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# Effect of Phytochrome on Development of Catalase Activity and Isoenzyme Pattern in Mustard (*Sinapis alba* L.) Seedlings

## A Reinvestigation

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*Summary.* In contrast to an earlier publication (Drumm *et al.*, Cytobiol. **2**, 335, 1970), a definite enhancement by phytochrome of the catalase level in mustard (*Sinapis alba* L.) cotyledons can be demonstrated. This response can be obtained either with continuous far-red light or with short red pulses, the effect of which is reversible by short far-red pulses. From the comparison of the time courses of catalase activity with the time courses of glyoxysomal (isocitrate lyase) and peroxisomal (glycolate oxidase, glyoxylate reductase) marker enzymes in dark grown and far-red irradiated cotyledons, there appears to be a close relationship between the catalase present in darkness and glyoxysomes and between the phytochrome-stimulated portion of total catalase and peroxisomes, respectively.

The isoenzyme pattern of catalase shows 3 strong and several weaker bands in dark grown cotyledons. Irradiation with white or far-red light leads to a more complex pattern with at least 12 detectable bands. The isoenzymes increased by light supplement rather than replace the isoenzymes present in darkness. This is true also in cotyledons and true leaves of white light grown plants which do not possess glyoxysomes. In the hypocotyl of the seedling, catalase formation is depressed by far-red light and no change in the isoenzyme pattern is observed.

It is concluded that the development of peroxisomes in the cotyledons is specifically controlled by phytochrome and that this subcellular differentiation also involves the control of catalase, a marker enzyme for both glyoxysomes and peroxisomes. The implications of these results with respect to the developmental origin of peroxisomes in cotyledons of fat-storing, potentially photosynthetically active cotyledons is discussed.

## Introduction

The fat-storing, potentially photosynthetic cells of the cotyledons of many epigealy-germinating dicotyledonous plants (e.g. mustard) possess the potential to produce two functionally different kinds of microbodies:

*Abbreviations:* $P_{fr}$: physiologically active form of phytochrome; DTE: dithioerytritol; CAT: catalase (EC 1.11.1.6); ICL: isocitrate lyase (EC 4.1.3.1); MS: malate synthase (EC 4.1.3.2); GO: glycolate oxidase (EC 1.1.3.1); GR: glyoxylate (hydroxypyruvate) reductase (EC 1.1.1.26).

(1) glyoxysomes (attached to fat bodies), which are engaged in fat mobilization during germination, and (2) peroxisomes (attached to chloroplasts), which are engaged in photorespiration (Tolbert, 1971). This type of cotyledon can be envisaged as a functional combination of endosperm tissue (containing only glyoxysomal microbodies) and true leaves (containing only peroxisomal microbodies) and thus provides an unique experimental system to study the developmental relationship between two types of microbodies housed in the same cell. So far, all attempts to physically separate glyoxysomes from peroxisomes *in vitro* were unsuccessful. Therefore, there seems to be no direct biochemical approach to answering the questions, whether or not these organelles are developmentally related, and how their formation (transformation ?) is controlled by the cell.

We became interested in these problems from a photomorphogenic point of view. From the investigation of the development of glyoxysomal (*e.g.* ICL, Karow and Mohr, 1967, see Fig. 2) and peroxisomal (*e.g.* GO and GR, Van Poucke *et al.*, 1970) marker enzymes during germination of mustard seedlings, it appears that formation of glyoxysomes is independent but that of peroxisomes is strongly influenced by light acting through phytochrome ($P_{fr}$). This conclusion is consistent with results derived from investigations on the effect of white light on microbody enzymes in a variety of other plants (Feierabend and Beevers, 1972a, b; Gerhardt, 1973; Gruber *et al.*, 1970, 1972, 1973; Schnarrenberger *et al.*, 1971; Trelease *et al.*, 1971a). The study of the effect of $P_{fr}$ on glyoxysomal and peroxisomal marker enzymes may provide a clue to the control mechanism of glyoxysome and peroxisome development.

Catalase is present in both glyoxysomes and peroxisomes. This enzyme has been shown to be stimulated by white light in leaf peroxisomes (*e.g.* Feierabend and Beevers, 1972a; Gruber *et al.*, 1972; Murray *et al.*, 1973). Electron microscopic examination of mustard cotyledons, treated with diaminobenzidine to localize CAT activity in the cell, indicate that the enzyme is detectable exclusively in the microbodies of this plant as well (Drumm *et al.*, 1970). Therefore, it may be expected that the time course of total CAT activity extractable from mustard cotyledons reflects a combination of $P_{fr}$-independent (glyoxysomal) and $P_{fr}$-dependent (peroxisomal) CAT activity. However, in a previous publication from this laboratory (Drumm *et al.*, 1970), only a slight enhancement by $P_{fr}$, produced and maintained by continuous far-red light, was found in mustard cotyledons. Due to the small, but nevertheless statistically significant differences in CAT activity from far-red treated and dark grown seedlings, it was not possible to demonstrate the involvement of phytochrome by red/far-red induction-reversion experiments. Furthermore, polyacrylamide gel electrophoresis revealed only a single,

broad band of CAT activity. These results led to the conclusion that "$P_{fr}$ does not exert any specific control on the temporal development of catalase". This paper reinvestigates the influence of $P_{fr}$ on the development of CAT activity using a different batch of mustard seeds and an improved procedure for electrophoretic separation of isoenzymes. It will be shown that the conclusion cited above is incorrect and that $P_{fr}$ indeed has a marked effect on the time course of catalase activity and isoenzyme pattern in mustard cotyledons. Our results are consistent with a general involvement of $P_{fr}$ in the transition of microbodies from glyoxysomal to peroxisomal function in fat-storing cotyledons which are transformed to photosynthetically active leaves in the light.

