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# Catalase Synthesis and Turnover during Peroxisome Transition in the Cotyledons of *Helianthus annuus L.*[1]

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## ABSTRACT

**Based on measurements of total catalase hematin and the degradation constants of catalase hematin, zero order rate constants for the synthesis of catalase were determined during the development of sunflower cotyledons (*Helianthus annuus* L.). Catalase synthesis reached a sharp maximum of about 400 picomoles hematin per day per cotyledon at day 1.5 during the elaboration of glyoxysomes in the dark. During the transition of glyoxysomes to leaf peroxisomes (greening cotyledons, day 2.5 to 5) catalase synthesis was constant at a level of about 30 to 40 picomoles hematin per day per cotyledon. In the cotyledons of seedlings kept in the dark (day 2.5 to 5) catalase synthesis did not exceed 10 picomoles hematin per day per cotyledon. During the peroxisome transition in the light, total catalase hematin was maintained at a high level, whereas total catalase activity rapidly decreased. In continuous darkness, total catalase hematin decreased considerably from a peak at day 2. The results show that both catalase synthesis and catalase degradation are regulated by light. The turnover characteristics of catalase are in accordance with the concept that glyoxysomes are transformed to leaf peroxisomes as described by the one population model and contradict the two population model and the enzyme synthesis changeover model which both postulate *de novo* formation of the leaf peroxisome population and degradation of the glyoxysome population.**

Studies on the transition of peroxisomes from glyoxysomal to leaf peroxisomal function in greening oil storing cotyledons have given rise to three models (1). The two population model (6, 7) and the "enzyme synthesis changeover" model (14, 15) postulate a breakdown of glyoxysomes and a *de novo* formation of leaf peroxisomes, whereas the one population model (18) assumes that glyoxysomes are transformed into leaf peroxisomes. In contrast to the two population model, both the enzyme synthesis changeover model and the one population model predict peroxisomes of intermediary character, *i.e.* organelles containing both glyoxysomal and leaf peroxisomal enzymes during the transition stage. According to the enzyme synthesis changeover model, leaf-type peroxisomes are formed as a consequence of a turnover of the whole organelles, whereas in the one population model individual enzyme turnover within the same organelle leads to a gradual replacement of glyoxysomal by leaf peroxisomal enzymes.

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The existence of peroxisomes of intermediary character has been demonstrated recently in immunocytochemical studies (10, 11, 17). These results provide strong evidence against the two population model which postulates two independent populations of peroxisomes functioning exclusively as either glyoxysomes or leaf peroxisomes. The question, however, whether individual enzyme turnover or organelle turnover accounts for the formation of the intermediary peroxisomes, could not be decided unequivocally.

Our analytical studies on the peroxisome transition are based on the premise that predictions on the turnover of enzymes (common to both types of peroxisomes) are different for the one population model than they are for the two other models. The most prominent common enzyme is catalase. The one population model predicts that, in the course of protein turnover, catalase present in the glyoxysomes is only partially replaced by newly synthesized catalase during peroxisome transition. The other two models imply complete degradation of glyoxysomal (not glyoxy-peroxisomal) catalase during the transition and complete de novo synthesis of catalase for leaf peroxisomes.

In a previous paper (5), we showed that the peroxisome transition in sunflower cotyledons was accompanied by a delay of catalase degradation. This result is in accordance with the one population model and incompatible with the two other models. Catalase synthesis has already been measured by density labeling in greening sunflower cotyledons (2) and by short time labeling of the cytosolic pools in greening cucumber cotyledons (8). These studies, however, did not provide all the data on catalase synthesis necessary for a complete quantitative assessment of catalase turnover. The density labeling experiments (2) allowed measuring enzyme activity which was due to catalase synthesis. Because the specific activity of catalase considerably decreases during the transition stage (4), the synthesis of catalase may have been underestimated by the density labeling method. Radioactive labeling of the cytosolic pools revealed the time course of catalase synthesis during the first hours of peroxisome transition (8). Rates of synthesis were measured in relative units (radioactivity incorporated into cytosolic catalase), therefore, the data did not give an idea of the amount of newly synthesized catalase.

Here we report quantitative determinations of catalase synthesis in sunflower cotyledons during different stages of peroxisome function. Results are based on determinations of amounts of catalase hematin, and with the results on catalase degradation (5), provide a complete quantitative description of catalase turnover. The data allow one to calculate to what

extent catalase synthesized during the different stages of peroxisome function contributes to the total catalase at any time of cotyledon differentiation.

