EFFECTS OF SODIUM FLUORIDE ON LEAF CATALASE ACTIVITY

D. W. NEWMAN

Department of Botany, Miami University, Oxford, Ohio

Treatment of leaf tissue with fluorides, either fumigation treatment or tissue culture treatment, may result in a respiratory stimulation or inhibition as measured by oxygen consumption (Meyerhof and Lohmann, 1926; Lohmann and Meyerhof, 1934; Tanko, 1936; Thimann, 1951; Warburg and Christian, 1941; Berger and Avery, 1944; Bonner and Wildman, 1946; Laties, 1949; James, 1953; McNulty and Newman, 1956, 1957; Hill et al., 1959; Applegate and Adams, 1959, 1960a, 1960b; Applegate et al., 1960). McNulty and Newman (1957) and Applegate et al. (1960) have indicated that at low concentrations, fluorides may stimulate oxygen uptake, whereas at high concentrations fluorides inhibit oxygen uptake.

The catalases are enzymes which cause the evolution of oxygen on attacking the substrate hydrogen peroxide. The exact amount of activity exhibited by catalases and the extent of the presence of these enzymes in plants are still matters of much speculation, since catalases have not been isolated from a great number of plant tissues (Burris, 1960). Hence, reference is made here to the catalytic decomposition of the substrate with a concomitant evolution of oxygen. Catalase activity is generally measured by determining the rate of decomposition of hydrogen peroxide (Appleman, 1951, 1952; Maehly and Chance, 1954; Appleman and Pyfrom, 1955). However, hydrogen peroxide may be attacked by many types of iron-porphyrin compounds (Burris, 1960). The net result of H_2O_2 decomposition by catalase or by other types of iron-porphyrin compounds would be to reduce the rate of apparent respiration. Since it is apparent that fluorides both stimulate and inhibit oxygen consumption, either the cytochrome system and/or other oxidative systems must exhibit a stimulation and subsequently an inhibition in the presence of fluorides. One possible explanation concerning this dual effect of fluorides would be an initial effect on some oxidative system other than the cytochrome terminal oxidase with a subsequent inhibition of the cytochrome system at increased fluoride concentration in the tissue. As indicated above, little is known concerning the actual function(s) of the catalases in plant tissues. However, recent views seem to favor the theory that catalases may also function peroxidatically (Burris, 1960). Here, then, is a fluoride sensitive, iron-porphyrin enzymatic system similar to the cytochromes with a dual function. Investigations of the influence of fluorides on leaf catalase activity under the conditions whereby McNulty (1959) demonstrated a fluoride stimulation of leaf oxygen consumption are the subjects of this paper.

METHODS AND MATERIALS

Bush beans (*Phaseolus vulgaris* L. 'Burpee Tender Pod') and Hubbard squash (*Cucurbita maxima* L. 'McCullough's Improved') were germinated and grown in vermiculite watered with 80 percent Hoagland's solution. All treatments were made under controlled temperature at 20° or 18.5° C and under constant and continuous light (60 ft-c Sylvania cool-white fluorescent). The leaf tissue was treated with fluorides by either of two methods: (a) leaf disks were cut with a number 11 cork-hole borer (1.8 cm in diameter) and placed in petri dishes containing calcium-free, but otherwise complete, culture solution with and without fluorides, or (b) the stems were excised 17 cm below the cotyledonary node and partially immersed in culture solutions. At intervals or at specified times, leaf tissue was removed from the cultures, homogenized with a mortar and pestle in 10 ml of pH 6.8, 0.1 M phosphate buffer and made to volume in the pH 6.8, 0.1 M phosphate

buffer. The homogenate was used directly. In general, the catalase activities of the homogenates were determined within 10 min after homogenization was completed. This was necessary because it was previously established that, under these conditions, the catalase activity of the homogenate declined upon storage (fig. 1; Landon, 1934; Galston, 1951).

