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# ELECTRON MICROSCOPIC STRUCTURE OF THE EPITHELIAL CELLS OF THE SCUTELLUM OF BARLEY II

## CYTOLOGY OF THE CELLS DURING GERMINATION

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ABSTRACT

During the germination of barley, the epidermal cells of the scutellum detach themselves from one another and grow to twice their former length.

Aleuron proteins are dissolved in the first 3–4 days and subsequently the lipid contents of the spherosomes are digested. This coincides with the development and temporarily increased activity of all metabolic organelles, viz. endoplasmic reticulum, mitochondria, dictyosomes (Golgi apparatus) and leucoplasts.

After ten days aqueous vacuoles have developed. After 21 days the cell organelles are smaller and fewer, though some spherosomes are still present.

## INTRODUCTION

The structure of the epidermal cells of the scutellum of mature barley seeds has been described in a previous paper (NIEUWDORP, 1963). In the present report the cytological changes during germination will be described. These changes were anticipated, firstly, since the scutellum is known to secrete both enzymes and gibberellins, which induce the aleuron-cells to produce and to secrete amylase and other enzymes (cf. NIEUWDORP, 1963 l.c. and BRIGGS, 1964), and secondly, since the scutellum must take in the products of enzymatic hydrolysis of the endosperm to transport them to the growing embryo.

## METHODS

Barley grains of the cultivar "Proctor" were soaked in constantly aerated water of 11° C for 72 h. The water was renewed after 24 h and 48 h. Obviously, germination had already started since the embryo's had begun to perforate the pericarp. The experiment was run at 11° C in a glass jar aerated with water-saturated air of the same temperature. Samples were taken on 6 consecutive days and further every other day up to 35 days. By that time the endosperm had become a small water-filled bag, but it was still attached to the embryo.

Longitudinal sections about 100 $\mu$ thick were prepared by hand from healthy grains. Fixation, embedding and sectioning as previously described. The osmium-fixed ultratome sections were contrasted with lead, according to KARNOVSKY (1961).

## RESULTS

### *Cells and cell-walls*

Our microscopic and electron microscopic observations on the changes of the cell shape confirm the description of GUILLIERMOND (1908). In the course of germination the epidermal cells elongate to about twice the original size. Simultaneously the middle lamellae of the side-walls (anticlinal walls) are loosened, so that the initially prismatic cells are disconnected and become cylindrical. Seemingly the diameter is reduced (Fig. 1).

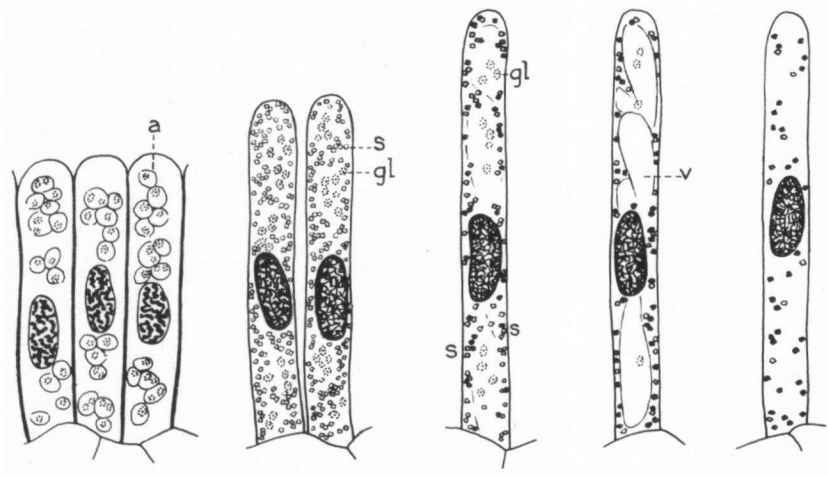


Fig. 1. From left to right: scutellum cells after 1, 5, 7, 10 and 21 days of germination, as seen under the light microscope, schematically.

During the first three days of germination the middle lamella of the anticlinal cell-walls shows a swelling up even to one third of the total thickness of the cell-wall (Fig. 6). This is revealed by increasing electron density resulting from reduction of permanganate. Obviously, the permanganate sensitive material is formed by biochemical changes within the lamella. The process of disconnection of epidermal cells takes about 5 days. The separate walls become a little thicker and more electron dense. Fine striations indicate the general direction of the cellulose microfibrils within it. Locally, tangential fissures may appear. Walls fixed and stained after about 30 days are as little electron dense as those so treated at the start of germination (Fig. 15). When eventually the endosperm walls have disappeared as a result of attack by the polysaccharase-complex cytase, the scutellar walls are still intact.

Obviously the plasmodesmata in the side-walls must have ruptured. Since we did not find any remnants of them within the walls (Figs. 3, 10 and 11), we must accept that they have disappeared, and, that they are used neither as ducts for the secretion of enzymes nor for the uptake of solutes.

*Nucleus*

As illustrated by Fig. 2 the chromosomes show up more clearly, but only in the earliest stages of germination. The nuclear membrane, during germination may be interconnected with the er (Fig. 4).

