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THE INFLUENCE OF ABSCISIC ACID ON THE RESPIRATORY ACTIVITY OF POTATO TUBER TISSUE

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Abstract

Potato tuber tissue was physiologically aged in the presence of abscisic acid (ABA). In addition, the influence of cyanide and malonic acid on the respiratory rates was measured. Changes in respiratory rates and in the sensitivities to these inhibitors were determined. Abscisic acid exhibited an inhibitory effect on the respiratory activity of the potato tuber tissue. ABA did not, however, alter the typical developmental pattern with regard to changes in sensitivities to cyanide and malonic acid.

The term "physiological aging" is used by plant physiologists to describe the events that occur when thin slices of storage tissue are incubated after being cut from a root or tuber. The slices are maintained in distilled water, tap water, or a dilute calcium salt solution and are usually aerated. It has long been known that these freshly cut tissues show an immediate increase in respiration over that of the intact organ. This rise in respiration has been referred to as "wound respiration" (Laties, 1957). Furthermore, these tissue slices exhibit an additional respiratory rise during one to several days of incubation. The respiratory metabolism of plant storage organs can be separated into two components, basal and induced (Laties, 1957). The latter respiratory rate may differ qualitatively and/or quantitatively from the basal respiration (Laties, 1959; 1962; Romberger and Norton, 1961). Several lines of evidence indicate that some biochemical pathways used by fresh tissue are distinct from those of aged tissue. The use of certain metabolic inhibitors allows these distinctions to be made. The effects of cyanide on terminal oxidation and malonic acid on tricarboxylic acid (TCA) cycle metabolism have been described by others. Their effects upon fresh and aged tissues are specific. Cyanide greatly suppresses oxygen uptake in fresh tissue but has little influence on aged tissue (Hackett et al., 1960). Cyanide is a specific inhibitor of cytochrome oxidase, the terminal enzyme of the electron transport chain. Aged tissue, with its high rate of oxygen consumption coupled with phosphorylating ability, may develop a method to circumvent the cyanide inhibition present in fresh tissue (Laties and Hoelle, 1965). Changes also occur in TCA cycle

metabolism during aging since malonic acid, an inhibitor of succinic dehydrogenase, lowers the oxygen consumption of aged tissue but not of fresh tissue (Webb, 1966).

Abscisic acid (ABA) has been identified as a plant hormone (Ohkuma et al., 1963; Cornforth et al., 1965) and as a component of the inhibitor B-complex from many different species (Cornforth et al., 1966; Milborrow, 1967). ABA has been claimed to be the most important growth-inhibiting component of the B-complex (Milborrow, 1967). In many situations the effects of ABA were quite transient, and repeated applications were necessary to elicit a response. Plant tissues are well adapted to ABA in that the normal metabolic processes can respond readily to the substance either with some observable change in activity or, in some circumstances, by a rapid inactivation of the applied ABA (Addicott and Lyon, 1969). The role of ABA, like that of the other plant hormones, is complex and it conceivably exerts a primary regulatory effect at more than one site (Milborrow, 1969). Knowledge of the influence of ABA on the synthesis and activity of enzymes in the plant is still relatively meager.

MATERIAL AND METHODS

Potato tubers of Solanum tuberosum L., var Burbank Russet were purchased locally in one hundred pound lots and stored in the dark at 5 ± 2 C. Twelve hours prior to the experiment, the tubers were removed from cold storage and allowed to come to room temperature. Cylinders of tissue having a diameter of 8.0 mm were removed from the tubers and cut into 1.0 mm slices with a hand microtome. The slices were placed immediately in sterile distilled water kept at 0 - 3 C. The slices were rinsed with additional cold, sterile distilled water to remove free starch and then randomly separated into required lots. All aging solutions contained 1 x 10^{-4} M calcium sulfate and were buffered with 2.5 x 10^{-2} M N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES, Sigma Chemical Co.), adjusted to pH 6.0. Abscisic acid (ABA, F. Hoffman-LaRoche and Co., Basle, Switzerland) was added as noted. Aging solutions were stored in the dark at 5 C until needed. Solutions were always made up fresh for each experiment.

The vessels used for aging potato tuber slices were a series of six sterile 150 ml glass Buchner funnels with a diameter of 6.5 cm. Each funnel was fitted with a coarse sintered glass bottom disk with a pore size of 40 - 60 microns. A compressed air line was attached to the stem of each funnel. To promote aseptic conditions air was first forced through a bacteriological filter and bubbled through sterilized water before it was allowed to enter the aging containers. The tissue was aged for a total of 48 hours at room temperature (25 ± 2 C) and under constant illumination by a cool white fluorescent tube. The tissue slices were agitated continuously by the sterile air bubbling through the aging solutions. The solutions were changed at 1, 2, 6, 12, 24, and 36 hour aging periods in order to maintain a constant concentration and pH, and to minimize microbial growth.