The catalase activities of the homogenates were determined manometrically at 30.3° C. The main compartment of the 16-ml capacity Warburg flask contained 2.6 ml of substrate (1.5 percent sodium perborate (w/v) adjusted to pH 6.8 with HCl) and 0.1 ml of pH 6.8, 0.1 M phosphate buffer. Also, the sodium perborate

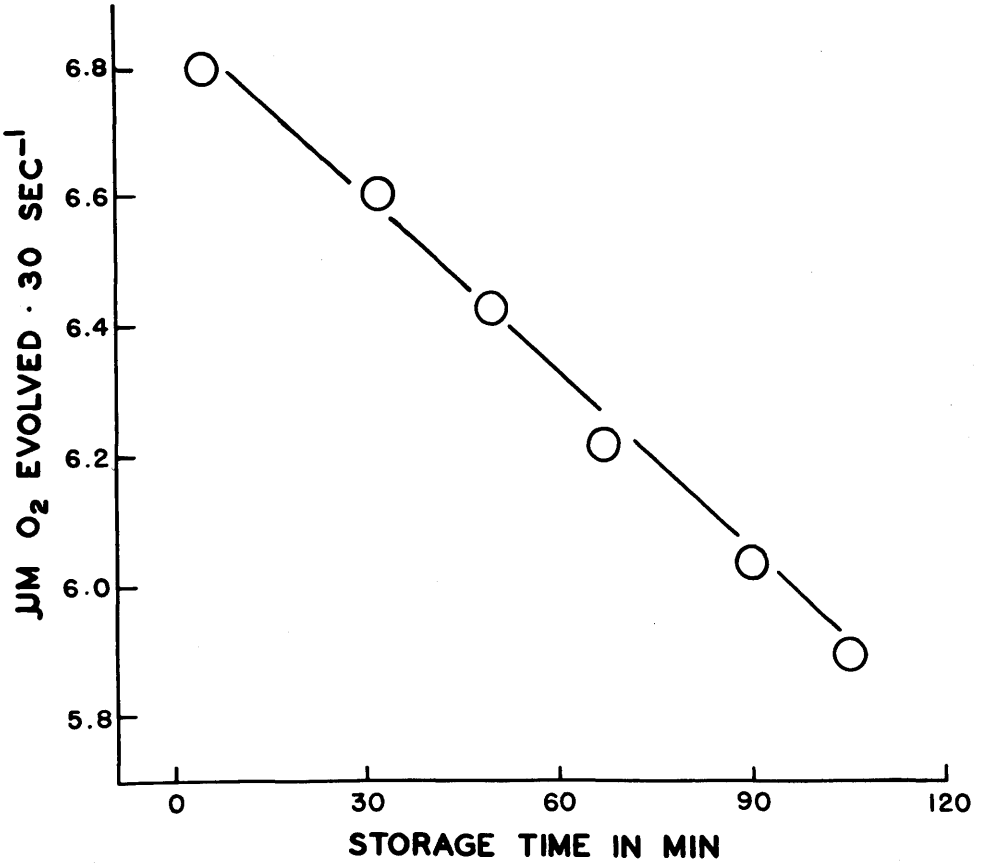

FIGURE 1. Effect of time of storage at 7° C on the catalase activity of bush bean leaf homogenates.

served as a rather effective buffer, although less effectively at this pH than at a higher pH. (DeKock et al., 1960; Feinstein, 1949). The side arm contained 0.3 ml of the buffered homogenate. A long stroke and fast shaking rate were used in order to maintain, as much as possible, equilibrium between the liquid and gaseous phases within the reaction flask. The materials in the reaction flask were temperature equilibrated for 7 min after which the contents of the side arm were tipped into the main compartment to start the reaction. The oxygen evolution was determined by "free manometry" using high-speed film (Goldstein, 1949a). The reaction rate was linear over a 50-sec period. In general, the slope during this 50-sec period per 0.1 g dry weight of material was taken as the rate of reaction.

