Acta Botanica Neerlandica **12** (1963) 295–301

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

THE STRUCTURE OF THE EPITHELIAL CELLS BEFORE GERMINATION

P. J. NIEUWDORP

(*Laboratory of General and Technical Botany, Delft*)

(*received May 27th, 1963*)

ABSTRACT

The cylindrical epithelial cells of the scutellum of ripe ungerminated barley were studied with the electron microscope. The most striking feature is the abundance of globular bodies of about 0.2 μ, probably containing lipids and provisionally called sphaerosomes. Most of them are situated along the cellwall and along several cytoplasmic bodies, which often lie in the vicinity of the nucleus. These bodies vary in shape and in size (1–3 μ) and they seem to be vacuoles filled with protein. Usually they have an inner cavity.

In other respects the structure seems normal for a cell in a resting-stage. Besides proplastids and mitochondria, a small number of Golgi apparatuses are to be seen. The endoplasmic reticulum is scarce.

INTRODUCTION

So far, the mechanism of secretion has only been studied electron microscopically with animal cells. We decided to study it in germinating barley, in which two kinds of tissues come into consideration. Firstly, the epidermal cells of the scutellum, which are known to secrete enzymes into the endosperm, both in ripening and in germinating seeds (BROWN, 1890; LEHMANN and AICHELE, 1931 and ENGEL, 1947). Recently was shown that the scutellum also secretes gibberellins. These activate the production of enzymes by the second secretorial tissues, viz. the aleuron layer (JOMO and ZINAMA, 1962; MACLEOD *et al.*, 1962 and BRIGGS, 1963).

This first communication is concerned solely with the cytoplasmic structure of the scutellum epidermis of ungerminated barley. Studies of the aleuron layer and of both tissues in germinated barley are in progress. The literature on the secretion of enzymes by the scutellum of cereals may be mentioned briefly.

In 1928, HORNING and PETRIE observed how, during the germination of various species of cereal grains, small bodies—which they referred to as "mitochondria"—were formed in the cytoplasm of the epidermal cells of the scutellum. These bodies, stainable with the

vital stain Janusgreen B, were supposed to migrate out of the epithelial cells into the starch-containing endosperm, via a strongly flattened layer of the endosperm which was devoid of starch.

As postulated by HORNING and PETRIE, the migratory "mitochondria" (together with the few "mitochondria" already present in the endosperm) might release the enzymes responsible for the breakdown of starch.

ENGEL and BRETSCHNEIDER (1947) however, did not find any correlation between mitochondria and amylase production. The experiments by ENGEL (1947a) on grains in the resting-stage show most of the amylase to be present in those cells of the starch-containing part of the endosperm which are closest to the aleuron layer (Fig. 1). In addition, a good deal of amylase is present in the scutellum.

ENGEL and HEINS (1947) find the aleuron layer rich in proteinase and dipeptidase, the endosperm on the other hand poor in these enzymes. The germ has but a small quantity of proteinase against a high concentration of dipeptidase, the latter enzyme being present especially in the root primordia and the epithelial layer of the scutellum.

It is commonly known, both among seed physiologists and among maltsters, that the process of dissolution of endosperm cellwalls and that of macerating of endosperm cells proceeds from the scutellum towards the tips of the germinating barley seeds. This strongly suggests that either cellwall attacking enzymes or activators of these are secreted by the scutellum (see LEHMANN and AICHELE, l.c.).

METHODS

Hand sections made of dry grains yielded bad fixations and the embedding in Vestopal was unsuccessful. The following method, however, yielded satisfactory results: grains were put in water of 0° C in a refrigerator for 24 hours. Then longitudinal hand sections of about 100 μ thickness were made and put in tap water. Under a dissecting microscope the embryos were taken out of these and immediately placed in a 2 % solution of $KMnO_4$, where they were kept for 2 hours at room temperature. Some other hand sections were fixed in OsO_4 buffered at pH 7.4. Thereupon they were rinsed with water, and put in 25 % acetone and increasing concentrations for dehydration.