*Endoplasmic reticulum (er)*

At the onset of germination there is only a small amount of er (Fig. 2). As becomes evident when Figs. 2, 3 and 8 are compared, the amount increases during the first 7–10 days. Later on the er slowly decreases again. Moreover, it changes in shape. Firstly, the number of local dilatations increases (compare Figs. 8 and 9). Secondly, points of contact with spherosomes (see below) increase (Fig. 9). Thirdly, the contents of the local dilatations become more electron dense especially in the vicinity of spherosomes, which is conspicuous after 17–21 days' germination (Fig. 14). The spherosomes seem to be dissolved and their products taken up and used, or at least transported, by the er.

In all stages of germination ribosome-covered (rough surfaced) er may be observed in osmium-fixed sections contrasted with lead (Fig. 8). No contact of the er with the plasmalemma was ever observed.

*Vacuoles*

In the soaked ungerminated grain we described irregularly shaped protein containing vacuoles with one or two electron transparent round spots "internal cavities" (NIEUWDORP, 1963).

Within 18 h these vacuoles become more electron transparent and smaller. This process continues until, after about 10 days, vacuoles appear with apparently aqueous contents. These aqueous vacuoles gradually become larger and eventually occupy a great part of the cell (Fig. 11). They may either derive from the protein vacuoles or they may be formed *de novo*.

POUX (1963) demonstrated that in embryonic cells of wheat, bodies similar to our "internal cavities", contain phosphates and that acid phosphatase occurs either in these bodies or nearby. Thus she could identify these bodies as globoids, and the protein vacuoles as aleuron grains. The behavior of the "internal cavities" during germination of barley confirms that they are globoids too. After about three days of germination, permanganate fixation reveals internal structure of the globoids, namely a granular electron dense periphery and sometimes a dense central part (Figs. 4 and 5) which suggests that they are attacked and slowly dissolved. They are found in the reduced "protein vacuoles" even after ten days of germination (Fig. 13).

Another phenomenon that should be mentioned is the appearance of very dark globules of about 150 m$\mu$ within the aqueous vacuoles (Figs. 10 and 11). These usually occur after ten or more days of germination. They are found near the tonoplast and often in the vicinity of disintegrating spherosomes. Probably they arise from the latter (Fig. 12).

*Spherosomes* [1])

These are vesicles in the scutellar epidermis of ungerminated barley grains. They are situated near the cell-wall and around the aleuron grains. We named them provisionally spherosomes (1963) because of their probably high lipid content, and in fact we found that they stain with lipophilic reagents such as Sudan black and Nile blue. A high refractive index, apparent with phase contrast and with dark field illumination, is another indication for a high lipid content. Meanwhile similar bodies have been studied by FREY–WYSSLING *et al.* (1963) in maize seedlings, *Allium* scales and some oil seeds. These authors showed that the vesicles contain lipids and probably also proteins and identified them as spherosomes. Similar bodies were also found by BUTROSE (1963) in endosperm and aleuron-cells of wheat. Spherosomes in root tips of *Pisum* were observed to move centripetally when centrifuged (BOUCK, 1963). Although there is some confusion as to the definition of spherosomes (cf. BUVAT, 1963) we will keep to this name for the lipid bodies of 0.3–0.5 $\mu$ diameter, enveloped by a single limiting membrane, as found by us and by the authors mentioned above.

After the very first day of germination many of the spherosomes move away from the cell periphery and from the vicinity of the aleuron grains to become more evenly distributed in the cytoplasm. During germination they are reduced both in number and in size as a result of chemical changes and dissolution. This process begins at the periphery. After 3 days they show electron dense surfaces after permanganate fixation; some of them even show this change throughout (Figs. 3 and 4). The endoplasmic reticulum also becomes more electron dense. This seems to indicate that the membranes of the spherosomes disappear and that the cisternae of the er have taken up the contents (Figs. 9 and 14).

Morphologically the process of the disappearance of the spherosomes is the reverse of that which occurs in their formation as described by FREY–WYSSLING *et al.* (1963) (see also DRAWERT and MIX, 1962). It should be noted that, as early as 1904, REED observed in the same cells during germination the appearance of basophilic corpuscles that subsequently decreased in number. It seems likely that these were spherosomes being attacked and dissolved. Probably, the staining with basic dyes is due to fatty acids, produced as a result of hydrolysis of fats (Fig. 1).

*Proplastids or leucoplasts*

As described by BUVAT (1963), proplastids differ morphologically from mitochondria in being slightly larger, and in having less structured contents. Leucoplasts differ from proplastids in being still larger and besides in the possible presence of lipid droplets or of starch grains. Since the "proplastids" described by us (1963) in

[1]) In our previous paper we have written "sphaerosomes", however the English spelling is "spherosomes".

ungerminated barley were much larger than the mitochondria, they should, according to this definition, have been called leucoplasts, although lacking starch and lipids.

During germination the leucoplasts show several changes. Firstly, many of them acquire starch grains after about three days (Fig. 7) and they increase in size. The starch grains may disappear again, but they may show anew afterwards. Internal cisternae develop and are often arranged parallel in the way of onion scales (Figs. 3, 9 and 10). Frequently connections between these internal membranes and the inner layer of the double membrane delimiting the plastid are observed (Fig. 13). After 20 or more days of germination the leucoplasts have become nearly similar in appearance to those present at the start of germination.