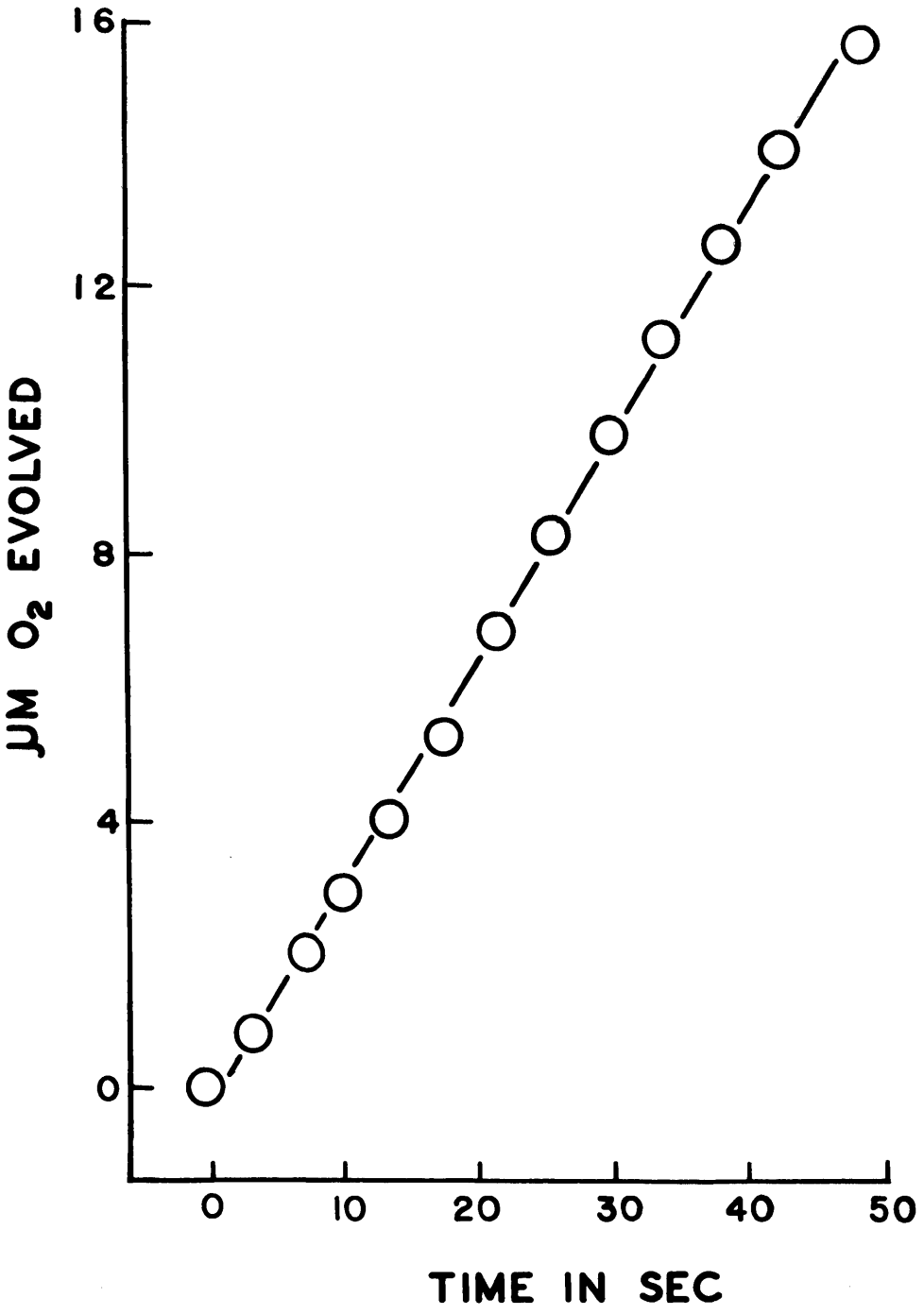

FIGURE 2. Graph of the quantity of oxygen evolved with respect to the time, in seconds, of the experiment.

Since these measurements were made during a comparatively short period of time, little or no substrate inactivation of the enzymatic system occurred (fig. 2).

RESULTS

Figure 3 is a graph of the reaction velocity with respect to the relative enzyme concentration. The reaction velocity with respect to the relative enzyme concentration is a linear function to a velocity of approximately 6 μM (micromoles) of oxygen evolved per 30 sec. However, this experiment was conducted using only 2.2 ml of the substrate instead of the usual 2.6 ml of substrate, and therefore the reaction rate with respect to the enzyme concentration should remain linear for a reaction velocity greater than 6 μM oxygen evolved per 30 sec. In general, the enzyme concentration was adjusted in each experiment such that the reaction velocity was maintained below 10 μM oxygen evolved per 30 sec.

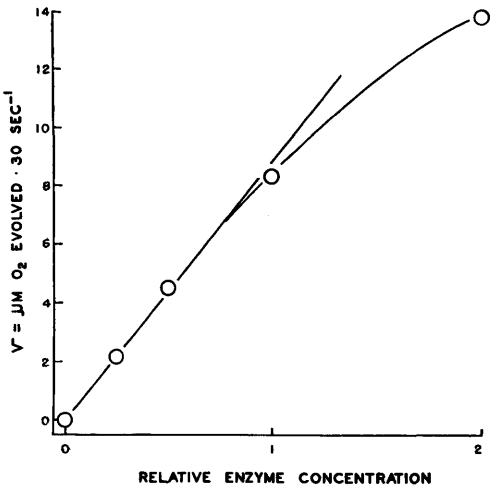

FIGURE 3. Graph showing the relative reaction velocity with respect to the relative enzyme concentration.

