ULTRASTRUCTURE OF THE DEVELOPING WHEAT ENDOSPERM

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Summary

The application of modern electron-microscope techniques to the developing endosperm of the variety Gabo wheat (*Triticum vulgare*) demonstrates that before cell walls form between the close-packed free nuclei some undifferentiated spherical bodies are present although none can be classified as plastids, mitochondria, or Golgi complexes. Apparently the initial development of cell walls does not take place by an alignment of vesicles along a plane as occurs in normal meristematically dividing plant tissues. Soon after cell wall formation organelles become recognizable, although their origin is still obscure. Extensive sac-like proliferations of the outer nuclear membrane are seen and a connection with the endoplasmic reticulum membrane system is postulated. A blebbing of the inner nuclear membrane also occurs; nuclear material appears in the proliferations of the outer nuclear membrane and, it is supposed, this material becomes continuous with the ground cytoplasm.

Large starch granules were observed only within plastids, which have a well-defined membrane system organized within the plastid stroma. Small starch granules appear first between large granules and their enclosing plastid membrane, which then extrudes and constricts, releasing the small granules, it would seem, into the cytoplasm. Protein deposits are first observed as spherical granules within membrane-enclosed spaces. Evidence is adduced of a role for the Golgi apparatus in the formation of these deposits.

I. INTRODUCTION

Morphology of the wheat endosperm is of great interest for two reasons. On the one hand endosperm arises from a triple nuclear fusion and there is free nuclear division before cells are formed. Thus it would not be surprising if the morphogenesis of cell constituents differed from that of normal diploid tissues. On the other hand wheat endosperm forms the basis of foodstuffs of great importance, and further knowledge of its development and structure promises greater control over such foodstuffs.

Many detailed microscopical studies of wheat grain morphology have been made, and the work of Bradbury *et al.* (1956) provides a recent example. More specific investigations of starch (Sandstedt 1946) and protein (Hess and Mahl 1954) deposition have also been made with the light microscope, but this instrument can provide only limited information. Hess and co-workers (Hess and Mahl 1954; Hess *et al.* 1955) have gone further and employed the electron microscope to study starch granules and protein in the mature grain. A preliminary report of a combined biochemical and electron-microscope study of wheat endosperm gluten has also appeared (Graham *et al.* 1962). In developing wheat grain, electron-microscope studies appear

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to have neglected endosperm tissues since only embryo (Setterfield, Stern, and Johnston 1959) and scutellum (Hrsel, Wolfova, and Mohelska 1961) tissues are so far recorded.

The present electron-microscope investigation of wheat endosperm development stemmed from the need for more information about the early development of triploid tissue, and the course of starch and protein deposition. Only observations considered characteristic of the tissue studied are reported.

II. Methods

A good quality wheat (*Triticum vulgare* cv. Gabo), extensively grown in southern Australia, was chosen as experimental material. Plants were grown in rich potting compost in earthenware pots in a glasshouse. Dates on which anthers appeared on ears were noted so that endosperm age could be specified. Samples for electron microscopy consisted either of the whole endosperm sac (in the case of endosperms up to 10 days old) or a small block of tissue removed from the centre of endosperm "cheeks" half way along the endosperm length. Material was fixed at 2°C in osmium tetroxide buffered at pH 7·4 with acetate–veronal buffer, or in similarly buffered potassium permanganate at 2°C or 20°C. Following subsequent dehydration it was embedded in either "Methacrylate" or "Araldite" according to standard practice and thin sections prepared and viewed in an electron microscope.

III. Observations

(*a*) *General Development*

The more obvious events following formation of the initial triploid endosperm nucleus are well recorded (Yampolsky 1957). In summary the nucleus divides rapidly without the formation of any enclosing cell walls, so that initially the endosperm sac is lined with free nuclei. At about 2 days after fertilization, cell walls first begin to form between nuclei and subsequently the endosperm rapidly becomes cellular, and further increase in cell number occurs by normal cellular division.

Early stages in endosperm development are shown in Plate 1, Figures 1 and 2. The membranes in such micrographs are not, in general, as clearly defined as those seen in later stages of development (e.g. Plate 1, Fig. 3; Plate 2) and it is impossible to decide whether this is entirely due to inherent structural differences which have been faithfully preserved, or partly to poor fixation of very young tissue. However, the ability to resolve the nuclear envelope to a double membrane (Plate 1, Fig. 1, inset) and the sharp appearance of some membranes in Plate 1, Figure 2, indicate that fixation was adequate.

A stage just before cell wall formation is illustrated in Plate 1, Figure 1. Nuclei are closely packed and there are relatively few discrete bodies in the restricted internuclear spaces. The spherical to oval bodies in such micrographs lack a recognizable fine inner structure such as permits a classification to mitochondria or plastids in other plant tissues. There is some resemblance of the membrane complex indicated by a double arrow to Golgi complexes, but the resolution of the membranes is inadequate to see whether they represent sections of cisternae. The isolated membrane

profiles do not have the double unit membrane organization typical of endoplasmic reticulum (cf. Plate 1, Fig. 3), and consequently have not been classified as parts of this system. Plate 1, Figure 2, shows a slightly later stage of development, when cell wall formation between nuclei has begun. At this stage spherical bodies and membrane profiles between nuclei and cell walls can be no better classified than those of earlier stages. When wall formation is complete cells increase rapidly in volume and easily recognizable plastids, mitochondria, and Golgi complexes appear. Portion of a young expanding cell is shown in Plate 2. It is noteworthy that endoplasmic reticulum profiles as normally seen in plant cells (Whaley, Mollenhauer, and Leech 1960) have not yet appeared. A still later stage in development is illustrated in Plate 1, Figure 3, which shows a small area at the border between two cells. All inclusions normally present in plant cells can now be seen—plastids containing tubular membrane structures and starch granules, mitochondria with cristae, Golgi complexes, and profiles of the endoplasmic reticulum. During further development until maturity endosperm cells fill almost entirely with starch and protein deposits, a consequence of which is the technical difficulty of obtaining preparations for electron microscopy. An example of portion of a cell at about the latest stage at which a reasonably clear image can be obtained (3 weeks after fertilization—maturity reached at 6 weeks) is shown in Plate 3, Figure 1. Starch and protein (see Section III(*e*)) deposits occupy most of the space.

(*b*) *Organelle Development*

The largest bodies (up to 2 μ diameter) seen in preparations such as Plate 1, Figure 1, are often immediately adjacent to the nuclear envelope, but no connections between their limiting membrane and that of the nucleus have been observed (e.g. Plate 1, Fig. 1, inset). The electron-translucent areas seen in many bodies in Plate 1, Figures 1 and 2, might be taken to represent starch, which, under similar fixation conditions, normally appears electron-translucent in the electron microscope (see Plate 1, Fig. 3). However, these areas lack a clear rounded outline normally associated with starch deposits, and it is felt they represent either some other carbohydrate material or highly aqueous areas. On this ground the bodies concerned cannot be definitely classed as plastids. The similarity in appearance of the peripheral, denser material in these bodies and membranous structures adjacent to developing walls (Plate 1, Fig. 2) permits the speculation that they have a role in cell wall deposition (cf. pectin vesicles of Buvat and Puissant 1958).

