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GLYCERIDE METABOLISM AND GLUCONEOGENESIS IN BARLEY ENDOSPERM

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Key Word Index—*Hordeum distichon*; Gramineae; barley; endosperm; gibberellic acid; aleurone; glycerides; gluconeogenesis; glutamine.

Abstract—Endosperms of quiescent barley grains contained, on average, 54.5 μg of neutral glyceride-glycerol, equivalent to *ca* 480 μg glyceride. Of this probably 90% was located in the aleurone layer. During germination the level of glyceride-glycerol declined. It also declined in degermed grains and aleurone layers incubated *in vitro*. The fall was accelerated by GA_3, but indoleacetic acid, kinetin and glutamine were without effect. Increases in the levels of malate synthase and isocitrate lyase from very low initial values, and the results of incorporation studies with [^{14}C]-labelled substrates, indicate that the glyoxylate cycle functions to convert glycerides to sucrose in germinating grains and degermed grains incubated with GA_3, but not in degermed grains without the hormone. In the absence of GA_3 the glyceride could be a respiratory substrate in degermed grains. The aleurone layers converted exogenous glucose to sucrose. Little label from [^{14}C]-amino acids appeared in sucrose but in some cases considerable incorporation occurred into glutamine.

INTRODUCTION

Barley grains contain 3–4.6% (dry basis) lipids, of which the largest class is neutral glycerides [1–3]. About two-thirds of the petrol-soluble lipid occurs in the endosperm (aleurone layer + dead starchy endosperm) [4]. Microscopic and other studies show that aleurone cells are rich in lipids, which occur in spherosomes [5–7]. The fate of the aleurone lipid during germination is in doubt, but the spherosomes decrease in number during germination, so presumably the lipid is utilized [5].

During germination the respiratory quotient (R.Q.) of the tissue may be as low as 0.3–0.4, a value consistent with the conversion of triglycerides to carbohydrates [8]. Further non-reducing acid-labile carbohydrates (sucrose or fructosans) accumulate in the aleurones of germinating grains [9]. The sucrose content of the endosperm increases during germination, and sucrose is a major sugar in the endosperm of germinated grain [10]. These observations suggest that the glyoxylate cycle may be functioning to convert lipids to carbohydrates. Although glyoxysomes have been isolated from aleurones the activity of isocitrate lyase, a key enzyme, was too low to measure [11]. Glyoxysomes do occur and the levels of enzymes of the glyoxylate cycle do increase in the aleurone layers of germinating wheat [12]. The aleurone layer converts other sugars into sucrose [13] but the changing levels of sucrose in the endosperm of germinating grains do not closely parallel the levels of other sugars, suggesting that there is another metabolic source of this disaccharide [10].

The work described was undertaken to determine the fate of glycerides in the aleurone.

RESULTS

The endosperms of quiescent grains contained, on average, 54.5 μg of neutral glyceride–glycerol. The proportions of fatty acids in the triglyceride fraction, estimated by GLC of the Me esters, were 14:0, 0.5%; 16:0, 16.6%; 18:0, 1.5%; 18:1, 17.8%; 18:2, 59.7%; 18:3, 4.0%, values in agreement with published data [4]. By TLC the glyceride fraction contained 80% triglyceride and 15% monoglyceride and diglyceride; no free glycerol was detected. Others reported that whole barley neutral glyceride contained 80.7% triglyceride, 7.6% diglyceride and 11.7% monoglyceride [1]. Calculation, assuming a uniform mean MW of 277 for the fatty acids, and that monoglycerides and diglycerides were present in equal amounts, give a mean MW of 806 for the glycerides. Thus each endosperm contained *ca* 0.48 mg of glyceride.

As aleurones, free from starchy endosperm, could not be separated dry from quiescent grains indirect estimates of their glyceride contents were made by measuring the differences between the complete endosperm and starchy endosperm (separated by dissection), and by separating aleurone layers from starchy endosperm after a 3-day incubation in a solution of pectinase [14]. The discrepant results obtained, that the aleurone contained at least 76 or 91% respectively of the glyceride–glycerol of the endosperm, are not surprising since the dissection process was crude, and with the pectinase technique the assumption has to be made that glyceride levels decline equally in endosperm halves incubated with or without pectinase. Thus after 66 hr incubation endosperms contained 31.6 μg glyceride–glycerol while separated aleurone