*Mitochondria*

During germination these show changes analogous to those shown by leucoplasts. After about three days the cristae increase in number and in size, extending to about half the diameter of the mitochondrion (Figs. 4 and 5). Later on they are reduced again (Fig. 14). In number and size the mitochondria do not change.

*Golgi apparatus or dictyosome*

Neither do the dictyosomes change in number, but they show conspicuous signs of activity simultaneously with the er, the leucoplasts and the mitochondria. The number of cisternae is usually about five. They increase in surface, become less curved and evidently produce more vesicles (Fig. 10). After 21 days they return to their original size and shape. Golgi vesicles in contact with plasmalemma were never observed.

*Ribosomes*

These are distributed through the whole cell, attached to or free from the endoplasmic reticulum (Fig. 8).

## Discussion

Our results, as far as microscopically visible organelles are concerned, like aleuron grains, vacuoles and spherosomes, are in line with those of Reed (1904) and Guilliermond (1908). The changes observed in the course of germination summarized in Table 1 may be related to the known production and secretion of enzymes and gibberellins during the first three days. This process coincides with the rapid dissolution of the proteins in the aleuron grain, the nuclear changes, probably reflecting the production of the necessary nucleic acids, and the development of the endoplasmic reticulum. Subsequently its function is mainly the uptake and transport of sugars and probably, to a minor extent, amino-acids from the endosperm.

Uptake and transport, either with or without concomitant chemical changes, of hydrolysis products from the endosperm probably follows.

TABLE 1

Changes in the epidermal cells of the scutellum of germinating barley

| | 1 day | 3 days | 7 days | 10 days | 21 days |
|---|---|---|---|---|---|
| Cells | middle lamella swells | sidewalls detached | | | |
| | | length doubled | | | |
| Nucleus | chromosomes more apparent | less apparent | | | |
| Endoplasmic reticulum | | increase in size and local dilations | | | decrease in size |
| | | | contact with and uptake of spherosomes | | |
| Aleuron grains and vacuoles | protein disappears | | | appearance of aqueous vacuoles | |
| | | globoids are attacked and disappear | | | |
| Spherosomes | | distributed over entire cells | | | |
| | | attacked from periphery | | | |
| | | | | | emptied in er |
| Leucoplasts | | starch grains appear and disappear | | | |
| | | | internal cisternae increase | | and reduce again |
| Mitochondria | | | cristae increase | | and reduce again |
| Golgi apparatus | | | cisternae increase and become less | | curved and reduce again |

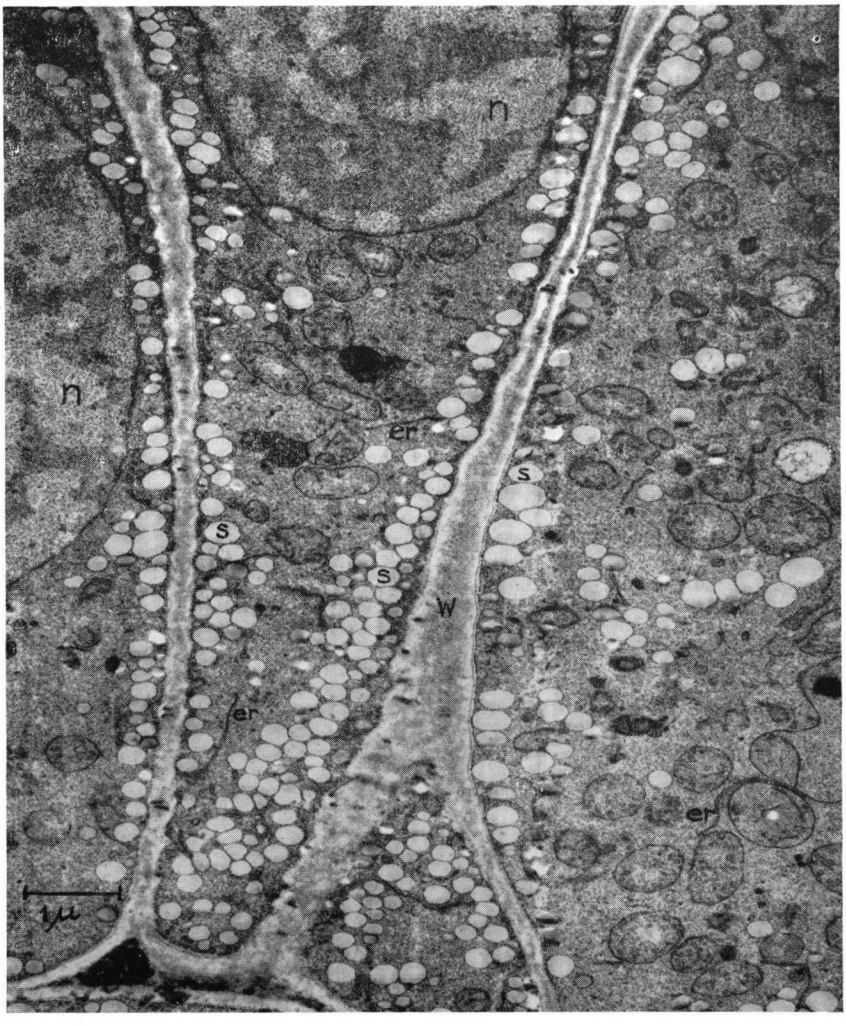


Fig. 2. After 3/4 days. Nucleus with chromosomes. A small amount of er. $KMnO_4$ fixation.

