ULTRASTRUCTURE OF THE DEVELOPING ALEURONE CELLS OF WHEAT GRAIN

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Summary

The developing aleurone layer cells of the wheat kernel have been investigated by electron microscopy and the results compared with those of light microscopy. Two weeks after flowering vacuoles appear in the cells and deposits accumulate in these until maturity when the cells are filled with the resulting "vacuolar units" 2–3μ in diameter, corresponding to the aleurone grains of light microscopy. The wheat aleurone grain consists of a bounding membrane (of vacuole origin) enclosing a matrix in which are embedded spherical deposits. Some of these deposits are translucent and others opaque to electrons after potassium permanganate and osmium tetroxide fixation. At all stages examined the cytoplasm of aleurone cells contained large numbers of small unidentified bodies with irregular outline and dense contents. At first they are dispersed, but towards maturity are organized as a monolayer over the surface of each aleurone grain and the inner surface of the cell walls. The apparent specificity of these structures to aleurone cells is discussed in relation to future chemical and physiological studies of the tissue.

I. Introduction

Since the advent of the electron microscope a close examination has been made of some tissues of the wheat grain, namely the embryo (Setterfield, Stern, and Johnston 1959), scutellum (Hrsel, Wolfova, and Mohelska 1961), and starchy endosperm (Buttrose 1963). However, the excellent but more restricted findings of light microscopy must still be relied on for knowledge of other tissues, and since plant physiologists are currently concerned with one of these, the aleurone layer of cereal grains and especially its function during germination (see MacLeod and Millar 1962), a more detailed knowledge of the cellular morphology of this tissue could be advantageous.

The aleurone tissue of wheat forms a layer one cell thick over the periphery of the starchy endosperm, and derives its name from the protein-rich aleurone grains which fill the cells. These grains were the main concern of light microscopists when studying the tissue and over the years views have changed as to their structure. Lüdtke (1890, quoted from O'Brien 1895) considered that in the Gramineae these grains were undifferentiated in contrast to those of legume and oil seeds [which, according to Pfeffer (1872), have both what were termed globoid and crystalloid portions], consisting of a homogeneous mass (corresponding to a globoid) and a surrounding membrane. Later Wieler (1943) described cereal aleurone grains as composed of a clear envelope enclosing a granulated substance, whereas quite recently Oksijuk (1961) claimed the presence of a globoid, attached crystalloid,

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and basic protein material within wheat aleurone grains. This last concept is similar to Pfeffer's (1872) classical description of the aleurone grains of *Ricinus* seeds, but Oksijuk did not publish any micrographs which would allow an independent interpretation of his preparations. As regards origin, workers in the past have favoured vacuoles as the site of deposition of aleurone grains (reviewed by Wieler 1943) and more particularly of the aleurone grains of cereal kernels (see Chaze 1934 and Oksijuk 1961). However, this is not universally accepted as Wieler (1943) considered the possibility of grains arising rather as a coacervate in the cytoplasm, and Muschik (1953) favoured a plastid origin. The present electron microscope study was aimed at investigating the origin and nature of wheat aleurone grains.

II. Methods

Wheat (*Triticum vulgare* cv. Gabo) was grown in a glasshouse. Dates on which anthers first appeared on ears were noted, and kernels harvested at 10, 18, 25, 35, and 40 days (maturity) after anthesis. Thin (0·5 mm) transverse slices were cut midway along the long axis of kernels and fixed immediately in either 2% osmium tetroxide solution, buffered at pH 7·4 with acetate–veronal, or in similarly buffered 2% potassium permanganate solution, at room temperature. Following dehydration, small aleurone-containing portions which were situated laterally in the original kernel were removed from the slices and embedded in "Araldite". Sections cut from the resulting blocks normally contained some pericarp layers, the aleurone layer, and a small amount of starchy endosperm. For light microscopy, free-hand sections were cut, mounted in glycerine, and observed by phase-contrast microscopy.