## MATERIAL AND METHODS

### Plant Growth Conditions

Following removal of the seed coat and of one of the cotyledons from soaked (16 h) sunflower achenes (*Helianthus annuus* L. cv Spanners Allzweck) the embryos were grown in plastic flats containing moist vermiculite at 30°C for 2.5 d in the dark and thereafter for 5.5 d (at 30°C) in continuous white light (150 $\mu$E $m^{-2}$ $s^{-1}$). Counting of the days of development started with the planting of the embryos. At d 0, 2.5 $\mu$L of a 1.2 mM solution of 5-amino levulinic acid, and at d 1.5, 2.5 $\mu$L of a neutralized 30 mM solution of 5-amino levulinic acid were applied to each cotyledon. All growth conditions and manipulations were the same as used in the experiments for determination of the degradation constants (5).

### Preparation of Cotyledonary Extracts and Purification of Catalase

Cotyledonary extracts were prepared as described previously (5). Catalase was purified according to Eising and Gerhardt (4). The last purification step (gelfiltration on Sephadex G-200) was omitted because non-catalase hematin was not present prior to this step (4, 5).

### Determination of Catalase Hematin per Cotyledon

The amount of catalase hematin in the cotyledons was calculated according to Equation 1.

$$\text{pmol hematin} \times \text{cotyledon}^{-1} = (\mu\text{kat} \times \text{cotyledon}^{-1}) : (\mu\text{kat} \times \text{pmol hematin}^{-1}). \quad (1)$$

The total catalase activity ($\mu$kat × cotyledon$^{-1}$) was determined in cotyledonary extracts, and the hematin based activity of catalase ($\mu$kat × pmol hematin$^{-1}$) was determined on the purified enzyme. It was shown previously that the prerequisites for determining the amount of catalase hematin in the cotyledons by Equation 1 were fulfilled (5).

### Determination of Catalase Synthesis

According to the general equation describing enzyme turnover (12), changes in the concentration of catalase hematin in sunflower cotyledons can be described by Equation 2.

$$\frac{dH}{dt} = S - kH \quad (2)$$

where $H$ is the amount of catalase hematin in the cotyledons (pmol × cotyledon$^{-1}$), $S$ is the zero order rate constant of synthesis of catalase hematin (pmol × $d^{-1}$ × cotyledon$^{-1}$) and $k$ is the first order rate constant ($d^{-1}$) for the degradation of catalase hematin. Integration of Equation 2 and solving it for $S$ gives Equation 3:

$$S = \frac{(H_t - H_0 \cdot e^{-kt})\, k}{1 - e^{-kt}} \quad (3)$$

where $H_t$ and $H_0$ are the amounts of catalase hematin at time $t$ and time 0, respectively. The rate constant $S$ was obtained from Equation 3 by determining $H_0$, $H_t$, and $k$. The determination of $k$ was reported previously (5).

### Assays

Catalase activity was measured spectrophotometrically at 22°C whereby the destruction of $H_2O_2$ was followed at 230 nm (4). Catalase hematin was determined according to Eising and Gerhardt (4).

### Statistical Analyses

The level of significance for differences was 5% in all cases. Significance of differences between means and of differences between regression coefficients was determined by Student's $t$-test.

## RESULTS

### Time Course of Catalase Activity and Catalase Hematin

To determine the change in catalase hematin, the hematin based activity of purified catalase ($\mu$kat × pmol$^{-1}$) and the total catalase activity ($\mu$kat × cotyledon$^{-1}$) were measured during cotyledon development (Fig. 1). Total catalase hematin (pmol × cotyledon$^{-1}$) was calculated according to Equation 1. Cotyledon development was separated into three stages based on the function of the peroxisomes in the cotyledons (5). In stage I (d 0–2.5), peroxisomes with glyoxysomal function were present exclusively. The transition from glyoxysomal to leaf peroxisomal function of the peroxisomes took place during stage II (d 2.5–5), and in stage III (d 5–8) the cotyledons contained completely developed leaf peroxisomes.

