3}H-uridine incorporation in the cap zones of aged (18 and 20 days) embryos.

●——● Type 3 aged embryos; x——x Type 2 aged embryos;
▲ - - - ▲ Type 3 aged embryos showing precocious senescence.

FIGURE III.G.2e. Illustrates silver grains which indicate ^{3}H-uridine incorporation over a cell of the zone of differentiation, which is immediately adjacent to the innermost rank of senescent cells in which no incorporation is evident. (x 2 520).

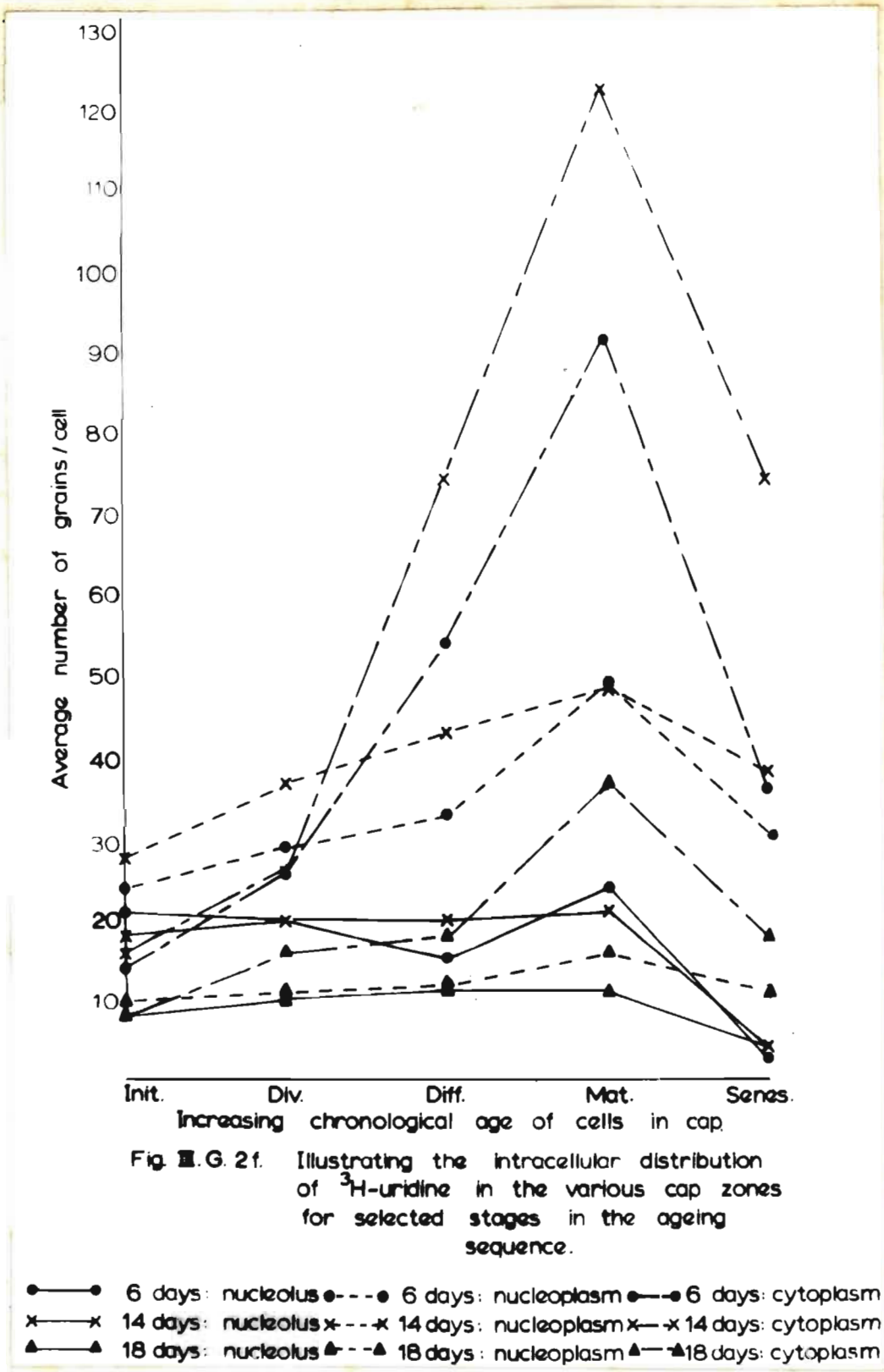


Fig. III. G. 2 f. Illustrating the intracellular distribution of ^{3}H-uridine in the various cap zones for selected stages in the ageing sequence.

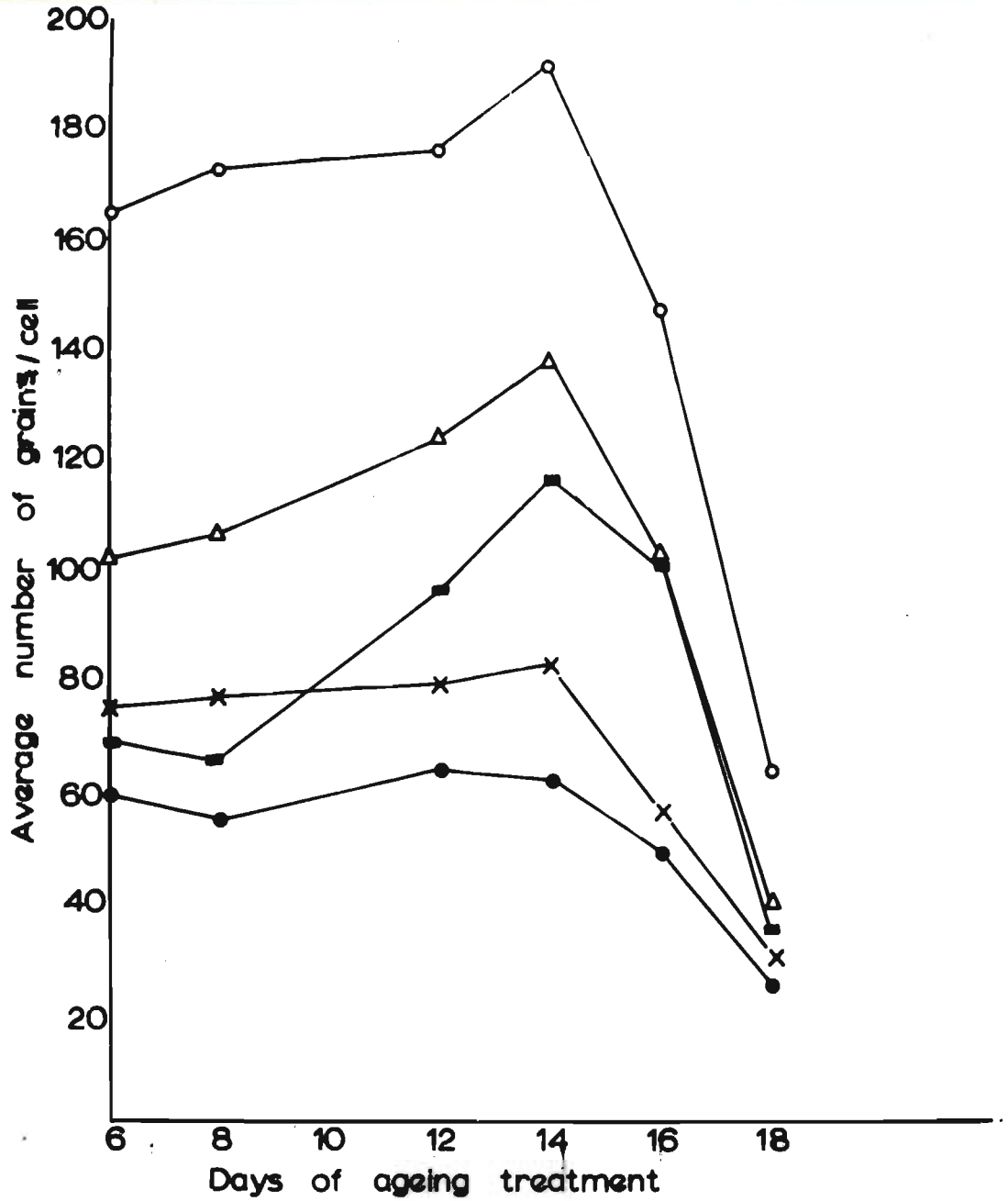


Fig. III G. 2g. Illustrating the relative incorporation of ^{3}H-uridine per cap cell type, with increasing age of the embryos.

●——● initials ×——× division

△——△ differentiation o——o maturity

■——■ senescent

declined in all the cell types in older material

Electron Microscopy

Electron microscopic observations show that there was a general incorporation of the label in the cytoplasm of cap cells of embryos which had received 14 days of the ageing treatment. In addition, silver grains appear over the mitochondria in thin sections of this material (Fig.III.G.2h).

In cells of type 3 aged embryos, the pattern of distribution of the label was similar to that described for 14-day material (Fig. III.G.2i). In those type 3 aged embryos which showed precocious senescence, silver grains were localised generally over the nucleus and cytoplasm of the viable cells immediately adjacent to cells in which the protoplast was completely disorganised. There is no evidence of ^{3}H-uridine incorporation in the latter cell type (Fig. III.G.2j).

