3. It is suggested that red light somehow triggers an exchange reaction between the 4-hydroxycinnamic acid glucose derivative and the kaempferol hexaglucoside, with the resultant formation of 3-(4-hydroxycinnamoyltriglucosyl)kaempferol.

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Intracellular Components Associated with Protein Synthesis in Developing Wheat Endosperm

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Almost all plant seeds accumulate storage proteins which are utilized by the developing embryo on germination. In wheat grain, the protein is formed predominantly in the endosperm tissue. Jennings & Morton (1963*a*, *b*) and Graham, Morton & Simmonds (1963*a*) have described the changes in the protein, carbohydrate, nucleic acids and other components during development of the endosperm of several varieties of wheat (*Triticum* spp.) grown under field conditions in South Australia.

During development of the endosperm from about 10 days after flowering until maturity at about 40 days there is rapid synthesis of protein; in variety Dural, for example, the protein nitrogen increased from 54μg./grain at day 12 to 558μg./grain at day 26 after flowering (Graham *et al.* 1963*a*). There is very little cell division in the endosperm during this period, but there is considerable cell expansion (Jennings & Morton, 1963*b*). The proteins formed during endosperm development may be fractionated by differential extraction into components soluble in 0·01M-sodium pyrophosphate, pH 7·4, in 0·05N-acetic acid and in 0·1N-sodium hydroxide respectively (Graham *et al.* 1963*a*). From about day 15, relative to the other protein components those soluble in acetic acid increase at the greatest rate, and thus represent the greater portion of the storage proteins of the grain. These storage proteins accumulate in distinctive granules called 'protein bodies' which have been isolated from homogenates of developing endosperm (Graham, Jennings, Morton, Palk & Raison, 1962; Graham, Morton & Raison, 1963*b*). The protein components soluble in pyrophosphate buffer remain in the supernatant fraction on sedimentation of the intracellular granules.

This and the succeeding papers (Morton & Raison, 1964; Morton, Raison & Smeaton, 1964) describe the fine structure and the enzymic features of intracellular components that incorporate amino acids into endosperm proteins.

MATERIALS AND METHODS

Wheat endosperm. This was obtained from developing grain of *Triticum vulgare* (cultivar. Gabo, Insignia, or Kashmir no. 5) or of *T. durum* (cultivar. Dural) grown in plots at Adelaide. Endosperm used for isolation of protein-body and supernatant preparations was obtained by the rolling method (Graham *et al.* 1963*b*) and that used for electron microscopy was isolated by dissection by hand.

Electron microscopy. Sections of intact endosperm were cut by hand with a razor blade and fixed in 1% osmium

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tetroxide, 2% potassium permanganate or 3·5% formaldehyde, each in Locke's solution at pH 7·4 and at 2°. The period of fixing was varied as necessary to give the optimum preservation of fine structure. Tissue fixed in formaldehyde was further treated either with 2% potassium permanganate for 2 hr. at 0°, or with a saturated solution of lead hydroxide for ½–2 hr. at room temperature. The fixed tissue was dehydrated and embedded, and thin sections were cut as described by Jennings, Morton & Palk (1963).

Homogenates and isolated cell fractions were treated similarly, pellets being collected by centrifuging as necessary.

In some studies, pieces of endosperm were disintegrated by ultrasonic vibration either in 0·4 M-sucrose or in 3·5% formaldehyde in Locke's solution at pH 7·4 by using a Disontegrator (Ultrasonic Industries Inc., New York) operating at 80 kcyc./sec. and at 60 w for 1–5 min. at about 2°.

Photographs were taken with an Elmiskop I electron microscope (Siemens und Halske, Germany) at 5000–40 000 magnifications, and photographically enlarged as necessary.

Chemical analysis. A protein-body preparation obtained as described by Morton & Raison (1964) was dialysed against water for 16 hr. at 2°, freeze-dried and dried to constant weight over P_2O_5 *in vacuo*. Purified protein bodies were prepared by density-gradient centrifugation as described by Graham *et al.* (1963*b*); the preparation was dialysed and dried as indicated for the protein-body preparation.

The dried preparations were ground to powders, and lipids, nucleic acids and proteins were estimated essentially as described by Martin & Morton (1956). Phosphorus was estimated by the method of Weil-Malherbe & Green (1951).

Sedimentation analysis. Sedimentation-velocity experiments were carried out with a Spinco model E analytical ultracentrifuge.