Aging was considered to begin when the tissue was removed from the chilled distilled water and added to the appropriate buffered solution at room temperature. Periodically sufficient slices were removed for respiratory measurements. Any particular tissue sample was measured

only once. Oxygen consumption was determined polarographically with a Clark oxygen electrode (Yellow Spring Instrument Co.). The measuring vials contained 8.0 ml of 5 x 10^{-2} M HEPES buffer at either pH 5.6 or pH 7.2. Temperature was maintained at 30 C (± 0.05 C) by means of a Haake constant temperature circulator. Data were recorded on a Varian (Model G-14 A) recorder set at 0.5 inches per minute. Rates for fresh and aged tissue were obtained using lots of eight and five slices, respectively.

Each tissue lot removed from the aging apparatus was first rinsed in distilled water, blotted dry, rapidly weighed to the nearest one tenth of a milligram and then equilibrated in vials with air-saturated buffer for 5 minutes prior to measurement. Where the influence of malonic acid or cyanide was studied, rate curves for uninhibited tissue were first established. The inhibitors were then added directly to the vials and the respiratory rates determined for an additional 10 minutes. The final concentration of malonic acid was 5 x 10^{-2} M at pH 5.6 and of cyanide was 5 x 10^{-3} M at pH 7.2. The two different pH's were used in order to obtain maximum responses to the two different inhibitors (Hanebuth, 1969). Duplicate samples were measured at each aging period.

RESULTS

Oxygen Consumption

Typical results of physiologically aging tissue slices in the presence of various concentrations of ABA, ranging from 10^{-6} to 10^{-3} M are shown in Figures 1 and 2. The data presented represent the mean from five experiments for the various ABA concentrations. The data presented in Figure 1 are the respiratory rates of fresh and ABA-aged tissues

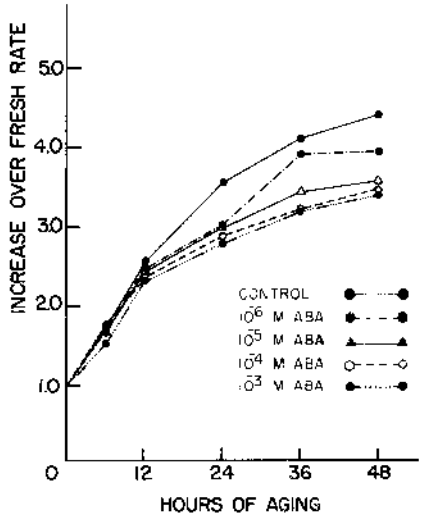

FIGURE 1. - Respiratory values of tissue slices measured at pH 7.2 after incubation in abscisic acid. The rate of fresh tissue was 1.66 ± 0.21 µmoles O_2 consumed/hr·g fresh weight.

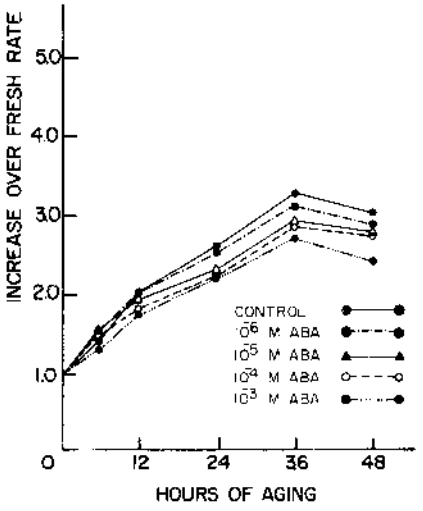

FIGURE 2. - Respiratory values of tissue slices measured at pH 5.6 after incubation in abscisic acid. The rate of fresh tissue was 1.83 ± 0.22 µmoles O_2 consumed/hr·g fresh weight.

measured at pH 7.2. In general, these values run 10 to 25 per cent higher than comparable rates determined at pH 5.6 (Fig. 2). As aging progressed, changes occurred in the oxygen consumption of the control tissues similar to that previously reported (Laties, 1957; Hanebuth, 1971). The respiratory rates showed the greatest increase during the first 12 hours of the aging period and the influence of ABA at all concentrations used was not apparent at this time. After 12 hours the slopes of the respiratory curves tend to level off with increasing aging time.

