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A MORPHOMETRIC ANALYSIS OF ULTRASTRUCTURAL
CHANGES IN THE SHOOT APEX OF GLYCINE MAX
DURING THE MORPHOGENETIC SHIFT FROM
QUIESCENCE TO GERMINATION

by

Saba Mebrahtu

A thesis submitted
in partial fulfillment of the requirements for the
degree Master of Science
Major in Biology
South Dakota State University

1984

A MORPHOMETRIC ANALYSIS OF ULTRASTRUCTURAL
CHANGES IN THE SHOOT APEX OF GLYCINE MAX
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This thesis is approved as a creditable and independent investigation by a candidate for the degree, Master of Science, and is acceptable as meeting the thesis requirements for this degree but without implying that the conclusions reached by the candidate are necessarily the conclusions of the major department.

— Thesis Advisor / Date

Head, Biology Department Date

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sbm

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ABSTRACT

This research is a study of a morphogenetic shift of the soybean shoot apex from quiescence to germination. The minute changes in the organelles were recorded with the use of stereological methods. The ultrastructural data was statistically analyzed. The ultimate goal was the recognition of relationships between the cell's functional units and its structural units, with the identification of morphogenetic regulation mechanisms.

Morphometric image analysis of cellular organelles indicated that storage reserves were transformed from lipid bodies to plastid starch. Proplastids and promitochondria, so difficult to differentiate in early stages of germination became obvious by 96 hours postgermination.

Significant differences were found in mean organellar volume fraction percent with reference to time at the four stages of development studied.

The Waller-Duncan K-ratio T-test for organellar volume fraction percent by development time were significantly different in the 48 and 96 hour groups.

Relationships between development time and each of the seven organelles studied were developed using regression. Cyclic fluctuations were observed in volume fraction percents of plastids, vacuoles and nuclei. A sharp rise in starch and sharp drop in lipid was observed. No change was observed in mitochondria and cell walls.

INTRODUCTION

Relatively old mathematical rules, stereological methods, recently have found application in the biological sciences. They enabled biologists not only to qualitatively analyze the ultrastructural changes of cells during a developmental period but also to quantify those minute changes in terms of organellar volume fraction, surface density and size distribution.

Morphometric information is obtained by application of stereological methods in light and electron microscope tissue sections (Weibel, 1973). The stereological methods are applied based on random sampling and geometrical probabilities. For that, it is sometimes called the geometrico-statistical method. Basically stereological methods consist of a body of formulas which have the aim of obtaining information about three-dimensional structure from two dimensional images. It can also be regarded as a means of extrapolation from a two to a three-dimensional space.

In the biological field of ultrastructural studies, electron micrographs have been used to obtain the two-dimensional configuration of the cell. Stereological methods interpret given information into three dimensions.

During a developmental change the morphometric information may be compared to other biochemical changes allowing the examination of any direct correlations between the fine structure and function of organelles. In this area especially, stereologic methods have found much application and have offered great contributions towards better

understanding about the ultrastructural and functional changes of the cell.

A growth period where there are dramatic changes, ultrastructurally and biochemically as well as morphologically, is germination. It has long been a subject of high interest among the botanists and biologists. It is a morphogenetic shift where the embryo (seed) assumes once again metabolic processes that have been halted or suspended during quiescence or dormancy (Yoo, 1970). Studies carried out at this particular stage of the life cycle have shown direct correlations between functional and structural changes within cells.

The oil seeds and the legumes have been of special interest simply because during germination the storage materials in the form of lipids, protein and starch have shown great fluctuation. The question that has preoccupied researchers has been what exactly the triggering mechanism is, especially in quiescent seeds where it's been indicated that metabolic actions start right after complete water imbibition. The nuclear material has been studied to determine any fluctuations in DNA and RNA synthesis. Also, using stereological methods the organelles are now being measured to determine any fluctuation in configuration that could easily be related to its function.

Already, the ultrastructural and biochemical changes that occur in the embryo right after water imbibition has been described in chronological order in several types of seeds in several publications. Use of stereological methods in this particular field has not been very extensive. Although, in its embryonic stage as a tool in the biologi-

cal sciences, there has been a lot of advancement in stereological methods, uses and application over a few years.

The objective of this project was to quantify developmental stages in organellar volume fraction, in the promeristematic tissue of the shoot apex of Glycine max (L.) Merr. at four progressive developmental stages, 12, 24, 48 and 96 hours after initial water imbibition. With the aid of statistical analysis, the morphometric information will then be used to make inferences about the functional changes of the organelles during early development.

LITERATURE REVIEW

I. Stereological Methods

Much anatomical and morphological work using both light and electron microscopy has been almost wholly descriptive, making little or no attempt to quantify any of the cellular and subcellular changes occurring in development. The first step towards quantification in anato-morphological research was taken over a decade ago. Stereological methods were introduced into the field and since then several researchers have used this mathematical method. The predominant aim of these studies was to strive for the most direct parameter of structure-function correlation, indicating the close linkage between functional units of cell metabolism and specific structural units of cell organization. Furthermore, Loud (1962) stated that quantitative analysis of subcellular architecture revealed by electron microscopy was a logical step in the investigation of the normal functioning of cells and their pathological alterations. For the evaluation of the relative areas occupied by formed structures, such as nuclei, mitochondria and inclusion bodies and for comparing cells in which only relatively small qualitative differences in composition were found, it was essential to have a sensitive systematic technique for the estimation of structural volumes. Therein, stereology has played a great role, in that it enabled biologists to explore the developmental changes in three-dimensional space when only two-dimensional sections through bodies or their projections on a surface was available.

Stereology was first developed in 1847 by Delesse. Delesse's principal demonstration was that volumetric composition of tissue was

equivalently represented in the planimetric density of profiles on random sections (Weibel & Elias, 1967a). This method first found application in geography to mean quantitative descriptions of geographical features and also in metallurgy (Weibel & Elias, 1967b). Delesse stated that the volume fraction V_{V_i} of a component i in the tissue can be estimated by measuring the area fraction A_{A_i} of a random section occupied by intersections of i . Delesse principles also demonstrated that volumetric composition (V_{V_i}) of tissue was equivalently represented in the planimetric (L_{L_i}) density of profiles on random sections. In other words, $V_{V_i} = A_{A_i} = L_{L_i}$. Another concept was added later on, in 1933. Glagoleff found that planimetry could be done by superimposing a regular point lattice on the section and counting the points which lie on transections of the structures. The fraction P_{P_i} of points lying on transections of i would be an estimate of V_{V_i} , i.e. $V_{V_i} = P_{P_i}$. Thus, $V_{V_i} = A_{A_i} = L_{L_i} = P_{P_i}$. (Weibel & Elias, 1967a).

The random probe used for these stereological measurements was the plane obtained by random sectioning of tissue blocks, and also included lines and points. Some parameters were estimated by a variety of probes. Volumes were measured planimetrically on random planar probes or by linear integration along random lines or by counting the fraction of a net of test points that lies within the structure (Weibel & Elias, 1967a). Hillard (1968) clarified the concept, by stating that all methods of estimating volume fractions from measurements on a two-dimensional section depend on an equivalence between the volume fraction

and the intercepted area (A_T), line (L_T) or point fraction (P_T). Out of the three test probes, Weibel (1973) concluded that point count procedures were superior to the more laborious methods of area or line measurement. However, Weibel (1981) later modified his comment by stating, stereological point counting methods, when assisted by computerized data acquisition systems, were very efficient and could only be surpassed by fully automatic image analyzer.

The quantitative relationship between A_T , L_T and P_T was derived and proven by Delesse (Weibel, 1973). Table 1 lists symbols that have been accepted by the International Society for Stereology. The Delesse principles involved:

- 1) P_T : Set of points for analysis of volume density $V_V = P_P = P_i/P_T$.
- 2) L_T : Test line for estimating S_V by the formula $S_V = 2 \cdot I_L = 2 \cdot I_i/L_T$.
- 3) A_T : Test area for estimating M_V by the formula $M_V = 2 \cdot Q_A = 2Q_i/A_T$

and for estimating N_V by any formula involving $N_A = N_i/A_T$.

Weibel's explanation (cf. Briarty, 1975) helped to visualize exactly what was involved in the procedure. If a cube of tissue containing a certain component of any shape whose volume density (V_V) was to be measured, it was cut into thin serial sections of constant thickness t . These thin slices of the tissue contained slices of the test component. On each section, the area of the profile a and the area of the section A (which remained constant) could be measured. All the profile areas were added and multiplied by t , which equalled the component volume. All the section areas were added and multiplied by t , which equalled the volume of the cube. The ratio of the two would obtain the volume

Table 1. List of basic symbols for stereology.

Symbol		
V	Volume of structure or test volume	cm ²
S	Surface of structure	cm ²
M*	Length of linear feature of structure	cm
N	Number of structures (particles)	cm ⁰
D	Linear dimension of structure ("caliper diameter")	cm
T	Section thickness	
A	Area on section (planar)	cm ²
L	Length of test line	cm ⁰
P	Number of test points	cm ⁰
B*	Profile boundary length on section	cm
I*	Intersections of surface trace with test line	cm ⁰
Q*	Transsections of linear feature with test area	cm ⁰
A _T	Test area	
L _T	Test line	
P _T	Test point set	
VV _i	Volume density of component i	cm ⁰
SV _i	Surface density of component	cm ⁻¹
MV _i	Density of length in volume	cm ⁻²
N _V	Numerical density	cm ⁻³
A _A	Profile density on test area	cm ⁰
B _A	Boundary density on test area	cm ⁻¹
Q _A	Density of transsections on test area	cm ⁻²
N _A	Numerical profile density on area	cm ⁻³
L _L	Intercept density on test lines	cm ⁰
I _L	Intersection density on test lines	cm ⁻¹
N _L	Intercept number on test lines	cm ⁻¹
P _p	Point density	cm ⁰

Weibel (1973) has introduced four additional letters (M, B, I, Q).

density.

Thus, $V_V = E(a) \cdot \underline{t} / E(A) \cdot t = A_A$. With this Weibel concluded that the density of profiles on sections was an unbiased estimate of the volume density of structures.

Schaefer (1970) developed some principles that needed to be considered for a clearer understanding of the methods and specific steps involved:

First Principle: The relative area fractions of the components A & B in a section through a body are the same as the relative volume fractions of the components in the whole body. If sections are used, cuts should be as thin as possible.

Second Principle: The relative area fractions of the components A & B in the total of a number of sections through a body are the same as the relative volume fractions of the components A & B in the whole body. The sections together amount to a representative sample of the whole body.

Third Principle: If an area F consisting of the components A & B is fully overlaid by a grid of P points, and the number P_A of points falling on the component A is then counted, the point fraction P_{P_A} (i.e., P_A/P) of component A is equal to the area fraction F_{F_A} (i.e., F_A/F) of that component.

Fourth Principle: The mean relative error of the result obtained for the contribution of a given component is inversely related to the volume fraction of that component and is also inversely related to the total number of points counted. According to this principle, a more repre-

sentative sample is obtained if the counting is carried out not on just a single section but on several. Therefore, the variance (square of s. d.),

$$V(\bar{\bar{y}}) = \frac{e_1^2}{n_1} + \frac{e_2^2}{n_1 \cdot n_2} + \frac{e_3^2}{n_1 \cdot n_2 \cdot n_3} + \frac{e_4^2}{n_1 \cdot n_2 \cdot n_3 \cdot n_4}$$

where,

e_1^2 = variance of individual m pieces, n_1 their number

e_2^2 = variance of individual sections, n_2 their number per m pieces

e_3^2 = variance of individual fields view, n_3 their number per section

e_4^2 = variance of individual points, n_4 their number per field of view

Note these relationships; an increase in the number of points counted per field of view (n_4) depresses only the fourth term. An increase in the number of fields (n_3) depresses fourth and third terms and an increase in the number of m pieces, or to a lesser extent an increase in the number of sections per m pieces decreases the value of $V(\bar{\bar{y}})$ substantially.

Other sets of stereological principles were considered by Weibel & Elias (1967b).

1) Measuring with random probes -- A stereological random probe is a well defined test system whose systems are known to the investigator. It is confronted with the objects under investigation in any random manner, whereby their confrontation is considered to occur at random if

the object does not influence the positioning of the probe. Probe and object are statistically independent and it is evident that any given confrontation probe and object will occur with a certain probability. There are three basic types of probes; planes, lines and points.

2) Requirements for derivation of stereologic principles -- There is a relationship between the number of spherical sections on the unit area of a test plane (random section) and the number of spheres contained in the unit volume of tissue. The derivation of such precise mathematical relationships involves rigorous considerations on geometrical probability, since both the tissue elements and the probes are geometrical objects which can combine at random.

3) Requirements for application of stereologic principles in morphology -- Once a suitable test system is defined, practical application will depend on its proper confrontation with the objects. In all test systems the thickness must be of negligible thickness.

Steer (1981) indicated that the effectiveness of stereological methods was governed by the design of the analytical system. That is, the sampling methods or the selection of the most suitable combination of micrographs depends on a thorough understanding of the three-dimensional organization of the tissue and cells under consideration. This means that sections must be taken in appropriate planes at appropriate levels in the tissue with appropriate test grids. Grid systems are used for point counting. The grid system of points serves as a statistical sample to estimate the quantities of various components of a tissue or organ (Berlyn & Miksche, 1976). Several sets of

points from several sections are integrated and an accurate estimate of the size, percent composition, etc., of the component or components could be determined.

Future progress in stereological analysis, surprisingly, is not only the design of more sophisticated tracing devices but the elaboration of rules on how best to divide the effort among the samples (Weibel, 1981). Nicholson (1978) admitted that the role of statistics in stereology has been minimal. Even though stereological point counting methods when assisted by computerized data acquisition systems were very efficient and could only be surpassed by fully automatic image analysis, they would still not be applicable to the real statistical problem encountered. The value and necessity of advanced statistical analysis is being realized more and more in the field. Basically, statistics is concerned with evaluation of the accuracy of the measurement process which is a topic just now being addressed by experimental stereologists (Nicholson, 1978). Use of statistics was limited mostly to sampling for the mean, standard deviation and binomial distribution as a model for a point counting fraction P_p . Only a few included confidence intervals using the t-distribution. Actually parameter standard deviation estimates and joint confidence regions measured lab precision. For the future the potential role of statistics lies in sampling, estimation and multivariate analysis (Nicholson, 1978). Nicholson (1978) further elaborated these potential roles of statistical analysis:

Sampling:

Systematic sampling is the probabilistic basis in stereology for

point counting and linear intercept analysis procedures using stereological test systems.

Stratified random sampling provides the theoretical framework for stereological estimation which gives equally valid results for homogeneous and heterogeneous specimens.

Subsampling provides the framework for the evaluation of the universal practice of sampling materials with sections followed by subsampling sections with fields and possible subsampling fields with several applications of a test system.

Estimation:

Historically, size distribution estimation was based on either the diameter of features on the section or the length of a chord through a feature resulting from a linear intercept analysis. Many stereologists felt that the chord method was superior because of its mathematical simplicity. One outcome of the linear functional estimation theory is that chord length estimates fail to produce unbiased estimates in some situations.

Multivariate Analysis:

Multivariate analysis is a form of multiple regression, principal components, factor analysis, or a cluster analysis, all of which has been used sparsely in the past by stereologists. The need for application and understanding of multivariate analysis methods will be greater in the future with increased usage of automatic image analyzers.

Weibel (1973) also stressed that proper use of statistical

methods play a central role in stereology. In summary;

- (1) they produce average values of parameters characterizing the structures,
- (2) they indicate that stereological methods must deal with increasing numbers of related structures,
- (3) the procedure of sectioning allows only a restricted sample of the structures to be analyzed, and
- (4) the principles involved were all based on considerations of geometric probabilities.

Weibel (1973) further divided the sampling methods into four different types:

- (1) dicing of organs into tissue cubes,
- (2) decreasing the number of those cubes which are cut into sections (thin slices), one of which is used for analysis,
- (3) recording of electron micrographs from each section usually comprising only a fraction of the section area, and
- (4) applying point counting procedures which sacrifice an increased part of the information contained in the micrographs, because all those components not hit by test probes are disregarded, including;
 - (a) randomly dispersed structures, not specifically oriented with respect to the plane of section,
 - (b) layered structures, and
 - (c) fasciculated structures.

Weibel (cf. Briarty, 1975) concluded that it was advisable in proper sampling techniques, to have at least one representative sample

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from each section and at least five or more sections. Steer (1981) added that in quantitative microscopy the number of individual animal or plants examined was usually very low (1-5), thus causing problems in the reliable estimation of error at the uppermost level of replication because the mean squares were based on only a few degrees of freedom. He suggested a more reliable estimate using the mean square from the next lowest level of replication which would have a larger degree of freedom. In addition to having adequate sampling, magnification of the micrographs selected should also be high enough to allow unambiguous localization of test points within organelles or easy counting of intersections between membrane traces and test lines. Steer (1981) developed an effective system he called split analysis, which analyzed large scale features at low magnification and efficiently examined main components of interest at high magnification. Application of this system was based on four levels of magnification:

- (1) 500-2000X light microscopy of tissue thickness 0.5-1.0 μm . This level would be used to establish volume fractions of each cell type in the whole tissue and surface densities of their cell and nuclear boundaries. Also, large-scale features of vacuoles could be determined at this level;
- (2) 3000-7000X using electron microscopy in studying the cell type of interest;
- (3) 10,000-20,000X using electron microscopy. This level would study the contribution of individual cell components to the whole cytoplasm taking micrographs only over the cytoplasm of the

required cell type and ignoring nuclei and vacuoles; and

- (4) 30,000-80,000X using electron microscopy. This would concern components within a particular organelle such as internal membranes.

This system was convenient in its interpretation because information from the highest level (i.e. greatest magnification) could be related to the whole tissue by using estimates obtained at successively lower levels for increasing scales of structures. As Steer (1981) indicated, areas of membrane per unit volume of cytoplasm could be converted to area per unit volume of the cell at the next lower level using volume fraction of cytoplasm in the cell, and to area per unit volume of tissue using the volume fraction of the cells in the whole tissue. Consequently, this way estimates of the total volume, surface area and perhaps the number of particular cell components in a whole tissue could be estimated reliably and compared with the biochemical or physiological activities of that tissue.

II. Ultrastructural and Functional Changes During the Morphogenetic Shift, Germination.

The direct correlation between the fine structure and function of organelles has been shown in several studies over the years, especially significant developmental changes during germination. During this period, the embryo resumes growth, and is activated from its previous resting stage. Drastic changes occur in the organization of the membrane system and the organelles of the cell. Jann and Amen (1977) defined germination morphologically as the transformation of an embryo into a seedling. Physiologically they defined it as the resumption of

metabolism and growth which were earlier depressed or suspended, and the switching on of the transcription of new portions of the genetic program. Biochemically it was defined as a sequential differentiation of oxidative, and synthetic pathways and the restoration of biochemical pathways typical of vegetative growth and development. In agreement with this, Yoo (1970) stated that seed germination represented the termination of dormancy and the resumption of active embryo growth. He also suggested that at the cellular level the process involved the development of pre-existing organelles, at the molecular level it involved primarily the synthesis or activation of mRNA. Jann and Amen (1977) further elaborated the activities that were associated with germination and they classified the events into anatomic, genetic, metabolic and hormonal. Germination thus encompassed a multitude of biochemical and physiological activities which were intricately related.

Of all the components in the soybean embryo, lipid has the highest economic significance followed by protein and then starch. Hence, the majority of the studies on soybean emphasize those storage components. These studies showed considerable fluctuation in the storage materials during the development of the soybean embryo. Other organelles of about equal importance that are associated with the storage materials, either during their synthesis or decomposition, are plastids, mitochondria, vacuoles, cell walls, microbodies, dictyosomes and nuclei. Therefore, the relationship between these organelles and storage components has been closely studied especially during quiescence and during germination.

Cragg and Willison (1980) studied the ultrastructural components of the quiescent bud of Tilia europaea. They observed plentiful oil droplets, smaller quantities of other storage material (protein, starch), inactive dictyosomes, and an unusual arrangement of endoplasmic reticulum (E.R.) particularly a peripheral reticulum. They also found that the oil droplets were not bounded by a membrane. In agreement with another study done by Kidwai and Robards (1969), Cragg and Willison indicated that the presence of many seemingly inactive dictyosomes and the absence of a large central vacuole appeared consistently in quiescent apical meristems. Also these quiescent tissues contained many oil droplets but the quantities of other storage materials varied between species. Linden buds examined in Cragg and Willison's study contained both starch and (presumed) protein bodies, but in much smaller quantities than oil. Their results strongly supported the contention of Bergfeld et al. (1978) that storage oil droplets were not surrounded by a typical unit membrane. This contradicted statements previously made by Kidwai and Robards (1969) and Kupila-Ahvenniemi et al. (1978).

There has been much confusion and contradictory interpretations on the ontogeny of the lipid bodies, which has led to confusing terminology. The terms oil droplet, spherosome and oleosome have been coined each with their structural definition. The crux of the argument is the fact that the oil body sometimes seems to have a membrane surrounding it while at other times no apparent membrane was associated with it. Frey-Wyssling et al. (1963) came up with a theory that oil

bodies were formed from spherosomes which themselves were derived from the E.R. He also added that both structures were surrounded by a unit membrane. Sorokin (1967) concluded that spherosomes and oil bodies represented separate entities, and that spherosomes were a common feature of most vegetative cells in higher plants, even in cells which did not produce oil. However, oil bodies were restricted to cells producing oil. She further differentiated between the two structures by stating that spherosomes had a limiting membrane whereas oil bodies did not. Schwarzenbach (1971) agreed with Frey-Wyssling (1963) that spherosomes were formed from the E.R. but changed the terminology, referring to spherosomes as prospherosomes and oil bodies as spherosomes. The author indicated that the spherosomal membrane underwent differentiation during seed maturation, resulting in a separation of the unit membrane into an inner and outer layer, between which lipid synthesis took place. The inner half of the unit membrane eventually became invisible, leaving the outer half of the unit membrane to form the limiting membrane of the spherosome. This evidence suggesting a half-unit membrane surrounding the spherosome (oil body) was further substantiated by Yatsu and Jacks (1972) who described an unusual single membrane surrounding the spherosome in contrast to the usual tripartite membrane. In an earlier publication Yatsu et al. (1971) compared the spherosomes from non-oil synthesizing tissues with oil bodies from oil bearing seeds, and concluded that the two structures were identical and should be renamed oleosomes. Smith (1974) presented evidence concerning the site of origin of the oil body. He found spherosomes and

oil bodies to be separate entities, with different sites of origin and function in the cell. The spherosomes, which were shown to be present in the cell before the onset of oil body formation, were formed from the E.R., and contained acid-glycerophosphatase. Smith indicated that one of the functions of the spherosome was to supply a hydrolytic enzyme to the vacuoles in which the aleurone grains subsequently developed. Smith also found out that oil bodies developed from small areas of particulate material in the cytoplasm 8-10 days after petal fall, and the oil body function appeared to be sites of an oil storage product (triglyceride) which was synthesized during seed development.

Rest and Vaughan (1972) added another dimension to the confusion. They presented their observations on the storage oil of Sinapis alba. The oil bodies were first seen in the cytoplasm as a few spherical bodies with stainable contents at 18 days from petal fall. These oil bodies did not have a densely staining limiting membrane, but neither did they appear to coalesce with adjacent oil bodies. The oil bodies seemed to arise directly from the cytoplasm. There was some E.R. present at this stage, but they could not find any evidence to suggest that the oil bodies arose from E.R. as described by Frey-Wyssling et al. (1963).

Schwarzenbach's (1971) theory for the origin of spherosomes was that the lipid bodies were produced by a local accumulation of triglyceride within the lipophilic middle layer of the tripartite unit membrane of the E.R.

Sorokin (1967) elaborately differentiated the oil droplets from

spherosomes. In earlier papers it was found that the spherosomes contained phospholipids, of uniform diameters and had a limiting unit membrane at the boundary of each spherosome, which in living cells prevented their coalescence. In contrast to the spherosomes, the reserve oil droplets combined neutral fat, had a wide range of diameters, and easily coalesced into larger aggregates. Nevertheless, spherosomes and oil droplets were frequently confused in the literature, particularly in electron micrographs and the regular reserve lipids of the oil bearing seed were often referred to as spherosomes. Sorokin further observed spherosomes as fairly constant in size, ranging from 0.8-1.0 μm in diameter. She also stated that smaller spherosomes of about $1/4$ - $1/3$ the diameter of the larger forms had been described in a number of plant species but the dimensions of each type of spherosome remained constant, within the species and no transitional forms between them had been observed. The reserve oil bodies on the other hand, varied widely in size ranging from the limit of visibility to very large globules and aggregates.

