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CARBONIC ANHYDRASE IN CERTAIN SPECIES OF PLANTS

THOMAS E. BROWN AND H. CLYDE EYSTER

Charles F. Kettering Foundation, Yellow Springs, Ohio

The first evidence of carbonic anhydrase in leaves was obtained by Neish (1939), who observed activity in both the crude chloroplast and cytoplasm fractions of four species. Several investigators failed to find any evidence of carbonic anhydrase in leaves (Burr, 1936; Mommaerts, 1940; Roughton, 1934; Van Goor, 1948) both before and after Neish's observations were reported. Evidence for the general occurrence of carbonic anhydrase in leaves was presented by Bradfield (1947), with which all of the more recent work is in agreement. The following studies of plant carbonic anhydrase have recently been reported: distribution among different species (Bradfield, 1947; Day and Franklin, 1946; Osterlind, 1950; Sibly and Wood, 1951; Steeman et al., 1949; Waygood and Clendenning, 1950, 1951), intracellular localization (Day and Franklin, 1946; Waygood and Clendenning, 1950, 1951), pH and temperature relations (Day and Franklin, 1946; Sibly and Wood, 1951; Waygood and Clendenning, 1950, 1951), stability of leaf extracts (Bradfield, 1947; Day and Franklin, 1946; Sibly and Wood, 1951; Waygood and Clendenning, 1950, 1951), purification (Sibly and Wood, 1951; Sirois and Waygood, 1952; Wood and Sibly, 1952; Yen and Tang, 1951), zinc content (Sibly and Wood, 1951; Wood and Sibly, 1952), kinetics (Bradfield, 1947; Byerrum and Lucas, 1952; Steeman et al., 1949; Waygood and Clendenning, 1951; Wood and Sibly, 1952), including linked reactions with plant decarboxylases (Hansl and Waygood, 1952), effects of cysteine (Bradfield, 1947; Sibly and Wood, 1951; Waygood and Clendenning, 1950, 1951), effects of inhibitors (Day and Franklin, 1946; Sibly and Wood, 1951; Waygood and Clendenning, 1950, 1951; Wood and Sibly, 1952), and relative activity of extracts prepared from albino, etiolated, and normal leaf tissue (Waygood and Clendenning, 1950, 1951).

MATERIALS AND METHODS

Carbonic anhydrase catalyzes the hydration and dehydration of both carbonic acid and ammonium carbamate, involving both the uptake and output of carbon dioxide depending upon the pH of the medium. The reaction as used in the present work measured the carbon dioxide evolved from a given NaHCO_3 solution by contact with carbonic anhydrase containing plant macerates.

The method used in the present work was a modification of the boat method of Meldrum and Roughton (1933) which has served as the basic method of most workers to date. A standard, rectangular Warburg constant temperature bath (15°C) was fitted with an arm shaker driven by a $\frac{1}{2}$ h. p., 1750 rpm. DC motor with a chain drive and attached gear box to stabilize shaking speed. The shaking rate was controlled by a rheostat and measured with a tachometer.

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The reaction vessel was a 50 ml. Erlenmeyer flask divided into two compartments by forcing the bottom upward with a knife edge to form a partition across the diameter of the vessel (Hove et al., 1940). A 2 cm. deep-wedged partition was found to provide two compartments which could hold up to 4 ml. each without accidental mixing and yet allowed complete mixing of the reactants upon shaking. Connections between vessel and manometer were combinations of glass and rubber pressure tubing. Two manometers were used; one a standard Warburg manometer from which the side arm had been removed (as used by Waygood and Clendenning, 1950) and the other a simple large-bore U-shaped manometer. The Warburg manometer was used in the majority of the activity measurements. The large-bore manometer was employed on the most highly active preparations.

All plant tissues whose carbonic anhydrase activity was to be determined, with the exception of the algae, were prepared by macerating 1.5 g. of fresh, healthy leaves with 3 g. banding sand (Central Scientific Co.) and either 5 ml. distilled deionized water or 5 ml. 0.01 M 1-cysteine solution (as specified) in a small porcelain mortar using an oversize pestle. As an aid to maceration the leaves were cut into fine pieces with scissors before weighing. This technique provided a homogeneous sample after three minutes of grinding. The suspension was filtered with suction through 51 gauge nylon placed over a Gooch extraction funnel. Employing a water pump, filtration was rapid with all but the most viscous samples. The sand was retained almost entirely by the nylon. Determinations of enzyme activity were started immediately.