ABA had a measurable inhibitory effect on the respiratory activity of potato tuber tissues. This inhibition increased with increasing ABA concentrations. At 24 hours of aging the ABA inhibition reached 15 to 22 per cent when measured at pH 7.2 and 4 to 15 per cent when measured at pH 5.6 (Figure 3). At 48 hours of aging, the inhibition ranged from 10 to 23 per cent at pH 7.2 and 5 to 20 per cent at pH 5.6 (Figure 4). The data presented in Figures 3 and 4 are expressed as the percentage of the control value at 24 and 48 hour aging time, respectively. The level of the control treatment was used as 100 per cent activity.

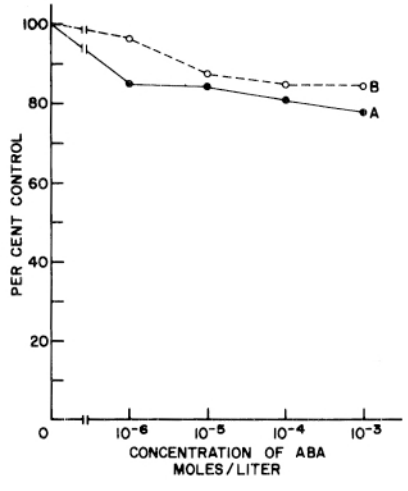

FIGURE 3. - Per cent control respiratory rates of tissue slices after incubation in abscisic acid for 24 hours. The level of control treatment is used as 100 per cent activity. The rates were measured at pH 7.2 (A) and pH 5.6 (B).

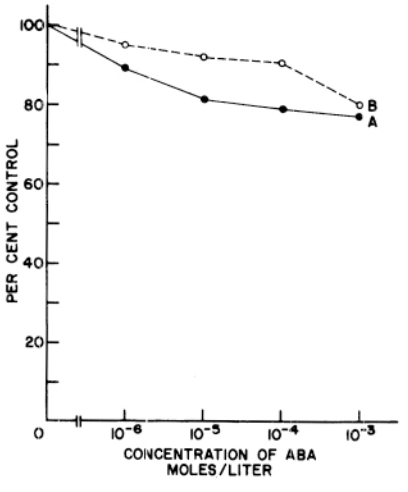

FIGURE 4. - Per cent control respiratory rates of tissue slices after incubation in abscisic acid for 48 hours. The level of control treatment is used as 100 per cent activity. The rates were measured at pH 7.2 (A) and pH 5.6 (B).

The Effect of Cyanide on ABA Aged Tissues

Addition of 5 mM potassium cyanide (pH 7.2) to fresh tissue reduced the respiratory rate of potato tuber tissue to 40 per cent of the cyanide-free control (Figure 5). By 36 hours of aging the control tissue became essentially cyanide insensitive. The data presented on the

respiratory rates of ABA-aged tissues were measured in the presence of 5 mM cyanide at pH 7.2. Compared with the cyanide-free measurement (Figure 1), during the first 24 hours of aging the comparable respiratory rates were lowered by 30 to 50 per cent.

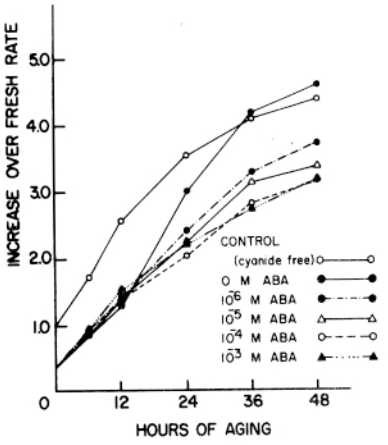

FIGURE 5. - The effect of potassium cyanide on the respiratory rate of tissue slices after incubation in abscisic acid. Control value is taken from Figure 1. Slices were aged in various concentrations of ABA and the rates of respiration were measured in 5 mM potassium cyanide at pH 7.2.

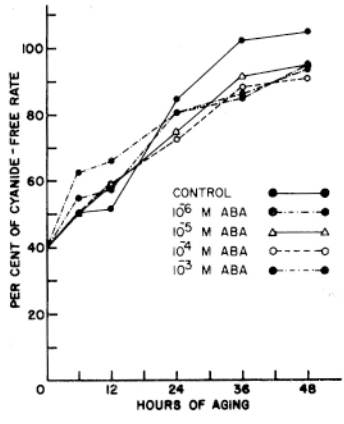

FIGURE 6. - Changes in cyanide sensitivity of abscisic acid aged tissue slices with time. The percentages are derived from the respiratory rates in the presence of potassium cyanide divided by the comparable rates in the absence of potassium cyanide.

As shown in Figure 6, ABA at all concentrations used had little effect on the loss of cyanide-sensitivity of the tissue with aging. These values are obtained by dividing the respiratory rates in the presence of cyanide by the rates in the absence of cyanide.