The term oleosome was later introduced in an attempt to eliminate all the confusion between oil droplets and spherosomes. Wanner and Theimer (1978) regarded spherosomes and oleosomes as the same entities. They stated that spherosomes (oleosomes) of cotyledons of rape, sunflower and watermelon seedlings were delimited by a $1/2$ -unit membrane that appeared to be continuous with each of the osmiophilic layers of a tripartite unit membrane forming a handle like appendix to the spherosomes. Vigil (1970) called oleosomes lipid-rich spherical

bodies. He found that they developed within the cytoplasm of wheat flag leaves before anthesis and persisted throughout leaf maturation and senescence. Oleosomes in the older leaves were found to be up to 14 μm in diameter and bounded by an osmiophilic layer rather than a unit membrane, and were readily seen in flag leaf sections. They were composed mainly of triacylglycerol and sterol. According to Bergfeld, et al. (1978), electron microscopic and biochemical investigations of developing embryonic mustard cotyledons provided no evidence for the widely accepted hypothesis that oleosomes of fat-storing tissues originated from the E.R. and were surrounded by a unit or 1/2-unit membrane. They observed the first lipid droplets appearing (about 12-14 days after pollination) in the ground cytoplasm near the surface of plastids. These nascent lipid droplets, which lacked any detectable boundary at this stage, became encircled by cisternae of rough E.R. At the same time an osmiophilic coat of about 3nm thickness became detectable at the lipid water interface. They also observed that in the cotyledon cells of germinating seedlings, a centrifugally moving front of fat degradation moved from the central vacuole(s) towards the periphery, leaving behind collapsed coats of oleosomes. They were depleted of their lipid contents (sacculles), appeared tripartite in cross section and were structurally different from the E.R. membranes. The oleosome coats could be isolated from oleosome preparation by extracting lipids with organic solvents. They concluded that the oleosome lipids accumulated in the ground cytoplasm and were bounded by a lamellar structure originating de novo from proteinaceous elements synthesized by specific

regions of the E.R. Yatsu and Jacks (1972) prepared ghosts of oleosome coats from peanut cotyledons in which they concluded that the single-line oleosome coat visible under E.M. represented an atypical biological membrane (corresponding to a 1/2-unit membrane) rather than a nonspecific absorption layer.

Gunning and Steer (1975), stated that the spherosome consisted of droplets of lipid which were abundant in some cells and sparse or absent in others. Their numbers and distribution fluctuated according to the metabolic state of rehydration and germination of seeds. They also indicated that the lipid content was usually homogeneous in appearance, although exposure to fixatives such as O_5O_4 may introduce non-homogeneity. They added that the spherosomes were not bounded by a membrane, but a surface skin consisting of an outer layer of oriented lipid molecules formed in response to the surrounding aqueous environment of the cytoplasm.

In order to gain a better understanding of the actual metabolism occurring during germination, researchers have studied dried embryos (seeds) and compared them to the imbibed seeds. Srivastava and Paulson (1968) commented that ultrastructural studies on dry embryos were rare, since fixatives and embedding media fail to penetrate, either in part, or completely, desiccated seed tissues. They overcame some of the difficulties by using aldehyde fixatives and small pieces of tissue and extended the period of embedding. They found that dry embryos of Lactuca sativa (var. Grand Rapids) were filled with reserve proteins and lipids, and that nuclei, mitochondria, ribosomes and some membranes

showed the best modifications in the dry state while other organelles were poorly filled. They continued the research and further studied the changes during germination. They examined changes on fine structure and reserve materials of dormant embryos of Lactuca sativa during the first 4 days of germination and discovered that activation of cells began soon after the embryos were soaked with a progressive loss of reserve materials. Simultaneously, there was an increase in clarity of organelles and membrane systems. They admitted that they did not know the first steps in activation of a dormant embryo, but suggested several pointers which implicated hormones and template DNA in the activation of germination.

Bechtel and Pomeranz (1978) examined all tissue of ungerminated, unimbibed rice germ, and found similar plastids, contained osmiophilic globules and phytoferritin in all cells. Some displayed a limited thylakoid system and all contained cytoplasmic tubular and vesicular inclusions formed by invaginations of the outer plastid membranes. They also discovered that cells having inclusions such as protein bodies and numerous lipid bodies were scattered throughout the cytoplasm. In general, they thought that many of the cells were immature and would undergo extensive differentiation during germination.

Horner and Arnott (1966) tried to follow the metabolic activities during pre- and post-germination of Yucca in chronological order. Their results showed that the ungerminated seeds combined large amounts of proteins and oils but did not contain starch. Protein and oil were in membrane-bound bodies within the cells of both the embryo and the

perisperm. In addition reserve carbohydrates were found in thick perisperm. Unlike the findings of Pazur et al. (1962) in sunflower and soybean, Horner and Arnott found that the first changes that occurred in the Yucca seed after hydration was the disappearance of protein bodies which were stored in procambial cells. They reasoned that after hydrolysis the proteins were transported through the embryonic conducting system to regions of growth. They presumed that the hydrolysis products were transported to growth centers where they were utilized in the synthesis of new protoplasm. In addition, no decrease in carbohydrates was observed in the cell walls of the embryo. However, there was starch formation and accumulation shortly after germination which was most closely correlated with protein hydrolysis and breakdown of lipids. Yoo (1970) suggested that imbibition marked the beginning of germination which appeared to be simply the activation of cellular organelles and development of vacuoles and dictyosomes during the process of germination. He found very little change in the nucleus by 48 hours soaking, but cytoplasmic organelles underwent developmental changes. He also discovered that there was a proliferation of E.R., the appearance of dictyosomes and an inward migration of lipid bodies. Most of these changes he observed within 8 hours after soaking started. Plastids, mitochondria, protein bodies and protein crystalline bodies were also identified, and followed their own developmental changes. Plastids and nuclei showed the least change. Cardemil and Reñero (1982) found changes in the starch and protein reserves of Araucaria araucana embryo and megagametophyte tissue during the first 90 hours

of the start of imbibition. They discovered that: (1) the starch content of the embryo (30%) decreased rapidly until radicle protrusion (40 hours of germination) and remained relatively constant ($\approx 9\%$); after that, (2) starch content of the megagametophyte decreased slowly throughout 90 hours; (3) amylase activity and soluble carbohydrate content of the embryo peaked rapidly at 20 hours, declined rapidly until germination and increased slowly thereafter; and (4) total amylase activity of the megagametophyte did not change markedly. In addition they found that the main reserves for embryo growth and development in A. araucana seeds were carbohydrates.

Hallum et al. (1972) elaborated on the biochemical and fine structural changes during germination. For convenience they divided the imbibition stages into three phases: Phase I was a short period (10 minutes) of physical wetting; Phase II was a longer period (1 hour) when little further imbibition occurred; and Phase III was in continuous water uptake. They suggested that the last phase coincided with an increase in respiration which coincided with an increase in the number of mitochondria and of their cristae. Changes in fine structure became evident in all organelles in Phase III, after 2 hours of imbibition. In the unimbibed embryo, endoplasmic reticulum was found to be present as short crescents associated with electron lucent bodies, but in Phase III the E.R. proliferated to form many surrounding circlets. One hour later these circlets became fewer and the E.R. was then seen in close association with the nuclear membrane. They indicated that from studies on monocot embryos protein synthesis was known to start within 30

minutes after water became available. However, their studies showed that nucleic acid (NA) synthesis was delayed for some hours. They also indicated that components of early protein synthesis were stored within the embryo in the dry state but nucleic acid synthesis was dependent upon a preparatory process occurring during the early hours of imbibition.

Hallam et al. (1972) went further into finer details on the organellar changes during progressive hours of water soaking. After 2 hours they found out that with the exception of an increase in the number of cristae within the mitochondria there was no apparent difference in fine structure from that of the unimbibed embryo. Up to this time they discovered small crescents of rough endoplasmic reticulum (RER) associated with electron lucent bodies and ribosomes attached to the membranes. Three hours later, they observed electron lucent bodies enriched by ER and dictyosomes appeared well defined with numerous vesicles associated with them. By contrast, however, Yoo (1970) found that dictyosomes in Pisum remained as flattened vesicles up to 8 hours of imbibition. Four hours later they showed mitochondria with extensive cristae, and some were in apparent division. At 7-24 hours they found that the proliferation of ER from the nuclear membrane increased rapidly, and by 9 hours extensive membranous sheets were formed. They also found that up to this time numerous lipid bodies which were aligned along the plasmalemma became considerably reduced in number. At these later stages many lipid bodies were found distributed throughout the cytoplasm. Durzan et al. (1971) studied the metabolism

and subcellular organization of the Jack pine embryo (Pinus banksiana) during germination. In agreement with most studies, they found that the cytoplasm of dormant cells of the cotyledon was packed with nutrient reserves composed mainly of protein, lipids and carbohydrates. Cytoplasm apart from storage reserve was sparse and dehydrated. Dormancy was evidenced by the irregular outline of nuclei and by wide distribution of nutritive storage bodies. After 12 hours of imbibition expanded nuclei and indentations created by the packed cytoplasm disappeared and protein bodies were undergoing digestion. Twenty-four hours later protein bodies appeared hydrated, and no elaborate endoplasmic reticulum (ER) was apparent. By 48 hours, mobilization and digestion of nutrient reserves was advanced as polysome-like strands and particles appeared around the nucleus, mitochondria, unidentified bodies and proplastids. Storage bodies remained dispersed throughout the cytoplasm. By 92 hours the decrease of total soluble protein corresponded to the occurrence of large activities in protein bodies and storage protein also disappeared.

In agreement with other authors, Harrison (1977) found that unimbibed seeds contained numerous protein and lipid bodies but not appreciable carbohydrate reserves. He found that lipid reserves were utilized in seeds, slowly in seeds sown aseptically onto medium lacking a carbohydrate source, but more rapidly on medium containing sucrose. He also found degenerating lipid bodies often closely associated with mitochondria, but glyoxysomes were not evident at any time during germination. Neither dictyosomes nor rough ER were found in the cells of

seedlings grown on sugarless medium.

Several authors have used the resting cambium to study the structural and functional aspect of organelles at the resting stage, to develop a better understanding of quiescence and dormancy. Some similarities between quiescent apical meristems and resting cambium in their cellular ultrastructure were observed. For instance the same electron opaque substance found within cotyledons of Brassica napus and S. alba observed by Frey-Wyssling (1963) was observed in resting cambium of Fagus sylvatica studied by Kidwai and Robards (1969). They recorded a similar type of description of cellular fine structure which had also been found among quiescent cells. According to their findings, cells of the resting cambium contained strands of smooth ER which was often arranged as a series of parallel membranes. Single membrane bound bodies of different types including lipid droplets, protein bodies many different intermediate forms, empty structures and vacuoles were present. They suggested that vacuoles were formed by the progressive dissolution of stored lipids and protein, during the onset of cambial activity. According to this study, resting cambium plastids contained numerous starch grains which were also common in willow and ash. In contrast to the vesiculate smooth ER of willow (Robards and Kidwai, 1969), the ER here was cisternal and less smooth. Oil droplets were also found. The presence of protein and lipid bodies at various stages suggested the formation of vacuoles by a degenerative process and suggested that the material described had just started to become active. Studies such as Kidwai and Robards' not only help differen-

tiate between quiescence and dormancy but also add another dimension to the understanding of resting cells, the metabolic activities and fine structures involved during embryogenic activation.

The orientation of lipid bodies has also been of great interest in resting cambia. Robards (1968) found electron opaque lipid bodies initiated in the cisternae of the ER and moved toward the wall. O'Brien (1967) suggested the possibility that lipids may locally inhibit wall synthesis. If this could be substantiated, large deposits of this material at the sites of the differentiating pits may indicate the local inhibition of secondary wall synthesis, thus contributing to the further elaboration of pits. In Robards' study of resting cambial cells, he found smooth ER, free ribosomes, oil droplets and protein bodies. He also found that they were comparatively surrounded by a cluster of plastids and mitochondria, that active cambial cells and young differentiating xylem elements were highly vacuolate and contained rough ER and polyribosomes. The Golgi apparatus was active in the production of vesicles and the distribution of organelles was a function of vacuolation of the cell. Robards suggested that the lipid droplets and protein bodies were storage materials which were required during the first stages of differentiation at the beginning of the growing period. Just like quiescent buds, Robards noted that the cells in the resting cambium were not completely dormant. One of the indications was that the resting cambium was not highly vacuolate but the differentiating cells rapidly became so.

Beakes (1980) elaborated on changes in organellar status and

associations of Saprolegnia ferax prior to and during germination. He noticed a steady accumulation of lipid and dense body reserves during oosphere differentiation and at the same time the overall status quo of other organelles was maintained. At the onset of germination the inner oosphere wall was rapidly digested and normal levels of cytoplasmic organelles were restored. In addition the neutral lipid fraction progressively declined indicating that fatty acids were highly efficient energy stores, yielding twice as much energy on oxidation as either carbohydrates or proteins. Lipid droplets represented the cell's major energy investment. Beakes believes both microbodies and mitochondria were directly involved in neutral lipid metabolism, and that β -oxidation of fatty acids could have occurred within mitochondria with the resulting acyl-CoA being oxidized via the citric acid cycle. This could have been the main site of lipid catabolism especially when mitochondria and reticular bodies became associated with lipid droplets and dense-body vesicles in advanced germlings and gemmae. Beakes' conclusions compared favorably with Srivastava and Paulson's (1968) study of the embryo of Lactuca sativa during germination. They studied the embryo during the first four days of germination and discovered that activation of cells began soon after the embryos were soaked. They also found that soaking involved a progressive loss of reserve materials, increased clarity of organelles and membrane systems, and development of structures either absent or present in a highly modified state in the dormant embryo. Bimpong and Hickman (1975) made similar observations on germinating cysts of Phytophthora palmivore, whereby as

germination proceeded, lipid bodies coalesced with each other and with adjacent crystalline vesicles. They observed lipid breakdown products appeared as osmiophilic network-like material, and the contents of disintegrating crystalline vesicles resembled those of lipid bodies. They also noticed a central vacuole being formed inside the body of the cyst. Bimpong (1975) conducted another study whereby lipids, carbohydrates, proteins and enzymes were particularly assayed during a progressive period of germination. He concluded that the utilization of lipids for motility and germination raised the possibility of the involvement of the glyoxylate cycle in conversion of fats to carbohydrates. This conclusion explained the rapid decrease of lipid bodies but significant increase of carbohydrates and proteins during germination. Ruben and Stanghellini (1978) studied morphological changes during the early stages of germination of Pythium asphanidermatum, whereby the central globule distorted in shape and became partially surrounded by vacuoles. They thought these changes indicated the storage reserves of the globule had begun to be metabolized. They had also noticed that storage bodies eventually became densely osmiophilic, indicating that the lipid contents had undergone an increase in the degree of unsaturation.

One particular problem encountered in studying the shoot apex ultrastructurally was the comparison of cytological zonation to anatomical zonation of the growing point. Many classifications of anatomical zonation have been discussed in the literature (Popham, 1966; Gifford and Corson, 1971). There has been concern regarding

ultrastructural cytological variation within these zones. Some authors have claimed that the cytoplasmic content was identical in all the zones. Sawhney, et al. (1981) studied the ultrastructure of the central zone of cells of the shoot apex of Helianthus annuus. They showed that in most respects the cytoplasmic components of the central zone cells were similar to those of the mitotically active peripheral zone cells. Similarity of structure and distribution among mitochondria, dictyosomes, ER, ribosomes and microtubules across zones also existed. The only striking difference that they found was the presence of starch containing plastids in the central zone and the two tunica layers, and their absence in the peripheral and immediately subjacent regions of the pith rib meristem. Starch containing plastids were also observed in the differentiating pith cells. They concluded that their study provided confirmation of the observations of other workers that the fine structure of quiescent meristem cells added little to our understanding of quiescence. In most respects the central zone cells of H. annuus did not differ from those of the more mitotically active peripheral zone. They possessed the same cytoplasmic components apparently in the same functional state. Central zone cells were not inactive mitotically, merely less active than the peripheral and subjacent cells. Davis et al. (1979) confirmed Sawhney's study in that they also found that the cytologically distinctive central zone of the vegetative shoot apex of Helianthus annuus had a mitotic frequency considerably lower than that of the surrounding peripheral zone in intact plants. Langenauer et al. (1974)

and Steeves et al. (1969) also carried out extensive studies on the ultrastructure of H. annuus. Both discovered that quiescent cell populations in apical meristems exhibited a quiescent central zone. Steeves et al. had observed that the vegetative shoot apex of H. annuus contained a central zone in which the cell nuclei were relatively large and stained faintly with the Feulgen reaction. Booker and Dwiredi (1972) discovered that the lateral bud meristems of Tradescantia paludosa showed a characteristic cytohistological zonation during dormancy. They noticed that at the extreme tip of the bud apex the cells rarely synthesized nuclear DNA or underwent mitotic division. This zone they termed 'zone of inhibition'. However, so far no evidence has indicated any variation in the cytoplasmic content.

There have been reports of structural changes occurring during the early hours of germination in embryos of Pisum (Yoo, 1970); and Pinus (Durzan et al., 1971). As for soybean seeds, there has been several studies published. Adams et al. (1981) found that the developing embryo accumulated starch during development and utilized it before maturity, so that the final mature seed had about 2% starch. In an earlier study Adams et al. (1980) had indicated that immature soybean seeds accumulated starch as a transient reserve material which was later utilized in development. They hypothesized that germinating seeds accumulated starch reserves as a result of gluconeogenesis from storage lipid. Developing beans showed a rapid increase in β -amylase activity which continued into early germination before declining. A rapid increase in starch content of germinating soybean was reported by

Von Ohlen (1931). Starch grains were observed in soybean cotyledons after 20 minutes of imbibition by Webster and Leopold (1977). It is interesting to note that oil content in germinating soybean cotyledons rapidly declined after the second day of germination as indicated by McAlister and Krober (1951). Most probably the newly formed starch was produced by gluconeogenesis using precursors from oil reserves. Parish and Leopold (1977) studied the soybean during water imbibition and discovered that several very significant physical and biochemical processes were taking place in the first few minutes of water uptake. They found that there was an inrush of water accompanied by a rapid loss of solutes from the cells and a release of absorbed gas. The cell membranes reorganized from a presumably porous condition into effectively functioning semipermeable barriers. This find was in agreement with Webster and Leopold (1977) who elaborated on ultrastructural changes. They compared the dry seed and the imbibed seed and discovered that the plasma membrane in cells of dry seeds was disorganized and disrupted which became relatively intact and continuous upon imbibition into a network of ER vesicles and tubules. No evidence of such a network was discerned in dry seeds where membranes were dispersed throughout the cytoplasm and around the margin of protein bodies. Mitochondria, which in dry tissue were distorted and nearly devoid of internal structure, were round or oval and were bound by an inset membrane enclosing numerous cristae and a dense stroma in imbibed cells. Starch grains were developed in proplastids of imbibed cells. They also observed that, as cells became hydrated, the architecture of

the membrane changed from a relatively porous, "hexagonal" state to a more stable "lamellar" configuration. The latter structure precludes free diffusion of solutes and thus would slow down the rate of imbibition. They concluded that the swiftness with which membranes and organelles were structurally altered during imbibition was a reflection of their effectiveness in rapidly modifying solute loss and solvent entry. Obendorf and Hobbs (1970) studied the physical aspect of imbibition of soybean in terms of temperature sensitivity during imbibition. They found that rates of imbibition were affected more by temperature than by seed moisture content. Pazur et al. (1962) studied the biochemical aspect during germination especially in the metabolism of oligosaccharides and found that sucrose, raffinose and stachyose were utilized rapidly in the metabolic processes of germinating soybeans. Of the constituent units from the oligosaccharide, D-fructose and D-glucose were readily detectable in extracts from the beans but only traces of D-galactose could be found. They interpreted this result to indicate a rapid utilization of the galactose from the oligosaccharides.

Overall, Khan (1977, 1982) has provided great documentation that elaborates and summarizes the possible activities that could take place in the seed embryo during germination. He has documented the physiology and biochemistry of seed development, dormancy and germination. Weibel (1981) indicated that biochemical and physiological studies such as Khan's have to be correlated to and compared with the morphometric information obtained from the cell's ultrastructure. Then and only

then can one attempt to attain as complete knowledge as possible on the function structure relations of the cell especially during a developmental stage where the organism is undergoing a morphogenetic shift, (such as from quiescence to germination).

III. Quantifications of ultrastructural studies using stereological methods and statistical analysis

The designs for biological research seems directed toward more sophistication, not only in the use of highly sensitive measuring devices but also in the elaboration of rules on how to best divide the effort among the sampling stages and statistical analysis (Weibel, 1981).

Stereological methods have been one of the means of sophistication in ultrastructural studies and has just recently been used in quantification of ultrastructural data. Stereological methods consist of a body of mathematical rules relating three dimensional measurements obtained on sections of a structure. The advancement is now leading toward more refined, three dimensional, accurate measurements of the cell's ultrastructure.

One of the first authors who saw the importance of more quantification in ultrastructural research was Loud (1962). He indicated that quantitative analysis of the subcellular architecture revealed by electron microscopy was a logical step in the investigation of the normal functioning of cells and their pathological alterations.

For comparing cells in which relatively small quantitative differences in composition are found, it was necessary to have a systematic

technique for the estimation of structures. Stereological methods were found to be a suitable systematic technique. By definition, qualitative measurements from two dimensional cross-sections can be extended to the real three-dimensional structure of the cell. Also, the volume fraction of the cytoplasm occupied by formed bodies and membrane space is numerically identical with the area (Loud, 1962).

Hans Elias (cf. Underwood, 1970), then the president of the International Society for Stereology, defined stereology by stating: "Stereology, sensu stricto, deals with a body of methods for the exploration of three-dimensional space, when only 2-dimensional sections through solid bodies or their projections on a surface are available. Thus, stereology could also be called extrapolation from two-to-three dimensional space."

Historically, one can hardly discuss stereological methods without mentioning Weibel (1966, 1973, 1981), who has studied and developed some of the stereological methods for ultrastructural studies. Weibel (1973) claimed that morphometric information was obtained by application of stereologic methods in light microscopy and electron microscopy of tissue sections, thus allowing a quantitative definition of organ models. These serve to correlate structure and function quantitatively and provide insight into internal functional processes.

Weibel (1967a and b) foresaw several applications for morphometric analysis: to study problems of morphogenesis quantitatively and be content with results indicating growth rates with good accuracy; to apply quantitative techniques in the analysis of experimental studies;

and to do comparative studies where the structural adaptation of an organ to different environmental conditions forms part of the adaptation of the entire organism to different functional requirements. Weibel also stated that the true interior of a system was inaccessible to direct investigation and since it applied to all domain research, stereological methods had a very wide application in all fields of research. Weibel (1967a) indicated that it was necessary to obtain the most complete knowledge possible of structural composition and functional behavior of the 'black-box region', and he suggested that the information should include a determination of all elements in terms of biochemical, biophysical and morphological characteristics, geometric properties and dimensions in terms of biological behavior. Furthermore, an exact knowledge of the architecture of an entire system, of the spatial relations and of the relative importance of the elements would form an essential basis for the considerations. Weibel (1967a) concluded that the functional characteristics of these models are defined by theory and experiment, while their structural properties are given by morphometry.

Underwood (1970) has studied extensively in this field, and developed some of the basic stereological methods and formulas. He stated that quantitative stereology attempted to characterize numerically the geometrical aspects of those features of the microstructure of interest. Underwood, Weibel and other major authors in this field have carried out several quantitative ultrastructural studies in the process showing the applications of stereological methods. These par-

ticular studies encompassed a variety of research areas thus portraying the wide applications of these methods.

A great concern for stereologists is in the proper use of a grid system. It is one of the crucial aspects in getting accurate results. Berlyn and Miksche (1976) described the use of a grid system for point counting, the points of which serve as a statistical sample to estimate the quantities of various components of a tissue. Steer (1981) elaborated more on the type of grid systems: the point counting grids — a lattice of 100-400 points/8x10 inch sheet; the square double lattice grids which are characterized by the ratio of major (coarse) points to minor (fine) points which are the most common; and line intersect grids with closely spaced lines leading to a direct increase in the amount of work involved in scanning along them with only a small increase in information.

Moore et al. (1977) reported their design of a simple, relatively inexpensive grid which permitted the quantification of cellular constituents from light and electron micrographs. They indicated its applicability to a variety of morphometric and stereologic techniques and required no electronic equipment. In contrast to the use of grids in the photographic process, it did not require additional points to be made specifically for stereologic analysis. This grid was especially useful among probes associated with the meaningful comparison of light and electron micrographs of normal and experimental tissues. However further research needs to be done to validate such comparison. Boyde et al. (1974) also used a special grid method that has been evolved for

scanning electron microscopy. They used a self-generated superimposed grid which compensated for the non-linearity of the CRT image, since CRT display which was photographed in the SEM may suffer from considerable non-linearity. There are differences in the magnification of different parts of the SEM image according to their situation on the CRT image. In an earlier paper, Boyde (1970 cf. Boyde et al., 1974) showed that a similar grid could be generated and superimposed on each photograph by making use of the reliable frame and line speeds on the SEM scan generator. He also indicated that even if there was no distortion of the raster in the SEM column the self-generated unmodulated grid pattern of the second CRT could also be used as the stereological grid. Boyde et al. (1974) illustrated the application of this method to a problem which had not been resolved satisfactorily by light or transmission electron microscopy. They showed that superimposition of a standard grid on an image recorded with the SEM used in their study would have introduced errors due to the distortion at the edges of the field. They discovered that their method of using the self-generated grid for a simple stereological analysis had the advantage that the grid intersections occurred at equal spacings on the specimen even though not on the recorded photograph. Thus, the use of self-generated, superimposed grids of the type suggested by Boyde as simple stereological nets should serve to tide us over the period during which the practicing electron microscopist must suffer the presence of non-linear CRT displays.

