THE *IN VIVO* UPTAKE AND INCORPORATION OF RADIOISOTOPES INTO PROTEINS OF WHEAT ENDOSPERM

By JANET S. D. GRAHAM,* THE LATE R. K. MORTON,* and J. K. RAISON*†

[*Manuscript received September* 20, 1963]

Summary

A procedure is described for the quantitative isolation of the protein bodies from developing wheat endosperm. The changes with time in specific activity of the storage proteins (present in the protein bodies) and of the soluble proteins (present in the high-speed supernatant fraction) have been followed in "pulse"-labelling experiments. Excised wheat heads were exposed to [^{35}S]sulphate for periods of 5 min to 1 hr; dilution of the ^{35}S-radioactivity within the wheat head before the incorporation of radioactivity into the endosperm proteins resulted in a constant ratio of the specific activity of the soluble proteins to that of the storage proteins.

A comparison was made of the uptake of [^{35}S]sulphate, [^{14}C]proline, and [^{14}C]leucine; the specific activities of the soluble and storage protein fractions could be correlated with the amino acid composition of each fraction. The higher specific activity of the storage proteins compared to the soluble proteins after incorporation of [^{14}C]proline and [^{14}C]leucine showed that the soluble proteins are not precursors for the synthesis of storage proteins.

The selective inhibition with sodium fluoroacetate after exposure to [^{35}S]sulphate demonstrated that the synthesis of storage proteins takes place independently of the synthesis of soluble proteins.

I. INTRODUCTION

The storage proteins of wheat endosperm accumulate in granules called "protein bodies" (Graham *et al.* 1962; Graham, Morton, and Raison 1963; Jennings and Morton 1963*a*; Jennings, Morton, and Palk 1963) which may be isolated from endosperm homogenates (Graham, Morton, and Raison 1963). Cytological studies have shown that protein bodies occur also in other plant seeds, such as sorghum grain (Duvick 1955, 1961, Watson *et al.* 1955), peanut (Altschul *et al.* 1961; Dieckert *et al.* 1962; Bagley *et al.* 1963; Morton and Raison 1963), peas (Varner and Schidlovsky 1963) and cottonseed (Yatsu 1963). However, there is little information concerning the biosynthesis of protein bodies. It was suggested by Graham *et al.* (1962) that protein bodies of wheat endosperm are formed by "internal secretion" of pre-formed protein which accumulates within a lipoprotein membrane. Little information was available to establish whether precursors (other than amino acids) were common for synthesis of storage and cytoplasmic proteins, or whether the synthesis of storage and of cytoplasmic proteins takes place simultaneously and

* Department of Agricultural Chemistry, Waite Agricultural Research Institute, University of Adelaide.

† Present address: Division of Plant Industry, CSIRO, Canberra.

independently from amino acids. Previous experiments (Graham and Morton 1963; Graham, Morton, and Raison 1963) showed that excised wheat heads rapidly take up [^{35}S]sulphate and [^{14}C]glycine and that the radioactivity is incorporated into the endosperm protein. However, from these results it was not possible to determine whether the soluble cytoplasmic proteins are precursors of the storage proteins.

This paper describes changes in the specific activities of the soluble and storage proteins of wheat endosperm as determined by brief exposure of intact wheat heads to radioactive compounds followed by exposure to similar but non-radioactive compounds ("pulse"-labelling experiments). The effects of treatment with chloramphenicol and with sodium fluoroacetate are described. The results show that the incorporation of radioactivity into the storage (protein body) proteins takes place simultaneously with, and relatively independently of, incorporation into the soluble (cytoplasmic) proteins, thus indicating the presence of two independent systems for the snythesis of the storage and soluble proteins of wheat endosperm.

II. Materials and Methods

(*a*) *Plant Material*

Wheat heads of *Triticum vulgare* cv. Bungulla, *T. vulgare* cv. Gabo, and *T. durum* cv. Dural were harvested at approximately 18 days after flowering as previously described (Graham and Morton 1963).

At the completion of the exposure and incubation periods the heads were stored at −15°C, and the endosperm was isolated either in the frozen state by hand dissection (Graham and Morton 1963), or by mechanical rolling at 2°C (Graham, Morton, and Raison 1963).

(*b*) *Radioactive Compounds*

[^{35}S]sulphate (carrier free), [^{14}C]leucine (uniformly labelled, specific activity 6 c/mole), and [^{14}C]proline (uniformly labelled, specific activity 33 c/mole) were obtained from the Radiochemical Centre, Amersham, England.

(*c*) *Exposure to Radioisotopes*

Heads (approximately 200) were packed upright into a cylindrical container (9·5 in. diameter by 4 in. high) cut from a section of polythene tubing. The base of the container was covered by fibre-glass mesh and fitted into a well cut in the surface of a polythene base. The solution (150 ml) containing the radioactive compound was placed in the well and covered the base of the container. With six containers, approximately 1200 heads could be exposed to the radioisotope simultaneously.

After exposure to the radioisotope, the bottom of the container and the wheat heads were thoroughly rinsed with water before being placed in the incubation solution. When a large number of treatments were required each container was conveniently divided into quadrants with Perspex cross-pieces.

Details of the conditions used in each experiment are shown in Table 1.

