# The Oxidizing Enzymes of Sugarcane: Cytochrome C Oxidase

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INTRODUCTION

Of the many oxidative reactions taking part in plant respiration, "terminal oxidation" has been studied more than any other. It is here that electrons from substrate oxidation are at last combined with oxygen and H^+ ions to yield water. Enzymes catalyzing the final reaction are called "terminal oxidases," and these bear a major responsibility for the ultimate consumption of oxygen.

In animal tissues evidence heavily favors an iron-porphyrin protein, cytochrome oxidase[2], as the probable catalyst in the overall reaction:

$$2 \text{ cytochrome } A_3 - (Fe^{2+}) + 2H^+ + \tfrac{1}{2} O_2 \rightarrow 2 \text{ cytochrome } A_3 - (Fe^{3+}) + H_2O$$
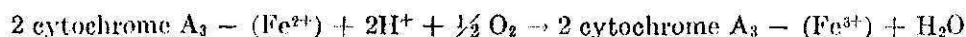
Two molecules of reduced cytochrome A_3 furnish electrons needed for reduction of one oxygen atom, and two protons are required to balance the reaction (*8*, p. 221)[3]. More specifically, a series of cytochromes is believed to react in sequence. Cytochrome B is the first reduced. Then follows cytochrome C_1, cytochrome C, and finally cytochrome A[4].

The mechanism of terminal oxidation is less clearly understood in higher plants. A number of oxidases have been suspected on the basis of varying requirements among different plant species and tissues (*6*, p. 200, *16*). Peroxidase, ascorbic acid oxidase, tyrosinase, and cytochrome C oxidase have all been considered as potential catalysts.

Initial work at this laboratory has revealed a cytochrome C reductase in leaves of sugarcane, but no clear evidence of cytochrome C oxidase could be found (*1*). More recently, cane peroxidase (*3*) and tyrosinase (*4*) have been studied in an effort to clarify relationships between sugar content and respiratory reactions. Much of the importance attributed to these

[2] Cytochrome oxidase is also commonly designated cytochrome A_3.

[3] Italic numbers in parentheses refer to Literature Cited, pp. 144–5.

[4] Even today the nature and functions of the cytochromes are not clearly understood, yet ironically these pigments were known long before biologists were ready to accept them as having significant roles. MacMunn studied them from 1882 to 1886 but his views were not accepted. According to Fruton and Simmonds (*12*, p. 349), in a book published posthumously, MacMunn wrote "A good deal of discussion has taken place over this pigment, and the name of Hoppe-Seyler has prevented the acceptance of the writer's views. The chemical position is undoubtedly weak, but doubtless in time this pigment will find its way into the text-books".

enzymes was based on the assumption that no cytochrome oxidase was active in cane. This paper concerns a water-soluble cytochrome C oxidase which has recently been extracted from roots of immature sugarcane.

MATERIALS AND METHODS

PREPARATION OF ENZYME

Cytochrome C oxidase was prepared with root tissues from 8-month-old plants of the sugarcane variety P.R. 980. The canes had been grown in quartz sand contained in 3-gallon, polyethylene containers, each of which received 1 liter of "complete"[5] nutrient solution every second day, and 1 liter of tapwater on alternate days. Root tissues were obtained by removing the nearly potbound plant cluster from the container, and trimming the fresh root growth which was now readily accessible. Root samples consisted of the young, white, turgescent segment extending 2 or 3 inches back from the growing point[6].

After thorough washing, about 50 gm. of root material were blended for 2 minutes in 125 ml. of distilled water and crushed ice. The mixture was then expressed through four layers of absorbent gauze, chilled to 1°C., and clarified by centrifuging for 10 minutes at 3,500 r.p.m. A few drops of 0.25 N NaOH was used to bring the supernatant liquid to pH 7. Sufficient solid ammonium sulfate was then added to achieve 80-percent saturation. The salt was added slowly with constant stirring over a 30-minute period. Each sample was then refrigerated for 3 hours at 2°C. to increase protein yield.

Following removal of the 0 to 80-percent protein fraction by centrifuge, more ammonium sulfate was slowly added until the solution was 95-percent saturated. The suspension was refrigerated for 3 additional hours. The 80- to 95-percent protein fraction was removed by centrifuge, taken up in 5 ml. of distilled water, and the protein suspension was again clarified by centrifuge at 3,500 r.p.m. Most of the root cytochrome C oxidase activity was present in the protein concentrate, which, in turn, was refrigerated until dilutions were required just prior to running individual assays.

ASSAY OF CYTOCHROME C OXIDASE

The cytochrome C oxidase procedure employed here was based on the method of Cooperstein and Lazarow (9), and of Webster (23), in which

[5] Nutrient concentrations, expressed as milliequivalents per liter, were provided as follows: Nitrate, 10; phosphate, 6; potassium, 5; calcium, 3; magnesium, 2; and sulfate, 2. Microelements, expressed as parts per million, were supplied in the following concentrations: Boron, 0.05; copper, 0.02; manganese, 0.50; zinc, 0.05; molybdenum, 0.01; and iron, 1.0. The letter M is used in this paper as an abbreviation for "molar."