A close study of Plate 2 revealed that the outer nuclear membrane (*NE*) extends at places (single arrows) to a greater or lesser extent into the cell away from the nucleus (this extension is also demonstrated in Plate 3, Fig. 2). In three dimensions this outer nuclear membrane probably forms voluminous sacs accounting for a considerable proportion of the cell volume. Within these sacs lie what in section appear to be numerous vesicles (av. diam. about 1 μ) containing material denser to electrons than the sac ground substance. Moreover, the contents of these apparent vesicles appear denser than the ground cytoplasm (in which lie plastids, mitochondria, and Golgi complexes), which might be interpreted as meaning that they are not sections of tubular extensions of ground cytoplasm into nuclear envelope

sacs but distinct vesicular inclusions. However, these vesicles sometimes show organized membrane structures (*I* and *II*), and in this respect have an affinity to cytoplasm. At some points (e.g. double arrow) there appear to be continuities through cell walls, and thus a continuity between nuclear envelope sacs of adjacent cells is possible.

In Plate 3, Figure 3, a somewhat more developed cell is illustrated (a portion of which may be seen in greater detail in Plate 3, Fig. 2). The extent of the outer nuclear membrane extrusion is greatly reduced, and frequently only narrow profiles are seen in section. The whole can be envisaged as a continuous reticular system and is tentatively classified as endoplasmic reticulum. In these young endosperm cells quite complicated extensions of the nuclear envelope have been observed, involving either both membranes or only the outer one (Plate 4, Fig. 1). The endoplasmic reticulum itself, after the stage illustrated in Plate 3, Figure 3, appears next as fragmentary cisternal elements (Plate 4, Fig. 2) or as more flattened profiles as seen in Plate 1, Figure 3, and Plate 4, Figure 3. An example of the extreme concentration of endoplasmic reticulum occasionally noticed in endosperm cells is seen in Plate 4, Figure 3. In stages as advanced as this (7 days after fertilization) continuities between endoplasmic reticulum and nuclear envelope are seldom seen.

In Plate 3, Figure 2, there is seen what is interpreted as an extrusion or bleb of the inner nuclear envelope (see arrow), presumably enclosing nuclear contents, into the sac formed by extension of the outer membrane. This raises the question as to whether other vesicles within this sac, as well as similarly situated vesicles of Plate 2, arise in a similar way by blebbing of the inner nuclear membrane.

(*c*) *Cell Wall Development*

The first indications of cell wall formation may be recognized in Plate 1, Figure 1, where relatively electron-lucid, elongated areas (single arrows), often with membrane profiles oriented parallel to them and in close proximity, lie between adjacent nuclei. Plate 1, Figure 2, shows a later stage of cell wall development. Again there are only a few membrane profiles tending to be parallel to the forming wall, and behind these membranes are those bodies suggested earlier as conceivably concerned in wall formation. There is no evidence that wall initiation in these cells is represented by a confluence of vesicles in a plane, as observed in mitotically dividing cells (Buvat and Puissant 1958; Porter and Caulfield 1960). The middle lamella in older endosperm cell walls is unusual in often appearing in high contrast to the remainder of the wall, after both potassium permanganate and osmium tetroxide fixation (Plate 4, Figs. 4 and 5).

(*d*) *Starch Granule Development*

From light-microscope studies it is known that wheat starch granules are oblate spheriods in shape, so that views of them may be described as "side-on" or as "face-on". Plate 4, Figure 6, offers an example of a side-on view of a young starch granule which appears as a bivalve, and so it is interpreted that a deep groove runs round its periphery in conjunction with membrane complexes in the adjacent plastid

stroma. A face-on view, when the granule appears circular, is shown in Plate 5, Figure 1, and it is observed that a membrane complex extends round the circumference between granule and bounding plastid membrane. Another micrograph showing the membrane complex in greater detail appears in Plate 5, Figure 2. The plastid envelope consists of two single (unit) membranes, the inner one being invaginated into the plastid stroma at points, giving rise to a possibly continuous interconnected membrane reticulum. This extensive proliferation of the inner plastid membrane

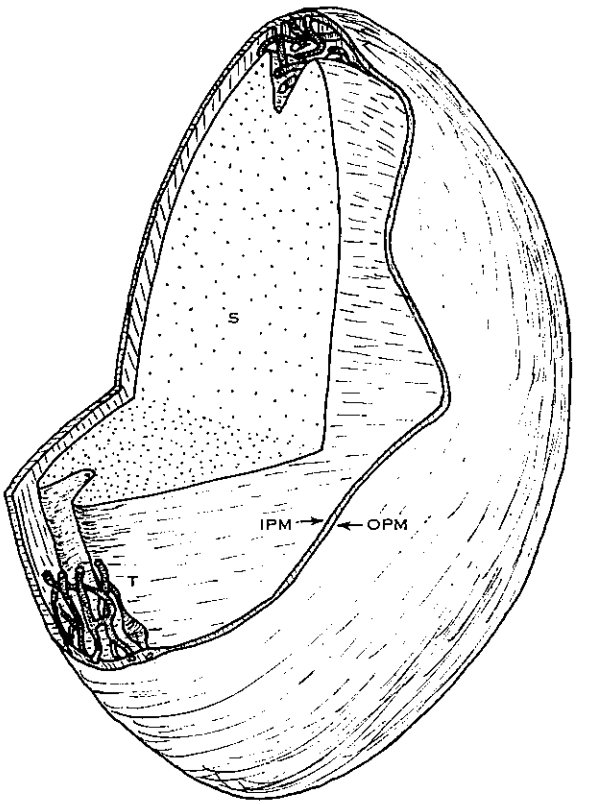

Fig. 1.—A three-dimensional diagram illustrating the structure of the developing wheat endosperm amyloplast. Features indicated are starch (*S*), outer plastid membrane (*OPM*), inner plastid membrane (*IPM*), and tubular invaginations of the inner plastid membrane (*T*).

is confined to a very limited portion of the amyloplast. The structure of the developing wheat amyloplast, as interpreted from the micrographs, is illustrated diagrammatically in Figure 1. The findings are essentially the same as those recorded for starch granules of the barley endosperm (Buttrose 1960).

In each plastid of newly formed cells there occurs the initiation of one starch granule which grows rapidly in size, followed later (at about 2 weeks after fertilization) by the initiation of small granules in the stroma space (see Plate 3, Fig. 1;

and Buttrose 1960). The amyloplast membrane extrudes into the cytoplasm carrying small granules which are released from the parent amyloplast by membrane constriction. Plate 5, Figure 3, illustrates an extrusion, and Plate 5, Figure 4, shows small granules after release. Further examples may be found in Plate 3, Figure 1.

(e) *Development of Protein Deposits*

Reference has already been made to protein deposits shown in Plate 3, Figure 1. These deposits have been classified as protein because of their medium electron-scattering power (cf. zymogen granules of Sjöstrand and Hanzon 1954), and because they occupy a volume consistent with the protein content of wheat endosperm (15% on a dry weight basis—see Kent-Jones and Amos 1957). Normally the contents of deposits are homogeneous (Plate 3, Fig. 1; Plate 6, Figs. 3 and 4; Plate 7, Fig. 1), but occasionally portions with higher electron-scattering power are found embedded in them (Plate 6, Fig. 1, arrows). The deposits usually appear to have a granular fine structure, with individual particles of approximately 100 Å diameter (Plate 7, Fig. 1). Protein deposits in the developing endosperm appear as spherical to oval granules from $0 \cdot 1\ \mu$ up to $8\ \mu$ in diameter, and usually appear to be enclosed loosely within large sacs delimited by a single membrane (e.g. Plate 6, Fig. 2).

