BRIEF PAPERS

CYTOCHROME OXIDASE IN CORN EMBRYOS[1]

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In connection with a study of iron metabolism in corn, it was necessary to establish the presence of the cytochrome system in corn embryos.

Evidence has been presented for the functioning of the cytochromes in the embryos of wheat (**1**), oats (**2**), and barley (**3**). It would be logical to assume, therefore, that corn embryos also contain cytochrome oxidase. However, in view of the present state of knowledge of plant respiratory enzymes, it would be unwarranted to accept such an assumption without proof. A search of the literature failed to reveal any studies designed to indicate the nature of the terminal oxidase in corn embryos.

Air dry corn kernels of the hybrid WF9 × M14 were selected with regard to integrity and weight (250 ± 10 mg.). The seeds were rinsed briefly with 0.2% mercuric chloride and then flushed with a large volume of sterile water. Sterile technique was used throughout the experiments with whole kernels, and there has been no observable instance of mold or bacterial growth. Individual seeds were placed in Warburg flasks lined on the bottom with filter paper. For the control vessels, 0.5 ml. sterile water was added. The same amount of various solutions containing respiratory inhibitors was added to the experimental flasks. The concentrations of the inhibitors were selected as being likely to show appreciable inhibition of iron or copper containing enzymes, the selections being based on concentrations used in studies referred to in the bibliography. All solutions were adjusted to pH 7.0. In the center wells, 0.2 ml. 20% KOH was used with a fluted section of filter paper, except for the cyanide inhibition study, in which KCN was used as the carbon dioxide absorbent (**4**). Oxygen uptake was measured, in an atmosphere of air and at 37° C, for 60 minute periods at various intervals, usually extending to 25 hours. By this time the control seeds exhibited a large oxygen consumption and roots 10 to 11 mm. in length.

In table I it may be seen that the profound effects of cyanide and azide leave little doubt as to the involvement of a heavy metal enzyme. In these cases germination was completely arrested. The results obtained with phenylthiourea and diethyldithiocarbamate suggest that the copper enzymes tyrosinase and ascorbic acid oxidase might not be involved in the corn seed's respiration at this stage of development (**5, 6**). However, ger-

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mination did not proceed at the normal rate in the presence of the latter two inhibitors, since the root length was reduced to 3 to 6 mm. at the end of 25 hours.

These inhibition effects naturally pertain to the respiration of the endosperm as well as of the embryo, but one would expect that major portion of the respiratory activity to be centered in the growing and differentiating embryo. In the case of wheat, it has been stated that the magnitude of embryo respiration is 10 to 15 times greater than that of the endosperm (**7**).

In order to obtain more direct evidence for the activity of the cytochrome system in the embryos, homogenates were prepared from embryos with 10 to 11 mm. roots. Ten embryos (free of endosperm) were selected which had a total weight of 1.0 ± 0.1 gm. These were crushed in a mortar with 2.0 ml. of ice cold sodium phosphate buffer, 0.1 M, pH 7.0, another

TABLE I

OXYGEN UPTAKE BY CORN KERNELS

SOLUTION	MM.^{3} O_2 PER SEED DURING INDICATED HOUR*		
	6–7	18–19	24–25
Controls	31 (25–33)	146 (127–162)	166 (140–184)
0.002 M PTU†	35 (21–54)	157 (142–158)	165 (159–175)
0.01 M DDC††	39 (35–46)	104 (97–108)	125 (107–141)
0.005 M NaN_3	3 (1–4)	6 (4–7)	4 (1–8)
0.001 M KCN	0	0	0

* Average is given first, followed by range of six determinations.
† Phenylthiourea.
†† Sodium diethyldithiocarbamate.

2.0 ml. of the buffer was added and the suspension was homogenized in a glass apparatus (**8**). Aliquots of these preparations were used for the experiments summarized in table II. Oxygen uptake from an atmosphere of air was measured in a conventional Warburg apparatus at 37° C and a shaking rate of 120 two centimeter strokes per minute. The cytochrome c used was prepared from horse heart by the method of KEILIN and HARTREE (**9**). The iron content of the preparation, as determined by the method of DRABKIN (**10**), was 0.28%. The concentrations of solutions of the pigment were determined in a Beckman quartz spectrophotometer at 550 mμ after reduction with hydrosulphite, using the extinction coefficient reported by THEORELL and AKESSON (**11**). Higher concentrations than that shown in the table did not increase the oxygen uptake, so that it may be assumed that the oxidase present in the homogenate was saturated at this level. In all of the experiments reported, the rate of oxygen uptake was essentially linear with time over the 60 minute period.

Initially, ascorbic acid was investigated as a reducing agent for the added cytochrome c (**12**). However, as indicated in the table, there was

TABLE II

OXYGEN UPTAKE BY CORN EMBRYO HOMOGENATES*

FLASK CONCENTRATION OF ADDITIONS	MM.^{3} O_2 UPTAKE PER HOUR†
(1) None	4 (2–9)
(2) 0.00003 M cytochrome c	10 (9–12)
(3) 0.01 M p-phenylenediamine	51 (48–58)
(4) As in (3), but homogenate boiled	26 (20–31)
(5) 0.00003 M cytochrome c + 0.01 M p-phenylenediamine	148 (132–174)
(6) As in (5), but homogenate boiled	21 (20–22)
(7) As in (5), + 0.001 M KCN	22 (18–25)
(8) As in (5), + 0.005 M NaN_3	26 (22–31)
(9) 0.02 M ascorbate + 0.00003 M cytochrome c	357 (310–406)
(10) As in (9), but homogenate boiled	249 (200–299)
(11) 0.0025 M 3,4-dihydroxyphenyl-L-alanine	5 (0–8)
(12) 0.003 M p-cresol	0

* Each flask contained 1.0 ml. 0.1 M sodium phosphate buffer, pH 7.0; 0.5 ml. homogenate; 0.2 ml. 20% KOH and filter paper in center cup; and water to make a total volume of 3.0 ml. Substrates tipped from side arm after temperature equilibration.

† Each value is the average of at least three determinations, the range being indicated in parentheses.

a high rate of non-enzymatic catalysis of the oxidation of ascorbic acid, and in further experiments p-phenylenediamine was used. It should be emphasized that the conditions used in these experiments are not necessarily optimum. The results show, nevertheless, that addition of the specific substrate for cytochrome oxidase, reduced cytochrome c, causes a marked stimulation of oxygen uptake by the homogenate. Furthermore, this increase in activity is substantially abolished by cyanide and azide, two known inhibitors of cytochrome oxidase.

Under the conditions of these experiments, no significant cresolase or polyphenol oxidase activity was apparent.

CHEMISTRY SECTION
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