Type 1 aged embryos do not incorporate ^{3}H-uridine at all. This is in keeping with the apparently non-viable condition of the cells.

G.3. ^{3}H-Leucine Incorporation

Light Microscopy

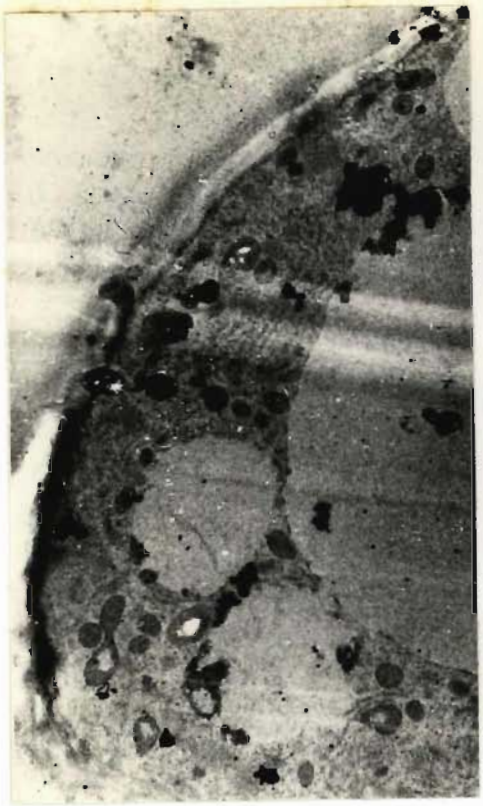
Silver grains were counted over 10 cells in each of the cap zones for selected stages of the ageing sequence. Counts of silver grains for material which had received 6 days of the ageing treatment were similar to those obtained for unaged material in all the cap zones.

Figure III.G.3a illustrates ^{3}H-leucine incorporation in the various cell zones for selected stages of the ageing sequence. It is seen that the silver grain count increased for all cap cell types, generally reaching a maximum in the

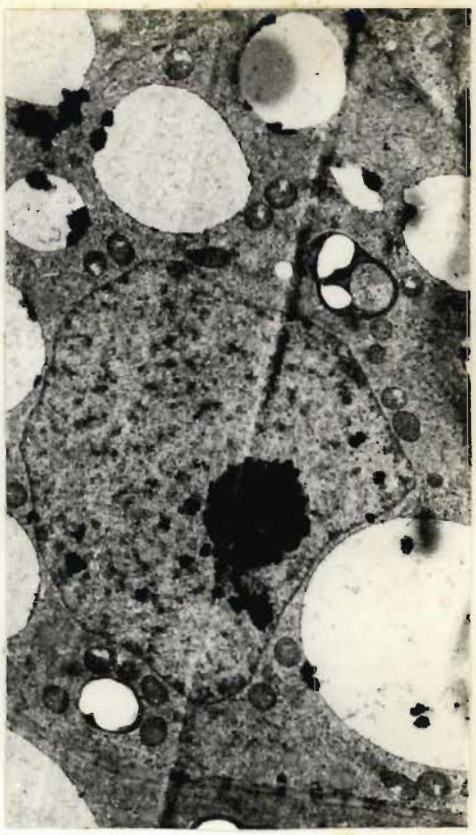
FIGURE III.G.2h. Illustrates that there is general incorporation of ^{3}H-uridine into the cap cell cytoplasm in material which received 14 days of the ageing treatment. Note that silver grains also appear over mitochondria. (x 7 700).

FIGURE III.G.2i. Illustrates incorporation of ^{3}H-uridine into a cap cell of a type 3 aged embryo. (x 8 100).

FIGURE III.G.2j. Illustrates that ^{3}H-uridine is incorporated into viable cells which are immediately adjacent to the innermost rank of senescent cells in a type 3 aged embryo which shows precocious senescence of the root cap. (x 5 500).



h



i

j

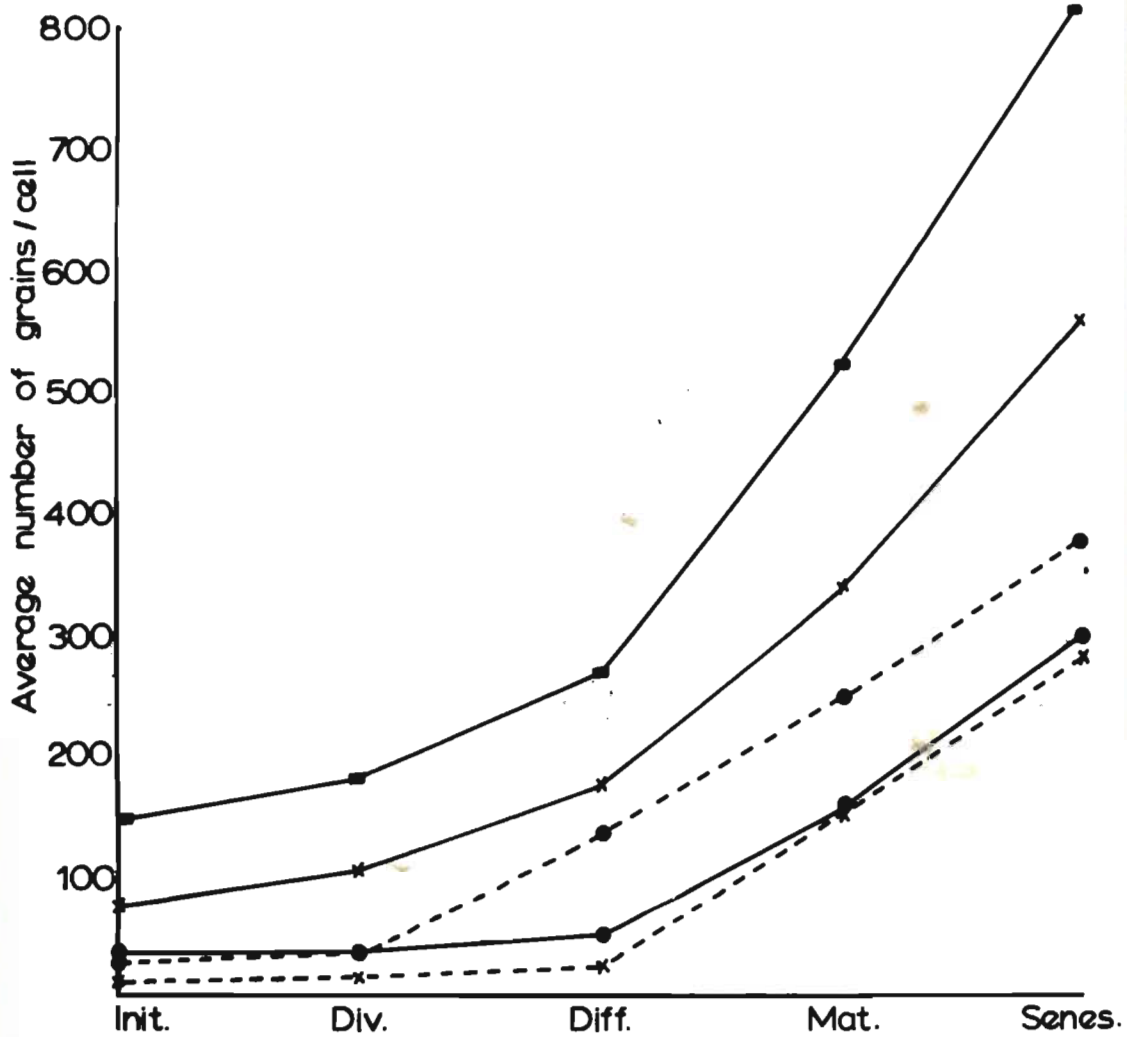


Fig. III.G. 3a Illustrating ^{3}H-leucine incorporation in the various cap cell zones, for selected stages in the ageing sequence.

●——● 6 days ×——× 10 days

■——■ 14 days ●- - -● 16 days

×- - -× 18/20 days type 3 aged embryos

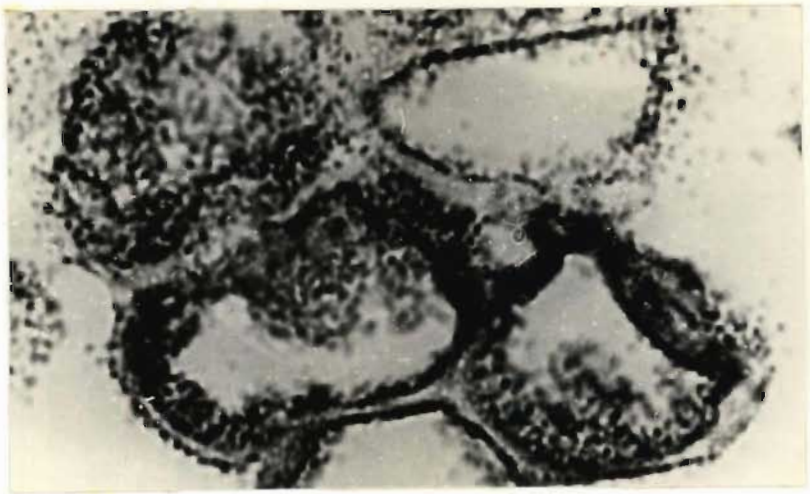
14-day material. A general drop in grain count occurred after this stage, reaching a minimum in aged, but viable material (see below). Figures III.G.3b and 3c are light micrographs which illustrate silver grains over outermost cells of material having received 6 and 14 days of the ageing treatment, respectively. Figure III.G.3d shows the pattern of ^{3}H-leucine incorporation over the entire root cap of an embryo subjected to 14 days of the ageing treatment.