Protein granules as defined here can be recognized at 1 week after fertilization or earlier, when structures, such as illustrated in Plate 6, Figure 2, appear. In section these deposits ($1\ \mu$ diameter) are normally surrounded on the periphery by small ($< 0 \cdot 1\ \mu$) vesicles, which must therefore occur over the entire surface of the granule. Sections interpreted as surface or near-surface views of protein granules are indicated (single arrows) in Plate 6, Figures 2, 4, and 5. An association of small vesicles with the surface of developing granules appears invariable.

Most protein granules in Plate 6, Figure 2, lie within a single membrane (*SM*), but one towards the top right-hand corner is not enclosed. A further example of granules without an enclosing membrane is seen in Plate 6, Figure 3, the conclusion being based on the observation that a mitochondrion appears in the same space and this organelle has never been observed within a larger membrane-enclosed space. Such cases, however, form a minority.

Osmium tetroxide fixation of endosperm material is generally unsatisfactory, but for comparison an example with four protein granules within a large bounding membrane is shown in Plate 6, Figure 4. Ribonucleoprotein granules in the surrounding cytoplasm are poorly defined, but examination of this and many other micrographs has not revealed an association of any ribonucleoprotein granules and membranes enclosing several protein granules (see also Graham *et al.* 1962).

Small vesicles similar to those already noted as being associated with developing protein granules have also been observed in other situations. Thus they are often seen outside the membrane enclosing protein granules (double arrows in Plate 6, Fig. 2) or within it but at some distance from the deposits (double arrows, Plate 6, Fig. 5). Similar vesicles are also commonly associated with the Golgi apparatus, for example the cisternae of the Golgi complex near the centre of Plate 7, Figure 2, have the usual vesiculated terminations, one of which (see arrow) clearly contains a

dense deposit. Adjacent to this complex are numerous vesicles with similar dense inclusions. Again, Plate 7, Figure 3, illustrates similar vesicles at higher magnification and it is considered that their dense contents (see single arrows) are similar in appearance to contents of protein granules. Further support for this conclusion can be drawn from Plate 7, Figure 4, which shows at still higher magnification a recognizable protein deposit close to a small vesicle (arrow). Similar vesicles are indicated in Plate 6, Figures 2 and 3 (double arrows). A possible relationship between these vesicles and protein deposition is discussed below.

IV. Discussion

(*a*) *General Development*

Electron-microscopic study confirms the findings of light microscopy on the grosser aspects of endosperm development. Furthermore significant new information concerning organelle, cell wall, and reserve deposit formations is provided, and these subjects are now discussed separately.

(*b*) *Organelle Development*

Comparison of well-differentiated mitochondria of Plate 1, Figure 3, and Plate 4, Figure 2, with the simpler form seen in Plate 2 suggests that endosperm mitochondria undergo marked development in the early stages. It is thus conceivable that the smaller bodies seen for example in Plate 1, Figure 1 (bottom left), represent pro-mitochondria. Similarly it must be conceded that the larger bodies seen in Plate 1, Figures 1 and 2, might be plastid initials. Were this so, plastids and mitochondria might be derived by division from organelles associated with the original polar nuclei of the ovary, conforming with the continuity theory of Schimper (1883). However, the observed morphogenetic development would be equally in keeping with a *de novo* origin of organelles, as proposed by Mühlethaler and Frey-Wyssling (1959). Furthermore certain observations are consistent with a third proposal made recently by Mühlethaler and Bell (1962), namely that plastids and mitochondria may arise as blebs from egg cell nuclei (see also Hoffman and Grigg 1958). Thus bodies in Plate 1, Figures 1 and 2, may not be organelle initials, whereas vesicular inclusions within nuclear envelope sacs, which might be derived as blebs from the nucleus (Plate 3, Fig. 2), sometimes contain organized membrane structures (Plate 2, *I* and *II*) which might be organelle initials. It is worthy of note that both egg nuclei and the first endosperm nuclei are to some extent comparable, in that they are associated with the initiation of new genetic tissue, and thus differ from those of meristematic cells. These three possibilities have been mentioned because it would seem that very young endosperm tissue shows features of organelle development which promise, when studied in greater detail, to be valuable in deciding the organelle origin controversy.

Development of the endoplasmic reticulum, as interpreted from the evidence of electron micrographs such as the series Plate 1, Figure 1, Plate 2, and Plate 3, Figure 3, to Plate 4, Figure 2, is illustrated diagrammatically in Figure 2. Further study is needed to reveal the sequence between Figure 2*a* and Figure 2*c*. During the

free nuclear stage the nuclear envelope appears to consist of a compact double membrane (Plate 1, Fig. 1 and inset), but at two points (labelled *A*) there appear to be spaces between the two membranes, and possibly these represent beginnings of outer nuclear membrane proliferations. Whereas the nucleus appears to contribute material (possibly ribonucleoproteins) within vesicles to this sac, the evidence is insufficient to decide whether the contents of all such vesicular inclusions derive from the nucleus. While the sum total of vesicular inclusions increases in volume there is a concurrent decrease in remaining space within nuclear membrane sacs until these appear as thin layers or profiles, which in turn may break away from the nucleus. As a speculation such profiles are proposed as initials of the endoplasmic reticulum system. If this interpretation is correct, and if vesicles within nuclear

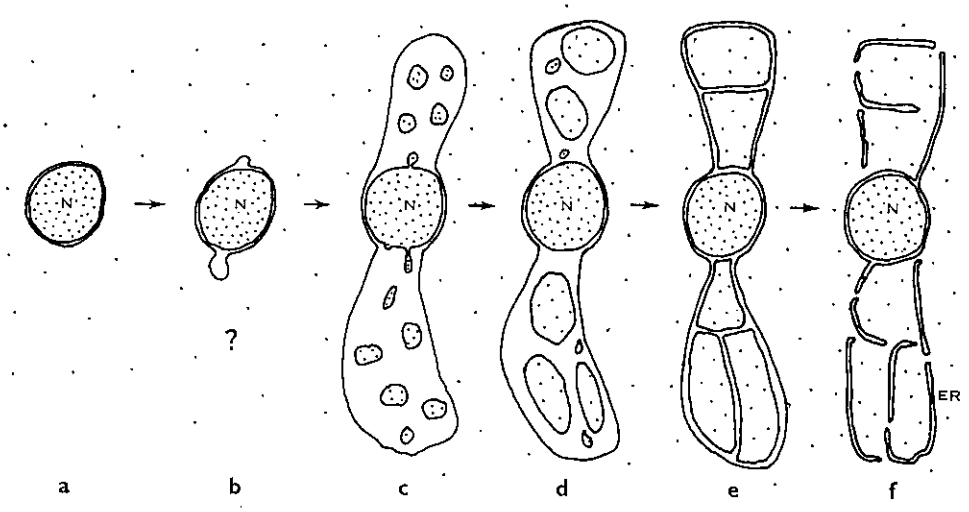

Fig. 2.—Diagram illustrating the postulated formation of endosperm endoplasmic reticulum (*ER*) from the nuclear (*N*) membrane.

membrane sacs derive at least in part as nuclear blebs, one membrane of an endoplasmic reticulum profile may be derived from the original outer nuclear membrane, and the other from the original inner nuclear membrane. In this case it would be equally possible for part of the outer nuclear membrane of fully differentiated cells (Fig. 2, stages *e* and *f*) to be derived indirectly from the original inner membrane.

