Plant Physiol. (1978) 62, 449–453

Effect of Gibberellin A_3 on the Endoplasmic Reticulum and on the Formation of Glyoxysomes in the Endosperm of Germinating Castor Bean[1]

Received for publication February 15, 1978 and in revised form May 19, 1978

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ABSTRACT

Seedlings of castor bean (*Ricinus communis* cv. Hale) were exposed to a range of concentrations of gibberellin A_3 (GA_3). Treatments for 20 hours with GA_3 concentrations of 0.5 μM or higher resulted in increased levels of NADH-cytochrome *c* reductase, phosphorylcholine glyceride transferase, and malate synthase in endoplasmic reticulum (ER) isolated from endosperm on linear sucrose gradients. GA_3 treatment also resulted in increased RNA associated with ER. Malate synthase and catalase in crude homogenates were enhanced by 1 to 100 μM GA_3 concentrations. Isocitrate lyase, citrate synthase, malate synthase, catalase, and glycolate oxidase in isolated glyoxysomes were enhanced by 60, 20, 18, 40, and 28%, respectively, over controls. Treatment with abscisic acid led to decreased levels of glyoxysomal enzymes and reduced glyoxysomal protein. The effect of GA_3 and abscisic acid on the specific activities of glyoxysomes of different densities suggests that GA_3 influences enzyme levels and glyoxysome assembly.

The exogenous application of gibberellin (GA_3) to the endosperm of germinating seeds of castor bean has been shown to affect the subcellular development of enzymes (21, 22, 28). Studies on barley (13) and wheat (3) aleurone layers, as well as on castor bean (20–22, 28) have shown that enzymes of the glyoxylate cycle and of fatty acid β oxidation are particularly responsive to exogenous GA. In fatty seeds such as castor bean (1), and in barley aleurone (13), glyoxylate cycle enzymes are housed in glyoxysomes.

In recent years a body of evidence points to an origin of glyoxysomes on the ER. Early electron microscope observations (26) on germinating endosperm suggested that the RER which was frequently seen in close proximity to, but seldom in continuity with, glyoxysomal membranes might play a role in glyoxysomal assembly. Subsequent studies (14, 16, 17, 24) showed that the origin of glyoxysomal membrane phospholipids was the ER. Further work (7) established that the glyoxysomal enzymes malate synthase and citrate synthase were found in high proportion on the ER during very early germination and that the proportion decreased as germination progressed. These authors (7) also found that the ER also contained several proteins which were recognized by an antiglyoxysomal-matrix-proteins antibody. Lord and Bowden (15) have provided conclusive evidence that the RER is the site of synthesis of malate synthase and that the malate synthase nascent protein is vectorially discharged into cisternae of the RER.

The question of the role of GA on organelle biogenesis and ER function is an obvious and important one. An attempt to answer this question was undertaken by Wrigley and Lord (28). They found that long term exposure of castor bean seedlings to GA_3 (0.3 mM) led to enhanced levels of mitochondrial and glyoxysomal enzymes and an increase of the total mitochondrial protein. Since these workers could not detect an increase in glyoxysomal protein nor an increase of choline phosphotransferase (an ER marker), they concluded that GA does not affect the assembly of glyoxysomes nor the proliferation of the ER.

In this report I provide evidence of the possible role of GA_3 on glyoxysomal enzyme syntheses, glyoxysomal assembly, and on the differentiation and functional segregation of the ER.

MATERIALS AND METHODS

Seeds of castor bean (*Ricinus communis* cv. Hale; McNair Seed Co., Plainview, Tex.) were soaked in running tap water for 24 hr. The imbibed seeds were transferred to moist Vermiculite and incubated at 30 C in darkness in a Percival germinator. RH was maintained constant at 90%.

Seedling Selection and Hormone Treatment. Seedlings germinated for 48 to 60 hr were sorted to yield a uniform size class with radicles between 2 and 5 mm long. These seedlings were surface-sterilized with 5% commercial bleach for 5 min and then thoroughly rinsed in distilled H_2O. Seedlings were grouped in sets of nine (or 12). Each group was placed on seven layers of cheesecloth in a Petri plate and carefully overlaid with one additional layer of cheese cloth and 5 ml of the treatment solution. The plates were then covered and incubated at 30 C for the appropriate time period. All treatments were carried out in duplicate.