There have been several authors who have used stereological

methods to quantitatively analyze their ultrastructural data. Fossum and Coutrik (1977) compared the volume density of dictyosomes and the volume and surface density of the RER of untreated rat pituitary cells with thyroliberin treated cells. They took EM pictures at 10,000X from all cells present in one grid square selected at random. For statistical analysis, they used a non-parametric rank test of Wilcoxon to compare the different cell populations, whereby each measurement from the two populations to be compared was given a rank number. In their stereologic analysis, they expressed V_V (volume density) as the ratio of the number of test points (P) falling on cytoplasm ($V_V = \frac{P\text{-organelle}}{P\text{-cytoplasm}}$), surface density (S_V) of ER was calculated as 2X the ratio between the number of intersections between test lines and PER (I) and the total length of test lines falling on cytoplasm (L) ($S_V = 2I\text{-RER}/L$) at the 95% confidence interval. Fossum and Cautvik thus compared the volume density of Golgi complexes and the volume and surface densities of RER in untreated and thyroliberin treated cells. They discovered that all three morphological parameters increased after the thyroliberin treatment in cells secreting prolactin only (GH_4C_1). They also observed increased in prolactin synthesis correlated the release with the morphological effects of thyroliberin treatment. From these studies, Fossum and Gautvik implied that the increase of prolactin secretion, at least in part, was due to increased prolactin synthesis.

Rather than using point count, Considine et al. (1982) used the linear method and regression analysis. They carried out stereological analysis of cell proliferation during early floret development and com-

pared developmental processes in fertile and sterile floret sites. They also examined the proposition that nascent meristems were formed prior to formation of a new axis by anticlinal cell divisions. They carried out two sets of regression analyses, one on nucleic acid content and the other on epidermal cell number, whereby the independent variable, epidermal cell number, was derived from stereological consideration of the probability of intersecting a cell in a section from assumptions concerning morphology of the floret. They also used the number of cells intersected in each subtending lemma primordium N as a sensitive index of developing nucleic acid content of sections of spikelets plotted against N . They then fitted linear regression and analyzed data with an analysis of covariance. Considine et al. claimed that a similar approach has been adapted to compare rate of cell proliferation in the two genotypes, fertile Gabo and base-sterile St_3 varieties of wheat, whereby the independent variable, epidermal cell number, was derived from stereological considerations of the probability of intersecting a cell in a section and from assumptions concerning morphology of the floret. Considine et al. then claimed that a resarch of that kind gave a proper appreciation of the actual differences in rate of development which would have been somewhat masked by use of linear intercepts. The results from their stereological analysis showed that cell number in the floret meristems of Gabo increased exponentially during development. By contrast, in St_3 , while there was a significant increase in cell number it was at a drastically reduced rate. They also observed that the intercept values were close to zero,

indicating that only one or two cells initiated floral meristem development. From this they concluded that the fertility alleles exerted their effect prior to the appearance of a visible floral primordium, and probably after initiation of the lemma.

Landay et al. (1977) used point counts for their stereological analysis but used a three-way analysis of variance instead of non-parametric. They did a stereological analysis of the stratified epithelium of normal human buccal mucosa. Sampling in the tissue block was random but in electron micrographs it was based on a model of stratification. For their point counts they used a coherent double lattice test system with 99 heavy and 891 light volumetric test points. They estimated the volume (V_V) and surface density (S_V) as well as volume and surface ratios of the various structural components in which they obtained by applying respective stereologic formulas. For comparative purposes Landay et al. calculated all data in relation to 1 cm^3 of epithelial stratum or 1 cm^3 of epithelial cytoplasm respectively, and for the significance test they did a 3-way analysis of variance. From this investigation they found the Buccal epithelium to be 0.48 mm thick, and interdigitated by long, slender connective tissue papillae, and comprised of a narrow basal and suprabasal, and a broad, homogeneously structured spinous and surface compartment. They also observed that from basal to surface layers the epithelium displayed a differentiation pattern different from that of keratinizing epithelia. They suggested that the dense meshwork of filaments which filled 70% of the epithelial cytoplasm in a broad subsurface and surface layer, served as

a functional matrix for epithelial distensibility.

Meyer et al. (1975) used stereologic point counting and performed significance tests using the classical F-test to examine the keratinizing epithelium of normal hard palate. They used a coherent double lattice system as their grid; they estimated the relative volumes (V_V) of mitochondria (Mi) aggregate of non-membrane bound free ribosomes (rib), smooth membrane cisternae, including the golgi apparatus and pinocytic vesicles (sms), lysosome-like bodies (ly), membrane coating granules (mcg), cytoplasmic filaments organized into tonofilament-like bundles (fib), keratohyalin (kh), and cytoplasmic ground substance or residue. They carried out the analysis at two levels. They estimated volumetric (V_V) and surface density as well as the surface to volume ratio (S/V) of the various structural components which were in turn obtained by applying basic stereological formulas. For comparative purposes, they calculated all data in relation to 1 cm^3 volume of epithelial stratum, or 1 cm^3 of epithelial cytoplasm respectively. Meyer et al. initially had a problem with sampling method since they merely wanted to examine the epithelial cells of human hard palate. To overcome this problem their E.M. sampling fields did not include the middle portion of the epithelial ridges. They finally reasoned that since this portion consisted only a small fraction of the total epithelial volume and because the cells of the stratum spinosum had been shown to constitute a rather well defined population which underwent major, differentiation changes at the level of the termination of connective tissue papillae, the restriction of sampling

to the stratum basale and the upper stratum spinosum would still appear to have detected the major steps in cellular differentiation. From this study, they found in general that the thickness of the palate epithelium was 0.12 mm (over papillae) and 0.31 mm (in ridges), the epithelium was distinctly stratified, and homogeneously orthokeratinized. They also observed that from basal to granular layers, the composition of strata revealed decreasing densities of nuclei, mitochondria, membrane-bound organelles and aggregates of free ribosomes. Also keratohyalin bodies and membrane coating granules increased and cytoplasmic filaments with a constant diameter of about 85A increased from 14-30% of cytoplasmic unit volume. They noted that the cytoplasmic ground substance occupied a stable 50% of the epithelial cytoplasm, in all strata.

Rearen, E. P. et al. (1977) also used point counting stereologic techniques to estimate the fraction of the cytoplasmic volume occupied by lipid droplets, microtubules and various organelles. They placed transparent grids with lattice dimensions appropriate to the structures being examined over each photographic enlargement (X3) of randomly obtained electron micrographs, and they recorded the number of points (P) of the lattice that fell on the cytoplasm. Then they obtained the fractional volume (volume density) of the structure within the cytoplasm from the ratio, $P(\text{str.})/P(\text{cyto})$. They also discovered that the estimation of microtubules by the point-counting method appeared to be highly reproducible. Finally, in that analysis they found out that microtubule content of the apical cytoplasm of absorptive cells

decreased after colchicine administration. They concluded that the decrease in microtubules appeared to be related to the dose of colchicine given and had also observed an accumulation of lipid in the apical region of these cells.

Wasserman, F. et al. (1967) used the stereologic technique to analyze the size distribution of fat cells in adipose tissue of different ages. They randomly selected photographs of sections taken at known magnification and they chose areas of the tissue by superimposing a grid of the size of the photo divided into 25 rectangles on the photographs. They then picked both the picture and area of the grid taken in each case from a table of random digits. They based their estimation of size distribution on the diameter of the features. They determined the diameters of the vacuoles appearing in the rectangles of the grid by 10 circles of equally spaced diameters with lengths of 10-100 micrometers and by assigning each vacuole to a size class of the circle that fitted closest to the outline of the vacuole. In each age group they chose sections from 2-25 animals and measured 200 vacuoles in each case and thereby constructed the size distribution of the fat droplets from plane sections. After performing the stereologic analysis Wasserman et al. concluded in old age, small droplets reappeared and the original fine droplets grew with age.

Elias and Henning (1967) by means of stereologic methods also examined the glomeruli of human kidneys of different ages. They especially had problems with sampling because the glomeruli were not evenly or randomly distributed over the entire kidney for medulla and

medullary rays did not contain any glomeruli and even in the cortex there were β -glomerular zones. After shifting to Weibel's method of sampling, satisfactory morphometric data was obtained.

Mauseth, J. D. (1980) correlated morphogenetic changes, using morphometric analysis (based on stereologic methods), with biochemical activity or changes in the shoot apical meristems of Echinocereus engelmannii at germination. His aim was: (1) to determine and compare the organellar structures of the tunica and corpus, (2) to evaluate the constancy of these structures so as to determine the precision with which organellar composition was controlled and (3) to determine the types and rates of changes that occurred within the cells of a particular zone as the meristem went from a state of quiescence to one of growth. Mauseth used the point counting technique to determine the relative volume fraction of organelles. He studied 69,886 dots and calculated relative volumes of cell components; nucleus, nucleolus, vacuole, mitochondria, chloroplasts, dictyosomes and hyaloplasm during 24 hours after water imbibition and recorded it as the percent of the protoplast volume. The results Mauseth obtained showed that at germination the shoot apical meristems of Echinocereus engelmannii were discs with a volume of $666,000 \mu\text{m}^3$ and were composed of a unistratose tunica (volume: $260,000 \mu\text{m}^3$) and a corpus which was two-cell layers thick (volume: $406,000 \mu\text{m}^3$). Four days after germination he found that the nucleus constituted 28.9% of the volume of the cell, and the vacuole constituted 24.5%. The mitochondria were 13.3% of the volume of the tunica cytoplasm, the chloroplasts 9.4% and the dictyosome only

1.2%. He also noted that the organelles of the corpus were identical in size and shape to those of the tunica but there were statistically significant differences in their cellular and cytoplasmic densities. According to his findings, the more distal corpus (C1) was less vacuolate (16.6% of the cell volume), and both corpus layers contained more chloroplasts, 12.0% of the cytoplasmic volume in the distal corpus layer (C1) and 14.3% in the more proximal corpus layer (C2). During the first four days after germination there was a dramatic increase in the size of the central vacuole (e.g. from 15.4% to 24.5% in the tunica), and the mitochondria increased in density from 10.2% of the cytoplasmic volume to 13.3%. Chloroplast density also increased in all meristem layers, but the dictyosome density decreased as much as a 30% loss in C2. There was also a highly significant reduction in the number of cisternae per dictyosome, from 5.47 to 4.77. Mauseth concluded that the organellar structure of the corpus cells were distinctly different from that of the tunica cells, and as the apical meristem became active after germination, the changes which occurred were not uniform in the meristem but rather were zone specific.

In a later publication, Mauseth (1981) used stereological methods but this time had to analyze the mature zonate apical meristem of Echinocereus engelmannii (Cactaceae). He examined each meristem of 20 plants, and picked meristems from the central mother cell zone (corpus), peripheral zone, pith rib meristem, central tunica and peripheral tunica. For his statistical analysis he used a paired sample statistics. Upon comparison of zones, Mauseth found that zones

were not identical on a structural basis. Each zone significantly differed from each other zone in some aspect of its ultrastructure.

Interestingly, the study revealed minor but statistically significant differences in the ultrastructure between zones of the adult shoot apical meristems. Furthermore, these differences were almost identical to those found in the seedling apex on a volume fraction percent basis.

Niklas and Mauseth (1981) examined the relationships among shoot apical meristem ontogenic features in Trichocereus pachanoi and Melocactus metanzanus (Cactaceae) using stereologic methods. They carried out percent volume measurements of shoot apical meristems. In their paper they described the ontogenic relationships within shoot apical meristem zones and their constituent cells. From their analysis, they showed two kinds of variations -- the seed due to intra- and interspecific differences reflecting the biological variance in apical configuration, and experimental error due to the uncertain position of zone boundaries. Their measurements of central zone cells, pith rib meristem and peripheral zone for individual apices gave standard variations of less than 2% for each zone. Except for the earliest seedling stages the observed intraspecific variations in shoot apical meristem parameters were insignificant thus reflecting a remarkable constancy during ontogeny of the youngest seedling stage.

Mauseth (1982a, 1982b) did two more follow-up studies of his earlier research, the morphometric study of the ultrastructure of Echinocereus engelmannii (Cactaceae) (Mauseth, 1980, 1981). In the first follow-up study, Mauseth (1982a) did a stereological morphometric

study of leaf primordia of Echinocereus engelmannii. He found that primordia were significantly different ultrastructurally, on both a protoplasmic and cytoplasmic basis, from the shoot apical meristem tissues (tunica and peripheral zone) that produced the primordia. He found a greater portion of the protoderm protoplasm consisted of nuclear material than was true for tunica protoplasm, significant at the 5% level. The protoderm was more vacuolate than tunica cells (2.7% vs. 10.4%). Volume of hyaloplasm was also increased. However he also found that mitochondria, chloroplast and dictyosome relative volumes were not different enough to be significant.

In his second follow-up study, Mauseth (1982b) computed the relative and absolute volumes of organelles in shoot apical meristems of Trichocereus pachanoi and reported and compared similar values published for three other species in two other families. He compared meristems of E. engelmannii, with meristems of Trichocereus pachanoi. Mauseth determined the relative volumes (V_V) by using a plastic overlay coherent point grid, with three sets of points spaced to optimize counting for objects of large diameter (nuclei, vacuoles, hyaloplasm), medium diameter (mitochondria, chloroplasts) and small diameter (dictyosomes, microbodies, lipid droplets). He used the following stereological formulas:

$$P_t = (0.453)(1 - V_V) / [V_V \times E^2(V_V)] = \text{total points}$$

$$N_v = (N_a \ 3/2) / (V_V \ 1/2)$$

to obtain relative values with errors (E) of less than \pm 3.3%. To determine the proper value for , he measured organelle profiles since

the long axes of mitochondria were usually more than 2X the length of the short axes. It was assumed that mitochondria were cylinders with the length about 5X greater than the diameter and thus $\beta = 3.0$. He finally computed mean organellar volume as V_V/N_V .

Moore et al. (1983) carried out a morphometric analysis of cellular differentiation in the root cap of Zea mays, in order to quantify the ultrastructural changes associated with cellular differentiation. They photographed random areas from random radial sections of a minimum of seven samples. But measurements were limited to a column of cells extending from the cap junction to the outermost peripheral cells associated with the root cap. They used the point counting technique, and used Snedecor and Cochran's (cf. Moore et al. 1983) student's t-test to evaluate the significance level of difference in means. They discovered that the relative volumes of the nucleus, nucleolus, and mitochondria in the protoplasm gradually decreased as the cell moved through the root cap, and the relative volume of plastids increased 240% during the differentiation of calyptrogen cells into columella cells. They concluded that since the relative volume of plastids as well as starch in plastids decreased markedly as columella cells differentiated into peripheral cells, the increase in the previous relative volumes was transient. They also discovered that dictyosomes and spherosomes increased more gradually than plastids, peaking in relative volume in the innermost peripheral cells. Interestingly, the relative volume of the vacuome decreased as calyptrogen cells differentiated into columella cells, after which it

increased during the differentiation of peripheral cells. In addition, they observed by the time the outermost peripheral cells (PCIII) were sloughed from the cap, the relative volume of the vacuome had almost tripled. These results indicated that each cell type comprising the root cap of Zea mays was characterized by a distinctive ultrastructure. Furthermore, they added, the ultrastructural changes associated with the differentiation of the cells are organelle specific.

Orr (1981) conducted a mitotic index study on median longitudinal sections of shoot apical meristems of Brassica campestris during transition from vegetative to the reproductive condition. He used stereological techniques to facilitate rapid quantitative and comparative statistical analysis of data obtained from microscope slides of apical tissue sections. He determined relative volume measurements by using a plastic overlay that contained a Weibel-type morphometric grid of points (Weibel, Kistler and Scherle, 1966. cf. Orr, A., 1981). He placed the overlay of grid points over a camera tube drawing, at standard magnifications, of apical zones, and when viewed through a microscope, points of the grid system that occurred in each zone or intercept tissue component were scored. Orr used Schweter's formula to determine standard deviation of a mean volume fraction value (p): $a = [P_p (1-P_p)/P]^{1/2}$ where $a = \text{s.d.}$ He defined P as a proportion of space and a whole shoot apical meristem or whole zone as P_p .

Orr found out that the relative volume and cell population of each zone remained constant from the vegetative to the reproductive stage. Volume fraction occupied by the nucleus and nucleolus remained

constant within each zone during the same time period. In each zone Orr discovered the volume of the nucleus was significantly correlated to the volume of the nucleolus.

Buckhout et al. (1982) did ultrastructural analysis of aleurone cells of wheat grains during germination. They examined electron micrographs of whole cells or portions of whole cells under transparent overlays containing dots spaced 1 cm apart, and determined organelle composition of the cells on a volume basis by counting the dots that were superimposed over specific cell components. However the analysis was confined to the cytoplasmic area of the cell. From each of three different aleurone preparations they analyzed a minimum of five cells per time and identified the ER, mitochondria, microbodies, Golgi apparatus, spherosomes and leucoplasts (plastids). Their studies revealed the aleurone cells of wheat underwent changes during development that encompassed two phases, a quiescent early phase and a synthetic late phase. During the early phase (8-14 hours of germination), the cells underwent changes that included the appearance and disappearance of lamellar bodies which were predominantly associated with protein bodies and ER. A precursor-product relationship between protein-body associated and ER-associated lamellar bodies and between lamellar bodies and ER was consistent with their morphometric data. They concluded that the differential distribution of lamellar bodies in aleurone cells may be an illustration of their general involvement in a unique arrangement, organization, or state of membrane lipids.

Reynolds (1984) obtained random sampling sections by making use

of the random orientation of pollen grains and by taking micrographs of cell profiles irrespective of the position in which the section cut through the cells. Only sections showing obvious distortions such as large holes or knife tears were not photographed. He used different levels of magnification due to the range in size of the cellular components to be measured. He also used point count data to provide a direct estimation of relative volumes of cellular components while line intersection counts provided estimates of relative membrane surface areas. Weibel (1969, 1973 cf. Reynolds, 1984). Volume density ($V_V X/Y$) expressed the volume occupied by the component x per unit test volume Y and was given in units of $\mu\text{m}^2/\mu\text{m}^3$. Reynolds selected nuclear and cytoplasmic changes of pollen grains of Hyoscyamus niger during normal gametophyte development and embryonic development, induced by anther culture, and analyzed and compared them ultrastructurally using stereological methods. From this study Reynolds discovered that nonembryonic pollen in vitro and in vivo possessed prominent nucleolar fibrillar zones and low ratios of dispersed to condensed chromatin. These differences he thought may have reflected changes in nuclear activity in potentially embryonic pollen grains during early stages of culture. He also noted that the volume fraction of the cytoplasm occupied by mitochondria and plastids and the area fraction of the cytoplasm occupied by mitochondria and plastids and the area fraction occupied by RER and Golgi cisternae differed in the generative cells of potentially embryonic and nonembryonic pollen. Finally Reynolds concluded that these results supported the idea that embryonic induc-

tion of H. niger took place at the uninucleate stage of development and that subsequent nuclear and cytoplasmic changes were essential for continued sporophytic development.

Briarty (1980a) thoroughly studied the quantitative changes in organelle volume of Phaseolus cotyledon cell during development following Weibel's (1973) methods of stereologic analysis. He made three types of measurements on cotyledons sections: volumetric analysis of cell and tissue compartments (e.g., cell walls, mitochondria) analyses of surface and interface areas (e.g., area of endoplasmic reticulum), and analysis of numbers of structures present (e.g., mitochondria). Due to the wide range in the size of structures that were measured, he analyzed them at different levels of magnification, X1000 (light microscopy), X3000 and X10,000 (electron microscopy). At the X1000 level Briarty intended to measure the large components, cytoplasm and vacuoles while smaller components were measured at the subsequent levels of X3000 and X10,000. Using techniques of Kister and Scherle (1966 cf. Briarty 1980a), he calculated volume, surface and numerical density and also calculated volume ratio $(S/V)V$. From this study Briarty found very few differences from a comparison of volume density estimates using means of quotients, and quotients of means. Also from the mean of the quotients and standard error, he was able to get the idea of the value of a parameter and also of its degree of accuracy. Briarty added that the measurements that he obtained directly could serve as the basis for deriving secondary parameters. He showed that by knowing the number of plastids and mitochondria per

cell and their volume density, mean volume for the individual organelles could be calculated. The numerical density was defined as the number per cm^3 of test volume, and he had measured the number of organelles per cell from which he was able to obtain the mean volume for the individual organelle.

Briarty (1980b) studied the quantitative changes in organelle volume, number and area during cotyledon cell development in Phaseolus vulgaris during the early period of reserve protein synthesis. He specified his period of study from 18-25 days after flowering. He discovered that subsequent increase in ER paralleled an increase in protein. Also, mitochondrial volume per cell increased as cell enlargement proceeded, but at a slower rate. There was no apparent decrease in mean mitochondrial volume since the number/cell increased more rapidly than the volume per cell. He also observed that increase in nuclear volume during development was paralleled by an increase in nuclear DNA content; however not with a close relationship which Scharpe and Van Parijs (1973, cf. Briarty, 1980b), also noted in ripening pea cotyledons. Briarty (1980b) noticed that the cell volume:nuclear volume ratio increased from 17:1 to 26:1 throughout the period. To that Briarty suggested the possibility that the increase in this ratio might have resulted solely from an increase in sequestered reserves which was not supported, since abstracting the vacuole and plastid/starch compartments from the protoplast volume gave a somewhat similar ratio increase. It was interesting to note that Briarty found that plastids varied considerably during development, according to cell division.

But he weakened any signs of correlation among any organelles. He stated that one general point to emerge from these data was that, while the trends of both biochemical and structural changes in the mitochondria and RER were in the same direction, the extent and the rates of these changes in any one organelle were by no means closely correlated.

Arias et al. (1983) used Loud's (1962) simple stereological method of linear integration and line intersect counting to determine the numbers of organelles and surface area of various cytomembranes per unit volume of cytoplasm. They determined, following Loud (1962), the membrane profile concentration (mean micrometers squared of membrane surface per micrometers cubed of cytoplasm) for plasma membrane, endoplasmic reticulum (ER), tonoplast and mitochondrial outer membrane. Arias et al. converted the amounts of cytomembranes and organelles in toxin-treated cells into percentage of control values. Their main aim was to detect changes in the quantities of cytomembranes and numbers of organelles in the outer root cap cells of sorghum seedlings treated with the host-specific toxin produced by Periconia circinata. In so doing, they extended their observations of qualitative cytological responses to the toxin. For their statistical methods, they used analysis of variance and separation of means by Duncan's multiple-range test for determination of differences at the 5% level of significance. Arias found that in seedlings susceptible to the pathogen, brief (0.25h) treatment with the toxin resulted in a marked and permanent decrease in the amounts of secretory vesicle membrane. In the 2 hr

treatment, they found that only secretory vesicle membrane was decreased. However, they also discovered that longer treatments led to an increased amount of endoplasmic reticulum (4h), which later decreased together with the amount of dictyosome membrane. Along with that they found that the amount of tonoplast increased (8h). On the other hand they detected that, in resistant seedlings treated with the toxin, there was early but transient increase in the quantities of plasma membrane, secretory vesicle membrane, dictyosome membrane, and endoplasmic reticulum and in the number of dictyosomes. Finally they concluded that insensitivity to the toxin may, indeed, involve the ability of resistant genotypes to recover from the toxin effect.

Fletcher (1983) presented a qualitative and quantitative ultrastructural observation on the development of oogonia of Saprolegnia terrestris from cultures grown under conditions of Ca^{2+} sufficiency and Ca^{2+} deficiency and contrasted them with his previous observations (1979a, cf. Fletcher, 1983) on oogonia of S. diclina. He used stereological methods to determine from electron micrographic prints the percentage volume densities of organelles relative to the peripheral protoplasm to volume densities and to total oogonial volume. He then used student's two sample t-test to determine significant differences between pairs of volume density. From his research, Fletcher found that as the central vacuole system enlarged, volume densities of dense-body vesicles, peripheral vacuoles, lipid bodies and cytoplasmic matrix decreased relative to total oogonial volume (peripheral protoplasm volume plus central vacuole volume), while the volume density

of nuclei increased and that of mitochondria remained constant. He also found that relative to the peripheral protoplasm only, volume densities of dense-body vesicles, lipid bodies and mitochondria increased and volume densities of peripheral vacuoles and of the cytoplasmic matrix decreased while the volume density of nuclei increased during central vacuole enlargement but subsequently decreased during formation of oospore initials.