The cell membrane in animal cells has been suggested as a source of endoplasmic reticulum (Palade 1955) but most evidence points to the nucleus as the origin (Haguenau 1958). An apparent unity of nuclear and endoplasmic reticulum membranes has been well demonstrated in mitotically dividing plant cells by Porter and Machado (1960). In such material, however, endoplasmic reticulum membranes are continuously present, and the evidence supports the suggestion of development of nuclear envelope from endoplasmic reticulum, rather than vice versa. The present work, while confirming the close relationship of the two membrane systems, supports the alternative suggestion, namely that endoplasmic reticulum develops from the nuclear membrane.

(c) *Cell Wall Development*

The observations that middle lamella formation in the endosperm and its staining properties are different from other cells is not unexpected, since anomalies based on chemical and histological investigation have been reported already by MacLeod and McCorquodale (1958). These workers found that pectic substances are absent from endosperm cells of *Bromus* spp., and that intercellular cementing material may be either lacking or very water soluble. They also found pectin absent from barley endosperm cell walls, and the intercellular cementing material was in certain cases concluded to be proteinaceous. The well-defined middle lamellae observed in the present study may then possibly represent a layer of water-soluble or proteinaceous material.

(d) *Starch Granule Development*

The present findings for wheat endosperm are similar to those for barley which were reported and discussed in an earlier paper (Buttrose 1960).

(e) *Development of Protein Deposits*

Dalton and Felix (1956) suggested that the Golgi apparatus had a generalized function in removing water and condensing, into visible deposits, molecules synthesized elsewhere. There are many instances of the Golgi apparatus being involved in condensation of protein secretions in animal tissues (Sjöstrand and Hanzon 1954; Burgos and Fawcett 1955; Farquhar and Wellings 1957; Bargmann and Knoop 1959; Hollmann 1959; Sano and Knoop 1959; Wellings and Deome 1961; Rhodin and Terzakis 1962), and recently Wellings and Deome (1961) have proposed a scheme in which the Golgi apparatus condenses protein molecules synthesized in the vicinity of the endoplasmic reticulum, and discharges the deposits within Golgi vesicles. Reports have also appeared of the probable role of the Golgi apparatus in formation of secretions in plant cells (Schnepf 1960, 1961; Vogel 1960; Mollenhauer, Whaley, and Leech 1961). The evidence of the present study, that protein deposition is linked through the small vesicles to the Golgi apparatus, provides yet another instance wherein a condensing function of this apparatus may be postulated. The small vesicles covering the surface of protein deposits vary greatly in size, shape, and staining properties, and to what extent they are derived from the Golgi apparatus is uncertain. Nevertheless it is significant that membranes of the Golgi apparatus may appear thick and in high contrast after potassium permanganate fixation (Mollenhauer and Zebrun 1960), since vesicle membranes (e.g. Plate 7, Fig. 1) often show these same characteristics whereas most other cell membranes do not.

Although it is clear that protein granules are often formed within spaces enclosed by single membranes, and so perhaps isolated from the general cytoplasm, it is not clear how these membranes arise. Golgi complexes have been observed in other studies within similar enclosed spaces and Golgi vesicles were noted above to lie within sacs containing protein granules (Plate 6, Fig. 2; Plate 7, Figs. 3 and 4), suggesting that the ground substance of such sacs may correspond to cytoplasm rather than for instance to endoplasmic reticulum contents. Further, the bounding

membrane appears free of associated ribonucleoprotein granules, so that the deposits cannot be equated with the intracisternal granules observed in barley endosperm under certain conditions (Buttrose, Frey-Wyssling, and Mühlethaler 1960). In addition, more confusion is introduced by the observation that some deposits are formed, and lie apparently free, in the cytoplasm. The problem is therefore raised for further study as to whether lack of an enveloping membrane is fortuitous, or whether naked deposits represent a different form of protein.

It is now recognized (Pence, Mecham, and Olcott 1956) that the protein separated from wheat flour (gluten) can be characterized into more specific fractions than the two protein types, gliadin and glutenin, defined by Osborne (1907), and recently Simmonds and Winzor (1961) have separated 11 distinct protein fractions from acetic acid-soluble proteins of wheat flour. In the electron microscope, however, no morphological variations have been observed corresponding to such heterogeneity, either within a protein granule or between granules. Only in rare cases (Plate 6, Fig. 1) is evidence of heterogeneity observed, and apart from this type the only other variation noted involves the presence or absence of an enveloping membrane. It is therefore likely that a range of different molecular types is deposited together in the one protein granule. A similar proposal has been made for protein granules of maize (Duvick 1961).

The importance of lipoprotein films in contributing to the properties of wheat gluten (see Grosskreutz 1961) warrants a consideration of lipid origin. Young endosperm cells contain no discrete lipid deposits, as are often found (Whaley, Mollenhauer, and Leech 1960) in plant tissue. Although such deposits may be laid down at a later stage, it is equally possible that the phospholipids occurring in crude gluten are derived from membranes (lipoproteins) in the cell. It can be understood from Plate 3, Figure 1, how, as protein granules grow together, membranes may become entrapped within the whole deposit. Cases have also been observed of small vesicles, such as those associated with protein granule surfaces, becoming similarly entrapped. These membranes may account for at least some of the phospholipid.

From light- and electron-microscope studies Hess and co-workers (Hess and Mahl 1954; Hess *et al.* 1955) concluded that wheat endosperm protein could be classified as either wedge (Zwickel) or adhesive (Haft) protein. Wedge protein refers to the wedge-shaped deposits separated from flour and which lay between starch granules, whereas adhesive protein refers to fibrillar deposits adhering to starch granule surfaces. Hess (1960) has further proposed a structural relationship of protein, lipid, and starch in wheat flour, in which wedge protein deposits are surrounded by a lipoid (and lipoprotein) layer, beyond which in turn lie adhesive protein layers and corresponding starch granules. Comparing these proposals with the observations of the present study leads to the suggestion that wedge protein of flour corresponds to the discrete protein deposits, while adhesive protein corresponds to a combination of desiccated plastid stroma, plastid membrane with its proliferations, and possibly in addition adjacent cytoplasmic membranes and ground substance. The fibrillar structure of adhesive protein observed by Hess and Mahl (1954) is doubtless due to desiccation of non-fibrillar soluble proteins and lipoprotein membranes. The clean separation often obtained of wedge protein, which according

to Hess' (1960) scheme might be due to a lipid or lipoprotein layer surrounding it, could, according to the present proposals, be due to the membrane (lipoprotein) surrounding discrete protein deposits. Finally, attention is drawn to the fact that no evidence has been obtained to substantiate the claim of Hanssen (1958) that wheat endosperm protein has a lamella structure.

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VI. References

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EXPLANATION OF PLATES 1–7

Except where stated, fixation was made with potassium permanganate. *ER*, endoplasmic reticulum; *G*, Golgi apparatus; *GV*, Golgi vesicle; *M*, mitochondrion; *N*, nucleus; *NE*, outer membrane of nuclear envelope; *P*, plastid; *Pl*, plasmalemma; *PM*, plastid membrane; *Pr*, protein deposit; *S*, starch; *SM*, protein granule sac membrane; *W*, cell wall

PLATE 1

Fig. 1.—Portion of the endosperm at the free nuclear stage. Single arrows indicate the first signs of wall formation, the double arrow a complex resembling a Golgi apparatus; in addition expanded areas between the two nuclear membranes (*A*) are indicated. ×5000. Inset ×17,000.

Fig. 2.—Portion of a very young endosperm at a slightly later stage than that shown in Plate 1, Figure 1. Cell walls, indicated by arrows, are becoming evident. ×5000.

Fig. 3.—View of a more differentiated stage in which most inclusions usually encountered in plant cells are seen. ×10,000.