## Materials and Methods

*a) Treatment of Seedlings.* Standard techniques for photomorphogenic research on mustard seedlings (*Sinapis alba* L.) were used (Mohr, 1966). The mustard seeds were purchased in 1969 from Asgrow Company (Hamburg, Germany). The seedlings were grown at $25 \pm 0.3°$ C in the dark and irradiated as indicated in the figures and tables. The standard far-red source (Mohr, 1966), which maintains a low $P_{fr}/P_{tot}$ ratio in the seedlings (Hanke *et al.*, 1969), was used at an irradiance of 3.5 $W \cdot m^{-2}$. The standard red source (Mohr *et al.*, 1964), which maintains a $P_{fr}/P_{tot}$ ratio of about 0.8, was used at an irradiance of 0.675 $W \cdot m^{-2}$. In some experiments, seedlings were grown in white fluorescent light (7000 lux). Older mustard plants were grown in the greenhouse on soil (14 h natural light per day) for 3 weeks.

*b) Determination of Catalase Activity.* Extraction and assay of CAT activity was performed as described earlier (20 pairs of cotyledons or 40 hypocotyls extracted with 6 ml of 150 mM phosphate buffer (pH 7.2) in the presence of 0.5 g Divergan; photometric assay of $H_2O_2$ decomposition; cf. Drumm *et al.*, 1970). A molar extinction coefficient $\varepsilon_{240} = 39.4\ l \cdot mol^{-1} \cdot cm^{-1}$ for $H_2O_2$ was used (Nelson and Kiesow, 1972).

*c) Separation of Isoenzymes.* For electrophoresis extracts were prepared as usual but with 5 mM DTE included in the extraction buffer. Discontinuous electrophoresis (Poulik, 1957) was performed not later than 6 h after extraction using conventional horizontal starch gel slabs (12%, 18 cm long) with 76 mM tris/5 mM citrate gel buffer (pH 8.6) and 300 mM Na borate electrode buffer (pH 8.5) at 180 V for 15 h. Before casting the gel, a small volume of gel buffer with DTE was added to the starch slurry, giving a final concentration of 1 mM DTE in the gel. 5–40 µl of CAT extract, adjusted to a constant amount of enzyme (corresponding to 10 µl of extract from 60 h old etiolated cotyledons), were applied to $5 \times 10$ mm strips of cellulose acetate foil (Sartorius membrane filter SM 11200). In some cases, the extract was concentrated using Centriflo membrane cones (Amicon). The strips were maintained under a stream of cool air until the free liquid disappeared and then rapidly (before drying) inserted in a vertical cut 5 cm from the cathodal end of the gel. Due to an extremely narrow starting zone, this procedure gives better resolution of isoenzyme bands than conventional sample application techniques using filter paper strips or preformed gel slots. All CAT isoenzymes moved to the anode. Visualization of CAT isoenzyme bands on the gel was achieved by cutting the gel slab transversely with a steel wire and treating the inner surface first with 0.01% $H_2O_2$ for 5 min and then, after a short water rinse, with a mixture of $FeCl_3$

and $K_3[Fe(CN)_6]$ (1% each) for 5 min. This highly specific negative staining procedure (Woodbury *et al.*, 1971) yields sharp clear zones, indicating CAT activity, on a deep blue background which does not fade with time. The stained gels were photographed on a light box.

*d) Isolation of Microbodies.* The methods of Huang and Beevers (1971) were followed. Cotyledons from 500 plants were minced with razor blades and then ground using a hand-driven Potter homogenizer. The particulate fraction was pelleted and subjected to isopycnic sucrose density gradient centrifugation. Fractions of the gradient were collected and assayed for fumarase, MS, GO, and CAT activity to localize the microbody band.

*e) General Remarks.* In accordance with related investigations in the mustard seedling, the biological unit (pair of cotyledons; hypocotyl) was used as a system of reference (*cf.* Mohr, 1974). Mean values given in Figures and Tables are based on 8 to 12 independent parallels. Standard errors range between 2 to 5%.

In methodological experiments, the following results, not described in the earlier publication (Drumm *et al.*, 1970), were obtained: (1) Light treatment of the seedlings did not influence the enzyme activity in the extract through the formation of inhibitors or activators as demonstrated in mixing experiments with pure catalase. (2) Addition of 0.2% Triton X-100 to the extraction buffer changed neither the extractable enzyme activity nor the isoenzyme pattern in either light or in dark grown seedlings (*cf.* Holmes and Master, 1969). (3) We could not detect any influence of DTE (5 mM) on extractability or *in vitro* activity of CAT. However, extracts without DTE gave distinct isoenzyme bands without extensive tailing only if electrophoresis was performed immediately after extraction. DTE sharpened the bands considerably without either altering the pattern or the relative strength of the bands. There was no influence of the redox state of CAT on the isoenzyme pattern, leading to disappearance of bands under the influence of DTE, as described by Heidrich (1968) and Holmes and Masters (1969). The typical zymograms presented in this paper have been reproduced many times within a period of one year.

## Results

### *1. Development of Catalase Activity in Mustard Cotyledons is Controlled by Phytochrome*

Continuous far-red light, considered to operate exclusively via phytochrome (Mohr, 1972) produces an increase of CAT level in the cotyledons of mustard seedlings (Fig. 1). These data have been obtained with a seed population different from the one used previously (Drumm *et al.*, 1970; cf. Fig. 1). The results with the two seed batches are qualitatively similar. However, the batch used in the present paper showed a considerable greater responsiveness to far-red light with respect to CAT formation, permitting a successful investigation of the influence of $P_{fr}$ on the level of this enzyme. The data in Table 1 indicate that continuous far-red light can be largely replaced by a dark period, interrupted by a sequence of 5 min red irradiations. The effect of these red irradiations is fully reversed by subsequent far-red irradiations (Table 1). Thus, the involvement of phytochrome ($P_{fr}$) in the control of total CAT level can now also be shown by conventional induction-reversion experiments.