Polito et al. (1984) conducted his stereological analysis according to Weibel et al. They determined relative volume fractions of nuclei from adult and juvenile meristems and young leaves by covering each electron micrograph with a plastic overlay that contained 2 Weibel-type point grid with a density of 4 points/ μ^2 and counting the total number of points lying over the entire nucleus, the electron-dense heterochromatin regions and nucleolus. They then analyzed their results by analysis of variance, and recorded both the actual and percentage measurements. From this study they noted that nuclei from shoot apical meristems and young leaves of the two growth forms central-zone nuclei were larger and less dense than peripheral zone nuclei. Between growth forms only minimal differences were found in heterochromatin contents of nuclei from young leaves. They also discovered that differences between growth forms were found only in the nuclei of the pith rib meristems. Here the juvenile form had smaller nuclei with greater density than comparable nuclei from the adult shoot apex. In conclusion it was suggested that this difference was probably related to differential rates of cell division, and that alterations in

the degree of chromatin compaction or heterochromatin/euchromatin ratios, detectable at the light or electron microscope levels, could not be considered causal factors in phase change in the English Ivy.

Pedro et al.'s (1984) quantitative methods were not exactly stereologic methods. They called the quantitative method, spatial statistics. They based their spatial statistical methods on Ripley's (1977; cf. Pedro et al., 1984) theoretical background for the description of the properties of a stationary point process by two functions K and P which they later defined. Using this quantitative method they analyzed the nuclear pore pattern in rat ventral prostate nuclei isolated from adult animals. In this particular investigation they searched for statistical evidence of a typical pore organization on the prostate nuclei of mature rats. They aimed at the description of such pore pattern by using a suitable model. Their results showed that pores on prostate nuclear membranes were not randomly distributed and the data sets, obtained from different micrographs were inconsistent with the same statistical model thus suggesting the existence of a typical pore distribution. Williams et al. (1983) also used quantitative methods other than stereology. They basically computed the average diameter (μm) of nuclear bodies in several species of germinating seedlings. They made the measurements on sections viewed in the electron microscope. They found the nuclear bodies in interphase nuclei of root apices of several species of germinating seedlings showed differences in structure and position relative to the nucleolus. Barlow, P. W. (1983) measured the diameter of the nuclei he was exa-

mining and the diameter of the nucleolus using a calibrated filar micrometer from electron micrographs. He considered only uncut nuclei. From the diameter measurement he computed the volumes and compared the averages among different species of root meristems. From the results he found that the number of bodies within a nucleus varies from species to species, but in general dense bodies were relatively numerous and lie in the nucleoplasm. The nucleolus associated body was usually solitary and lied on the surface of the nucleolus. Using nuclear volume as an indicator of the age of the nucleus since mitosis, he found that the number of dense bodies and nuclear associated bodies were related to the nuclear cycle.

Fagerberg (1984) based his computations of the relative volume of the cell occupied by organelles on Underwood's (1970) formula $V_v = a/A$ where a was the number of points falling on the organelle of interest and A was the number of points falling on the total profile. Surface to volume ratios (S_v) he determined by another of Underwood's formulas: $S_v = 2P$, where P was the number of intersections of a random set of test lines of total length with membrane systems within the chloroplast or mitochondrial profiles. For numerical density or number of organelles per unit volume (N_v) Fagerberg (1984) used Weibel and Gomez's relationship, $N_v = K / \beta \cdot N_{32}/P$; where N was the number of organelle profiles per unit area, P was the volume density, β was a coefficient based on profile axial ratios and geometry of the organelle profile and K was a size distribution coefficient. From this study Fagerberg noted that chloroplast and nuclear compartment V_v values changed significantly in

the early stages when cells were dividing. Mitochondrial and vacuolar compartments showed no significant change in V_v value during the development process. He also discovered that the surface to volume ratios of the chloroplast membranes increased significantly throughout all stages of the leaf development while mitochondrial cristae S_v values did not change. In addition he discovered organelle replication rates appeared to be independent of changes in cell volume with each organelle exhibiting a specific replication pattern. His conclusions suggested two possible mechanisms for the control of cell structural development involving both intrinsic and extrinsic factors. Bendich et al. (1984) used a 220 mm square transparency containing a grid of 99 large points, overlaying each electron micrograph to be analyzed stereologically. As they described, each large square defined by 4 large points was subdivided into 9 smaller squares by a grid of small points. They determined for each micrograph, the frequency with which these points fell within protoplast, nuclei, plastids and mitochondria. Then estimates were made of the volumes of protoplasm, nuclei, chloroplast and mitochondria for each tissue and extrapolated to the volumes of an average cell in that tissue. They indicated that they made more than 100 measurements in each case from random sections, and computed the S.d. by dividing the micrographs subjected to morphometric analysis randomly into sets of ten. They then examined several species and several tissues using this method. According to their findings, for a given tissue there was little species to species difference in volume of the cells' nuclei, total plastids and total mitochondria (the

chondriome), although for each species the volumes were widely variable for cell and plastids and somewhat variable for nuclei and the chondriome. Several other authors have held the same theory as Bendich et al. regarding mitochondria (Pellegrini, 1980; Gaffal and Schneider, 1980; Johnson et al., 1980; Blank et al., 1980; cf. Bendich et al., 1984). They indicated that mitochondria were morphologically diverse but biochemically similar organelles in eukaryotic cells. They also stated that according to light and E.M. studies that mitochondria revealed varied morphology from large, lobed organelles to elongated, branched or unbranched units to smaller, cylindrical, ellipsoidal or ovoid units. They also claimed that these different morphological forms could be interchangeable, the larger forms fragmenting and smaller forms coalescing.

The technology of devices used in carrying out stereologic methods are advancing at a rapid rate. Silage et al. (1984) proposed the use of a touch-sensitive screen as a tool to facilitate acquisition of data both in point counting and morphometric planimetry. As Rigant et al. (1983; cf. Silage et al., 1984) indicated, planimetric measurements are promoted in a variety of commercial instruments which utilize the joystick, trackball, digitizing tablet, or light pen to outline the boundaries of structures. Silage et al. stated that except for the light pen, all other planimetric instruments yield only a correspondence image. They reasoned, because the tracing or editing of a boundary performed by the investigator was not accomplished on the same surface where the combined image was displayed. With the touch-

sensitive screen that Silage et al. were proposing, labelling or tracing and display occurred on the same surface, which eliminated the correspondence image. They specifically showed the advantage the touch-sensitive screen had over the light pen. It obviated the tracing cursor target of the light pen, as the coordinates of a location were read directly and the translation of the image was easily recorded. The increasing role of computer-assisted image analysis cannot be underestimated with advancements in technology. Teweski et al. (1983) used such devices to facilitate the measurements required to carry out a morphometric analysis. Nicholson (1978) described automatic image analyzers as a blessing but also a complicating factor to quantitative stereology. He concluded that the data generated by an automatic imaging analyzer were extremely well organized and were organized far beyond the usual with manual or semi-automatic collection. Not only devices but new concepts are being incorporated to the methods of stereology. Serra (1984) described the irregularity of non-planar surfaces and introduced two notions of flatness and roughness which referred to local properties of the profiles and of the surfaces. Serra's objective was to measure the roughness of surfaces, and to do that he proposed two parameters. He defined flatness as the ratio of the measure of the surface divided by its projection and roughness as the average of the square of the mean curvature of the surface per unit area. He indicated that both parameters were accessible from vertical plane sections.

The role of statistics in stereology which had been minimal

(Nicholson, 1978) is becoming more significant in the interpretation of quantitative data from ultrastructural studies. As Nicholson pointed out only the basic tools of statistics, standard deviations, confidence intervals and t-test were being used. He described some ways in which more complete use of statistics could improve quantitative microscopy, the potential role of statistics being in sampling, estimation and multivariate analyses. He also showed how one could use F-distribution and multiple comparison tests to show significant changes in placental microstructure. He also predicted that the need for application of and understanding of multivariate analysis methods would be greater in the future with increased usage of automatic image analyzers.

MATERIALS AND METHODS

Collection and processing of the soybean tissue specimen

Twenty-five Glycine max (L.) Merr. cv. Corsoy seeds were randomly selected and placed in a petri dish lined with filter paper in which the seeds remained completely covered with water for the first six hours for rapid imbibition. Then the water was drained and the filter paper was kept moist. Seeds were collected after a total of 12 hours of water imbibition. The twelve-hour collection served as controls since no cytological changes had occurred during early imbibition (Pease, 1972). The same collection procedure was followed for seedlings after 24 hours, 48 hours and 96 hours of growth.

The embryo of each seed was then excised using a single edge razor until only the growing point was saved. All the leaf primordia and the rest of the embryo was removed. The growing point (shoot apex) was then fixed immediately in cold buffered 5% glutaraldehyde and placed in a refrigerator overnight. The dissected growing points were then rinsed in phosphate buffer pH 7.2 (stock solutions: A. potassium phosphate dibasic 14.19 g/l liter double distilled water; B. potassium phosphate monobasic 13.60 g/liter of double distilled water. Working solutions included: 110 mls of B + 390 mls of A), allowing 10 minutes for each rinse. The growing points were placed at room temperature in 1% osmium tetroxide for 45-60 minutes under a fume hood using rubber gloves. Pipettes were disposed of by rinsing in water and discarding, and used OSO_4 was placed in a waste container marked for osmium. This was followed by four buffer rinses of ten minutes per rinse. The

tissue went through the alcohol dehydrating series of 25%, 50%, 75% and 95% of ETOH for 15 minutes in each step.

Three changes of 100% ETOH with 15 minutes per change was followed by three changes in 100% acetone, 20 minutes per change. Filtration of the tissue in Spurr involved the following series of changes: 2 parts acetone -- 1 part resin (Spurr) for 15 minutes, 1 part acetone -- 1 part resin for 30 minutes, 1 part acetone -- 2 parts resin for 1 hour and finally in pure resin for overnight. For embedment the tissue was placed in pure resin. Each tissue was carefully placed with the proper orientation in a Been capsule or molds, labels were added written in pencil only, the capsules were then put in a 70° C embedding oven for 10 hours.

Trimming of specimen blocks

The tissue blocks were carefully taken out of the capsules by excising the capsules on the side with a razor blade. The tissue blocks were then ready for trimming under the dissecting scope.

The goal was to obtain a trapezoid with the top (~0.2 mm) and the bottom (~0.5 mm) parallel and the height ~0.5 mm. The top and bottom edges of the trapezoid had to be parallel in order to obtain a ribbon of sections.

Sectioning

An MT-2B Porter-Blum ultramicrotome was used in sectioning of tissue blocks. Glass making is the most critical aspect of ultramicrotomy. In fact, the quality of the knife determines the overall quality

of the micrograph. Knives were made from strips of glass using the LKB Knife Maker 7800B. Each knife made was examined under a dissecting scope to make sure that the cutting edge did not have fine nicks. About 1 cm below the cutting edge some melting wax was applied to retain some water on the top surface. The glass knife was at a 4° clearance angle on the ultramicrotome. The trimmed block was mounted and oriented in the microtome so that the longer base of the trapezoid was at the bottom. After aligning the microtome, thick sections were cut manually to finely trim the tissue block. Sections 700-1000 Å thick of yellow, gold and silver were obtained, which floated in the trough filled with water. The thin sections were picked up from the trough with a 200-mesh copper grid. Excess water was absorbed on filter paper grids and stored in filter paper lined petri dishes and labeled. At least 10 grids were collected from each specimen block.

Staining

Approximately 300 ml. double distilled water was boiled and cooled. A petri dish with four quadrants was then prepared. The first quadrant was filled 1/4 full with sodium hydroxide (NaOH) pellets. The second quadrant was filled with 2% uranyl acetate drops, to accommodate all the grids (1 drop/grid). The preparation of the 2% Uranyl acetate solution was as follows: 2 grams of uranyl acetate was added into enough double distilled water to yield a total of 100 mls. The third quadrant was then filled with lead citrate drops to accommodate all the grids (1 drop/grid). The preparation of the lead citrate solu-

tion was as follows: first double distilled water was boiled to remove carbon dioxide (CO_2), and cooled. A 100-ml volumetric flask was partially filled with the boiled double distilled water, 0.5 grams of lead citrate and 8 ml of 2N NaOH was added. It was mixed until the solution cleared. The volume was then brought to 100 mls with boiled double distilled water. It was divided into aliquots in snap cap vials, covered with mineral oil and stored at 3°C . Before each use the prepared solution had to be brought to room temperature. Finally the fourth quadrant was filled $1/4$ full with double distilled water.

The following procedure was used in staining the grids. The grids were first allowed to float on uranyl acetate, 1 grid/drop, with the section on the stain for 1 hour. Two beakers were filled with boiled double distilled and cooled water and the grids were rinsed 20 dips in each beaker. Grids were dipped vertically (up and down) breaking the surface on each dip. The grids were allowed to float section side down in lead citrate for 10 minutes. The grids were rinsed in four beakers filled with boiled double distilled water, 20 dips per beaker. Finally, the grids were left to dry in a petri dish lined with filter paper and stored in a grid holder. While staining, it was important to keep the petri dish covered when not actually handling the grids, also avoiding breathing on grids as any exposure of lead citrate to CO_2 will yield a lead carbonate precipitate.

Sampling method

The shoot apex was examined at four different levels of germination -- 12, 24, 48 and 96 hours of growth. For every sampling

level, five samples of tissue blocks of the shoot apex were picked at random. Five micrographs were obtained from each sample. Thus for each hour level there were twenty-five micrographs taken at a magnification of 5,000.

The micrographs were selected from the central zone cells two cells in from the tunica. All specimens were examined on the Hatachi HU-12 transmission electron microscope in the Veterinary Science Diagnostic Laboratory.

Morphometric analysis

Morphometric planimetry (areal analysis) was the stereological method used to carry out the quantitative analysis of the cell's ultrastructure. This was done with the Apple IIe microcomputer, and graphics tablet as a semi-automatic image analyzer using an Optomax image analysis program (Optomax, Inc., Hollis, NH). The stereologic methods used within the program were based on Underwood (1970). The electron micrograph was placed on the graphics tablet, upon which a transparent grid overlay was placed. A stylus outlined the boundaries of the organelles.

Before any measurement was carried out, the micrograph had to be calibrated, and the graphics tablet overlay standardized. This was done using a standardized 200 mesh waffle type grid. The length of each line in the grid was 416.7 nm. To detect any fluctuations in the electron microscope a picture of the grid was taken periodically. Also in making photographic prints of the grid and the other micrographs to be analyzed the Besseler 45MX enlarger had to be placed at the same

position every time to keep the enlargement constant. The magnification used in the electron microscope was 5,000X. The length of the line on the grid was thus calculated at 5,000X and that measurement was used for calibration purposes. The image analysis program converted organellar measurements from projected size to actual size.

For every electron micrograph that was subjected to morphometric analysis an average area of $85.595 \mu\text{m}^2$ was randomly selected. The transparent plastic grid overlay was used to outline the boundary of the field selected. Every micrograph to be analyzed was covered by the transparent plastic grid overlay. While the tracing of the boundaries of the components to be measured was done on the graphics tablet, the image outline was displayed on the monitor allowing for more accurate outlining of the components.

The Optomax image analysis program allowed the measurements to be stored under different classifications. The classification, FILE was used to store the measurements of a particular component or organelle. The following components were measured; plastids, mitochondria, vacuoles, cell walls, lipid bodies, starch grains and nuclei. These components were measured at four developmental periods of 12 hours, 24 hours, 48 hours and 96 hours of growth after water imbibition. Thus there were a total of 28 files stored in the data disk. Each file was designated first by the hour and then by the organelle's identifying number which was from 1 to 7 respectively. The program also allowed each organelle measurement to be classified into 10 different classes. However, since a total of 25 micrographs were measured for every

developmental period, this data had to be stored in three different disks. The first 10 micrographs were stored in the first disk under the 10 classes, numbered from 0 to 9, the next 10 micrographs in the 2nd disk and the last 5 in the 3rd disk. Thus a total of 12 disks were used to store all the data obtained from all the 100 micrographs measured.

Statistical analysis

Measurements from the 100 micrographs based on relative volume of the components within a randomly selected field of an average area of $85.595 \mu\text{m}^2$ was stored in the data disks of an Apple IIe microcomputer. These data were printed out, all measurements of each component per field were added. Then these sums were transferred into the main frame IBM series 370-3031 computer. Using the SAS program as described in the SAS Basic User's Guide (1982), an analysis of variance and a regression analysis, which Sokal (1969) and Balam (1972) extensively defined, was carried out to interpret the data statistically.

The first step after the data had been transferred into the main frame, was to print it out on the basis of volume fraction percent. The printout was then checked for mistakes, and corrected.

The second step was to carry out an analysis of variance, in order to detect any significant difference in volume fraction percent of all the organelles with time and to determine any significant interaction between time and volume fraction percent of the organelles.

The third step was then to specify the relationship between organelles with the hourly stage of development during growth following

germination. A regression analysis was carried out, and the measurements of the organelles on the basis of volume fraction percent was related to hourly developmental stages of growth following germination. For each organelle the regression analysis was carried out at three different levels from the first to the third term of a polynomial regression analysis. The residual and regression lines and confidence limits were plotted and the best model was chosen for each organelle.

RESULTS

100 electron micrographs of promeristematic cells from the central zone (2 cells below the tunica), obtained from germinating soybean seeds at four developmental stages, 12, 24, 48 and 96 hours after water imbibition, were first qualitatively then quantitatively analyzed.

Qualitative Analysis.

Representative micrographs of the four stages are shown in Figures 1-4. Ultrastructural comparisons were made using the 12-hour stage as the standard, because the seedlings had not yet resumed full metabolic growth (Pease, 1972). The cells were hydrated and the architecture of the membrane had just changed from relatively porous "hexagonal" state to the more stable "lamellar" configuration (Webster and Leopold, 1977). As shown in Figure 1, the mitochondria and proplastids were fully rounded, the cristae and prethylakoidal bodies (as they were called by Srivastava and Paulson, 1968) were not developed (See Figure 3A). Not only were the mitochondria and proplastids immature at this stage, but the ER and dictyosomes were almost absent, or at a low level of development. However at this stage of development, the lipid bodies were abundant and highly concentrated along the plasmalemma and closely associated with the vacuoles. There were some starch grains within the proplastids. However, whenever the proplastids did not contain starch grains, it was extremely difficult to differentiate them from mitochondria, because at this stage the

Figure 1. A. Central zone cells in the soybean shoot apex 12 hours after initial water imbibition.

B. Central zone cells after 24 hours.

C - cell wall; M - mitochondria; N - nucleus; P - proplastid; V - vacuoles; * - lipid bodies surrounding vacuole.

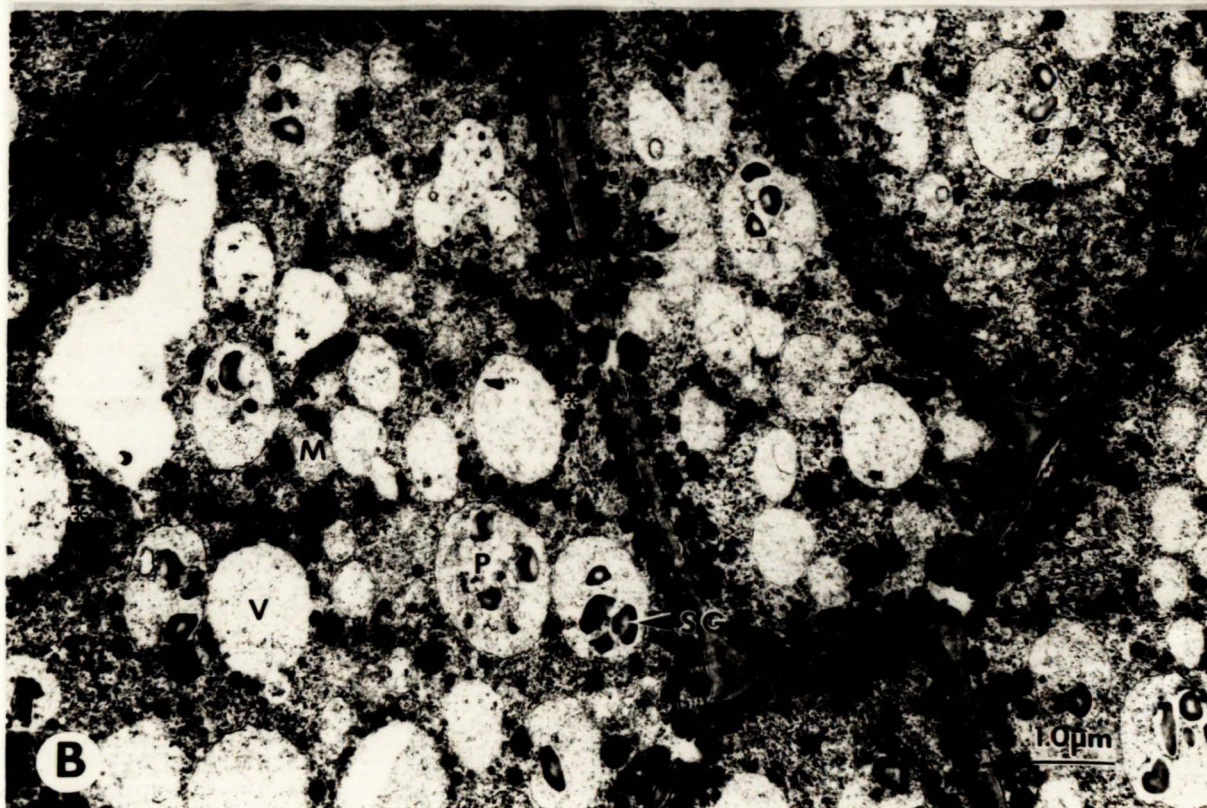
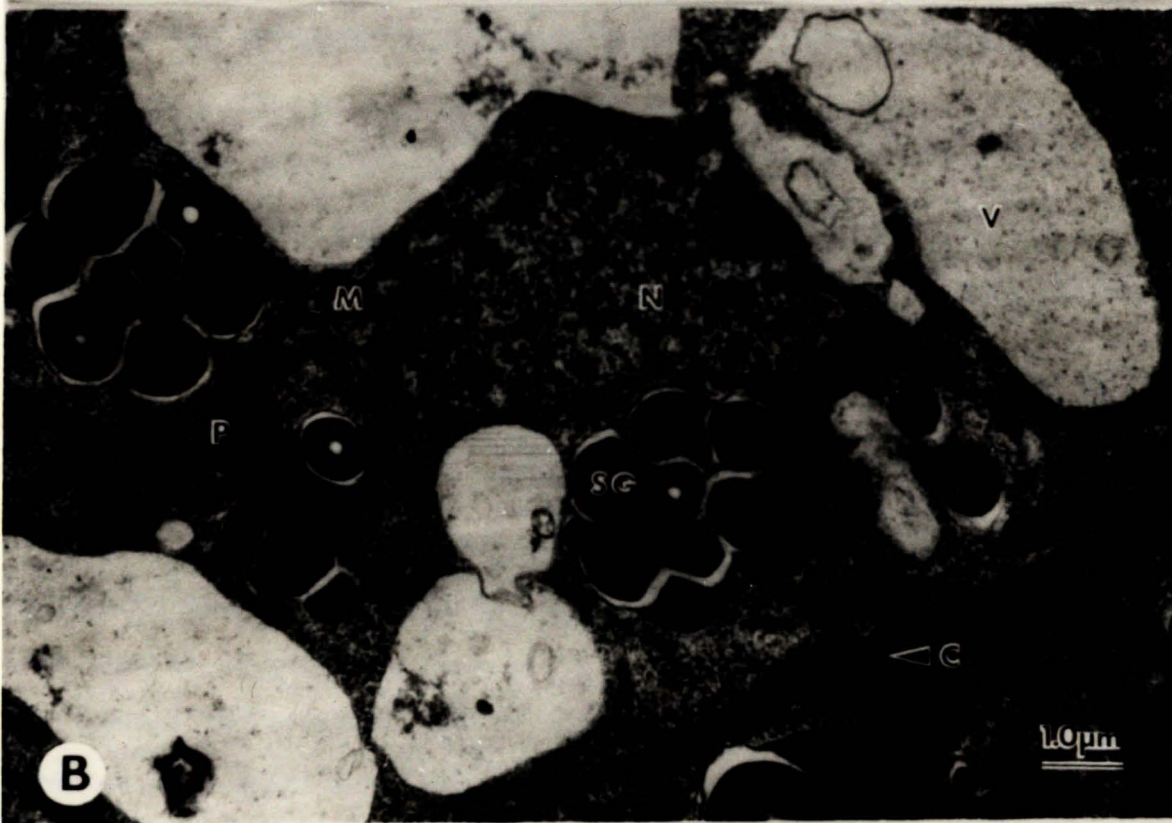


Figure 2. A. Central zone cells from the soybean shoot apex 48 hours after initial water imbibition.

