Plant Physiol. (1970) 46, 475–482

Glyoxysomes in Megagametophyte of Germinating Ponderosa Pine Seeds[1,2]

Received for publication December 8, 1969

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ABSTRACT

Decoated ponderosa pine (*Pinus ponderosa* Laws) seeds contained 40% lipids, which were mainly stored in megagametophytic tissue and were utilized or converted to sugars via the glyoxylate cycle during germination. Mitochondria and glyoxysomes were isolated from the tissue by sucrose density gradient centrifugation at different stages of germination. It was found that isocitrate lyase, malate synthase, and catalase were mainly bound in glyoxysomes. Aconitase and fumarase were chiefly localized in mitochondria, whereas citrate synthase was common for both. Both organelles increased in quantity and specific activity of their respective marker enzymes with the advancement of germination. When the megagametophyte was exhausted at the end of germination, the quantity of these organelles and the activity of their marker enzymes decreased abruptly. At the stage of highest lipolysis, the isolated mitochondria and glyoxysomes were able to synthesize protein from labeled amino acids. Both organellar fractions contained RNA and DNA. Some degree of autonomy in glyoxysomes is indicated.

It is known that fatty coniferous seeds convert triglycerides to carbohydrates in megagametophytic tissue and transfer the carbohydrates to embryos for growth (6, 14). The key enzymes of the converting step in the glyoxylate cycle, isocitrate lyase and malate synthase, are found to be correlated with the lipid content of coniferous seeds (13), and they have participated in germinating Italian Stone pine seeds (14). The association of glyoxylate cycle enzymes with the recently discovered glyoxysomes in castor bean endosperm (2, 4) and the *de novo* synthetic nature of isocitrate lyase and malate synthase in germinating peanut cotyledons (19, 28) suggest an investigation of the developmental pattern of glyoxysomes. An increase of number and size of glyoxysomes in the megagametophyte was observed in ponderosa pine seeds (Figs. 1, 2), so the pine seeds were chosen to elucidate the pattern.

MATERIAL AND METHODS

Materials. Three lots of ponderosa pine (*Pinus ponderosa* Laws) seeds collected in the Cascade mountains of Oregon with 80% or more germination capacity were used for the study. Seeds were soaked in a disinfectant (0.1% suspension of Semesan Jr. containing 1% ethyl mercury phosphate) for 2 hr, chilled at 4 C for 1 to 4 weeks (varied with seed lot to give comparable uniform germination speed) and then germinated on Sponge-Rok under a daily cycle of 30 C for 16 hr with 800 ft-c of fluorescent light and at 20 C for 8 hr in the dark.

Compositional and Isocitrate Lyase Changes during Germination. The general procedure for analyzing Douglas fir seeds (6) was followed for the estimation of total weight, lipids, sugars, and starch in megagametophytes and seedlings during germination. The total isocitrate lyase activity in tissue was completely solubilized and determined (20) in the soluble fraction extracted by grinding 10 megagametophytes or seedlings in 10 ml of tris-HCl buffer, 0.05 M, pH 7.5, which contained 10 mM mercaptoethanol and 1 mM disodium EDTA; centrifuging the slurry at 30,000*g* for 10 min; and treating the supernatant with charcoal (23).

