Cytochrome oxidase probably occurs in the tissues of most plants and mediates a major portion of terminal respiration. Webster (11, 12) recently demonstrated the presence of a cytochrome oxidase in the tissues of 54 plant species, representing 23 families of dicotyledonous plants. Investigations in the plant physiology laboratory at Rutgers University have indicated the presence of a cytochrome oxidase in sunflower, tomato, soybean, and wheat leaf, and in dodder (*Cuscuta subinclusa*) tissues.

There is evidence that a low or negligible cytochrome oxidase activity reported by some investigators for a number of plant species is at least partly due to the use of buffers at a molarity and a pH which are not optimum for the activity of this enzyme. It has been well established by Howell and Sumner (6) for urease, by Massey (7) for fumarase, and by Gilbert and Swallow (5) for Q-enzyme that the pH optimum for maximum activity is dependent upon ion concentration of the reaction mixture, and that definition of the pH optimum or ion concentration alone does not satisfactorily characterize an enzyme reaction. Smith and Stotz (9) reported that the rate of oxidation of ferrocytochrome c by rat liver, heart, kidney, and intestine homogenates increased slightly from pH 6.5 to 7.0. Bertrand and Gavard (1) found the optimum pH of beef heart cytochrome oxidase to be 8.0. Quinlan-Watson and Dewey (8) reported a pH optimum of 7.1 to 7.3 for various animal tissues. Ducet and Rosenberg (4) reported a pH optimum of 7.4 and a phosphate buffer concentration of about 0.05 M for potato leaf particulate material. Cooperstein, Eichel, and Wainio (2) found the optimum for beef heart cytochrome oxidase to be about 7.0. In a later study, Wainio et al (10) reported that the rate of oxidation of ferrocytochrome c is markedly influenced by the molar concentration of the Na$_2$HPO$_4$-KH$_2$PO$_4$ buffer used. Maximum activity of the beef heart cytochrome c-cytochrome oxidase system was found to increase and the optimum molarity of the buffer to decrease as the pH was changed from 7.4 to 6.2. Wainio and his co-workers in more recent, unpublished work, have demonstrated that desoxycholate preparations of beef heart cytochrome oxidase assayed spectrophotometrically show maximum activity in a phosphate buffer of pH 6.0 with a cation concentration of 0.112 M. They found that the activity of the enzyme is not related to total ionic strength or to the anion concentration of the buffer.

An investigation was carried out to determine the optimum pH and cation concentration at which the cytochrome oxidase of sunflower leaf tissues exhibited its maximum activity. The plants used (*Helianthus annuus* L.) were grown in culture solutions containing adequate levels of the essential inorganic nutrients. A pH optimum curve was determined by utilizing a series of 0.1 M phosphate buffers (Na$_2$HPO$_4$-KH$_2$PO$_4$), ranging in pH from 5.0 to 7.0. The optimum cation concentration was determined by using a series of phosphate buffers with cation concentrations ranging from 0.019 to 0.160 M, each at pH 6.2. Cytochrome oxidase of whole sunflower leaf homogenates was assayed spectrophotometrically by the method of Wainio et al (10) and Darby and Goddard (3). A similar method was also used by Webster (11). The leaf homogenate was prepared by grinding 1 gm of young leaf tissue in a cold mortar and pestle. Maceration was completed in a cold Ten Brock glass homogenizer. The homogenate was then diluted to 25 ml with cold, deionized water. Aliquots of 0.1 ml were used for activity determinations. Figure 1 indicates, in terms of the velocity constant (K sec$^{-1}$), that the pH optimum for the sunflower cytochrome c-cytochrome oxidase reaction is 6.0, and figure 2 indicates that the cation concentration optimum at pH 6.2 is about 0.130 M.

These values agree reasonably well with those found for animal tissues by Wainio and his co-workers. They do not agree, however, in all respects with the results of Ducet and Rosenberg (4). Whereas their cation concentration was not stated di-

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2 Paper of the Journal Series, New Jersey Agricultural Experiment Station, Rutgers University, the State University of New Jersey, Department of Plant Physiology and Bureau of Biological Research, New Brunswick.
directly, the optimum buffer concentration was approximately 0.05 M phosphate which would correspond to a cation concentration of about 0.1 M. This concentration falls in the vicinity of the optimum cation concentration found by us. However, there is some difference in pH optima found which may be due to their use of a manometric instead of a spectrophotometric method.

It appears from the work reported that a combination of a pH of about 6.0 and a cation concentration of the phosphate buffer of about 0.130 M is probably the most favorable one for the determination of cytochrome oxidase activity in plant tissues by the spectrophotometric method employed.

LITERATURE CITED


THE EFFECT OF CARBON MONOXIDE ON RESPIRATION IN HIGHER PLANTS

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Current studies (1, 2, 5) have indicated that a cytochrome oxidase is found in most of the tissues of higher plants. The widespread distribution of this enzyme has raised the possibility of its general participation in plant respiration in a manner similar to that in animals, yeast, and aerobic bacteria. Unquestionable proof of the participation of cytochrome oxidase in plant respiration is very difficult, but the relative importance of cytochrome oxidase and tyrosinase (or other copper containing enzymes) in respiration may be estimated by use of the carbon monoxide inhibition techniques of Warburg (4). It is well established (3, 4, 5) that the carbon monoxide inhibition of cytochrome oxidase activity is eliminated by light while the carbon monoxide inhibition of tyrosinase is insensitive to light. This difference in properties provides a means of differentiating between the activities of these oxidases in plant respiration.

In connection with some previously reported studies (5) on the occurrence of cytochrome oxidase in plants, the author had occasion to examine the effect of carbon monoxide and light on the respiration of a number of plant tissues. The respiration of root (1 cm sections) or other tissue (0.5 mm slices) in 0.05 M potassium phosphate buffer (pH 7.1) was measured at 25°C by standard manometric tech-

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1 Received March 15, 1954.