Table 1 gives the catalase activities (μM oxygen evolved for 30 sec per 0.1 g dry weight) of tissue homogenates of leaves which were attached to shoots partially immersed in flasks containing either calcium-free nutrient solution or calcium-free nutrient with 3×10^{-3} M NaF for a period of 76 hr. The plants had been etiolated previ-

TABLE 1

The comparative catalase activities of control and fluoride-treated (3×10^{-3} M NaF for 76 hr) bush bean leaf homogenates. The leaves were etiolated prior to and during part of the treatment and then were exposed to light for 27 hr prior to homogenization.

Sample	Mean μM oxygen evolved·30 sec^{-1}·0.1 g dry wt^{-1}
Control	24.2±1.93* (3)
Fluoride-treated	18.0 (3)

*Note—70% limits with pooled variance.

TABLE 2

The comparative catalase activities of control and fluoride-treated (3×10^{-3} M NaF for 8 days) bush bean leaf homogenates. The leaves were green prior to and during the entire experiment.

Sample	Mean μM oxygen evolved·30 sec^{-1}·0.1 g dry wt^{-1}
Control	9.35±0.66* (2)
Fluoride-treated	7.74 (2)

*Note—70% limits with pooled variance.

ously and then were placed in the light for 27 hr prior to the catalase measurements. The fluoride-treated leaf homogenates exhibited a lower rate of catalase activity as compared to the control, non-treated leaf homogenates. A similar result was obtained when previously greened shoots were immersed in aerated nutrient or aerated nutrient with fluoride (3 x 10^{-3} M NaF) under controlled light and temperature conditions for 8 days (table 2). In the former experiment the leaves were etiolated at the time of the initiation of the treatment and then were placed in the light, whereas in the latter the plants were germinated in the light and maintained under continuous light conditions. In both experiments the fluoride-treated leaf tissue exhibited a reduced catalase activity. However, the reduction in the catalase activity was not great. This is especially important since these are, comparatively, extended periods of treatment at relatively high fluoride concentrations.

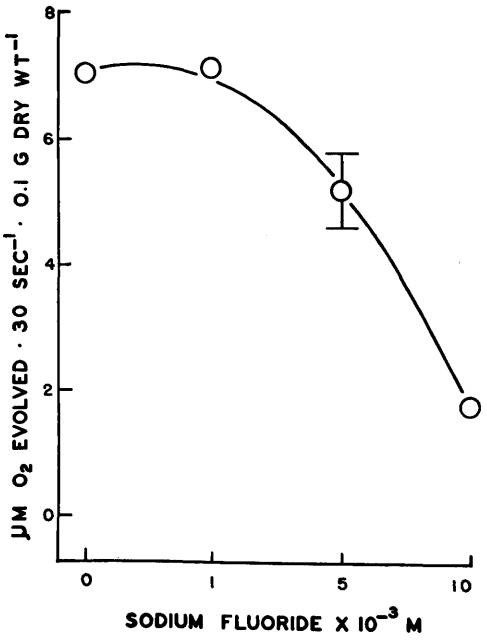

FIGURE 4. The relationship between reaction velocity of catalase in the homogenates and the sodium fluoride concentration in which the tissues were cultured.

Young squash leaf tissue was cultured in petri dishes containing various concentrations of fluorides from zero to 1 x 10^{-2} M NaF. Following 67 hr of treatment at 18.5° C under constant light at 60 ft-c the catalase activities of 3 samples of each treatment were determined. Figure 4 gives the results of this experiment. It can be seen that with increasing concentrations of fluorides in the culture medium, the catalase activity of the homogenized leaf tissue decreases, except at the lowest fluoride concentration used (1 x 10^{-3} M NaF). In this experiment flaccid tissue was observed on the leaf disks treated with 1 x 10^{-2} M NaF.

The effect of exposure time was investigated next. Leaf disks were cultured in nutrient or nutrient with sodium fluoride (1 x 10^{-2} M NaF) for various periods of time. At specific intervals during the course of the experiment 3 individual samples of treated tissue were homogenized and the catalase activities measured. Figure 5 is a graph showing the percentage of control activity that the treated tissue exhibited during the experiment. With increased time of exposure the fluorides caused a general reduction in the rate of catalase activity as measured in the homogenate.