III. Observations

The appearance of a wheat aleurone cell at 10 days after anthesis is illustrated in Plate 1, Figure 1. Structures normally seen in undifferentiated plant cells (nucleus, mitochondria, plastids with starch granules, Golgi structures, endoplasmic reticulum membranes) are present, although at this stage no vacuoles can be recognized. In addition there are many cytoplasmic inclusions (*U.B.*) seen, after potassium permanganate fixation, as vesicles (0·2–1·0 μ diam.) with bounding membrane, irregular outline, and electron-opaque contents although often a central portion is electron-translucent. These inclusions will be referred to as unidentified bodies (*U.B.*). Experience demonstrated that they were restricted to the aleurone cells and for this reason were useful as indicators when scanning sections.

A section from an aleurone cell at 18 days after anthesis appears in Plate 1, Figure 2. Preparations at this stage of development differed from those of 10 days in having no starch granules and in containing large (up to 2 μ diam.) relatively electron-translucent spherical bodies (*I*) lying within a matrix (*Ma.*) enclosed by a membrane (single arrows). This enclosing membrane is irregular in outline and is comparable with the membrane structure indicated by a single arrow and labelled *V*, which is classed as a vacuole (e.g. see Poux 1962). A further spherical inclusion lying within a matrix bounded by an irregular membrane is illustrated in Plate 1, Figure 3. This micrograph shows more clearly the single unit-membrane nature of this membrane, in contrast to the double unit-membrane of an adjacent endoplasmic

reticulum profile (E.R.). It is thus considered that the new structures are vacuoles which are nearly filled with spherical deposits, and these deposits will be referred to as vacuolar inclusions (*I*). No decision has yet been reached as to whether the membrane-like periphery (double arrows) of the vacuolar inclusions represents an interface effect or a membrane.

Plate 2, Figure 1, shows parts of adjacent aleurone and endosperm cells at the 18-day stage. Aleurone cells are characterized by the unidentified bodies (*U.B.*) and vacuoles containing spherical inclusions (*I*), whereas endosperm cells are characterized by large starch granules and protein deposits. The higher magnification micrograph of an unidentified body (*U.B.*) in Plate 2, Figure 2, reveals that the peripheral dense contents are granular in fine structure, as has been observed for endosperm protein deposits after similar fixation (Buttrose 1963). Plate 2, Figure 3, shows that osmium tetroxide fixation of kernel tissues gave disappointing preservation although the identity of the numerous irregular bodies less than 1 μ diameter with the unidentified bodies (*U.B.*) of potassium permanganate-fixed material is clear. They are not strongly osmiophilic (cf. contents of a vacuole *V* in the same micrograph) and thus are unlikely to be lipid in character.

Sections prepared from 25-day-old tissues differed so greatly in appearance that they were assigned, in roughly equal numbers, to one of two groups of which Figures 4 and 5 of Plate 2 are representative. In Figure 4 there are relatively electron-translucent circular bodies of 3–4 μ diameter which are probably the inclusions (*I*) of the 18-day vacuoles, and also some electron-opaque spherical bodies, possibly in the same vacuoles. Dotted between these large inclusions are numerous small (less than 1 μ diam.), spherical, electron-translucent areas. In Plate 2, Figure 5, both electron-translucent and electron-opaque inclusions (*I*) are again embedded in what is presumably a vacuolar matrix, but between these large inclusions there are no small spherical electron-translucent areas (of Plate 2, Fig. 4) but instead the unidentified bodies (*U.B.*) of earlier stages. The small (less than 1 μ diam.) spherical areas of Plate 2, Figure 4, correspond in size and number with the unidentified bodies (*U.B.*) of Plate 2, Figure 5, so that it is reasonable to equate the two, and it was the variation in appearance of these bodies between replicate preparations that was the outstanding feature of 25-day-old tissue. Their appearance in preparations at later stages of development (Plate 3, Fig. 1, at 35 days) was invariably similar to that of Plate 2, Figure 4, whereas at earlier stages (Plate 1, Fig. 2) to that of Plate 2, Figure 5. It would seem then that 25 days was a transition stage between young and maturing tissue. To test the postulation that the change in appearance of unidentified bodies might be due to changing reaction of aqueous fixation media with progressively more dehydrated tissue, mature aleurone grains were allowed to become hydrated under natural conditions (germination at 25°C for 1 day) and then fixed in potassium permanganate. Dense unidentified bodies (*U.B.*) of resulting preparations, as in Plate 3, Figure 2, are similar to those of younger stages (Plate 1, Fig. 2). Further indications that fixative induced a pronounced alteration in native structure of maturing aleurone cells are (see Plate 2, Fig. 4) the apparent disorganization (arrows) within vacuolar inclusions (*I*) and absence of cytoplasmic membranes. Membranes

appear in Plate 2, Figure 5, and Plate 3, Figure 2, where unidentified bodies (*U.B.*) are normal.