Figure III.G.3e shows that three different patterns exist for incorporation of ^{3}H-leucine into aged (18 to 20 days) material. Curve (a) is suggested to represent type 3 aged embryos as the pattern of incorporation is similar to that encountered in material which had been subjected to shorter periods of the ageing treatment (ref. Fig. III.G.3a). It is suggested that curve (b) represents type 2 aged embryos in which a lower incorporation of ^{3}H-leucine occurred, the pattern of which differed from that in younger material. Curve (c) is representative of certain type 3 aged embryos which showed precocious senescence in the cap. In this material (represented by curve (c)) the cells distal to the zone of differentiation have deteriorated and do not incorporate any ^{3}H-leucine. However, cells of the zone of differentiation exhibited a substantial incorporation of the label. Figure III.G.3f is a light micrograph illustrating the silver grains over cells of the zone of differentiation, immediately adjacent to senescent cells which have not incorporated the label. As with ^{3}H-thymidine and ^{3}H-uridine, there was no incorporation of ^{3}H-leucine into cells of type 1 aged embryos.

There was a progressive increase in ^{3}H-leucine incorporation in all cap cell types, which reached a peak in material which had been subjected to the ageing treatment

FIGURES III.G.3b & 3c. Illustrate the distribution of silver grains, and thus of ^{3}H-leucine incorporation, over the most distal root cap cells in material which received 6 and 14 days of the ageing treatment, respectively. (x 2 000).

FIGURE III.G.3d. Shows the pattern of ^{3}H-leucine incorporation over the root cap (from the initials to the outermost cells) for material which received 14 days of the ageing treatment. (x 500).



b

c

d

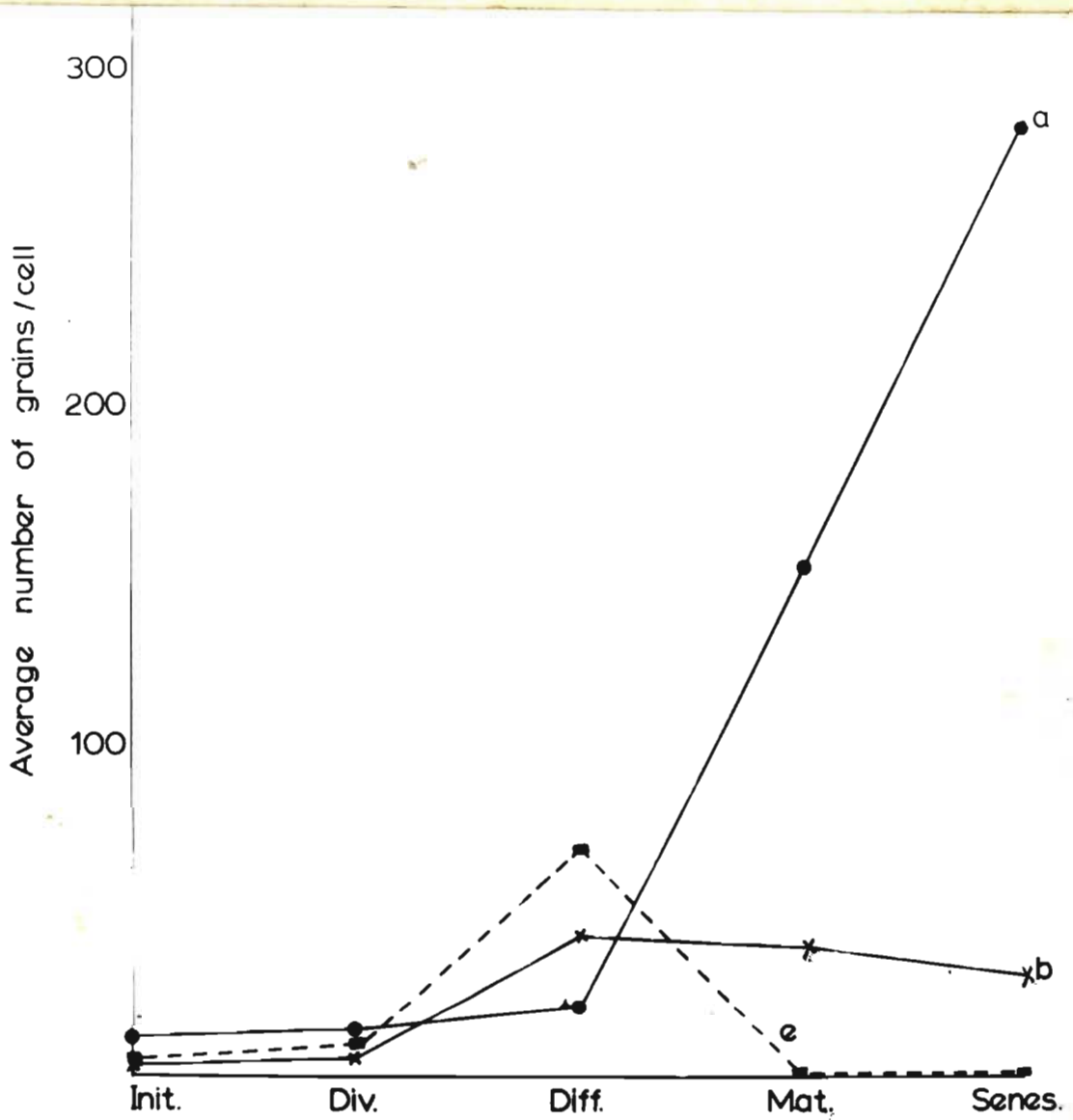


Fig. III G. 3e. Illustrating the various patterns of ^{3}H-leucine incorporation in the cap zones of aged (18 and 20 days) embryos.

FIGURE III.G.3f. Illustrates silver grains, which indicate ^{3}H-leucine incorporation over cells of the zone of differentiation which are immediately adjacent to senescent cells in a type 3 aged embryo which shows precocious senescence of the root cap. (x 1 600).

for 14 days (Fig. III.G.3g). There was a progressive decline in incorporation in all cap cell types of material which had received periods of the ageing treatment in excess of 14 days.

Electron Microscopy

^{3}H-leucine was incorporated into the nucleus and generally into the cytoplasm of material which had received a short period (6 days) of the ageing treatment (Fig. III.G.3h). The enhanced incorporation of ^{3}H-leucine in the cytoplasm of cap cells of the 14-day material is illustrated in Figure III.G.3i. Silver grains also appear over mitochondria in these cells. There is also an apparently enhanced incorporation of ^{3}H-leucine into the nucleus of cap cells of the 14-day material (Fig. III.G.3j).

Electron microscopic observations confirm that the general incorporation of ^{3}H-leucine was lowered in cells of type 3 aged embryos (Fig. III.G.3k) and that no incorporation of the label occurred in cells of type 1 aged embryos.

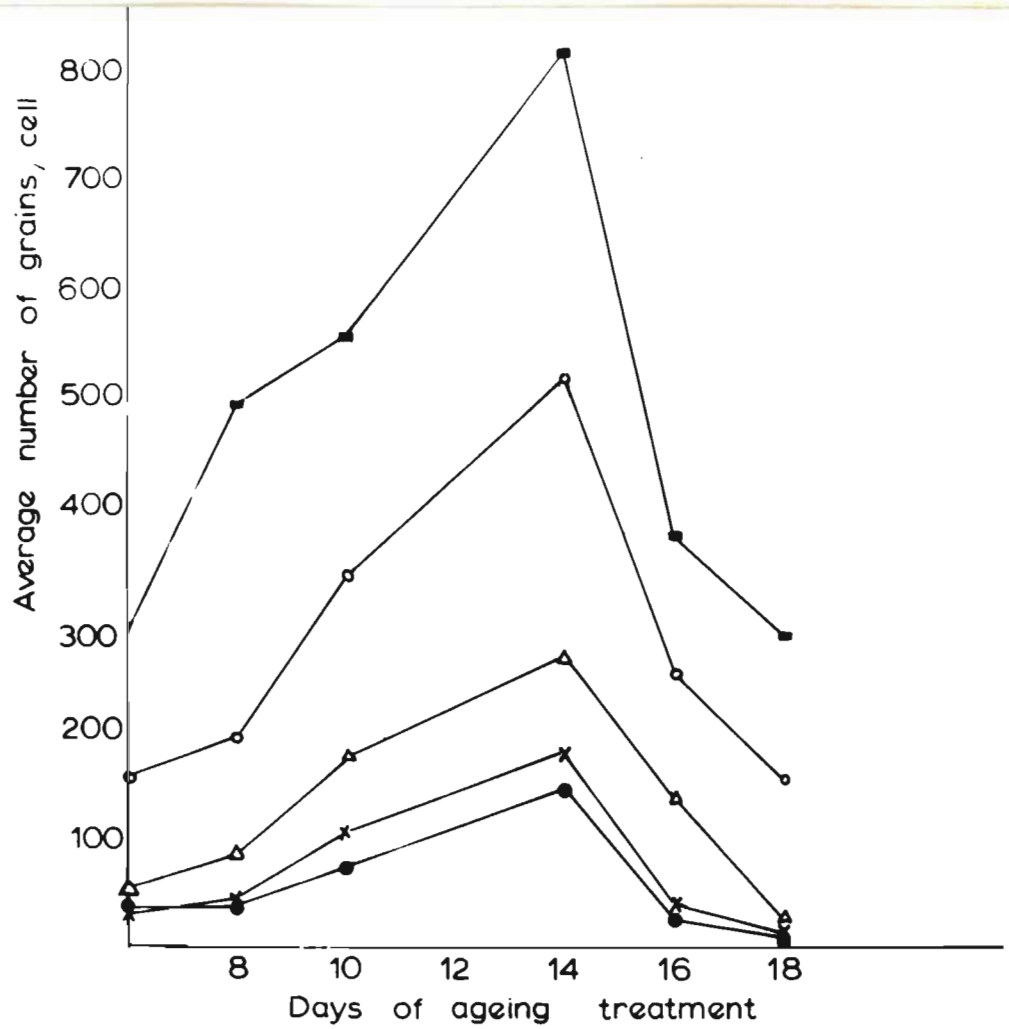


Fig III. G.3g. Illustrating the relative incorporation of ^{3}H-leucine per cap cell type with increasing age of the embryos.