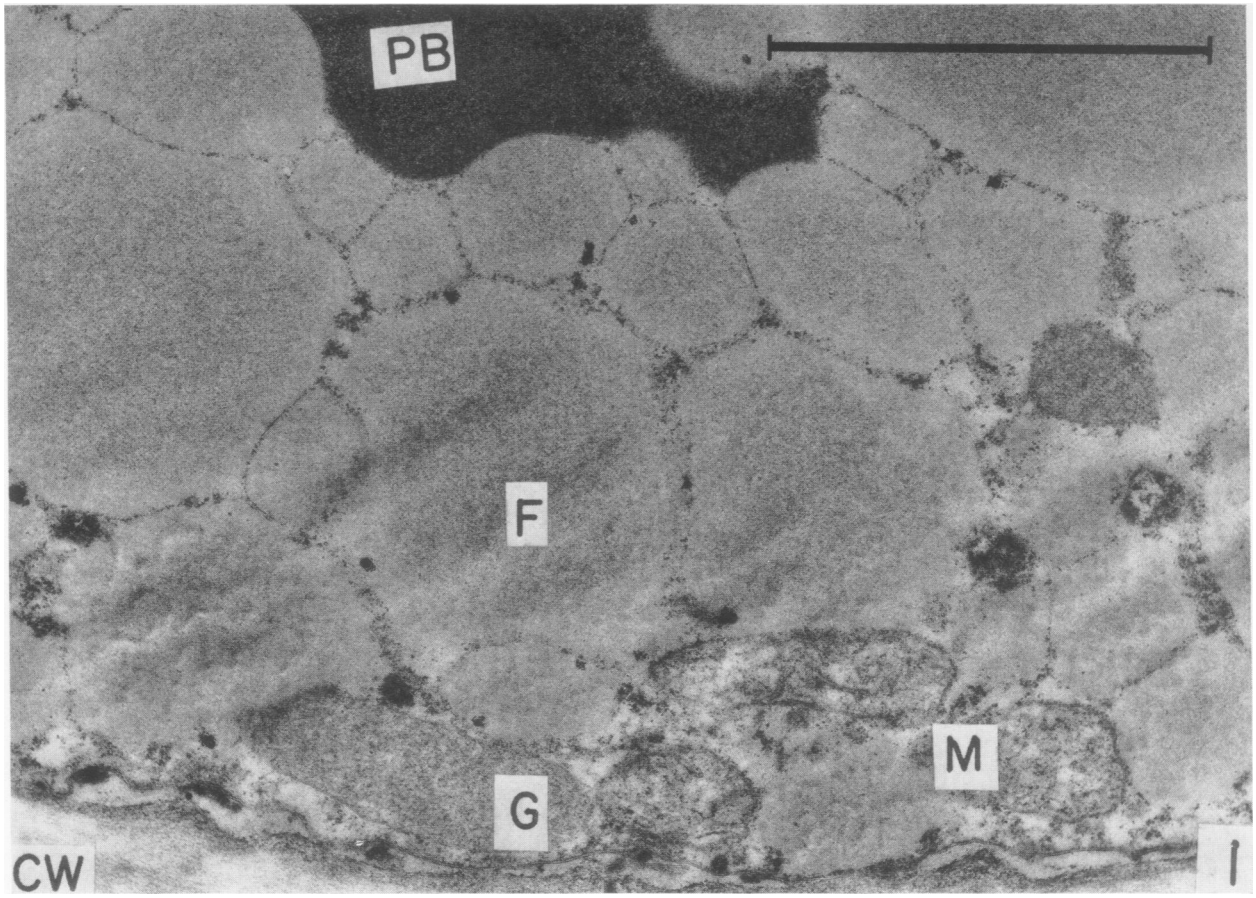
Isolation of Glyoxysomes. Using a constant weight of fresh material for isolation was found to be the key in obtaining reproducible results of organellar quantity and quality in the subsequent sucrose density gradient centrifugation, therefore, 7 g of megagametophyte tissue were used in all the experiments. The tissue was washed in 1% sodium hypochlorite for 10 sec; chopped with a razor blade for 2 min in 10 ml of homogenizing medium which contained tris-HCl buffer (0.05 M, pH 7.5), 0.4 M sucrose, 10 mM mercaptoethanol or DTT,[3] 10 mM potassium chloride, 1 mM disodium EDTA, 0.5 mM magnesium chloride, and 0.1% bovine serum albumin; and further homogenized in a mortar with a pestle for 30 sec with an additional 20 ml of medium. The homogenate was filtered through eight layers of cheesecloth and centrifuged at 500*g* in a Servall RC2 for 10 min. The supernatant was centrifuged at 10,000*g* for 10 min to sediment the crude pellet. The pellet was suspended in 2 ml of 32.5% sucrose, layered over a discontinuous sucrose density gradient, and centrifuged for 4 hr in a SW 25.2 rotor, at 105,000*g* in a Spinco L2-HV ultracentrifuge. The gradient was composed of 15 ml of 60% (w/w) sucrose as a cushion in the bottom, then 8 ml each of 55, 50, 45, and 40% sucrose and 5 ml of 35% sucrose. All the sucrose solutions contained 10 mM mercaptoethanol or DTT and 1 mM disodium EDTA. After centrifugation, 25 to 30 1-ml fractions were collected from the gradient in the density range from 1.17 to 1.27 with the aid of a Harvard Peti-pump which displaced a 63% sucrose solution at a speed of 2 ml/min from the bottom of the gradient tube placed in an ISCO (Instrumentation Specialties Co.) tube holder. The sucrose concentration of the fractions was estimated in the odd numbered tubes isolated from a blank gradient using an Abbé refractometer at 20 C. All the isolation procedures were conducted and fractions stored at 0 to 5 C. Enzyme assays were conducted in every other tube within 2 days after isolation in a definite order.

Protein Content and Enzyme Assays. Protein content in every other fraction was estimated by Lowry's method with crystalline bovine serum albumin as a standard (29). The interference of sucrose was corrected for calculation (15).

[1] Instruments were provided by National Science Foundation grants.

[2] Oregon Agricultural Experiment Station Paper 2800.

[3] Abbreviation: DTT: dithiothreitol.