The dehydrated sections were then embedded in a layer of Vestopal of $\pm$ 2 mm thickness, which was lying on a piece of dry gelatin sheet folded in a small petri dish. After polymerization at 60° C for a week, the embedded sections were cut out with the aid of a fret-saw. A platelet obtained in this manner was clamped between the halves of a Vestopal cone and fixed in the holder of an L.K.B.-ultratome. The top of the pyramid could now be sharpened with a razor-blade under the dissecting microscope so as to expose the epithelium of the scutellum. Finally, sections of about 600 Å were made with a glass knife and photographed in an electron microscope.

Description of the cells of the scutellar epithelium

From studies it is well known that the epithelial layer of the scutellum is composed of cylindrical cells standing normal to the scutellum surface (Figs. 1 and 2). In the resting barley grain these cells are about four times as long as they are wide. The upper ends of the cells are rounded so that triangular intercellular spaces appear between these rounded tops and the flattened layer of the endosperm (Figs. 2 and 3). The nucleus is distinctly visible, and in the protoplast many little dots are observed.

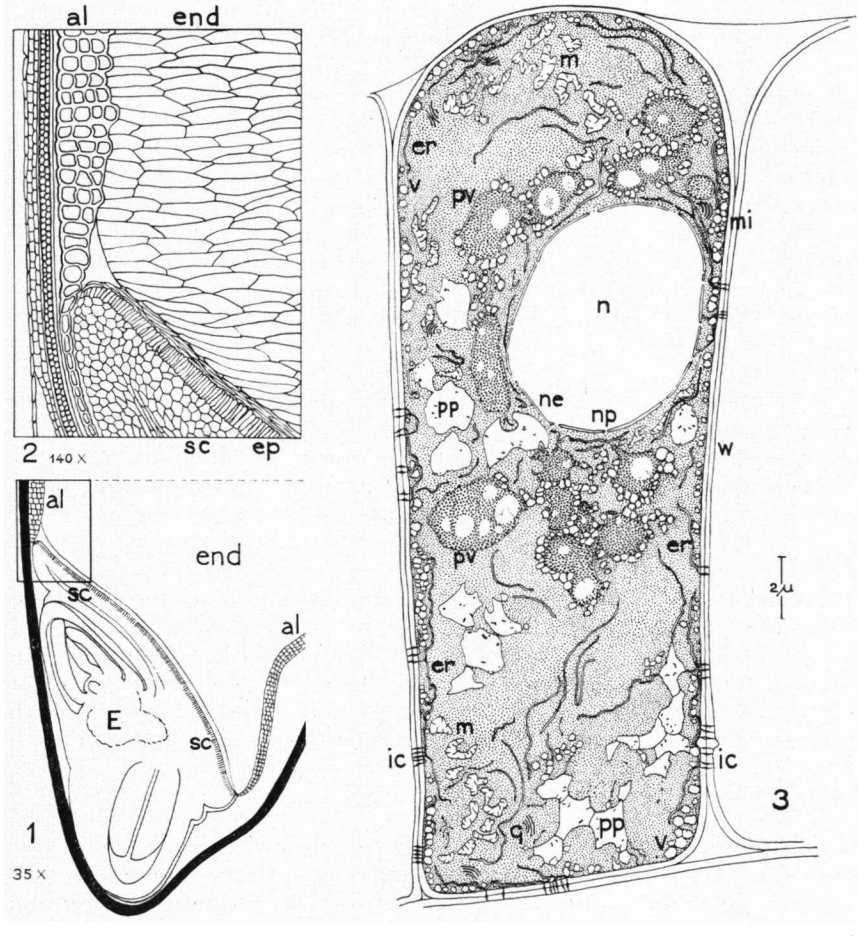

Fig. 1. Part of a longitudinal section with embryo (E), scutellum (sc), endosperm (end) and aleuron layer (al).

Fig. 2. Part of fig. 1 enlarged, to show the place where the sections for the E.M. are made.

Fig. 3. Scheme of an epithelium cell with the plasma organelles which are observed in the E.M. micrographs.