cap cell zones

initials

division

differentiation

senescent

maturity

FIGURE III.G.3h. Shows incorporation of ^{3}H-leucine into a cap cell of material which received 6 days of the ageing treatment. (x 13 050).

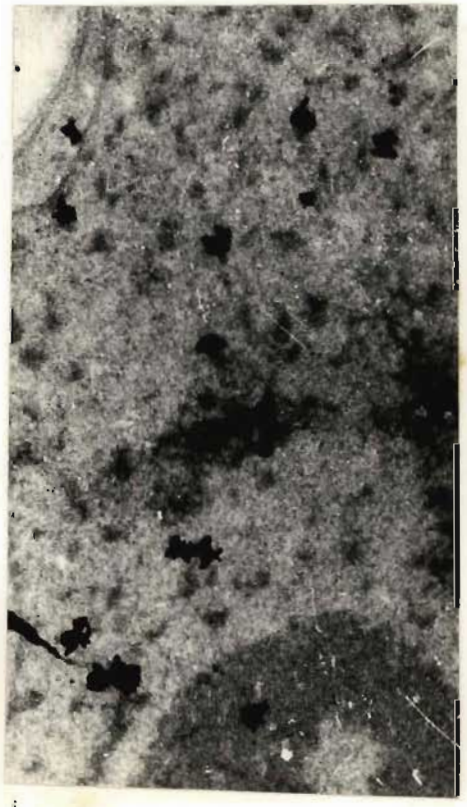
FIGURE III.G.3i. Illustrates silver grains over the cytoplasm of a cap cell of material which received 14 days of the ageing treatment. (x 10 350).

FIGURE III.G.3j. Shows incorporation of ^{3}H-leucine into a cap cell nucleus of material which received 14 days of the ageing treatment. (x 16 100).

FIGURE III.G.3k. Shows that there is a lowered general incorporation of ^{3}H-leucine into cap cells of type 3 aged embryos. (x 11 500).

h

i



j

k

PART IV - DISCUSSION

A. Aspects of Senescence in the Root Cap of Unaged Material

Senescence of the outermost cells of a root cap is of a type where a discrete part of a tissue degenerates, as distinct from senescence of an entire tissue. It is possible that friction between the outermost cap layer and the soil particles alone, would suffice to remove the former, but this would be a random process, varying in efficiency with the soil type and its moisture content and with the firmness of attachment of the outermost cap cells. Endogenous processes which facilitate removal of these cells are advantageous to the functional efficiency of the root cap. Therefore processes causing senescence and removal of the outermost cap cells may be described as useful and necessary and in this respect comparable to morphogenetic cell death which accompanies embryogenesis (Saunders, 1966; 1966a).

Ultrastructural observations made on root caps of unaged embryos after the imbibition phase show that degenerative changes have occurred in the outermost cell layer. Mitochondria and plastids appear to be swollen and disorganised, dictyosomal cisternae are absent, short disorganised ER profiles occur and no polysomes are formed. In addition, apparent dissolution of the bounding membranes of lysosomes has occurred. However, in those mature cells immediately adjacent to the outermost layer there are no visible signs of deterioration.

The intimate association of the ER profiles with fully-formed first-phase lysosomes occurs characteristically in mature cap cells approximately 12 hours after the start of imbibition. It is possible that this association might be significant in the formation of second-phase lysosomes observed in the outermost root cap cells at this germination stage. The ER-lysosome association which apparently immediately preceeds swelling of the

lysosomes occurs only in imbibed material. It is possible that ER-associated enzymes may synthesize material which is transported into the lysosome. These molecules, or the products of their hydrolysis would appreciably increase the particulate concentration of the lysosome, followed by osmotic uptake of water and associated swelling of this organelle. Another possibility is that a compound elaborated by an ER-associated system and transported into the lysosome might be an hydrolase or what is more likely, an enzyme activator. There is yet another possible explanation of the ER-lysosomal association. If the plant cell has a pathway of electron transport alternative to cytochrome oxidase, and this pathway is linked up with cytochrome b_7 associated with the ER (W.D.Bonner, 1961) then it is possible that the close ER-lysosomal association may represent a localised energy source.

Possibly the ER-lysosomal association represents a combination of two or more of these, or other phenomena. It has been reported that ER profiles occur in close association with certain hepatic microbodies (Novikoff and Shin, 1964; Shnitka, 1966). As a result of these observations Novikoff and Shin (1964) suggested that microbodies form from peripheral dilations of the ER. However, this is unlikely to be the interpretation of the ER-lysosome association in these mature cap cells, as this association occurs only between fully-formed first-phase lysosomes and not during the first developmental phase itself.

Acid phosphatase activity occurs to a limited extent both in the fully-formed first-phase lysosomes and the second-phase lysosomes, as well as in the cytoplasm of the outermost cap cells. Senescence of the outermost cap cells may be described as precipitous and it is suggested that the changes involved in the process are brought about by the action of hydrolases normally confined inactive in the cap cells in the quiescent seed, becoming activated in distal cap cells during imbibition.

No cell division is observed and there is no apparent separation of the outermost cap cells during the period 12-24 hours after the start of imbibition. During this period there has been a general increase in development of the various cap cell types, evidenced by the development and apparent activity of their organelles. The mature cells immediately adjacent to the outermost layer also show signs of enhanced activity with no visible changes suggestive of physiological failure which might be interpreted as senescent change.

However, at the 24-hour germination stage degradative processes appear more advanced in the outermost cells than at the 12-hour germination stage. Plastids and mitochondria if intact, are swollen and disorganised, while some of these organelles are in a state of apparent breakdown. ER profiles have completely disappeared from the cytoplasm of these cells.

Acid phosphatase activity occurs throughout the cytoplasm of the outermost cap cells at the 24-hour germination stage. It is suggested that the activity of this and other hydrolases have brought about further degradative changes during the period 12-24 hours after the start of imbibition. These cells are judged to be in an advanced state of senescence, and totally disorganised.

Incorporation of ^{3}H-thymidine indicates that DNA replication occurs in meristematic cap cells and ultrastructural observations confirm that cell division takes place in the root cap at the 48-hour germination stage. The senescent cap cells described for the 12- and 24-hour material have been sloughed off at 48 hours after the start of germination.

Although cap cells of the mature zone appear to be highly active certain changes occur which are associated with their subsequent senescence. Although senescence of outermost root cap cells has been described above as precipitous, such changes are in

keeping with the suggestion of Weiss (1966) that some events associated with development may be described as contributing to the ageing process. The incorporation of vesicles produced by hypersecretory dictyosomes into lysosomal vacuoles is one such change. Lysosomal vacuoles swell very markedly at this stage, and the swelling is attributed to osmotic activity resulting from an increased concentration of particles derived from incorporated dictyosomal secretion. In this respect Morré et al. (1967) have demonstrated that the dictyosomal secretion is polysaccharide, and Jones and Morré (1967) showed that acid hydrolysis of this secretion yields predominantly glucose and galactose units. Matile (1968) has shown that carbohydrases occur in the lysosomal vacuole. It is possible that these enzymes could account for hydrolysis of the polysaccharide secretion, thereby further increasing the concentration of solutes within the lysosomal vacuole and enhancing osmotic activity. Subsequent to the marked lysosomal swelling, dissolution of the bounding membrane of this organelle occurs in outermost cap cells.

Another such change is the incorporation of dictyosomal secretions between the plasmalemma and cell wall, as this secretion is thought to accelerate separation of the outermost cells. In this respect Morré et al. (1967) have shown that the secreted material moves through the cell wall to appear as a droplet adhering to the root tip.

Therefore, the hypersecretory activity of dictyosomes in mature cap cells is suggested to be an ageing change, instrumental both in lysosomal function and in the cell separation process. The change in staining reactions of the middle lamella region of cell walls in the distal part of the mature zone may also be interpreted as a change associated with ageing.