The increase of catalase hematin during stage I coincided

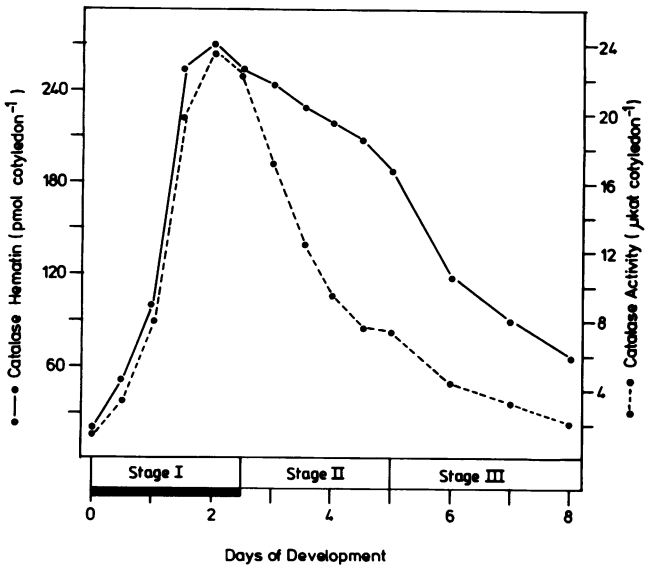


**Figure 1.** Time course of changes in catalase hematin and catalase activity in the cotyledons of sunflower seedlings. After 2.5 d of growth in darkness the seedlings were exposed to continuous white light. The results shown were obtained from one flat of seedlings.

with the increase of catalase activity (Fig. 1). The decrease of catalase activity during stage II, however, was much more rapid than the decrease of catalase hematin. Semilogarithmic plots of catalase activity and of catalase hematin *versus* time (day of development) showed that both the decrease of activity and the decrease of hematin followed first order kinetics during stage II and III (Fig. 2A). Therefore, the kinetics can be described by Equation 4 and 5.

$$A_t = A_{2.5} \cdot e^{-m(t - 2.5)} . \quad (4)$$

In Equation 4, $A_t$ and $A_{2.5}$ are the catalase activities ($\mu$kat × cotyledon$^{-1}$) at the time $t$ and $t$ = 2.5 d, respectively, and $m$ is the first order rate constant ($d^{-1}$) for the decrease of catalase activity.

$$H_t = H_{2.5} \cdot e^{-c(t - 2.5)} . \quad (5)$$

In Equation 5, $H_t$ and $H_{2.5}$ are the amounts of catalase hematin (pmol × cotyledon$^{-1}$) at time $t$ and $t$ = 2.5 d, respectively, and $c$ is the first order rate constant ($d^{-1}$) for the decrease of catalase hematin. However, this decrease must be described by biphasic kinetics because statistical comparison of the regression coefficients, calculated separately for stage II and III, revealed that they were significantly different. Therefore, $c$ in Equation 5 must be substituted by $c_{II}$ or $c_{III}$ which denote the first order rate constants in stage II and III, respectively. Analyses of the decrease of catalase activity revealed no significant difference between the regression coefficients calculated for stage II and III.

When the seedlings were kept in continuous darkness (after d 2.5), both the decrease of catalase activity and of catalase hematin followed monophasic first order kinetics between d 2.5 and 8 (Fig. 2B). The first order rate constants are denoted $m_d$ (for catalase activity) and $c_d$ (for catalase hematin).

The first order rate constants $m$, $c_{II}$, $c_{III}$, $m_d$ and $c_d$ were determined using cotyledons from replicate flats of sunflower seedlings. The mean values of the rate constants were compared by Student's $t$-test (Table I). The results indicated that m was significantly higher than $c_{II}$. This showed that the rapid decline of catalase activity during the peroxisome transition was not only effected by a reduction in the catalase content of the cotyledons, but also by a mechanism which impaired the catalytic activity of the enzyme. The comparison of $c_{II}$ and $c_d$ (Table I) revealed that a high content of catalase hematin was maintained during the elaboration of leaf peroxisomes, whereas catalase hematin considerably decreased in the dark grown cotyledons. In contrast to the time course for catalase hematin, the time course for catalase activity was not significantly influenced by light (difference between $m$ and $m_d$ not significant; Table I), *i.e.* the change in activity was independent of the formation of leaf peroxisomes. This result confirms the observation reported by Schnarrenberger *et al.* (13).

