# An Oxalic Acid Oxidase in the Leaves of *Bougainvillea spectabilis*

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Oxalic acid undergoes enzymic transformation along diverse channels in cell-free preparations from micro-organisms and plants. In *Collyvia veltepes* oxalate undergoes a simple decarboxylation (Shimazono, 1955; Shimazono & Hayaishi, 1957), whereas in other micro-organisms the decarboxylation is preceded by the formation of the CoA derivative of oxalate (Jakoby, Ohmura & Hayaishi, 1956; Kimura, Katayama & Sasakawa, 1956; Quayle, Keech & Taylor, 1961). In plants the decarboxylation of oxalate is of an oxidative type. Studies by Houget, Mayer & Plantefol (1927, 1928), Franke & Hasse (1937), Franke, Schumann & Bannerjee (1943), Niekerk-Blom (1946) and Datta & Meeuse (1955) indicated that oxalate underwent oxidative decarboxylation by an enzyme preparation from moss according to the scheme:

$$(CO_2H)_2 + O_2 \longrightarrow 2CO_2 + H_2O_2 \text{ (oxidase); R.Q. = 2}$$
$$\underline{H_2O_2 \longrightarrow H_2O + \tfrac{1}{2}O_2 \text{ (catalase)}}$$
$$(CO_2H)_2 + \tfrac{1}{2}O_2 \longrightarrow 2CO_2 + H_2O; \text{ R.Q. = 4}$$

Laties (1950) reported that cell-free homogenates of spinach leaves exhibited a cyanide-insensitive oxidase reaction with R.Q. greater than 1·4. It would appear that this reaction was due to a particulate-associated oxalic acid oxidase acting on endogenous oxalate. Arnon & Whatley (1954) and Finkle & Arnon (1954) reported that the plastid fraction of sugar-beet leaves contained an enzyme system, which on mixing with boiled leaf extract, containing oxalic acid, led to oxygen uptake and carbon dioxide evolution. The gas exchange of the overall reaction was consistent with the scheme shown above. Meeuse & Campbell (1959) demonstrated that sugar-beet roots also contained oxalic acid oxidase.

The present study reveals the presence of a plastid-bound oxalic acid oxidase in the leaves of *Bougainvillea spectabilis*.

## MATERIALS AND METHODS

*Preparation of homogenates.* The plants used in the present investigation were raised on the Laboratory premises. Freshly harvested leaves of *Bougainvillea spectabilis* were freed from petioles, washed with distilled water and ground for 3 min. with the appropriate medium in a Waring Blendor, the bowl of which was kept externally chilled with ice. The medium was water or 0·5M-sucrose in water or 0·5M-sucrose in 0·05M-phosphate buffer, pH 7·2. The ground material was filtered through muslin and the filtered suspensions made to volume to correspond to approximately 20% (w/v) on fresh-weight basis. Homogenates prepared with water had a pH value in the range 6·1–6·3; when buffered sucrose was employed, the homogenate was pH 6·9. Homogenates of the leaves of the other plants used in the investigation were prepared in water.

*Enzyme assay.* Oxygen consumption and carbon dioxide evolution were measured over a 60 min. period in conventional Warburg manometers at 37°. The assay system consisted of 1·0 ml. of enzyme preparation and 1·3 ml. of 0·1M-phosphate buffer of appropriate pH in the main compartment of the reaction vessel and 0·5 ml. of 0·2M-potassium oxalate in the side arm. In experiments where the effect of added material was to be tested, 0·1 ml. of the solution was added to the main compartment, the amount of buffer being reduced to 1·2 ml. The centre well contained strips of filter paper in 0·2 ml. of 20% (w/v) potassium hydroxide or calcium cyanide–calcium hydroxide mixture or water. When the potassium hydroxide or calcium cyanide was replaced by water, the net gaseous exchange (which was always a positive value) represented the difference between the oxygen consumed and the carbon dioxide evolved. The amount of carbon dioxide formed was calculated by adding the value of oxygen consumption obtained in the first step to the net gas exchange found in the second step. A correction was applied for the retention of carbon dioxide in buffer according to Umbreit, Burris & Stauffer (1957). Suitable controls, in which externally added oxalate was withheld, enabled corrections to be applied for endogenous reactions.