In general, organelles in mature cap cells at the 48-hour germination stage appear to be structurally intact and highly active,

and there are no signs of degenerative changes per se in these cells. However, in the outermost cap cells (of SA 4) at this stage there are early signs of deterioration. The lysosomal membranes are discontinuous in places, and some of the mitochondria have a darkened matrix. The darkening of the matrix of some of the mitochondria is in keeping with the results of Öpik (1965) who interpreted this to be a degenerative change accompanying senescence of cotyledons.

Treffry et al. (1967) and Butler (1967), in studies on senescing cotyledons, reported that tonoplast breakdown apparently preceeds final destruction of the protoplast. Cell vacuoles have been suggested to be homologous with secondary lysosomes (Matile,1968; Matile and Moor, 1968) and results obtained by the present writer are in keeping with this concept. Thus breakdown of the tonoplast in senescing cotyledons probably represents the release of hydrolytic enzymes and is analagous to dissolution of the lysosomal membranes in the outermost root cap cells which is suggested to precede general autolysis of the cells concerned. Acid phosphatase activity occurs within all the fully-formed first-phase lysosomes and in the second-phase lysosomes in cap cells 48 hours after the start of imbibition. In addition, localised activity of this enzyme occurs in the cytoplasm of the outermost cap cells (of SA 4), which are considered to be in the early stages of senescence. The separation process generally removes the outermost cap cells (of SA 4) in this early stage of senescence.

Ultrastructural observations made on the maize variety Hickory King by the present writer (Berjak, 1968) show that senescence of the outermost cap cells is more precipitous than in the hybrid SA 4. Diffuse hydrolase activity occurs throughout the cytoplasm of the outermost cap cells in which there is no suggestion of organisation, or even of intact organelles. These cells are immediately adjacent to the apparently highly-organised distal cells

of the mature zone, which resemble comparable cap cells of the variety SA 4 in all respects. These results are in keeping with light microscopic observations of Gahan and Maple (1966) who observed that acid phosphatase activity is confined to particulate sites in the meristematic cap cells of Vicia faba whereas outermost cells showed only a diffuse reaction for the activity of this enzyme.

Autoradiographic observations which were carried out on SA 4 at the 48-hour germination stage indicate that both RNA and protein synthesis increase with increasing chronological age of the cells within the cap, up to the mature zone. In the outermost cells which are in the early stages of senescence in this variety there is a drop in RNA systhesis, but a further increase in protein synthesis. This suggests that part of the m-RNA involved in protein elaboration in the outermost cap cells was synthesized while these cells were in the mature zone i.e. that some of the m-RNA in these cells persists as it has become longer-lived. This is in keeping with the suggestion of Medvedev (1967) who suggested that the longevity of RNA molecules might increase with age. Note that despite the localised activity of certain hydrolases in the cytoplasm, polysomes persist in these cells. Protein synthesis in these outermost cells is not confined to any one organelle, but occurs throughout the cell. It is suggested that at least part of the RNA, and thus part of the protein synthesized in the outermost root cap cells results from template activity of newly-derepressed genes which control the process of senescence.

The type of senescence occurring in outermost root cap cells of unaged material is apparently a rigidly controlled process. There are few visible signs of senescent change in the cells shortly before their apparent death. Although hypersecretory activity of the dictyosomes is thought to be a change connected with senescence, this is not a degenerative change. The first

deleterious changes are dissolution of the lysosomal membrane and the appearance of hydrolase activity in the cytoplasm.

This type of senescence implies genetic control. It is suggested that a group of genes exists controlling this process, and that these genes remain repressed during differentiation and establishmnet of the mature cap cells. However, certain events in the microenvironment of the cells of the distal part of the cap trigger the derepression of these genes, resulting firstly in hypersecretory activity of the dictyosomes, followed by dissolution of lysosomal membranes and release of hydrolases into the cytoplasm. By these means autolysis and separation of the outermost cap cells are suggested to occur.

It seems more likely that a group of genes which controls senescence becomes derepressed than that progressive decrease in the availability of information occurs by ordered gene repression. The latter process has been suggested to control senescence in leaves (e.g. Woolhouse, 1967; Osborne, 1967), where this process is progressive and involves the entire organ, rather than the precipitous process of senescence described for outermost root cap cells.

B. Ultrastructural Changes which Occur with the Increasing Age of Seed.

1. Significance of these Changes in Imbibed Material.

Various changes in subcellular structures which appear with increasing age of the seed are apparent in the imbibed material. These changes reflect damage which has accumulated during the ageing treatment. Such damage is suggested to be limiting in certain cases, culminating in the production of apparently non-viable type 1 aged embryos.

There is an increasing tendency for nuclear lobing to occur with increasing age of the seeds. The lobing which is first

apparent only in the most mature cap cells of unaged seeds later occurs throughout the zones of this organ in aged seeds. In addition, nuclear lobing becomes more marked with increasing age. Measurements of the average cross-sectional diameter show that the envelope becomes compressed as lobing occurs, the perinuclear cisternae becoming narrower. Nuclear lobing is suggested to result from fundamental changes in the nature of the membrane, the nucleoplasm bulging and causing stretching of the envelope. Senescent change of the nuclear envelope has been observed by Shaw and Manocha (1965) who reported that the nuclear membrane became crenated, irregular and vesiculated in senescing wheat leaves. Barton (1966) also reported vesiculation of the nuclear membrane in senescing Phaseolus mesophyll cells. Although these age-related changes are also membrane phenomena, the marked nuclear lobing reported in the present work does not appear to have been previously encountered, judging from the literature.

A change in the staining reactions of the chromatin to potassium permanganate occurs with increasing age of the seed. This is interpreted as a change in the chromatin, or in its reactivation during imbibition, which has occurred during the ageing process,

Plastids are amongst the first organs to show changes as a result of the ageing treatment. These changes are reflected in distortion of the inner and outer membranes of these organelles. Several investigators have reported that plastid degeneration occurs in senescing leaf cells. Shaw and Manocha (1965) described swelling of the intergrana lamellae in chloroplasts of senescing wheat leaves. These authors and also Barton (1966) reported that chloroplasts decreased in size in senescing leaf cells. R. Barton (1966) and Butler (1967) reported the accumulation of globules, presumed to be lipid in nature. Barton (1966) suggested that a specific enzyme system localised in the chloroplasts caused thylakoid breakdown at the onset of senescence in mesophyll cells

of Phaseolus, and Butler (1967) reported that the final phase of plastid degeneration involved rupture of the outer membrane of this organelle. Treffry et al. (1967) reported that plastids in senescing soybean cotyledons became extremely swollen prior to their rupture.

Plastid degeneration occurs in root cap cells of embryos with increasing age of seed and the changes are reflected in membrane distortions. This is suggested to indicate that changes have occurred in the molecular configuration of the membranes. However, senescent changes in plastids of cap cells do not involve localised rupture of these organelles. In cells of type 1 aged embryos, where marked degeneration of the protoplast has occurred during storage, the plastids are extremely swollen, with a marked lessening in the density of their contents. This is suggestive of loss of transport control across the outer membrane with subsequent swelling of this organelle.

Senescence in leaves (e.g. Woolhouse, 1967) and in cotyledons (e.g. Varner, 1961) is suggested to be genetically controlled, but the senescent changes described for seeds of increasing age are a result of environmental processes. However, it is pertinent that the ageing changes in the plastids of foliage leaves, cotyledons and embryos from stored seed, involve the lipoprotein membranes.

Dictyosomes apparently become disorganised with increasing age of the seed. Early degeneration of this organelle apparently involves 'unstacking' of the cisternae followed by their apparent loss from the cytoplasm. It is possible that individual cisternae persist, but are not recognised as such. In senescent cells of type 1 aged embryos no structures are visible which are recognisable as dictyosomes, or as individual cisternae. In this respect Butler (1967) reported that no dictyosomes were present in the cytoplasm of cucumber cotyledons in the late

stages of their senescence.

Distortion of ER profiles was seen with increasing age of the maize embryos. Irregularities were first visible in cap cells of material which had received 12 days of the ageing treatment, where an increase in the diameter of the cisternal lumen was seen. In some of the aged embryos (18 to 20 days) the ER profiles became longer and thinner than was usual in the unaged material. These changes are interpreted to result from loss of the normal properties of the membranes, and pressure of the enchylema is suggested to cause profile distortion. In this respect, Shaw and Manocha (1965) reported that the earliest visible ultrastructural change in senescing wheat leaves was swelling of the ER cisternae.

In cells of type 1 aged embryos only short, distended ER profiles remain. It is probable that initial breakdown of the ER occurs as a result of fundamental changes in the molecular configuration of the membranes in these senescent cells. However, the final stages of degradation of this organelle might have occurred during imbibition, as lysosomes with ruptured bounding membranes were encountered in cells of type 1 aged embryos. In addition, there was acid phosphatase activity scattered in the cytoplasm of these cells. In this respect, Treffry et al. (1967) reported that the ER is probably represented by vesicles and isolated membrane fragments in the final stage of senescence of soybean cotyledons. These authors described these final senescent changes to follow tonoplast breakdown. The cell vacuole is thought to be homologous with the secondary lysosome (e.g. Matile, 1968; Matile and Moor, 1968) and thus the final degradation of the ER in senescing cotyledons described by Treffry et al. (1967) could have been brought about by hydrolytic action, that is, by a mechanism similar to that suggested for senescent cells of type 1 aged embryos during imbibition.