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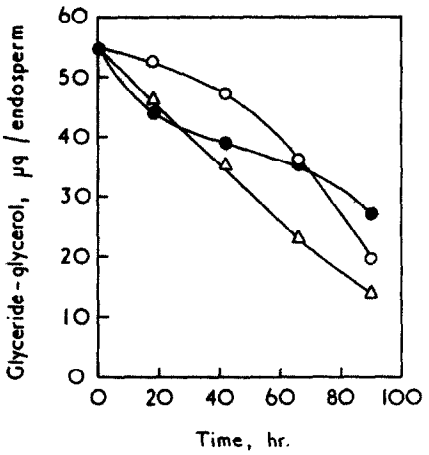

Fig. 1. Glyceride–glycerol content of endosperms of grains germinated at 25° and of endosperm halves incubated in buffer, with or without GA_3 (2 μg/ml). Results are the means of three determinations with 20 endosperm halves used for each determination. O——O endosperms of germinating grains; ●——● endosperm halves; △——△ endosperm halves + GA_3.

layers (pectinase technique) contained 28.7 μg. The corresponding values when gibberellic acid (GA_3; 2 μg/ml) was present were 18.3 and 14.5 μg/grain part; thus the hormone accelerates triglyceride utilization. The weight of intact aleurone layers from quiescent grain cannot be determined directly, but if the estimate of 7.2% (w/w) of the grain is accepted [15] then *ca* 13–15% (w/fr. wt) of the aleurone (*ca* 2.9 mg) is due to glyceride.

During germination the glyceride content of the aleurone declined, as it did in degermed grains (endosperms) incubated *in vitro* (Fig. 1). While the decline in endosperms was continuous in the presence of GA_3, the rate of decline decreased in the absence of this hormone. Anaerobiosis prevented alterations in glyceride levels under all the incubation conditions tested. Antibiotics (penicillin, streptomycin and nystatin) were used in experiments with separated endosperms, since when asepsis failed microbial contamination occurred and reduced or prevented the response to GA_3. The antibiotics did not alter the response to added GA_3. It was previously shown that under specified conditions they did not harm other aspects of aleurone metabolism [14]. The same alterations of glyceride levels occurred whether incubations were in water, in phthalate buffer, or in succinate buffer.

α-Indole acetic acid (IAA), kinetin and glutamine, used at levels found to be optimal for altering lipid metabolism in wheat aleurones [16,17], had no effect on the levels of glycerides attained in barley endosperms incubated *in vitro*, with or without GA_3. Barley aleurone layers were prepared from endosperms by the pectinase technique; during the 3-day incubation period their glyceride–glycerol level fell to 20.7 μg/aleurone. On further incubation, with or without GA_3, glyceride–glycerol contents fell to 3.9 and 10.0 μg/aleurone respectively, so a decline can occur in the absence of starchy endosperm. A stimulation of lipid decline by GA_3 in isolated aleurone layers incubated for 24 hr has been found by Firn and Kende [18]. In the absence of a method for preparing intact, dry aleurone layers it is impossible to test whether the starchy endosperm has an inductive effect on triglyceride decline, as observed in wheat [16].

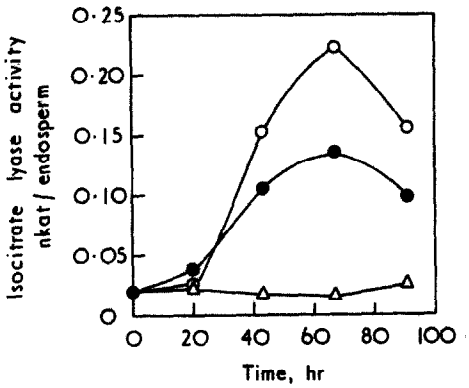

Fig. 2. Isocitrate lyase activity of endosperms of grains germinated at 25° and of endosperm halves incubated in buffer with or without GA_3 (2 μg/ml). Results are the means of three determinations with 20 endosperm halves used for each determination. O——O endosperms of germinating grains; ●——● endosperm halves + GA_3; △——△ endosperm halves.