Two milliliters of 0.2 N solutions of c. p. NaHCO_3 made up in 0.02 N solution of c. p. NaOH were placed in one compartment of the vessel, and 2 ml. of 0.2 N phosphate buffer (pH 6.70) plus 1 ml. of plant tissue macerate were placed in the other. The reaction vessel was connected to the shaker arm and manometer and was then equilibrated for five minutes, a time period found adequate in preliminary tests. The contents of the two compartments were then mixed and readings were taken at 15 sec. intervals during continuous high speed shaking (200 oscillations per min., 6-inch thrust, 75 degree arc).

RESULTS AND DISCUSSION

Carbonic Anhydrase Activity of Different Plant Species

The carbonic anhydrase activity of several species and varieties is reported in figure 1. All species but New Zealand spinach are reported on here for the first time. New Zealand spinach was used as it was desired to include a species of known high activity for comparison (Waygood and Clendenning, 1950). With the exception of *Nostoc muscorum*, all plants were macerated in water. No activity could be found in *Nostoc* preparations except when macerated in 0.01 M cysteine, an ultrasonic generator being used to rupture the cells.

The question arose as to whether the physiological changes occurring in leaves preceding autumnal coloration would affect the carbonic anhydrase activity of such tissues as well. Mature green and senescent yellow leaves from the same branch of *Liriodendron tulipifera* L. were tested for their carbonic anhydrase activity. Carbonic anhydrase activity of the yellow leaves was about 55 percent lower than the activity for the green leaves. Autumnal leaf coloration in this species was thus accompanied by a considerable decrease in carbonic anhydrase activity.

With leaves of certain plant species the addition of cysteine to the grinding medium increased the carbonic anhydrase activity in agreement with Bradfield (1947), Waygood and Clendenning (1950) and Sibly and Wood (1951). An increase in activity of about 150 percent was noted on "low activity" plants when 0.01 M 1-cysteine was used as the macerating medium. On "high activity" plants the increase was only 3-11 percent. These increases in activity were

corrected for the effect of the cysteine solution on the control. Addition of 60 mg. solid cysteine to the system to provide 0.1 M concentration immediately before equilibration did not result in a significant increase in enzyme activity although the rate of CO_2 production was slightly higher both in the presence and absence of macerated leaf tissue. Apparently the effect of cysteine will occur only when it is brought in contact with the plant material at the time the cells are ruptured. This effect appears to be one of stabilization of the enzyme during preparatory handling. That it may be due, in whole or in part, to limiting substrate seems reasonable but this study does not provide proof since the basis of specie comparison was equal amounts of plant macerate which did not lend itself to variation of enzyme concentration.