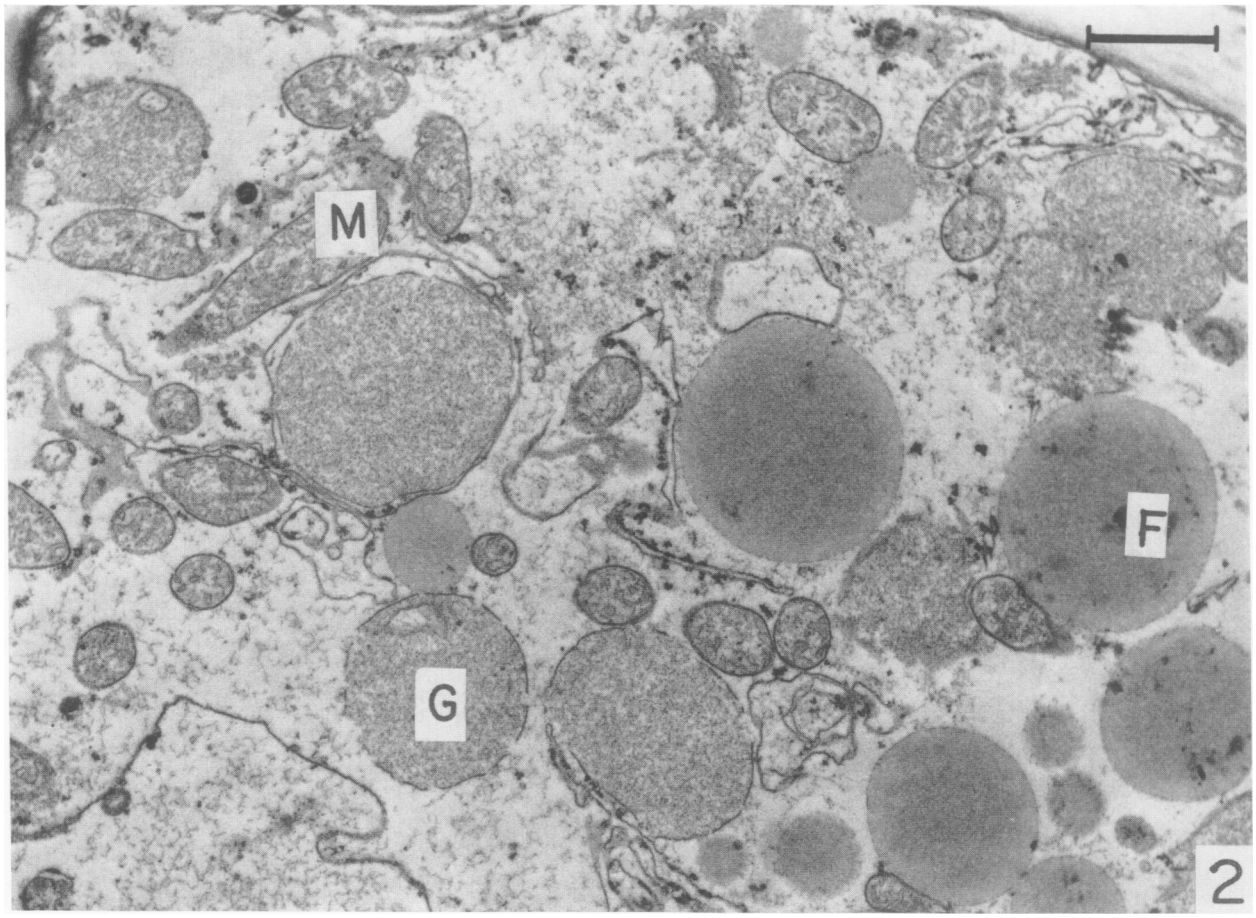


FIG. 1. A portion of a gametophytic cell in a hydrated ponderosa pine seed. CW: Cell wall; PB: protein body; F: fat body; M: mitochondria; G: glyoxysome. Scale: 1 μ.

FIG. 2. A portion of a gametophytic cell in a ponderosa pine seed germinated for 7 days. Legend the same as Figure 1.

Isocitrate lyase was assayed in a total volume of 1 ml that contained 100 mM tris-HCl buffer, pH 7.4; 10 mM cysteine hydrochloride; 10 mM $MgCl_2$; 100 μl of enzyme preparation; and 20 mM trisodium DL-isocitrate as substrate at 30 C for 5 min. Glyoxylate was determined by the mercuric acetate method (22) with a comparable reaction mixture without substrate for the blank.

Malate synthase was assayed in a total volume of 1 ml that contained 50 mM tris-HCl buffer, pH 7.4; 10 mM $MgCl_2$; 0.025 mM acetyl-CoA; 5 mM sodium glyoxylate; and 10 μl of enzyme preparation. The reaction rate was recorded at 232 nm for 2 min at 25 C by a Cary model 11 spectrophotometer with a blank composed of above chemicals except acetyl-CoA (11). Deacylase did not interfere in such a dilute enzyme concentration.

Citrate synthase was assayed in the same way as malate synthase with oxalacetate as the substrate (11).

Fumarase was assayed in a total volume of 2 ml that contained 100 mM tris-HCl buffer, pH 7.4; 50 mM sodium L-malate; and 100 μl of enzyme preparation (35). The reaction was recorded at 240 nm for 2 min at 25 C by a Beckman DB with a blank containing everything but substrate.

Aconitase was assayed similar to fumarase with trisodium isocitrate as the substrate (35).

Catalase was assayed in a total volume of 2 ml that contained 10 mM potassium phosphate buffer, pH 6.9; 2 mM hydrogen peroxide; and 10 μl of enzyme preparation at 25 C. The reaction was recorded by a GME oxygraph model KM by the following procedure; 2 ml of buffer containing hydrogen peroxide was first pipetted in the reaction chamber and its oxygen tension was adjusted as 100% span of the recorder. Nitrogen was bubbled into the reaction chamber to reduce the oxygen to 10% of saturation. Then enzyme was added and recording continued for 5 min.

Electron Microscopy. After density gradient centrifugation, one top fatty layer and five bands were observed in the tube. The top layer and bands were collected separately. Glutaraldehyde was added to each collection to a final concentration of 2.5%. The mixture stood at 0 C for 15 min. Three volumes of 0.5 M sucrose solution were added to each collection, and the collections were centrifuged at 37,000g for 30 min. The pellets were dehydrated, infiltrated with Epon, sectioned, stained with uranyl acetate and lead citrate, and photographed in a Philips EM-300 electron microscope (7).

Protein Synthesis. For determining the protein synthetic ability of glyoxysomes and mitochondria, bands were collected separately in bulk. From 2 to 3 volumes of homogenizing medium were added and mixed well. The diluted fractions were centrifuged at 105,000g for 15 min. The pellets were suspended in a small volume of 0.1 M tris-HCl buffer, pH 7.5 (25 C), that contained 1 mM DTT, 1 mM EDTA, and 0.4 M sucrose. An aliquot of the washed bulk preparations was incubated at 30 C with U-^{14}C-amino acids (50 mc/milliatom C) or ^{3}H-phenylalanine (5 c/mmole) with cofactors and energy supply under probably hypotonic conditions (about 0.3 M sucrose) in a total volume of 0.5 ml (see legend of Fig. 8 for composition of the reaction mixture). After incubation, 0.5 ml of 20% (w/v) trichloroacetic acid was added to the incubated reaction mixtures, and the mixtures were centrifuged at 6,000g for 3 min. The pellets were extracted with hot 5% trichloroacetic acid for 15 min at 90 C to hydrolyze aminoacyl-tRNA, cooled, centrifuged, and washed once with 10% trichloroacetic acid. The resulting pellets were dissolved in 0.2 ml of 1 N NaOH, 0.1 ml of which was counted in Bray's solution, and the other 0.1 ml was assayed for protein by Lowry's method.