Endosperms of quiescent barley grains contain low levels of malate synthase and isocitrate lyase. During germination the activities of these enzymes rise until the second and third days, then decline (Figs. 2 and 3). The enzyme levels in separated endosperms incubated *in vitro* do not alter appreciably unless GA_3 is included in the medium; however, the peak levels observed with GA_3 were less than those detected in germinating grain (Figs. 2 and 3). These results are similar to those obtained with wheat [12]. Both malate synthase and isocitrate lyase were found only in the aleurone layer, when this and the starchy endosperm were separated after 3 days germination. The same changes of enzyme activity were found whether endosperms were incubated in water or in succinate buffer. It was calculated that enzyme activities were sufficient to metabolize all the C-2 units that could be derived from the glyceride disappearing during the experiments.

Oxygen uptake was more uniform when antibiotics were included in the medium. After 3 days incubation of endosperms, with or without GA_3, oxygen uptake averaged respectively, 447 and 153 nmol O_2/endosperm/hr at 25° while in the absence of starchy endosperm,

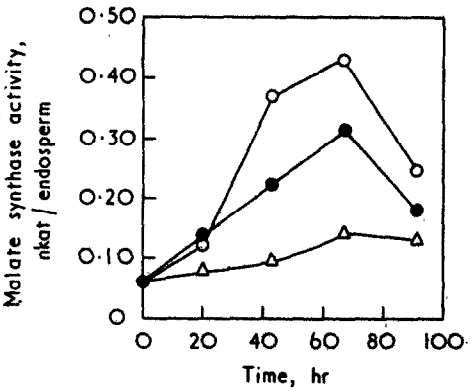

Fig. 3. Malate synthase activity of endosperms of grains germinated at 25° and of endosperm halves incubated in buffer with or without GA_3 (2 μg/ml). Results are the means of three determinations with 20 endosperm halves used for each determination. O——O endosperms of germinating grains; ●——● endosperm halves + GA_3; △——△ endosperm halves.

Table 1. The incorporation of label from radioactive acetate into various fractions, by barley aleurone layers peeled from germinating grain. Half aleurone layers (10) peeled from grains germinated for 2 days, were incubated for 2 hr at 25° in (Expt I), succinate buffer (20 mM, pH 5.3) containing acetate-[1-^{14}C] (0.26 μCi; 0.1 mM) or acetate-[2-^{14}C] (0.28 μCi; 0.1 mM), or (Expt II) distilled water containing acetate-[1-^{14}C] (0.56 μCi; 0.1 mM) or acetate-[2-^{14}C] (0.58 μCi; 0.1 mM). In Expt I the tissue and medium were analysed together. Aleurone layers were rinsed with unlabelled sodium acetate (50 mM) before analysis

Substrate	Acetate-[1-^{14}C] Incorporation (%) in			Acetate-[2-^{14}C] Incorporation (%) in		
Experiment	I Pooled	II Tissue	II Medium	I Pooled	II Tissue	II Medium
Neutral fraction	4.6	8.8	1.9	12.2	15.1	3.8
Basic fraction	8.0	9.5	1.7	13.7	—	3.0
Acidic fraction	3.0	4.4	4.4	5.5	3.4	6.3
Chloroform-soluble fraction	1.1	—	—	1.3	—	6.3
CO_2	—	20.0		—	3.2	

removed after softening with pectinase during the incubation period, the figures were 467 and 194 nmol O_2/aleurone/hr. These results were obtained in succinate buffer, containing antibiotics. Thus, as expected, gibberellic acid increased the respiration rate of the aleurone layer [19]. After 3 days incubation in water, phthalate buffer, or succinate buffer the respiration of endosperms averaged 163, 172 and 155 nmol O_2/endosperm/hr respectively. When the incubation media contained GA_3 (2 μg/ml) the aleurone layers separated, and the respiration rates were respectively 254, 382 and 482 nmol O_2/aleurone layer/hr. Thus in the presence of GA_3 phthalate and succinate allowed more respiration than water. Succinate buffer was used in most experiments because of its proven ability to control the pH of the medium [14]. Oxidation of fatty acids could account for the observed decline in glycerides in endosperms incubated with GA_3, if these were the only respiratory substrates. These values are tentative since respiration was not measured at all times during the incubation period.