It is concluded that at 25 days aleurone cells are largely filled with "vacuolar units", as in Plate 2, Figure 5, consisting of a matrix with either electron-translucent or electron-opaque spherical inclusions (*I*), an enclosing vacuolar membrane, and covering the entire outer surface a single layer of unidentified bodies (*U.B.*). From this stage on, these bodies are almost wholly confined to the exterior surface of vacuolar units and the inner surface of the plasmalemma (this latter location may be seen in Plate 3, Figs. 1 and 4).

The appearance of aleurone cells at 35 days after anthesis, by which time dehydration was advanced, is shown in Plate 3, Figure 1. The prominent features are what are considered to be vacuolar units which fill most of the space between nucleus and cell wall. Essentially there is no difference between 25-day and 35-day samples, except a deterioration in the quality of structure preservation obtained. The vacuolar units seen in maturing aleurone cells (Plate 3, Fig. 1) contain inclusions either relatively translucent or opaque to electrons, but the "dark" inclusions often appear damaged, with translucent (empty) portions within them. No bounding vacuolar membrane can be seen here, and so to learn whether such a membrane might exist in mature grains, wheat kernels were germinated on moist filter paper for 24 hr at 25°C to hydrate the cells before fixation. The appearance of the expanded vacuolar units after this pretreatment is seen in Plate 3, Figure 2. A membrane immediately interior to the dense unidentified bodies is clearly evident, and it is reasonable to infer that it was present in the mature vacuolar unit and represents the original vacuolar membrane.

It remains to determine which structure seen in the electron microscope corresponds to the aleurone grain of light microscopy. For this reason a phase-contrast light micrograph of mature aleurone grains is shown in Plate 3, Figure 3, and for comparison an electron micrograph at the same magnification in Plate 3, Figure 4. The electron micrograph was made from a preparation of a 1-day-germinated kernel in order to obtain a clearer image than for instance that of Plate 3, Figure 1, but was of aleurone cells which were not noticeably swollen. The dark, round, separated bodies in Plate 3, Figure 3 (arrows), are aleurone grains. They have a diameter of from 2 to 3 μ and seem to be embedded in a ground substance which appears as bright material between the grains. From Plate 3, Figure 4, it is clear that the corresponding unit in electron micrographs is the 2–3 μ vacuolar unit described above, with one reservation. What is questionable is whether the layer of dense unidentified bodies immediately exterior to the vacuolar membrane constitutes part of an aleurone grain, or not. In this connection (cf. Plate 2, Fig. 5; Plate 3, Figs. 1, 2, and 4) it appears that the dense unidentified bodies (*U.B.*) attached to adjacent vacuolar units are often in contact, so that at these points there appears no cytoplasmic ground substance between units. In the light microscope, though, aleurone grains are always clearly separated from each other by the bright ground substance (see Plate 3, Fig. 3), an observation which applies both to dry mature cells and to those following 1-day germination. The inference is that the layer of dense unidentified

bodies does not constitute part of the aleurone grain distinguished by the light microscope but belongs to the ground substance.

IV. Discussion

The evidence of this study suggests that wheat aleurone grains originate as deposits within vacuoles in young aleurone cells at about 2 weeks after flowering, and develop steadily in size and complexity. This conforms with general opinion of the origin of aleurone grains, and does not support the suggestion of Wieler (1943) of a coacervate origin in the cytoplasm, nor that of Muschik (1953) of plastid origin. However, further study will be needed to learn to what degree development of cereal aleurone grains is similar to that of grains in other plants and tissues.