Catalase was a freeze-dried preparation (from liver; 100 units/mg.) obtained from Sigma Chemical Co., U.S.A. A stock solution containing 10 mg./ml. was prepared in 0·1M-phosphate buffer, pH 6·8, and stored frozen. Calcium cyanide, used in studies on the effect of hydrogen cyanide, was prepared according to the method of Robbie (1948).

One unit of enzyme activity corresponded to the absorption of 1 $\mu$l. of oxygen, or evolution of 1 $\mu$l. of carbon dioxide, in 60 min. at 37° and pH 6·8. Specific activity has been expressed as units/mg. dry weight or units/mg. of total nitrogen.

*Other assays.* Dry weights were determined by heating to constant weight at 80–90°. Protein was determined by the method of Lowry, Rosebrough, Farr & Randall (1951), after trichloroacetic acid precipitation and solution of the precipitate in dilute alkali. Nitrogen determinations were carried out by the micro-Kjeldahl method. Chlorophyll assays were carried out by extracting samples of whole homogenates or isolated fractions with 80% acetone and

determining the intensity colorimetrically (Arnon, 1949). Total oxalate was estimated by the method of Baker (1952) on portions of homogenates prepared for enzyme assay.

*Localization by differential centrifuging.* An International refrigerated centrifuge, model PR-2, was used in the experiments. The 'larger particles' were collected as a single fraction by spinning at 1600*g* for 30 min. The sedimented material was washed once on the centrifuge with a small volume of the grinding fluid, the wash liquid combined with the main supernatant and the residue suspended in a small volume of the grinding fluid. In other experiments homogenates prepared in buffered sucrose were separated into a number of fractions.

Whole chloroplasts isolated from a homogenate prepared in 0·5 M-sucrose containing 0·05 M-phosphate buffer, pH 7·2, were suspended in fresh medium and ground in a VirTis homogenizer, supplied by Arthur H. Thomas Co. Ltd., U.S.A. The bowl had a capacity of 250 ml. and was kept externally chilled in crushed ice. The grinding period was 3 min. with the rheostat set at 40 on the dial. The resulting suspension was then centrifuged at 15 000*g* to separate the grana from the stroma.

## RESULTS

The homogenates were usually assayed within 30 min. after preparation. However, storage at 0–4° for 12 hr. was found not to affect the enzyme activity significantly. There was about 60 % loss in activity when homogenates were held at 60° for 15 min. The enzyme was completely inactivated during this period at 80–100°.

*pH–activity relationship for enzymic activity.* Phosphate buffer, 0·1 M, was used in the range pH 5·3–8·0 for oxygen-absorption measurements. Carbon dioxide evolution was studied only in the range pH 5·3–7·0, because of the high solubility of the gas above pH 7·0. A fairly sharp optimum in the region pH 6·8–7·0 was obtained (Fig. 1).

*Relative rates of oxygen uptake and carbon dioxide evolution.* The data plotted in Fig. 2 show that oxygen uptake and carbon dioxide evolution ran approximately parallel. The R.Q. was between 2 and 3.

*Effect of catalase.* The source of enzyme was a suspension of the particles sedimenting at 1600*g* (30 min.) from the homogenate in water. Catalase (0·1 ml.) was added to the main flask at the commencement of the run. The data for the gas exchange are reported in Table 1.

The results showed that the R.Q. registered an increase of 15–18 %. The addition of catalase led to an actual increase in the absorption of oxygen and liberation of carbon dioxide.

*Effect of inhibitors and activators.* The effect of various reagents on the oxidation of oxalic acid is summarized in Table 2. The figures given for percentage inhibition are those for oxygen uptake, but identical values were obtained for carbon dioxide evolution.

Cyanide and fluoride did not inhibit the enzyme, but azide was a powerful inhibitor. EDTA inhibited, but 8-hydroxyquinoline at a final concentration of 1·1 mM had no effect, whereas at higher concentrations (3·3 mM) the reaction rate was increased. Arsenate and arsenite were without effect. Sodium chloride and sodium nitrate inhibited, but only when present in high concentrations. Molybdate exerted a powerful inhibitory effect on the enzyme.