PLATE 2

Showing a stage when cell walls are fully formed and cells are starting to expand. Points at which the outer nuclear membrane extends into the cytoplasm are indicated by single arrows, and continuities between resulting sacs of different cells by double arrows. Labels *I* and *II* indicate vesicular inclusions of these large sacs which themselves contain organized membrane structures. ×8000.

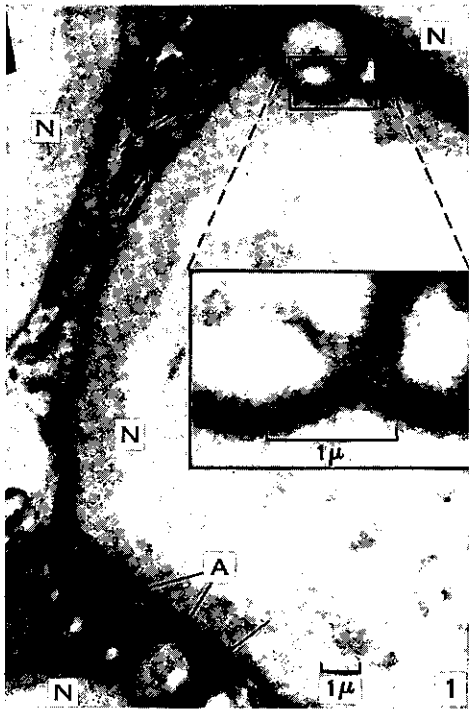
PLATE 3

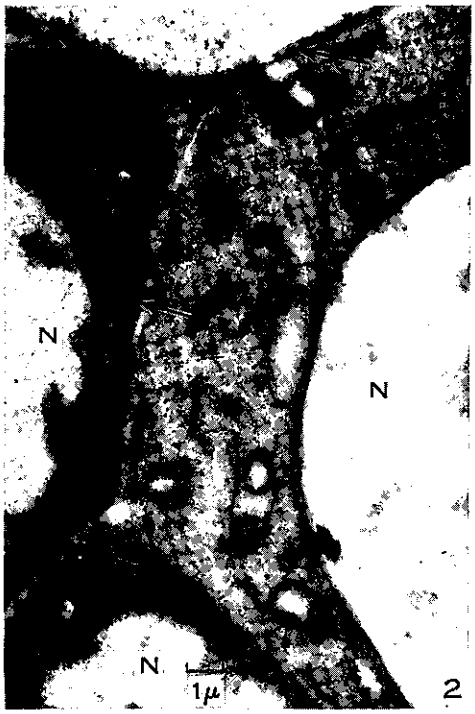
Fig. 1.—Typical appearance of a more advanced endosperm cell, showing the extent of starch and protein deposition. ×12,000.

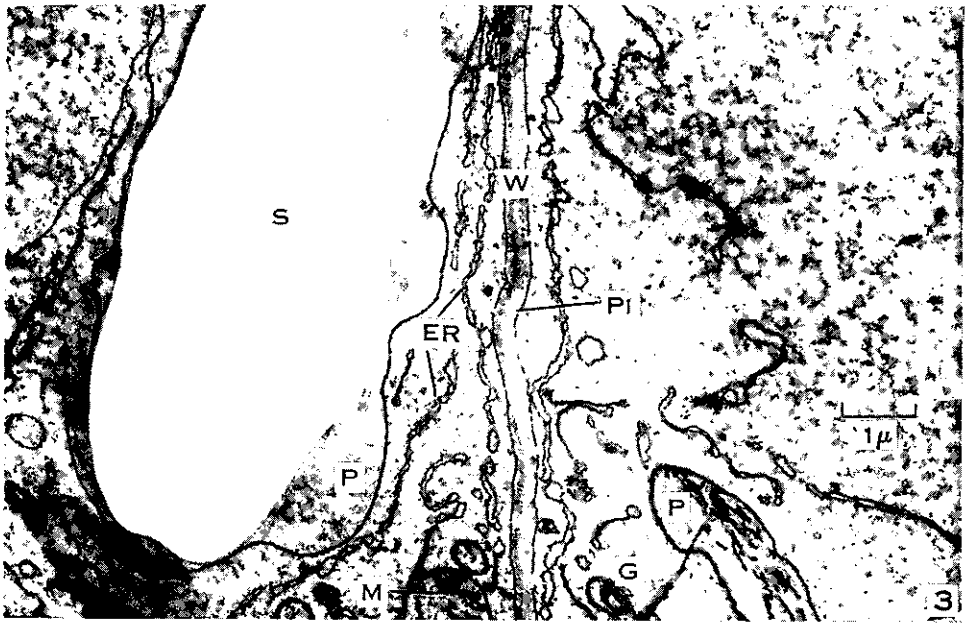
Fig. 2.—The edge of a nucleus (same section as Plate 3, Fig. 3) showing proliferation of the outer nuclear membrane, and a bleb arising from the inner membrane (arrow). ×10,000.

Fig. 3.—View of a nucleus and associated "endoplasmic reticulum" at a slightly later stage than in Plate 2. ×6000.

ULTRASTRUCTURE OF DEVELOPING WHEAT ENDOSPERM







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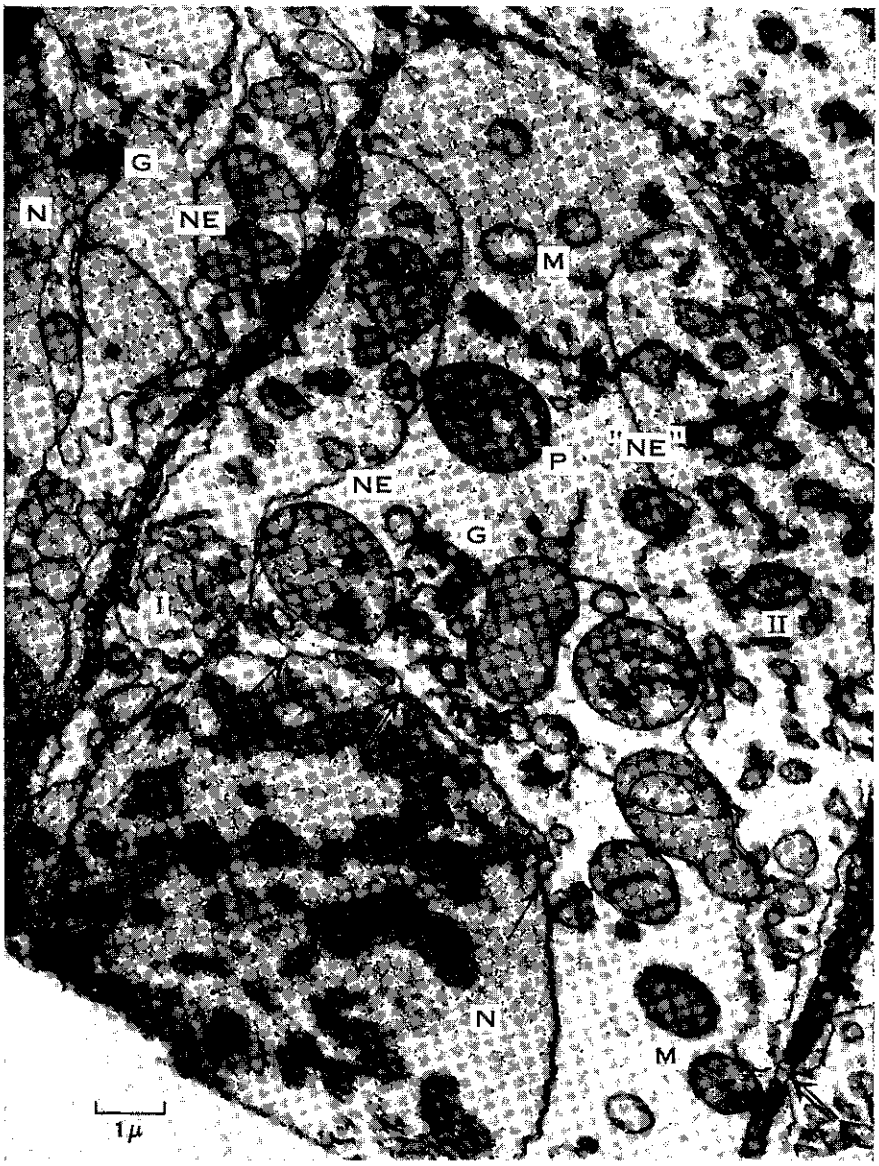