B. Central zone cells after 96 hours

C - cell wall; D - dictyosome; ER - endoplasmic reticulum membrane; M - mitochondria; N - nucleus; P - plastid; SG - starch grain; V - vacuole

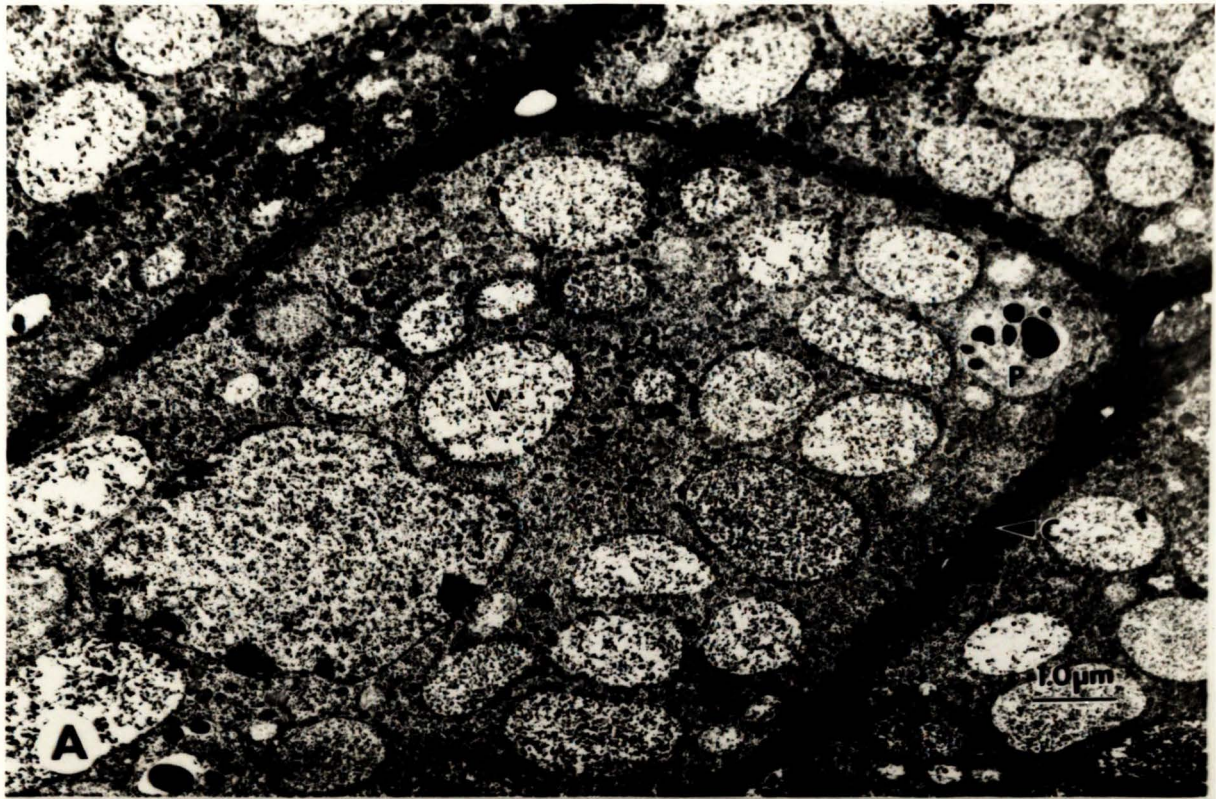


internal membrane system was poorly developed. The cells in general were not highly vacuolated compared to the later stages. The vacuoles were greater in number but were relatively smaller in size and had amorphous, granular content (See Figures 3A-3B). The cytoplasm also appeared saturated with free ribosomes but this finding was not unique to this particular stage of development. Microbodies, ER, and dictyosomes were extremely difficult to identify due to their lack of development.

At the 24 hours stage of development (See Figure 1B), there were several indicators that suggested the initiation of metabolic activities. The lipid bodies were still closely associated with vacuoles and the plasmalemma. The vacuoles at this stage had relatively decreased in number but increased in size (See Figures 1B and 4A), due at least in part to coalescence. Starch grains were accumulating in the plastids. The mitochondria at this stage were easier to identify, since their cristae were more well developed and prethylakoidal bodies were developing (See Figure 4B). Dictyosomes were also more visible at this stage.

At the 48 hours stage of development (See Figure 3), almost all the lipid bodies had moved from the plasmalemma and decreased in number. The plastids were developing their internal membrane system. The starch grains were also more numerous and had increased in size (See Figures 2A and 5A). Some plastids had an irregular outline. The vacuole decreased in number, but increased in average size. Vacuoles were still in the process of coalescing (Figure 5B). There

- Figure 3. A. Vacuoles from central zone cells of soybean shoot apex showing amorphous content and close association with lipid bodies 12 hours after initial water imbibition.
- B. Vacuoles 12 hours after initial water imbibition, showing the vacuolar content and close association of lipid bodies.
- C - cell wall; LB - lipid body; P - proplastid; V - vacuole



- Figure 4. A. Vacuoles showing coalescence and decreasing content of amorphous material 24 hours after initial water imbibition.
- B. Proplastid showing developing prethylakoid membranes 24 hours after initial water imbibition.

C - cell wall; LB - lipid body; P - plastid; PTH - prethylakoid; SG - starch grain; V - coalescing vacuoles.

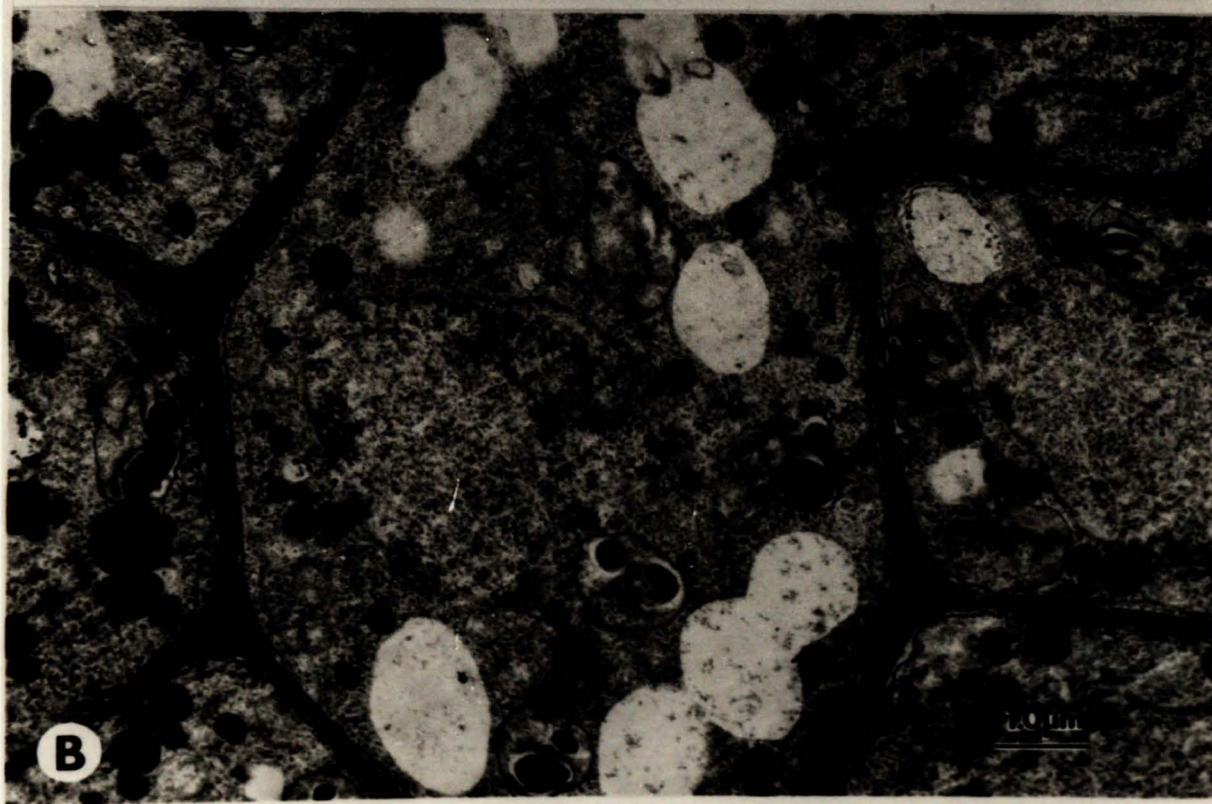
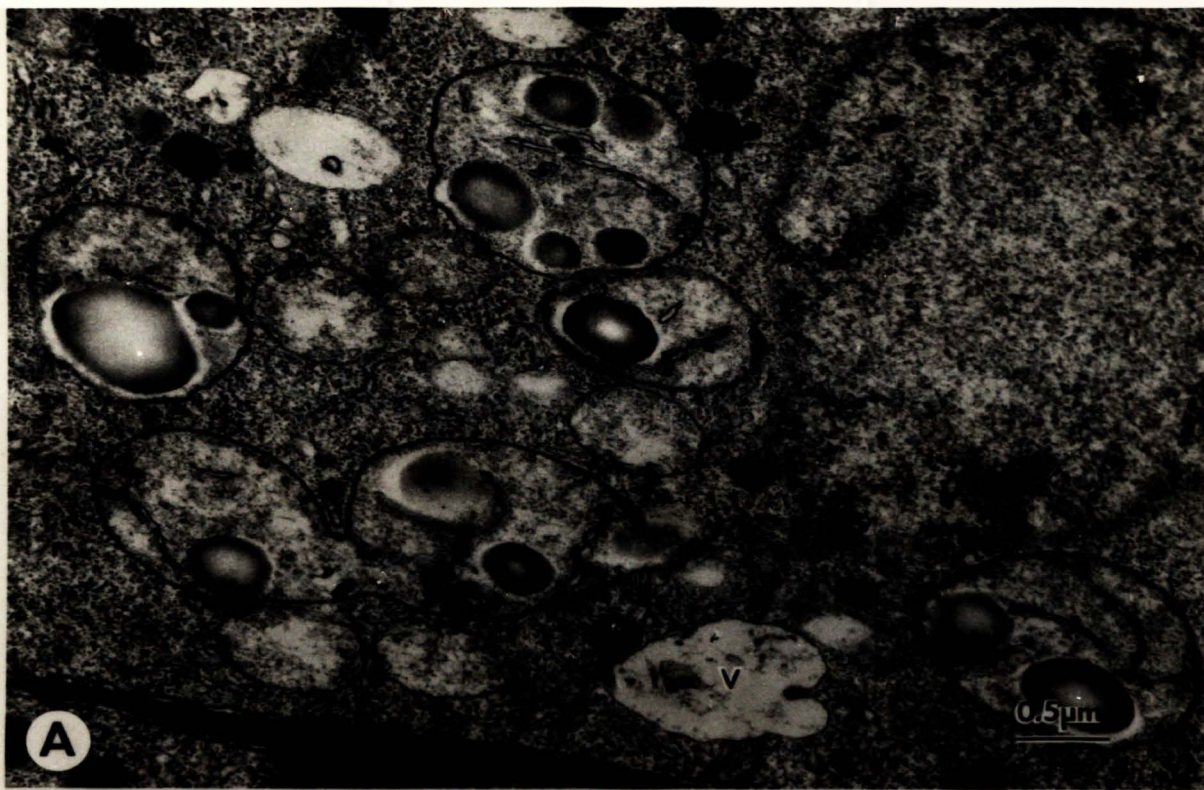
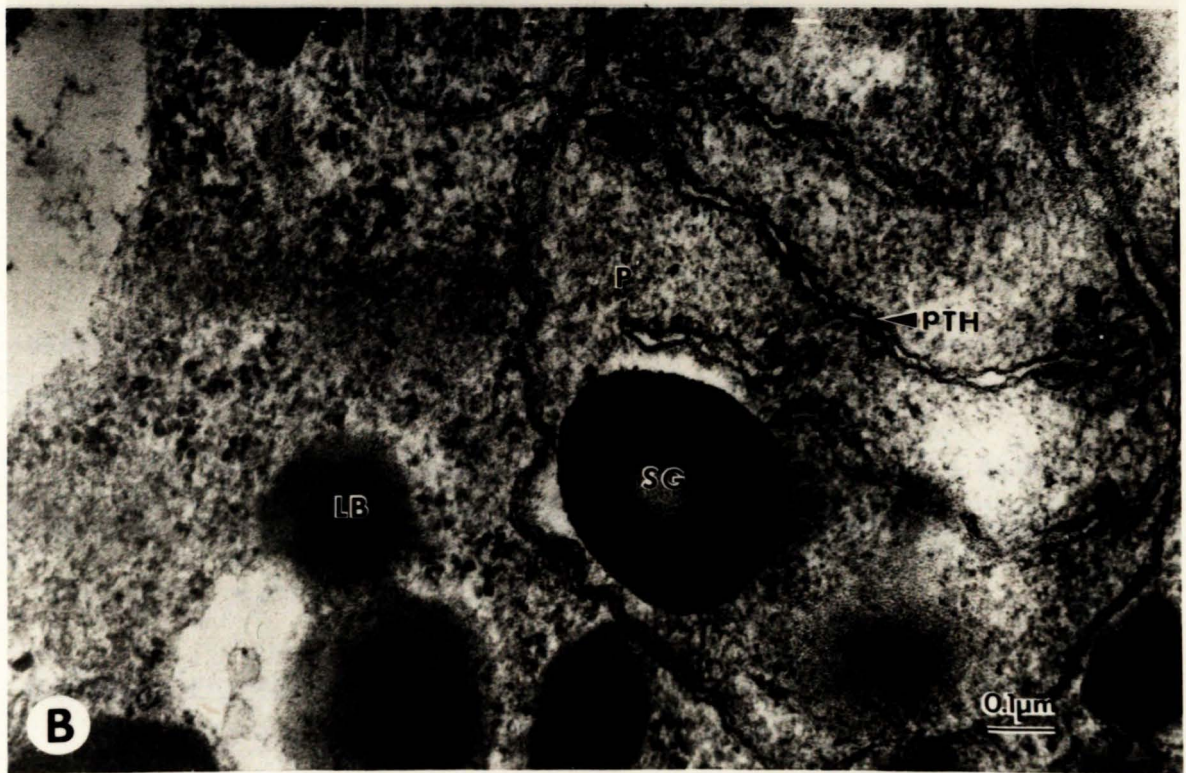
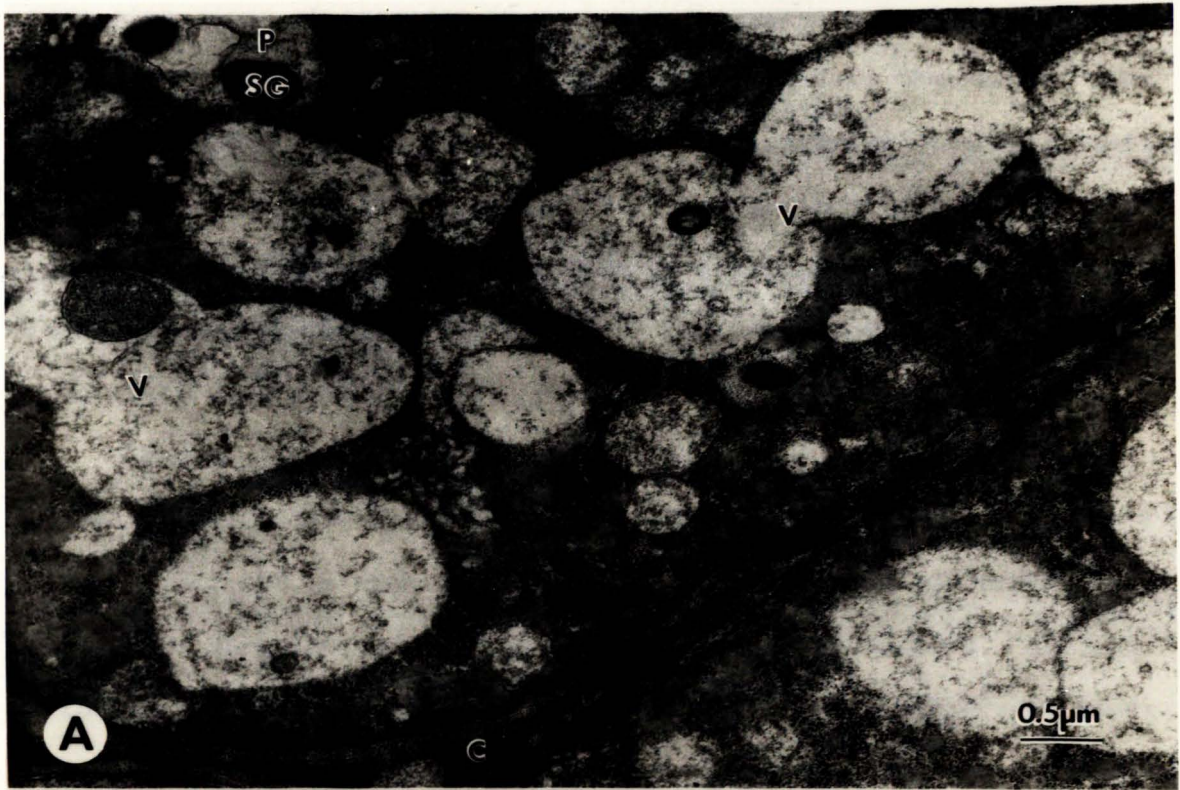
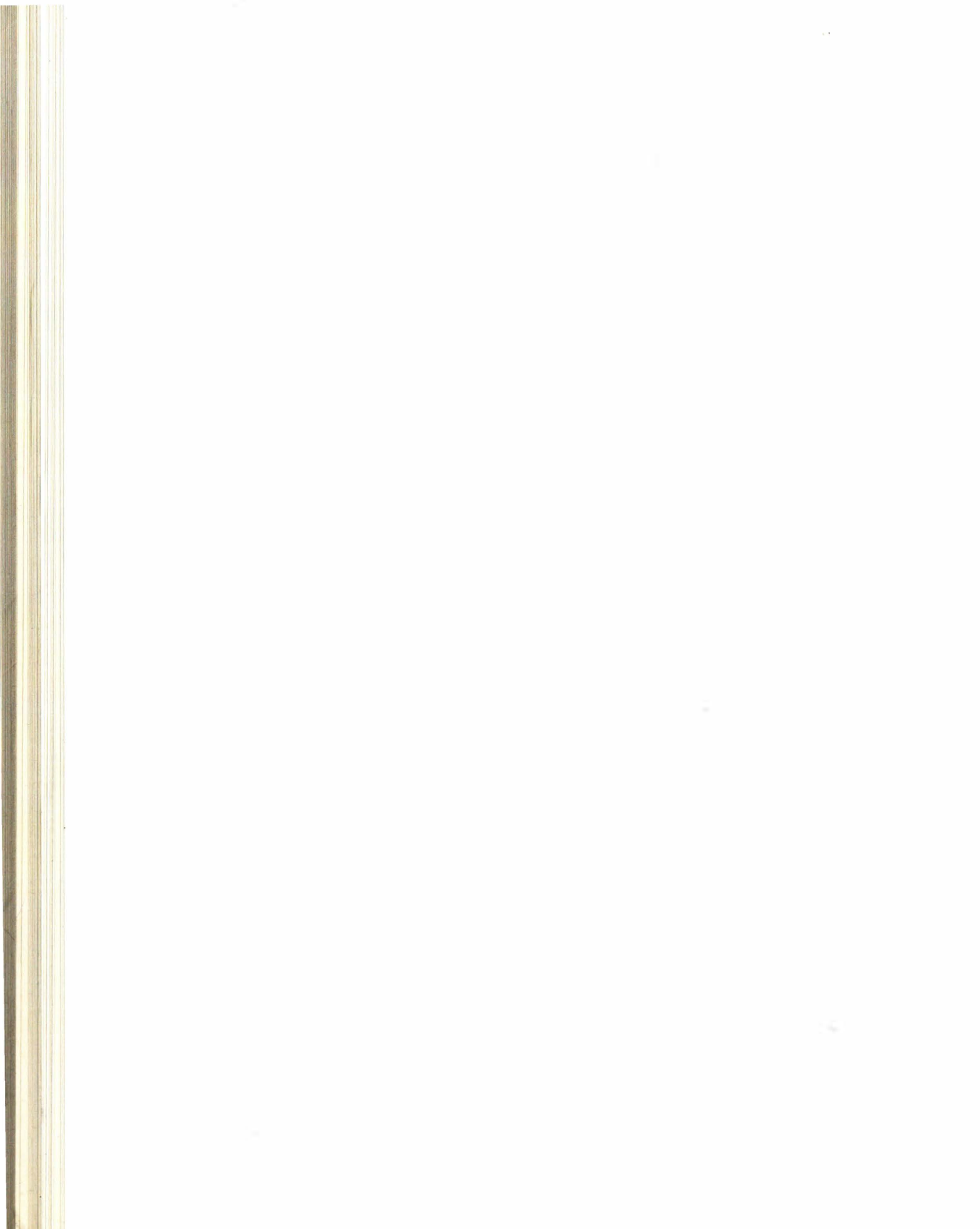


Figure 5. A. Plastids showing irregular shapes and increasing starch content 48 hours after initial water imbibition.

B. Coalescing vacuoles with decrease in amorphous contents after 48 hours after initial imbibition. Note the decrease and random arrangement of the lipid bodies.

LB - lipid body; V - vacuole.





were also signs of increase in membranous storage material within some vacuoles (See Figure 6A). The amorphous contents in the vacuole were disappearing. The dictyosomes were more vesicular and the ER cisternae was more prominent at this stage. As the lipid bodies decreased in concentration, the starch grains were increasing. The mitochondria internal membrane system appeared more well developed. There were also relatively more pyrenoids within the mitochondria.

The lipid bodies at the 96 hour stage had essentially disappeared as shown in Figures 2B and 6B. The vacuoles occupied almost 1/3 of the cell, and by this time almost all the vacuoles had coalesced into large vacuoles and had lost their granular appearance. The mitochondria and plastids had well developed internal membranes (See Figure 7A). There were relatively more dictyosomes and ER, and the starch grains were most conspicuous.

Overall, the cell wall and the nucleus did not show any significant fluctuations during these developmental stages. The plastids and mitochondria also developed in terms of their internal membrane system with time, but there was no apparent change in size. As lipid bodies disappeared, starch grains in the plastids increased. Amorphous material in the vacuoles became much reduced.

The plastids and mitochondria were surrounded by a double membrane, the vacuoles by a single membrane and the lipid bodies by an interfacial membrane (See Figure 7B).

Quantitative Analysis.

Volume fraction percents were determined using areal analysis

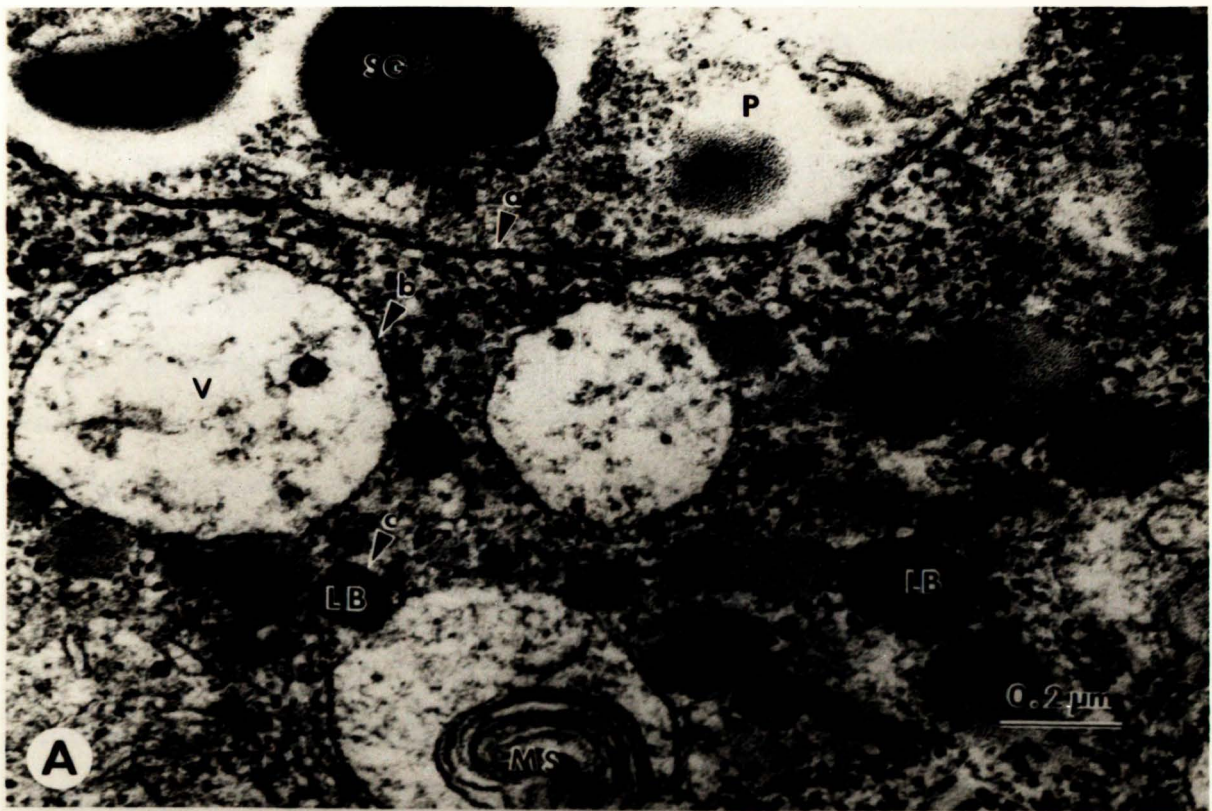
- Figure 6. A. High magnification of a vacuole showing a membranous storage material.
- B. Central zone cells 96 hours after initial water imbibition showing; relative increase in the size vacuoles (V), relative decrease in the content of amorphous material within vacuole (V), relative increase in starch grain, SG, content within plastids, and absence of lipid bodies.
- C - cell wall; P - plastid; SG - starch grain; V - vacuoles.