Polysome formation was apparent in cap cells of embryos

which had received 6 and 12 days of the ageing treatment, 12 hours after the start of imbibition. However, in aged embryos the ribosomes were still largely disaggregated as monosomes at this germination stage. This change is thought to result from general slowing down of various cellular processes during germination of the ageing, but apparently viable embryos. Type 1 aged embryos are considered to be non-viable and it is therefore of the greatest interest to note that the ribosomes persist as monosomes within their cells.

Although the protoplasts are disorganised the ribosomes are still present and apparently entire. These observations are in keeping with those of Öpik (1966) who reported that ribosomes showed no signs of degeneration in senescing cotyledons of Phaseolus. However, Shaw and Manocha (1965) reported that ribosomes disappeared from the cells of senescing wheat leaves, and Butler (1967) reported that free ribosomes were absent from cells of senescing cucumber cotyledons. It is difficult to account for these conflicting reports. However, in cells of type 1 aged embryos, most of the visible deleterious changes are at the membrane level and ribosomes which are composed of RNA and protein are apparently unaffected, at least from an ultrastructural viewpoint.

Ultrastructural changes in mitochondria are among the earliest age-related changes to appear in cap cells of embryos from the stored seed. The profiles of the mitochondria in thin section become irregular, and in addition there is apparent disorganisation of the inner membrane system of this organelle. It is suggested that the visible mitochondrial damage results from fundamental changes in the molecular configuration of the membranes.

Mitochondria in cap cells of type 1 aged embryos show signs of extreme degeneration. These organelles have little internal structure and the earlier membrane distortion appears

to be reversed. However, the mitochondria in these cells are swollen and have a marked reduction in matrix density. It is suggested that complete loss of transport control across the mitochondrial membranes has occurred. Swelling is probably due to loss of transport control, and the earlier membrane distortions are suggested to have been reversed only as a consequence of the swelling.

It is suggested that damage to the mitochondria is the primary cause of cell death in type 1 aged embryos. In this respect, Throneberry and Smith (1955) suggested that loss of viability in corn seeds was closely associated with respiratory failure, and Varner (1961) suggested that a decrease in cellular repiratory efficiency was caused by gradual loss of mitochondrial function during senescence.

The maize seeds investigated by the present writer were stored at 40°C and 14% moisture content. In addition the oxygen content of their gaseous environment was that of the atmosphere at the start of the ageing treatment, falling somewhat as a result of respiratory exchange during storage. Roberts and Abdalla (1968) demonstrated that oxygen even in relatively low concentrations had a deleterious effect on stored seed.

It is suggested that the effect of oxygen could be on the lipoprotein cell membranes. Membrane lipids have unsaturated fatty acid components, and these have been suggested to be subject to peroxidative processes under aerobic conditions (Harman, 1962). This type of process involves initial removal of hydrogen (e.g. by reaction with endogenously-formed free radicals). As a result the lipid molecule becomes a free radical, which in turn can react with oxygen to form a peroxide. Such reactions could change the physical and chemical properties of membranes.

Therefore it is suggested that the visible membrane damage which accompanies the ageing process might be a result of

a peroxidative process. It is further suggested that accumulation of such damage in the mitochondria would result in disturbance of respiratory assemblies which are believed to be firmly embedded in the inner membrane (e.g. Lehninger, 1964; Roodyn, 1967) as well as of the inner membrane subunits thought to be associated with the enzymes of oxidative phosphorylation (Racker et al., 1964). As a result of such disturbances respiratory efficiency would progressively decline to a level where the energy requirements of the cell could no longer be met. Loss of viability as seen in type 1 aged embryos is suggested to result.

It is pertinent in this respect that biochemical studies on ageing soybean seeds have shown that mitochondrial efficiency dropped with increasing age of the seed (Abu-Shakra, 1965; Abu-Shakra and Ching, 1967). These authors suggested that degradation of the mitochondrial membranes probably resulted in uncoupling of the electron transport and phosphorylative systems.

Mitochondria in cap cells of type 1 aged embryos are markedly swollen. Various investigators have reported that mitochondrial swelling occurs in senescent cells e.g. Phaseolus mesophyll cells (Barton, 1966) and wheat leaf cells (Shaw and Manocha, 1965). It is suggested that swelling of mitochondria in cap cells of type 1 aged embryos probably occurs once phosphorylative efficiency has dropped. In this respect the suggestion that mitochondrial swelling normally occurs in the absence of a non-phosphorylated high-energy intermediate of oxidative phosphorylation may be pertinent (Stoner and Hanson, 1966). In addition, loss of transport control probably occurs once the degree of membrane damage has reached a certain limit.

Lysosomes show a degree of swelling in cap cells of type 1 aged embryos. In addition, the bounding membranes of some of these organelles appear to be incomplete. Acid phosphatase activity is located within the lysosomes and also in the cytoplasm. However,

in this case it is suggested that lysosomal membrane dissolution is a result, not a cause of cellular degeneration. Most of the other organelles are intact, although distorted in cells of type 1 aged embryos, 12 hours after the start of imbibition. In addition, it is unlikely that hydrolytic activity occurs, or that hydrolases could diffuse through the cytoplasm to any extent in the relatively dehydrated cells of embryos in stored seed. It is probable that lysosomal membranes also undergo molecular alteration during the ageing process, although these membranes are presumed to have properties differing somewhat from other cell membranes. Swelling and membrane rupture are suggested to be a consequence of such changes and acid phosphatase activity is apparently enhanced in cells of type 1 aged embryos compared with unaged material, 12 hours after the start of imbibition. It is possible that this enzyme normally occurs predominantly in a bound (inactive) form at this germination stage, but that its activity is somehow enhanced during ageing.

Most of the visible degenerative changes which occur with increasing age of the stored seed appear to result from membrane damage. In this respect there is a similarity to the situation in ripening (senescing) fruit tissue in which, although senescence is considered to be genetically programmed changes in membrane permeability are reported to accompany this process (e.g. Sacher, 1967). Ultrastructural observations on senescing fruit tissue demonstrated swelling of organelles to accompany the process (Bain and Mercer, 1964). It is suggested that the cells of type 1 aged embryos represent a stage where membrane damage of the organelles generally, and particularly of the mitochondria, has a limiting effect resulting in complete senescence of the cells concerned.

2. Age-related Changes at the 24- and 48-hour Germination Stages in Type 1 Aged Embryos.

Degenerative changes are more advanced in the cells of type 1 aged embryos 24 hours after the start of imbibition than at the 12-hour stage. There is evidence of general organelle breakdown and few intact lysosomes are visible. It is thought that these further degenerative changes have been brought about by the activity of hydrolases released from the ruptured lysosomes, and tests for acid phosphatase have demonstrated the activity of this enzyme to be wide-spread in the protoplast.

By 48 hours after the start of imbibition, progressive destruction of all the subcellular components can be traced in cap cells of type 1 aged embryos. This process is generally accompanied by the progressive accumulation of a granular deposit thought to represent hydrolytic reaction products between the wall and plasma membrane. In the final stages of protoplast destruction fragments of the ruptured plasma membrane, and the occasional swollen mitochondrion are visible within the mass of homogeneous granular deposit. Acid phosphatase activity is associated with cellular remains during all the progressive stages of protoplast destruction.

It is suggested that cells of type 1 aged embryos lose their viability in the stored seed as described above. When the seeds are imbibed with water, leakage of hydrolytic enzymes from damaged lysosomes is thought to occur, and it is suggested that final, complete intracellular breakdown results from the action of these hydrolases. It is pertinent that no incorporation of ^{3}H-thymidine, ^{3}H-uridine or ^{3}H-leucine occurs in cells of type 1 aged embryos, supporting the view that these cells are non-viable, as no activity occurs at the molecular control level.

3. Repair and Compensatory Mechanisms and Type 3 Aged Embryos

Many of the ultrastructural abnormalities visible in imbibed cap cells appear to be reversed in certain material 24 and 48 hours after the start of imbibition.

Ultrastructural abnormalities which were visible in mitochondria and plastids in cap cells of imbibed 6- and 12-day and type 3 aged embryos appear to be reversed by the 24-hour germination stage. It is possible that the damage which was evident at the 12-hour germination stage did not represent a fundamental change in the molecular configuration of the membranes. However, this is unlikely to be the case. It is more likely that specific membrane repair systems might operate. Such systems, under genetic control, could be operative during the 12 - 24 hour period after the start of imbibition, and could act to repair membrane damage. In this respect, specific enzyme systems which eliminate damage at the DNA level have been described (Setlow and Carrier, 1964) and processes of repair are known to occur at various organisational levels. It is suggested that the reversal of mitochondrial and plastid membrane abnormalities results primarily from the action of repair systems under direct control of the genome. In addition, these organelles are known to contain DNA. Mitochondria contain DNA and a DNA-dependent RNA polymerase (Roodyn and Wilkie, 1968) and O'Brien and Kalf (1967) demonstrated the presence of ribosomes within these organelles. According to Roodyn and Wilkie (1968) mitochondria contain all the necessities for protein synthesis and labelled amino acids are incorporated into peptide chains within these organelles. Proteins synthesized by mitochondria are apparently of an insoluble structural nature (Roodyn and Wilkie, 1968). Thus it appears probable that repair of mitochondrial membranes in maize embryos might be partly controlled by the individual mitochondria. It is pertinent in this respect that silver grains are encountered over mitochondria in cap cells

of senescing embryos, illustrating localised incorporation of both ^{3}H-uridine and ^{3}H-leucine to have occurred. This did not appear to be the case in unaged material where general cytoplasmic localisation of both these labels occurred. It is suggested that a measure of RNA and protein synthesis occurs in these organelles with increasing age of the material and that these syntheses may at least partly represent systems involved in membrane repair.