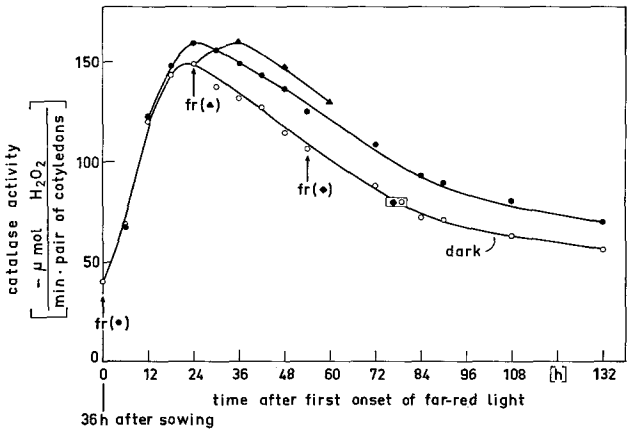



Fig. 1. The development of catalase activity in the cotyledons of mustard seedlings in darkness (○) and under continuous far-red light. Onset of irradiation (indicated by arrows) 36 h (●), 60 h (▲), and 90 h (♦) after sowing

Table 1. The stimulating effect of short red light pulses on catalase activity in mustard cotyledons and its reversal by far-red light pulses. The enzyme was assayed 114 h after sowing

| Treatment between 54 and 90 h after sowing | Catalase activity $\left[\frac{-\mu\text{mol } H_2O_2}{\text{min} \cdot \text{pair of cotyledons}}\right]$ |
|---|---|
| 36 h dark | $65 \pm 1$ |
| $3 \times$ (5 min red+12 h dark) | $77 \pm 2$ |
| $3 \times$ (5 min red+5 min far-red+12 h dark) | $66 \pm 2$ |
| $3 \times$ (5 min far-red+12 h dark) | $67 \pm 2$ |

## 2. *Correlation of Catalase Kinetics with the Kinetics of Glyoxysomal and Peroxisomal Marker Enzymes*

Fig. 1 shows detailed kinetics of CAT activity in cotyledons of dark grown seedlings which were irradiated with continuous far-red light starting 36, 60, and 90 h after sowing. The influence of the far-red light seems to be restricted to a "light sensitive" period starting at approximately 48 h and possibly lasting up to 90 h after sowing. The dark kinetics show a sharp rise followed by a slower decay. There is a striking coincidence between development of CAT and ICL activity in dark grown cotyledons. Both enzymes display maximum activity at about 60 h after sowing (Fig. 2). In contrast to CAT, the kinetics of the glyoxysomal marker enzyme ICL is not influenced by phytochrome (Karow and Mohr, 1967). On the other hand, the peroxisomal marker enzymes, GO and GR, are strongly increased by phytochrome (continuous far-red

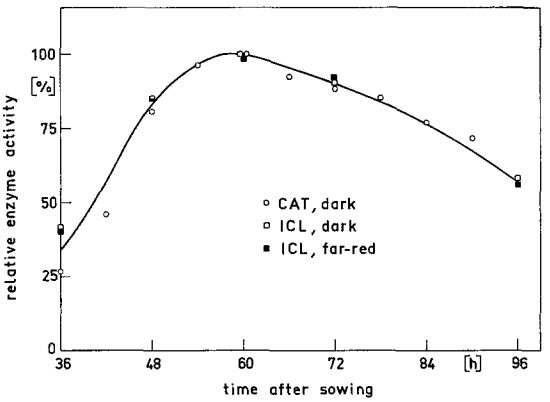



Fig. 2. The correlated development of catalase (○) and isocitrate lyase (□) activity in darkness and of isocitrate lyase activity in continuous far-red light (from the start of sowing, ■) in the cotyledons of mustard seedlings. The values are normalized to 100% at 60 h after sowing. (The isocitrate lyase data are adopted from Karow and Mohr, 1967)

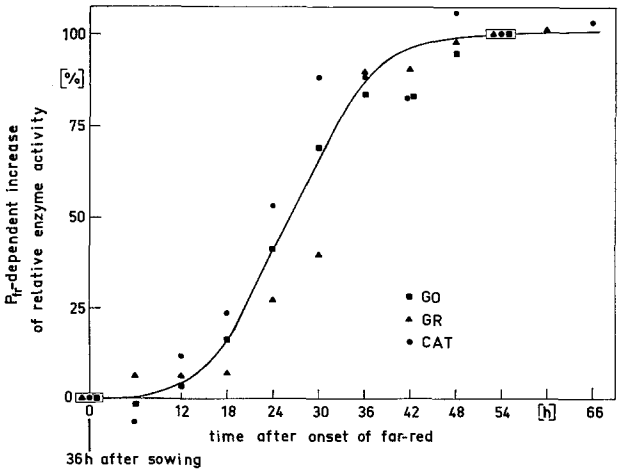



Fig. 3. The correlated development of catalase (●), glycolate oxidase (■), and glyoxylate reductase (▲) activity under the influence of continuous far-red light in the cotyledons of mustard seedlings. Onset of irradiation 36 h after sowing. The differences between far-red and dark kinetics are plotted on a percent scale, normalized to 100% difference 54 h after onset of irradiation. (The glycolate oxidase and glyoxylate reductase data are adopted from Van Poucke *et al.*, 1970. These authors used whole seedlings for their experiments. However, it has been shown that the far-red effect is restricted to the cotyledons)