From a morphological standpoint a wheat aleurone grain appears to consist of a peripheral bounding membrane within which is enclosed a ground substance of medium electron-scattering power containing a number of spherical inclusions. In mature tissue there are always both electron-translucent and electron-opaque spherical inclusions, suggesting a heterogeneity in aleurone grain content. There is no obvious indication of what classical aleurone grain descriptions (Pfeffer 1872; see also Oksijuk 1961) refer to as a distinct globoid and crystalloid, but it is clear that the grains are more complex than suggested by Lüdtke (1890, quoted from O'Brien 1895), and conform more closely to the description given by Wieler (1943).

The present study has demonstrated the existence of an unknown structure in wheat aleurone cells, namely the small, dense, unidentified bodies. These bodies are present in large numbers in aleurone cells from very young stages onwards, and towards maturity they are positioned in an orderly way in the cell. Similar bodies are not apparently a prominent feature of other tissues, although comparable cellular inclusions have appeared occasionally in published micrographs, for example of meristematic root cells or root cap cells, when the authors have designated them as unidentified bodies or inclusions (see Mollenhauer 1959; Whaley, Mollenhauer, and Leech 1960; Mollenhauer, Whaley, and Leech 1961; Fabergé and Lewis 1962). The reaction with osmium tetroxide indicates that their contents are not lipoid, and their electron opacity following potassium permanganate treatment points to a proteinaceous or carbohydrate nature. A possibility of the bodies being proteinaceous, on the one hand, is suggested by the recent work of MacLeod and Millar (1962), who postulated the presence of lysosomes which could release hydrolytic enzymes during germination. These authors have pointed out that lysosomes are somewhat lighter than mitochondria, and it will be noted that the unidentified bodies are smaller than mitochondria. Another speculation is that the unidentified bodies have a ribonucleic-compound content, leading from Vazart's (1960) report of these substances in barley aleurone grains. In considering carbohydrate content, on the other hand, certain observations in the present study are worthy of note. It was observed that whereas young aleurone cells are capable of storing carbohydrate in the form of starch granules (Plate 1, Fig. 1), by 18 days starch was rarely seen and at later stages examined it was invariably lacking. That the unidentified bodies contain a non-starch carbohydrate reserve is thus one possibility that might be considered in future chemical and physiological studies.

V. Acknowledgments

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VI. References

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Explanation of Plates 1–3

Except where stated, fixation was made with potassium permanganate. *E.R.*, endoplasmic reticulum; *G*, Golgi structure; *I*, vacuolar inclusion body; *M*, mitochondrion; *Ma.*, vacuolar matrix; *N*, nucleus; *P*, plastid; *Pr.*, protein body; *S*, starch; *U.B.*, unidentified body; *V*, vacuole; *W*, cell wall

Plate 1

Fig. 1.—Portion of an aleurone cell at 10 days after anthesis. The contents are typical of undifferentiated cells, except for the presence of many unidentified bodies (U.B.). ×9000.

Fig. 2.—Portion of an 18-day aleurone cell. Vacuoles with inclusion bodies (*I*) are seen at this stage. Single arrows indicate vacuolar membrane and double arrows the peripheral boundary of vacuolar inclusions. ×8000.

Fig. 3.—Portion of a vacuole and inclusion body (*I*) from an 18-day aleurone cell. Single arrow indicates vacuolar membrane, and double arrow the periphery of the inclusion body. ×18,000.

Plate 2

Fig. 1.—Portions of adjacent aleurone (right-hand side) and endosperm cells at 18 days. Differentiation is striking. ×3000.

Fig. 2.—Detail of an unidentified body (*U.B.*) from an 18-day aleurone cell. × 45,000.

Fig. 3.—Portion of an 18-day aleurone cell. Unidentified bodies (*U.B.*) are not markedly osmiophilic. Osmium tetroxide fixation. ×6000.

Fig. 4.—Portion of a 25-day aleurone cell. Native structure was possibly disrupted badly during fixation. Arrows indicate disruption in vacuolar inclusions. ×2500.

Fig. 5.—Another example of a 25-day aleurone cell, with probably less disruption during fixing. × 10,000.