Another procedure (experiment C in Table II), directly with fractions that represented the peak of mitochondrial and glyoxysomal bands in the sucrose density gradient, was adapted to reduce possible breakage due to washing and to estimate the participation of bacteria, broken nuclei, and cytoplasmic ribosomes in the incorporation of amino acids. An aliquot of the gradient fractions containing 50 to 100 μg of protein was incubated with 0.25 μc of ^{3}H-phenylalanine (5 c/mmole) under conditions identical to those for the washed bulk preparation. After incubation the procedure of Parenti and Margulies (33) was followed to solubilize organellar protein by adding Triton X-100. Since Triton X-100 did not dissolve bacteria or nuclei, the protein synthesized by bacteria and broken nuclei during the incubation was filtered on a Schleicher and Schuell B6 filter and counted. Another group of reaction mixtures were incubated in the presence of ribonuclease which hydrolyzes interorganellar RNA and thus eliminates the incorporation due to the contamination of cytoplasmic ribosomes. Ribonuclease does not enter mitochondria or glyoxysome under the experimental conditions.

RNA and DNA Determination. Washed bulk preparation of mitochondria and glyoxysomes isolated from 6-day-old germinants was fractionated by the procedure of Shibko *et al.* (36), and RNA and DNA were estimated by the orcinol (27) and diphenylamine method (5). To another comparable preparation, DNase (40 μg/ml) was added and the preparation was incubated at 37 C for 4 hr to verify the authenticity of DNA (18).

RESULTS AND DISCUSSION

Both glyoxysomes and mitochondria could only be found along the outer cellular and inner nuclear membranes in ungerminated seeds (Fig. 1). The frequency was low for both, usually 5 to 15 per gametophytic cell, and their size was about 0.5 to 1.0 μ in length and 0.25 to 0.5 μ in diameter in the fully hydrated tissue. Upon germination, both organelles increased exponentially in number, but glyoxysomes also enlarged in size by 2- to 3-fold (Fig. 2).

The participation of the glyoxylate cycle in converting lipolytic products of acetyl-CoA to carbohydrate in germinating megagametophytes of ponderosa pine seeds is clearly indicated in Figure 3 where reduction of lipids was accompanied with an increase of isocitrate lyase activity. As the lipids were exhausted in the later stages of germination, the enzyme activity continued for awhile then abruptly dropped. A parallel increase of glyoxylate cycle key enzymes has been observed in germinating Italian Stone pine, peanut cotyledons, and watermelon seedlings (14, 20, 30). The converted carbohydrates did not accumulate in the gametophyte but were transported to the growing seedling for growth and energy supply as in castor beans (24, 39, 40). A further experiment in which a wick of moist filter paper was used to absorb the diffusate in the gametophytic cavity after the removal of the seedling showed an average diffusion rate of 12 μg of sugars/hr·seed at the peak of lipid utilization. This continuous removal of sugars from the gametophytic tissue probably controls the opera-