light, Van Poucke *et al.*, 1970). The far-red dependent increase in CAT activity (Fig. 1) appears to be closely correlated with the far-red mediated increase in GO and GR activity when the kinetics of the 3 enzymes are compared on a per cent basis (Fig. 3). A similar correlation is also apparent with respect to the duration of the "light sensitive" period during seedling development in these enzymes. Especially noteworthy is the unusually long lag phase of about 12 h which is displayed by all 3 enzymes when 36 h old etiolated seedlings are irradiated. While CAT of dark grown seedlings, as well as ICL, shows an extensive decrease in later stages of development, the increment in CAT activity produced by phytochrome represents an apparently constant amount of enzyme similar to that which has been shown for GO and GR (Van Poucke *et al.*, 1970; Van Poucke and Barthe, 1970). Thus, the kinetic behaviour of CAT in far-red light and darkness as shown in Fig. 1 is consistent with the idea that CAT activity present in darkness is essentially associated with microbodies acting as glyoxysomes and that the CAT produced under the influence of phytochrome (far-red light) is essentially associated with microbodies having the properties of potential peroxisomes.

*3. Phytochrome Changes the Isoenzyme Pattern of Catalase in the Cotyledons*

Since the existence of CAT isoenzymes in plants is well established (*e.g.* Scandalios, 1968), we reinvestigated the mustard CAT using a starch gel techniques with high resolving power. The rational of these experiments was to find out whether or not "glyoxysomal" and "peroxisomal" CAT (in the operational meaning justified above) can also be discriminated on the isoenzyme level. Fig. 4 shows that it is indeed possible to demonstrate 3 strong and several weaker bands on zymograms obtained with extracts from mustard cotyledons. This result disproves and replaces the earlier finding (Drumm *et al.*, 1970) where CAT appeared to be homogeneous using polyacrylamide gel electrophoresis of low resolution in the absence of DTE, the presence of which greatly improves the detectability of mustard CAT isoenzymes (cf. Methods). Fig. 4 shows that phytochrome changes the isoenzyme pattern of CAT. In cotyledons from dark grown seedlings, only a rapidly migrating group of 3–5 bands (depending on age) is clearly detectable. Irradiation with far-red or white light leads to the appearance of at least 7 additional bands of various strengths, which are below the limit of detectability if an equal amount of CAT activity units from dark grown cotyledons is subjected to electrophoresis[1]. It appears from these data that the action of phyto-

1 In fact, the effect of irradiation seems to be a quantitative rather than a qualitative one. Using extract concentrated several fold, we were able to detect traces of at least some of the far-red induced isoenzymes also in 120 h old dark grown cotyledons. This agrees with the fact that the peroxisomal marker enzymes GO and GR are also present in low activity in this material.

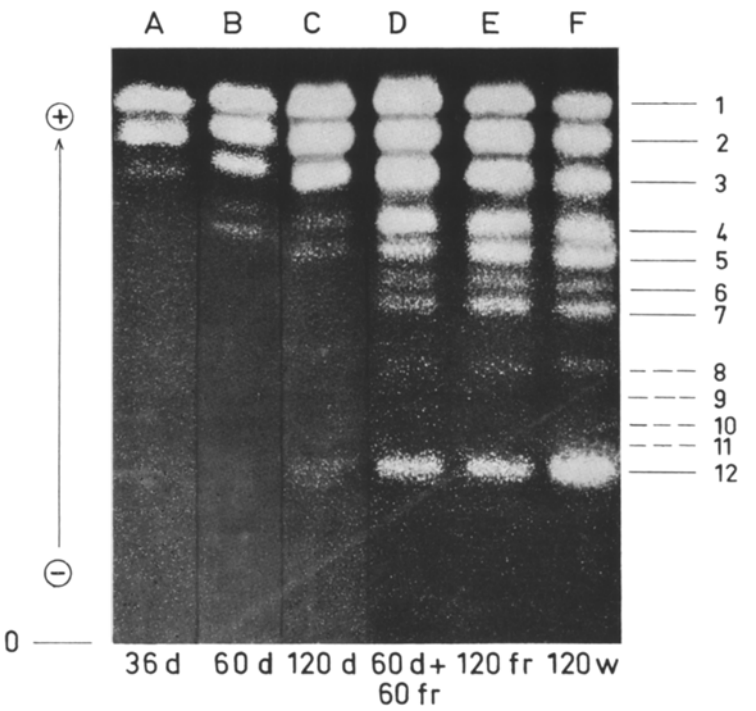



Fig. 4. Separation of catalase isoenzymes by starch gel electrophoresis. Equal amounts of total catalase activity units from cotyledons of dark grown (*d*, *A–C*), far-red (*fr*, *D–E*), and white (*w*, *F*) irradiated seedlings were used (time from sowing as indicated). With extracts from light grown cotyledons, a total of 12 bands could be detected by eye, however the bands 8, 9, 10 and 11 are too weak to be reproduced in the photograph. In dark grown cotyledons, only bands 1, 2, 3 are always clearly visible; under suitable conditions (old seedlings) however, bands 4, 5, and 12 are faintly visible

chrome is not to replace one set of isoenzymes by another but, rather, to supplement the "glyoxysomal" isoenzymes by additional molecular species of CAT. The zymograms shown in Fig. 5 support this suggestion.