Abbreviations used in figures.

al — aleuron layer
E — embryo
end — endosperm
ep — epithelium
mi — middle lamella
sc — scutellum
er — endoplasmic reticulum
Ga — Golgi apparatus
Gv — Golgi vesicle
ic — intercellular connections, plasmodesmata
m — mitochondrion
n — nucleus
ne — nuclear envelope
np — nuclear pore
pp — proplastid
pv — protein vacuole
ri — ribosomes
w — cellwall
v — vesicle (sphaerosomes)

In the electron micrographs, these long-stretched cells are mostly cut obliquely. Their most striking feature is that they evidently are in a resting-stage (Fig. 3). The queer-shaped proplastids show but little of internal membranes. Of the endoplasmic reticulum, not much is visible. All other protoplasmic organelles appear to be shrivelled up and are more or less reduced in size. There are two salient points: the resting cells are crowded with many small vesicles, probably filled with lipids, and with several larger vacuoles, probably filled with protein. Possibly these facts are associated with the cells' function, production of enzymes.

The different parts may be described in more detail as follows.

Cellwall

The thickness is 0.125–0.25 μ. The middle lamella is clearly visible (Figs. 4 and 9), as are the plasmodesmata (Figs. 4 and 8). The latter may be situated in any part of the cellwall, provided the cells are bordering on other scutellar cells. They never occur at the upper ends, where the intercellular spaces are to be found (Fig. 7).

Protoplast

Adjacent to the cellwall is the protoplasmic membrane, which is singular, cut through here and there by plasmodesmic connections of the endoplasmic reticulum. When fixation with $KMnO_4$ is employed, the cytoplasm shows little detail, but the membranes appear clearly. No ribosomes (Palade bodies) are perceptible. However, when OsO_4 is used as a fixative, ribosomes are visible in abundance (Figs. 9 and 10).

Nucleus

This often lies at about the middle of the cell. Its contents show no details, it is evidently in a resting-stage. It is enfolded by the nuclear envelope, which as usual consists of a double membrane locally perforated by pores (Fig. 5).

Vacuoles

In the protoplasm we frequently see bodies of 1–3 μ, slightly darker than their surroundings, irregularly shaped and often with many protuberances (Figs. 3, 4 and 5). Not uncommonly they lie in the

vicinity of the nucleus and they are not seldom surrounded by rows of vesicles of unknown origin. We take the former to be vacuoles (perhaps protein vacuoles). Their membrane is a single layer as is characteristic for vacuoles (POUX, 1962; GRAHAM, JENNINGS *et al.*, 1962). After fixation in $KMnO_4$ these vacuoles are shown provided with internal cavities. There are no connections leading from these to the surface. Their occurrence cannot be due to incomplete penetration of the fixative, as the cavities often lie very close to the surface of the vacuole. Within them, most distinctly near the margin, we see a sediment, as often is found in vacuoles. Sometimes the margin is covered by what seems to be a porous membrane. However, this might have arisen as a result of the formation of the sediment (Figs. 3, 4 and 5).

Similar vacuoles, though without the cavities, are found in the electron micrographs, of root cells of other Gramineae, by FABERGÉ (1962) and WHALEY (1960). They called these "lipoid vacuoles".

Vesicles or sphaerosomes

In this category, we will for the present include many bodies of about 0.5–0.3 μ diameter. Their abundance in the scutellar cells is very remarkable. In large majority, they are situated along the cell-walls and in close proximity of the vacuoles; sometimes they form clusters in the vicinity of the latter. They are more or less spherical in shape, often indented probably due to shrivelling, which fact might be a result of bad fixation and dehydration.

In material fixed with $KMnO_4$ and dehydrated with acetone the vesicles often are empty, or at least colorless, although in some of them a little sediment may occur (Figs. 5 and 7). The wall is a single membrane.

However, when we use OsO_4-fixation, which results in very poor micrographs (Figs. 9 and 10), the vesicles are not empty and the membrane is not so strongly shrivelled. This fact raises the question whether or not these bodies are filled with a lipid, that is removed by the acetone employed after $KMnO_4$-fixation. DRAWERT and MIX (1962) described perhaps a similar plasma organel.

Similar vesicles seem to be present in the micrographs of WHALEY (1959 and 1960), who refers to them as "unidentified protoplasmic bodies". To find out the origin of the vesicles it seems necessary to study the epithelial cells of the scutellum in an earlier state of development of the grain.

Golgi apparatus

Neither this, nor the vesicles proceeding from it, are represented in great numbers (Fig. 6); nor are the cisternae. As compared to the above-mentioned sphaerosomic vesicles, the Golgi vesicles are much less numerous and much smaller (Fig. 7). The contents are of a dark hue with $KMnO_4$-fixation. It is obvious that the Golgi apparatus is in a resting-stage.