Ribosomes from the supernatant preparation were obtained as follows. The precipitate remaining after removal of the high-speed supernatant fraction obtained from the supernatant preparation (see Fig. 2 of Morton & Raison, 1964) was resuspended in 0·1 M-tris–HCl buffer, pH 7·4, containing 1% (v/v) of detergent (Nonidet P40) and magnesium chloride (6 mM), and centrifuged at 105 000*g* for 60 min. The pellet was suspended in 0·1 M-tris–HCl buffer, pH 7·4, containing magnesium chloride (6 mM). Ribosomes from the protein-body preparation were obtained by first centrifuging the disrupted protein bodies (see Morton *et al.* 1964) at 20 000*g* for 30 min. and then centrifuging the supernatant at 105 000*g* for 60 min. The pellet so obtained was suspended in 0·1 M-tris–HCl, pH 7·4, containing magnesium chloride (6 mM).

Enzymic activities. For measurement of oxygen uptake, homogenates were prepared in 0·25 M-sucrose containing sodium phosphate (0·1 M) and magnesium chloride (3 mM), final pH 7·4, either with a Potter–Elvehjem homogenizer or by grinding with acid-washed sand in a mortar. Fractions were then isolated by differential centrifugation of the homogenates. Endogenous respiration and succinate-dependent respiration were measured manometrically at 30°. Ribonucleic acid-depolymerase (ribonuclease) activity was estimated as described by McDonald (1955).

Nonidet P40. This non-ionic detergent was obtained from Shell Chemical Co. (Aust.) Pty. Ltd.

RESULTS

Fine structure of intracellular components of endosperm tissue. The occurrence and distribution of protein bodies and of other intracellular components of wheat endosperm, and the changes that occur during development, were studied by electron microscopy. Plates 1(*a*)–1(*d*) show the fine structure of some components of endosperm cells. Plate 1(*a*) is a structure observed in endosperm at about day 19 after flowering. The electron-dense protein body (PB) has a fine membrane (PrM) at the periphery of the body. In several regions, this membrane merges with an outer membrane (PlM) which appears to consist of several electron-dense layers. In one region the outer membrane appears to form a network with associated electron-dense particles (R) which are probably ribosomes (see the Discussion section). The area of low electron density (v) is possibly a vacuole between the protein body and the outermost membrane (PlM). The vesicles (Ve) at parts of the periphery of the protein body itself may be formed by rupture of the protein-body membrane (PrM).

The spherical area of uniform density (DA) within the protein body itself is a characteristic feature of many of the protein bodies observed in this tissue. The dark region across the central portion of the protein body is almost certainly an artifact arising from compression during cutting of the thin section. This type of artifact has been frequently observed and is probably due to the density of the protein of the protein body.

Protein bodies vary from about 0·2 to 20 μ in diameter in tissue at any stage of development. Bodies of about 20 μ in diameter were frequently observed in tissue at about day 30 whereas bodies of 0·2–5 μ were predominant at about day 19 after flowering. Plate 1(*b*) shows a portion of the periphery of a large body. The protein body (PB) is closely adpressed to the outermost membrane (PlM) which consists of multiple layers thus forming a lamellar structure. The double membrane (StM) encloses a large starch grain, and is distinct from the lamellar structure at the periphery of the protein body. The absence of a vacuolar space between the membrane (PlM) and the protein body (PB) probably reflects better preservation of fine structure than was achieved in Plate 1(*a*).

In addition to the structure shown in Plate 1(*a*), endosperm contained granules with internal cristae and which were considered to be mitochondria; Plate 1(*c*) is representative. Such granules were not very abundant.

Plate 1(*d*) shows tubular elements which probably form part of the endoplasmic reticulum. These elements are particularly abundant in immature endosperm and may surround areas within which

protein bodies arise (see Plate 4 of Jennings *et al.* 1963). In tissue fixed with osmium tetroxide, electron-dense particles corresponding in size to ribosomes were frequently observed in association with these lipoprotein elements.

Fine structure of isolated cellular components. Plates 2(*a*)–2(*e*) show electron micrographs of components isolated from disrupted endosperm cells (and from disrupted protein bodies). Plate 2(*a*) is representative of the components of a protein-body preparation, and Plate 2(*b*) is representative of components present after ultrasonic disruption of endosperm. In both Plate 2(*a*) and Plate 2(*b*) the protein-body membrane, as observed in intact tissue (Plate 1*a*), appears to have been retained in association with the protein bodies. Some of the protein bodies in the protein-body preparations had dense areas similar to that shown in Plate 1(*a*). Plate 2(*b*) also shows regions of membranous material, vesicular membrane elements associated with protein bodies, and some granules which are apparently not typical protein bodies.

Plates 2(*c*) and 2(*d*) show greater detail of components obtained by ultrasonic disruption of endosperm. Plate 2(*e*) shows fragments of lipoprotein membranes, the predominant components of the supernatant preparation.