Comparisons between the CAT patterns obtained with equal activity units from cotyledons of far-red treated seedlings and those from cotyledons of older plants grown under white light demonstrate the survival of the "glyoxysomal" isoenzymes in the aged cotyledons in which the period of glyoxysomal activity has been surpassed. Furthermore, it becomes evident that the microbodies of true leaves, which are peroxisomes by definition, also contain the complete set of CAT isoenzymes characteristic of light grown cotyledons. Thus, the following conclusions may be justified: (1) Glyoxysomal microbodies (from etiolated cotyledons)

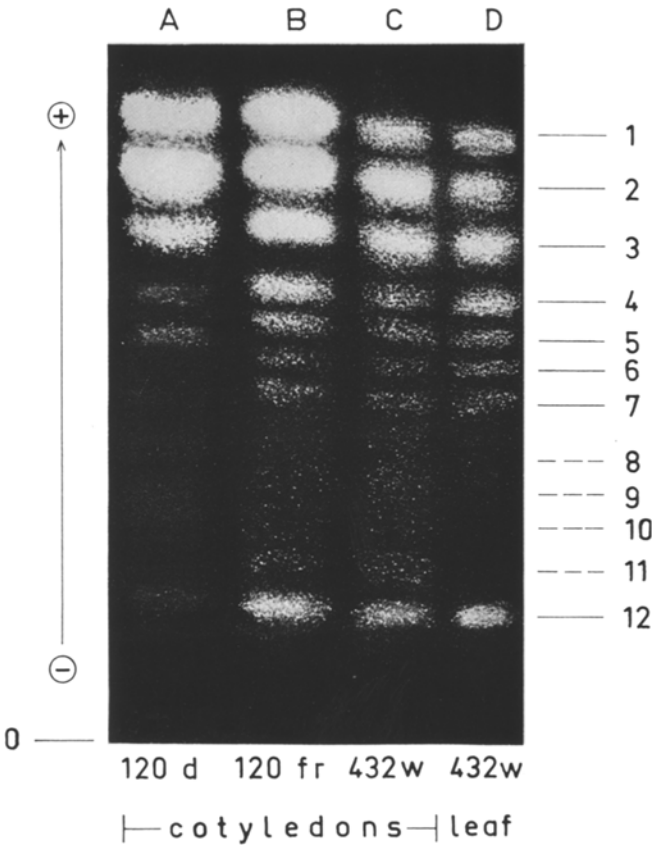



Fig. 5. Zymograms of catalase isoenzymes from cotyledons of 120 h old seedlings kept in darkness (*A*) or irradiated with far-red light (*B*), and from cotyledons (*C*) and true leaves (*D*) of green mustard plants grown for 3 weeks under natural white light (*cf.* legend of Fig. 4)

are characterized by a relatively simple pattern of CAT isoenzymes while peroxisomal microbodies (from green leaves) have a more complex pattern which includes the "glyoxysomal" isoenzymes. (2) Light acting through phytochrome mediates the transformation of the glyoxysomal to the peroxisomal pattern of CAT isoenzymes in the cotyledons.

In a further experiment, microbodies of equal amounts of cotyledons from dark grown and far-red irradiated seedlings were prepared by conventional sucrose density gradient centrifugation (cf. Methods). After fractionation, the microbody band was located by assaying for CAT, GO, and MS. Aliquots of the CAT peak fractions and from the upper end of the sucrose gradients were subjected to electrophoresis. The

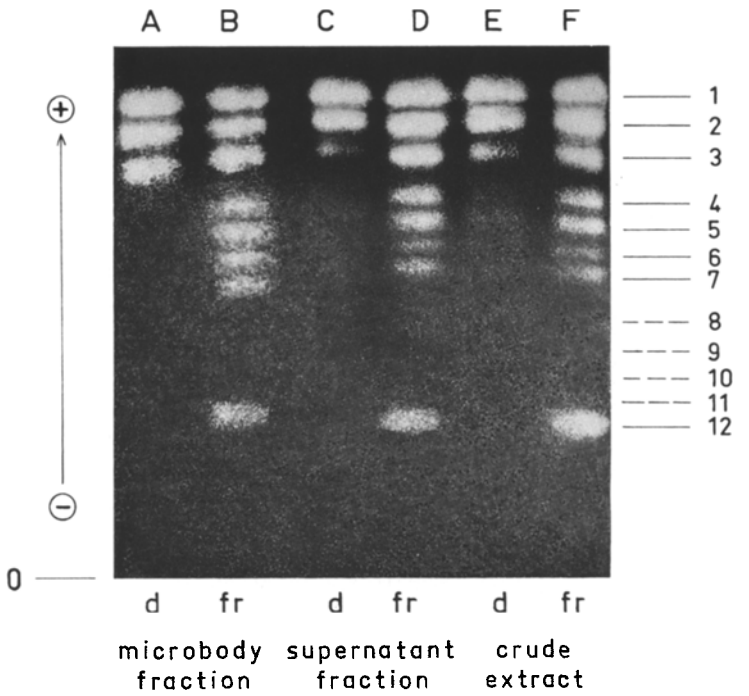



Fig. 6. Zymograms of catalase isoenzymes from microbody preparations. A crude particulate fraction obtained from equal amounts of cotyledons of 120 h old seedlings (grown in darkness or irradiated with far-red light from 36 to 120 h after sowing) was subjected to sucrose gradient centrifugation. Aliquots of the microbody peak fraction (*A*, *B*) and the top fraction of the gradient (representing broken microbodies, *C*, *D*) were used for electrophoresis. Crude protein extract prepared by the standard procedure was used as a control (*E*, *F*)

results shown in Fig. 6 provide the justification for attributing the phytochrome-dependent changes in the CAT isoenzyme pattern, obtained with crude extract (*cf.* Figs. 4, 5), to the microbody fraction.