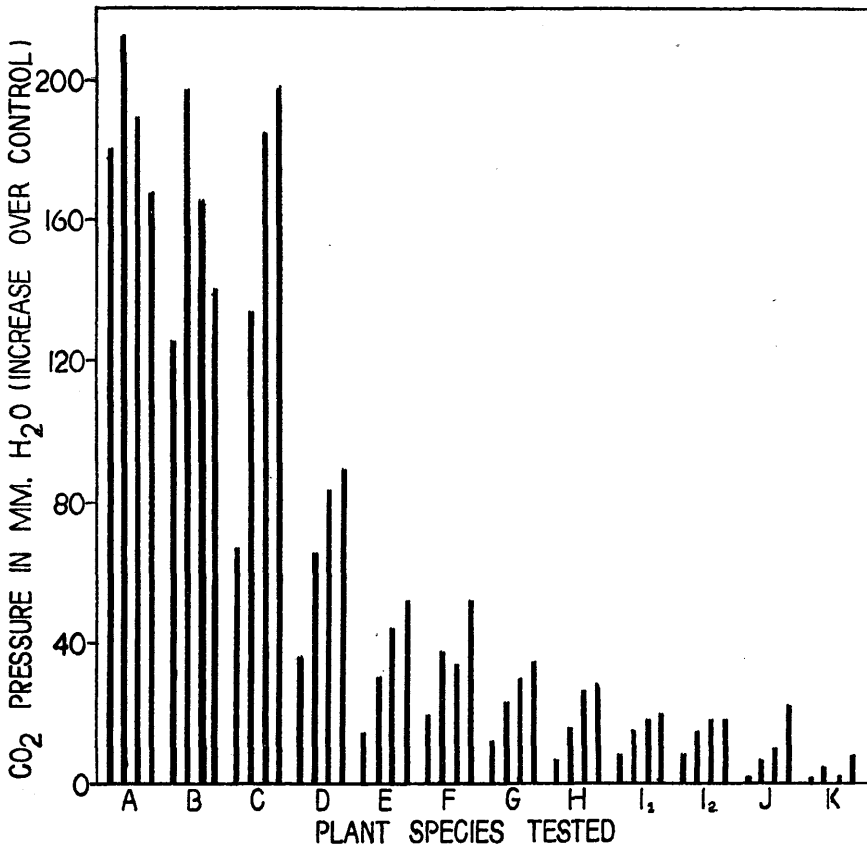


FIGURE 1. Carbonic Anhydrase Activity in Certain Plants (15, 30, 45, 60 sec. readings left to right in each group)

KEY:

- | | | | |
|---|-----------------------------------|----------------|--|
| A | <i>Tetragonia expansa</i> Murr. | G | <i>Nostoc muscorum</i> Ag. |
| B | <i>Ipomoea purpurea</i> L. | H | <i>Polystichum acrostichoides</i> Schott |
| C | <i>Amaranthus retroflexus</i> L. | I ₁ | <i>Zea mays</i> L., green seedlings |
| D | <i>Liriodendron tulipifera</i> L. | I ₂ | <i>Zea mays</i> L., albino seedlings |
| E | <i>Maclura pomifera</i> Schneid. | J | <i>Plantago rugelii</i> Dcne. |
| F | <i>Verbascum thapsus</i> L. | K | <i>Portulaca oleracea</i> L. |

Carbonic anhydrase activity of different chloroplast pigment types of Zea mays L., and the effect of etiolation

Pedigreed strains of corn, segregating green and albino, green and yellow, and green, yellow and albino respectively, were tested for their carbonic anhydrase activity. Albino and "green" seedlings were grown from seed in both light and dark and pairs of samples were harvested at 10-14 days. All samples were macerated in 0.01 M cysteine to enhance the low carbonic anhydrase activity (figure 2).

The carbonic anhydrase activity of the light-grown plants belonging to four different pedigrees varied from a very low to a moderately high level of activity. The enzyme activities of albino plants from three different pedigrees were more uniform and were intermediate to the extremes observed on macerates of the green plants in the same pedigrees. The carbonic anhydrase activity of the green plants thus was higher or lower than that of the albino plants, depending upon the strain which was employed. No carbonic anhydrase activity could be found in macerates of either "green" or albino corn plants which were grown from seed in the dark. Two distinct pedigrees of yellow corn seedlings showed an even greater difference in carbonic anhydrase activity than was observed among the green plants in these pedigrees. Among the nine pedigrees of corn examined the highest and lowest enzyme activities were observed on the two yellow varieties which were grown in light. These findings present an interesting problem in genetics. The levels of carbonic anhydrase activity observed were a characteristic of the strain employed and were thus subject to genetic control. However, there is no simple relationship between the genetic control of pigmentation and carbonic anhydrase activity as observed on the leaf macerates *in vitro*.

Bradfield (1947) has reported that plants showing high carbonic anhydrase activity in their leaves do not show detectable activity in their roots. Waygood and Clendenning (1950) have observed much lower carbonic anhydrase activities in albino than in normal green barley leaf extracts as well as in the white regions of variegated *Tradescantia* leaves. Etiolation of *Tropaeolum* and *Petroselinum* leaves was also associated with a decrease in carbonic anhydrase activity (Waygood and Clendenning, 1950). Our observations on corn varieties are confirmatory with respect to the effect of etiolation. However, the large variation in levels of activity observed between green plants of different pedigrees and the yellow plants of different pedigrees suggests genetic control of enzyme activity which may bear no relation to chlorophyll content or photosynthetic capacity.