Proplastids

These are in a resting-stage as well. The dimensions are about equal to those of the vacuoles; the shape is very irregular (Figs. 3 and 4). With $KMnO_4$-fixation the contents are of a lighter hue than those of the vacuoles and thus show hardly any contrast with the surrounding protoplasm. The outer membranes are distinctly double and the ones within are reduced to small vesicles and short pieces of double membrane. We don't see any trace of amylum in them.

Mitochondria

These also have irregular shapes; their outer membranes obviously are double. The cisternae, though not highly developed, are as a rule better discernible than the membranes within the proplastids. Even though the mitochondria are much smaller than the proplastids, and their cisternae are more distinct, there is some resemblance between the two (Figs. 3, 4 and 7).

Endoplasmic reticulum

This is very scarce and undeveloped. Mostly we trace pieces along, or at least in the neighborhood of, the cellwall. Sometimes these seem to merge into sphaerosomic vesicles as well as into membraneless protoplasmic parts, of a dark shade (Fig. 7). The endoplasmic reticulum here and there crosses the cellwall as *plasmodesmata*. These are circular in cross section, and bordered by a distinct membrane (Fig. 8).

Ribosomes

These organelles can only be seen after fixation with OsO_4. They are very numerous (Figs. 9 and 10) and can sometimes be observed arranged along the membranes of the endoplasmic reticulum (rough-surfaced endoplasmic reticulum).

ACKNOWLEDGMENTS

I wish to thank Miss M. C. Buys for her technical assistance, Miss H. Kern for making the drawings and Professor Dr. P. A. Roelofsen for the suggestion to make our investigations on barley seeds.

REFERENCES

BRIGGS, D. E. 1963. J. of the Institute of Brewing **60**: 13.
BROWN, H. T. and G. H. MORRIS. 1890. J. Chem. Soc. **57**: 458.
DRAWERT, H. und M. MIX. 1962. Berichte der Deutsch. Bot. Gesellsch. **75**: 128.
ENGEL, CHR. 1947a. Biochimica et Biophysica Acta **1**: 42.
————. 1947b. Biochimica et Biophysica Acta **1**: 278.
———— and L. H. BRETSCHNEIDER. 1947. Biochimica et Biophysica Acta **1**: 357.
———— and J. HEINS. 1947. Biochimica et Biophysica Acta **1**: 190.
FABERGÉ, A. C. and C. W. LEWIS. 1962. The J. of Cell Biology **15**: 579.
GRAHAM, J. S. D., A. C. JENNINGS, R. K. MORTON, B. A. PALK and J. K. RAISON. 1962. Nature **196**: 967.
HORNING, E. S. and A. H. K. PETRIE. 1928. Proc. of the Royal Society of London **102**: 188.
JOMO, H. and H. ZINAMA. 1962. J. Agr. Biol. Chem. **26**: 201.