### *4. The Influence of Phytochrome on the Activity Level and Isoenzyme Pattern of Catalase in the Hypocotyl*

The hypocotyl of the mustard seedling contains only traces of storage fat, ICL, and GO (Hock *et al.*, 1965; Karow and Mohr, 1967; Van Poucke and Barthe, 1970), and does not become photosynthetic in the light. Nevertheless, microbodies are detectable in electron micrographs from this organ also (B. Steinitz, personal communication). These particles may resemble non-specialized microbodies with limited enzyme composition

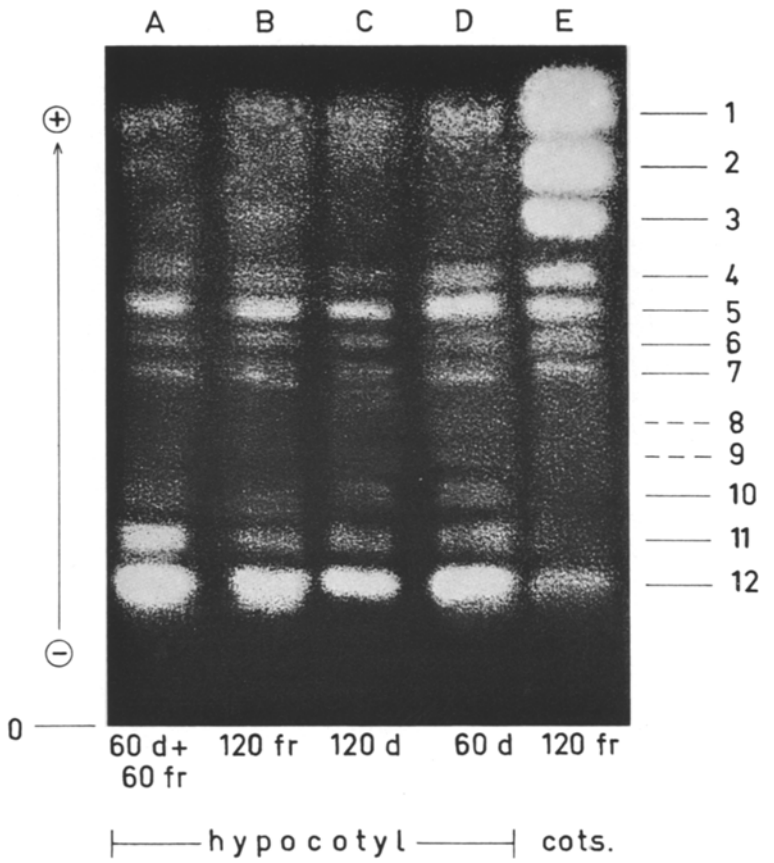
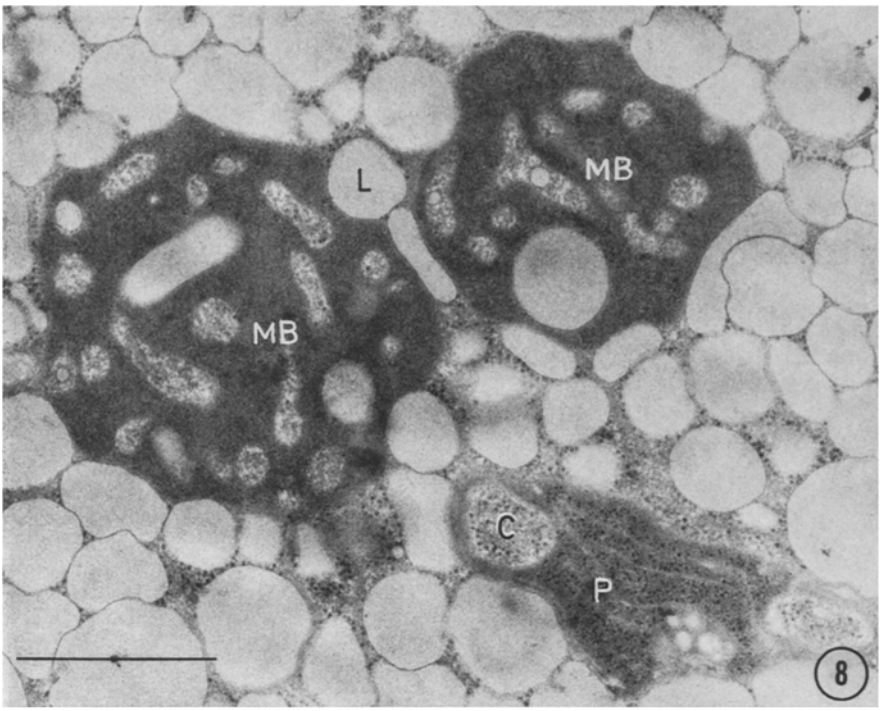