Sedimentation properties of isolated ribosomes. The ultracentrifuge patterns obtained on sedimentation of the ribosome fractions obtained from the protein-body preparation and from the supernatant preparation respectively are shown in Figs. 1(*a*) and 1(*b*). The sedimentation coefficients of the components shown in Fig. 1(*a*) were 107, 76, 53 and 35 S, and of the components shown in Fig. 1(*b*) were 120, 80, 60 and 40 S. The $E_{260\,m\mu}/E_{280\,m\mu}$ ratio was about 1·9 for both the ribosome preparations in 0·1 M-tris–hydrochloric acid buffer, pH 7·4.

Chemical composition of the protein bodies. Table 1 shows the chemical composition of a protein-body preparation and of purified protein bodies.

Enzymic activities of isolated cellular fractions. Table 2 shows the endogenous respiration, and the succinate-dependent respiration of endosperm, of homogenates and of fractions prepared from the homogenates. Table 3 shows the distribution of ribonuclease activity among fractions of an homogenate of endosperm obtained without detergent.

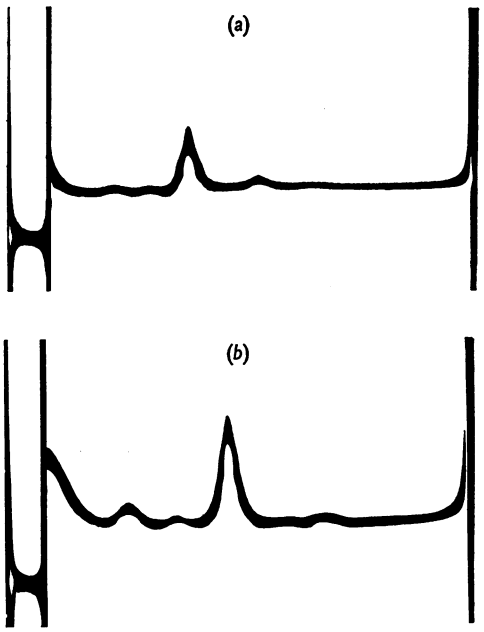
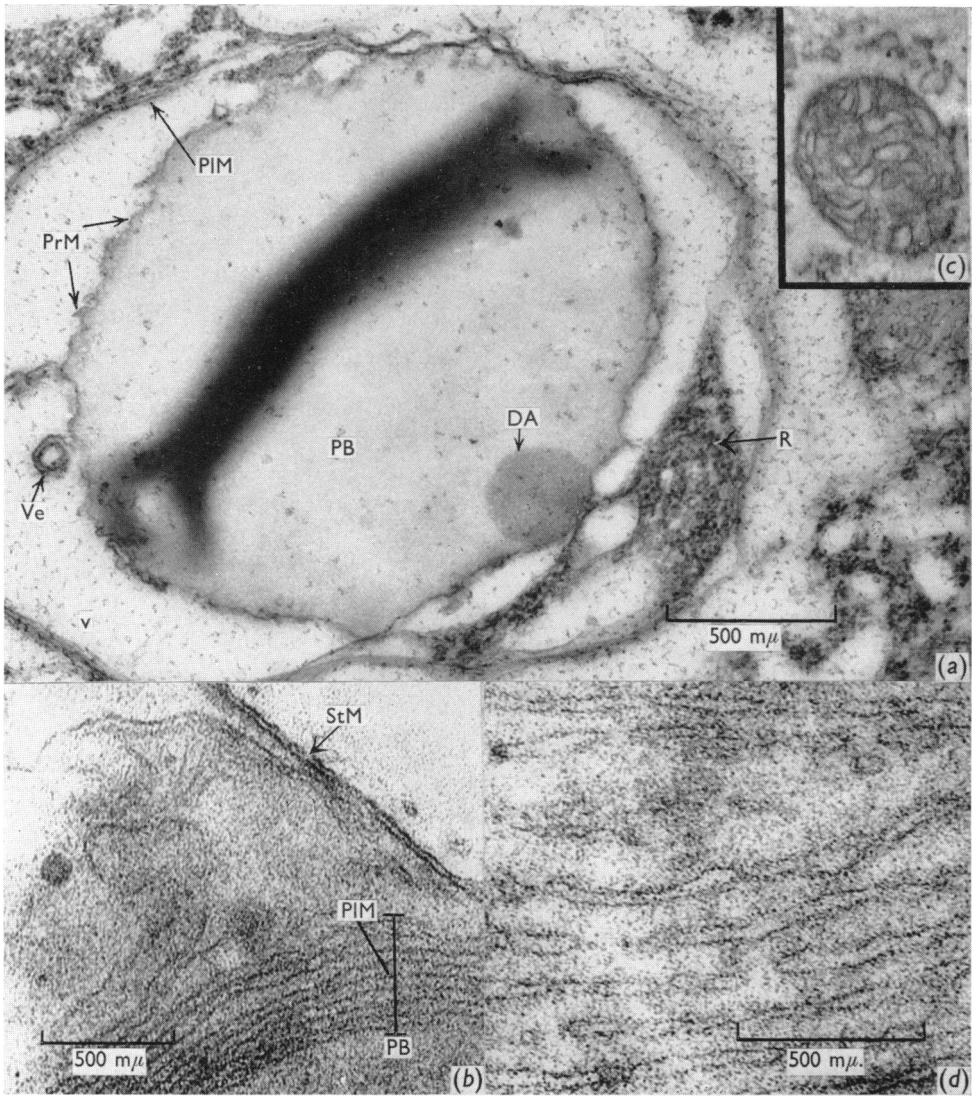