Figure 7. A. Central zone cells showing a double membrane around the plastid (a), a single membrane around the vacuole (b), and an interfacial membrane (c) separating the vacuole from the hyaloplasm.

B. A proplastid and a mitochondrion in the 96 hour stage of development showing well developed internal membranes.* Endoplasmic reticulum cisternae becoming more obvious.

CR - cristae; LB - lipid body; M - mitochondria; MS - membranous storage material; P - plastid; SG - starch grain; TH - thylakoid; V - vacuole; ER - endoplasmic reticulum.





obtained from a semi-automatic image analyzer using stereologic methods.

The ultrastructural data was statistically analyzed. The average volume fraction percent of each organelle at the four different stages of development are in Table 1.

An analysis of variance was carried out to detect any significant difference in organellar volume fraction percent. There was a highly significant difference in organellar volume fraction with reference to time (developmental stages) according to the F-test (Table 2). There were also significant interactions between time and organellar volume fraction percent. In addition there was significant fluctuation in organellar volume fraction percent from sample to sample readings.

To summarize the overall organellar changes quantitatively, during this development, a Waller-Duncan K-Ratio T-test was carried out and the results are shown in Tables 3 and 4.

The results from Table 3 indicated that there were significant differences among the organelles in mean volume fraction percent without considering time. The grouping specially pointed out to the organelles which were significantly different from each other in the basis of mean volume fraction percent. The results from Table 4 did not show difference with significance as high as in Table 3 in average volume fraction percent of all organelles at specified developmental stages. However, there was a significant decline in the mean volume fraction percent of total organelles at the 48 hours stage of

Table 1. Average volume fraction percent of the organelles from 25 samples for each collection period following imbibition showing \pm standard deviation and, in parentheses, the coefficient of variation.

Hours of development	Plastids	Mitochondria	Vacuoles	Cell wall	Lipid bodies	Starch grain	Nucleus
12	5.59 +2.40 (42.94)	2.64 +1.71 (64.73)	3.05 +2.46 (80.65)	5.94 +3.15 (52.95)	18.70 +6.74 (36.06)	0.58 +0.69 (119.35)	16.80 +14.02 (83.43)
24	7.68 +4.76 (61.97)	2.34 +1.31 (55.89)	15.60 +11.95 (76.60)	8.65 +4.05 (46.81)	14.51 +7.54 (52.00)	1.85 +1.70 (91.74)	7.49 +7.03 (93.83)
48	6.87 +3.97 (57.72)	1.77 +1.04 (58.82)	7.53 +5.97 (79.31)	7.92 +2.46 (31.11)	5.67 +3.53 (62.27)	2.15 +2.19 (101.63)	12.75 +11.30 (88.69)
96	7.31 +4.30 (58.81)	2.17 +0.90 (41.38)	19.26 +14.77 (76.69)	10.45 +14.30 (136.77)	0.10 +0.23 (231.10)	3.23 +2.64 (81.72)	16.79 +14.45 (86.04)

Table 2. ANOVA table showing significant difference among the means in volume fraction percent of organelles with time in hours after initial water imbibition.

Source	Df	Sum of squares	Mean square	F value
Hours of development (time)	3	474.44	158.15	2.47*
Organelles	6	11465.87	1910.98	29.83**
Time * organelle	18	10821.23	601.18	9.39**
Sample (time * organelle)	112	7174.35	64.05	1.33*
Error	560	26926.25	48.08	
Corrected total	699	56862.14	81.35	

C.V. = 90.15

Table 3. Waller-Duncan K-ratio T test for organellar volume fraction percent by organelle.

Grouping*	Mean volume fraction %	N	Organelles
A	13.46	100	Nucleus
B	11.36	100	Vacuoles
C B	9.74	100	Lipid bodies
C D	8.24	100	Cell wall
D	6.86	100	Plastids
E	2.23	100	Mitochondria
E	1.95	100	Starch grain

*Means with the same letter were not significantly different.

K ratio = 100 MSE = 64.06 DF = 112 F = 29.83
 Critical value of T = 1.78 Min. significant difference = 2.01

Table 4. Waller-Duncan K-ratio T test for organellar volume fraction percent by development time.

Grouping*	Mean volume fraction %	N	Time (hours of development)
A	8.47	175	96 hours
B A	8.30	175	24 hours
B A	7.61	175	12 hours
B	6.38	175	48 hours

*Means with the same letter were not significantly different.

K ratio = 100 MSE = 64.06 DF = 112 F = 2.46
 Critical value of T = 2.27 Min. significant difference = 1.94

development.

Therefore, there were strong indications, from the statistical analysis carried out thus far, of organellar fluctuations based on volume fraction percent during the development of the germinating seedlings. Hence, to determine the exact relations between these three dimensional measurements with the developmental stages in hours after water imbibition, regression analysis was carried out for each organelle. The regression model was chosen which best fit each organelle's change based on volume fraction percent with developmental stages in hours after water imbibition. The regression lines with confidence of 95% limits were plotted in graphs as shown in Figures 8-14.

PLASTIDS

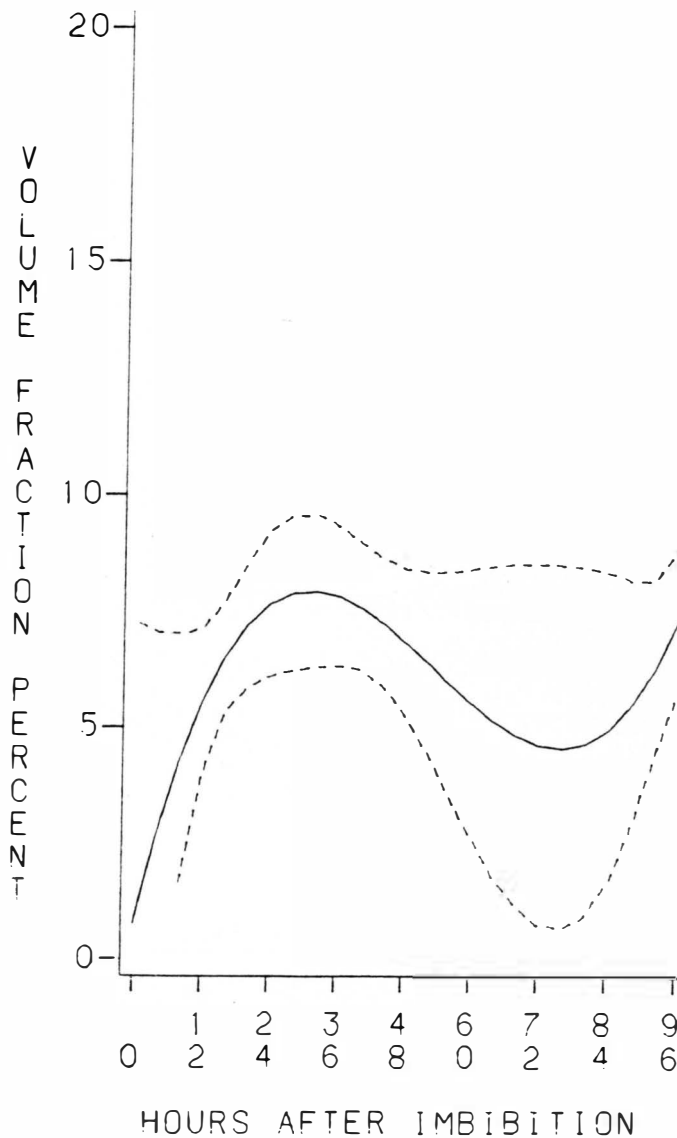


Figure 8. Plastids from central zone cells of the soybean shoot apex showing: changes in volumetric density V_V^P of plastids, in percent, with hours after initial water imbibition. $R^2 = 0.04$, S.D. = 3.96, $Y_{V_V^P} = 0.78 + 0.54X - 0.01X^2 + 0.00008X^3$

MITOCHONDRIA

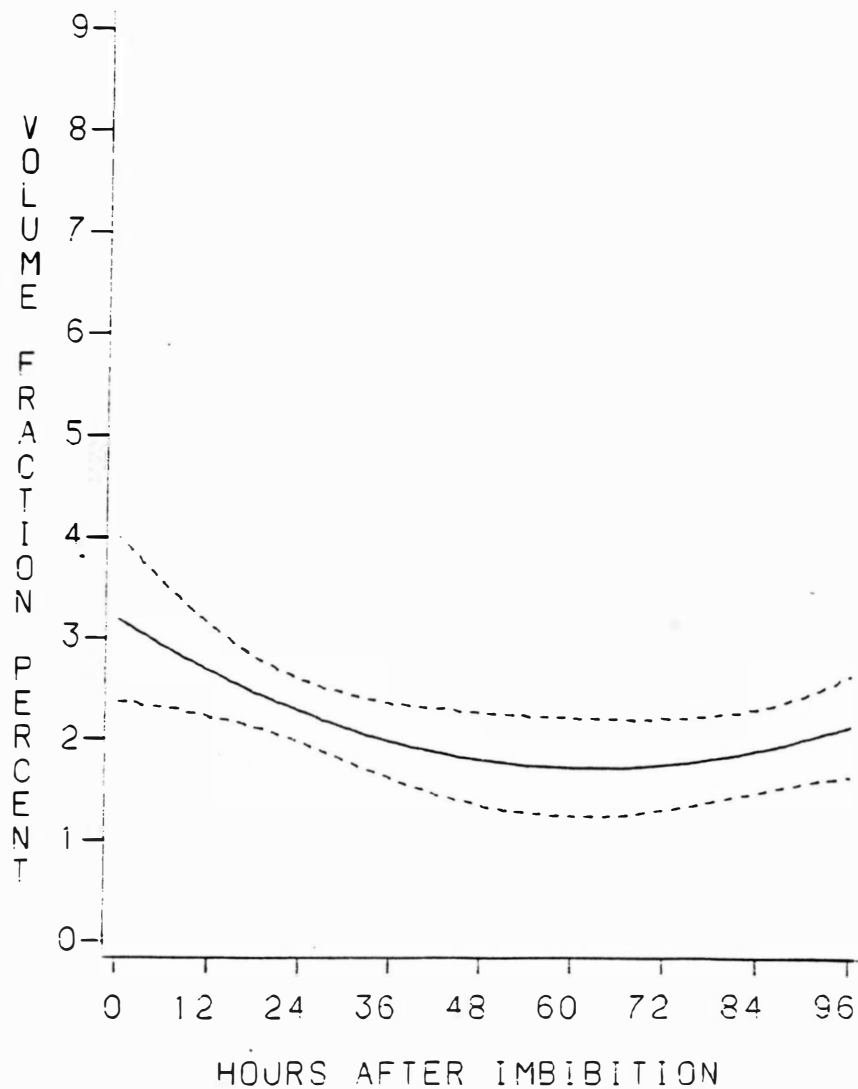


Figure 9. Mitochondria: Changes in volumetric density, V_V^M of mitochondria, in percent, with hours after initial water imbibition.

$$R^2 = 0.06, \text{ S.D.} = 1.27, Y_{V_V^M} = 3.19 - 0.05X + 0.0004X^2$$

VACUOLES

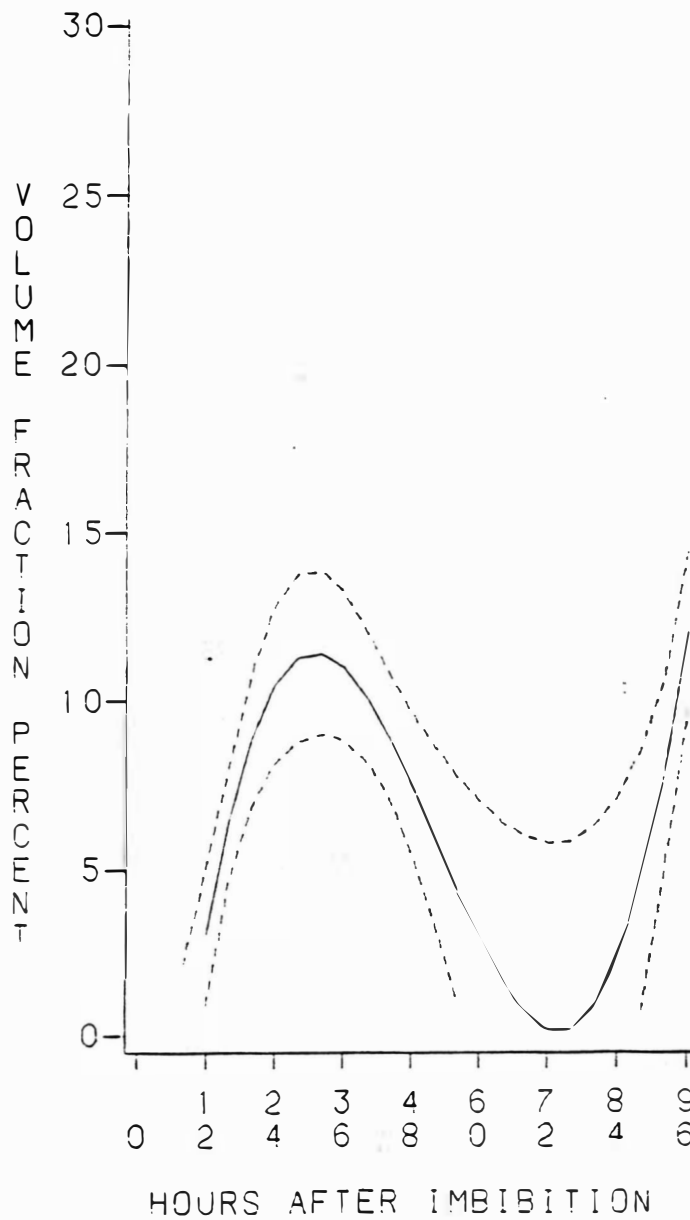


Figure 10. Vacuoles: Changes in volumetric density. V_V of vacuoles, in percent, with hours after initial water imbibition.
 $R^2 = 0.30$, S.D. = 10.03, $Y_{V_V} = 28.19 = 3.54X - 0.08X^2 + 0.006X^3$

CELL WALL

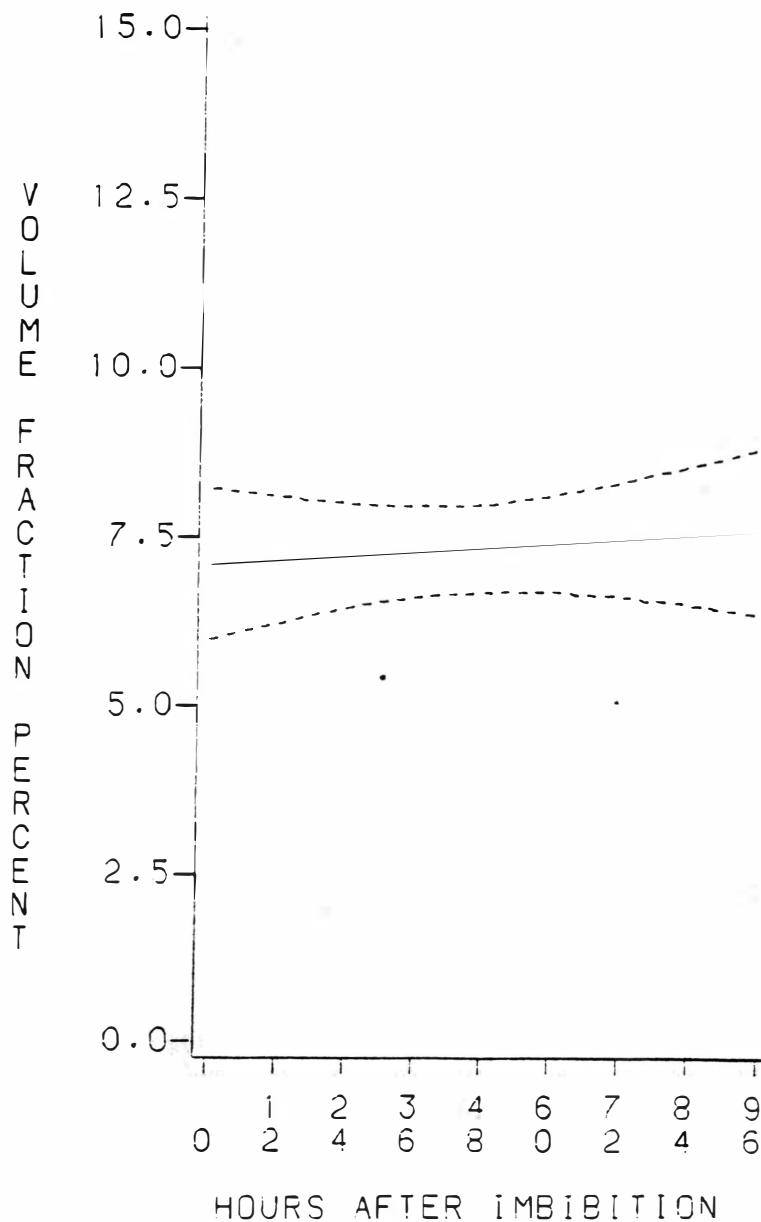


Figure 11. Cell wall: Changes in volumetric density. V_V^C of the cell wall, in percent, with hours after initial water imbibition. $R^2 = 0.30$, S.D. = 7.66, $Y_{V_V^C} = 6.29 + 0.04X$

LIPID BODIES

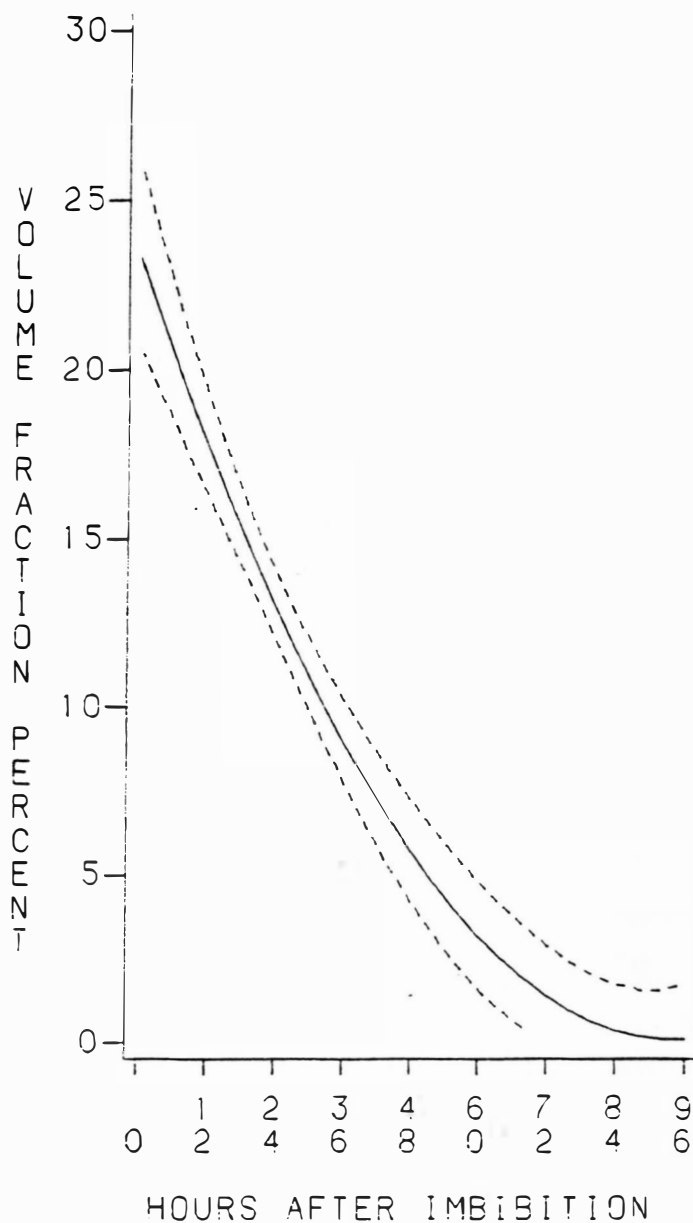


Figure 12. Lipid bodies: Changes in volumetric density, $V_{V, LB}$, in percent, with hours after initial water imbibition.
 $R^2 = 0.66$, S.D. = 5.35, $Y_{V, LB} = 25.06 - 0.53X + 0.003X^2$

STARCH GRAINS

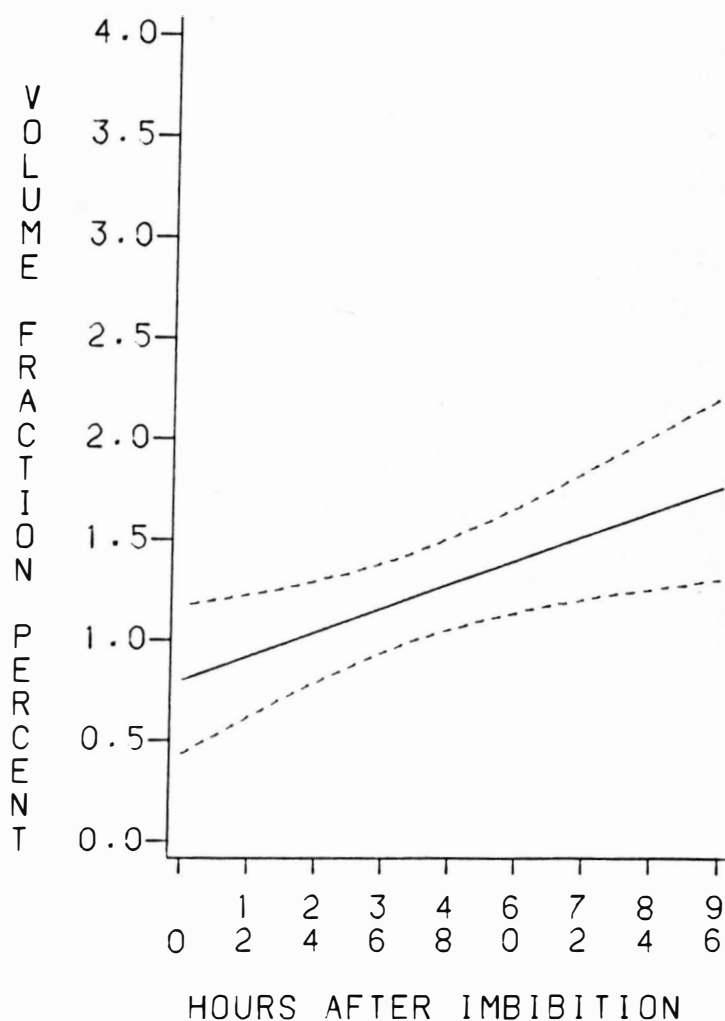


Figure 13. Starch grain: Changes in volumetric density, V_V^{SG} , of starch grains, in percent, with hours after initial water imbibition. $R^2 = 0.17$, S.D. = 1.96, $Y_{V_V^{SG}} = 0.72 + 0.03X$

NUCLEUS

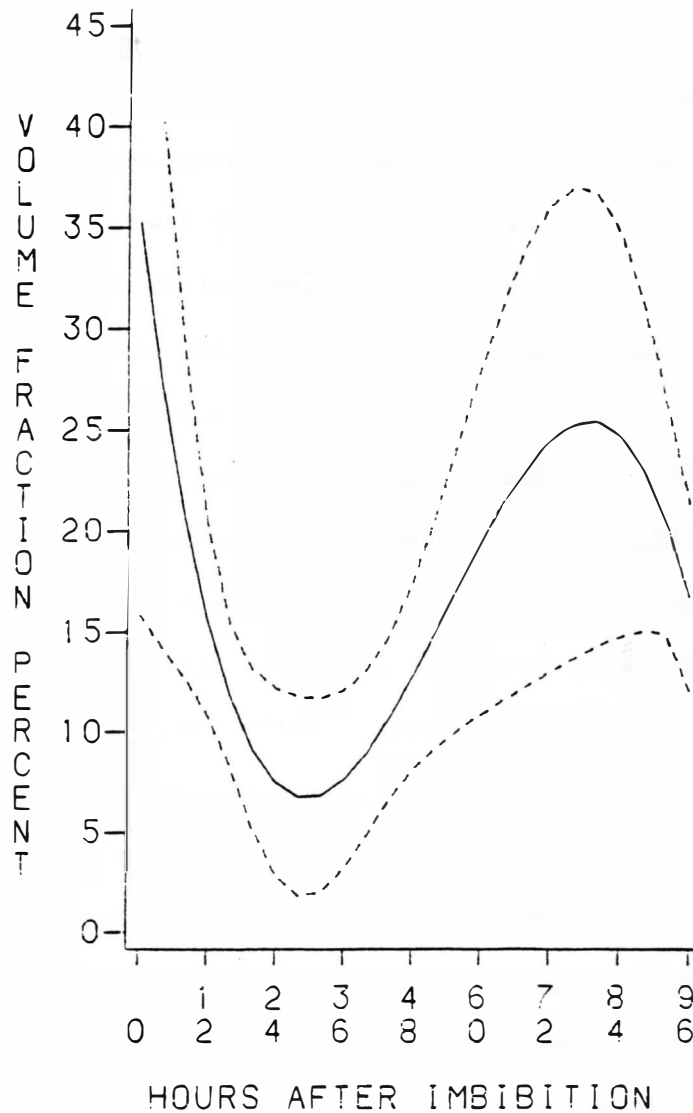


Figure 14. Nucleus: Changes in volumetric density, V_V^N , of nucleus, in percent, with hours after initial water imbibition. $R^2 = 0.09$, S.D. = 12.09, $Y_{V_V^N} = 38.94 - 3.48X + 0.06X^2 - 0.0004X^3$

DISCUSSION

Qualitative

Unlike dormant seeds, the quiescent soybean needed only to be hydrated to resume activity. Laidman (1982) indicated that this was because the necessary enzymes of intermediary metabolism were present. Jann and Amen (1977) went a little further and elaborated on the differences between quiescence and dormancy. They defined quiescence as the suspension of growth imposed by unfavorable environmental conditions and dormancy as the suspension of growth by active endogenous inhibition. They also added that quiescent seeds were readily germinable with no specific trigger agents such as sufficient moisture and favorable temperature. However, dormant seeds were those which would not germinate even under conditions which were normally favorable for growth, and that dormant seeds required a specific environmental stimulus which was not constant but which triggered germination. Several authors, however (Ackerson, 1984a; Ackerson, 1984b; Walton, 1980) have indicated that, in quiescent seeds, hormones such as abscisic acid played a role in germination as triggering agents.