The Effect of Malonic Acid on ABA Aged Tissues

Addition of 50 mM malonic acid at pH 5.6 to fresh tissue, in contrast to cyanide, had essentially no effect on the respiratory rate (Figure 7). With aging the tissues showed an increase in malonite sensitivity. This occurred in both the control and the ABA treated slices. However, as shown in Figure 8, ABA at all concentrations used had little influence on the development of this sensitivity, just as it had an insignificant effect on the development of cyanide insensitivity (Figure 6). As before, these values were obtained by dividing the respiratory rates in the presence of malonic acid by the rates in the absence of malonic acid.

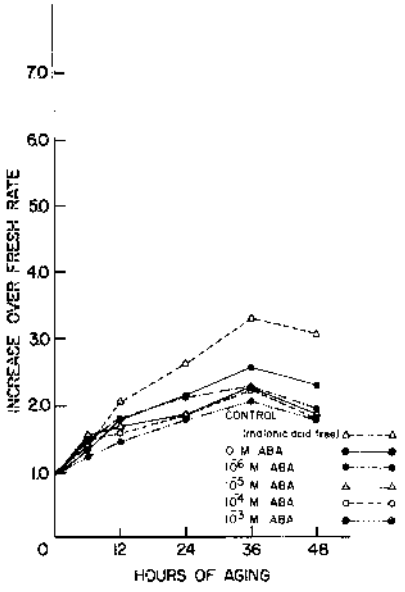

FIGURE 7. - The effect of malonic acid on the respiratory rate of tissue slices after incubation in abscisic acid. Control value is taken from Figure 2. Slices were aged in various concentrations of ABA and the rates of respiration were measured in 50 mM malonic acid at pH 5.6.

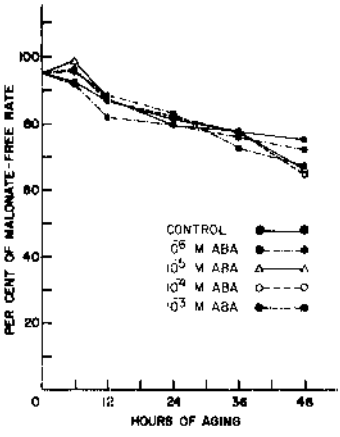

FIGURE 8. - Changes in malonate sensitivity of abscisic acid aged tissue slices with time. The percentages are derived from the respiratory rates in the presence of malonic acid divided by the comparable rates in the absence of malonic acid.

DISCUSSION

Although marked increases in respiratory activity have been observed in a wide variety of sliced storage organs, little is known about the mechanisms which control this enhancement. In recent years, it has become increasingly evident that growth regulating substances play a central role in the regulation of metabolic processes in plants. The enhancement of the respiratory rate of aged tissue can be influenced by many factors, e.g., thickness of the slices (Laties, 1962), the aging temperature (MacDonald and Dekock, 1958), and the washing and aeration conditions (Reed and Kolattukudy, 1966). Since respiration includes many enzymatic reactions, hydrogen ions may play a significant role in the reaction. As shown in this investigation, the respiratory rate was significantly different in two different hydrogen ion concentrations.

Considerable evidence already exists in the literature to show that ABA inhibits many physiological processes in plant tissue. One would expect that the ABA-treatment would reduce the respiratory activity of potato tuber tissue. From this investigation it is evident that ABA had a measurable inhibitory effect on the respiratory activity of the tissue, as great as 20 per cent after 48 hours. This inhibition increased with increasing ABA concentration. While the exact mode of the action of ABA is not known, there have been at least two possible mechanisms proposed

to explain this inhibitory phenomena. Based on the regulatory model of Jacob and Monod (1961), it has been suggested that ABA might exert its effect: (1) at the level of translation (Srivastava, 1968; Haber et al., 1969, or (2) at the level of transcription (van Overbeek et al., 1967; Wareing et al., 1968). Both have the same ultimate effect on protein (enzyme) synthesis. In this investigation the inhibitory effect of ABA may be due to a reduction in the synthesis of respiratory enzymes with a consequent reduction of the respiratory activity of the potato tuber tissue.

The cyanide and malonate inhibition studies may provide some evidence for the mechanism of the effects of ABA on the respiratory activity of potato tuber tissue. ABA had no significant effect on the tissue in terms of the development or loss of sensitivity to both inhibitors. Two possibilities for ABA's effect are: (1) ABA and the inhibitors (malonate and cyanide) act at the same level or the same point in the respiratory process, or (2) ABA acts at the enzyme synthesis level and inhibitors act at the enzyme activity level. From a comparison of the chemical structures and properties of these compounds, the possibility that they might react at the same point or level of the respiratory system seems fairly remote. That ABA might inhibit the synthesis of respiratory enzymes of potato tuber tissue is quite reasonable.

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