Aleurone layers, peeled from germinating grains, incorporated label from acetate -[1-^{14}C] and -[2-^{14}C], into the neutral fraction, whether incubations were carried out in distilled water or succinate buffer (Table 1). About 90% of the activity in the neutral fraction occurred in sucrose, which was characterized by PC, both before and after hydrolysis with invertase or dilute acid, and by GLC of the TMSi ether and trapping the radioactivity. Small quantities of glucose and fructose were also present. Isolated aleurone cell walls are reported to hydrolyse sucrose slowly [13]. The radioactivity in the chloroform-soluble material, from the experiment with acetate-[1-^{14}C] (Table 1), separated by TLC was distributed as 17.7% in polar materials, 25.3% in triglycerides and 12.1% in hydrocarbons compared to 16.2, 29.0 and 11.5% respectively for the experiment with acetate-[2-^{14}C]. Aleurone layers incubated with labelled acetates released labelled materials into the medium. CO_2 was more heavily labelled from acetate-[1-^{14}C] than acetate-[2-^{14}C] (Table 1). Aleurone layers peeled from grain, germinated for 70 hr, incorporated more label from acetate-[2-^{14}C] (0.49 μCi, 0.1 mM) into the neutral fraction in a 2-hr incubation (18.5%), than aleurone layers separated from grain after 47 hr germination (10.4%) or 94 hr germination (11.9%). Sucrose comprised 89–93% of the neutral fractions. With increasing periods of incubation aleurone layers incorporated a progressively higher proportion of label into sucrose and less into monosaccharides (Fig. 4).

When aleurone layers, (separated after a 3-day digestion of endosperms with pectinase with or without GA_3, or with GA_3 and no pectinase) were incubated with labelled acetate incorporation into the neutral fraction was stimulated relatively more by the hormone than incorporation into the basic or acidic fractions, or into CO_2 (Table 2). Taken together the results suggest that in the initial stages of germination, or in endosperms or aleurone layers incubated without GA_3, glyceride is mainly a respiratory substrate, but that in germinating grains, or in endosperms or aleurone layers treated with GA_3, an increasing proportion is probably metabolized by the glyoxylate cycle, with the production of sucrose.

However when aleurone layers from grains germinated for 3 days were incubated with glyoxylate-[2-^{14}C] (1.50 μCi; 0.1 mM) for 2 hr there was only 0.006% incorporation of label into the neutral fraction and 0.6% into CO_2. When similar aleurone layers were incubated for 2 hr with palmitate-[1-^{14}C]-albumin complex, (0.38 μCi;

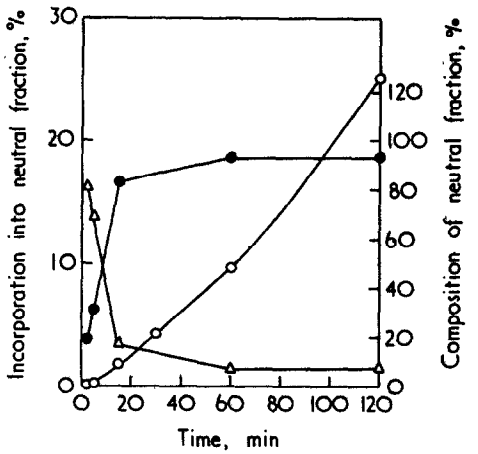

Fig. 4. Incorporation of radioactivity into the neutral fraction of half aleurone layers, peeled from grains germinated for 3 days at 25°, incubated with acetate-[2-^{14}C] (2 ml; 0.51 μCi; 0.1 mM). The tissue and medium were pooled for analysis. Each point is the means of two determinations with 10 half aleurone layers used for each determination. O——O incorporation into neutral fraction; ●——● sucrose; △——△ monosaccharide.

Table 2. Incorporation of radioactive acetate into various fractions by aleurone layers prepared *in vitro* in the presence or absence of GA_3. The tissues and media were pooled for analysis. Half aleurone layers (10) were prepared by incubating endosperms halves in succinate buffer containing antibiotics, with or without pectinase or GA_3 (2 μg/ml) (in the absence of either aleurone layers could not be separated). Incubations were for 2 hr, at 25°, in water containing acetate-[1-^{14}C] (0.56 μCi; 0.1 mM) or acetate-[2-^{14}C] (0.57 μCi; 0.1 mM)

Method of aleurone preparation:	Acetate-[2-^{14}C] incorporation (%) Pectinase (no GA_3)	Pectinase + GA_3	GA_3 only
Basic fraction	6 0	17 2	18.1
Acidic fraction	0.3	1 1	0.7
Neutral fraction	0 2	3.4	5 6
CO_2	2 2	3 5	2 0
Acetate-[1-^{14}C] CO_2	14.7	18.9	15.0

3.2 mM) which could give rise to acetyl-[1-^{14}C]-CoA by β-oxidation, label was incorporated into the neutral fraction (0.35%), the basic fraction (0.38%), the acidic fraction (0.24%) and CO_2 (2.2%). In a comparable trial with palmitate-[16-^{14}C] (0.9 μCi; 7.6 mM), which would give rise to acetyl-[2-^{14}C]-CoA by β-oxidation, incorporation into the neutral fraction was 0.56%, and 0.14% into carbon dioxide. In each case sucrose was the main component of the neutral fraction.