PLATE 3

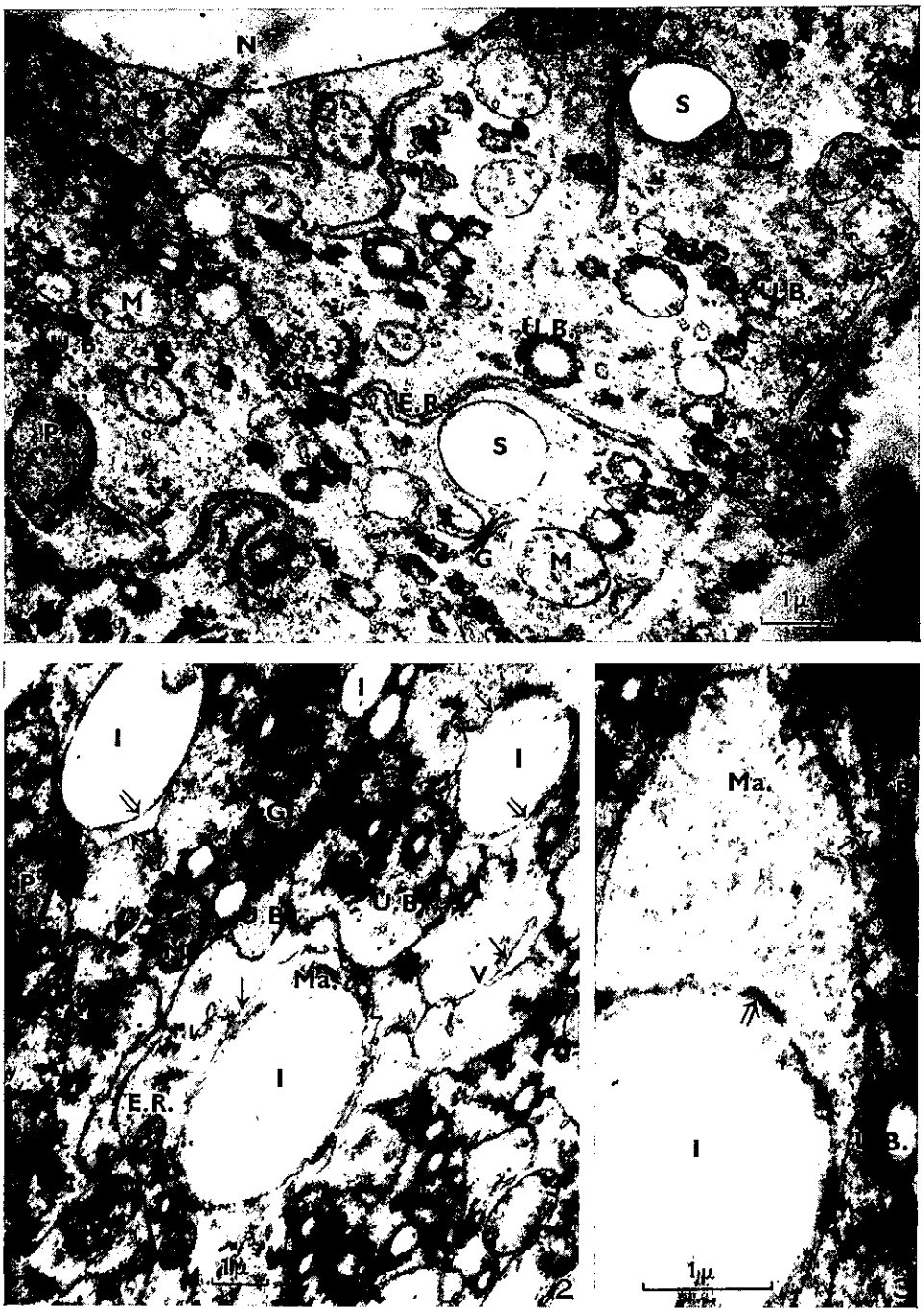
Fig. 1.—Portion of a 35-day aleurone cell. Vacuolar units are prominent between nucleus and cell wall but structural details are not preserved. ×3000.

Fig. 2.—Portion of an aleurone cell from a 1-day-germinated mature wheat kernel. Unidentified bodies (*U.B.*) resemble those seen at 10 and 18 days. Arrows indicate vacuolar membrane. ×7000.

Fig. 3.—Phase-contrast micrograph of a section from the aleurone layer. Arrows indicate aleurone grains. ×2500.