Homogenization and Fractionation of Endosperm. Eighteen endosperm halves were ground to a paste in a solution containing 0.067 M K-phosphate (pH 7) and 0.1% Triton X-100. The paste was filtered through one layer of Miracloth. The homogenate volume was adjusted (nine beans/10 ml) and then centrifuged at 10,000*g* for 15 min. The pellet and fat pad were discarded.

The ER was isolated from shallow, 20 to 40% sucrose gradients. For experiments in Figures 3 and 4, gradients were collected in 0.3-ml fractions. In Figure 4, larger amounts of membrane material for RNA extraction were obtained by pairing contiguous 0.3-ml fractions and pooling corresponding paired fractions from an identical duplicate gradient. Thus, a sample contained a volume of 1.2 ml.

Organelle fractionations were carried out on 20 to 60% sucrose gradients as described previously (7). The grinding solutions contained 20% sucrose (w/w), 100 mM Tricine (pH 7.5), 10 mM KCl, 1 mM EDTA, and 1 mM $MgCl_2$. Fractions, 0.9 ml, were collected from the top of the tube in an Isco density gradient fractionator and UA5-UV monitor (Instrumentation Specialties Co., Lincoln, Nebr.).

Enzyme Assays. Catalase (19), malate synthase (10), isocitrate

[1] This research was supported in part by National Science Foundation Grant PCM 76-81484.

lyase (10), citrate synthase (10), hydroxypyruvate reductase (11), glycolate oxidase (5), and CDP-choline phosphatidyl glyceride transferase (23) were assayed as described previously. NADH-Cyt *c* reductase was assayed in 0.08 M Tricine buffer (pH 9.0), 8×10^{-5} M Cyt *c*, 2×10^{-4} M KCN, and 5.6×10^{-4} M NADH. The reduction of Cyt *c* was followed at 550 nm. The final assay volume was 0.25 ml. Assays were carried out at 30 C in triplicate in a Beckman model 25 kinetic system spectrophotometer.

Analytical Determinations. RNA from RER isolated on sucrose gradients was extracted with 1% SDS, buffer-saturated phenol and 0.05% 8-hydroxyquinoline and precipitated with 2 volumes of cold absolute ethanol and 0.2 M NaCl. After standing overnight at −20 C, the RNA was pelleted at 30,000*g* for 30 min in a JA-20 rotor of the Beckman J21-B centrifuge. The RNA was dissolved in 2 mM Tris-EDTA (pH 8.0) and its 260:280 ratio was determined on a Beckman model 25 spectrophotometer. Amounts of RNA were calculated from the nomograph by Warburg and Christian (27). Sucrose concentrations were determined refractometrically. Protein determination was according to Lowry *et al.* (18) using BSA as standard.

Phytohormones. GA_3 and ABA were obtained from Sigma Chemical Co. as gibberellin (GA_3) grade III, and (±) *cis-trans* abscisic acid, grade IV.

RESULTS

NADH-Cyt *c* Reductase in Crude Homogenates. The level of NADH-Cyt *c* reductase in endosperm homogenates is enhanced when intact seedlings are exposed to GA concentrations of 0.5 μM or higher (Fig. 1). The largest increase in activity, 40%, was attained when seedlings were exposed to concentrations of 50 μM or greater. Concentrations as high as 1 mM did not lead to higher activities and did not result in decreased enzyme levels.

Effect of GA on RER. The endosperm of treated or control seedlings was homogenized with a razor blade and the resulting homogenate was fractionated on linear sucrose gradients. Figure 2 shows a portion of a typical A_{280} profile of membranes in a shallow, 20 to 40%, sucrose gradient. These membranes have been characterized and have been shown to conform to established criteria for RER (17). The mobility of the membranes through a gradient depends on two conditions: the age of the seedling and the concentration of Mg^{2+} ions in the grinding medium. For the conditions of this study, 1 mM $MgCl_2$ in the grinding medium has been found to be optimal for retaining integrity of the RER. Increasing the $MgCl_2$ concentration to 2 mM does not result in further migration of the membranes into the gradient. Centrifugation of the gradients for 3 hr in a Beckman SW 27 rotor at 20,000 rpm is sufficient to permit these membranes to reach their isopycnic equilibrium.