Fig. 1. Sedimentation pattern of ribosomes obtained from the protein-body preparation (*a*) and the supernatant preparation (*b*) as described in the Materials and Methods section. Sedimentation was from left to right. The photographs were taken 24 min. (*a*) and 32 min. (*b*) after reaching 29 000 rev./min. The concentration of nucleoprotein was approx. 3·5 mg./ml., the temperature was 4° and the magnesium chloride concentration was 6 mM.

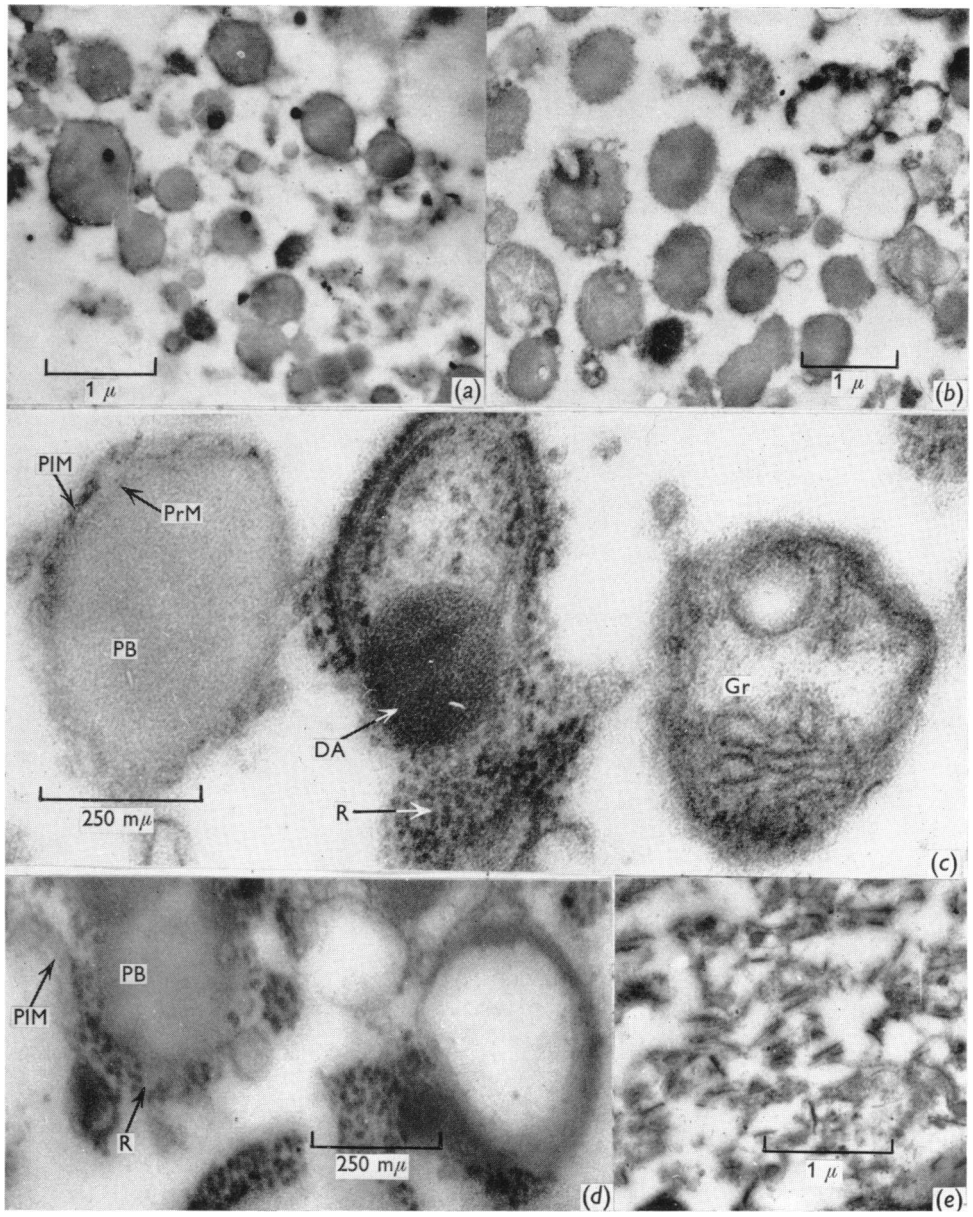
DISCUSSION

Protein bodies and associated structures of wheat endosperm. Protein bodies and starch granules are prominent intracellular components of cells of developing wheat endosperm when suitably treated tissue is observed with an electron microscope, but the protein bodies are largely obscured by the starch granules when sections of tissue are examined by light-microscopy (Jennings *et al.* 1963). From observations on sections of isolated protein bodies, it is concluded that most of these are oblate spheroids.

The analyses reported in Table 1 show that the protein-body preparation contained protein, lipid, RNA and phytic acid (see below); contamination with small starch granules accounted for 36·8 % of the dry weight. Thus the protein bodies in the preparation consist predominantly of protein (68 %), lipid (22·7 %, most of which is phospholipid), nucleic acid (5·5 %) and phytic acid (4·0 %). The amino acid composition of this type of preparation is in good agreement with the composition of the storage protein of wheat endosperm (Jennings & Morton, 1963*c*), and indicates that the protein bodies contain mostly storage protein, as was also shown by starch-gel electrophoresis (Graham *et al.* 1963*b*). These results are supported by analyses on a selected fraction of protein bodies purified by density-gradient centrifugation (see Graham *et al.* 1963*b*). This fraction, containing bodies 0·1–0·3 μ in diameter, represented only about



Electron micrographs of structures observed in intact endosperm of wheat grain. (*a*) Portion of endosperm of *T. durum* cultivar. Dural at about day 19 after flowering; the tissue was fixed with formaldehyde and the section was treated with lead hydroxide. (*b*) and (*c*) Different areas of sections of *T. vulgare* cultivar. Kashmir no. 5 at about day 22 after flowering; the tissue was fixed with formaldehyde and subsequently treated with potassium permanganate. (*d*) Area of a section of *T. vulgare* cultivar. Insignia