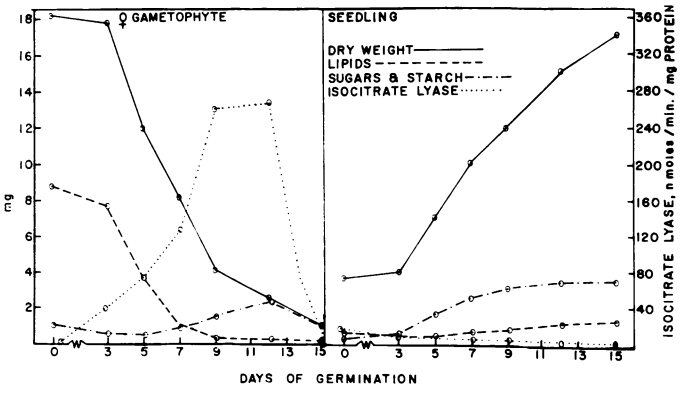
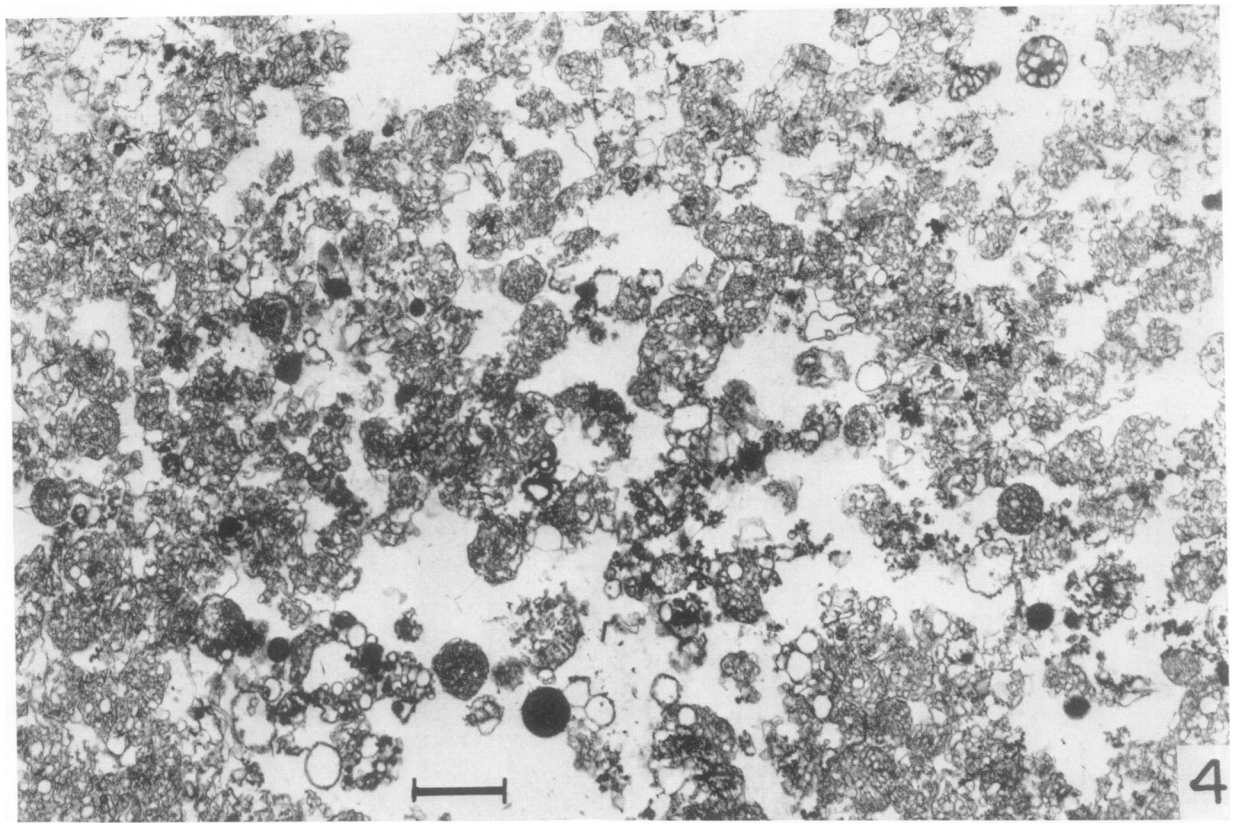
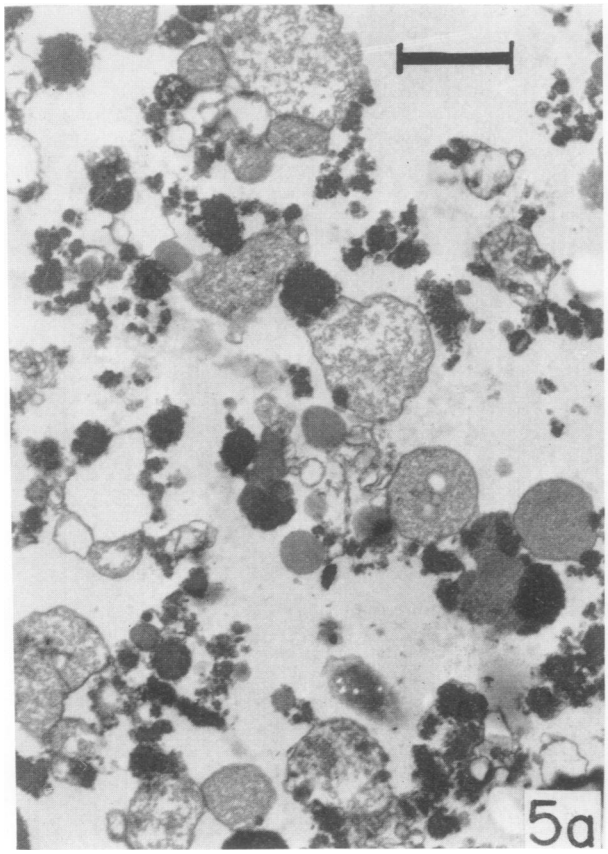


FIG. 3. Changes of dry weight, contents of lipid, sugar and starches, and specific activity of isocitrate lyase in megagametophyte and seedling of germinating ponderosa pine seed.





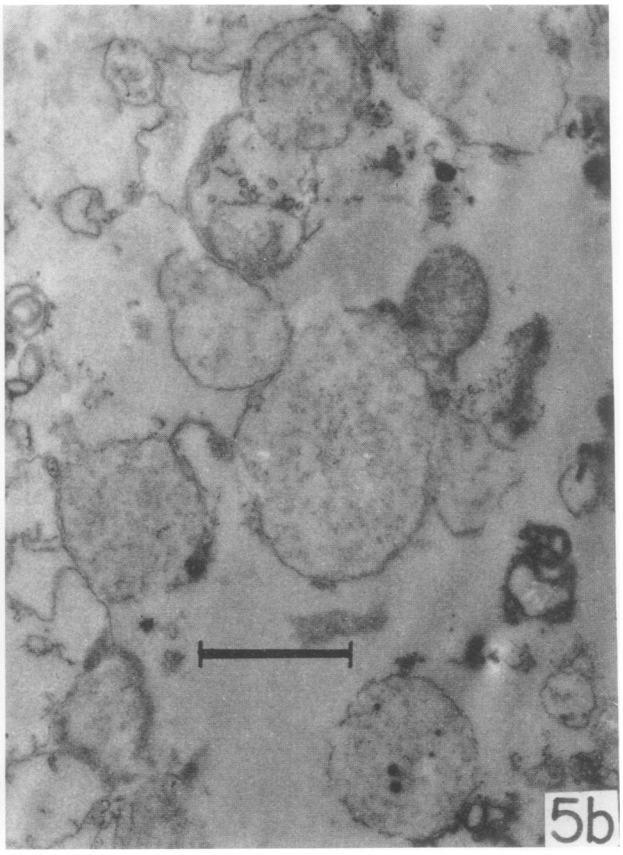


FIG. 4. Electron micrograph of isolated mitochondrial fraction.
FIG. 5. a: Electron micrograph of isolated glyoxysomal fraction; b: electron micrograph of washed glyoxysomal fraction.