Aleurones incorporated glycerol-[1-^{14}C] (2 hr; 0.62 μCi; 9.5 mM) into mono- and di-saccharides (0.80%), materials that stayed on the PC baseline (0.17%), and chloroform-soluble material (0.18%). When aleurone layers were incubated with glycerol-[1-^{14}C], (2 hr; 7.17 μCi; 110 mM) incorporation into the basic fraction (0.22%) and acidic fraction (0.26%) was also detected. When the neutral fraction was separated by PC in addition to the sucrose (0.34%) and unidentified material that remained on the base-line (0.08%) another unidentified product "A" (0.13%) was detected between glucose and galactose (R_G 0.88). GLC of the TMSi derivative of material "A" yielded radioactivity that emerged after all the derivatives of monosaccharides, where no mass peak could be detected. After acid hydrolysis (1.5 M H_2SO_4, 2 hr, 100°) compound A gave one radioactive spot that moved with glycerol on PC. On GLC the TMSi derivative of authentic monogalactosyl glycerol emerged a little before "A". On PC monogalactosyl glycerol had an R_G of 0.82. Incubation of aleurone layers with glucose-[U-^{14}C] did not give rise to labelled "A". Thus "A" was tentatively identified as a glycerol-monoglycoside. After partial acid hydrolysis (0.5 M H_2SO_4, 30 min, 100°) material from the base line of the PC also gave rise to a substance moving with glycerol on PC.

When aleurone layers, peeled from grains germinated for 3 days, were incubated with glucose-[U-^{14}C] (2 hr, 9.07 μCi; 1.5 mM) the greatest incorporation of radioactivity was into sucrose (2.6%) compared to 1.0% into the acidic fraction and 0.44% into the basic fraction. The neutral fraction also contained unidentified radioactive substances; at the baseline (0.09%) and two areas close to the baseline (0.11 and 0.17%). In similar experiments the incorporation into CO_2 was 1.5%, suggesting that the conversion of exogenous glucose into sucrose is more important than its oxidation. Thus the most important fate of exogenous glucose appears to be conversion to sucrose, confirming the results of Chrispeels *et al.* [13].

When aleurone layers were incubated in the dark in a medium containing sodium bicarbonate-[^{14}C] (2 hr, 54.4 μCi; 0.55 mM) label appeared in the basic (0.38%), acidic (0.025%) and neutral (0.62%) fractions. In the neutral fraction sucrose contained 93.4% of the label, glucose 3.1% and fructose 2.0%. The non-photosynthetic incorporation of labelled bicarbonate into many tissues, e.g. barley roots, takes place by reversible carboxylation reactions giving rise to high levels of labelling in organic acids such as malate, citrate, aspartate and glutamate, and no activity in sugars [20]. The incorporation of most label into sugar by the aleurone layer is consistent with the operation of the glyoxylate cycle [21].

Aleurone layers, peeled from germinating grain, incorporated relatively little label from a mixture of amino acids-[U-^{14}C] into the neutral (0.82; 80% sucrose) or acidic (0.17%) fractions, or carbon dioxide (0.63%). The incorporation into glutamine, isolated from the basic fraction, was appreciably greater (4.8%). Incorporation of individual amino acids-[U-^{14}C] into neutral fraction was small (0.04–0.77%). However incorporations into glutamine ranged from massive (glutamic acid, 43.4%; proline, 39.6%; phenylalanine, 31.7%; alanine, 18.2%; leucine, 15.0%; isoleucine, 5.9%; glycine, 4.7%) to slight (tyrosine, 0.67%; serine, 0.62%; valine, 0.59; and arginine, 0.25%). The low incorporation into the neutral fraction contrasts with the findings with castor bean endosperm, in which label from certain amino acids was largely incorporated into sucrose [22].