GA treatment results in higher levels of all enzyme markers assayed in isolated ER. The 27% enhancement of NADH-Cyt *c* reductase seen in isolated membranes (Figs. 3 and 4), of 70 μM GA_3-treated seedlings, is in agreement with values obtained from crude homogenates (Fig. 1) of seedlings treated with similar GA

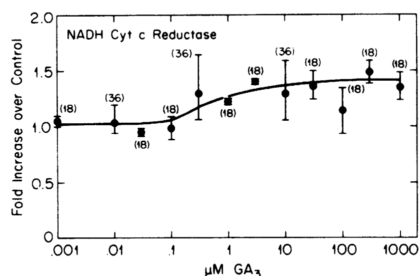

FIG. 1. Effect of GA_3 on the activity of NADH-Cyt *c* reductase in crude extracts from endosperm. Numbers in parentheses refer to total number of seedlings used. Vertical bars represent range of values obtained.

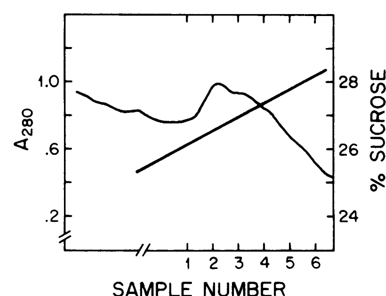

FIG. 2. Tracing of 280 nm absorbance profile of membranes in a linear sucrose gradient. Profile of GA_3 gradient shown in Figure 4.

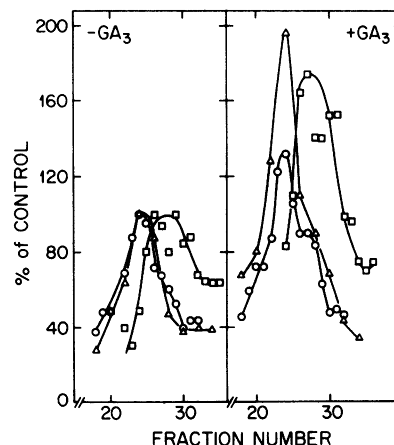

FIG. 3. Enzyme activities associated with ER. ER from control and GA_3-treated (0.07 mM) seedlings was isolated on linear sucrose gradients. Values at 100% are equivalent to: malate synthase, 62 nmol/min·fraction (□); NADH-Cyt *c* reductase, 127 nmol/min·fraction (○); phosphorylcholine glyceride transferase, 24,247 cpm/75-μl extract (△).

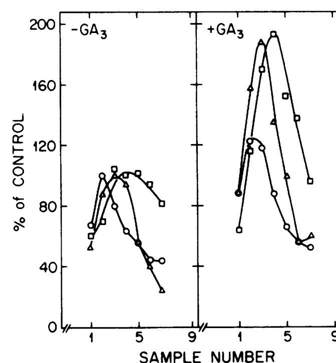

FIG. 4. Enzyme activities and RNA associated with ER. ER from control and GA_3-treated (0.07 mM) seedlings was isolated on linear sucrose gradients. Values at 100% are equivalent to: malate synthase, 205 nmol/min·sample (□); NADH-Cyt *c* reductase, 355 nmol/min·sample (○); RNA, 145 μg/sample (△).

concentrations. The level of NADPH-Cyt *c* reductase (data not shown) is enhanced to the same extent as the NADH-Cyt *c* reductase. CDP-Choline glyceride transferase is enhanced by GA (Fig. 3), with increases ranging from 30 to 100%. The most predictable and reproducible GA effect is on the level of the glyoxysomal enzyme, malate synthase, which is associated with the ER (Figs. 3 and 4). The level of malate synthase in the isolated membranes is enhanced 50 to 70% over control values. Total RNA (Fig. 4) associated with the ER is similarly enhanced. After 20 hr of treatment, RNA was enhanced by 70% in membranes from treated seedlings.

RNA detected in membranes is predominantly ribosomal RNA

(unpublished data). Therefore, an increase in RNA reflects increased numbers of ribosomes (polysomes) associated with the ER. The number of ribosomes associated with membranes affects the apparent density of the membranes, *i.e.* membranes with fewer ribosomes are less dense (17). The increase of polysomes on membranes can be detected by the altered density of membranes in the gradients. Figure 5 shows the position of membranes at the beginning of a treatment period, (0 hr) and the position of membranes after 18 hr in the presence and absence of GA. Under controlled conditions, the normal, subcellular development during the 20-hr period results in progressively denser membranes. The apparent density has been observed to range from 1.117 g/cm^3 at zero hr to 1.132 g/cm^3 at 18 hr. Membrane density shifts as a result of treatment become observable after 8 to 10 hr of treatment.