TABLE 1

CONDITIONS FOR RADIOISOTOPE-INCORPORATION EXPERIMENTS

Experiment Reported in Figure No.:	Plant Material		Conditions of Pretreatment		Conditions of Exposure Treatment			Conditions of Incubation Treatment	
	Cultivar	Days after Flowering	Solution	Period (min)	Isotope	Radio-active Solution (μc/ml)	Period (min)	Solution	Period (hr)
1	Bungulla	16	—	—	[^{35}S]Sulphate	10	60	Water	0,1,3,6,12, and 24
2	Gabo	18	—	—	[^{35}S]Sulphate	10	60	Water	0,1,3,10,20, and 48
3	Gabo	18	—	—	[^{35}S]Sulphate	7·3	5, 10, 20, 30, 40, and 60	Water	6
4	Dural	15	—	—	(*a*) [^{35}S]Sulphate	10	60	Water	0,5·5,10,20, and 48
					(*b*) [^{14}C]Proline	10	60	Water	
					(*c*) [^{14}C]Leucine	10	60	Water	
5	Dural	19	—	—	[^{35}S]Sulphate	13·5	60	(*a*) Water	0,6,12, and 24
								(*b*) Chloramphenicol (0·066M)	
								(*c*) Sodium fluoroacetate (0·05M)	
6	Dural	19	(*a*) Water (*b*) Chloramphenicol (0·066M) (*c*) Sodium fluoroacetate (0·05M)	60	[^{35}S]Sulphate	13·5	60	Water	0,4, and 8

(*d*) *Starch-gel Electrophoresis*

Samples to be examined by starch-gel electrophoresis were exhaustively dialysed against water and freeze-dried. Electrophoresis was carried out as previously described (Graham 1963). Radioautographs of the components separated by starch-gel electrophoresis were prepared as previously described (Graham and Morton 1963).

(*e*) *Fractionation of Endosperm*

Three methods of fractionating the endosperm were used:

(i) *Soluble Extracts.*—Proteins soluble in sodium pyrophosphate, acetic acid, and sodium hydroxide respectively were extracted from freeze-dried endosperm as previously described (Graham, Morton, and Simmonds 1963).

(ii) *Isolated Cellular Components.*—Protein bodies were isolated by density-gradient centrifugation and the soluble proteins as the high-speed supernatant after the method of Graham, Morton, and Raison (1963).

(iii) *Isolated Fractions.*—Endosperm (5 g wet weight), obtained by either hand dissection or by mechanical rolling, was homogenized in 35 ml of 0·2M phosphate buffer (pH 7·2) containing 0·006M magnesium chloride and 1·5% (v/v) of detergent (Nonidet P40) as previously described (Graham, Morton, and Raison 1963). The mixture was centrifuged at 12,000 *g* for 15 min. This extraction was repeated twice by resuspending the precipitate in 5 ml of phosphate buffer medium and centrifuging at 12,000 *g* for 10 min. The supernatants were combined. The precipitate was finally washed with 30 ml of water to remove detergent and freeze-dried. This fraction was designated as the "12,000 *g* precipitate".

The combined supernatants were centrifuged at 105,000 *g* for 90 min. The supernatant was exhaustively dialysed against water to remove traces of detergent to give the "supernatant fraction".

The proteins present in these two fractions were examined by starch-gel electrophoresis as previously described (Graham 1963; Graham and Morton 1963).

(iv) *Specific Activity Measurements.*—Radioactivity was measured with a scintillation counter as previously described (Graham and Morton 1963), liquid samples being mixed with Diotol and solid samples with Thixin gel. Nitrogen was estimated by a microKjeldahl procedure.

III. Results

(*a*) *Starch-gel Electrophoresis of Proteins*

Starch-gel electrophoresis was used to compare the protein components of the 12,000 *g* precipitate and the supernatant fraction prepared from cv. Bungulla with the protein components extracted from the same endosperm with sodium pyrophosphate and acetic acid. Plate 1 shows the results. The 12,000 *g* precipitate contained predominantly slow-moving components and showed a similar distribution of fast- and slow-moving components to that obtained with protein bodies isolated as described by Graham, Morton, and Raison (1963) from cv. Gabo. The supernatant fraction contained no slow-moving components; the fast-moving components corresponded to the components extracted with sodium pyrophosphate.

These results show that the quantitative separation of particulate and soluble protein by the method described also provided a satisfactory qualitative separation of the storage and the cytoplasmic proteins.

(*b*) *Incorporation of* [^{35}S]*Sulphate into cv. Bungulla*

Excised heads of cv. Bungulla were exposed for 1 hr to [^{35}S]sulphate and were then transferred to water. Measurements were made of the changes in specific activity of the endosperm proteins with increasing periods of incubation in water; the proteins were fractionated by the three methods described above (see Section II). The changes in specific activity of the different fractions are shown in Figures 1(*a*), 1(*b*), and 1(*c*).

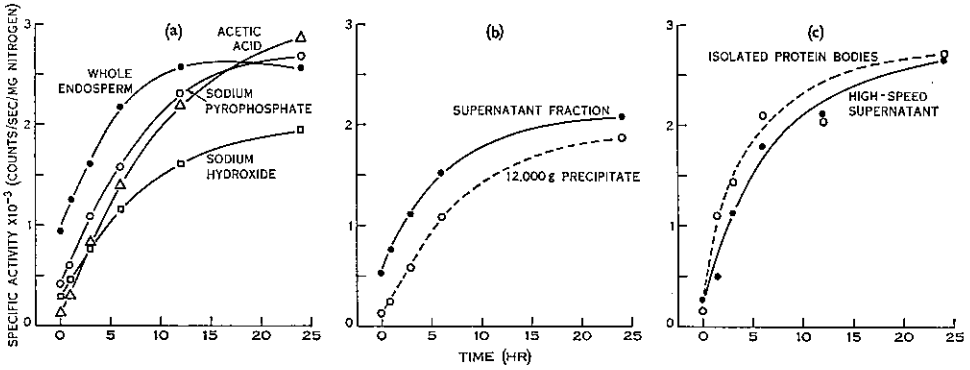


Fig. 1.—Change in specific activity of whole endosperm from cv. Bungulla and of the protein fractions obtained from the endosperm by the methods described in Section II(*e*), after the uptake of [^{35}S]sulphate into excised wheat heads followed by increasing periods of incubation in water.