DISCUSSION

The initial decline in endosperm glycerides during germination and in degermed grains incubated without GA_3 are consistent with their acting as respiratory substrates. However, in germinated grains, as in degermed grains incubated with GA_3, incorporation studies with labelled acetates, palmitates, glycerol and bicarbonate, and the alterations in enzyme levels show that the glyoxylate cycle is functioning and is converting glyceride to sucrose. This is consistent with some published data for barley and wheat [12]. However, GA_3 was said to have no effect on levels of wheat lipids, and the "inductive" effects of nitrogenous compounds and kinetin reported for wheat could not be detected in barley [16,17]. Failure to obtain high levels of incorporation of exogenous glyoxylate-[2-^{14}C] into sucrose is disturbing, but has been found before in tissues synthesising sugar from acetate [23, 24]. The slower initial rate of decline in glycerides in whole grains, compared to degermed grains, is probably due to a slower rate of hydration. The lower levels of malate synthase and isocitrate lyase activity in endosperms incubated with GA_3, relative to whole grains, requires further investigation. The failure of others to demonstrate a functional glyoxylate cycle in aleurone tissue is due, in part, to insufficient time being allowed for the enzymes to develop [11]. It is surprising that a tissue of an "amyliferous" seed, containing 60–65% starch, should metabolize triglyceride by a route thought typical of "oleaginous" seeds. The present observations suggest two new functions for the aleurone in germinating barley; (i) the supply of sucrose for the germinating embryo at the expense of aleurone lipid, and (ii) the ability to convert some amino acids to glutamine. The ability of the aleurone to convert glucose to sucrose has been confirmed [13]. Thus sucrose synthesised in the

aleurone may originate from glyceride and/or glucose produced by the breakdown of starch and cell walls. Other functions of the aleurone are to supply mineral salts to the embryo [25], and to supply some hydrolytic enzymes and activate others to degrade the reserve substances of the starchy endosperm [19].

EXPERIMENTAL

Barley preparations. Grains of barley (*Hordeum distichon* cv. Proctor) between 2.5 and 2.8 mm in width, were selected, decorticated, and sterilized [26]. Endosperms were prepared by removing the embryo with a scalpel and dividing the endosperm longitudinally. Aleurone layers were peeled from endosperms of grains, germinated for at least 2 days, or from endosperms treated with GA_3 for 2 days, or from quiescent grain by the 3-day pectinase digestion technique [14]. As far as possible asepsis was maintained. Grains were germinated at 25° on a wet substratum as described in Ref. [10].

Incubation conditions of endosperm halves and aleurone layers. Endosperm halves (20, equivalent to 10 degermed grains) or separated half aleurone layers (equivalent to 5 grains) were incubated in buffer (2 ml, Na succinate 20 mM; $CaSO_4$, 1 mM; to pH 5.3 with H_2SO_4) and where appropriate with GA_3 (2 μg/ml). Incubations were in conical flasks (25 ml) on an orbital shaker, at 25°, in the dark. Sometimes antibiotics (benzyl penicillin, 0.04%; streptomycin sulphate, 0.08%; and nystatin, 0.04% were added to the medium [14]. Solns were sterilized by Millipore filtration, but nystatin, which forms an insoluble suspension, was added as a sterile powder.

Glyceride determination. Glyceride was extracted with $CHCl_3$–MeOH (2:1; *ca* 5 ml; 5°) from tissue disrupted with a Potter–Elvehjem homogenizer [27]. The extract was added to petrol (1 ml, bp 40–60°) to settle starch. The residue was re-extracted (×2; $CHCl_3$–MeOH, 2:1). After centrifugation the combined supernatants were evaporated to dryness. Glyceride–glycerol in the extract was determined using a Boehringer, "Test Combination for Neutral Fat and Glycerol", a method in which glycerol, freed by saponification, is assayed using coupled enzyme reactions that ultimately oxidize $NADH^+$. Glycerides were also determined after separation by TLC on plates of Si gel PF 254 + 366 (Merck; containing fluorescent indicator; layers, 0.5 mm). Using as solvent Et_2O–C_6H_6–EtOH–HOAc (40:50:2:0.2) followed by Et_2O–hexane (6:94) [47] the glycerides were located under UV, then eluted from scrapings [28].