Since no good technique was found to prepare quiescent, unhydrated embryos, the assumption was made in this study that, during the early hours of imbibition (12 hours) the apical meristematic cells from the central zone did not show any ultrastructural changes from their quiescent state in terms of organellar development. There was no sign of mobilization of the most obvious nutrient reserve (lipid bodies). Jann and Amen (1977), in their review, indicated that the

embryo axis cells of lima bean embryos during the early hours of imbibition resembled those of immature developing embryos rather than those actively growing seedlings. Pease (1972) indicated that at this early stage the seed had not resumed full metabolic growth.

In soybean cotyledon parenchyma cells, Webster and Leopold (1977) observed extensive changes in membranes and organelles after 20 minutes of imbibition. But their description of some membrane systems and organellar substructure of the dry seed resembled the 12 hour imbibition stage in the embryo apex in this study. Thus the 12 hour stage was used as a basis or standard for comparison in this study.

The presence of abundant lipid bodies, premature or underdeveloped organelles and sometimes little starch reserves appear to be common among quiescent apical meristematic cells which further supported using the 12 hour stage as a control. The mitochondria and proplastids appeared fully rounded, indicating that they were hydrated but the cristae and prethylakoid bodies were not developed indicating that these organelles were not ready to carry out full scale metabolic reactions. The dictyosomes were flattened and without vesicles and the E.R. were dispersed in small segments, and in most cases both were absent indicating that cells were highly inactive at the early stage. Also the proplastids and mitochondria were extremely underdeveloped so that the distinction between them was difficult. Frey-Wyssling et al. (1963) also encountered this difficulty. Cragg (1980) indicated that the ultrastructural components of quiescent bud of Tilia europaea contained plentiful lipid bodies, smaller quantities of other storage

material (protein, starch) inactive dictyosomes and unusual arrangements of E.R. Cragg added that the presence of many seemingly inactive dictyosomes and the absence of large central vacuoles tended to appear consistently in cryptobiotic (quiescent) apical meristems. Similar findings were recorded by Kidwai and Robards (1969) in Fagus sylvatica. Robards and Kidwai (1969) in willow, Cecich (1977) in Jackpine and Harrison (1977) in Orchid. In the soybean, central zone cells of the shoot apex showed all characteristics of quiescence at the 12 hr stage except they were hydrated.

The major activity taking place at the early hours of water imbibition (up to 12 hrs), is the organization of the membrane systems from the disorganized at the dessicated state to organized at the hydrated state (Buttrose, 1973; Chabot, 1982; Webster and Leopold, 1977; Seewaldt, 1981). As Webster and Leopold (1977) stated, the architecture of the membrane had just changed from relatively porous "hexagonal" state to the more stable "lamellar" configuration.

Vacuoles were growing in size through coalescence and expansion. At 12 hours they were relatively small, but numerous and were surrounded by a single membrane tonoplast (Figure 1A), at a later stage (24 hrs) vacuoles started coalescing (Figure 4A) and continued coalescing at 48 hours (Figure 5B). By 96 hours they were full grown into large central vacuoles (Figures 2B and 6B). This observation was reported in active apical meristematic cells by several authors (Healey et al., 1965; Davis et al., 1979 and Klein and Ben-Shaul, 1966). Large central vacuoles seem to be characteristic of either mature or active

plant cells indicating that the soybean embryo had fully resumed metabolic activities by 96 hours, which was relatively inactive at earlier stages (12, 24 and 48 hrs.).

The site of the vacuoles origin was, however, not determined by this study. At the earliest stage of development examined (12 hrs), the vacuoles were not seen in close association with dictyosomes (vesicles) or E.R. (cisternae), which is the available current evidence on the site of their origin. It still, however, remains disputed as to whether vacuoles originated from vesicles or from the cisternae of the E.R. Gay et al. (1971) indicated that vacuoles originated from dense body vesicles, by stating that the oogonia of Saprolegnia furcata contained distinctive granules in the dense body vesicles and in the central vacuole which formed and expanded during cleavage. Dainty (1968) in agreement with the findings of Gay et al. (1971) also indicated that vacuoles originated by being budded off, as vesicles, from the Golgi complex and subsequently growing and coalescing to produce the final large central vacuole. Buvat (1963, cf. Dainty, 1968) presented a similar origin of vacuoles from endoplasmic reticulum. This study however, conforms to Davis's (et al. 1979) observation, for they indicated that there was no evidence in their study suggesting that the vacuoles had originated from cisternae of E.R. or vesicles of dictyosomes. In this study, the tonoplast was rarely observed in continuity with either the smooth or rough E.R. which could suggest that the vacuoles may have originated from there.

Interestingly, the increase in average size of the vacuoles was paralleled with the decrease in their amorphous granular content. As seen in Figures 3A and 3B, the vacuoles were totally filled with amorphous granular substance. By 24 hours (Figure 4A), the concentration had decreased and by 48 and 96 hours it had essentially disappeared (Figures 5B and 6B). Klein and Ben-Shaul (1966), in the lima bean root and hypocotyl at the beginning of imbibition also found amorphous substances in the vacuoles. By 24 hours the vacuolar content had disappeared. They suggested that the contents may be phenolic compounds or proteins. As amorphous granular material, membranous storage materials appeared in some vacuoles within 24-48 hours (Figures 6A and 7B). These observations suggest roles that vacuoles play during germination; storage, secretory and excretory.

No storage protein in the form of protein bodies was found in the apical meristematic cells. This was rather surprising, because soybean has a high protein content compared to the low protein content of pea pods (Madison et al., 1981). Unless of course all the storage proteins were restricted to the cotyledons, which is most probable in this case. Adams et al. (1982) studied the biochemical nature of proteins in soybeans during maturation. They harvested the seeds at various stages of development and allowed them to dry in intact pods. They noted that, after undergoing maturation, the seeds showed an accumulation of buffer-soluble polypeptides in the molecular weight range of 43-94 KD. Therefore he thought maturation may be associated with the synthesis of specific polypeptides having a molecular weight of

about 85 KD. Interestingly, however, alkali-soluble proteins, which represented the storage proteins, did not show any responses to maturation. With these results and those of earlier studies done by Adams and Rinnie (1981 cf. Adams et al., 1982) they concluded that there was lack of involvement of the alkali-soluble proteins in maturation and germination. In addition Adams et al. (1982) showed that soybean seeds retained considerable soluble proteins and soluble sugars throughout maturation. They also indicated that the soluble sugars were converted from the final reserve carbohydrates of the seed during maturation. Biochemical evidence in the highly complex molecular content of the soybean seed could possibly be correlated with the ultrastructural observations in this study. It's most probable that the soluble proteins and sugars are stored in the vacuoles after maturation, and even before the lipid bodies are degraded these soluble proteins and sugars are used first as energy sources which are readily and easily available. This would thus explain their fast disappearance (within 12 and 24 hours). Note, the amorphous granular material started disappearing before the mobilization of lipid bodies (within 24-48 hours).

At the later stage of development (24 hours), the cristae were somewhat developed and easier to identify. Now, as metabolic activities and rates of cellular respiration increase dramatically, like they do during germination, one would naturally expect the average volume of the mitochondria to also increase significantly, because the organelle is directly related to cellular respiration. To the contrary, no such evidence was found. Instead, with subsequent development of the

embryo, the cristae appeared more and more developed, but the relative size remained almost constant, thus indicating a direct correlation between the cristae and cellular respiration rather than the average volume of the mitochondria. Frey-Wyssling et al. (1965), in agreement with this evidence, stated that in view of the mitochondrial inner structure, a small number of large mitochondria could be as effective as a large number of small mitochondria. Gunning and Steer (1975) added their support claiming that the increase/decrease in volume fraction of mitochondria did not necessarily parallel with an increase/decrease in the rate of respiration. Rather, the development of cristae determined the rate of respiration. Briarty (1980b), also arrived at the same conclusion, stating that changes in mitochondrial volume did not parallel the changes in respiration rate.

As shown in Figure 5A, the inner membrane of the plastids were developing, which indicated that they were maturing (48 hours). Some took irregular shapes, which reflected the fluctuations observed in average size of plastids. Some appeared that they were dividing by budding. Briarty (1980b) had claimed that the average volume of plastids varied considerably during development according to cell division. Frey-Wyssling et al. (1965) also indicated that the plastids increased in number by division, but then only after differentiation of these organelles had begun and concluded. This may indicate that the plastids observed at 48 hours were fully mature and had started dividing.

The oils found in the cytoplasm as droplets have been called

variously, oil or lipid bodies, oil or lipid droplets, oil or lipid vesicles, oleosomes and spherosomes (Yatsu, 1971; Fry-Wyssling et al., 1963; Laidman, 1982; Cragg, 1980; Kidwai and Robards, 1969; Robards and Kidwai, 1969; Kupila-Ahvenniemi, 1978; Schwarzenbach, 1971 Sorokin, 1967; Sorokin and Sorokin, 1966; Yatsu and Jack, 1972; Rest and Vaughn, 1972; Wanner and Theimer, 1978; Bergfeld et al., 1978; Smith, 1974; Bauer et al., 1975; Galatis et al., 1978; Harwood et al., 1971).

Still the lipid bodies remain an enigma. Their mixed nomenclature reflects the uncertainty concerning their cellular origins and structure. There has been much dispute as to whether the lipid bodies are surrounded by a phospholipid bilayer membrane or by a monolayer half-membrane, or indeed whether they have a limiting membrane at all. In this study, the lipid bodies were closely examined at magnification ($\sim 80,000$) so as to clarify their nature. As shown in Figure 7A, none of the lipid bodies were seen surrounded by either a phospholipid bilayer membrane or a monolayer half-membrane. Rather, there was an interfacial membrane separating the lipid body from the hyaloplasm. Cragg (1980) and Bergfeld et al. (1978) also made the observation, that the lipid bodies (which they called oil droplets) were not bounded by a membrane. However, there were several other authors who had contradictory reports (Kidwai, 1969; Kupila-Ahvenniemi, 1978; Yatsu et al., 1972; Fry-Wyssling et al., 1963; Pihakaski, 1966, 1968 and 1972), all reported either a phospholipid bilayer or monolayer surrounding the lipid bodies. However, the three components, spherosomes (surrounded by phospholipid bilayer), oleosome (surrounded by monolayer

half-membrane) and lipid bodies (surrounded by interfacial membrane), may indeed be separate entities, all with different sites of origin, function and structure (Smith, 1974).

Mollenhauer et al. (1971a) decided to call the lipid bodies lipid vesicles because the interfacial structure appeared relatively dense to them which led them to think that it was a bounding membrane. However, they found no evidence that would support the origin of the lipids from E.R. In contradiction with Mollenhauer et al., the interfacial membrane (as seen in Figure 7A), did not appear dense enough to be called a surrounding membrane. Therefore, the lipid bodies retained their name rather than lipid vesicles. However, in agreement with Mollenhauer et al.'s findings, in this study, no pools of reserve lipids appeared inside the E.R., and none were continuous with, or appeared to be budding from the E.R. Therefore, it seemed more reasonable to assume that lipid synthesis occurred external to, or on the surface of, the E.R. or even in spherosomes as suggested by Fry-Wyssling et al. (1963). Also, in contradiction with Mollenhauer et al. (1971b) and Allen et al. (1971), only one kind of lipid body was observed. These lipid bodies ranged widely in diameter and they associated strongly among themselves. In fact, there was some coalescence witnessed (Figures 7A, 5B, 4A 4B, 3A, 3B, 2A, 1A and 1B). This observation was in agreement with Sorokin (1967) where the lipid bodies observed also had a wide range of diameters and easily coalesced into larger aggregates.

The significance of lipid bodies in energy metabolism during

germination was obviated by their disappearance during early germination. Apparently, it is a common pattern of quiescent seeds, with lipids as the major reserve. The apical meristematic cells of soybeans examined in this study certainly followed this pattern. The lipid bodies were abundant at the early hours, then gradually decreased. This was paralleled with an increase in starch grains in plastids. Ching (1963) also found a similar pattern in the Douglas fir seed. The major food reserve was fats, and he observed an increase in carbohydrates accompanied by a rapid decrease of glycerides during the germination of the seed. As for the great contribution of fats for high energy storage and metabolism, the triacylglycerol molecules represented substantial energy store which could be readily utilized following relief of drought stress. As the molecules made little demand on the cell water for their storage, the water that was present could be used for the maintenance of hydration and protein stability (Douglas and Paleg, 1982 cf. Murphy et al., 1984). Berrie et al. (1982), in addition, indicated that fatty acids were highly efficient energy stores, yielding twice as much on oxidation as either carbohydrates or proteins and thus they concluded that lipid droplets represented the cell's major energy investment.

In addition to being degraded during germination, the lipid bodies moved within the cell. This mobilization of lipid bodies was also reported by Sorokin (1958). At 12 and 24 hours they were predominantly located very close to the plasmalemma, and by 48 hours they were found randomly scattered in the hyaloplasm. The mobilization in soybean

apical meristematic cells apparently started within 24 to 48 hours. Laidman (1982) reviewed several studies that showed lipid mobilization during germination in seedlings of several species. The mobilization observed in barley coincided with that of soybean which was also within 24-48 hours of commencement of imbibition. This may reflect barley and soybean germination at about the same rate, or that the embryo develops at about the same rate during germination. However, Laidman found some evidence that the scutellum of maize showed mobilization in the first day of germination. And in wheat, he found that the embryo axis mobilized hybrids after about 12 hours following imbibition.

Several biochemical studies on lipids have been carried out, reporting the actual biochemical constituents of the lipid bodies and the metabolic pathways involved during the decomposition of the nutrient reserves during germination. Murphy et al. (1984) claimed that the lipid bodies (which he called oleosomes) consisted mainly of triacylglycerols (50-60% by weight) and esters (15-40% by weight). He also defined the oleosomes as lipid-rich spherical bodies which were composed of triacylglycerol and sterol or wax esters which could function as energy-rich and osmotically-inert storage material.

Ohlrogge et al. (1984) claimed that most of the lipid in soybean seeds was stored in cotyledons in the form of triglyceride packages in specialized structures, sometimes called oil bodies or spherosomes. Similar findings were reported by several authors (Galliard et al., 1975; Privett et al., 1973; Bewley et al., 1978; Gurr et al., 1972; Hsu et al., 1973; Vigil, 1969; Yazdi, 1977; Bimpong, 1975). The biosynthe-

sis of the lipids during seed maturation has also been closely studied (Ohlrogge et al., 1984; Porra et al., 1976). During seed development Porra et al. (1976) found that cotyledons showed an increase in total endogenous lipid, and changes in the composition of the fatty acid component as the soybean seed matured. Ohlrogge et al. (1984) also discovered that during soybean seed development, the rate of lipid biosynthesis per seed increased greatly. However, as the seed reached maturity, they found that lipid synthesis declined. In addition they discovered that the control of lipid biosynthesis was an acyl protein carrier (biosynthetic proteins). Yazdi (1977) also observed an increase in oil production during seed development in soybean seeds. Bimpong (1975) suggested the possibility of the involvement of the glyoxylate cycle in conversion of fats to carbohydrates. The zoospore and germinating cysts extracts in his study exhibited isocitrate lyase activity, but only trace amounts of malate synthase were detected. Bimpong, in his biochemical assay, measured the lipid bodies as acyl glycerides and free fatty acids. Adams et al. (1980) claimed that germinating soybean seeds accumulated starch reserves, probably as a result of gluconeogenesis from storage lipid. They found that developing seeds showed a rapid increase in β -amylase activity which continued into early germination. However, they also found that the distribution of β -amylase activity was not consistent with its supposed role in starch degradation. In addition, soybean seeds were also found to contain δ -amylase and δ -glucosidase activities which could be responsible for starch mobilization. McAlister (1951), Wai (1947),

Rubel (1972), Hsu (1973) also found that soybean seeds contained large amounts of oil and somewhat lesser amounts of soluble carbohydrates and indicated that during germination, protein and oil were major sources of energy for the developing embryo.

Although microbodies were not identified in this study, they apparently seem to play a major role in the catabolism of lipid bodies (Berrie et al., 1972). Norikoff et al. (1968) identified microbodies both in light and electron microscopy and Choinski et al. (1977) described them as subcellular respiratory organelles. They claimed that the microbodies enclosed a finely granulated matrix and in general contained enzymes associated with oxidative degradation. Choinski et al. also observed that microbodies were in close association with mitochondria lipids and/or segments of rough E.R. Galatis et al. (1976) seemed to have found a new function of microbodies which they saw in close association with cytoplasmic tubules but they also indicated that this new function was yet unknown. Vigil (1970) in agreement with Berrie et al. (1982) claimed that seedling development during germination paralleled the disappearance of lipid bodies which also coincided with an increase in microbodies (glyoxysomes), mitochondria and plastids with an elaborate network. Schnarrenberger et al. (1971) further confirmed this by stating that glyoxysomes (microbodies) were present in germinating tissues which were converting storage fat to sugars and they also stated that these particles contained α -oxidation system acids and glyoxylate. Berrie et al. (1982) also claimed that there was good reason to believe both microbo-

dies and mitochondria were directly involved in a neutral lipid catabolism.

Despite all these studies showing close associations between lipid bodies, mitochondria, plastids, and microbodies, no indication was found in this study supporting this evidence. The lipid bodies, however, showed close association with vacuoles (Figures 3A and 3B), especially at the early hours of imbibition. Not only were the lipid bodies surrounding the tonoplast but also the plasmalemma at this early stage. The possibility of the correlation between these obvious associations of lipid bodies with the tonoplast and plasmalemma cannot be overlooked. By 24 hours the lipid bodies were still in the vicinity of both the tonoplast and the plasmalemma. The function of the lipids at that early stage may be a barrier (Cragg, 1980; Robards and Kidwai, 1969) restricting intracellular (vacuole to cytoplasm via tonoplast) and intercellular (cell to cell via plasmodesmata) movement. This restriction then apparently was eliminated as the lipid bodies moved away from the plasmalemma, as they were being degraded. Of course by that time the cells were more active and transport within and between the cells was most efficient. Sorokin (1955) also observed more lipid bodies around the vacuoles rather than the mitochondria or the plastid. In this study, as shown in Figures 5B and 2A, once in a while lipid bodies would be seen in close association with plastids and even less frequently with mitochondria.

Several authors have claimed that during germination there was a turnover of proteins and amino acids, with the balance between synthe-

tic and degradative processes determining the resultant pattern (Chen et al., 1978; Konopska, 1983). Klein (1955, cf. Kylen et al., 1975) presented evidence of protein synthesis, and he reported a rise in the amino acid content of lettuce seeds during germination. Young and Verner (1959 cf. Vanderstoep, 1981) reported net synthesis of enzymic protein during germination and they indicated that was possibly accounting for a major portion of the reported protein increase. In peas, during the period of rapid axis growth (3-8 days following germination), the nitrogen content of the cotyledons declined rapidly with an accompanying increase in the nitrogen in the developing axis, which was probably a result of the translocation of the products of proteolysis (Beevers and Guernsey, 1966 cf. Vanderstoep, 1981). Fordham et al. (1975) reported that on a dry weight basis, germinated seeds contained approximately 100% of the original seed protein, whereas sprouts alone contained about 210%. The results obtained from the ultrastructural analysis in this study are certainly consistent with the available evidence. The vacuolar contents, amorphous granular material, coincided with Klein and Ben-Shaul's (1966) amorphous substance, containing phenolic or protein compounds were visible at 12 hours after initial water imbibition. The soluble proteins which were synthesized during maturation (Adams et al., 1982) were probably stored in the vacuoles, which appeared somewhat granular in the electron micrographs (Figures 3A and 3B). Before the lipid bodies had mobilized (within 24 to 48 hours) the vacuoles started emptying their constituents (within 12 to 24 hours), the proteins were possibly being

degraded for energy metabolism. Then active protein synthesis probably started as soon as the E.R. was well developed and active (within 48 to 96 hours), hence, the turnover of proteins and amino acids). Wai et al. (1947) determined 54 hours of germination to be the optimum period for soybeans to contain quality proteins and vitamins. This could explain the mobilizing of amorphous contents out of vacuoles (protein) and its increase in RER for protein synthesis.

Over the years not only have soybeans attracted agronomists for their high economic value of oil but they have also been of great interest to nutritionists for their high nutritive value of proteins. Special attention has also been turned to the mobilization or synthesis of the nutrients during germination. Several reports concerning the nutritive value of germinating soybeans have been found, showing the effects on several vitamins and amino acids (Johnston et al., 1977; Bates et al., 1977; Chen et al., 1978; Fordham et al., 1975; Ferrel, 1978; Kongeska, 1983; Kylen et al., 1975; Vanderstoep, 1981; Wai et al., 1947; Wu et al., 1953). The authors through their studies, have shown along the way that the ancient practice of sprouting mung beans and soybeans was a means of greatly improving the nutritional worth of the seeds. Chen et al. (1975) showed that germination caused an increase in a number of vitamins. Ascorbic acid content of soybeans, mung beans and peas increased from 4-20 fold. Wai et al. (1947) also reported the increase took place almost linearly over the first 54 hours. Studies of Kylen et al. (1975) and Chen et al. (1975) showed an increase of 2.5-4.5 times the Riboflavin in a variety of seeds as a

result of germination. However, Chen et al. (1975) pointed out that thiamine concentration remained practically unaltered (Kylén et al., 1975), while niacin increased during germination. The majority of the studies, in addition to increase in certain proteins and vitamins, also showed a decrease in fats or triglyceride contents of the embryo during germination (Bates et al., 1977; Kylén et al., 1975).

The nuclei found in the central zone cells occupied a major fraction of the cell. And at the four subsequent stages during germination, there was no relative change in average size. It appeared more granular than the surrounding hyaloplasm, but there was no apparent change with time. Pihakaski (1966) had also made similar observations in the meristematic cell of the shoot apex of Bazzania trilobata.

The cell wall also did not show major changes during the developmental stages during germination. Characteristic of the meristematic cells, it was relatively narrow in width while many cells appeared in early interphase. The 12-hour phase reflected the nature of the apex during quiescence while later stages reflected active division.

Arias et al. (1983) used simple stereological methods, described in Loud (1962), of linear integration and line intersect counting, to determine the numbers of organelles and surface area of various cytomembranes per unit volume of cytoplasm. They determined the membrane profile concentration (mean micrometers squared of membrane surface per micrometers cubed of cytoplasm) for plasma membrane, endoplasmic reticulum (ER), tonoplast and mitochondrial outer membrane. They used ana-

lysis of variance and separation of means by Duncan's multiple-range test for determination of differences at the 5% level of significance. They found that in seedlings susceptible to the pathogen, brief (0.25 hr) treatment with the toxin resulted in a marked and permanent decrease in the amounts of secretory vesicle membranes. In 2 hour treatments, they found that only secretory vesicle membrane was decreased. However, they also discovered that longer treatments led to an increased amount of endoplasmic reticulum (4 hr), which later decreased together with the amount of dictyosome membrane. They also noted that the amount of tonoplast increased (8 hr). In resisting seedlings treated with toxin, on the other hand, they observed that there were early but transient increases in the quantities of plasma membrane, secretory vesicle membrane, dictyosome membrane, endoplasmic reticulum and in the number of dictyosomes. Finally Arias et al. concluded by stating that insensitivity to the toxin may, indeed, involve the ability of resistant genotypes to recover from the toxin effect.