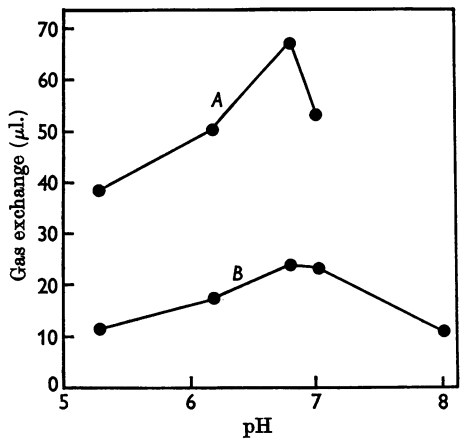


Fig. 1. pH–activity relationship for oxalic acid oxidase. The enzyme preparation was an homogenate of leaves in water. 0·1 M-Phosphate buffer of different pH values was used for assay. pH values higher than 7 were not used for carbon dioxide evolution because of high solubility of gas. *A*, Carbon dioxide evolution; *B*, oxygen consumption.

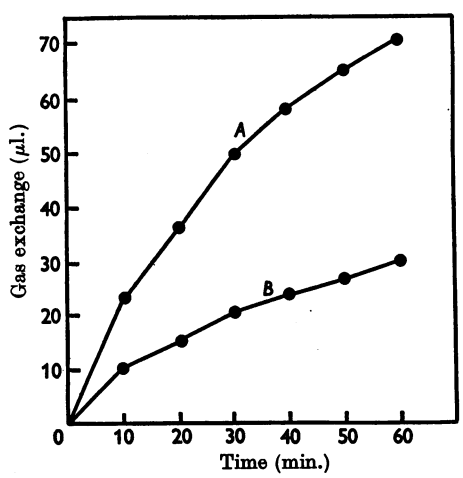


Fig. 2. Relative rates of oxygen uptake and carbon dioxide evolution. The enzyme preparation was an homogenate of leaves in water. 0·1 M-Phosphate buffer, pH 6·8, was used for assay. *A*, Carbon dioxide evolution; *B*, oxygen consumption.

Table 1. *Effect of catalase on the gas exchange of oxalic acid oxidase*

Enzyme was prepared by centrifuging a water homogenate of leaves at 1600*g* for 30 min. and suspending the resulting particles in water. A portion (0·1 ml.) of catalase was added to the main compartment of the Warburg flasks before the commencement of the run. 0·1 M-Phosphate buffer, pH 6·8, was used.

| | Activity without catalase | | | Activity with catalase | | |
|---|---|---|---|---|---|---|
| Period of incubation (min.) | Evolution of $CO_2$ ($\mu$l.) | Consumption of $O_2$ ($\mu$l.) | R.Q. | Evolution of $CO_2$ ($\mu$l.) | Consumption of $O_2$ ($\mu$l.) | R.Q. |
| 15 | 43·5 | 20·4 | 2·0 | 68·8 | 28·9 | 2·3 |
| 30 | 73·9 | 34·0 | 2·2 | 125·3 | 47·6 | 2·6 |
| 45 | 95·0 | 42·5 | 2·2 | 165·8 | 62·9 | 2·6 |

Table 2. *Effect of inhibitors and activators on oxalic acid oxidase*

Enzyme preparation was an homogenate of leaves prepared in water. 0·1 M-Phosphate buffer, pH 6·8, was used for assay. Reagent to be tested was added to the main compartment of the Warburg flasks in the final concentrations noted below and preincubated for 10 min. before the addition of substrate. In the HCN experiment, 0·63 M-$Ca(CN)_2$–$Ca(OH)_2$ was used in place of KOH in the centre well and neutralized KCN in a final concentration of 2·2 mM was used in the main compartment.

| Reagent tested | Final concn. (mM) | Activation (+) or inhibition (−) (%) |
|---|---|---|
| KCN | 2·2 | 0 |
| $NaN_3$ | 1·1 | −61 |
| | 5·5 | −77 |
| | 11·0 | −87 |
| EDTA | 6·7 | −44 |
| $KNO_3$ | 0·3 | 0 |
| | 33·0 | −50 |
| NaCl | 100·0 | −50 |
| Hydroxylamine | 3·3 | −100 |
| 8-Hydroxyquinoline | 1·1 | 0 |
| | 3·3 | +100 |
| *o*-Phenanthroline | 4·1 | 0 |
| NaF | 11·0 | 0 |
| $NaAsO_3$ | 3·3 | 0 |
| $NaAsO_4$ | 3·3 | 0 |
| $NaMoO_4$ | 1·0 | −60 |

There was no significant change in the activity of the homogenates on dialysis for 24 hr. in the cold at 0–4° against distilled water. The addition of riboflavin and flavin mononucleotide in final concentration of 0·01 mM and 0·3 mM was without significant effect on the activity of either fresh or dialysed homogenates.