Fig. 7. Zymograms of catalase isoenzymes from hypocotyls (*A–D*). For comparison, extract from the cotyledons (*E*) of far-red grown seedlings has been co-electrophoresed. The enzyme samples were adjusted to equal activity units (*cf.* legend for Fig. 4)

observed in other plant tissues (Huang and Beevers, 1971; Ruis, 1971), the metabolic role of which is not known at present. We used the hypocotyl to demonstrate the specificity of the light effect on the CAT of cotyledons. Table 2 shows that the CAT activity in the hypocotyl of 60 h old dark grown seedlings is about one order of magnitude lower than in the cotyledons. The enzyme level in the hypocotyl continues to rise during the period of CAT net decrease in the cotyledons (cf. Fig. 1). Far-red light considerably suppresses the increase of enzyme activity. However, there is no detectable difference in the isoenzyme pattern between dark grown and far-red irradiated tissue (Fig. 7). In both cases,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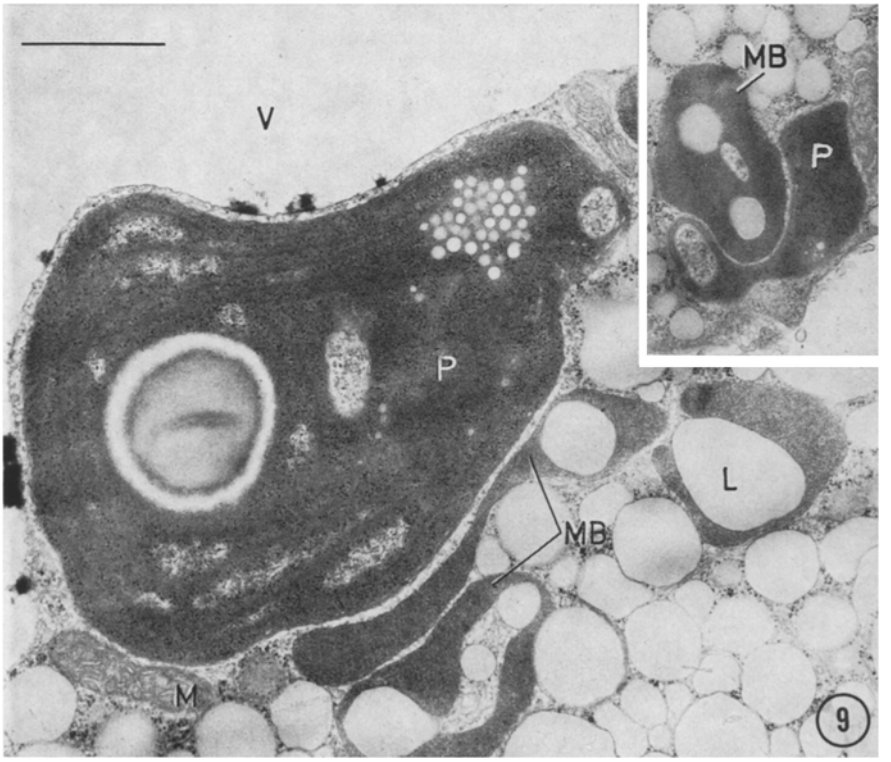
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Table 2. The influence of continuous far-red irradiation on the extractable catalase activity of hypocotyl and cotyledons in the mustard seedling

| Treatment after sowing | Catalase activity $\left[\frac{-\mu\text{mol } H_2O_2}{\text{min} \cdot \text{organ}}\right]$ | |
|---|---|---|
| | Pair of cotyledons | Hypocotyl |
| 60 h dark | $141 \pm 3$ | $14.3 \pm 0.7$ |
| 120 h dark | $89 \pm 5$ | $19.6 \pm 0.5$ |
| 120 h far-red | $94 \pm 2$ | $8.9 \pm 0.3$ |
| 60 h dark+60 h far-red | $95 \pm 4$ | $15.8 \pm 1.1$ |

the pattern shows a predominance of those bands which are increased by light in the cotyledons. No further bands in addition to those observed in the cotyledons could be detected. Obviously, the CAT of hypocotyl microbodies, as compared to the cotyledon enzyme, responds in a qualitatively different way to phytochrome. It appears that the level of total CAT activity parallels the longitudinal growth of the hypocotyl, which is strongly inhibited by phytochrome (*cf.* Schopfer and Oelze-Karow, 1971). Thus, the response in the hypocotyl contrasts with that in the cotyledons where the CAT activity increases in response to $P_{fr}$.

## Discussion

Our data provide further evidence for the involvement of phytochrome in the light-dependent developmental changes of microbodies

Fig. 8. Typical microbodies (glyoxysomes) in spongy parenchyma cells of 84 h old dark grown mustard cotyledons. Note the numerous inclusions of cytosol and lipid material. An association of microbodies and plastids is not apparent. *MB* microbody; *P* plastid (etioplast); *L* lipid body; *C* inclusion of cytosol.—Electron micrograph by Dr. H. Falk. The tissue was fixed in green safelight in 3% glutaraldehyde, buffered with 50 mM Na cacodylate/HCl, pH 7.2, containing 10 mM $MgCl_2$ and 200 mM KCl and postfixed in 2% $OsO_4$ in the same buffer. Blocks stained with 2% uranylacetate. After sectioning, double staining with uranylacetate and Pb citrate (Magnification $\times 26500$)

Fig. 9. Typical microbodies in spongy parenchyma cells of cotyledons of 84 h old mustard seedlings irradiated with continuous far-red light from 36 to 84 h after sowing. Note the large, starch-containing plastid ("far-red etioplast") with few primary thylakoids. (In other plastids, large prolamellar bodies are detectable.) A large fraction of the microbodies appears attached to or—as in the insert—even interdigitated with a plastid. Most of these microbodies contain lipid bodies.—Electron micrograph by Dr. H. Falk (for methods, see legend for Fig. 8). *MB* microbody; *M*: mitochondrion; *L*: lipid body; *P* plastid; *V* vacuole (Magnification $\times 19000$; inset $\times 16500$)

in fat-storing cotyledons. Since the far-red light source used in these experiments to activate the phytochrome system does not appreciably support chlorophyll synthesis (Masoner *et al.*, 1972), a contribution of photosynthesis to the observed photoresponses can be disregarded. This is a crucial prerequisite to avoid the interference of an indirect inhibitory light effect on the time course of glyoxysomal enzymes as has been observed in several investigations (*e.g.* Hock, 1969). After reaching the maximum rate of lipid mobilization (about 60 h after sowing, *cf.* Hock *et al.*, 1965), there appears to exist a period of slowly declining glyoxysomal activity as well as a simultaneous build-up of potential peroxisomes in the cotyledons of mustard seedlings. This phytochrome-mediated intracellular differentiation revealed by the time courses of specific glyoxysomal and peroxisomal marker enzymes can also be demonstrated with the common marker enzyme, CAT.