Schwarze (1954) found an increase in peroxidase activity concomitant with a defect in chlorophyll production. He indicated that in the absence of chlorophyll protoporphyrin may become available for peroxidase synthesis. An investigation of the influence of sodium fluoride on leaf catalase activity was made on leaf tissues grown in the dark and under continuous illumination. Chlorophyllous squash leaf disks were cultured in calcium-free nutrient or calcium-free nutrient containing sodium fluoride (5 x 10^{-3} M NaF). One-half of the petri dishes with and one-half of the petri dishes without sodium fluoride were placed in the dark during the culturing period. All of the petri dishes were placed in the plant growth

chamber at 18.5° C for 93 hr prior to the catalase measurements. The results of this experiment are given in table 3. The light-grown fluoride-treated disk homogenates exhibited less catalase activity than did the light-grown control disk homogenates. The dark-grown fluoride and control leaf disk homogenates exhibited approximately equal activity. Also, the dark-grown leaf disk homogenates exhibited higher catalase activities than did the light-grown leaf disk homogenates. This finding is in agreement with that of other investigators (Appleman, 1952; Eyster, 1950b).

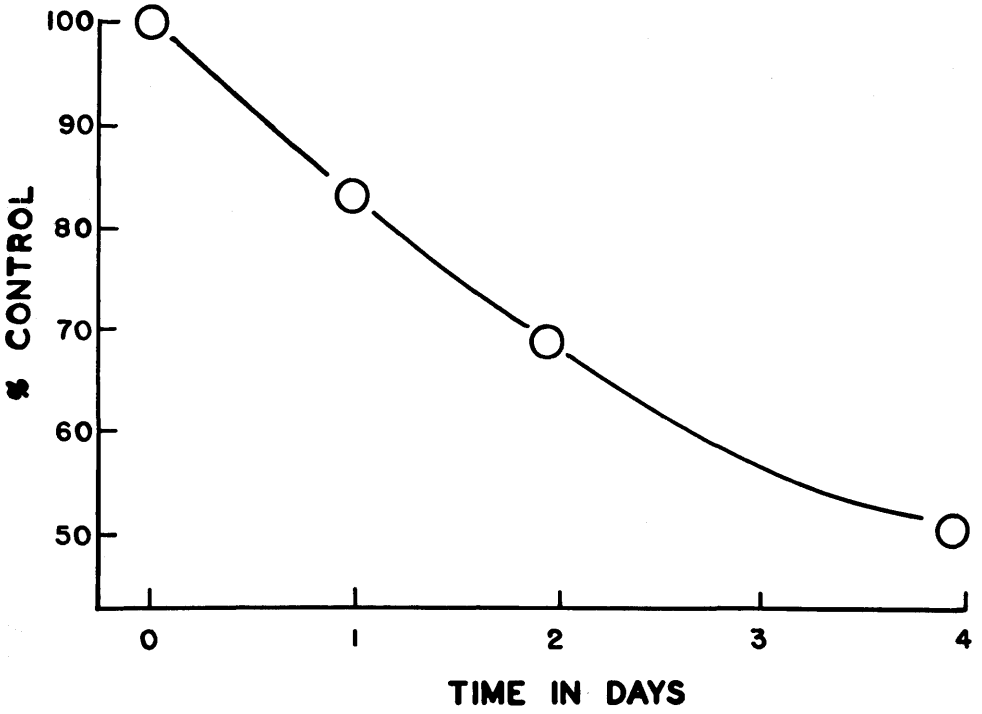

FIGURE 5. Graph showing the effect of sodium fluoride addition on the relative catalase activity with respect to duration of treatment.

TABLE 3

The comparative catalase activities of squash-leaf homogenates of control light-grown leaf disks, fluoride light-grown leaf disks, control dark-grown leaf disks, and fluoride dark-grown leaf disks.

Sample	Mean μM oxygen evolved·30 sec^{-1}·0.1 g dry wt^{-1}	
Light-grown Control	1.82±0.255*	(3)
Light-grown Fluoride	1.38	(3)
Dark-grown Control	3.30	(3)
Dark-grown Fluoride	3.34	(3)

*Note—70% limits with pooled variance.