Fig. 4.—Electron micrograph of a section from the aleurone layer. Vacuolar units (arrows) correspond to aleurone grains. ×2500.

ULTRASTRUCTURE OF DEVELOPING ALEURONE CELLS



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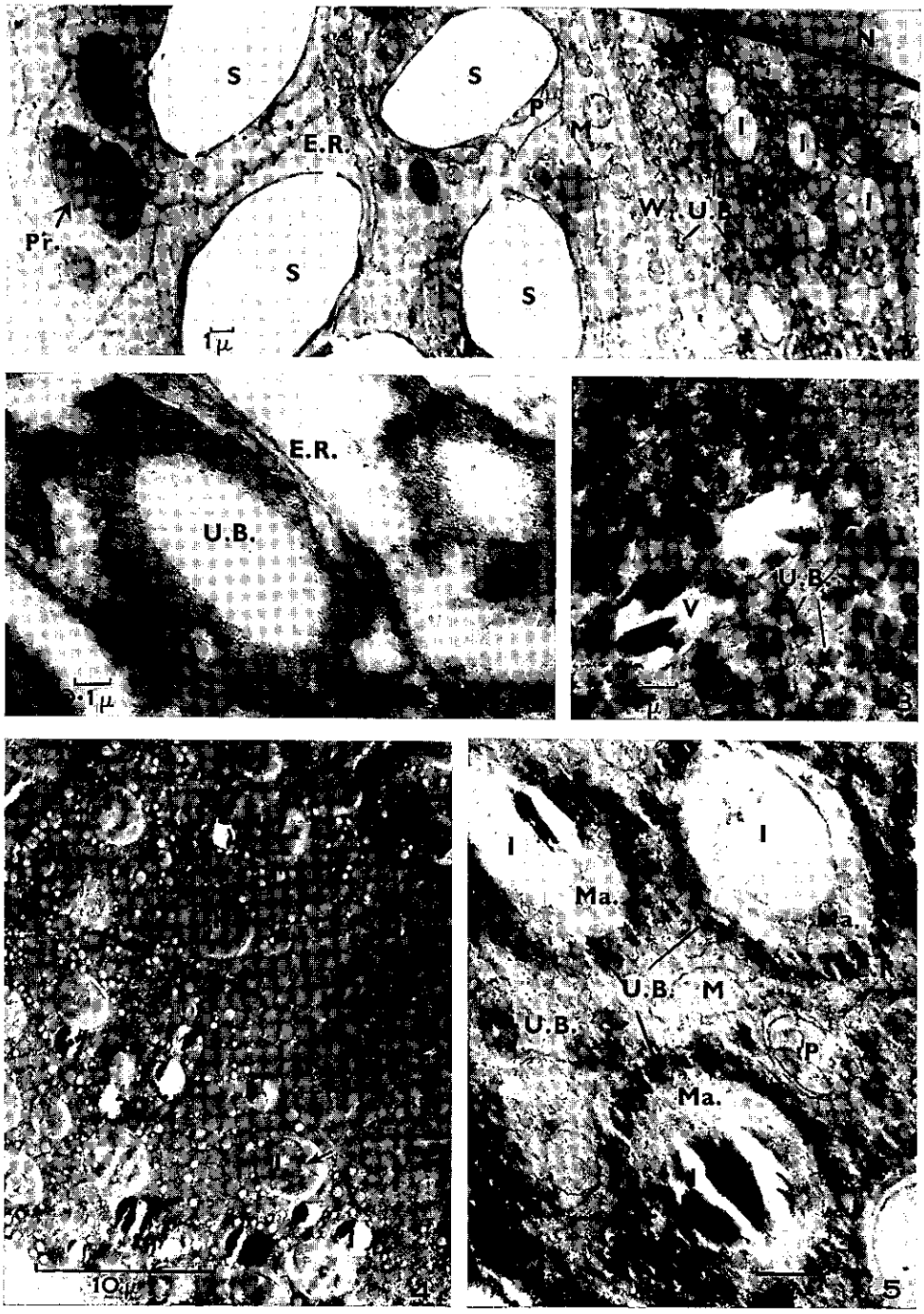

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