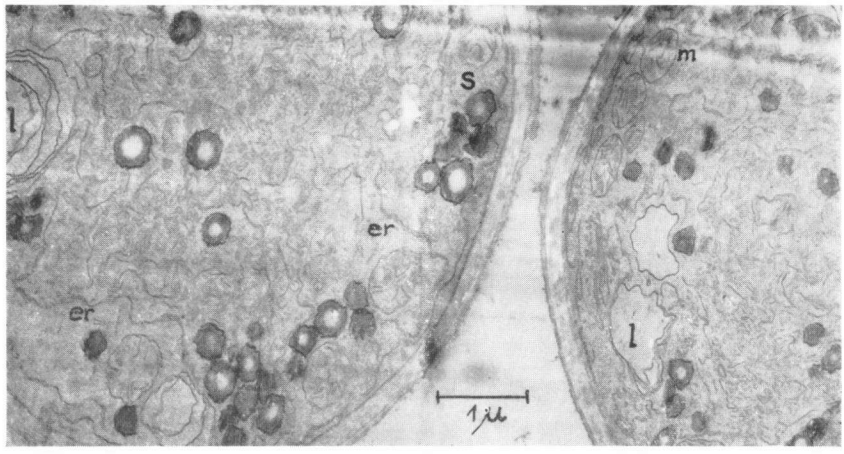


Fig. 3. After 4 days. Increased amount of er with local dilatations. Spherosomes with electron dense surfaces. Leucoplasts with cisternae. $KMnO_4$.

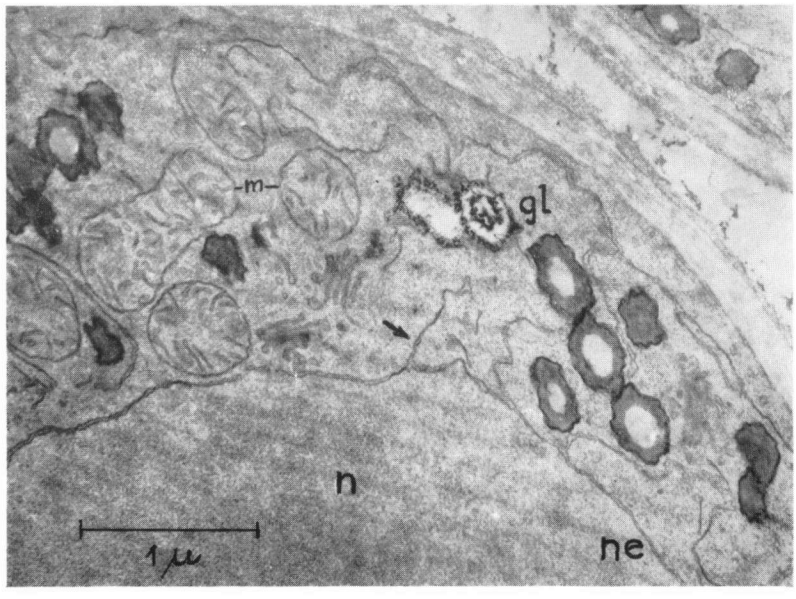


Fig. 4. After 3¼ days. Nuclear membrane with probable interconnections with the er (arrow). Reduced aleuron grains with globoids. $KMnO_4$.

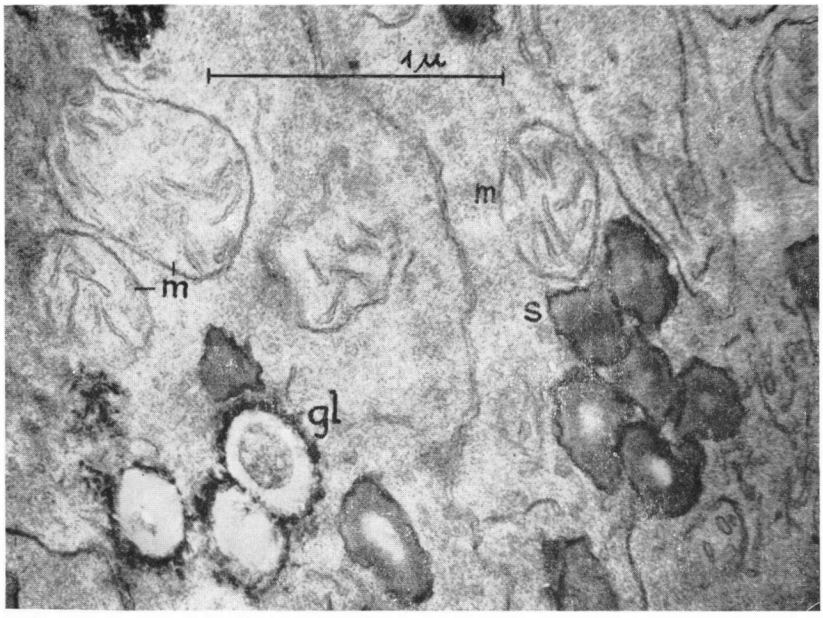


Fig. 5. After 3¼ days. Spherosomes with electron dense rings. Mitochondria with lengthened cristae. Globoids. $KMnO_4$.