at about day 16 after flowering; the tissue was fixed with osmium tetroxide. PB, Electron-dense protein body; PrM, membrane of protein body; PlM, outer membrane of plastid; R, electron-dense particles; v, area of low electron density, possibly a vacuole between protein body and outer membrane; Ve, vesicles; DA, spherical area of uniform electron density within the protein body; StM, double membrane enclosing starch grain.



Electron micrographs of components isolated from endosperm of *T. durum* cultivar. Dural at about day 19 after flowering. (*a*) Representative area from a pellet of the protein-body preparation fixed with osmium tetroxide. (*b*), (*c*) and (*d*) Areas from pellets obtained from endosperm disintegrated by ultrasonic vibration as described in the Materials and Methods section. (*e*) Representative area from a pellet obtained from the supernatant preparation fixed with osmium tetroxide. PB, Electron-dense protein body; PrM, membrane of protein body; PlM, outer membrane of plastid; R, electron-dense particles; DA, spherical area of uniform electron density within the protein body; Gr, granule.

Table 1. *Chemical composition of the protein-body preparation and purified protein bodies*

The protein-body preparation was obtained from endosperm of *T. durum* cultivar. Dural harvested at 18 days after flowering. The purified protein bodies were obtained from endosperm of *T. vulgare* cultivar. Gabo harvested at 18 days after flowering. The successive extractions and the analyses were carried out as described by Martin & Morton (1956). Carbohydrate was determined by the indole method described by Dische (1955). The RNA contents were calculated from $E_{260\ m\mu}/E_{280\ m\mu}$ ratios as described by Warburg & Christian (1942).

Protein-body preparation

Extraction procedure	Component determined	Composition (% dry wt.)	Composition (% on carbohydrate-free basis)
Methanol, chloroform–methanol, butan-1-ol and ether at room temperature	Total P	1·8	—
	Lipid (by wt.)	14·3	22·7
	Lipid (from P × 6·2)	11·6	—
0·2 N-$HClO_4$ at 0°	Total P	0·75	—
	Acid-stable P	0·55	—
	Inorganic P	0·17	—
	Phytic acid (from P × 4·42)	2·4	4·0
	Amino acids	+*	—
10 % (w/v) KCl at 100° for 20 min.	Inorganic P	0	—
	Total P	0·37	—
	RNA (from $E_{260\ m\mu}/E_{280\ m\mu}$ ratio)	3·4	—
	RNA (from P × 9·4)	3·5	5·5
N-KOH at 100° for 15 min., then N-H_2SO_4 for 15 min.	Total P	0·6	—
	Total N	7·4	—
	Protein (from protein N × 5·75)	42·5	68