With respect to the developmental origin of peroxisomes, these results do not permit any conclusive decision. In principle, the data can be reconciled with a $P_{fr}$-controlled appearance of peroxisomes, which is independent of the rise and fall in glyoxysomes (*cf.* Beevers, 1971), as well as with a $P_{fr}$-mediated transformation of glyoxysomes to peroxisomes (Trelease *et al.*, 1971a, b; Gerhardt, 1973). However, there are several lines of evidence which tend to support the second alternative: (1) It is interesting to note that the $P_{fr}$-mediated appearance of peroxisomal enzymes is limited to the period of decay in glyoxysomal enzymes (see also Trelease *et al.*, 1971a; Fig. 2). This kind of correlation would hardly be expected if the two types of microbodies were developmentally unrelated. (2) The change in CAT isoenzyme pattern tends to support the notion that microbodies, present already in the dark grown cotyledons, can be transformed to peroxisomes by $P_{fr}$. It is evident from Figs. 4 and 5 that those isoenzymes which are characteristic for glyoxysomes are not *replaced* by other isoenzymes when peroxisomes are formed in cotyledons (or true leaves) but rather persist and become supplemented by additional CAT species. A comparison with Fig. 7 shows that the peroxisomes of the leaf appear to be more closely related to the microbodies of the cotyledons than to the microbodies of the hypocotyl, at least with respect to the CAT isoenzyme pattern. Taken together, these observations provide further circumstantial evidence for the hypothesis (advanced by Trelease *et al.*, 1971a, b) that in fat-storing cotyledons at least some preformed microbodies can be transformed into peroxisomes by specifically losing certain enzymes and acquiring others without loss of their compartmental integrity. Recently, Lazarow and De Duve (1973) have found direct biochemical evidence for a rapid import of CAT subunits into pre-existing rat liver peroxisomes.

Electron micrographs from cotyledons of dark grown and far-red irradiated mustard seedlings also contribute some information to these findings. In 84 h old etiolated cotyledons, the microbodies are expanded in volume and show numerous invaginated pockets (or inclusions) of cytosol containing ribosomes and sometimes even mitochondria (Fig. 8). Similar pictures have been published by Gruber *et al.* (1970) and Trelease *et al.* (1971 a, b), who investigated cucumber cotyledons during the transitory phase of microbody function. In the mustard cotyledons, lipid bodies can often be detected in these invaginations, emphasizing the close functional relationship between the two cellular components. Only in rare cases are microbodies located near the plastids. Also, cotyledon cells of 84 h old far-red irradiated seedlings (Fig. 9) contain appreciable amounts of lipid and most microbodies still show a close association with lipid bodies. In many cases, however, a close spatial relationship between microbodies, some of which carry lipid bodies, and plastids can be seen. In regard to peroxisomes, an association with plastids has to be expected (Frederick and Newcomb, 1969). Obviously, these cotyledons are still in the transitory phase of change from glyoxysomal to peroxisomal function. This is in agreement with the kinetic data of Figs. 1–3 which suggest a substantial overlap in time of the two microbody functions in the mustard cotyledons.

The question whether the "peroxisomes" of far-red treated cotyledons are active in photorespiration has not yet been investigated. The plastids present in these cotyledons are very similar to etioplasts of dark grown cotyledons (Kasemir *et al.*, 1974). However, the presence of large starch grains in these "far-red etioplasts" indicate an active carbohydrate metabolism, possibly related to gluconeogenesis.

In the present investigation, the isoenzyme pattern of CAT has been used as a molecular marker which allows discrimination between different types of microbodies. The biochemical basis of CAT heterogeneity in the mustard seedling has not been studied in any detail to date. However, it is likely that the isoenzyme bands separated by starch gel electrophoresis (Figs. 4–7) are produced by a permutation of different types of monomeric polypeptide subunits (obviously produced in unequal amounts) in the tetrameric CAT molecule (*cf.* Scandalios, 1968). Since radom combination of two types of subunits would lead to a pattern with a maximum of 5 bands, based on the expansion of $(a + b)^4$, one has to assume a minimum of 3 types of subunits, coded for by different allelic or non-allelic genes, to explain the complex pattern of 12 detectable bands in the mustard seedling. The expansion of $(a + b + c)^4$ leads to 15 terms. This number would be in agreement with our results if the existence of 3 further isoenzymes, not detectable by the methods used,

is taken into account. The differences of the CAT pattern shown in Figs. 4, 5, and 7 are quantitative rather than qualitative. Under appropriate conditions, the presence of all 12 bands can be demonstrated in dark or light grown cotyledons and hypocotyls. Therefore, it seems to be the relative amounts of the different subunits which are responsible for the differences observed in these zymograms. It is tempting to speculate that the $P_{fr}$-stimulated bands (Fig. 4) partly originate from the enhanced formation of one type of subunit which is present only in traces in etiolated cotyledons. However, this point requires further clarification. One has to bear in mind that the seeds used in this investigation are probably not genetically homogeneous; therefore, the CAT isoenzyme patterns observed are representative for a population of plants rather than for an individual genotype.

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