Since the number of ribosomes on membranes is also affected by the Mg^{2+} concentration, an absolute density characterization of membranes is not possible. Yet, at any Mg^{2+} concentration from 0 to 1 mM, for any given seedling group, membranes from treated seedlings always band at a higher density than membranes from nontreated seedlings.

The apparent effect of treatment with ABA is the opposite of the GA effect. ABA treatment reduces glyoxysomal enzyme levels and glyoxysomal protein in isolated glyoxysomes. In the presence of ABA, the ER has a lower apparent density, presumably reflecting decreased levels of polysomes (Fig. 6). In the experiment shown (Fig. 6), ABA treatment also resulted in decreased levels of NADH-Cyt *c* reductase associated with the ER membranes.

Effect of GA_3 on Glyoxysomal Enzymes in Crude Endosperm Homogenates. Intact seedlings and excised endosperm were exposed for 20 hr to a range of GA_3 concentrations. At least two glyoxysomal enzymes (Fig. 7), malate synthase and catalase, are affected by GA_3 concentrations ranging from 1 to 100 μM. GA_3 concentrations below 1 μM do not lead to enhanced enzyme activity while concentrations above 300 μM appear to have an inhibitory effect on these enzymes. There is no significant difference in enzyme levels resulting from application of GA_3 either to the intact seedling or to the excised endosperm.

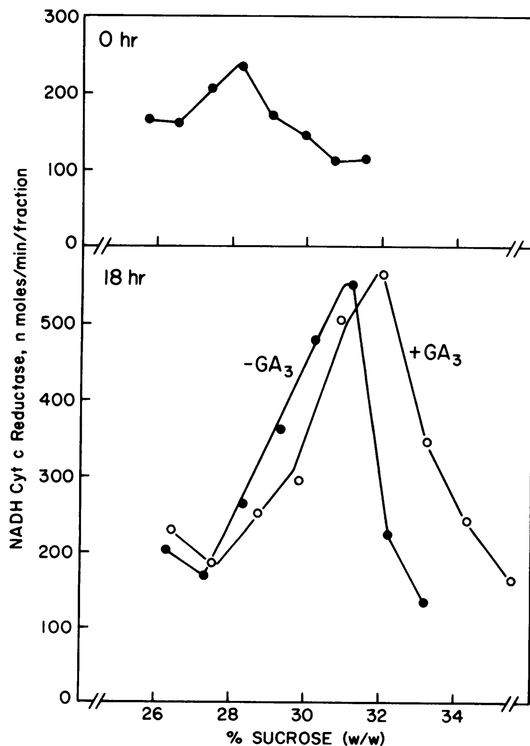

FIG. 5. Activity of NADH-Cyt *c* reductase in ER membrane of 48-hr germinated seedlings (0 hr) and in seedlings incubated for an additional 18 hr in water or in 0.07 mM GA_3.

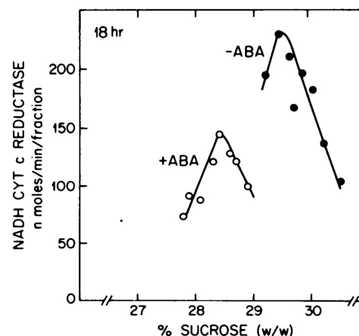

FIG. 6. Activity of NADH-Cyt *c* reductase in ER membranes of seedlings exposed to water or to 0.07 mM ABA.

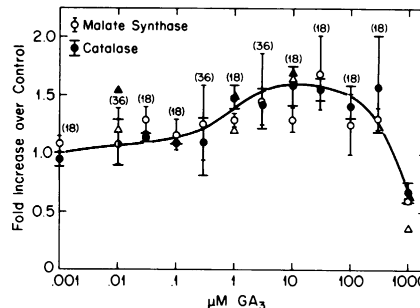

FIG. 7. Effect of GA_3 on enzyme activities in crude extracts of endosperm. Malate synthase (○) and catalase (●) in crude extracts after 20-hr exposure of seedlings to GA_3. Numbers in parentheses refer to total number of seedlings used. Vertical bars represent range of values obtained. Activity in extracts of endosperm treated directly by GA_3 is represented by ▲, △.