*Effect of bivalent metal ions.* The various salts were added in final concentration of 1 mM and were incubated with the enzyme preparation for 10 min. before addition of oxalate. Owing to the extreme insolubility of calcium oxalate, considerably less than 1 mM-$Ca^{2+}$ ions would have been present in the test system. Since control experiments in which sodium chloride was employed showed that $Cl^-$ ions did not inhibit at this dilution, the effects of added metal chlorides may be attributed to the cations. Whereas $Ca^{2+}$ ions inhibited the reaction by 40 %, $Mn^{2+}$, $Co^{2+}$, $Zn^{2+}$, $Mg^{2+}$ and $Ni^{2+}$ ions had no effect; $Ca^{2+}$ ions inhibited only the carbon dioxide evolution, the uptake of oxygen being unaffected.

*Localization of the oxalic acid oxidase.* Typical results obtained by differential centrifuging of the homogenates are reported in Table 3.

Over 90 % of the total activity of dialysed or undialysed homogenates was found in the particulate fraction when this was collected as a single fraction sedimenting at 1600*g*, irrespective of the medium employed for grinding and whether the pH varied from 7 to 6. When differential centrifuging was employed practically the entire activity was removed below 1000 *g*. There was a rough parallelism between the chlorophyll content of the sedimented fractions and their enzyme activity. The specific activity of some of the fractions was two- to three-fold that of the whole homogenate.

When a suspension of the whole plastid fraction was subjected to high-speed grinding in a VirTis homogenizer there was about 20 % loss in activity. The grana fraction obtained by high-speed centrifuging contained 50 % of the total activity present in the ruptured chloroplast preparation, and the stroma fraction was totally inactive. The loss in activity was not restored when the grana fraction was supplemented with the stroma fraction. The R.Q. of the grana fraction remained the same as that of the whole homogenate and that of isolated parent fraction, and the specific activity in the grana fraction was equal to that of the whole plastid fraction.

*Attempts at solubilization of enzyme.* Repeated freezing and thawing of the sedimentable fraction, freeze-drying or acetone treatment and subsequent treatment with water or phosphate buffer did not solubilize the enzyme. Treatment of the freeze-dried material or acetone-dried powder with digitonin or deoxycholate was also ineffective, but since the latter reagent solubilized some inert protein the

Table 3. *Localization of oxalic acid-oxidase activity*

The medium used for preparing the homogenate was 0·5 M-sucrose containing 0·05 M-phosphate buffer, pH 7·2. Differential centrifuging at various speeds was carried out. The particles were resuspended in the grinding fluid without washing. 0·1 M-Phosphate buffer, pH 6·8, was used for assay.

| | Activity recovered (%) | | Nitrogen recovered (%) | Specific activity (units/mg. of nitrogen) | | Chlorophyll recovered (%) |
|---|---|---|---|---|---|---|
| | Evolution of $CO_2$ | Consumption of $O_2$ | | Evolution of $CO_2$ | Consumption of $O_2$ | |
| Whole homogenate | (100) | (100) | (100) | 57 | 23 | (100) |
| Particulate fraction at | | | | | | |
| 100*g*, 5 min. | 42 | 42 | 41 | 60 | 23 | 39 |
| 250*g*, 5 min. | 19 | 19 | 7 | 153 | 60 | 10 |
| 500*g*, 5 min. | 16 | 16 | 7 | 139 | 54 | 12 |
| 1000*g*, 5 min. | 7 | 6 | 3 | 151 | 58 | 5 |
| 1600*g*, 30 min. | 3 | 3 | 4 | 45 | 18 | 8 |
| Supernatant | — | — | 39 | — | — | 17 |

Table 4. *Diurnal variation in oxalic acid-oxidase and oxalate content*

Samples of 30–40 leaves were collected at random and pooled every 4 hr. during a 24 hr. period. The enzyme preparation was an homogenate in water. 0·1 M-Phosphate buffer, pH 6·8, was used for assay. Samples for the determination of dry weight and total oxalate were taken from the homogenates prepared for enzyme assay.

| Hour of collection of samples | Total oxalate (mg./100 mg. dry wt.) | Specific activity (units/mg. dry wt.) | |
|---|---|---|---|
| | | Evolution of $CO_2$ | Consumption of $O_2$ |
| 8 a.m. | 4·50 | 3·87 | 1·53 |
| 12 a.m. | 5·32 | 2·75 | 1·04 |
| 4 p.m. | 6·00 | 2·12 | 0·69 |
| 8 p.m. | 4·62 | 3·85 | 1·59 |
| 12 p.m. | 4·05 | 4·80 | 1·94 |
| 4 a.m. | 3·38 | 3·93 | 1·68 |

specific activity of the enzyme in the residual particles was increased about twofold.