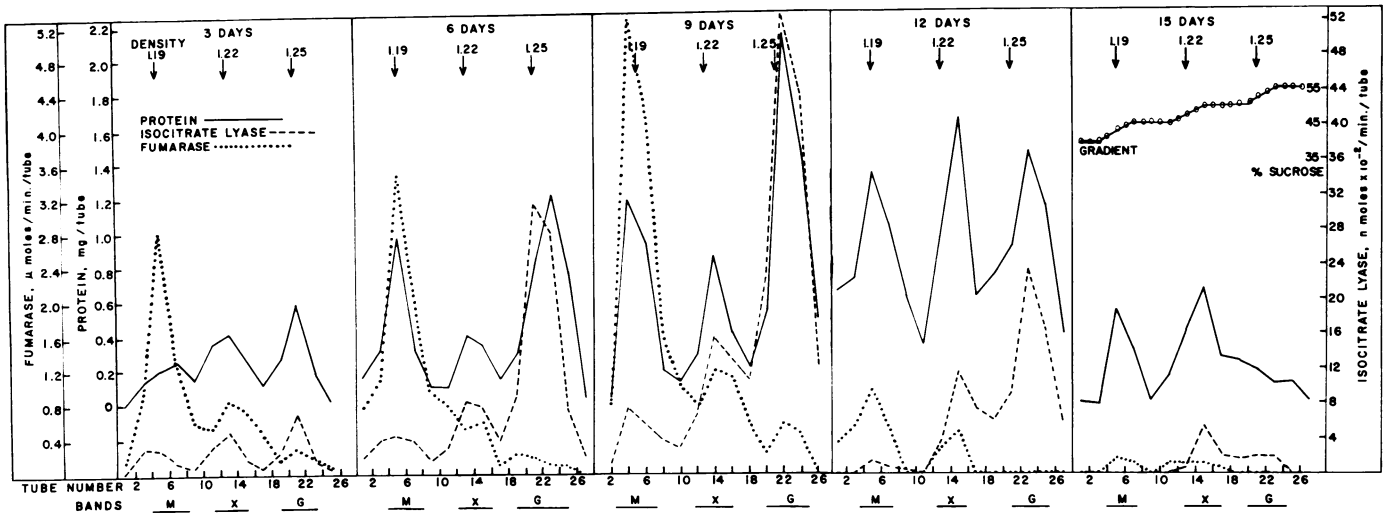


FIG. 6. Changes in protein content and activity of fumarase and isocitrate lyase with germination stages in fractions isolated by sucrose density gradient centrifugation of the crude pellet from 250 gametophytes. M: Mitochondria; X: mixture; G: glyoxysomes. For fractionating procedure and assay method see "Materials and Methods."

Table I. *Protein Content and Enzyme Specific Activity of Mitochondria and Glyoxysomes Isolated from 100 Megagametophytes of Germinating Ponderosa Pine Seeds*

The data are the mean and standard deviation of four replications.

Days of Germination	Protein Content		Specific Activity[1]	
	Mitochondria	Glyoxysome	Fumarase	Isocitrate lyase
	μg	μg		
3	153 ± 11	690 ± 93	2.97 ± 0.40	0.12 ± 0.01
6	373 ± 82	1258 ± 176	3.75 ± 0.13	0.21 ± 0.06
9	645 ± 193	1503 ± 105	4.97 ± 0.51	0.35 ± 0.06
12	1087 ± 93	1411 ± 132	0.85 ± 0.21	0.17 ± 0.04
15	479 ± 105	339 ± 29	0.27 ± 0.04	0.06 ± 0.01

[1] Specific activity was estimated in the peak fraction of respective bands as micromoles of fumarate or glyoxylate produced per milligram of organellar protein per minute.

tional rate of the glyoxylate cycle, as indicated by the continuously rising activity of isocitrate lyase until substrate was exhausted at the end of germination. The embryos had some activity of isocitrate lyase, but the activity was negligible in seedlings throughout the rest of the germination process, which indicated a lack of conversion of fats to sugars after the exhaustion of embryo fat reserves.

Six bands were usually formed after the gradient centrifugation of the crude pellet. The top was a fatty layer; the second one was a small band with a peak density of 1.16 and contained broken pieces of mitochondria, protein bodies, and glyoxysomes; the third thick band with a peak density of 1.19 was composed mainly of mitochondria (Fig. 4); the fourth narrow band with a peak density of 1.22 was made chiefly of aggregates of whole or broken mitochondria and glyoxysomes, fat droplets, and occasionally proplastids. The fifth thick and dense band with a peak density of 1.25 contained glyoxysomes, some protein bodies, and very small fat droplets (Fig. 5a). The last thin band with a peak density of 1.28 was composed mainly of protein bodies (7, 38). The purity of the bands could be improved somewhat by varying magnesium and EDTA concentrations in the homogenizing medium, longer chopping and less grinding of the material, and longer centrifugation time. Nuclei were seldom found in the crude pellet by routine staining of pellets with Feulgen reagent and examining with a microscope.

A developmental pattern of mitochondria and glyoxysomes in gametophyte of germinating ponderosa pine is demonstrated in Figure 6. Not only did the total quantity of these organelles as measured by protein content increase with the advancement of germination, but the specific activity of their respective marker enzymes was elevated (Table I). In the later stages of germination, the enzyme activity was reduced as lipids and protein reserves were exhausted (Fig. 3). Similar biogenetic pattern of mitochondria had been reported in germinating peanut cotyledons (2) and castor bean endosperm (26). A parallel developmental pattern of glyoxysomes has been observed in the endosperm of germinating castor beans (17).