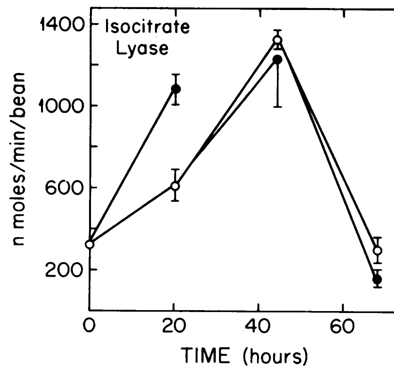

FIG. 8. Seedling age *versus* sensitivity to GA_3. After 48-hr germination (0 hr) seedlings of uniform size and development were resown in Vermiculite. At intervals, as indicated, seedlings were removed for treatment with GA_3 (●, 70 μM GA_3; ○, water control) for 20 hr in Petri plates. All points represent an average of two sets of nine seedlings.

The seedling's ability to respond to GA_3 appears to depend upon germination stage. Figure 8 shows the response of seedlings exposed to GA_3 at intervals during germination. Seedlings lose their ability to respond to GA_3 some 24 to 30 hr after radicle emergence (Fig. 8), *i.e.* the isocitrate lyase activity of older seedlings was not significantly affected by GA_3 treatment.

The kinetics of development of enzyme activity as a response to GA_3 treatment was also determined. Figure 9 shows the development of catalase activity and malate synthase in seedlings treated with 70 μM GA_3 or with distilled H_2O. As expected the levels of activity of the two enzymes increased rapidly over a 20-hr period in the nontreated seedlings. The application of GA_3 leads to an acceleration of the increase in activity of the two enzymes. The final total increase after 20 hr is similar to the increase over control

observed at this GA_3 concentration shown in Figure 7.

Enzyme Levels in Isolated Glyoxysomes. The activities of various enzymes in isolated glyoxysomes are affected by GA as well as by ABA. The data in Table I show that the effect on the various glyoxysomal enzymes is not identical for all enzymes. The mean increase in total activity as a result of GA_3 treatment is 28%, while the mean inhibition of ABA is 20%. Closer inspection reveals that the glyoxylate cycle enzymes appear to be more sensitive to both GA_3 and ABA than the peroxisomal enzymes. The strongest effect was on isocitrate lyase.

A somewhat different pattern emerges when the effect of the *specific* activity of these enzymes is determined (Table II). GA_3 leads to a 25% increase in mean specific activity of all of the enzymes measured (from Table II) while the effect of ABA on the specific activities of these same enzymes is less than 10%. These results suggest that both GA_3 and ABA affect glyoxysome assembly. This hypothesis was explored by means of an experiment to determine the effect of these phytohormones on total glyoxysomal protein and enzyme specific activities in glyoxysomes. Figure 10 shows a detailed analysis of protein content and isocitrate lyase activity in glyoxysomes isolated on sucrose gradients. Surprisingly, GA_3 had no significant effect on the protein-per-fraction profiles shown in Figure 10. Not only does GA_3 treatment result in a 162% increase in the total activity of isocitrate lyase, but there is also a shift of the activity profile toward the denser side of the glyoxysome band. ABA treatment resulted in decreased protein in the glyoxysomes as well as a proportional decrease in isocitrate lyase activity. That is, the profile of specific activity of isocitrate lyase of the control and that of the GA_3-treated gradient are not congruent, while the profiles for the specific activity of isocitrate lyase in the control and ABA gradient are very much the same. This observation is illustrated more clearly in Figure 11 which shows a plot of the ratio of specific activity of enzyme in a fraction from a GA_3-treated seedling gradient to the specific activity of that enzyme in the corresponding fraction from a control seedling gradient. Such treatment of the data reveals that the effect of GA_3 on the specific activity of glyoxysomes is much more dramatic than the effect on peroxisomal enzymes within these same particles. In contrast the effect of ABA on the specific activities of enzymes remains fairly constant throughout the range of glyoxysome densities.

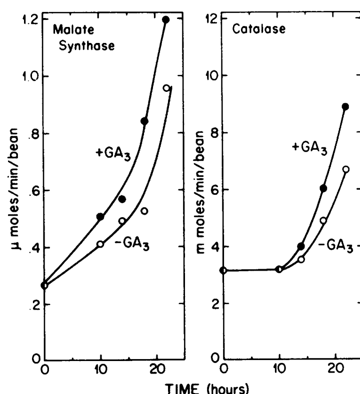

FIG. 9. Development of enzymic activity after GA treatment. Seedlings were germinated in Vermiculite and removed for treatment at 48 hr (O hr). Seedlings were exposed to water (○) or to 70 μM GA_3 (●) until harvested at the times indicated. Enzyme activities were determined in crude extracts of endosperms.