The acetone-dried powders prepared from the whole leaves did not show any oxidase activity, but those prepared from the separated particles retained about 60 % of the activity of the particles.

*Diurnal variations in oxalic acid oxidase and oxalate content.* Results obtained in a typical experiment are shown in Table 4. There was a more than twofold diurnal variation in the enzyme activity, with minimum at 4 p.m. and maximum at 12 p.m. When the homogenates from samples collected at 4 p.m. and 12 p.m. were mixed in equal volumes, the resulting activity was the same as the mean of the individual activities, showing the absence of any metabolic inhibitor or activator. The total oxalate content also showed a significant diurnal variation, but in a direction opposite to that of the enzyme. Thus the oxalate content was 30 % lower at 12 p.m. than at 4 p.m. and reached a minimum at 4 a.m. There was no marked fluctuation in the dry weight, total nitrogen or protein content. The pH of the homogenates did not vary with time of homogenization.

The above results were obtained in the month of March with freshly sprouted leaves. In experiments conducted in February, when only mature leaves were available, the pattern of change of enzymic activity and oxalate content remained the same, but the magnitude of increase in enzymic activity at night was less.

*Oxalic acid oxidase in other species of plants.* Homogenates of the leaves of *Colocasia antiquorum*, *Alocasia decipiens*, *Portulacca oleracea*, *Oxalis corniculata* and *Beta vulgaris*, which are known to be rich in oxalate (Srivastava & Krishnan, 1959), were also assayed for enzyme activity, but the results were negative. The apparent absence of enzymic activity was not due to the presence of any naturally occurring inhibitor, because assays in which the homogenate of *Bougainvillea* leaves was mixed with an equal volume of an homogenate of the leaves of these plants showed activities exactly half that of the *Bougainvillea* enzyme.

## DISCUSSION

The oxalic acid oxidase of moss has been purified (Franke *et al.* 1943; Datta & Meeuse, 1955), but the enzyme of higher plants has not been characterized. The enzymic activity of *Bougainvillea*-leaf homogenates has a pH optimum near neutrality and is heat-labile, contrasting with the optimum pH 2–5 and heat-stability of purified moss enzyme. Nitrate was reported to be characteristically inhibitory for moss and also beet-root oxidase, but the inhibition of *Bougainvillea* enzyme was obtained only when higher concentrations were employed. EDTA, which enhances the activity of moss enzyme, inhibited the *Bougainvillea* enzyme. Fluoride had no effect on the activity in *Bougainvillea*-leaf homo-

genate, whereas the particulate preparations from sugar beet (Arnon & Whatley, 1954) and spinach leaves (Laties, 1950) were reported to be inhibited. The enzyme from mosses showed no inhibition by azide in catalase-free preparations. On the other hand, Finkle & Arnon (1954) reported that azide inhibited oxalic acid oxidation by beet-leaf preparation, but the inhibition was reversed by the addition of catalase, showing that only the catalase component was inhibited by azide and not the oxidase proper. With *Bougainvillea*, however, it was found that both carbon dioxide evolution and oxygen consumption were equally inhibited, indicating that oxidase itself was inhibited by azide.

The experiment of Laties (1950) and of Arnon & Whatley (1954) point to the particulate nature of the oxalic acid oxidase in the leaves of spinach and sugar beet. Shimazono (1955) quoted unpublished experiments of M. Nagahisa & A. Hattori, claiming the 'isolation' of 'oxaloxidase' from the grana of the chloroplasts of higher plants. The method of preparation of moss enzyme, involving as it does extraction for several hours in a ball mill with water or sodium chloride (Franke & Hasse, 1937), does not permit a definite conclusion about the soluble or particulate nature of the enzyme. Meeuse & Campbell (1959) obtained evidence that the enzyme in sugar-beet roots was associated with mitochondria. There was reason to believe that the oxalic acid oxidase of *Bougainvillea* leaf was localized in the chloroplasts, since there seemed to be a more or less constant ratio between the enzymic activity and the chlorophyll content in various fractions sedimenting on centrifuging below 1600*g*. Further disintegration of the chloroplasts showed that the activity was present in the grana fraction. Attempts to solubilize the enzyme from the plastid fraction have not been successful.