Fatty acid composition of triglyceride. Glyceride was extracted as above but with $CHCl_3$–MeOH (2:1) containing butylated hydroxytoluene (BHT, 0.01% w/v) to inhibit fatty acid oxidation [29]. Triglyceride was isolated by TLC using as solvent petrol (bp 40–60°)–Et_2O–HOAc (80:10:1) [30]. The Me esters of the component fatty acids were formed by transesterification of the triglyceride on the TLC adsorbent [31]. The Me esters were separated by GLC on a 1.5 m × 4 mm column of polyethylene glycol adipate (10%) at 190° with N_2 at 30–35 ml/min using FID. Peak areas were calculated by triangulation.

O_2 *uptake* was measured using an O_2 electrode (Yellow Springs Instruments Co., Ohio) with an electrode bridge of saturated KCl solution separated from the reaction medium by a Teflon membrane [32]. Half endosperms or half aleurones (20) were placed in a Perspex reaction vessel (17 ml) containing H_2O which was continuously stirred and kept at 25°. O_2 uptake was calculated using the O_2 content of H_2O saturated with air at 25° as 0.25 μmol/ml [33].

Assays of malate synthase (E.C. 34.1.3.2) and isocitrate lyase (E.C. 4.1.3.1). Enzymes were extracted with buffer (2 ml, Na Pi 0.1 M; $MgCl_2$, 10 mM; EDTA, 10 mM; pH 7.5, at 4°), from tissue disrupted with a Polytron PT 10 homogenizer (3 × 5 sec at setting 10). After centrifugation enzyme activities were determined in the supernatants. When isocitrate lyase was determined dithiothreitol (DTT to 10 mM) was added immediately after centrifugation. Enzyme activities were determined, at 25°, by the methods of Ref. [34]. For malate synthase the reaction mixture (2.8 ml) contained buffer (Tris–HCl, 0.1 M; pH 8.0); 5.5-dithiobis-(2-nitrobenzoic acid) 1.5 mM, $MgCl_2$ (10 mM) and acetyl-CoA (0.2 mM). Enzyme soln (0.1 ml) was added and the reaction started by the addition of sodium glyoxylate (0.1 ml; 0.6 M). A blank contained H_2O instead of glyoxylate. Formation of the 5-thio-2-nitrobenzoate anion was followed at 412 nm. Calculations were made using a molar extinction coefficient of 1.36×10^4 l./mol/cm for the anion. For isocitrate lyase the reaction mixture (2.8 ml) contained buffer (Na Pi 0.1 M; pH 6.9), $MgCl_2$ (10 mM), DTT (5 mM) and freshly prepared phenylhydrazine hydrochloride (10 mM). Enzyme soln (0.1 ml) was added and the reaction started by addition of DL(+)-sodium isocitrate (0.1 ml; 0.39 M). A blank contained H_2O instead of isocitrate. Formation of the glyoxylate–phenylhydrazone complex was followed at 324 nm. Calculations were made using a molar extinction coefficient of 1.7×10^4 l./mol/cm for the complex. Acetyl-CoA was prepared by the method of Ref. [35] and assayed by the method of Ref. [36]. The identity of glyoxylate as the reaction product in the assay of isocitrate lyase was confirmed by trapping it with semicarbazide and then TLC of its dinitrophenyl-hydrazone [37–39].