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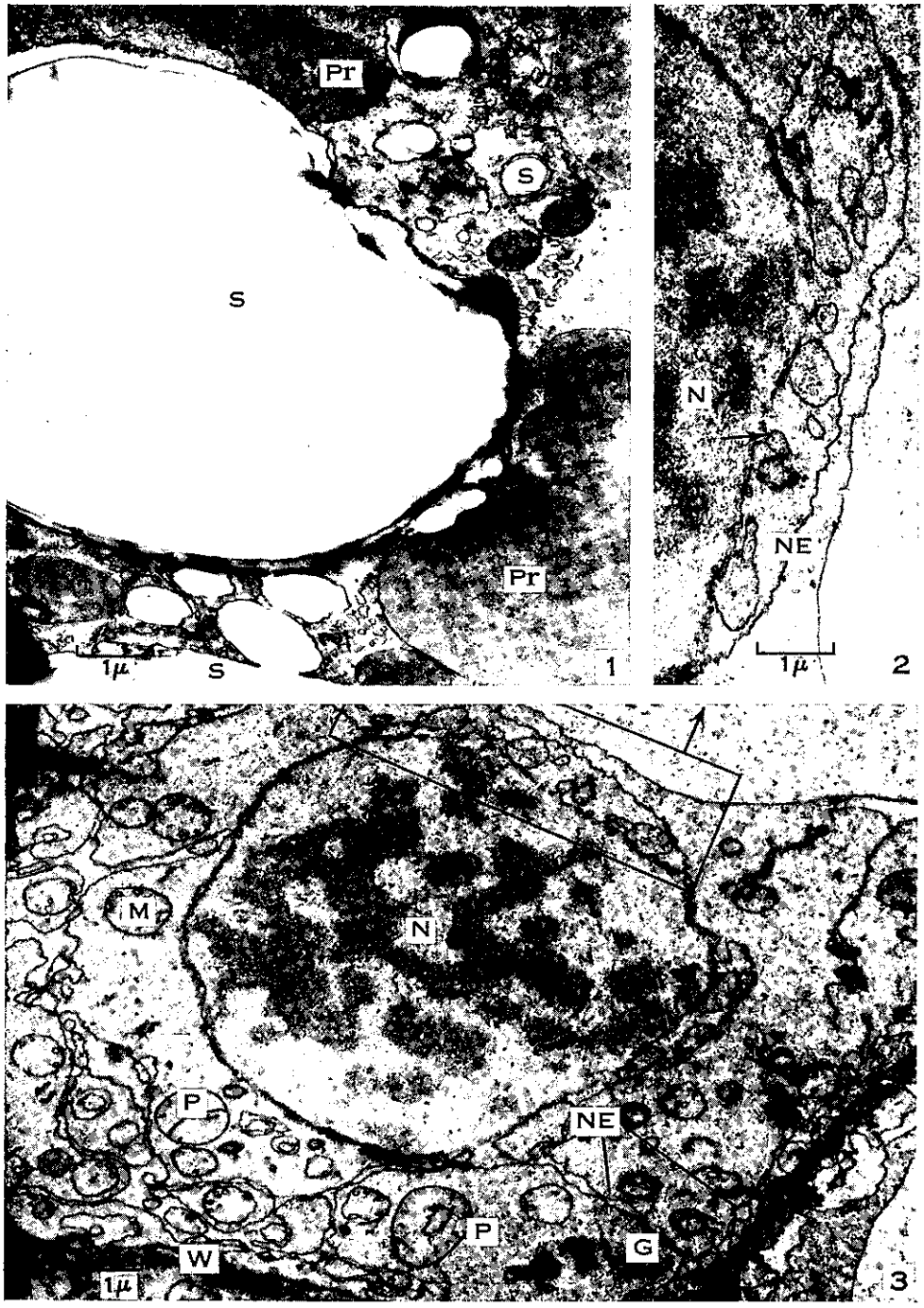

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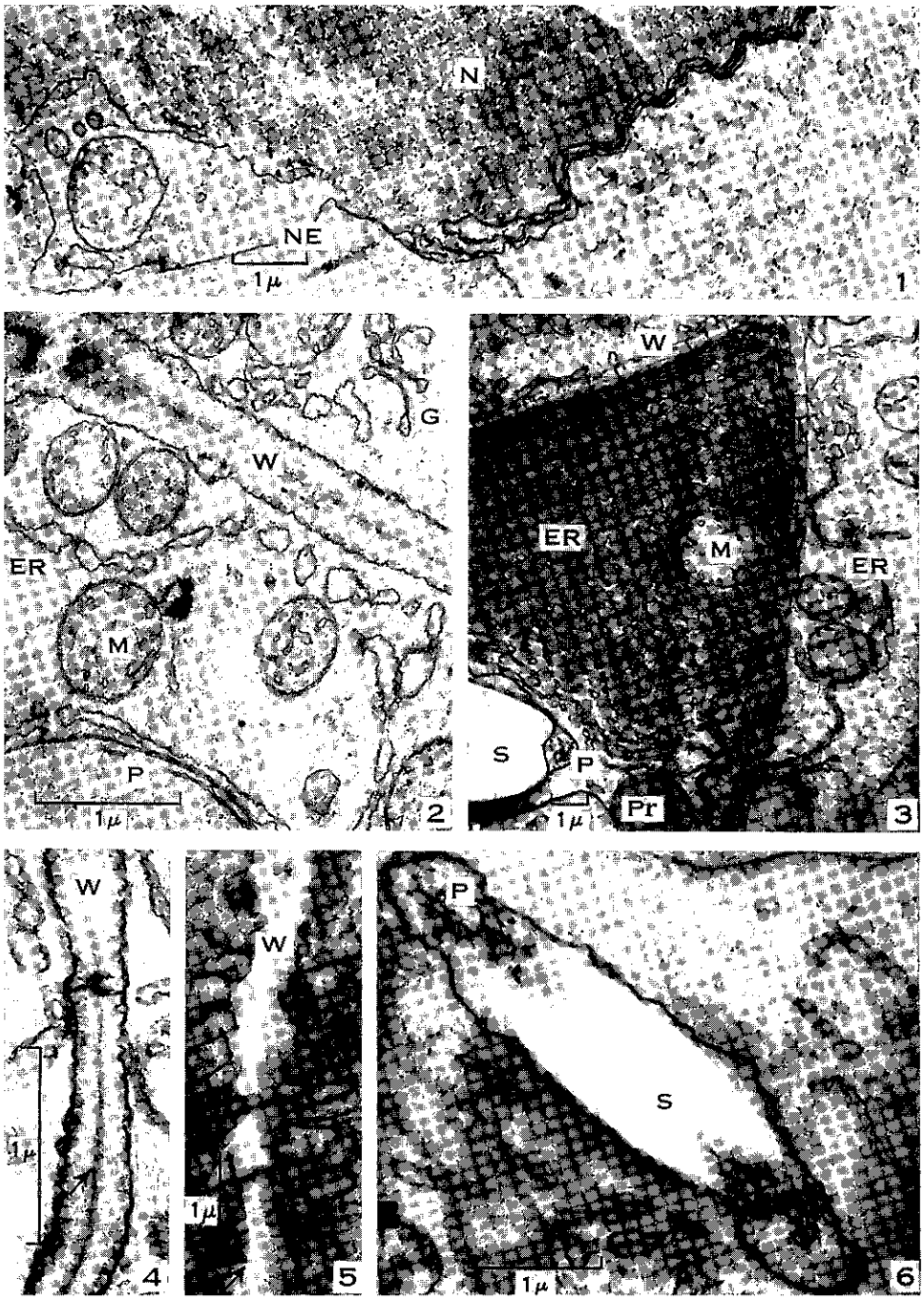