### Synthesis of Catalase.

Zero order rate constants for the synthesis of catalase hematin (pmol × $d^{-1}$ × cotyledon$^{-1}$) were calculated according to Equation 3 at time intervals of 0.5 d for stage I through III. $H_0$ and $H_t$ then corresponded to the amounts of catalase

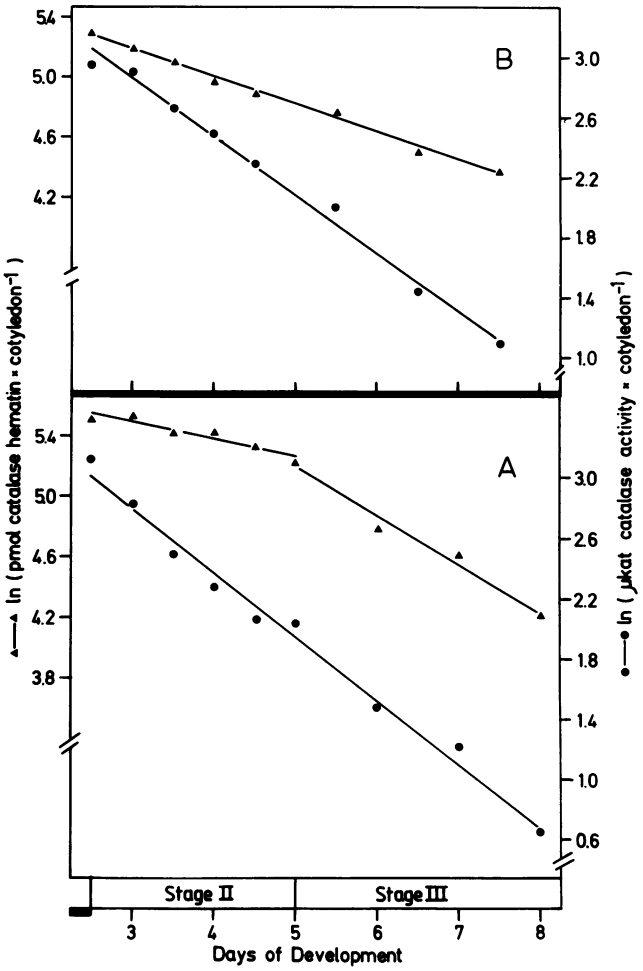


**Figure 2.** Semilogarithmic plot of the decrease of catalase hematin and catalase activity in sunflower cotyledons during stages II and III. Individual values for the amounts of catalase hematin (pmol × cotyledon$^{-1}$) were calculated according to Equation 1. Catalase activity was measured in cotyledonary extracts. The straight lines were determined by linear regression. A, Seedlings kept in continuous light between d 2.5 and 8 (standard development); B, seedlings kept in continuous darkness.

hematin at the beginning and the end of each 0.5 d interval. Therefore, $H_0$ in Equation 3 was substituted by $H_{t-0.5}$. The values for $H_{t-0.5}$ and $H_t$ were calculated according to Equation 5 using the values of the rate constants $c_{II}$, $c_{III}$, and $c_d$ shown in Table I. The amount of catalase hematin at d 2.5 (*i.e.* $H_{2.5}$ in Eq. 5) was measured for the cotyledons of 16 replicate flats of seedlings, and the mean value 290 pmol × cotyledon$^{-1}$ (SE = ±10.4) was used as the initial value for the calculations of $H_{t-0.5}$ and $H_t$. The following example shows the calculation of the rate constant $S$ for the time interval between d 4 and 4.5.

$$S_{4-4.5} = \frac{(H_{4.5} - H_4 \cdot e^{-k_{II} \cdot 0.5}) k_{II}}{1 - e^{-k_{II} \cdot 0.5}}$$

$H_4$ = 259 pmol × cotyledon$^{-1}$ and $H_{4.5}$ = 249 pmol × cotyledon$^{-1}$ were calculated by Equation 5 with $H_{2.5}$ = 290 pmol × cotyledon$^{-1}$ and $c = c_{II}$ = 0.076 $d^{-1}$ (Table I). The