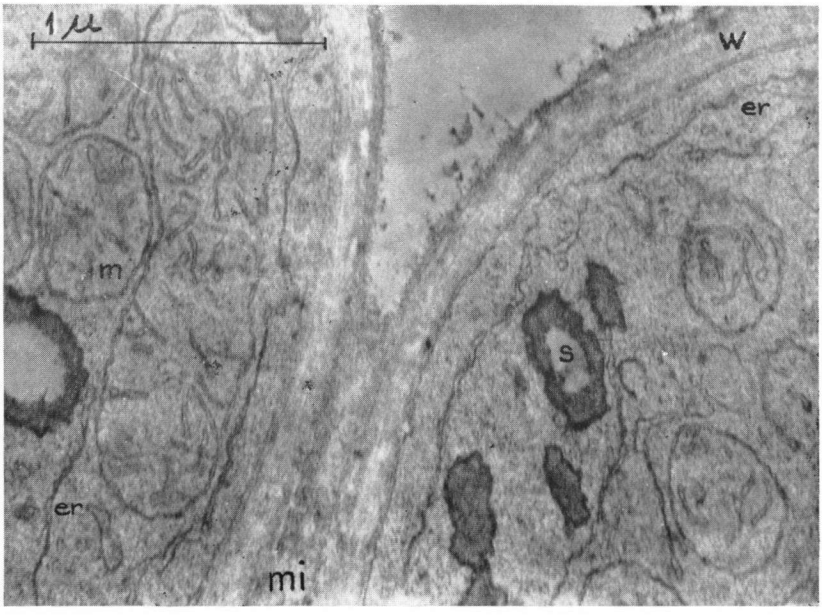


Fig. 6. After 3½ days. Middle lamella swollen, the cell-walls detaching. $KMnO_4$.

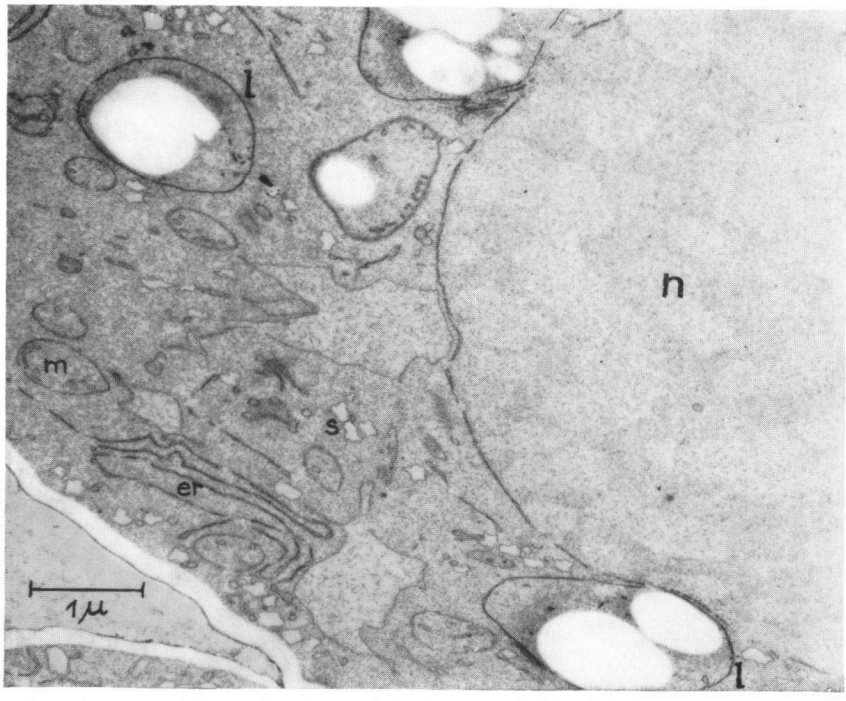


Fig. 7. After 2¾ days. Starch grains within the leucoplasts. $KMnO_4$.