Quantitative

The morphometric information, a three-dimensional measurement expressed in volume fraction percent, obtained from the quantitative analysis were statistically tested. These tests were carried out in an effort to correlate the ultrastructural measurements (obtained by using stereological methods) with the qualitative analysis and functional changes, based on the biochemical studies during the morphogenetic shift from quiescence to germination, which have been discussed in

detail in the previous sections. The quantitative study provided a more precise analysis than the qualitative, even though the two analyses were complementary to one another. Qualitative analysis became more valid and sound when supported by a statistically based quantitative analysis. Several stereologists have recently shown great interest in quantitative ultrastructural comparisons using statistics (Mauseth, 1980, 1981, 1982a, 1982b; Moore et al., 1983; Miklas et al., 1981; Orr, 1981; Reynolds, 1984). Nicholson (1978) has indeed been proven right for predicting that statistics would be playing a greater role in stereology.

In this study, the analysis of variance F-test (in Table 2), showed a highly significant difference in the mean organellar volume fraction percent among the seven organelles examined; plastids, mitochondria, vacuoles, cell wall, lipid bodies, starch grains and nuclei. According to the Waller-Duncan K-ratio T-test for organellar volume fraction percent by organelle (in Table 3), the mean volume fraction percent of the organelles with the same letter (under the grouping) were not significantly different. For instance, the mean volume fraction of mitochondria and starch were not significantly different from each other while, with the rest of the organelles, they showed significant difference. The nucleus as shown in Table 3, was the major component of the cell (46%), which is a common characteristic of meristematic cells (Pihakaski, 1966). Next to the nucleus, the vacuole occupied a major volume (averaging volumes of all development times), 11.36%. The minor components were mitochondria and starch

grains with 2.23% and 1.95% respectively. Lipid bodies could also be called a second major component (9.74%) next to the nucleus. There was no significant difference between vacuoles and lipid bodies (see Table 3). These quantitative results support the qualitative description of the cells described already in previous sections, even though the measurements were averaged over the four development times — 12, 24, 48 and 96 hours after initial water imbibition (Cragg, 1980; Booker et al., 1972; Cardemil et al., 1982; Durzan et al., 1971; Horner et al., 1966).

According to the F-test (0.05) there was a significant difference in the mean organellar volume fraction percent with reference to time. This indicated that there were fluctuations in the mean organellar volume fraction percent at the four stages of development examined, considering the volume fraction percent of all organelles. According to the Waller-Duncan K-ratio T-test for organellar volume fraction percent by development time specifically, there was significant difference between the two stages of development, 48 and 96 hours. The rest of the hours tested did not have significant differences. According to this test, the mean volume fraction percent of all organelles was 7.61% at 12 hours and 8.30% at 24 hours. There was a slight increase but was statistically insignificant. There was a significant decrease at 48 hours (6.38%), followed by another minor increase (8.47%) at 96 hours. The results indicated that the fluctuation in the mean organellar fraction percent was cyclic rather than linear as there was an increase between 12 and 24 hours, significant decrease at 48

hours and followed by another increase at 96 hours. We had no way of knowing what exactly happened between 48 and 96 hours for no data was taken during the period from 48-96 hours. However, a prediction was made using a regression analysis with the available data which will be discussed later. In addition, the F-test showed highly significant interactions between organelles and times of development. Thus, there was at least one of the organelles examined that showed great fluctuations during the water imbibition for 12, 24, 48 and 96 hours. From there a regression analysis was carried out to specifically show the fluctuation of each organellar volume fraction percent with time, which will be discussed later.

Table 1 shows the average volume fraction percent of the organelles with \pm S.D. and coefficient of variation in parenthesis. There was a significant amount of variation within the samples collected, a total of 25 subsamples from 5 samples for all the organelles. This may have been because the area of the field selected, $85.595 \mu\text{m}^2$, was not large enough. For areal analysis, the precision would increase with an increase in field area. Steer (1981) indicated 5 samples were adequate representation for estimating a population under study. It was certainly not due to zonation, because all the cells were selected from the central mother zone which showed common ultrastructural characteristics (Mauseth, 1980, 1981). The seeds were all germinated under the same laboratory conditions, and throughout the study the same variety, Corsoy, was used. Therefore, the most probable cause of variation was the fact that the total field area was not large enough. The chances of including all the organelles being studied within the

field at one time was low. It was especially a problem with the major components; nucleus, vacuoles and cell wall, and with lipid bodies (at 96 hours) which were few in number and aggregated. The following components showed the greatest variation; starch grains with the highest coefficient of variation, 119.35% at 12 hours; nucleus, 93.83% at 24 hours; lipid bodies, 231.10% at 96 hours; cell wall, 136.77% at 96 hours; and vacuoles 80.65% at 12 hours. Plastids and mitochondria showed relatively low variation which was to be expected because they were the minor components and were also randomly scattered throughout the cytoplasm. The chances of including representative samples of the two components within every field was relatively high.

However, the data still fully supported the observations recorded in the qualitative analysis of this study and other authors (Cragg, 1980; Cecich, 1977; Harrison, 1977; Kidwai and Robards, 1969; Robards and Kidwai, 1969). Indeed, the common pattern of quiescent cells, with abundant lipid bodies was in full agreement with these results. Lipids were found to be major components with mean volume fraction percent, 18.70% at 12 hours. With embryo development time, the average volume fraction percent decreased from 18.70% at 12 hours, to 14.51% at 24 hours, 5.67% at 48 hours and finally 0.10% at 96 hours. The decline of the lipids was statistically significant. Lipids were major energy stores and that they were decomposed due to the high energy demands during the active stage of germination. These data are in full accord with the qualitative findings and the biochemical results discussed in the previous section. Also in accordance with

earlier findings, there was very slight amount of starch grain at the early stage (0.58%). The volume fraction percent of starch grains in mature soybeans (Am Soy 71) was relatively higher than in quiescent seeds. Wilson (1978) showed that at the mature stage soybeans contained $0.80 \pm 0.03\%$ of starch grains. The immature soybeans, however, contained more starch. Yazdi et al. (1977) reported that immature soybean seeds contained 4-5% starch and that the starch decreased to almost zero at maturity. As germination began, a significant increase in starch grains was observed, from 0.58% at 12 hours to 1.85% at 24 hours, 2.15% at 48 hours and 3.23% at 96 hours. This was a strong indication that starch was actively being synthesized, most probably the lipids, a high energy organic compound, was being broken down to a lower energy organic storage material which was easily accessible to the cell.

As mentioned in the previous section and in Frey-Wyssling et al. (1963), the distinction between proplastids and mitochondria was almost impossible. This may probably explain the fluctuations seen in the volume fraction percent of proplastids (5.59%) and mitochondria (2.64%) at 12 hours, compared to those recorded at 24 hours, proplastids (7.68%) and mitochondria (2.34%). At the later stages of development, 24-96 hours, the cristae were somewhat developed so mitochondria were easier to identify.

The average volume fraction percent of the vacuoles seen after 24 hours (15.60%) was five times greater than the vacuoles observed after 12 hours (3.05%). This indicated that the vacuoles were actively

coalescing and growing in size between 12 and 24 hours (Klein and Ben-Shaul, 1966). Even though further coalescence was still observed in the micrographs at 48 hours (see Figure 5B), the average volume fraction percent of the vacuoles significantly decreased to 7.53% and increased significantly to 19.26%. This once again suggested a cyclic type of activity rather than linear. There has not been adequate cytological or biochemical evidence to explain the morphometric phenomenon. However, in scanning micrographs taken at 4 hour intervals of germinating soybean apex, that leaf initiation took place at about 44-50 hours which would coincide with the significant decrease in vacuolar volume fraction. This suggests that there could be some relation between the two.

Excluding 12-24 hours, the volume fraction percent of proplastids did not fluctuate significantly at the later stages of germination; at 24 hours (7.68%), at 48 hours (6.87%); at 96 hours (7.31%). Similarly the average volume fraction percent of mitochondria, except for the slight fluctuation between 12 to 24 hours, did not fluctuate significantly during the development periods studied; 24 hours (2.34%), at 48 hours (1.77%) and at 96 hours (2.17%). As Gunning and Steer (1975), Briarty (1980b), Frey-Wyssling et al. (1965) and the previous qualitative discussion indicated, there was no direct correlation between the volume fraction of these two organelles and their rate of metabolism. Instead, the internal membrane system determined the functional state of the organelles. The quantitative results further confirmed the available evidence, which directly related the

function of the organelles with the development of inner membranes other than overall volume fraction percent.

The nucleus found in the central zone occupied a major fraction of the cell throughout the stages of development examined; at 12 hours, 16.80%; at 24 hours, 7.49%; at 48 hours, 12.75% and at 96 hours, 16.79%. According to these results the nucleus did not show significant fluctuation in the volume fraction percent except at 24 hours, where there was a significant decrease. However, the observation further confirmed the quantitative report in the previous discussion and it coincided with Pihakaski (1966) where he stated that the nucleus was the dominating organelle in the meristematic cell of the shoot apex of Bazzania trilobata.

The volume fraction percent of the cell wall showed a relatively minor increase even though it was not statistically significant. The volume fraction percent at 12 hours was 5.94%; at 24 hours, 8.65%; at 48 hours, 7.94% and at 96 hours, 10.45%. The results indicated that the meristematic cells were undergoing continuous cell division, therefore the cell wall did not add new volume.

In order to determine the specific relations between the organelles and the development time, three terms of regression analysis, linear, quadratic, and cubic were carried out for every organelle. The regression lines were expressed in organellar volume fraction percent with reference to time (hours after initial water imbibition). The goodness of fit was based on the highest R^2 values of the model, and randomness of the data distribution on the residual plot.

The plastid's best fit model was a cubic polynomial:

$Y_{V_V}^P = 0.78 + 0.54x - 0.01x^2 + 0.00008x^3$, where $R^2 = 0.04$, and S.D. = 3.96 (Figure 8). The sharp increase which showed in the graph (Figure 8) was probably due to the problem encountered at 12 hours in distinguishing the plastids from mitochondria. Note, in Figure 9, the volume fraction percent at 12 hours was relatively high and then decreased at 24 hours. At 12 hours, some of the proplastids may have been mistakenly recorded as mitochondria. Apart from that, the plastids did not show significant fluctuation. However, the estimated volume fraction percent of the plastids seemed to be relatively low between 60-84 hours. But no data was actually collected at those hours. At 96 hours there was another increase in plastid volume fraction percent. As explained in the previous discussion and by several other authors (Briarty, 1980; Prisham, 1968), the volume fraction percent of plastids did not reflect the functional state of the organelle. Rather the development of the thylakoid bodies predicted the increase/decrease in their activity.

The mitochondria best fit was a polynomial, quadratic model (Figure 9): $Y_{V_V}^m = 3.19 - 0.05x + 0.0004x^2$, where $R^2 = 0.06$ and S.D. 1.27. The mitochondria showed almost no fluctuation throughout except between 12 and 24 hours which, as already mentioned was due to the difficulty in distinguishing between proplastids and mitochondria. Similarly, the volume fraction percent of the mitochondria did not parallel its functional role, which would be active respiratory component of the cell during germination. However, the cristae develop-

ment was found to be the determining factor (Gunning and Steer, 1975; Briarty, 1975; and previous qualitative discussion).

The vacuole's best fit model was a cubic polynomial (Figure 10): $Y_{V_v}^V = 28.19 + 3.54X - 0.08X^2 + 0.006X^3$, with $R^2 = 0.30$ and S.D. = 10.03. The volume fraction percent from the collected data showed significant fluctuations with a sharp increase from the early stage at 12 hours (3.05%) to the later stage at 24 hours (15.60%). Another sharp fluctuation (decrease) was shown at 48 hours (7.53%) followed by an increase (19.26%) at 96 hours. However, the regression line shown in Figure 10 had a peak at 36 hours which declined sharply until 72 hours and was followed by an increase which went up to 96 hours. This was of course the estimated ($Y_{V_v}^V$) volume fraction percent obtained from the polynomial model (quadratic) which best fit the vacuoles and their direct relation with time (hours after initial water imbibition). In the overall, however, the result still supported the fact that the vacuoles did finally increase by 96 hours in volume fraction percent as the embryo underwent development following germination.

As expected the cell wall did not show much fluctuation in the regression line (Figure 11). The best fit model was linear: $Y_{V_v}^C = 6.29 + 0.04X$ where $R^2 = 0.30$ and S.D. = 7.66. This was an insignificant increase in the volume fraction percent of the cell wall during germination.

As shown in Figure 14, the nucleus appeared to have fluctuated significantly, unlike the qualitative observation in the previous section of this study. At 24 hours the mean volume fraction percent

significantly decreased to 7.49% from 16.80% at 12 hours and increased back to 12.75% at 48 hours followed by another increase at 96 hours (16.79%), as recorded in Table 1. However, the estimated regression line showed a low peak between 24-36 hours, followed by a sharp incline afterwards peaking at about 84 hours and declined again up to 96 hours. There has not been adequate cytological evidence supporting the morphometric changes. The best fit model for the nucleus was cubic polynomial: $Y_{V^N} = 38.94 - 3.48X + 0.06X^2 - 0.0004X^3$, where $R^2 = 0.09$ and S.D. = 12.09.

It was interesting to note how well Figure 12 shows the sharp decline of the major reserve (lipid bodies) during water imbibition (0-96 hours). The lipid bodies best fit model was a polynomial, quadratic model: $Y_{V^{SD}} = 0.72 + 0.03X$ where $R^2 = 0.17$ and S.D. = 1.96. Figure 13 displayed the sharp increase in starch grains, paralleling the lipid disappearance. The best fit model for the starch grain was linear: $Y_{V^{SG}} = 0.72 + 0.03X$ where $R^2 = 0.17$ and S.D. = 1.96. These results further confirm the available evidence on the common characteristic of quiescent cells becoming active (undergoing germination), whereby the major nutrient reserve is degraded for energy metabolism and converted to starch which would be another form of energy storage but easily and more readily available than lipids (Laidman, 1982; Bimpong, 1975; Adams et al., 1980; Berrie et al., 1982; Srivastava et al., 1968; Vigil, 1970; Hsu, 1973; Cragg, 1980; qualitative discussion in this study).

CONCLUSION

In summary, the biophysical activity (organization of membranes) in the central zones of the soybean embryo began after the seeds were soaked, 0-12 hours. The metabolic activity was then initiated between 12-24 hours, which continued at an increasing rate from 24-96 hours. This involved a progressive loss of reserve materials (lipid bodies and proteins) and was accompanied by carbohydrate synthesis and protein synthesis. There was an increased clarity in organellar membrane systems during the later hours (24-96 hours), and development of structures either absent or present in the highly modified state in the quiescent embryo. By 96 hours the organelles were highly developed and E.R. and dictyosomes appeared more frequently (Figures 2A, 2B and 7B). The mitochondrial cristae and plastid thylakoids showed considerable development, suggesting an increase in metabolic activities within the organelles (Figures 4B and 7B). The cells developed large central vacuoles, an indication of increased activity and maturation (Figure 6B).

The quantitative analysis recorded in this study mostly supported the qualitative observations, thus making the whole study valid statistically, more precise and detailed quantitatively. The morphometric information was expressed in terms of volume fraction percent for every component examined within the meristematic cell. Statistics were used to further summarize and interpret the data.

With the sophisticated quantitative methods now available for ultrastructural studies, minute changes in the fine structure that

could be detected qualitatively can now easily be used for comparisons with biochemical changes. In the process, close analyses tend to show relationships between the function and structure of the cell components. In the previous years biochemical studies, unlike morphometric analysis, were far more advanced and more common amongst biologists. However, the recent advancement in the morphometric studies will enhance better understanding of cellular activities in terms of biochemical and structural changes, at the organelle level especially, during a morphogenetic shift.

RECOMMENDATIONS

1. A histochemical study to determine the chemical nature of the amorphous material in vacuoles which disappears through early germination.
2. An ultrastructural study of the relationship between lipid bodies and plasmodesmata in terms of a possible barrier to intercellular movements of materials (especially water) during quiescence.
3. A study of mitochondria and plastids to determine possible reasons for the volume fraction percents remaining fairly constant and the internal membrane systems rather incomplete.
4. A linear density morphometric study of the membrane system (ER, mitochondria, plastids, dictyosomes).
5. A dry seed histological and ultrastructural study to determine the validity of the effects of early hydration (12 hours) on organellar volume fraction percent.
6. A study of the 72-hour development period to see if the regression line dip at this stage is a valid reflection in volume changes in many organelles.

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Appendix B. Organellar volume fraction percent at the 12 hours stage of development of germinating soybean seedlings.

Sample	Subsample	Plastids	Mitochondria	Vacuoles	Cell wall	Lipid bodies	Starch grain	Nucleus
1	1	7.45	2.72	2.04	10.00	11.18	9.88	4.81
	2	8.72	2.28	2.86	6.89	16.09	2.35	8.36
	3	6.62	2.41	2.65	8.88	20.90	2.51	4.56
	4	2.32	2.65	1.65	6.06	22.20	0.68	15.78
	5	9.11	6.20	3.88	4.47	19.91	0.15	0.00
2	1	5.84	3.15	3.21	12.47	20.62	0.78	16.97
	2	9.65	2.11	1.48	12.56	24.84	0.11	0.58
	3	8.26	1.73	1.90	4.79	21.20	0.00	26.73
	4	4.90	5.85	2.16	9.18	21.44	0.00	13.41
	5	5.99	2.49	0.74	9.02	25.23	0.06	7.76
3	1	5.56	6.69	6.72	4.09	14.94	0.27	6.68
	2	4.77	2.59	3.53	4.33	11.36	0.37	8.53
	3	0.77	0.75	0.28	4.40	13.20	0.43	45.77
	4	8.15	1.37	1.65	2.84	11.10	0.14	20.41
	5	6.47	1.96	2.31	2.76	11.86	0.89	36.68
4	1	7.77	2.68	2.57	5.00	16.45	0.65	17.96
	2	3.51	0.70	5.88	3.46	13.00	1.57	39.80
	3	2.48	0.39	2.10	6.93	21.24	0.33	22.35
	4	5.82	5.41	7.99	7.70	18.76	0.29	36.23
	5	4.24	1.97	4.11	4.15	20.81	0.33	0.00
5	1	6.51	1.13	1.39	2.89	15.49	0.00	37.90
	2	2.25	1.78	1.55	3.57	18.38	0.09	26.29
	3	2.22	1.78	10.93	0.00	25.88	0.00	4.33
	4	5.76	1.45	0.88	8.14	41.51	0.99	0.00
	5	4.52	3.74	1.87	3.95	9.88	0.30	18.17

Appendix B. Organellar volume fraction percent at the 24 hours stage of development of germinating soybean seedlings.

Sample	Subsample	Plastids	Mitochondria	Vacuoles	Cell wall	Lipid bodies	Starch grain	Nucleus
1	1	3.97	3.97	3.72	15.82	8.62	0.91	0.61
	2	5.49	3.14	6.97	9.15	9.25	1.22	14.24
	3	7.35	2.43	14.11	6.38	4.82	1.17	0.93
	4	14.93	2.79	10.60	5.11	3.07	2.94	10.74
	5	9.27	2.30	32.24	11.34	20.23	2.32	2.59
2	1	3.00	1.88	5.35	12.03	19.83	0.89	13.14
	2	6.71	2.37	3.49	13.41	7.51	0.09	13.25
	3	2.04	0.42	13.04	13.81	31.27	0.00	6.96
	4	4.21	1.64	5.74	13.29	20.26	0.36	9.99
	5	15.09	1.66	14.05	3.59	31.16	2.11	0.00
3	1	8.96	2.09	5.20	7.52	17.29	3.03	9.16
	2	7.64	1.60	12.73	10.20	14.90	1.74	8.07
	3	12.54	2.97	5.96	9.14	10.61	4.32	16.40
	4	12.97	2.79	12.82	13.29	17.85	3.15	8.21
	5	5.13	2.34	9.63	4.91	25.19	2.39	0.00
4	1	3.52	1.92	13.05	8.97	10.82	0.50	22.85
	2	11.16	1.73	31.03	7.72	20.98	2.79	0.00
	3	12.76	1.78	33.73	10.21	15.70	1.52	0.00
	4	3.31	7.34	11299	10.82	11.15	0.00	0.00
	5	3.65	1.41	15.25	12.06	6.27	0.33	0.00
5	1	6.55	1.27	47.50	1.69	13.62	1.55	5.01
	2	2.09	3.03	29.00	4.19	10.05	0.78	0.00
	3	19.83	2.31	12.43	2.16	14.25	7.90	1.89
	4	3.20	0.57	35.04	2.73	6.66	2.51	11.59
	5	7.64	2.76	4.17	6.64	11.27	1.73	22.68

Appendix B. Organellar volume fraction percent at the 48 hours stage of development of germinating soybean seedlings.

Sample	Subsample	Plastids	Mitochondria	Vacuoles	Cell wall	Lipid bodies	Starch grain	Nucleus
1	1	4.12	4.94	3.47	7.37	7.54	0.37	8.69
	2	4.21	1.82	1.30	6.85	6.03	0.74	4.52
	3	11.47	1.26	4.53	12.03	13.32	0.00	5.41
	4	4.86	1.78	16.51	11.78	5.29	1.14	0.00
	5	9.93	1.07	2.68	8.98	7.01	0.00	4.18
2	1	11.24	1.31	11.27	5.56	2.39	0.89	3.14
	2	5.47	2.84	6.27	10.62	5.33	0.68	0.54
	3	14.97	2.51	3.15	6.09	1.85	6.37	23.44
	4	11.18	3.17	6.46	5.42	0.77	1.99	20.18
	5	1.76	1.65	2.43	4.39	1.36	0.32	29.12
3	1	2.84	1.18	5.00	11.89	6.47	0.26	12.03
	2	6.04	1.60	4.59	8.19	8.80	2.06	4.37
	3	1.96	3.05	0.00	9.22	10.77	0.28	32.36
	4	7.91	1.75	6.45	5.51	5.29	4.02	11.15
	5	6.65	2.80	1.38	9.05	5.41	3.86	0.00
4	1	10.51	1.75	10.26	8.80	5.72	2.39	16.45
	2	7.59	0.61	3.79	7.41	3.12	3.39	3.75
	3	5.64	1.36	21.40	9.09	4.49	4.35	0.00
	4	15.95	1.38	9.226	4.65	5.47	8.79	5.25
	5	8.59	1.01	2.85	7.69	5.26	3.03	5.65
5	1	2.51	0.46	20.43	11.33	15.19	0.98	17.37
	2	4.52	2.68	15.61	6.65	6.59	0.88	25.44
	3	5.50	0.79	72073	3.47	0.97	2.52	10.36
	4	2.48	1.04	7.96	9.59	3.66	0.42	17.01
	5	3.90	0.39	14.18	6.27	3.59	0.91	31.37

Appendix B. Organellar volume fraction percent at the 96 hours stage of development of germinating soybean seedlings.

Sample	Subsample	Plastids	Mitochondria	Vacuoles	Cell wall	Lipid bodies	Starch grain	Nucleus
1	1	9.87	3.04	39.20	6.55	0.60	4.70	12.57
	2	15.35	2.06	8.90	18.76	0.15	9.05	1.14
	3	4.88	2.13	18.25	7.07	0.08	1.79	19.08
	4	3.29	1.75	11.00	5.84	0.00	0.70	44.41
	5	1.71	1.29	5.49	76.78	0.84	1.18	8.56
2	1	7.41	0.84	15.50	4.16	0.00	3.11	29.94
	2	4.52	2.10	12.66	8.38	0.00	1.58	34.65
	3	4.42	1.89	9.60	10.55	0.00	0.77	27.17
	4	10.64	3.17	35.36	2.77	0.00	7.23	15.40
	5	15.43	1.94	44.35	5.28	0.00	4.73	0.00
3	1	4.38	2.51	23.45	8.88	0.00	0.60	0.00
	2	3.12	1.23	29.19	4.70	0.00	1.00	18.25
	3	11.47	1.23	33.05	8.12	0.00	6.05	1.27
	4	8.14	1.55	14.60	3.76	0.00	4.88	41.08
	5	7.56	1.13	11.22	5.62	0.00	3.26	15.22
4	1	11.73	4.54	7.15	8.13	0.00	7.11	0.00
	2	6.39	1.88	3.91	6.79	0.67	2.82	25.70
	3	2.90	2.50	5.74	12.00	0.00	0.47	36.11
	4	9.40	3.05	6.78	12.23	0.00	3.88	3.72
	5	6.62	2.38	10.48	5.21	0.00	2.49	26.00
5	1	3.62	2.10	39.45	9.61	0.00	0.84	3.86
	2	4.00	1.42	12.08	6.55	0.00	0.69	33.04
	3	16.68	3.95	150.23	13.82	0.20	8.48	1.00
	4	6.74	2.90	8.81	6.95	0.00	2.47	19.98
	5	2.43	1.54	59.97	2.80	0.00	0.98	1.64