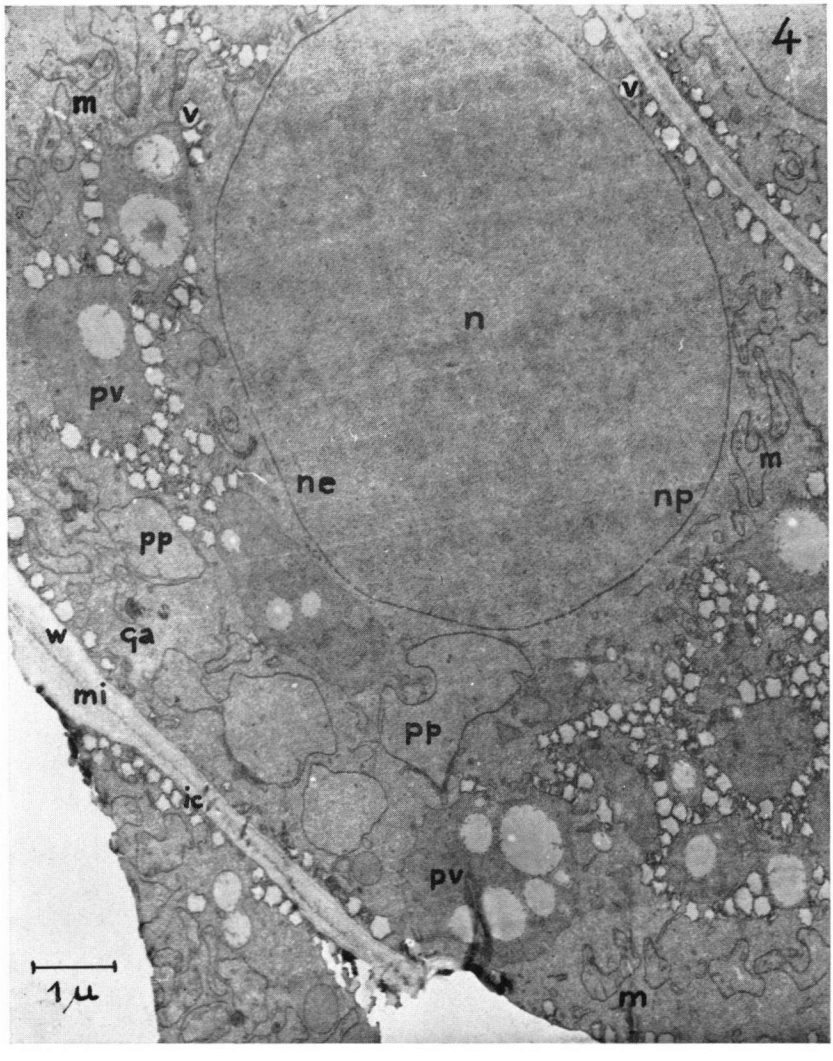

Fig. 4. E.M. micrograph, a survey. $KMnO_4$-fixation. Middle lamella (mi), intercellular connections, plasmodesmata (ic).