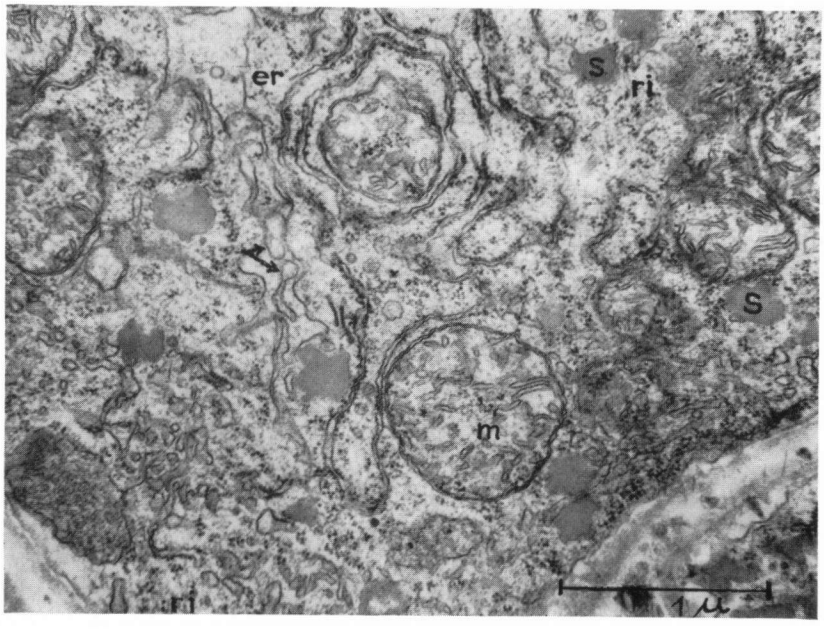


Fig. 8. After 6 days. er with ribosomes. Local dilatations of the er (arrows). Spherosomes electron dense. $OsO_4$, $Pb(OH)_2$.

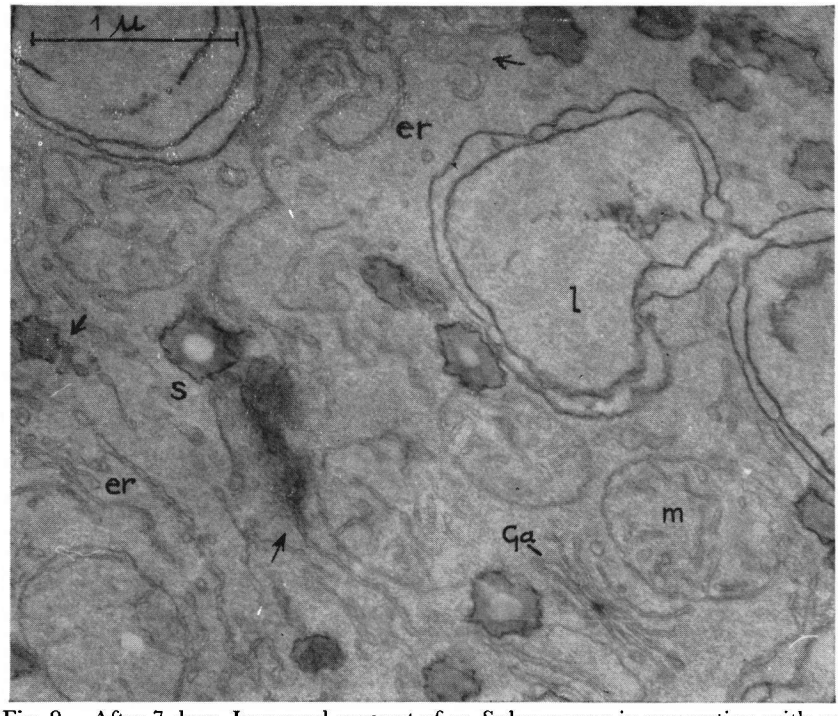


Fig. 9. After 7 days. Increased amount of er. Spherosomes in connection with er, of which the contents have become electron dense in the vicinity of the spherosomes (arrows). $KMnO_4$.

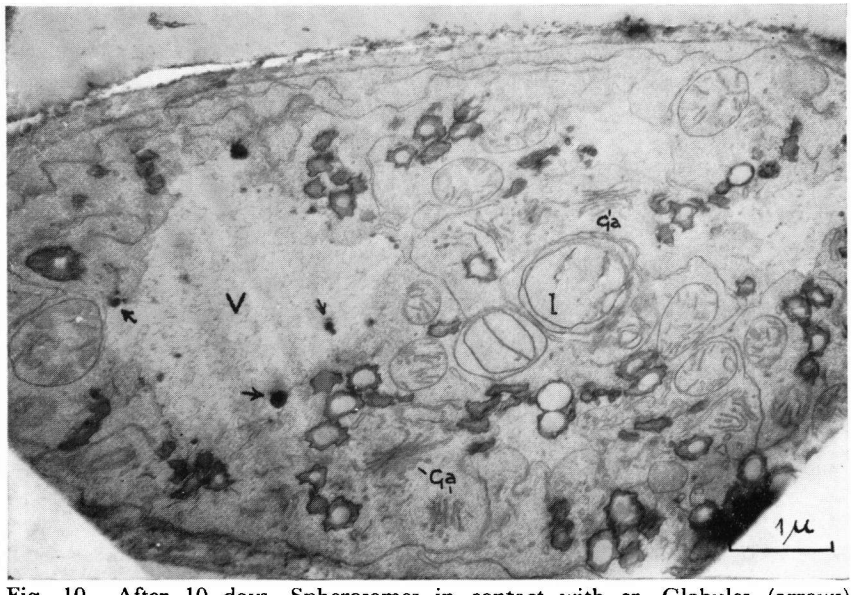


Fig. 10. After 10 days. Spherosomes in contact with er. Globules (arrows) within vacuoles. $KMnO_4$.