[6] These roots are equivalent to the "shoot roots" described by Van Dillewijn (22, pp. 53-4).

the decrease in optical density of a standard digest containing reduced cytochrome C is measured at 550 mμ. Reduced cytochrome C is pink and possesses visible absorption bands near 550 and 415 mμ, while oxidized cytochrome C is yellow and has a visible absorption band at 400 mμ. Figure 1 illustrates the absorption spectra of reduced, ox-heart cytochrome C[7] before and after being acted upon by the cane-root enzyme.

Other methods suitable for cytochrome C oxidase include the manometric technique of Stotz, et al. (20,19), requiring hydroquinone as a secondary substrate, and the colorimetric method of Smith and Stotz (18), in which a
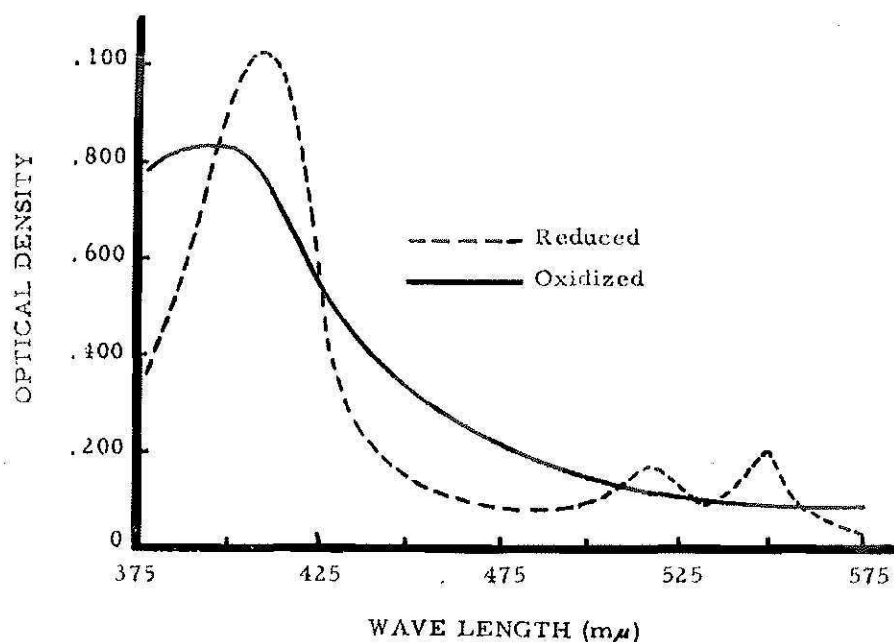

FIG. 1.—Absorption spectra of reduced and oxidized cytochrome C from ox heart. Reduced spectrum was obtained before exposure to cane cytochrome C oxidase, and oxidized spectrum was derived after 4 hours action by the sugarcane enzyme. Cytochrome C concentration was 1.5 × 10^{-2} μmol/ml.

leuco dye is oxidized to its colored form. The assay described below for cane oxidase was selected because of its relative speed and precision.

Cytochrome C oxidase was measured directly in duplicate, 1-cm. Pyrex cuvettes, employing a Beckman Model B spectrophotometer. Each cuvette received 1.0 ml. of 0.2-M acetate buffer (pH 4.5), 0.5 ml. of enzyme preparation, and 0.5 ml. of additive solution or distilled water. One milliliter of water was added to the control vessel and the optical density reading at 550 mμ was adjusted to 0. One milliliter of reduced cytochrome C solution was then placed in the test cuvette, optical density was measured and a stopwatch started immediately. Optical density was again recorded after

[7] Supplied by the Nutritional Biochemicals Corp., Cleveland, Ohio.

60 seconds had passed. Sufficient cytochrome C was used to make a final concentration of 0.1 µmol/ml. of digest, and all tests were conducted at laboratory temperature (22°C.). One cytochrome C oxidase unit was arbitrarily defined as the amount of enzyme causing an optical density decrease of 0.10 per minute. Protein content of the enzyme preparations was determined by the method of Sutherland *et al.* (*21*), and oxidase action was subsequently expressed as specific activity (units per milligram of protein).