Characteristic plastid DNA has been identified (Chun et al., 1963; Kirk, 1963) and RNA has been demonstrated to occur within plastids (e.g. Dyer and Leech, 1968). In addition, ribosomes have been identified within plastids of certain plant material (e.g. Eisenstadt and Brawerman, 1963). Thus protein synthesis can probably occur in these organelles.

However, plastids of maize root cap cells do not appear to contain ribosomes, thus localised protein synthesis is unlikely within these organelles. It is pertinent in this respect, that the autoradiographic studies of RNA and protein syntheses did not reveal particular incorporation of the label into these organelles. Thus membrane repair which is apparent in cap cell plastids of senescing embryos probably results from the action of a DNA-directed repair system, located in the nucleus.

Dictyosomal damage which accompanies ageing is thought to involve 'unstacking' of cisternae and possible loss of single cisternae. This damage is suggested to be primarily a disruption of the intercisternal region perhaps involving both the intercisternal elements and the bonding constituent proposed by Mollenhauer and Morré (1966). However, replication of these organelles or re-association of unstacked cisternae is apparent by the 24-hour germination stage in cap cells of embryos which have received 6 and 12 days of the ageing treatment, and by the 48-hour germination stage in type 3 aged embryos. Thus although dictyosomes appear to undergo structural damage with increasing age of the material,

the control systems for their replication and/or maintenance are apparently unaffected, at least in some of the aged embryos.

Distortion of the ER occurs in cap cells of material which has been subjected to an intermediate period (12 days) of the ageing treatment, at the 12-hour germination stage. However, by the 24-hour germination stage ER profiles are generally atypically long in this material. In this respect, Opik (1966) reported a tendency for the ER to become orientated in parallel lamellae in ageing Phaseolus cotyledons. It is possible that this apparent development of the ER may represent a type of compensatory mechanism.

W. D. Bonner (1961) suggested that all plant cells have a system of electron transport to oxygen which is alternative to the cytochrome oxidase system of the mitochondria. It is possible if such a system is linked up with cytochrome b_7 associated with the ER, that atypical development of the ER in cap cells of these embryos might represent a compensatory mechanism by which respiratory function is at least partly transferred to this organelle. It is interesting in this respect that Marsh and Goddard (1939) reported the respiration of young carrot leaves to be sensitive to carbon monoxide and cyanide (inhibitors of mitochondrial system), while respiration in old leaves carried on in the presence of these inhibitors to which cytochrome b_7 is insensitive (W.D. Bonner, 1961). In addition, the ER is reported to develop extensively under anaerobic conditions, which suggests a transfer of metabolic function to this organelle with the elimination of the mitochondrial system (e.g. Linnane et al., 1962).

By the 48-hour germination stage, development of the ER in cap cells of embryos which have been subjected to 12 days of the ageing treatment was similar to that described for unaged material. This suggests the unimpaired functioning of genetic systems which control development, repair and maintenance of the ER.

The pattern of ER development in cap cells of type 3 aged embryos is similar to that described for embryos which have been stored for 12 days at 40°C and 14% moisture content.

Although polysome formation is slower with progressive age of the seed characteristic aggregations of ribosomes have occurred by the 24-hour germination stage in cap cells of ageing and type 3 aged embryos.

It is probable that at least part of the long-lived m-RNA suggested to be present in quiescent seeds (e.g. Dure and Waters, 1965) may continue to be functional in cap cells of embryos with increasing age, thus accounting for some polysome formation at the 24-hour germination stage. In addition, synthesis of new m-RNA apparently occurs early in the germination process (Key, 1964).

Autoradiographic investigations using ^{3}H-uridine demonstrated that some RNA synthesis occurs in cap cells of senescing and type 3 aged embryos at the 48-hour germination stage. Thus it is probable that polysome formation in these embryos is partly dependent on long-lived m-RNA and partly on newly-synthesized RNA. In addition, the RNA is functional in supporting protein synthesis, as evidenced by ^{3}H-leucine incorporation.

Membranes bounding lysosomes do not appear to become distorted with increasing age of the material, as do membranes bounding, for example, the mitochondria and plastids. This might be a part-consequence of necessary differences in these membranes (Gahan, 1967), which normally function to isolate hydrolytic enzymes form the cytoplasm.

However, atypical, large second-phase lysosomes which are seen in cap cells of material which had received 12 days of the ageing treatment and in these cells of type 3 aged embryos, are thought to result from a precocious development of these organelles. In addition, remnants of organelles normally encountered in the

cytoplasm are visible within these lysosomal vacuoles in the zones of division and differentiation. This is an unusual feature for cap cells other than those of the mature zone, in unaged material. It is possible that the atypical development and activity of the lysosomes is the result of ordered control, representing a type of compensatory mechanism by which inefficient or non-functional organelles are eliminated from the cytoplasm.

The action of another controlled compensatory mechanism is suggested to result in the enhanced rate of mitochondrial replication described for cap cells of ageing, but apparently viable embryos. By means of this suggested compensation more efficient mitochondria might be produced to meet the energy requirements necessary for germination.

Although the feasibility of compensation in ageing cells is open to question (Medvedev, 1967) it is possible that such mechanisms do function in cells of certain senescing embryos. It is suggested that such mechanisms might function in embryos where damage has occurred, but where such damage is not too extensive and has not involved fundamental changes in those portions of the genome controlling vital systems. Those maize embryos in the cells of which compensatory mechanisms are suggested to operate are apparently viable.

Thus it is suggested that the cells of aged, but viable embryos retain a significant measure of genetic control evidenced by the action of membrane repair systems and possible compensatory mechanisms. It is further suggested that those aged embryos which germinate and establish growing seedlings can only do so by elimination of the sub-cellular damage accumulated during ageing, thereby implying largely unchanged, functional control mechanisms.

However, although the cells of type 3 aged embryos appear to be viable 12 and 24 hours after the start of imbibition, this

was not found without exception at the 48-hour germination stage. In some of the type 3 aged embryos at the 48-hour germination stage progressive, accelerated senescence is encountered in the root caps. This has been termed precocious senescence.

Precocious senescence in root caps of type 3 aged embryos appears to be similar to senescence of the outermost cap cells of unaged material with one notable difference. Senescent cells are not confined to the outermost layer, but the entire distal portion of the cap, which varies in extent from embryo to embryo shows marked senescent change. This type of senescence is sequential, progressing from the oldest region of the cap towards the youngest.

Apparently viable cells, which occur immediately adjacent to cells in which the entire protoplast is degenerating, show a marked degree of development and apparent activity irrespective of the cap zone in which this occurs. There is apparent reversal of the the ultrastructurally-visible damage encountered in cap cells of all senescing embryos 12 hours after the start of imbibition. In general, the various organelles within viable cells which border on senescent cells appear to be ultrastructurally normal, and there is evidence of their replication (e.g. mitochondria) and apparent activity (e.g. dictyosomes).

There is a peak in ^{3}H-uridine incorporation in these cells compared with other cells in these root caps indicating that enhanced RNA synthesis occurs and the ribosomes are aggregated to form polysomes. In addition the incorporation of ^{3}H-leucine and therefore protein synthesis, reaches a maximum in viable cap cells which are immediately adjacent to senescent cells. None of the senescent cells incorporate either ^{3}H-uridine or ^{3}H-leucine.

Lysosomes within the apparently viable cells immediately adjacent to the senescent cells are in their second developmental phase, but there is evidence of localised dissolution of the bounding membranes of these organelles.

Senescence occurs precipitously and extensive acid phosphatase activity is located in the protoplasts of these cells in which few organelles are recognisable. However, monosomes persist in degenerating protoplasts, indicating that m-RNA has probably been degraded, but that ribosomes are apparently more resistant to hydrolysis than other subcellular structures. This is in keeping with the results of Öpik (1966) who reported ribosomes to persist in senescing cotyledons of Phaseolus, but contrary to the reports of Shaw and Manocha (1965) and Bulter (1967) discussed above (P. 208).

The relationship between viable, apparently highly organised cells and cells which are senescent is similar to the relationship described for the distal cells of the mature zone and the outermost cells in root caps of unaged maize embryos, especially Hickory King.

It is suggested that the precipitous senescent changes which occur are a direct consequence of the release of hydrolases normally confined within the lysosomes. It is probable that a group of genes exists which controls senescence, and that these genes remain repressed during the ordered sequence of repression and derepression which controls normal differentiation and development of the root cap. It was suggested (see above, p.203) that senescence of the outermost root cap cells in unaged material is brought about by derepression of these genes, resulting in the release of lysosomal enzymes and autolysis of the cells concerned. It is suggested that, in material which shows precocious senescence this group of genes becomes derepressed in cap cells other than those comprising the most distal region of this tissue.