**Table I.** *First Order Rate Constants for the Decrease of Catalase Hematin and Catalase Activity in Sunflower Cotyledons*[a]

| | First Order Rate Constant | Statistical Comparison with the Other Rate Constants | | | | |
|---|---|---|---|---|---|---|
| | | $m$ | $c_{II}$ | $c_{III}$ | $m_d$ | $c_d$ |
| | $d^{-1}$ | | | | | |
| $m$ | 0.410 ± 0.013 | | + | − | − | UC |
| $c_{II}$ | 0.076 ± 0.020 | + | | + | UC | + |
| $c_{III}$ | 0.324 ± 0.047 | − | + | | UC | − |
| $m_d$ | 0.378 ± 0.010 | − | UC | UC | | + |
| $c_d$ | 0.265 ± 0.082 | UC | + | − | + | |

[a] Values of the rate constants are given as means ± SE. Each individual value for the calculation of a mean value was determined by linear regression of a semilogarithmic plot of catalase hematin × cotyledon$^{-1}$ or catalase activity × cotyledon$^{-1}$ *versus* time. *c*, first order rate constant for the decrease of catalase hematin; *m*, first order rate constant for the decrease of catalase activity. Indices of the rate constants refer to stages of cotyledon development (stage II, III; *d*, seedlings kept in continuous darkness from d 2.5 to 8). Significance of differences between the rate constants was determined by Student's *t*-test with P = 0.05. +, difference significant; −, difference not significant; UC, useless comparison.

rate constant of degradation $k_{II}$ amounted to 0.205 $d^{-1}$ (5). It follows:

$$S_{4\text{-}4.5} = 32.8 \text{ pmol} \times d^{-1} \times \text{cotyledon}^{-1}.$$

The values for $H_{t-0.5}$ and $H_t$ in stage I could not be calculated from Equation 5 because the amount of catalase hematin increased during this stage (Fig. 1). Therefore, the amount of catalase hematin was measured at 0.5 d time intervals during stage I using five replicate flats. The mean values were used for the calculations of $S$. Due to the time consuming pulse-chase labeling procedure, the degradation constant during stage I ($k_I$) could only be determined between d 1.5 and 2.5 (5). Therefore, the calculations of $S$ between d 0 and d 1.5 were done under the assumption that $k_I$ is also valid for the first 1.5 d in stage I.

The highest rate constant of catalase synthesis was reached in the interval between d 1 and 1.5 where it amounted to nearly 400 pmol hematin × $d^{-1}$ × cotyledon$^{-1}$ (Fig. 3). Thereafter it rapidly decreased and remained almost constant at a level of about 30 to 40 pmol hematin × $d^{-1}$ × cotyledon$^{-1}$ during stage II and the first part of stage III. When the seedlings were kept in continuous darkness between d 2.5 and 8, the rate constant of synthesis was considerably lower than in the light. It did not exceed 10 pmol hematin × $d^{-1}$ × cotyledon$^{-1}$.

The results on catalase synthesis and degradation (5) showed that light had a dual effect on catalase turnover during the formation of the leaf peroxisome population in stage II. On one hand catalase degradation was delayed, whereas on the other hand light was the stimulus which maintained catalase synthesis at a higher level than in the dark. Both the deceleration of degradation and the stimulation of synthesis led to the high level of catalase hematin which was maintained in the cotyledons during peroxisome transition. This was in contrast to the events observed in dark grown cotyledons (Fig. 2, Table I).

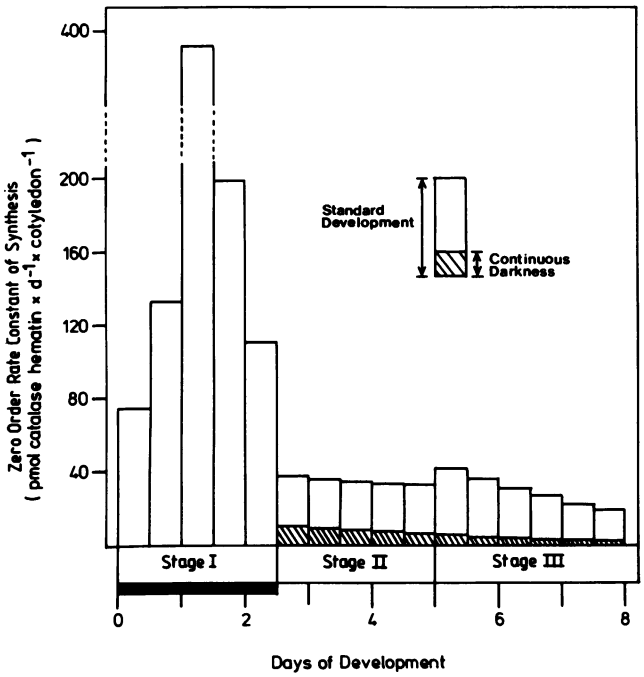


**Figure 3.** Zero order rate constants for the synthesis of catalase hematin in sunflower cotyledons. The rate constants were determined according to Equation 3.