Further investigation on the distribution of isocitrate lyase activity in postpellet supernatant and crude pellet was conducted with albumin-free homogenizing medium. In one experiment each of the three lots showed a consistent 28 to 32% isocitrate lyase activity in the supernatant regardless of growth stage and quantity of material used. Furthermore, the average specific activity of the enzyme in the supernatant was 0.045, 0.047, and 0.037 μmole/mg of protein · min for material obtained from 3, 6, and 9 days of germination, respectively, but in the crude pellet the average specific activity was 0.101, 0.145, and 0.281, respectively. Because of the consistency in distribution and specific activity in the supernatant, the isocitrate lyase found in the supernatant probably originated from broken particles and possibly was extracted out from the particles during isolation, as observed in germinating peanut and castor bean (30). A major portion (68–72%) of the isocitrate lyase activity, however, was particle-bound. These data comply with the results found in castor bean (3). Glyoxylate cycle enzymes are probably packaged in glyoxysomes which increase in quantity and in enzyme specific activity with increasing rate of lipolysis in germinating ponderosa pine seed.

Citrate synthase was found in mitochondria and glyoxysomes as expected with a specific activity of 0.75 μmoles/mg of protein · min for mitochondria and 0.66 for glyoxysomes in 8-day germinants (Fig. 7). Large variation in specific activity was observed in younger stages even though a biogenetic pattern was indicated. The variation probably was attributed to difficulty in sampling a 10-μl quantity of particulate suspension for enzyme assay. Triton X-100 will be incorporated for future work (31). Homogenizing the isolated fractions with sonication and freeze-thawing resulted in reduced enzyme activity.

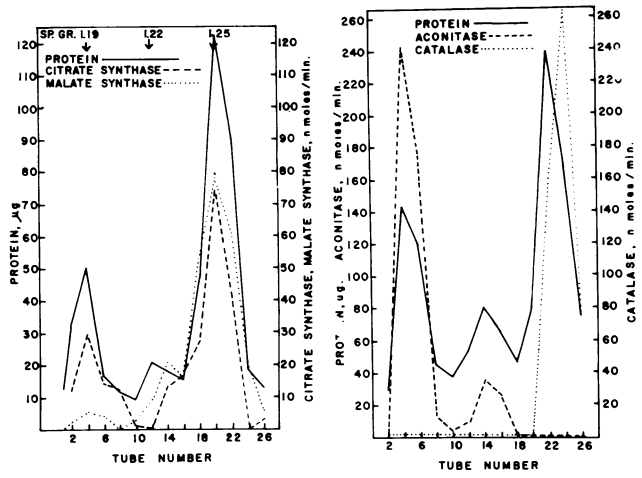


FIG. 7. Distribution of protein, citrate synthase, malate synthase, aconitase, and catalase in fractions isolated by sucrose density gradient centrifugation of 8-day-old material. Data in graphs show 1/10 of each tube.

Malate synthase was apparently glyoxysome-bound (Fig. 7), having a specific activity comparable to that of citrate synthase. Again the enzyme assay suffered the same technical difficulty as the citrate synthase. However, a developmental pattern comparable to isocitrate lyase activity was noted with the advancement of germination. Aconitase should be observed in mitochondria as well as in glyoxysomes if the general pathway of the glyoxylate cycle (25) was followed in this material. The enzyme, however, was found only in mitochondria (Fig. 7). Addition of —SH compounds in isolation media, addition of Fe^{2+} (0.1–1.0 mM), and variation of the buffer pH between 6.0 and 8.0 for reaction condition did not increase the enzyme activity in glyoxysome fractions. Extraction of the enzyme during isolation (30) or direct condensation of oxalacetate and acetyl-CoA to isocitrate in the glyoxysome may be the possible explanation. Perhaps the isolation procedure and assay condition used by Cooper and Beevers (9) might resolve this question. Isocitrate was found to be five to seven times more efficient than citrate as the substrate for mitochondrial aconitase.

The association of catalase with the glyoxysomal fraction is shown in Figure 7. The significance of such an association was recently proposed as a regenerating system of the reduced flavin adenine nucleotide formed in β-oxidation of fatty acids (10).

Both isolated mitochondria and glyoxysomes from 8-day germinants were able to synthesize protein when substrate, energy supply, coenzymes, and cofactors were provided (Fig. 8 and Table II). Glyoxysomes incorporated from 3- to 5-fold more amino acids than mitochondria and almost 20-fold more than the microsomal fraction plus dialyzed supernatant. The low protein-synthesizing ability of the microsome plus supernatant is not explainable at the moment. Organelles isolated from 4- to 5-day germinants had almost equal synthetic ability as that of 8-day material, while 12- to 13-day germinants were practically incapable of protein synthesis. The length of exposure of organelles to the concentrated sucrose solutions used in the density gradient reduced protein synthetic ability in mitochondria more than in glyoxysomes. Therefore, all preparations were used immediately after separation. Washing of the isolated particles decreased total protein by 30 to 50% in glyoxysomal preparations, whereas a 15 to 20% decrease was observed in mitochondrial preparations. This difference might account for the reduced specific incorporation in unwashed glyoxysomal fractions (experiment C in Table II). Washed glyoxysomal fraction had shown various degrees of dilation and breakage of the organelles (Fig. 5b), so the unwashed peak preparation was used for the estimation of contamination by cytoplasmic ribosomes, bacteria, and nuclei with RNase (32, 34) and Triton X-100 treatment (33). The data in Table II indicated that about 5% of the incorporation in unwashed mitochondrial preparation was caused by cytoplasmic ribosome, but 20% was in unwashed glyoxysomal preparation. Bacterial and nuclear contamination was low as a negligible radioactivity was retained after the treatment with Triton X-100 (30). Actual counts of viable bacteria in reaction mixtures were conducted by incubating the mixture in 10 ml of sodium albuminate or nutrient agar for 48 hr at 26 C. An average of eight colonies was found (range 1–33). Bacterial growth apparently was suppressed by the added antibiotics and the high osmotic concentration in reaction mixtures. The inhibition by chloramphenicol and the stimulation by polyuridylic acid in the incorporation of ^{3}H-phenylalanine point out the similarity in protein synthetic machinery in both mitochondria and glyoxysomes.