It may be that mutation of the genes controlling senescence retults in their derepression (e.g. Mevedev, 1967; Roberts et al., 1967). However, the fact that disorganisation progresses from the chronologically oldest cells towards the

youngest suggests that precocious senescence is more likely to be a consequence of acceleration of the genetically-controlled process suggested normally to bring about senescence of the outermost cap cells only. It should be noted that in this respect precocious senescence is confined to the root caps of these type 3 aged embryos, whereas all the other forms of senescent change observed in maize embryos have occurred in cells of the root apex as well.

It is suggested, however, that embryos showing precocious senescence of the root cap might possibly not establish viable seedlings. Once cellular autolysis brings about the death of the entire root cap, then the unprotected state of the root apex would probably result in physical injury and impaired efficiency of the root.

4. Damage at the Control Level and Type 2 Aged Embryos.

Environmental factors may bring about changes at the control level within cells which contribute to ageing. The increased incorporation of ^{3}H-thymidine which is mainly encountered in cap cells of embryos which have received intermediate periods of the ageing treatment, is suggested to be an ageing change. It is notable that the increased proportion of labelled cap nuclei is at least partly due to ^{3}H-thymidine incorporation into zones which are non-meristematic and do not incorporate this label in caps of unaged material. It is therefore suggested that mutation may have occurred in the genes which control DNA replication. These genes are normally repressed in non-meristematic cells, and probably become derepressed as a consequence of some structural change of the DNA. This is suggested to result in the synthesis of m-RNA and thus in the synthesis of DNA-polymerase, resulting in replication of the DNA.

Although ^{3}H-thymidine incorporation does not occur in the meristematic zones of any of the aged (18 to 20 day) embryos a measure of incorporation of this label occurs in non-meristematic cell zones of some of the aged embryos. If such embryos are viable (i.e. certain type 3 aged embryos) then the lack of incorporation of ^{3}H-thymidine in the meristematic cells could be an expression of the lowered rate of germination of aged embryos. However, the random incorporation of the label into the nuclei of non-meristematic cells probably results from derepression of the genes concerned, brought about by mutation.

This interpretation of the changed pattern of ^{3}H-thymidine incorporation into nuclei of cap cells with increasing age is in keeping with suggestions of Medvedev (1967) and Roberts et al. (1967) that mutation in repressed portions of the genome might interfere with the complementarity between cistron and repressor, thereby transforming a gene from the repressed to the active stage.

Cells of type 2 aged embryos are those in which general disorientation of the organelles has occurred. The disorientated organelles are crowded in the perinuclear cytoplasm, and only a relatively sparse scattering of organelles occurs near the cell peripheri. The nucleus itself shows signs of gross damage, as it consists of a mass of distorted processes with no recognisable central core.

There are no signs of membrane repair to mitochondria or plastids in these cells 48 hours after the start of imbibition. Generally, little activity of the organelles is evident, and no apparent organelle replication occurs.

It is suggested that failure to repair damaged organelles results from breakdown at the control level in these cells. Such failure might occur at the DNA, RNA or protein synthesis level. However, as this failure is general, and all the various apparently inactive organelles retain the damage accumulated during the ageing

treatment, it is most probable that breakdown of the control mechanisms occurs at the DNA level. Damage at the DNA level may take the form of mutation, or perhaps irreversible repression of parts of the genome.

However, the strikingly atypical development of the ER in cap cells of type 2 aged embryos at the 48-hour germination stage suggests thay some measure of molecular control exists. It is pertinent in this respect that cap cells of type 2 aged embryos show some incorporation of ^{3}H-uridine and ^{3}H-leucine. The pattern of incorporation differs from that usually encountered in that there is no marked peak in incorporation of ^{3}H-uridine in the mature cells and no increase in incorporation of ^{3}H-leucine in mature or senescing root cap cells. In addition, polysome formation has occurred in these cells by the 24-hour germination stage and polysomes persist at the 48-hour germination stage. The formation of polysomes at the 24-hour germination stage is probably largely dependent on the long-lived m-RNA present in mature seed (Dure and Waters, 1965). However, by the 48-hour germination stage some of the newly-synthesized RNA is probably involved in polysome formation.

Elaboration of the ER is suggested to be an example of translation of 'nonsense information' from RNA. If this is the case then molecular change may have occurred at the DNA level. Such change would probably involve mutation with subsequent derepression of the genes concerned. Genome mutations in somatic cells are widely considered to contribute to the ageing process (e.g. Curtis, 1966; 1967) and chromosome damage which has been found to be a reliable index of ageing (e.g. Curtis, 1963; Roberts et al., 1967) probably results from damage to DNA i.e. from mutations.

Another possibility is that part of the long-lived m-RNA suggested to be present in mature seed (e.g. Dure and Waters, 1965) might normally be involved with early ER develppment. Such m-RNA might persist and be active in these generally-disorganised cells.

This implies breakdown in a control system which normally limits the life of this m-RNA. In addition, this m-RNA might itself have been subject to molecular accident during ageing, thus accounting for atypical ER elaboration.

It is also possible that the atypical elaboration of the ER represents a compensatory mechanism by which respiratory function is transferred to an ER-associated system with the failure of the mitochondrial system. However, such a mechanism implies condiderable organisation at the molecular control level and it is improbable that this exists in these cells because their general appearance suggests that little organised molecular control exists.

Use of the Gomori method for acid phosphatase localisation showed that the activity of this hydrolase is confined to the lysosomes, all of which are apparently intact in cap cells of type 2 aged embryos.

It is suggested that the pattern of degenerative change which occurs in cells of type 2 aged embryos results primarily from damage to the membranes (discussed for type 1 aged embryos) and that general breakdown at the molecular control level also occurs. Some aspects of the disorganisation suggest non-function of the genetic pathways concerned, while others suggest the translation of 'nonsense information'.

5. Aspects of Chromosome Damage.

Chromosome damage is found to accumulate in non-dividing cells of the maize root tip with increasing age of the seed. These observations are in agreement with those of many investigators. This subject has been reviewed by Barton (1961) and it has been firmly established that chromosome aberrations are produced in non-dividing cells during the ageing of seeds. In this respect Abdalla and Roberts (1968) showed that any combination of temperature, moisture level and oxygen tension which led to loss of seed viability

also led to the accumulation of aberrent cells. These authors suggested that an upper limit of the frequency of aberrent cells in which cellular damage is reflected by chromosome breakage exists in the embryo, after which death of the embryo occurs. They also suggested that the value of this upper limit is peculiar to species.

Many investigators in the field suggested that chromosome aberrations result from the accumulation of automutagenic substances within the seed (D'Amato and Hoffmann-Ostenhof, 1956). However, much of the evidence reviewed by these authors is open to criticism, although the results of more recent, controlled experiments apparently relate the administration of leachates from old seeds to an increase in chromosome fragmentation (e.g. work on onion seed by Jackson, 1959). However, Roberts et al (1967) using aged and fresh seeds of peas, beans and barley, showed that concentrated aqueous extracts from young and aged seed did not induce chromosome aberration in fresh seed of the same species in each case. Thus the role of automutagenic substances in the production of chromosome abberration in seeds is still controversial.

It is likely that chromosome aberrations represent general damage to the DNA which has occurred at the molecular level. Nuclear division was absent in many of the aged embryos examined for chromosome aberration 60 hours after the start of imbibition. These are probably representative of type 1 aged embryos where death is thought to have occurred during storage. Those embryos having received 18 to 20 days of the ageing treatment, which showed nuclear division on cytological examination are thought to be representative of type 3 aged embryos, and possibly also of type 2 aged embryos.

Replication of DNA in non-meristematic cells is thought to result from mutation followed by derepression of that part of the genome involved. This visible manifestation of what is probably a mutation occurs at a stage where the average percentage

of aberrent cells per root tip is 8 although the average germination percentage is still 90%. Note that the upper limit of aberrent cells per root tip is 9% in the material investigated. Certain visible ultrastructural phenomena, for example, non-repair of organelles and atypical proliferation of the ER in type 2 aged embryos and the production of abnormal cristae in the mitochondria of apparently viable type 3 aged embryos have been interpreted to result possibly from mutation. It is probable that the molecular alterations underlying these and other mutations may result in microscopically visible chromosome damage. In addition, there is a multitude of pathways in the cell whose status cannot be ascertained by electron microscopy, thus it is probable that many mutations have not been detected. The fact that the staining reaction of the chromatin to potassium permanganate changes with increasing age of the material is interpreted as possibly resulting from a change in the nature of the chromatin which has occurred during the ageing process.

Therefore it appears probable that microscopically-visible chromosome damage is the result of molecular disturbances at the DNA level. This interpretation is in keeping with the suggestion of Roberts et al. (1967) that mutation in somatic cells probably underlies the microscopically-visible chromosome damage.

In conclusion it is suggested that not only can no one factor be cited as the cause of ageing, but that several patterns of senescent change exist at the cellular level, at least in the root cap cells of maize embryos.

Senescence of the outermost root cap cells is suggested to be a genetically-programmed event. The various ultrastructural changes which appear early in the ageing sequence may be a direct consequence of endogenously-produced free radicles with subsequent action of oxygen at the membrane lipid level. Such changes are

suggested to lead to complete loss of viability, probably resulting from respiratory failure in certain aged embryos. The patterns of senescence which in a study of the aged embryos also reveal probable failure at the molecular control level, manifested by apparent lack of activity or by inappropriate activity in certain cases, as well as an apparent acceleration of the usual pattern of cap senescence in other cases.

PART V - BIBLIOGRAPHY

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