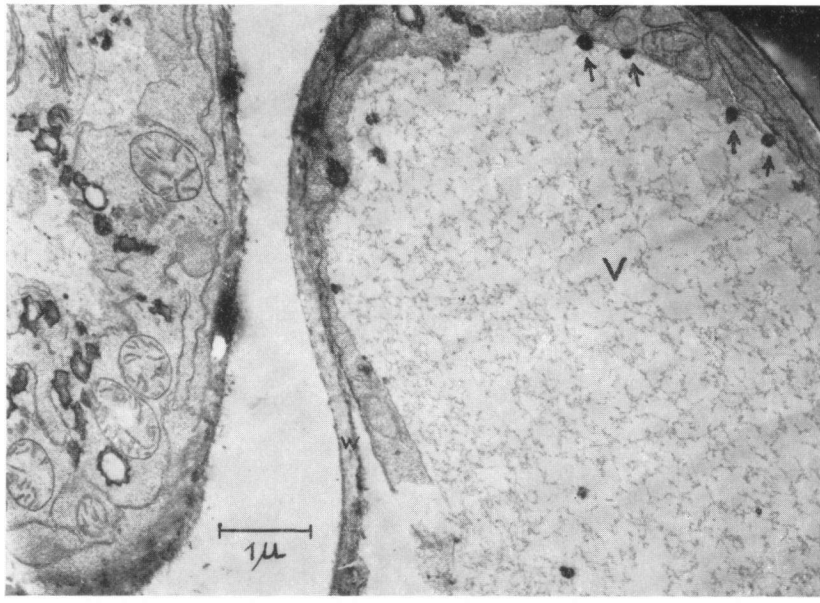


Fig. 11. After 10 days. Big aqueous vacuoles with globules (arrows). No plasmodesmata. $KMnO_4$.

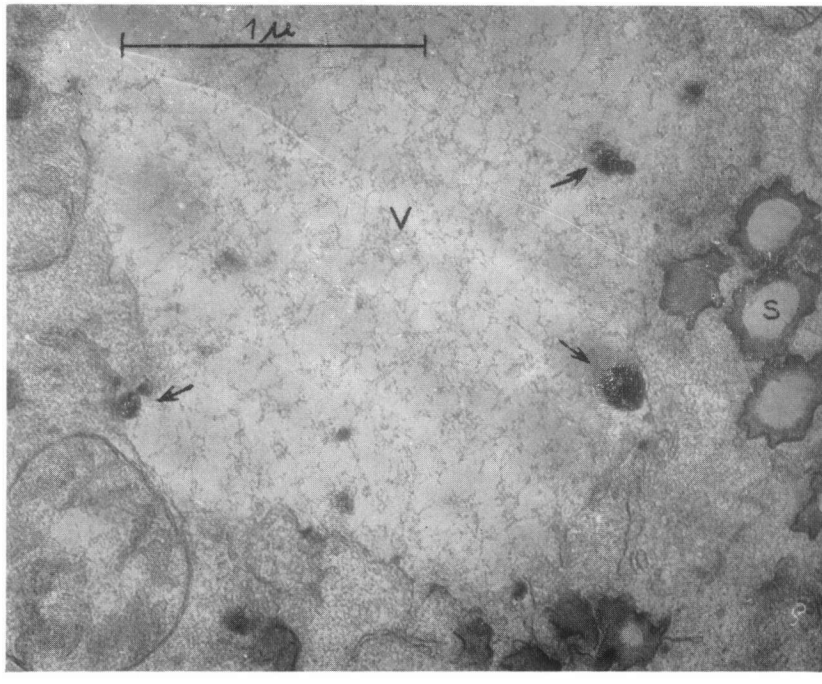


Fig. 12. After 10 days. Vacuole with globules (arrows). $KMnO_4$.