Purified protein bodies

Extraction procedure	Component determined	Composition (% dry wt.)	Composition (% on carbohydrate-free basis)
0·2 N-$HClO_4$ at 2°	Total P	0·96	—
	Phytic acid (based on total acid-soluble P, not corrected for inorganic P)	4·2	8·4
Ethanol and ethanol–ether	Total P	0·05	—
	Lipid (from P × 6·2)	0·43	0·83
0·2 N-$HClO_4$ at 90°	Total P	0·55	—
	RNA (from P × 9·4)	5·2	10·4
	RNA (from $E_{260\ m\mu}/E_{280\ m\mu}$ ratio)	5·5	—
N-KOH at 100° for 15 min., then N-H_2SO_4 for 15 min.	Total P	0·22	—
	Protein (from protein N × 5·75)	37·2	72·0

* Presence of free amino acids indicated by a ninhydrin-positive reaction.

5 % of the protein-body nitrogen of the endosperm at day 18 after flowering (Graham *et al.* 1963*b*). This probably accounts for the differences in the lipid and nucleic acid content (Table 1) and in the amino acid composition (Jennings & Morton, 1963*c*) from the protein bodies present in the protein-body preparation.

The appearance of electron micrographs of the protein-body preparation (Plate 2*a*) and of a fraction from disintegrated endosperm (Plates 2*b*–2*d*) is consistent with the chemical analyses and shows the protein-rich bodies with associated lipoprotein membranes (PrM and M in Plate 2*c*, for example) and electron-dense particles (R in Plates 2*c* and 2*d*) which are probably RNA-containing ribosomes. The protein-body preparation also contains transfer RNA (Morton *et al.* 1964).

The protein-body preparation contains 0·55 % of acid-soluble acid-stable organic phosphorus (Table 1). E. M. Lees, R. K. Morton & J. K. Raison (unpublished work) have directly demonstrated that phytate is a component of a protein-body preparation. If the acid-stable organic phosphorus is all present as phytic acid, as seems likely, then the protein-body preparation contains 2·4 % and the protein bodies contain 4 % of phytic acid (Table 1). Dieckert, Snowden, Moore, Heinzelman & Altschul (1962) have shown that the protein-rich fraction 2 ('aleurone grains') consisting of spheres 1–10 μ in diameter, isolated from cotyledons of peanuts (*Arachis hypogea*), contains 6·3 % of phytic acid (dry wt. basis). The 'aleurone grains' of peanut cotyledons described by Dieckert *et al.* (1962) and the protein bodies of wheat endosperm both

Table 2. *Distribution of endogenous and succinate-dependent oxygen consumption in fractions obtained from an homogenate of immature endosperm*

Endosperm obtained from grain harvested at 18 days after flowering was homogenized in 0·2 M-sucrose containing sodium phosphate (0·125 M) and magnesium chloride (3 mM), final pH 7·4, and centrifuged at 3000*g* for 15 min. The supernatant was removed and centrifuged at 10000*g* for 15 min. The precipitates were separately suspended in 0·2 M-sucrose containing sodium phosphate (0·125 M) and magnesium chloride (3 mM), final pH 7·4. Oxygen consumption was measured at 30° for 1 hr. with a Warburg respirometer. The reaction mixture (total vol. 3 ml.) contained 200 μmoles of sodium phosphate, pH 7·4, 1 ml. of the enzyme fraction (containing 150 μg. of N) and 180 μmoles of sodium succinate; 0·1 ml. of 20% (w/v) KOH was in the centre well.

Fraction	Oxygen consumption (μl./hr./mg. of N) With succinate	Without succinate
Homogenate	23	26
3000*g* precipitate	63	24
10000*g* precipitate	45	28
10000*g* supernatant	0	0

Table 3. *Ribonuclease activity in fractions obtained from an homogenate of immature endosperm*

An homogenate of endosperm tissue was prepared as described in the Materials and Methods section. The homogenate was centrifuged at 3000*g* for 15 min. The supernatant fraction was centrifuged at 10000*g* for 30 min. and the resulting supernatant centrifuged again at 60000*g* for 30 min. The precipitates were separately suspended as described in Table 2. Ribonuclease activity was estimated by the formation of acid-soluble nucleotides (McDonald, 1955) and is expressed as the change of $E_{260\,m\mu}$/20 min./mg. of N for a light-path of 1 cm.