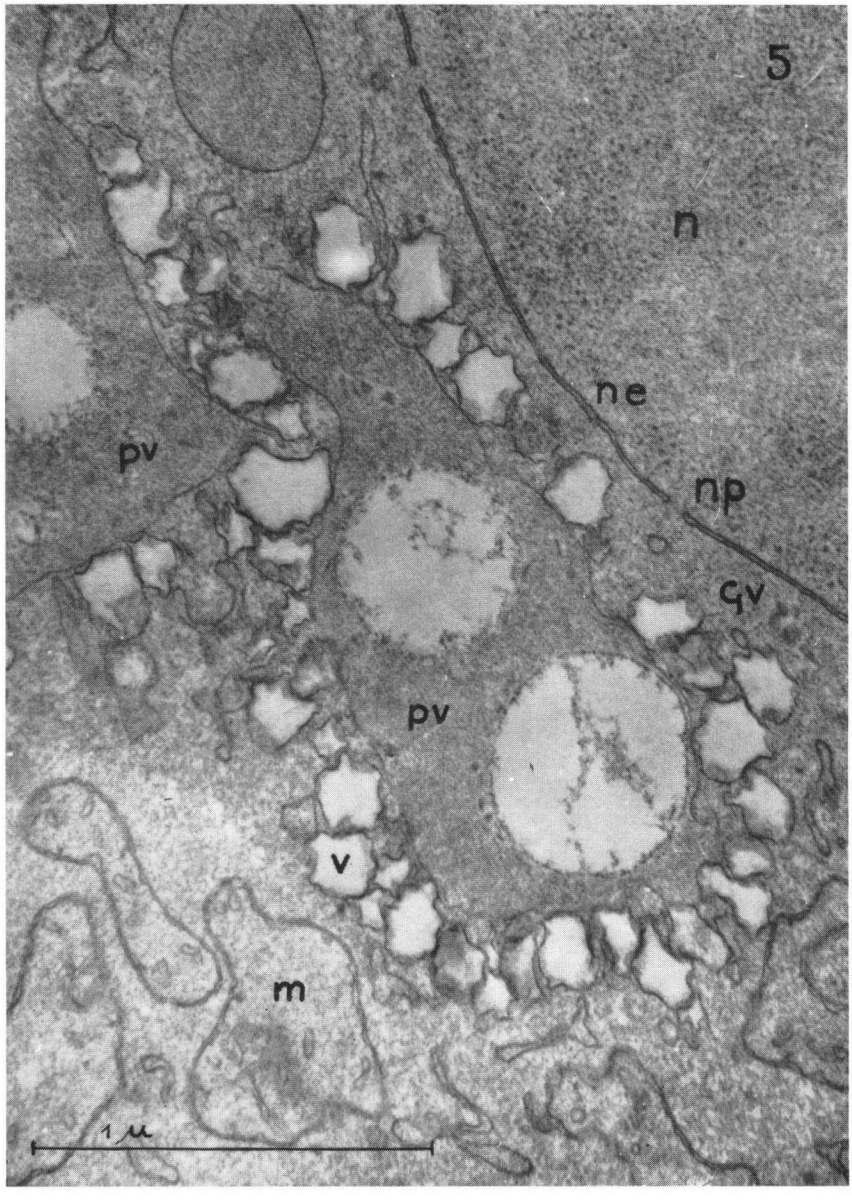

Fig. 5. Greater enlargement with nucleus (n), nuclear envelope (ne) and the pores (np), vacuole (pv), vesicles (v). $KMnO_4$-fixation.

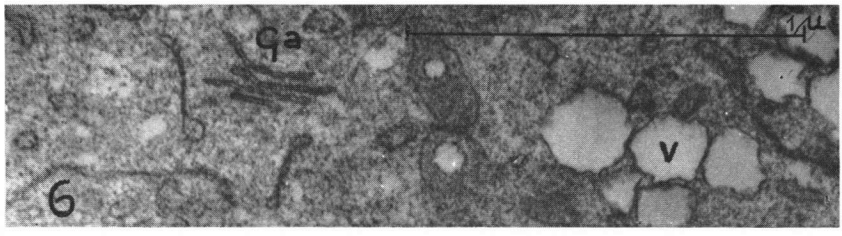

Fig. 6. Golgi apparatus. $KMnO_4$-fixation.

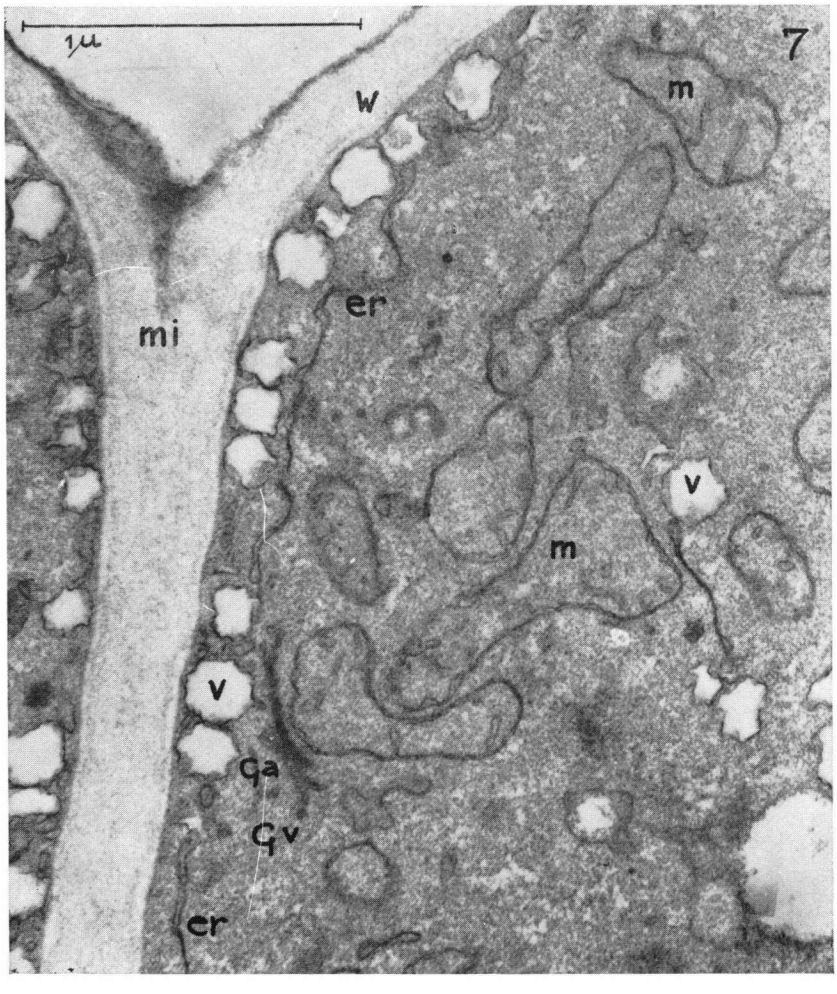

Fig. 7. A detail of the top of a cell with endoplasmic reticulum (er), vesicles (v), mitochondria (m). $KMnO_4$-fixation.