Table I. The change, expressed as per cent of control, in TOTAL ACTIVITY of enzymes in glyoxysomes from seedlings treated with either GA_3 or ABA.

Treatment[1]	Isocitrate lyase	Citrate synthase	Malate synthase	Catalase	OH-pyruvate reductase	Glycolate oxidase
	%	%	%	%	%	%
GA_3	162	120	118	140	99	128
ABA	71	77	77	92	--	81

[1]Treatment of intact seedlings with 0.07 mM GA_3 or 0.07 mM ABA for 20 hrs at 30 C.

Table II. The change, expressed as per cent of control, in SPECIFIC ACTIVITY of enzymes in glyoxysomes from seedlings treated with either GA_3 or ABA.

Treatment[1]	Isocitrate lyase	Citrate synthase	Malate synthase	Catalase	OH-pyruvate reductase	Glycolate oxidase
	%	%	%	%	%	%
GA_3	160	117	116	138	98	126
ABA	86	98	91	92	--	97

[1]Treatment of intact seedlings with 0.07 mM GA_3 or 0.07 mM ABA for 20 hrs at 30 C.

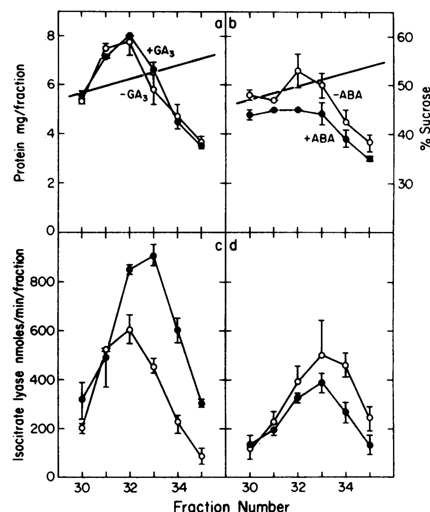

FIG. 10. Analyses of protein content (a, b) and isocitrate lyase activity in gradients (in duplicate) (c, d). Panels a and c compare glyoxysomal protein and isocitrate lyase activity in control (○) and 70 μM GA_3-treated seedlings (●). Panels b and d compare glyoxysomal protein content and isocitrate lyase activity in control (○) and 70 μM ABA-treated seedlings (●).

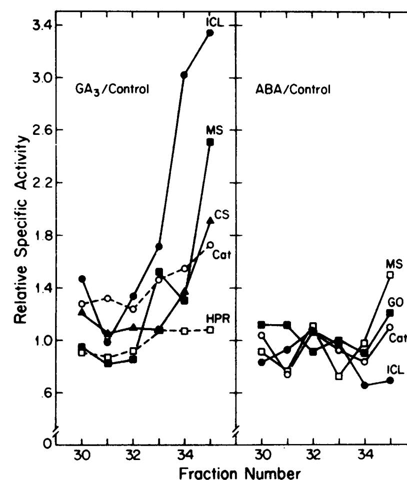

FIG. 11. Comparison of specific activities of enzymes: isocitrate lyase, ICL, malate synthase, MS; citrate synthase, CS; catalase, Cat; hydroxypyruvate reductase, HPR; glycolate oxidase, GO, in glyoxysomes. Ratio, for each fraction, of specific activity of hormone-treated tissue enzyme to specific activity of control is shown.

DISCUSSION

The activities of glyoxysomal enzymes in crude homogenates of endosperm as well as in isolated glyoxysomes are significantly enhanced as a result of treatment with GA_3. The present study corroborates and extends the previous observations of Marriott and Northcote (20–22) and of Wrigley and Lord (28). It also reveals that induction of glyoxysomal enzymes is not coordinate. Whether this result can be attributed to GA specificity or to some

more trivial explanation is not known at this time. One could suggest that the expression of each enzyme is sensitive to a unique threshold concentration of GA_3 or that each enzyme is sensitive to GA at a different seedling developmental stage (Fig. 2) or simply that the kinetics of enzyme synthesis, once induced, are different for each enzyme (Fig. 3). All three possibilities may provide the explanation for the range of enzyme activity increases observed.