There was a decline in the rate of increase in the specific activity of all the protein fractions between 12 and 24 hr which corresponded with the change in specific activity of the intact endosperm. In Figure 1(*a*) the storage proteins are represented by the sum of those soluble in acetic acid and sodium hydroxide (Graham, Morton, and Simmonds 1963). A comparison of the changes in specific activity of the sodium pyrophosphate-soluble proteins with the mean of the specific activities of the acetic acid- and sodium hydroxide-soluble proteins [Fig. 1(*a*)] show a similar trend to the changes found in the supernatant fraction and the 12,000 *g* precipitate [Fig. 1(*b*)]. At each time interval the specific activity of the soluble proteins exceeded that of the storage proteins. In contrast, results for the isolated cellular components [Fig. 1(*c*)] show that the specific activity of the protein body fraction exceeded that of the supernatant fraction (except for an initial lag).

Since the 12,000 *g* precipitate represents an almost quantitative recovery of protein bodies, and as the starch-gel electrophoresis results show that it contains predominantly slow-moving components, it was considered that this fraction was more representative of the total storage proteins than the small protein bodies isolated by density-gradient centrifugation. Moreover, the supernatant fraction

does not contain particulate proteins which are always present in small quantities in the sodium pyrophosphate extract (Graham 1963). Consequently in further experiments, changes in specific activities of the soluble and storage proteins were followed in the supernatant fraction and in the 12,000 *g* precipitate fraction.

(c) *Incorporation of* [^{35}S]*Sulphate into cv. Gabo*

If the soluble (cytoplasmic) proteins were precursors of the storage (protein body) proteins, then the specific activity of the soluble proteins would fall below that of the protein body proteins during incubation. A similar experiment to that shown in Figure 1 was therefore carried out with cv. Gabo, except that the period of incubation was extended to 48 hr. As shown in Figure 2, the specific activity of the soluble proteins declined after 30 hr but within the 48 hr period the specific activity

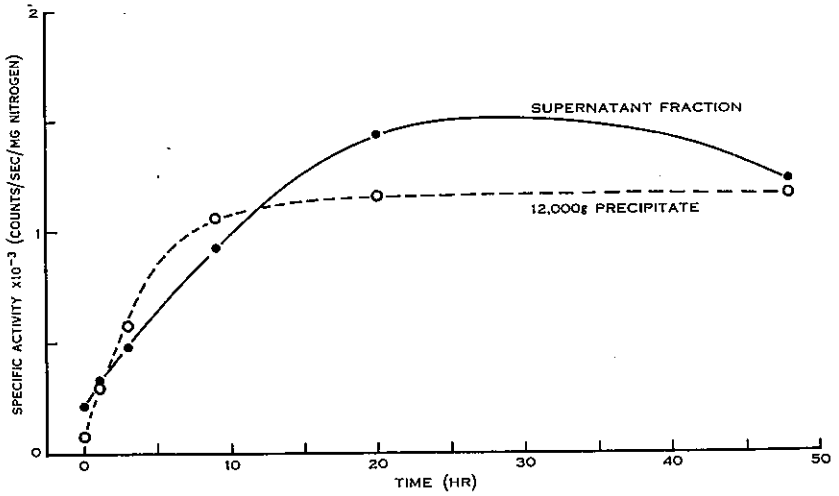


Fig. 2.—Changes in specific activity of the 12,000 *g* precipitate and of the supernatant fraction of cv. Gabo after exposure of excised wheat heads to [^{35}S]sulphate and incubation in water for the periods indicated.

of the soluble proteins did not fall below that of the storage proteins. It was apparent that a more rapid dilution of the isotope was required to follow the changes in specific activity between the two groups of proteins. Since it was considered undesirable to extend the period of incubation longer than 48 hr, the period of exposure to the isotope was reduced.

Heads of cv. Gabo were exposed to [^{35}S]sulphate for 5, 10, 20, 30, 40, and 60 min and were then incubated in water for 6 hr. The specific activities of the supernatant fraction and of the 12,000 *g* precipitate fraction are shown in Figure 3.

The results show that the ratio of the specific activity of the proteins of the supernatant fraction to that of the proteins of the 12,000 *g* precipitate remained approximately constant after exposure periods of 5–30 min; there was a decrease in the specific activity of the supernatant fraction of heads exposed for 40 and 60 min which was accompanied by a decline in the increase in the specific activity of the 12,000 *g* precipitate.

It therefore appears that [^{35}S]sulphate was taken up into the intact heads to form a pool which was only slowly diluted as the radioactivity from the sulphate was incorporated into the endosperm proteins. Consequently after 6 hr the specific activities of both the soluble and storage protein fractions reflected the amount of isotope incorporated during the period of exposure to [^{35}S]sulphate.

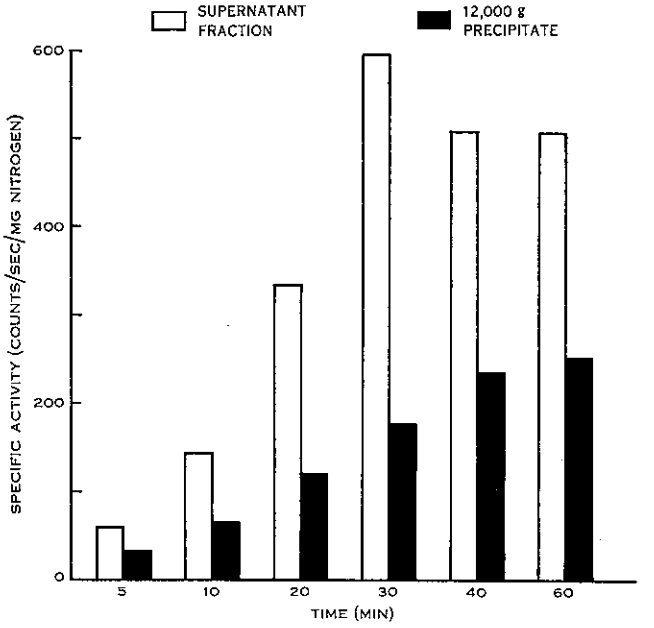


Fig. 3.—Specific activity of the 12,000 *g* precipitate and of the supernatant fraction obtained from cv. Gabo endosperm after exposure to [^{35}S]sulphate for varying periods and incubation in water for 6 hr.