The *Bougainvillea* oxidase reaction had R.Q. between 2 and 3, but it is doubtful whether catalase was operating with true oxidase, since it has not been possible to alter the gas exchange significantly by such diverse procedures as heat-treatment, separation of sedimentable fraction and its further treatment with a view to solubilization of the enzyme and by the absence of any effect on the addition of cyanide. The fact that the addition of catalase did not alter the R.Q. by more than 15–18% may be considered as evidence in support of the non-involvement of catalase action in the observed gas exchange. The possibility that hydrogen peroxide produced is utilized in a coupled oxidation without affecting the R.Q. cannot be ruled out, but appears unlikely. Separate experiments have shown that the particles contained catalase activity corresponding to about one-fortieth of that of rat liver, as assayed by the method of Feinstein (1949), both on fresh-weight basis. The striking increase in carbon dioxide production on addition of catalase is suggestive of a protective action of catalase on oxalate oxidation.

The diurnal periodicity in physiological processes is one of the important endogenous rhythms operating in plants and lower forms of life and has been reviewed by Bunning (1956) and by Hastings (1959). There have been only a few studies of any possible associated rhythmicity at the enzymic level (Ehrenberg, 1950, 1954; Venter, 1956; Hastings & Sweeney, 1957; Sanwal & Krishnan, 1960; Mukerji, Sanwal & Krishnan, 1961; Viswanathan & Krishnan, 1962; Viswanathan, Srivastava & Krishnan, 1962). The present investigation has revealed a distinct diurnal variation in the oxalic acid-oxidase activity and oxalate content in the leaves of *Bougainvillea*. The activity of glycollic acid oxidase in barley is controlled by its specific substrate (Tolbert & Cohan, 1953) and indolylacetic acid-oxidase activity in pea seedlings is under the regulating influence of its substrate (Galston & Dalberg, 1954). Galston & Dalberg (1954) believed that the adaptive formation and de-adaptive disappearance of an enzyme, together with the existence of a mechanism for substrate formation, provide the essential features of a self-contained mechanism of diurnal rhythmicity. Thus one can picture oxalic acid accumulating by day, probably as a consequence of photosynthetic activity, and this increase would induce the formation of oxalic acid oxidase. This in turn would lead to a lowering of the substrate concentration and a consequent lowering of the enzyme activity. When the enzyme activity is low the substrate would have a chance to accumulate and the cycle would continue.

## SUMMARY

1. Homogenates of the leaves of *Bougainvillea spectabilis* bring about an enzymic decomposition of oxalate, accompanied by uptake of oxygen and evolution of carbon dioxide. Enzymic activity could not be demonstrated in a number of other oxalate-bearing plant tissues.

2. The enzymic activity of homogenates of *Bougainvillea* leaves was associated with the particulate fraction sedimenting at 1600*g*. A rough proportionality between chlorophyll content and enzymic activity of separated fractions is indicative of localization of the enzyme in chloroplasts. Further disintegration of the chloroplasts in a VirTis homogenizer resulted in the association of part of the enzymic activity with the grana fraction, the stroma fraction being inactive.

3. The R.Q. for the gas exchange was between 2 and 3. It was not certain whether catalase formed a component of the oxidase system, because the R.Q. remained more or less constant during such

diverse procedures as heat-treatment, treatment with inhibitors or activators and addition of exogenous catalase.

4. The enzyme activity of the leaves showed a significant diurnal variation, being minimum at 4 p.m. and maximum at 12 p.m. The total oxalate content also showed a distinct diurnal fluctuation, but in a direction opposite to that of the enzyme.