Fraction	Ribonuclease specific activity
Homogenate	0·240
3000*g* precipitate	0·047
10000*g* precipitate	0
60000*g* precipitate	0·244
60000*g* supernatant	0·082

represent accumulations of protein and both contain phytic acid, and in these respects are similar cellular inclusions. However, the classical definition of aleurone grains limits their distribution to the aleurone layer and embryo of cereal grain. The spherical dense regions (DA in Plates 2*c* and 1*a*) are probably regions of high concentration of phytic acid. This conclusion follows from observations by Morton & Raison (1963), who showed that both phytic acid and calcium phytate give a very dense appearance to thin sections of gelatin fixed with potassium permanganate; gelatin without phytic acid or calcium phytate has only slight electron density. Phytic acid and calcium phytate also appear as dense areas in gelatin fixed with osmium tetroxide (R. K. Morton & B. A. Palk, unpublished work). Protein bodies in peanut cotyledons show similar dense areas to those observed in endosperm bodies (Morton & Raison, 1963). These localized phytate accumulations probably correspond to the spherical inclusions (globoids) observed in 'aleurone grains' (protein bodies) by light-microscopy (see Guilliermond, 1941, for example).

The electron micrographs shown in Plate 1 pertain to the intracellular localization and site of formation of the protein bodies. The structure shown in Plate 1(*a*) is considered to be a plastid, consisting of lipoprotein membranes which enclose the protein body (PB) and a region rich in ribosomes (R). The protein body has a dense area (DA) similar to that seen in Plate 2(*c*). The compression artifact shown in Plate 1(*a*) (see the Results section) may partly account for the vacuolar space (v) between the protein-body membrane (PrM) and the outermost membrane (PlM). No such vacuolar space was observed surrounding the very large protein body of which a portion is shown in Plate 1(*b*). In Plate 1(*b*) the protein is closely adpressed to the membrane (PlM) which consists of a lamellar structure having about nine dense layers. The lamellar arrangement of the membrane of the plastid was often seen in association with large protein bodies (10–20 μ in cross-section), and 20 dense layers were observed in the membrane associated with one protein body. However, only two or three layers are usually observed in association with small protein bodies. Plate 1(*a*) shows some separation of the layers of the membrane (PlM) of the plastid.

The protein-body preparation contains 3·5% of RNA (see above, and Table 1), much of which is probably present in ribosomes. The region of ribosomes associated with a dense area and with membranes shown in Plate 2(*c*) could well have originated by disruption of a region of accumulation of ribosomes (R) seen in Plate 1(*a*). Plate 2(*d*) shows ribosomes apparently still in association with a protein body and its plastid membrane, after disruption of endosperm by ultrasonic vibration. The predominant component of the ribosomes (Plate 2*d* and Fig. 1*a*) isolated from the protein-body preparation has a sedimentation coefficient of 76 s (see the Results section). The component of 107 s is probably an aggregate of ribosomes; grouping of ribosomes has sometimes been observed in electron micrographs of endosperm fixed with formaldehyde.

The protein-body preparation is obtained after disruption of endosperm in the presence of an alkylphenylethylene oxide condensate (a non-ionic detergent known as Nonidet P40) which has

effects similar to those of sodium deoxycholate (see Graham *et al.* 1963*b*). Moreover, since ribosomes may only be isolated from the protein-body preparation after vigorous mechanical disruption, it is very unlikely that the ribosomes associated with protein bodies could result from contamination by the supernatant material. This is consistent with the electron micrographs (Plates 1*a* and 2*d*), which show that ribosomes are adjacent to the protein body itself and, together with the protein body, are enclosed by the plastid membrane.

As shown in the succeeding papers (Morton & Raison, 1964; Morton *et al.* 1964), the protein-body preparation contains the enzymes and nucleic acid components for the incorporation of amino acids into protein. Similar components are associated with other systems, such as that of the reticulocyte, which appear to catalyse protein synthesis. These findings, together with the observations described above, lead to the conclusion that the protein body is formed by accumulation of storage protein within the plastid membrane, and that the structure shown in Plate 1(*a*) itself is the site of formation of the protein body. Hence the plastid present in protein-body preparations has been called a 'protein-forming plastid' or 'proteoplast' (Morton & Raison, 1963).

Respiratory activity of endosperm. As shown in Plate 1(*c*), granules resembling mitochondria of other plant tissues are observed by electron microscopy of intact endosperm tissue. The respiratory activity of homogenates of endosperm prepared without detergent (Table 2) is therefore attributed to the presence of mitochondria, although there was little stimulation by the addition of succinate to washed precipitates containing the mitochondria, starch grains and protein bodies. There was considerable variation in the oxygen consumption of homogenates of endosperm, and very little endogenous or succinate-stimulated oxygen uptake was observed in endosperm older than 20 days after flowering. Some granules resembling mitochondria (see Gr in Plate 2*c*) were detected in the protein-body preparations. Whereas nuclei were observed in very young endosperm tissue (5–15 days after flowering), few were observed in later stages of development when the cells had undergone considerable enlargement to accommodate the starch grains, amyloplasts, protein bodies and other synthetic products.