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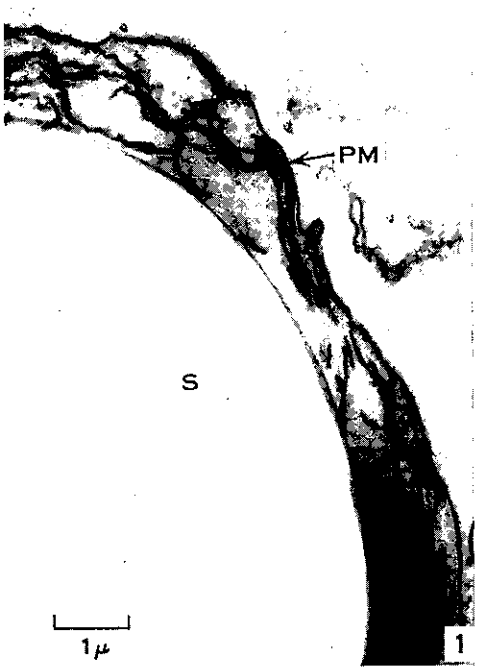

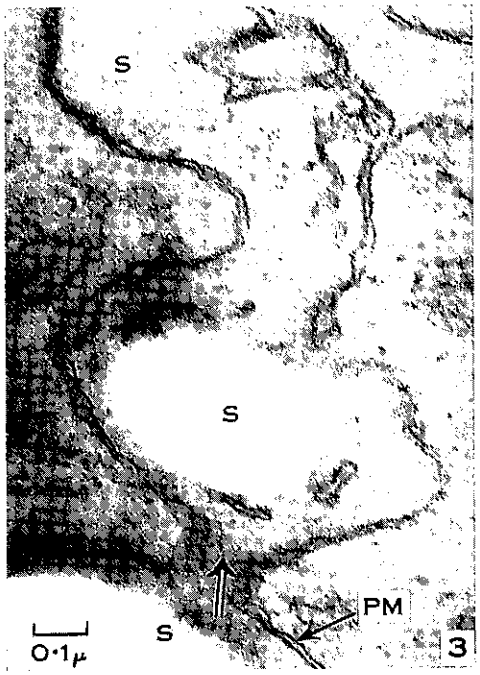

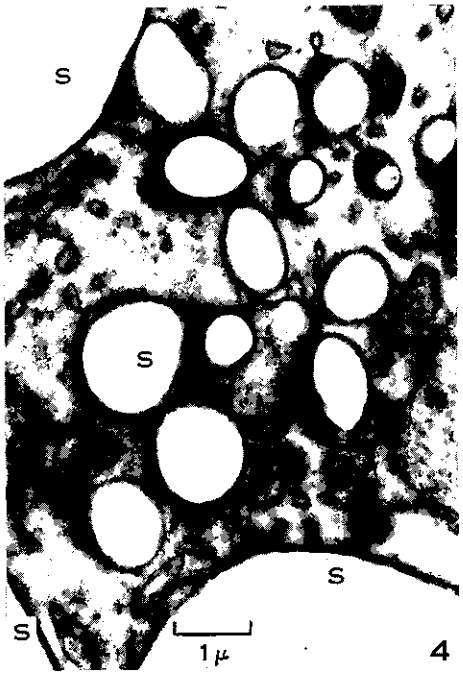

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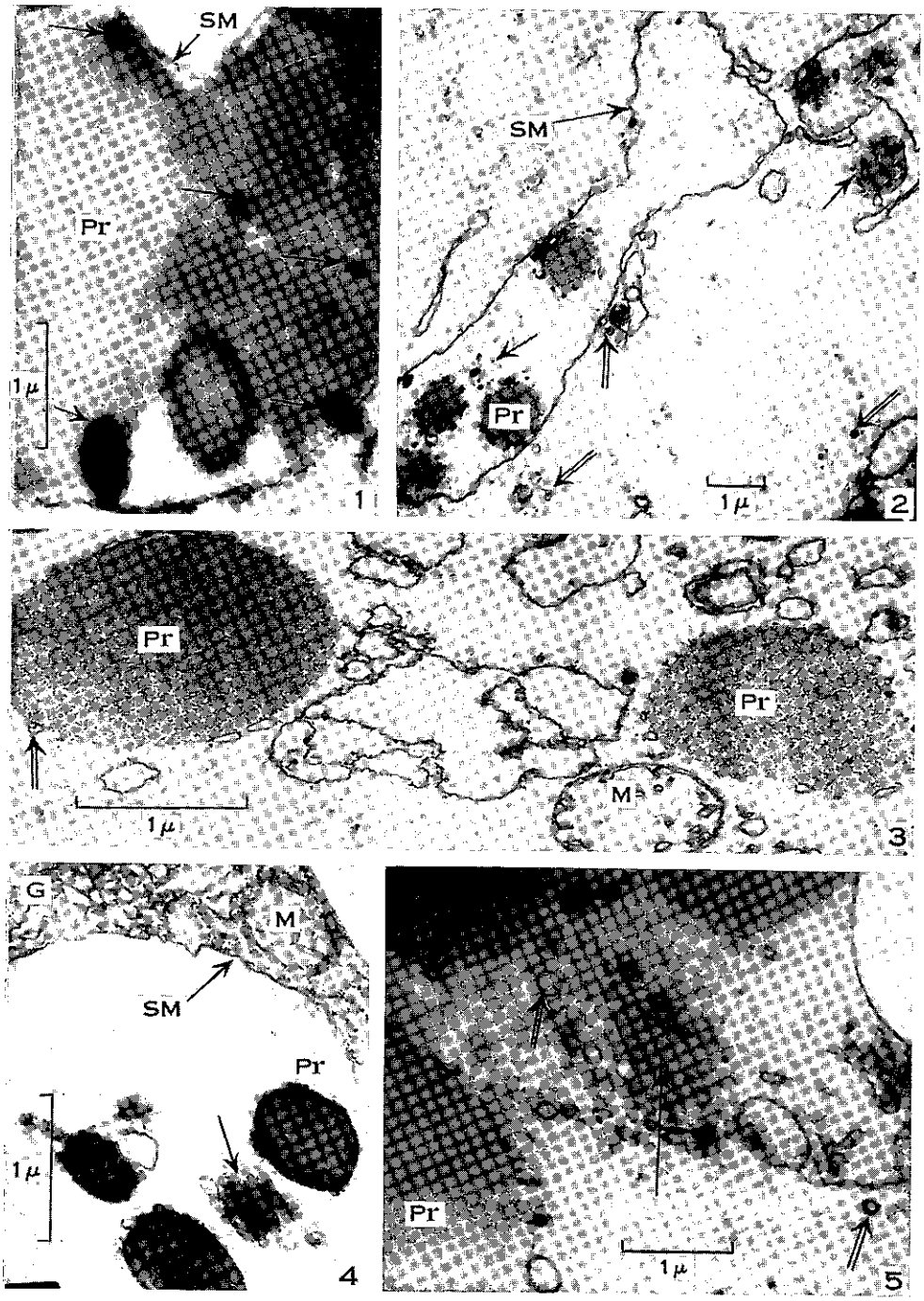