(*d*) *Incorporation of* [^{35}*S*]*Sulphate,* [^{14}*C*]*Proline, and* [^{14}*C*]*Leucine into cv. Dural*

In the previous experiments uptake of [^{35}S]sulphate by excised wheat heads and the subsequent incorporation of [^{35}S]amino acids into the endosperm proteins resulted in a higher specific activity in the soluble (supernatant) proteins as compared to the storage proteins. Amino acid analyses (Jennings and Morton 1963*a*) of the supernatant fraction and of the "10,000 *g* precipitate" prepared from cv. Gabo endosperm 18 days after flowering, showed that the relative amount of cysteine and methionine was greater in the soluble proteins than in the storage proteins. To examine the incorporation of labelled amino acids present in at least equal concentration in the storage and cytoplasmic proteins, excised heads of cv. Dural were exposed to [^{14}C]proline and to [^{14}C]leucine (as well as to [^{35}S]sulphate) for 1 hr and then incubated in water for various periods up to 48 hr. Figures 4(*a*), 4(*b*), and 4(*c*) show the changes in specific activities of the supernatant fraction and of the 12,000 *g* precipitate during incubation.

To demonstrate that the radioactivity from [^{14}C]-labelled amino acids and [^{35}S]sulphate was incorporated into the various protein components of the 12,000 *g* precipitate and the supernatant fraction, a radioautograph was prepared after the protein components were separated by starch-gel electrophoresis. Plate 2 shows that most of the protein components of the 12,000 *g* precipitate and the supernatant fraction revealed by staining with nigrosine were detected by radioautography; there was no qualitative difference in the distribution of radioactivity of samples labelled with [^{14}C]-amino acids or with [^{35}S]sulphate. With each radioactive compound, and in both protein fractions, the rate of increase in specific activity was constant for 10 hr and then declined until 30 hr, after which there was a decrease in specific

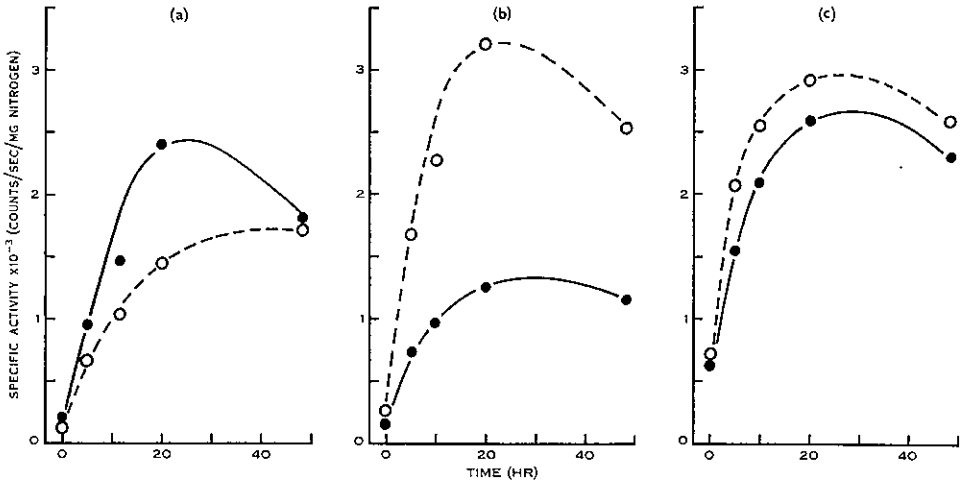


Fig. 4.—Changes in specific activity of the 12,000 *g* precipitate (○) and of the supernatant fraction (●) obtained from cv. Dural endosperm after exposure to: (*a*) [^{35}S]sulphate, (*b*) [^{14}C]-proline, and (*c*) [^{14}C]leucine, each for 1 hr. After exposure the excised heads were incubated in water for the periods indicated.

activity (Fig. 4). As in the previous experiments with other varieties, throughout the 48-hr period of incubation in water the incorporation of radioactivity from [^{35}S]sulphate resulted in a greater specific activity of the proteins of the supernatant fraction compared with that of the 12,000 *g* precipitate [Fig. 4(*a*)]. However, with both [^{14}C]proline and [^{14}C]leucine the specific activity of the proteins of the supernatant fraction was always less than that of the 12,000 *g* precipitate [Figs. 4(*b*) and 4(*c*)]. Thus the proteins of the supernatant fraction (cytoplasmic proteins) cannot be precursors of those of the 12,000 *g* precipitate (storage proteins).

(*e*) *Effect of Chloramphenicol and of Sodium Fluoroacetate on the Incorporation of Radioactivity from* [^{35}*S*]*Sulphate into Endosperm Proteins*

To examine whether a differential inhibition of the synthesis of soluble proteins and of the synthesis of storage proteins could be obtained, the effect of chloramphenicol and of sodium fluoroacetate on the incorporation of radioactivity from [^{35}S]sulphate into the soluble and storage proteins was studied.