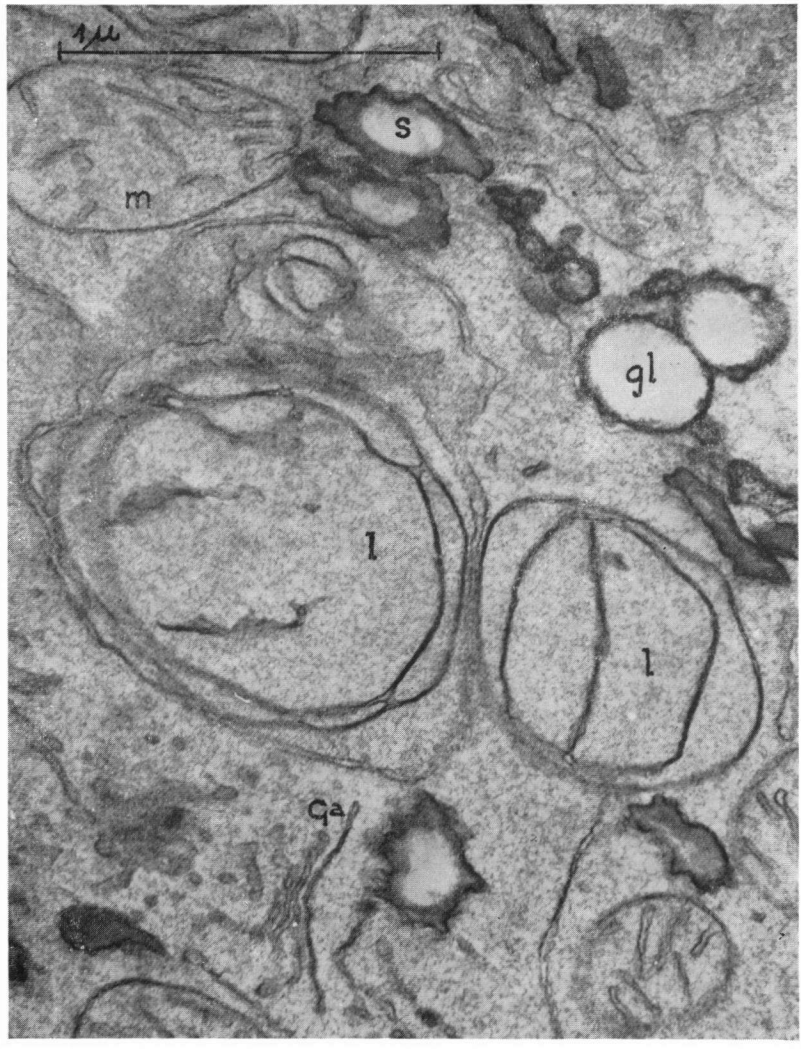


Fig. 13. Detail of Fig. 10. Leucoplasts with internal connections. Golgi apparatu less curved. Remains of globoids. $KMnO_4$.

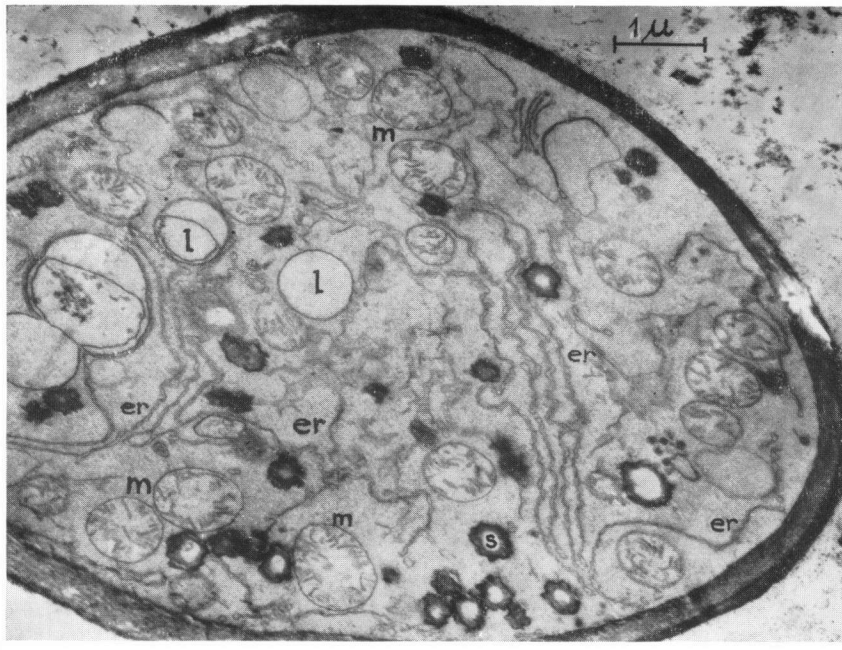


Fig. 14. After 21 days. er with dark contents. Plastids (leucoplasts) and mitochondria reduced. $KMnO_4$.

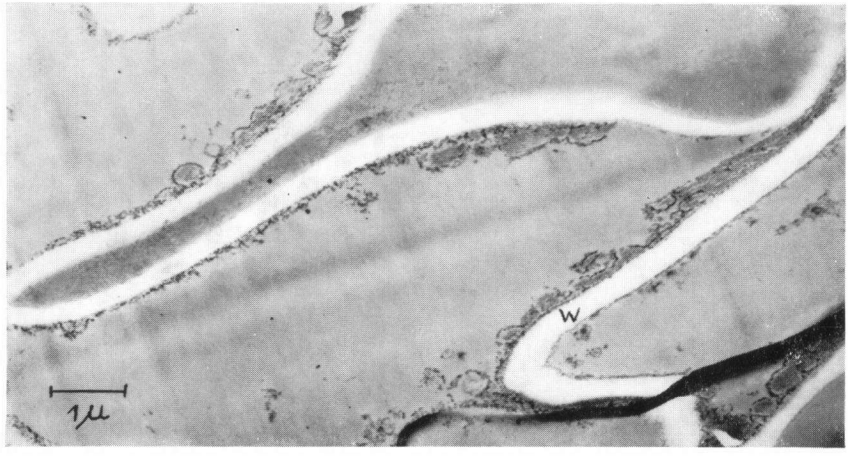


Fig. 15. After 35 days. Cell-walls low electron dense. $KMnO_4$.

This may coincide with the apparent development and activity of the leucoplasts, the dictyosomes and the endoplasmic reticulum and besides with the attack on and uptake of both the lipid contents of the spherosomes and the phosphates of the globoids. Although the cells in question constantly dispose of an abundance of metabolic materials from the endosperm, they seem to have and to use their own reserve materials.

*Abbreviations*

a – aleuron grain (protein vacuole)
er – endoplasmic reticulum
Ga – Golgi apparatus (dictyosome)
gl – globoid
l – leucoplast (proplastid)
m – mitochondrion
mi – middle lamella
n – nucleus
ne – nuclear envelope
ri – riboso mes
s – spherosomes (basophilic corpuscles)
v – vacuole
w – cell-wall

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