**Table II.** *Catalase Protein and Total Peroxisomal Protein in Sunflower Cotyledons*[a]

| Time of Development | Total Peroxisomal Protein | Catalase Protein | |
|---|---|---|---|
| *d* | *μg × cotyledon*$^{-1}$ | *μg × cotyledon*$^{-1}$ | *% of total peroxisomal protein* |
| 2 | 143 | 19.7 | 13.8 |
| 3 | 125 | 18.5 | 14.8 |
| 4 | 141 | 17.1 | 12.1 |
| 5 | 87.5 | 15.9 | 18.2 |
| 6 | 81.0 | 11.5 | 14.2 |

[a] Values for the total peroxisomal protein per cotyledon are the means of the values reported by Schuh and Gerhardt (16), except the value at d 6, which is from B. Schuh (unpublished result).

### Catalase Proportion of Peroxisomal Protein

Catalase from sunflower cotyledons contains 4 heme groups per molecule (4) and has a mol wt of 265,000 (3). Using these values, the amounts of catalase protein (μg × cotyledon$^{-1}$) during cotyledon development were calculated from the measured amounts of catalase hematin. The total peroxisomal protein of sunflower cotyledons was determined by Schuh and Gerhardt (16). Table II shows that catalase represented a rather constant portion of about 15% of total peroxisomal protein during cotyledon development.

## DISCUSSION

The results on the synthesis of catalase presented here supplement the data from previous studies on the degradation

of catalase (5). Now, all data for a quantitative description of catalase turnover in sunflower cotyledons are available, *i.e.* the first order rate constants of degradation, the zero order rate constants of synthesis (Fig. 3) and the time course of total catalase (Fig. 1, Table I). All these data are based on quantitative determinations of catalase hematin. As shown previously (5), a reutilization of hematin during the course of catalase turnover did not substantially influence the determination of the rate constants. We are currently investigating whether hematin and apoprotein of catalase have different turnover rates.

Development (differentiation) of glyoxysomes in stage I was accompanied by extensive changes in catalase synthesis exhibiting a sharp maximum between d 1 and 1.5. Thus, catalase synthesis was correlated with the elaboration of the glyoxysome population. From this result one would expect similar changes in catalase synthesis during stage II if this stage is characterized by a de novo synthesis of the leaf peroxisomes. However, the rate of catalase synthesis was nearly constant during stage II (Fig. 3). This result is in accordance with the observation of Kindl (8) who measured relative rates of catalase synthesis in greening cucumber cotyledons.

Since we determined absolute rates of synthesis, it was possible to calculate which proportions of total catalase in the cotyledons had been synthesized during the different stages of development (Fig. 4). At the end of stage II, when the development of the leaf peroxisomes has been completed, 73% of total catalase was still catalase I (*i.e.* catalase synthesized during stage I) and only 27% of total catalase was catalase II. At d 8, when isocitrate lyase activity was essentially gone (<2% of maximum activity at d 2) (5), catalase I amounted to 41%, catalase II to only 15% of total catalase, and catalase III was nearly half of the total catalase. These results clearly show that replacement of catalase I by catalase II was very low and was exceeded by replacement of catalase II by catalase III.