Excised heads of cv. Dural were exposed to [^{35}S]sulphate for 1 hr and were then incubated in either (1) water, (2) chloramphenicol (0·066M), or (3) sodium fluoroacetate (0·05M) for various periods up to 24 hr. The changes in specific activity of the supernatant fraction and of the 12,000 *g* precipitate are shown in Figures 5 (*a*), 5 (*b*), and 5 (*c*).

Chloramphenicol did not inhibit the incorporation of radioactivity into either protein fraction. However, after incubation for 6 hr the incorporation into the proteins of the supernatant fraction was inhibited in the heads which had been treated with fluoroacetate, whereas there was no effect on the incorporation of radioactivity into the proteins of the 12,000 *g* precipitate [Figs. 5 (*a*), 5 (*b*), and 5 (*c*)].

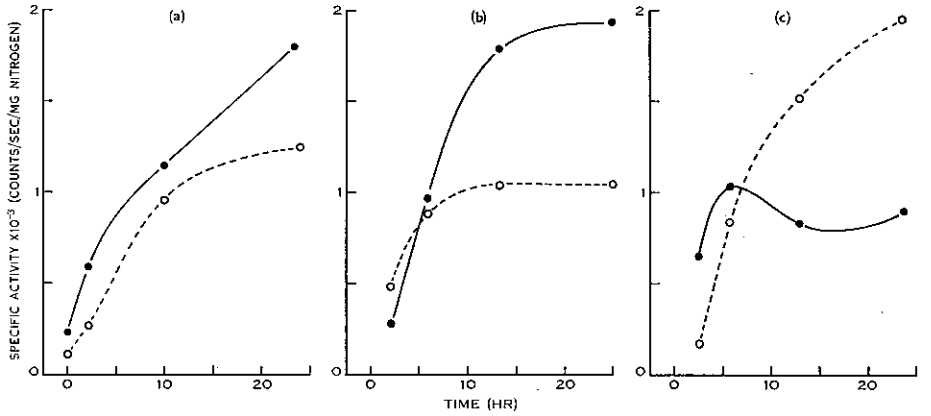


Fig. 5.—Changes in specific activity of the 12,000 *g* precipitate (○) and of the supernatant fraction (●) obtained from cv. Dural endosperm after exposure to [^{35}S]sulphate for 1 hr and subsequent incubation of the excised wheat heads in: (*a*) water; (*b*) chloramphenicol (0·066M); (*c*) fluoroacetate (0·05M).

In another experiment, the excised heads were pre-incubated for 1 hr in either (1) water, (2) chloramphenicol (0·066M), and (3) sodium fluoroacetate (0·05M) before exposure to [^{35}S]sulphate for 1 hr and further incubation in water for periods up to 8 hr. The changes in specific activity of the protein fractions are shown in Figures 6 (*a*), 6 (*b*), and 6 (*c*). Whereas the specific activities of the protein fractions in heads pretreated with chloramphenicol did not differ markedly from those of heads which had been pretreated with water only, pretreatment of the excised heads in sodium fluoroacetate resulted in a marked inhibition of the incorporation of radioactivity into both the supernatant and 12,000 *g* precipitate fractions.

IV. Discussion

The changes in the specific activities of the cytoplasmic (soluble) and of the storage (particulate) proteins of developing wheat endosperm after exposure to radioactive isotopes was studied in previous experiments (Graham and Morton 1963; Graham, Morton, and Raison 1963). There were marked differences in the specific activities of the proteins soluble in sodium pyrophosphate buffer, as compared with

those soluble in acetic acid and in sodium hydroxide (Graham and Morton 1963). Also, there were marked differences in the specific activities of the proteins of the high-speed supernatant fraction as compared with those of protein bodies separated by density-gradient centrifugation (Graham, Morton, and Raison 1963).

In the present study, the storage proteins were obtained in the 12,000 *g* precipitate and the cytoplasmic (soluble) proteins in the supernatant fraction. As shown by Plate 1 and in Section III, there was good qualitative and quantitative separation of the two groups of proteins. The proteins of the pellet sedimented at 105,000 *g* were not examined; this pellet consists mostly of microsomes (Morton and Raison 1964) and is quantitatively of little significance in the present study. The almost-quantitative recovery of the storage proteins in the 12,000 *g* precipitate contrasts

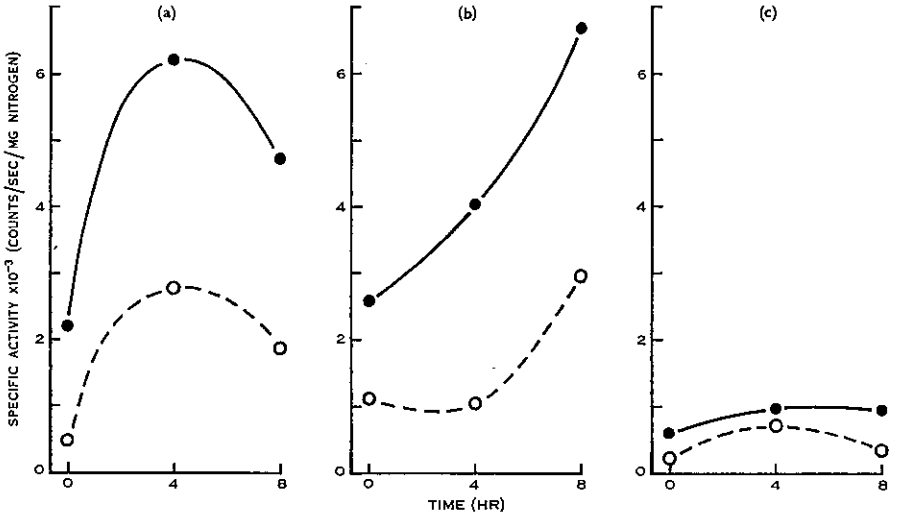


Fig. 6.—Change in specific activity of the 12,000 *g* precipitate (○) and the supernatant fraction (●) obtained from cv. Dural endosperm. Excised wheat heads were incubated for 1 hr in: (*a*) water, (*b*) chloramphenicol (0·066M), (*c*) sodium fluoroacetate (0·05M), exposed to [^{35}S]sulphate for 1 hr, and further incubated in water for the periods indicated.