Although the effect of GA_3 on seeds at stages prior to radicle emergence has not been determined in this study, the time of radicle emergence, which occurs 48 to 60 hr after planting, is the most interesting stage for study. This is the period of maximum phospholipid-synthesizing capacity (2). It also appears to precede the stage of maximum activity of enzymes which biosynthesize cyclic diterpenes [such as (-)-kaurene] which was described (25) in germinating castor bean. The synthesis of kaurene, which probably occurs in the endosperm (8), suggests that gibberellins may be present endogenously at the time when membrane proliferation and glyoxysome formation (2, 6, 7) is maximal.

Since Wrigley and Lord (28) found that long term application of 0.3 mM GA_3 did not result in an increase in glyoxysomal protein, they concluded that GA_3 did not affect glyoxysome assembly. Twenty-hr treatment with 0.07 mM GA_3 leads to enhanced enzyme levels but not to significant protein increase in the glyoxysome peak. Yet, an examination of specific activities of induced enzymes in glyoxysomes of different densities suggests that GA_3 does lead to an induction of a special class of glyoxysomes.

After short term treatment with GA, any significant enhancement or acceleration in glyoxysome formation over controls should be detectable as increased protein in the glyoxysome peak. This situation is not observed. Nevertheless, the pattern of relative specific activities for the various enzymes within the glyoxysome peak reveals that the induced enzymes were not incorporated into preexisting glyoxysomes. The pattern of relative specific activity ratios in fractionated glyoxysomes suggests that GA_3 treatment leads to the assembly of a special class of high density glyoxysomes with a high specific activity of isocitrate lyase. This glyoxysome population is superimposed upon glyoxysomes present prior to treatment within the normal density distribution. This pretreatment population should also be characterized by a constant enzyme specific activity ratio for all glyoxysomes in the normal density distribution. Evidence for this hypothesis comes from a comparison of the specific activity ratios (GA_3 gradient fraction specific activity/control gradient fraction specific activity) of the GA-inducible and of the noninducible enzymes. The ratios for hydroxypyruvate reductase remain constant throughout the density range but those of the GA-induced isocitrate lyase are seen to be highest in the dense region of the glyoxysome peak.

The results obtained after treatment with ABA are consistent with results reported by others (4, 9, 22). As in barley, the inhibiting effect of ABA suggests that endogenous GA is involved in the normal regulation *in vivo*.

The evidence provided here is consistent with the hypothesis that GA levels in castor bean endosperm influence subcellular development of organelles. GA applied exogenously to young castor bean seedlings results in enhanced levels of glyoxylate cycle enzymes and thus increased formation of glyoxysomes. As expected, GA affects the proliferation of the ER and results in an enhancement of the level of glyoxysomal enzymes associated with the ER (7). The concomitant increase in RNA content observed and the effects on membrane density all suggest that GA leads to an acceleration of glyoxysome-forming activities on the ER.

The position of glyoxysomal marker enzymes and RNA within the ER membrane peak deserves comment. As is the case with malate synthase and citrate synthase associations with the glyoxysomal membrane (12), these synthases have a similar affinity to the ER membranes of the 2-day seedlings (7). Yet it is evident that in *linear* gradients the malate synthase peaks do not exactly coincide with the NADH or NADPH-Cyt *c* reductase peaks. The malate synthase and RNA are associated with the leading edge of these membrane bands. The reason for this noncorrespondence is of potential, significant interest. Recent experiments by Lord and Bowden (15) suggest that the malate synthase is sequestered in ER cisternae or "a proteolysis-resistant space." This compartment can not represent a post-ER, glyoxysomal precursor since membranes obtained under conditions which do not preserve the integrity of the RER (no $MgCl_2$ and 2 mM EDTA in the grinding medium), show congruence of the ER markers and malate synthase activities in linear gradients. Lord and Bowden (15) also found that the additional processing of membranes, recovered from step gradients and subsequently treated with EDTA and applied to a linear gradient, resulted in coincident peaks of the ER markers and malate synthase.

All of these observations are consistent with the idea that only a portion of the ER membranes may be involved in glyoxysome assembly. It may be that this observed membrane heterogeneity is a result of functional differentiation of ER.