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## REFERENCES

Arnon, D. I. (1949). *Plant Physiol.* **24**, 1.
Arnon, D. I. & Whatley, F. R. (1954). *Physiol. Plant.* **7**, 602.
Baker, C. J. L. (1952). *Analyst*, **77**, 340.
Bunning, E. (1956). *Annu. Rev. Plant Physiol.* **7**, 71.
Datta, P. K. & Meeuse, B. J. D. (1955). *Biochim. biophys. Acta*, **17**, 602.
Ehrenberg, M. (1950). *Planta*, **38**, 244.
Ehrenberg, M. (1954). *Planta*, **43**, 528.
Feinstein, R. N. (1949). *J. biol. Chem.* **180**, 1197.
Finkle, J. B. & Arnon, D. I. (1954). *Physiol. Plant.* **7**, 614.
Franke, W. & Hasse, K. (1937). *Hoppe-Seyl. Z.* **249**, 231.
Franke, W., Schumann, F. & Bannerjee, B. (1943). *Hoppe-Seyl. Z.* **278**, 24.
Galston, A. W. & Dalberg, L. Y. (1954). *Amer. J. Bot.* **41**, 373.
Hastings, J. W. (1959). *Annu. Rev. Microbiol.* **13**, 297.
Hastings, J. W. & Sweeney, B. M. (1957). *J. cell. comp. Physiol.* **49**, 2009.
Houget, J., Mayer, A. & Plantefol, L. (1927). *C.R. Acad. Sci., Paris*, **185**, 304.
Houget, J., Mayer, A. & Plantefol, L. (1928). *Ann. Physiol. Physicochim. biol.* **4**, 123.
Jakoby, W. B., Ohmura, E. & Hayaishi, O. (1956). *J. biol. Chem.* **222**, 435.
Kimura, T., Katayama, H. & Sasakawa, T. (1956). *Symp. Enzyme Chem., Japan*, **11**, 119.
Laties, G. G. (1950). *Arch. Biochem. Biophys.* **27**, 404.
Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951). *J. biol. Chem.* **193**, 265.
Meeuse, B. J. D. & Campbell, J. M. (1959). *Plant Physiol.* **34**, 583.
Mukerji, S. K., Sanwal, G. G. & Krishnan, P. S. (1961). *Nature, Lond.*, **191**, 516.
Niekerk-Blom, C. J. (1946). *Proc. kon. ned. Akad. Wet.* **49**, 1096.
Quayle, J. R., Keech, D. B. & Taylor, G. A. (1961). *Biochem. J.* **78**, 225.
Robbie, W. A. (1948). *Meth. med. Res.* **1**, 307.
Sanwal, G. G. & Krishnan, P. S. (1960). *Nature, Lond.*, **188**, 664.
Shimazono, H. (1955). *J. Biochem., Tokyo*, **42**, 321.
Shimazono, H. & Hayaishi, O. (1957). *J. biol. Chem.* **227**, 151.
Srivastava, S. K. & Krishnan, P. S. (1959). *J. sci. industr. Res.* C, **18**, 146.
Tolbert, N. E. & Cohan, M. S. (1953). *J. biol. Chem.* **204**, 639.
Umbreit, W. W., Burris, R. H. & Stauffer, J. F. (1957). *Manometric Techniques*, p. 28. Minneapolis: Burgess Publishing Co.
Venter, J. (1956). *Z. Bot.* **44**, 59.
Viswanathan, P. N. & Krishnan, P. S. (1962). *Nature, Lond.*, **193**, 166.
Viswanathan, P. N., Srivastava, L. M. & Krishnan, P. S. (1962). *Plant. Physiol.* **37**, 283.

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# Studies on Experimental Thiamine Deficiency

## 3. GLYOXYLIC ACID, CITRIC ACID AND TISSUE METABOLISM*

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Glyoxylate is toxic to animals (Adler, 1893; Barnes & Lerner, 1943). Kleinzeller (1943) showed that inhibition of oxygen uptake of tissues by glyoxylate was related to a specific inhibition of decarboxylation of pyruvate. The degree of inhibition depended on the concentration of glyoxylate, as found also by Weinhouse & Friedmann (1951), Nakada & Weinhouse (1953), Weinhouse (1955) and D'Abramo, Romano & Ruffo (1957). D'Abramo, Romano & Ruffo (1958) studied the effect of glyoxylate on the oxidation of components of the tricarboxylic acid cycle, and Ruffo, Romano & Adinolfi (1959) suggested that glyoxylate condenses with a $C_4$ tricarboxylic acid-cycle intermediate to compete for aconitase. Their results showed both inhibition of oxidation and accumulation of citrate, which reached very high values only when oxaloacetate was the substrate. They suggested that both the depression of oxygen uptake and the formation of citrate are due to the two different ways in which oxaloacetate may react in the liver cells with acetyl-coenzyme A to form citrate

* Part 2: Liang (1962*b*).