with the low recovery of protein bodies by density-gradient centrifugation by which only small protein bodies are isolated (Graham, Morton, and Raison 1963); these small bodies differ in amino acid composition from the bodies isolated as described here (Jennings and Morton 1963). The somewhat greater increase in specific activity of the isolated protein bodies obtained by density-gradient centrifugation [Fig. 1(*c*)] as compared with that of the 12,000 *g* precipitate [Fig. 1(*b*)] may be due to more rapid synthesis of the protein of small protein bodies.

Similar changes in the specific activities of the soluble and storage proteins were found if excised wheat heads were exposed to [^{35}S]sulphate either for 48 hr (Graham and Morton 1963) or for 1 hr followed by 48 hr incubation in water (Fig. 2), indicating the presence of a large pool of substrates in which the [^{35}S]-radioactivity was diluted before incorporation into the endosperm proteins. This was confirmed in experiments with cv. Gabo where the ratio of the specific activity of the soluble proteins to that

of the storage proteins was the same at 6 hr after exposure to [^{35}S]sulphate for either 5 or 30 min (Fig. 3). These results indicate that excised wheat heads are not suitable for pulse-labelling experiments in relation to the synthesis of endosperm proteins. Attempts to obtain a more rapid dilution of the [^{35}S]sulphate within the head by incubating the heads in 0·05M potassium sulphate instead of water resulted in a decrease in the specific activities of both the soluble and storage proteins.

A comparison of the results obtained after incorporation of radioactivity from [^{35}S]sulphate, [^{14}C]proline, and [^{14}C]leucine [Figs. 4(*a*), 4(*b*), and 4(*c*)] shows that the ratio of the specific activity of the soluble proteins to that of the storage proteins depended on the isotope administered. Since it has been shown (Plate 2) that radioactivity from ^{14}C-labelled amino acids and from [^{35}S]sulphate is incorporated into similar protein components, the results obtained using the two isotopes can be compared. The ratios of the specific activities of the supernatant fraction to the 12,000*g* precipitate fraction at 10 hr for [^{35}S]sulphate, [^{14}C]proline, and [^{14}C]leucine were 1·64, 0·39, and 0·90. From the results of amino acid analyses (Jennings and Morton 1963) the ratios of the amino nitrogen content of the supernatant fraction and of the precipitate fraction in cv. Gabo, 18 days after flowering, for cysteine and methionine, proline, and leucine were 1·23, 0·32, and 1·1 respectively. A comparison of these two sets of ratios indicates that for each radioisotope, the relative specific activities of the two protein fractions at the 10 hr period were related to the relative amount of the amino acids present in each fraction.

The results of studies of the incorporation of [^{14}C]proline and of [^{14}C]leucine (Fig. 4) provided evidence that the soluble proteins were not precursors in the synthesis of storage proteins. The specific activity of the soluble proteins after incorporation of either [^{14}C]proline or [^{14}C]leucine never exceeded that of the storage proteins. If the supernatant fraction contained soluble precursors of the storage proteins, the specific activity of this fraction would initially have exceeded the specific activity of the 12,000 *g* precipitate. The results do not exclude the possibility of a precursor of the storage proteins which remained associated with a particulate component present in the 105,000 *g* precipitate.

For each radioisotope, parallel changes in specific activities of both the soluble and storage proteins were found, indicating that the synthesis of the soluble proteins and synthesis of the storage proteins may have been related. However, the results obtained after treatment of the excised heads with sodium fluoroacetate [Fig. 5 (*c*)] indicate that the synthesis of the soluble proteins and synthesis of the storage proteins takes place by independent systems.

Chloramphenicol at a concentration of 0·03M inhibits the incorporation of amino acids into bacterial protein (Gale and Folkes 1953) and the incorporation of amino acids into proteins in homogenates of wheat endosperm (Morton and Raison 1963). In the present experiments (Figs. 5 and 6) no inhibition of protein synthesis was observed suggesting that the chloramphenicol did not reach the site of protein synthesis. In contrast, under the same conditions sodium fluoroacetate strongly inhibited the synthesis of soluble proteins. Treble, Lamport, and Peters (1962) have demonstrated that aconitate hydratase (EC 4.2.1.3) of plant tissues is substantially inhibited by fluorocitrate arising from fluoroacetate. The inhibition of

aconitate hydratase would result in substantial inhibition of generation of adenosine triphosphate (ATP) by interference with oxidations involving the tricarboxylic acid cycle. Since the incorporation of amino acids into protein requires utilization of ATP (see Hoagland 1961), it is likely that the observed effect of fluoroacetate is due to decreased production of ATP. After exposure to [^{35}S]sulphate, treatment of the excised heads with sodium fluoroacetate had no effect on the rate of incorporation of radioactivity into the storage proteins, whereas the incorporation into the soluble proteins was inhibited after incubation for 6 hr [Fig. 5(*c*)].