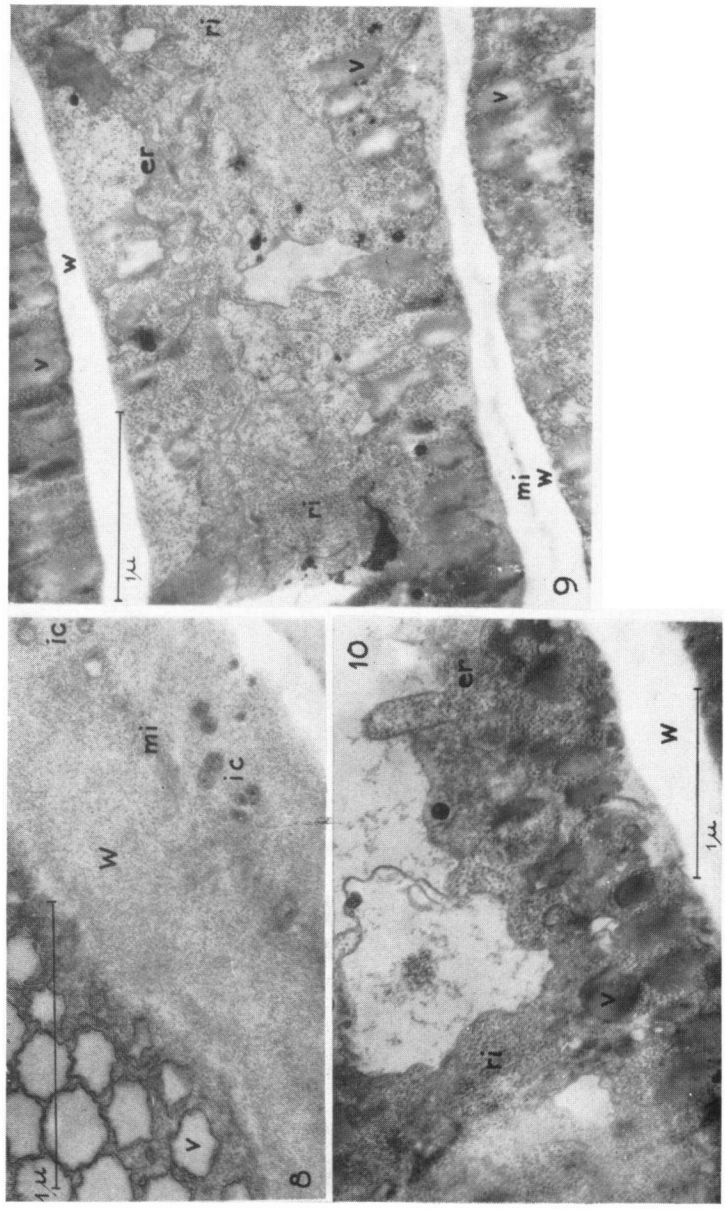

Fig. 8. Tangential section of a cell, with cellwall (w), vesicles (v) and plasmodesmata (ic). $KMnO_4$-fixation.
Figs. 9 and 10. OsO_4-fixation. Ribosomes (ri) and vesicles (v).

LEHMANN, E. und F. AICHELE. 1931. Keimungsphysiologie der Gräser. (Stuttgart).
MACLEOD, A. M. and A. S. MILLAR. 1962. J. Inst. Brewing, **68**: 322.
MOLLENHAUER, H. H., C. G. WHALEY and J. H. LEECH. 1961. J. of Ultrastructure Research **5**: 193.
POUX, N. 1962. J. de Microscopie **1**: 55.
WHALEY, W. G., H. H. MOLLENHAUER and J. E. KEPHART. 1959a. The J. of Biophysic. and Biochem. Cytol. **5**: 501.
————, ———— and ————. 1959b. Amer. J. of Botany **46**: 743.
————, ———— and J. H. LEECH. 1960. Amer. J. of Botany **47**: 401.