DISCUSSION

It had been determined previously that in vitro introduction of NaF in the homogenate system did not inhibit the activity of catalase to any great extent over the limited period of measurement—(1 min) at 1 x 10^{-2} M NaF concentration. Eyster (1953) found that NaCl in the concentration of 1 x 10^{-1} N reduced the catalase activity of corn-leaf preparations to 92 percent of that of the water treated preparations. Eyster found, in general, an inhibition of catalase activity upon the addition of inorganic salts, except for 1 x 10^{-1} N Na_2SO_4 in which case the ratio of catalase activity with solute to catalase activity without solute was found to be 103 percent. It appears that the reduction in the catalase activity of the fluoride-treated leaves is a result of inhibition of the enzymatic system or is a change in the actual catalase concentration in the fluoride-treated tissues as compared to the non-treated tissues. Santesson (1923, *In* Eyster, 1953) found that the inhibiting effect of fluoride on catalase compared to other anions was as follows: ClO_3^-, NO_3^-, Cl^-, I^-, Br^-, $CO_3^=$, $B_4O_7^=$, F^-, $HPO_4^=$, $SO_4^=$. Many other investigators have also found a fluoride inhibition of catalase (Beers, 1955; Agner and Theorell, 1946; Ogura et al., 1950a). Agner and Theorell (1946) concluded that iron atoms of the catalase have hydroxyl groups that may be replaced by low molecular-weight anions, in sufficient concentrations, such that catalase is correspondingly inhibited. However, the fluorides in vivo may be causing a reduction in the amount of catalase per se and not just a simple inhibition of the catalase system as measured in the homogenates. McNulty (1959) found a fluoride stimulation of bush bean leaf disk respiration up to 48 hr at 1 x 10^{-3} M NaF concentration. Therefore, it seems unlikely that the fluoride effect on catalase activity could adequately explain the fluoride stimulation and inhibition of respiration as measured by oxygen uptake. Otherwise, the catalase activity would have to be greatly inhibited during the early periods of fluoride treatment, when the fluoride respiration would be high, and the catalase activity would have to be greatly stimulated during the later periods of fluoride treatment, when the fluoride respiration is low. This is not apparent from the data presented. In addition, no evidence for a stimulation of catalase activity was evident.

ACKNOWLEDGMENTS

I wish to acknowledge the technical assistance of Miss D. A. Bell and Mr. J. A. McArthur and the financial support of the Miami University Loyalty Fund.

REFERENCES

Agner, K., and **H. Theorell.** 1946. On the mechanism of the catalase inhibition by anions. Arch. Biochem. 10: 321–338.

Applegate, H. G., and **D. F. Adams.** 1959. Effect of carbon dioxide concentration on respiration of fluoride fumigated bean plants (*Phaseolus vulgaris* var. *humilis.* (Abstr.) Am. Soc. Plant Physiol. (Western Section) 1959 Annual Meeting, San Diego, Calif. p. 4.

———. 1960a. Effect of atmospheric fluoride on respiration of bush beans. Botan. Gaz. 121: 223–227.

———. 1960b. Nutritional and water effect on fluoride uptake and respiration of bean seedlings. Phyton. 14: 111–120.

———, ———, and **R. C. Carriker.** 1960. Effect of aqueous fluoride solutions on respiration of intact bush bean seedlings. I. Inhibition and stimulation of oxygen uptake. Am. J. Botany 47: 339–345.

Appleman, D. 1951. Manometric determination of catalase activity. Apparatus and method. Anal. Chem. 23: 1627–1632.

———. 1952. Catalase-chlorophyll relationship in barley seedlings. Plant Physiol. 27: 613–621.

———, and **H. T. Pyfrom.** 1955. Changes in catalase activity and other responses induced in plants by red and blue light. Plant Physiol. 30: 543–549.

Beers, Jr., R. F. 1955. Equilibrium inhibition of the catalase—hydrogen peroxide system during the steady state. J. Phys. Chem. 59: 25–30.

Berger, J., and **G. S. Avery, Jr.** 1944. Glutamic and isocitric acid dehydrogenases in the **Avena** coleoptile and the effect of auxins on these enzymes. Am. J. Botany 31: 11–19.