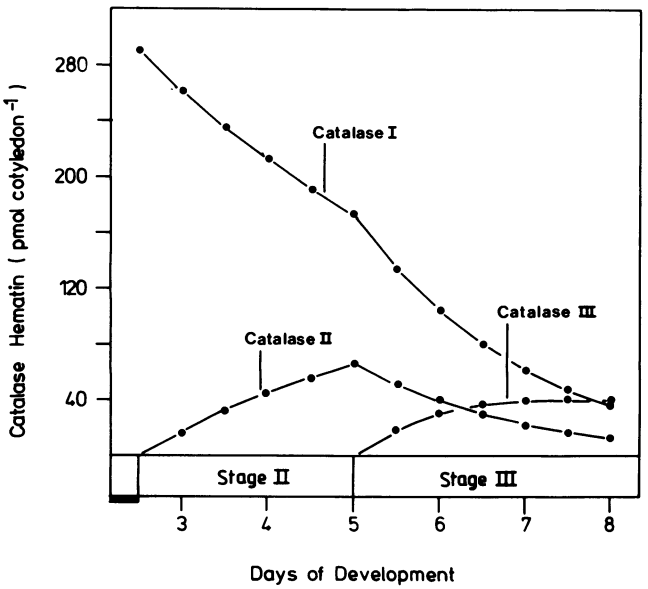


**Figure 4.** Time course of catalase hematin I, II, and III during cotyledon development. The denotion of catalase by I, II, and III refers to the stage of development in which the enzyme was synthesized. At d 2.5, total catalase (exclusively catalase I) amounted to 290 pmol hematin × cotyledon$^{-1}$. Using this initial value the amounts of catalase I, II and III were calculated as follows.

Catalase I in stage II: $290 \cdot e^{-k_{II} \cdot t}$.
Catalase I in stage III: catalase I (d 5) $\cdot e^{-k_{III} \cdot t}$.
Catalase II in stage II: total catalase − catalase I.
Catalase II in stage III: catalase II (d 5) $\cdot e^{-k_{III} \cdot t}$.
Catalase III: total catalase − (catalase I + catalase II).

$k_{II} = 0.205\ d^{-1}$ and $k_{III} = 0.515\ d^{-1}$ are the degradation constants of catalase during stage II and III, respectively (5). Total catalase was calculated according to Equation 5 with $H_{2.5}$ = 290 pmol hematin × cotyledon$^{-1}$, $c = c_{II}$ in stage II and $c = c_{III}$ in stage III (Table I).

The observation that catalase represented a rather constant percentage of total peroxisomal protein during development (Table II) indicated that the catalase content of a leaf peroxisome (or of a peroxisome of intermediary character) was similar to that of a glyoxysome. It follows that at d 5, when the leaf peroxisomal function was fully expressed and 27% of total catalase was catalase II, at most 27% of all peroxisomes could have been leaf peroxisomes formed *de novo*.

The results for catalase synthesis clearly contradict the concepts of the two population model and of the enzyme synthesis changeover model that the glyoxysome population is degraded and replaced by *de novo* synthesized leaf peroxisomes. On the other hand, the results are in accordance with the one population model which predicts that glyoxysomal catalase needs not be completely replaced by newly synthesized catalase during peroxisome transition. The observation that catalase synthesis is considerably lower in continuous darkness than in light, does not contradict this model. It is obvious that catalase synthesis has to be maintained at a higher level in greening cotyledons than in the etiolated cotyledons.

Catalase activity in sunflower cotyledons was not only controlled by synthesis and degradation, but also by an unknown mechanism which led to a decrease of the specific activity of the enzyme (4). In the light, this decrease was much higher than in the dark. From a value of 76 μkat × nmol hematin$^{-1}$ at d 2.5 the specific activity decreased to 33 μkat × nmol hematin$^{-1}$ at d 5 in greening cotyledons, but only to 57 μkat × nmol hematin$^{-1}$ at d 5 in cotyledons kept in continuous darkness. At d 7.5 it was 27 μkat × nmol hematin$^{-1}$ in green and 43 μkat × nmol hematin$^{-1}$ in etiolated cotyledons. Catalase in greening pumpkin cotyledons exhibits a similar decrease of the specific activity (19). It was shown that this decrease is due to the accumulation of an enzymically "low active" (low activity) form of catalase with subunits of 59 kD (20, 21). In the leaf peroxisomes this form is not processed to the fully active form with subunits of 55 kD. However, as in the cotyledons of mustard (22) and cotton (9), the low active 59 kD catalase has not been detected in sunflower cotyledons (22). As discussed previously (3), inactive (or low active) catalase in sunflower cotyledons could be formed by a modification of mature catalase, and the delay of degradation during peroxisome transition (5) would then lead to the accumulation of this inactivated catalase in the peroxisomes. Our results show that light had a modulating effect on the accumulation of inactive (or low active) catalase

because the difference between the decrease of catalase hematin and of catalase activity was much greater in the light than in the dark (Table I).

## ACKNOWLEDGMENT

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