LITERATURE CITED

1. Beevers H 1969 Glyoxysomes of castor bean endosperm and their relation to gluconeogenesis. Ann NY Acad Sci 168: 313–324
2. Bowden L, JM Lord 1975 Development of phospholipid synthesizing enzymes in castor bean endosperm. FEBS Lett 49: 369–371
3. Doig RI, AJ Colborne, G Morris, DL Laidman 1975 The induction of glyoxysomal enzyme activities in the aleurone cells of germinating wheat. J Exp Bot 26: 387–398
4. Evins, WH, JE Varner 1971 Hormone controlled synthesis of endoplasmic reticulum in barley aleurone cells. Proc Nat Acad Sci USA 68: 1631–1633
5. Feierabend J, H Beevers 1972 Developmental studies on microbodies in leaves. I. Conditions influencing enzyme development. Plant Physiol 49: 28–32
6. Gerhardt BP, H Beevers 1970 Developmental studies on glyoxysomes in *Ricinus* endosperm. J Cell Biol 44: 94–102
7. Gonzalez E, H Beevers 1976 Role of the endoplasmic reticulum in glyoxysome formation in castor bean endosperm. Plant Physiol 57: 406–409
8. Green TR, DT Dennis, CA West 1975 Compartmentation of isopentenyl pyrophosphate isomerase and prenyltransferase in developing castor bean endosperm. Biochem Biophys Res Commun 64: 976–982
9. Ho DT-H, JE Varner 1976 Response of barley aleurone layers to abscisic acid. Plant Physiol 57: 175–178
10. Hock B, H Beevers 1966 Development and decline of the glyoxylate-cycle enzymes in watermelon seedlings (*Citrullus vulgaris* Schrad.). Z Pflanzenphysiol 55: 405–414
11. Huang AHC, H Beevers 1972 Microbody enzymes and carboxylases in sequential extracts from C_4 and C_3 leaves. Plant Physiol 50: 242–248
12. Huang AHC, H Beevers 1973 Localization of enzymes within microbodies. J Cell Biol 58: 379–389
13. Jones RL 1972 Fractionation of the enzymes of the barley aleurone layer: evidence for a soluble mode of enzyme release. Planta 103: 95–109
14. Kagawa T, JM Lord, H Beevers 1973 The origin and turnover of organelle membranes in castor bean endosperm. Plant Physiol 51: 61–65
15. Lord JM, L Bowden 1978 Evidence that glyoxysomal malate synthase is segregated by the endoplasmic reticulum. Plant Physiol 61: 266–270
16. Lord JM, T Kagawa, H Beevers 1972 Intracellular distribution of enzymes of the cytidine diphosphate choline pathway in castor bean endosperm. Proc Nat Acad Sci USA 69: 2429–2432
17. Lord JM, T Kagawa, TS Moore, H Beevers 1973 Endoplasmic reticulum as the site of lecithin formation in castor bean endosperm. J Cell Biol 57: 659–667
18. Lowry OH, NJ Rosebrough, AL Farr, RJ Randall 1951 Protein measurements with the Folin phenol reagent. J Biol Chem 193: 265–275
19. Luck H 1965 Catalase. *In* HU Bergmeyer, ed, Methods of Enzymatic Analysis. Academic Press, New York, p 885
20. Marriott KM, DH Northcote 1975 The breakdown of lipid reserves in the endosperm of germinating castor beans. Biochem J 148: 139–144
21. Marriott KM, DH Northcote 1975 The induction of enzyme activity in the endosperm of germinating castor-bean seeds. Biochem J 152: 65–70
22. Marriott KM, DH Northcote 1977 The influence of abscisic acid, adenosine 3',5' cyclic phosphate, and gibberellic acid on the induction of isocitrate lyase activity in the endosperm of germinating castor bean seeds. J Exp Bot 28: 219–224
23. Moore, TS Jr 1976 Phosphatidylcholine synthesis in castor bean endosperm. Plant Physiol 57: 383–386
24. Moore TS, JM Lord, T Kagawa, H Beevers 1973 Enzymes of phospholipid metabolism in the endoplasmic reticulum of castor bean endosperm. Plant Physiol 52: 50–53
25. Robinson DR, CA West 1969 Biosynthesis of cyclic diterpenes in extracts from seedlings of *Ricinus communis* L. II. Conversion of geranylgeranyl pyrophosphate into diterpene hydrocarbons and partial purification of the cyclization enzymes. Biochemistry 9: 80–89
26. Vigil EL 1970 Cytochemical and developmental changes in microbodies (glyoxysomes) and related organelles of castor bean endosperm. J Cell Biol 46: 435–454
27. Warburg O, W Christian 1942 Isolierung und Kristallisation des Gärungsferments Enolase. Biochem Z 310: 384–421
28. Wrigley A, JM Lord 1977 The effects of gibberellic acid on organelle biogenesis in the endosperm of germinating castor bean seeds. J Exp Bot 28: 345–353