Radiochemical experiments. Radiochemicals were purified immediately before use by descending PC on Whatman No. 1 paper using the solvents: glyoxylate-[2-^{14}C], *n*-BuOH–H_2O–propionic acid (21:14:10); glycerol-[1-^{14}C], PrOH–EtOAc-H_2O (7:2:1); glucose-[U-^{14}C], PrOH–EtOAc–H_2O (7:1:2). Palmitic acid-[1-^{14}C] was 99.6% pure judged by chromatography on Whatman DE-81 paper, with $CHCl_3$–C_6H_6 (2:1) [40]. Palmitic acid [16-^{14}C] was purified by TLC on SiO_2 gel G using hexane–Et_2O–HOAc (70:30:1). Amino acids-[U-^{14}C] were isolated from an equimolar mixture of amino acids-[U-^{14}C]. Proline-[U-^{14}C] and glutamic acid-[U-^{14}C] were isolated by descending PC on Whatman No. 1 paper using the following sequence: (a) *n*-BuOH–HOAc–H_2O (12:3:5); (b) phenol–H_2O (500 g phenol in 125 ml H_2O); (c) solvent b–NH_3 (200:1). All other labelled amino acids were isolated by (1) PC with solvent a; (2) high voltage paper electrophoresis (3 kV for 90 min) on Whatman 3 MM paper with HCO_2H–HOAc–H_2O (1:4:45; pH 2); (3) PC with solvent c. Standard amino acids were visualized by spraying with ninhydrin in BuOH (0.5%) or by dipping through ninhydrin (2%) and cadmium acetate (0.06%) in Me_2CO. Radioactive areas on chromatograms were detected using a spark chamber or by autoradiography. Radioactive compounds were eluted from chromatograms with H_2O or the paper was cut into 1 cm strips and placed in scintillation vials with H_2O (1 ml) and toluene–triton scintillation fluid (10 ml). All results are reported as % incorporation *per* ten aleurone or endosperm halves. Scintillation soln consisted of PPO (0.4 g) and POPOP (0.05 g) per l. of toluene. For aq. samples this was mixed with half its vol of Triton X-100; counting was with automatic quench correction by the external standardization procedure. Radioactive compounds were eluted from chromatograms with H_2O or the paper was cut into 1 cm strips and placed into scintillation vials with H_2O (1 ml). Triton–toluene soln (10 ml) was added.

Incubation and extraction of aleurone layers. Aleurone layers (10 or 20 halves) were incubated in solns of radiochemical (2 ml) in 25 ml conical flasks which were shaken at 25°. The incubation was stopped, compounds extracted and separated into H_2O soluble and $CHCl_3$ soluble groups [41]. The aq. fraction was separated into neutral, basic and acidic fraction using columns of Dowex 50 (H^+, 8% cross linked, dry mesh 200–400; 8 × 0.9 cm i.d.) and Dowex 1 (Cl^-, 10% cross linked dry mesh 200–400; 8 × 0.9 cm i.d.) [42]. When glutamine was to be isolated from the basic fraction, the basic compounds were eluted from Dowex 50 with NH_3 (2 M; 40 ml) followed by H_2O (15 ml).

Separation of the neutral fraction. By descending PC, Whatman No. 1 paper with PrOH–EtOHc–H_2O (7:2:1). Sugars

were detected by the $AgNO_3$ staining method [43], but with $Na_2S_2O_3$ (10%) replacing NH_3 as the final wash. TMSi ethers of the neutral fraction components were separated by GLC on a 1.2 m × 5 mm column of 10% SE-30 at 224° with an argon pres. of 44.8 kPa using a β-ionization detector. Effluent from the column was trapped by glass wool packed loosely in glass tubes. Tubes were placed directly into scintillation vials.

Isolation of glutamine from the basic fraction. By high voltage paper electrophoresis (3 kV for 90 min) on Whatman 3 MM paper with HCO_2H–HOAc–H_2O (1:4:45, pH 2), followed by descending PC on Whatman No. 1 paper with *n*-BuOH–HOAc–H_2O (12:3:5). Its identity was confirmed by hydrolysis with H_2SO_4 (1.5 M for 4 hr) at 100° followed by PC to identify the resulting glutamic acid.

Lipid production with acetate-[^{14}C] as substrate. The $CHCl_3$ fraction was washed × 4 with NaOAc (0.05 M). The $CHCl_3$ was removed by evaporation under N_2 in scintillation vials.

With glycerol-[^{14}C] as substrate. The MeOH–$CHCl_3$–H_2O (12:5:3) extract [41] was adjusted to 12:11:10 with $CHCl_3$ and aq. KH_2PO_4 (2%), 2 clear phases were obtained by centrifugation. The $CHCl_3$ phase was washed × 4 with MeOH–H_2O (1:1) containing glycerol (1%), NaCl (2.5%) and KCl (1%). The $CHCl_3$ was removed by evaporation under N_2 in scintillation vials.

$^{14}CO_2$ collection. By the method of Ref. [44] except 2-phenylethylamine (50% in MeOH) replaced hyamine.

Preparation of palmitate-[^{14}C]-albumin complex. By the method of Ref. [45].

Preparation of monogalactosyl glycerol. Authentic monogalactosyl diglyceride was deacylated [46] and neutralized with Dowex 50 (H^+). PC on Whatman No. 1 paper with PrOH–EtOHc–H_2O (7:2:1) showed only one spot on staining with $AgNO_3$. GLC of the TMSi derivatives showed one peak.

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