Bonner, J., and **S. G. Wildman.** 1946. Enzymatic mechanisms in the respiration of spinach leaves. Arch. Biochem. 10: 497–518.
Burris, R. H. 1960. Hydroperoxidases (Peroxidases and catalases). 12(1): 365–400. *In* W. Ruhland (ed.), Encyclopedia of Plant Physiology. Springer-Verlag, Berlin.
DeKock, P. C., K. Commisiong, V. C. Farmer, and **R. H. E. Inkson.** 1960. Interrelationships of catalase, peroxidase, hematin, and chlorophyll. Plant Physiol. 35: 599–604.
Eyster, H. C. 1950a. Effect of temperature on catalase activity. Ohio. J. Sci. 50: 273–277.
———. 1950b. Catalase activity in chloroplast pigment deficient types of corn. Plant Physiol. 25: 630–638.
———. 1953. Effects of certain inorganic ions on corn leaf catalase. Ohio J. Sci. 53: 101–104.
Feinstein, R. N. 1949. Perborate as substrate in a new assay of catalase. J. Biol. Chem. 180: 1197–1202.
Galston, A. W., R. K. Bonnichsen, and **D. I. Arnon.** 1951. The preparation of highly purified spinach leaf catalase. Acta Chem. Scand. 5: 781–790.
Goldstein, A. 1949a. A "free manometer" technique for use with the Warburg apparatus. Proc. Am. Acad. Arts Sci. 77: 235–253.
———. 1949b. A "free manometer" method of using the standard Warburg apparatus. Science 110: 400.
Hill, R., J. R. Kimmel, and **E. L. Smith.** 1959. The structure of proteins. 28: 127. *In* J. M. Luck (Ed.), Annual Reviews of Biochemistry.
Hill, A. C., M. R. Pack, L. G. Transtrum, and **W. S. Winters.** 1959. Effects of atmospheric fluorides and various types of injury on the respiration of leaf tissues. Plant Physiol. 34: 11–16.
James, W. O. 1953. The use of respiratory inhibitors. 4: 59–90. *In* D. I. Arnon and L. Machlis (Ed.), Annual Review of Plant Physiol.
Landon, R. H. 1934. The effect of certain chemicals on the catalase activity in plants. Am. J. Botany. 21: 583–591.
Laties, G. G. 1949. The role of pyruvate in the aerobic respiration of barley roots. Arch. Biochem. 20: 284–299.
Lohmann, K., and **O. Meyerhof.** 1934. Uber die enzymatische Umwandlung von Phosphoglycerinsaure in Brenztraubensaure und Phosphorsaure. Biochem. Z. 273: 60–72.
Maehly, A. C., and **B. Chance.** 1954. The assay of catalases and peroxidases, 1: 357–424. *In* D. Glick (Ed.), Methods of Biochemical Analysis. Interscience Publishers, Inc., New York.
McNulty, I. B., and **D. W. Newman.** 1956. The effects of a lime spray on the respiration rate and chlorophyll content of leaves exposed to a fluoride atmosphere. Proc. Utah Acad. Sci. 33: 73–79.
———, and ———. 1957. Effects of atmospheric fluoride on the respiration rate of bush bean and *Gladiolus* leaves. Plant Physiol. 32: 121–124.
———. 1959. The influence of fluoride on leaf respiration. Proc. IX Intern. Botan. Congr. 2: 245.
Meyerhof, O., and **K. Lohmann.** 1926. Uber Atmung und Kohlenhydratumsatztierischer Gewebe. Biochem. Z. 171: 421–435.
Ogura, Y., Y. Tonomura, S. Hiro, and **H. Tamiya.** 1950a. Studies on the reaction between catalase molecule and various inhibitory substances. I. J. Biochem. (Tokyo) 37: 153–177.
———. 1950b. Studies on the reaction between catalase molecule and various inhibitory substances. II. J. Biochem. (Tokyo) 37: 179–204.
———, and **H. Hasegawa.** 1952. The mechanism of inhibition of the catalase action. VIII and IX. Symposia on Enzyme Chem. 7: 79–80.
Schwarze, P. 1954. Beziehungen zwischen Peroxydasereaktion, EiweBspiegel und Chlorophyllbildung. Planta. 44: 491–502.
Tanko, B. 1936. Hexosephosphates produced by higher plants. Biochem. J. 30: 692–700.
Thimann, K. V. 1951. Studies on the physiology of cell enlargement. Growth Symp. 10: 5–22.
Warburg, O., and **W. Christian.** 1941. Isolierung und Kristallisation des Garungsferments Enolase. Biochem. Z. 310: 384–421.