Components of the supernatant preparation. In addition to proteoplasts with contained protein bodies, amyloplasts, starch granules and mitochondria, cells of developing endosperm contain areas of tubular lipoprotein elements as shown in Plate 1(*d*) (see also Jennings *et al.* 1963; Buttrose, 1963). It is considered these are part of an endoplasmic reticulum. The ribosomes (Fig. 1*b*) and lipoprotein fragments (Plate 2*e*) found in the supernatant preparation probably arise mostly by disruption of the endoplasmic reticulum. Other than these components, no other prominent particle was detected in the supernatant fraction by electron microscopy.

The ribosome fraction obtained from the supernatant preparation (Fig. 1*b* and Plate 2*e*) has a major component with a sedimentation coefficient of 80s, and minor components of 60 and 40s, and thus resemble those obtained by Ts'o, Bonner & Vinograd (1956) from pea seedlings for which values of 80, 60 and 40s were reported. Under similar conditions however, the fundamental ribosome units from the protein-body preparation have lower sedimentation coefficients (76, 53 and 35s). It would appear that the fundamental units of the two ribosome preparations (having values of 60 and 40s in the supernatant preparation, and 53 and 35s in the protein-body preparation) are significantly different in physicochemical properties. Hall & Slayter (1959) and Huxley & Zubay (1960) have discussed the size and shape of these fundamental units of microsomes, and Gilbert (1963) and Goodman & Rich (1963) have described their functions in protein synthesis.

The supernatant preparation contains the soluble (cytoplasmic) proteins of the wheat endosperm. These proteins are distinguished from the storage proteins by differences on starch-gel electrophoresis (Graham *et al.* 1963*b*) and in amino acid composition (Jennings & Morton, 1963*c*). Whereas the storage proteins increase continuously in amount/grain during development of grain, the cytoplasmic protein increases in amount only slightly from about day 25 to maturity (Jennings & Morton, 1963*c*; Graham *et al.* 1963*a*). In mature endosperm, starch granules and protein bodies occupy most of the cell volume, and little endoplasmic reticulum can be detected by electron microscopy. These observations are consistent with the view that the site of synthesis of cytoplasmic proteins in endosperm cells is the endoplasmic reticulum, derivatives of which are found in the supernatant preparation (Morton & Raison, 1963, 1964; Morton *et al.* 1964).

SUMMARY

1. Electron microscopy of thin sections of suitably treated tissue was used to investigate the fine structure of components of wheat endosperm cells which accumulate storage proteins during development.

2. Protein-body preparations and supernatant preparations obtained by differential centrifuging of homogenates of endosperm were shown to contain different cellular components. Although some

granules resembling mitochondria and areas of lipoprotein membranes were present, protein bodies were the predominant cellular components of the protein-body preparations. Lipoprotein fragments and ribosomes were present in the supernatant preparation.

3. The fine structure of protein bodies in intact tissue and of bodies obtained by ultrasonic disruption of endosperm shows that the bodies and associated ribosomes are contained within a distinctive lipoprotein membrane. Evidence is presented to show that protein bodies are formed within proteoplasts.

4. Ribosomes isolated from the protein-body preparation have lower sedimentation coefficients than ribosomes obtained from the supernatant preparation under similar conditions. The significance of these observations is discussed.

5. The protein-body preparations contain protein, lipid, nucleic acids and phytic acid.

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The Separate Incorporation of Amino Acids into Storage and Soluble Proteins Catalysed by Two Independent Systems Isolated from Developing Wheat Endosperm

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With intact heads of immature wheat grain it has been shown that [^{35}S]sulphate and ^{14}C-labelled amino acids are taken up and accumulate in proteins associated with distinctive cellular components of the endosperm tissue (Graham & Morton, 1963). Each of the protein components separated by starch-gel electrophoresis was found to contain radioactivity (Graham & Morton, 1963; Graham, Morton & Raison, 1964). The cellular components of developing endosperm tissue include protein bodies and the associated protein-forming plastids (Morton & Raison, 1963; Morton, Palk & Raison, 1964*a*). These may be separated from the supernatant-protein fraction by differential centrifugation of suitable homogenates of endosperm tissue (Graham, Morton & Raison, 1963*b*). With intact heads, the incorporation of radioactivity

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