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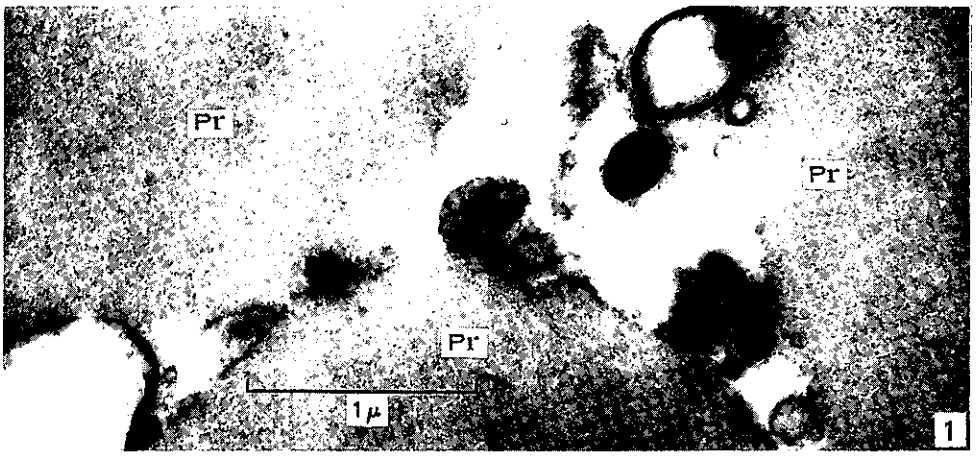

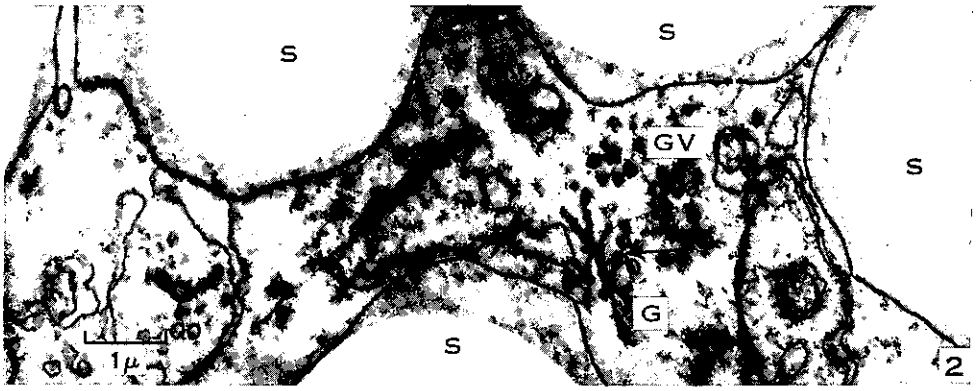

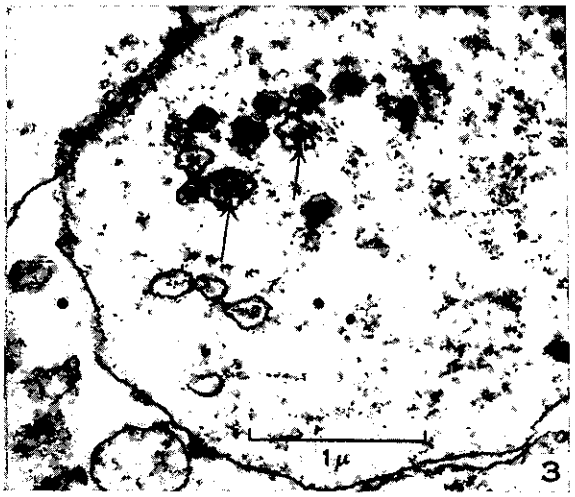

PLATE 4

Fig. 1.—Edge of a nucleus illustrating the complex configurations of nuclear membranes in young endosperm cells. ×10,000.

Fig. 2.—View showing, in particular, vesicular elements of the endoplasmic reticulum during the development of this system (cf. Plate 3, Fig. 3). ×20,000.

Fig. 3.—An example of endoplasmic reticulum concentration sometimes encountered in developing cells. ×5000.

Fig. 4.—Developing endosperm cell wall: the middle lamella (arrow) is well contrasted. ×27,000.

Fig. 5.—As for Plate 4, Figure 4, but after osmium tetroxide fixation. ×15,000.

Fig. 6.—A newly formed starch granule within its plastid, seen side-on. Note the invaginations at each end, with corresponding membranes complexes. ×18,000.

PLATE 5

Fig. 1.—Portion of a developing starch granule seen face-on. The membrane complex extends round the circumference between granule and plastid membrane. ×9000.

Fig. 2.—A higher-magnification micrograph to illustrate the continuity of the membrane complex of the plastid and the inner plastid membrane. ×50,000.

Fig. 3.—An example of a plastid membrane proliferation, carrying with it small starch granules. The parent plastid membrane is indicated by the single arrow, and the double arrow indicates the probable area of constriction before release. ×70,000.

Fig. 4.—Small starch granules, enveloped within plastid membrane derivatives, after release from parent amyloplasts. Large-type granules appear in three corners. ×10,000.

PLATE 6

Fig. 1.—Portion of a protein deposit including surrounding membranes and heterogeneities (arrows) within the deposit. ×18,000.

Fig. 2.—Protein granules in an early stage. Most lie within a space enclosed by a single membrane, though the granule towards the top right (arrow) may be free in the cytoplasm. The single arrows indicate a surface or near-surface section, and double arrows indicate small vesicles with dense contents. ×8000.

Fig. 3.—An example of protein deposits lying free in the cytoplasm. The double arrow indicates a vesicle with dense contents. ×24,000.

Fig. 4.—Osmium tetroxide-fixed material, including protein granules. The arrow indicates a near-surface view of one deposit. ×20,000.

Fig. 5.—View including portions of three protein granules, and the surface of a fourth (single arrow). Double arrows indicate small vesicles similar to those at the surface of granules but situated at some distance. ×15,000.

PLATE 7

Fig. 1.—Portions of large protein deposits, which may be seen to have a fine granular structure. Note the variation in size and appearance of vesicles associated with the surface of the granules. ×30,000.

Fig. 2.—Portion of a young endosperm cell showing a Golgi apparatus lying between amyloplasts. The arrow indicates a vesiculated end to a cisterna containing dense material. Neighbouring vesicles with similar inclusions are classed as Golgi vesicles. ×10,000.

Fig. 3.—Small vesicles with dense contents are seen within a membrane-bound space. The dense contents of small vesicles (arrows) are similar to protein deposits. ×23,000.

Fig. 4.—A recognizable protein deposit, and adjacent to it a small vesicle with dense contents (arrow) resembling Golgi vesicles. ×42,000.