Since ATP is certainly required for the synthesis of storage proteins as well as for the synthesis of cytoplasmic (soluble) proteins, it appears that ATP for incorporation of radioactivity into the storage protein is generated elsewhere than via the tricarboxylic acid cycle. This clearly establishes a difference in localization of the sites of synthesis of the storage and cytoplasmic proteins of the endosperm. The delay in fluoroacetate inhibition of incorporation into the soluble proteins is comparable with the slow development of inhibition of aconitate hydratase observed by Treble, Lamport, and Peters (1962) in tissue cultures. Pretreatment of excised heads with fluoroacetate before exposure to [^{35}S]sulphate caused substantial inhibition of the incorporation of radioactivity into both the soluble and the storage proteins [Fig. 6(*c*)]. This is attributed to the inhibition of the ATP-dependent uptake and transport of [^{35}S]sulphate to the site of formation of ^{35}S-labelled products (presumably cysteine and methionine), the incorporation of which into both soluble and storage proteins accounts for the incorporation of radioactivity from [^{35}S]sulphate.

Jennings, Morton, and Palk (1963) have described in detail the fine structure of protein bodies, and have shown their distinctive localization within a surrounding lipoprotein membrane. Buttrose (1963) has also reported on the cytology of the endosperm tissue. The observations described here are consistent with the interpretation of the functional activities of the components of wheat endosperm cells given by Morton, Palk, and Raison (1964) and Morton and Raison (1964) and indicate that the synthesis of storage proteins which accumulate in protein bodies occurs simultaneously with, and independently of, the synthesis of the soluble proteins present in the cytoplasm. The changes in relative rates of synthesis of storage (acetic acid-soluble) as compared with cytoplasmic (pyrophosphate-soluble) protein during development of the endosperm (Graham, Morton, and Simmonds 1963; Jennings and Morton 1963*b*) are attributable to a relative increase in the amount and activity per cell of the organelles (proteoplasts or protein-forming plastids, cf. Morton and Raison 1963) which specifically synthesize the storage proteins.

V. Acknowledgments

It is a pleasure to acknowledge the technical assistance of Miss M. Ryder and Mr. D. Morton.

Grateful acknowledgment is made of financial support from the Wheat Industry Research Fund. The work described in this and preceding papers from this Department concerned with developing wheat endosperm was greatly assisted by a grant from the Rural Credits Development Fund.

VI. References

Altschul, A. M., Snowden, J. E., Manchon, D. D., and Dechary, J. M. (1961).—*Arch. Biochem. Biophys.* **95**: 402–4.

Bagley, B. W., Cherry, J. H., Rollins, Mary L., and Altschul, A. M. (1963).—*Amer. J. Bot.* (In press.)

Buttrose, M. S. (1963).—*Aust. J. Biol. Sci.* **16**: 305–17.

Dieckert, J. W., and Snowden, J. E. (1960).—*Fed. Proc.* **19**: 126.

Dieckert, J. W., Snowden, J. E., Moore, A. T., Heinzelman, J. E., and Altschul, A. M. (1962).—*J. Food Sci.* **27**: 321–5.

Duvick, D. W. (1955).—*Amer. J. Bot.* **42**: 717–25.

Duvick, D. W. (1961).—*Cereal Chem.* **38**: 374–85.

Gale, E. F., and Folkes, J. P. (1953).—*Biochem. J.* **53**: 493–8.

Graham, Janet S. D. (1963).—*Aust. J. Biol. Sci.* **16**: 342–9.

Graham, Janet S. D., Jennings, A. C., Morton, R. K., Palk, B. A., and Raison, J. K. (1962).—*Nature* **196**: 967–9.

Graham, Janet S. D., and Morton, R. K. (1963).—*Aust. J. Biol. Sci.* **16**: 357–65.

Graham, Janet S. D., Morton, R. K., and Raison, J. K. (1963).—*Aust. J. Biol. Sci.* **16**: 375–83.

Graham, Janet S. D., Morton, R. K., and Simmonds, D. H. (1963).—*Aust. J. Biol. Sci.* **16**: 350–6.

Hoagland, M. B. (1961).—In "The Nucleic Acids." Vol. 3 p. 349. (Eds. E. Chargaff and J. N. Davidson.) (Academic Press, Inc.: New York.)

Jennings, A. C., and Morton, R. K. (1963*a*).—*Aust. J. Biol. Sci.* **16**: 384–94.

Jennings, A. C., and Morton, R. K. (1963*b*).—*Aust. J. Biol. Sci.* **16**: 318–331.

Jennings, A. C., Morton, R. K., and Palk, B. A. (1963).—*Aust. J. Biol. Sci.* **16**: 366–74.

Morton, R. K., and Raison, J. K. (1963).—*Nature* **200**: 429–33.

Morton, R. K., and Raison, J. K. (1964).—*Biochem. J.* (In press.)

Morton, R. K., Palk, B. A., and Raison, J. K. (1964).—*Biochem. J.* (In press.)

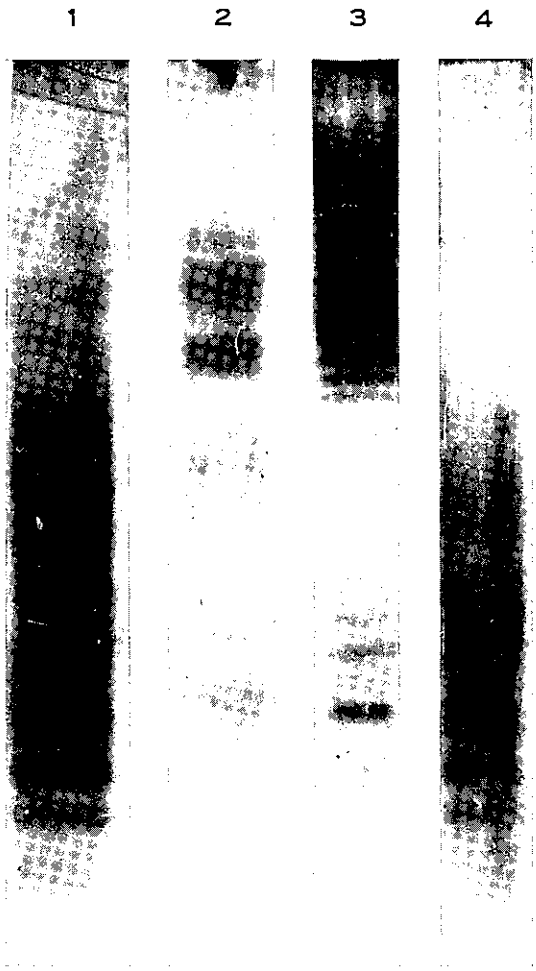
Treble, D. H., Lamport, D. T. A., and Peters, R. A. (1962).—*Biochem. J.* **85**: 113–5.