Washed mitochondrial preparation from 8-day germinants contained 20.2 ± 2.1 µg of RNA and 1.51 ± 0.30 µg of DNA per mg of organellar protein. These quantities agree with other plant materials (32, 33, 37). Washed glyoxysomal preparation consisted of 16.2 ± 0.9 µg of RNA and 1.36 ± 0.23 µg of DNA/mg organellar protein. After DNase treatment, there was no DNA present in the residue, which indicated that the DNA is authentic. Its origin is still unknown, however. The RNA content in glyoxysomes is of the same magnitude as found by Beevers' group in castor bean endosperm (16). The presence of DNA is entirely possible since a different kind of cytoplasmic DNA than mitochondrial DNA has recently been found in microsomes of mouse liver (1). A positive identification of the glyoxysomal RNA and DNA is in progress.

From the biosynthetic pattern *in vivo*, protein synthetic ability *in vitro*, the presence of RNA and DNA, ultrastructural changes, and other evidences (9, 10, 12, 19) one may speculate that the developmental pattern of glyoxysomes during germination is probably preprogrammed at seed maturation time. The program is perhaps installed as stable mRNAs and ribosomes in pre-existing glyoxysomes which synthesize functional enzymes when free

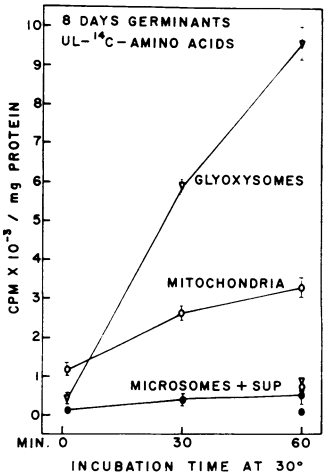


FIG. 8. Time course of amino acid incorporation by washed bulk preparation of glyoxysomes, mitochondria, and microsomes plus dialyzed supernatant. The three isolated markers at the lower right corner show the radioactivity of boiled samples. The microsome were sedimented from the postpellet supernatant at 105,000*g* for 2 hr, and the postmicrosomal supernatant was dialyzed for 4 hr at 0 C in 0.05 M tris-HCl buffer, pH 7.5, containing 10 mM DTT. In 0.5 ml of reaction mixture, 100 to 150 µg of organellar protein and 100 µg of supernatant protein, 0.25 µc of U-^{14}C-amino acids, 20 µg of pyruvate kinase. Others, in µmoles: tris, pH 7.5, 50; KCl, 25; $MgCl_2$, 4; ATP, 1; GTP, 1; phosphoenolpyruvate, 1, were added.

Table II. *Incorporation of ^{3}H-Phenylalanine into Protein by Isolated Glyoxysomes and Mitochondria from 8-day Germinants*
Data are mean counts per minute per milligram protein plus or minus standard deviation.

Incubation Condition[1]	Time	Glyoxysomes	Mitochondria
	min	*cpm/mg protein*	
Experiment A. 6 replications, washed bulk preparation			
Complete	0	111 ± 8	80 ± 4
Complete	20	1748 ± 394	443 ± 12
Complete	40	3600 ± 850	1023 ± 18
Complete	60	5153 ± 1254	1089 ± 78
Boiled	60	215 ± 11	208 ± 26
Experiment B. 3 replications, washed bulk preparation			
Complete	0	116 ± 17	89 ± 4
Complete	45	4533 ± 400	846 ± 34
Complete + chloramphenicol (150 μg)	45	526 ± 71	354 ± 43
Complete + poly U (50 μg)	45	4821 ± 411	1103 ± 170
Experiment C. 3 replications, peak preparation, isotonic incubation			
Complete	0	6 ± 1	11 ± 3
Complete	45	816 ± 60	777 ± 107
Complete + RNase (100 μg)	45	652 ± 58	742 ± 67
Complete + Triton X-100 after reaction	45	15 ± 4	25 ± 5

[1] Incubation condition was the same as for Figure 8.

amino acids, coenzymes, and cofactors become available during germination. The glyoxysomes then grow in size and divide to convert the mass flow of substrate (fatty acids) coming from fat bodies (7) to sugars. As substrate is exhausted in the later stages of germination, the glyoxysomes disintegrate either because of the loss of effector (substrate) (12) of the enzyme complex, which consists of enzymes in the β-oxidation helix and glyoxylate cycle (9, 10, 21) or because of lysosomal-like action common to storage tissue of seeds at the end of germination (6).

Acknowledgments—My thanks are to E. J. Trione for the use of the GME oxygraph and the procedure for catalase assay; to H. J. Evans for use of the Cary spectrophotometer; to M. W. Montgomery for use of the SW 25.2 rotor; to P. C. Scott and D. J. Reed for assistance in protein synthesis work and discussion; to K. C. Lu for the bacteria countings; to R. K. Dejmal for assistance in fractionation; and to I. Schoolcraft for able technical assistance.

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