SUMMARY

Carbonic anhydrase activity was found in macerates of ten plant species (eight species of Angiosperms, *Polystichum acrostichoides* and *Nostoc muscorum*) which have not previously been examined for the presence of this enzyme. A decrease in carbonic anhydrase activity of leaves was found to accompany the decomposition of chlorophyll which is associated with autumnal leaf coloration in *Liriodendron tulipifera* L. Carbonic anhydrase activity was observed in macerates of genetically green and albino corn seedlings which were grown in light but no activity was observed in leaf macerates of corresponding plants which were cultivated in darkness. There was little relationship between pigmentation and level of carbonic anhydrase activity observed in extracts of nine varieties of *Zea mays* L. The levels of carbonic anhydrase activity were a varietal characteristic. An increase in activity of about 150 percent was noted on "low activity" plants when 0.01 M cysteine was used as the maceration medium. No increase in carbonic anhydrase activity was observed on addition of solid cysteine to leaf macerates in the reaction vessel immediately before equilibration. Apparently the effect of cysteine will occur only when brought in contact with the plant material at the time the cells are ruptured.

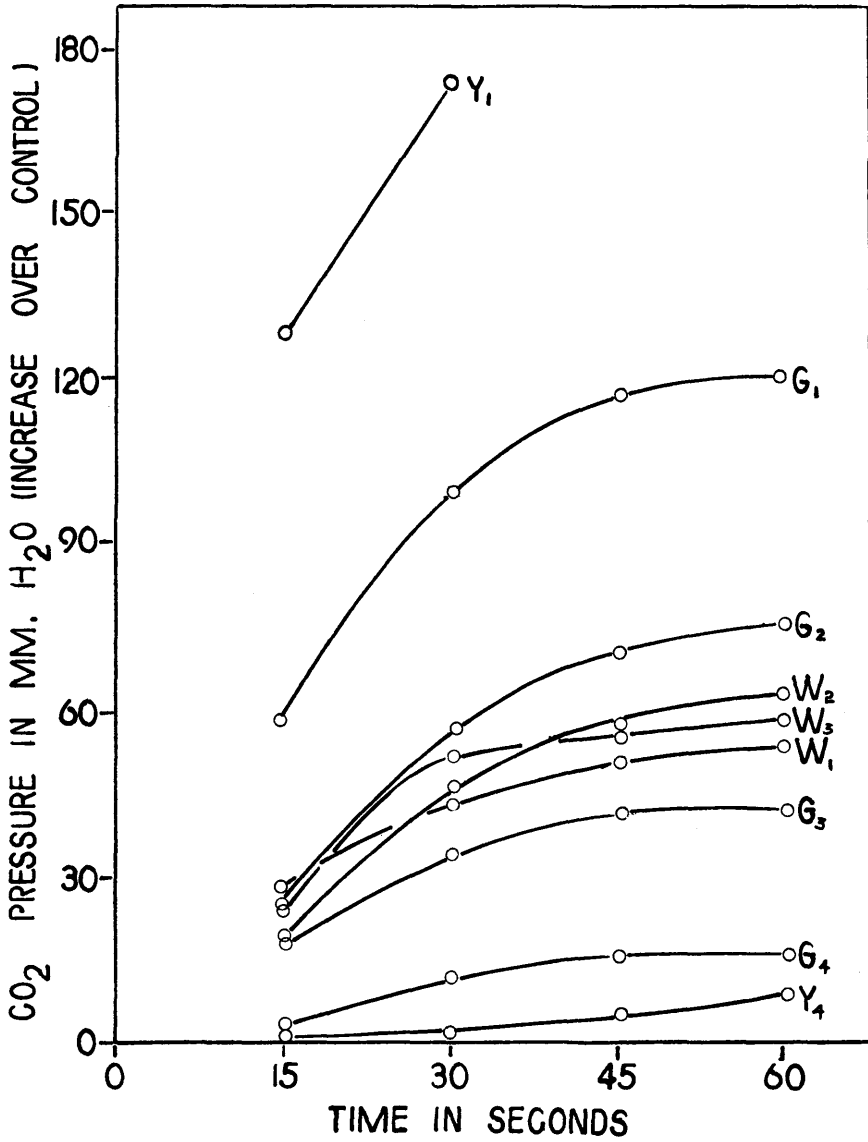


FIGURE 2. Carbonic Anhydrase Activity of Varieties of *Zea mays* L.

KEY:

Subscripts indicate pedigrees as follows: 1=4811; 2=482; 3=484; 4=485.

G. green seedlings

W. white seedlings (albino)

Y. yellow seedlings

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