Varner, J. E., and Schidlovsky, G. (1963).—*Plant Physiol.* **38**: 139–44.

Watson, S. A., Sanders, E. H., Wakely, R. D., and Williams, C. B. (1955).—*Cereal Chem.* **32**: 165–82.

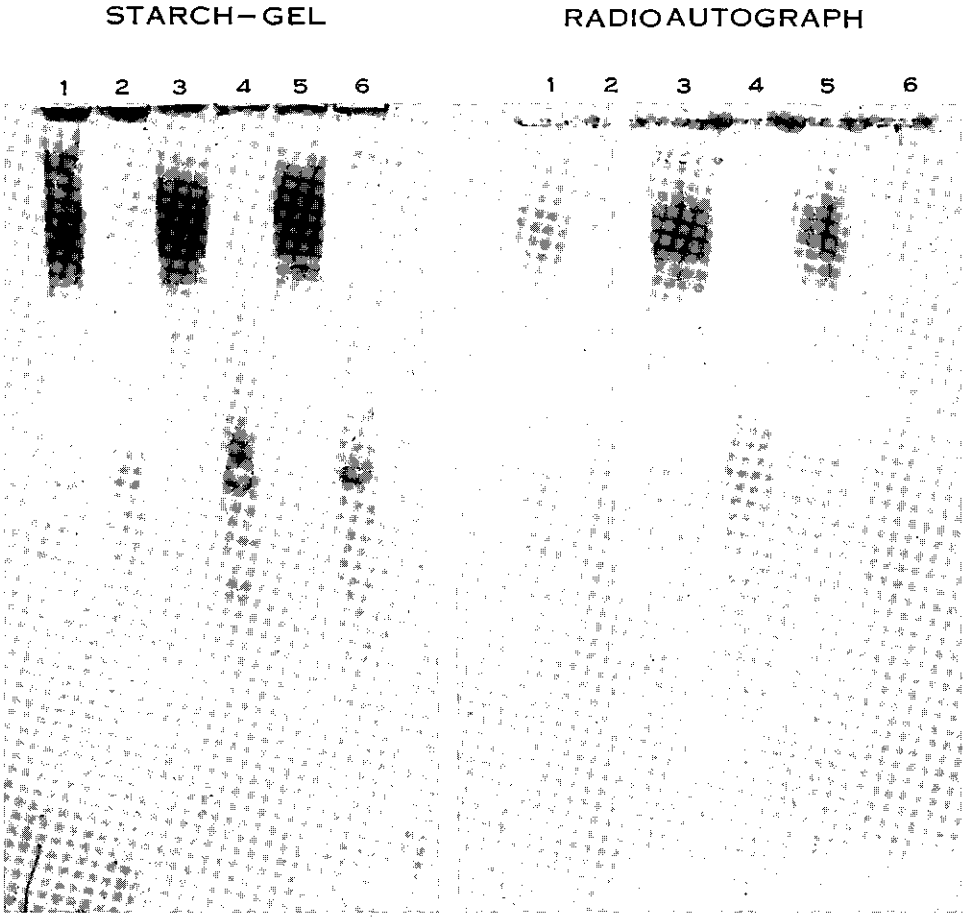
Yatsu, L. Y. (1963).—*J. Cell. Biol.* (In press.)

INCORPORATION OF AMINO ACIDS INTO WHEAT PROTEINS



The protein components of fractions of endosperm of *T. vulgare* cv. Bungulla as separated by starch-gel electrophoresis at 10 V/cm for 5·5 hr. The gel was prepared and stained as described by Graham (1963). Columns **1** and **2** show results for extracts of endosperm prepared as described by Graham, Morton, and Simmonds (1963); columns **3** and **4** show results for fractions prepared as described in Section II. **1**, 0·01M sodium pyrophosphate, pH 7·4; **2**, 0·05N acetic acid; **3**, the 12,000 *g* precipitate; **4**, the supernatant fraction.

INCORPORATION OF AMINO ACIDS INTO WHEAT PROTEINS



The protein components of the 12,000 *g* precipitate and the supernatant fraction obtained from cv. Dural endosperm as separated by starch-gel electrophoresis at 10 V/cm for 6 hr. The fractions were obtained from endosperm exposed to either [^{35}S]sulphate, [^{14}C]proline, or [^{14}C]leucine for 1 hr followed by incubation in water for 10 hr. The starch-gel was stained with 0·0125% (w/v) nigrosine for 16 hr. The radioautograph was prepared by exposing X-ray film to a filter-paper replica of the labelled protein components separated by starch-gel electrophoresis as described by Graham and Morton (1963). The photographic copy of the radioautograph was prepared by direct contact printing. Columns **1**, **3**, and **5** give results for the 12,000 *g* precipitate, columns **2**, **4**, and **6** give results for the supernatant fraction obtained from endosperm exposed to the various isotopes as follows: **1** and **2**, [^{35}S]sulphate; **3** and **4**, [^{14}C]proline; **5** and **6**, [^{14}C]leucine.