RESULTS AND DISCUSSION

EXTRACTION AND DISTRIBUTION AMONG CANE TISSUES

It now appears that our earlier failure to detect cytochrome C oxidase in cane was due to interference by tyrosinase, and to the fact that only leaf

TABLE 1.—*Cytochrome C oxidase activity among different tissues of 10-month-old sugarcane*[1]

Data classification	Results for tissues analyzed						
	Meristem	Leaf (−1 to +1)	Leaf (+3 to +5)	Sheath (+3 to +5)	Node (8 to 10)	Node (1 to 3)	Root
Specific activity	0.5	0.5	0	6.6	3.2	10.0	29.3
Oxidase units per gram of fresh weight	Trace	Trace	0	2.8	1.6	Trace	12.0

[1] Variety P.R. 980. The standard assay procedures were employed throughout as described under Materials and Methods above.

tissue was studied. Webster (*23*) pointed out that the high *in vitro* activity of certain copper oxidases will usually mask the activity of other oxidative enzymes. This investigator avoided tyrosinase by capitalizing on the relative insolubility of cytochrome C oxidase. He localized the latter enzyme in a particulate fraction of his plant materials, sedimented between 1,000 and 16,000 × *g*, while tyrosinase remained soluble in the supernatant liquid. Our own attempts to isolate cytochrome C oxidase among particulate fractions were negative. However, we did obtain from meristem extracts a weak oxidase activity by fractionating with ammonium sulfate, and subsequently discarding the tyrosinase-bearing portion which was precipitated between 0 and 80-percent saturation.

The meristem oxidase was not sufficiently strong to suggest real biochemical importance. The search was therefore extended to other tissues. Table 1 shows that of the various tissues studied, only roots contained strong activity, although some cytochrome C oxidase activity was found in sheath

and node preparations. Salt fractionation of root extracts produced the most active precipitates above 75-percent saturation (fig. 2). This is interesting in that tyrosinase is precipitated almost entirely below 75-percent saturation (4), although in roots there is little evidence of any tyrosinase at all.

PROPERTIES OF CANE CYTOCHROME C OXIDASE

Stability

Cytochrome C oxidase preparations gradually lost activity during prolonged storage under toluene (table 2). The enzyme was slightly more stable in dilute cysteine-HCl solution. Since these preparations were not
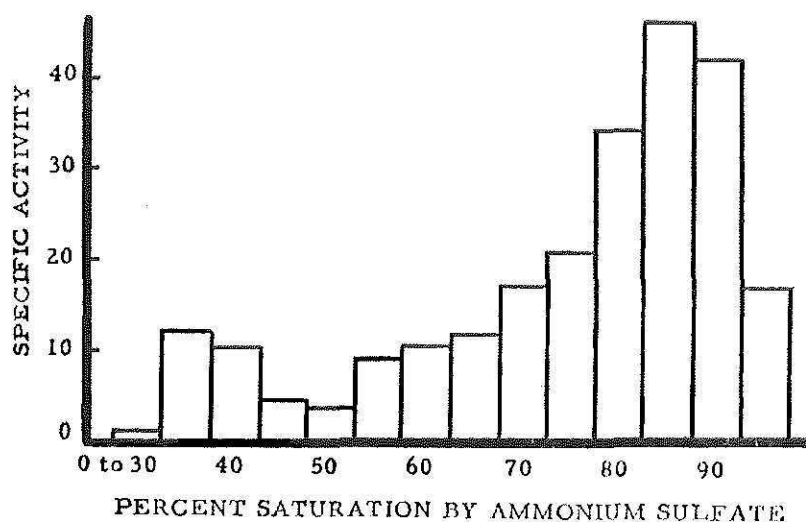

FIG. 2.—Cytochrome C oxidase activity among protein increments precipitated from extracts of sugarcane roots.

adversely affected by freezing, the usual procedure was to freeze a series of fresh aliquots and to thaw sufficient enzyme for a given day's work. The enzyme did not recover activity following inactivation by boiling.

Optimum pH and Temperature

Optimum pH for cytochrome C oxidase was found to lie between 4.0 and 4.5 (fig. 3,A). This value is unexpectedly low and accounts, in part, for our not detecting the enzyme during earlier work. Cooperstein and Lazarow (9) measured rat cytochrome C oxidase at pH 7.4, while Stotz et al. (20,19) and Hass et al. (14) assayed the enzyme at pH 7.2 and 7.3, respectively. The only other known sugarcane enzyme having such a low pH preference is an α-amylase which we encountered in leaf preparations while studying the Q enzyme (2).

Temperature effects were determined with the aid of a constant-temperature water bath, in a manner similar to that described for peroxidase (3) and tyrosinase (4). No great precision is claimed, since, unlike the standard assay which is run directly within the spectrophotometer, it was first necessary to transfer active reaction mixtures from the water bath to cuvettes

TABLE 2.—*Specific activity decline of cane cytochrome C oxidase stored at room, laboratory, and refrigerator temperatures*[1]

Storage temperature (°C.)	Results for indicated days following preparation			
	0	1	3	9
Room (28° 29°)	28.0	15.0	14.0	6.0
Laboratory (21°–22°)	28.0	21.0	15.0	8.0
Refrigerator (2°)	28.0	22.0	15.5	12.4

[1] Standard assay procedure was employed as described under Materials and Methods, pp. 116–7.
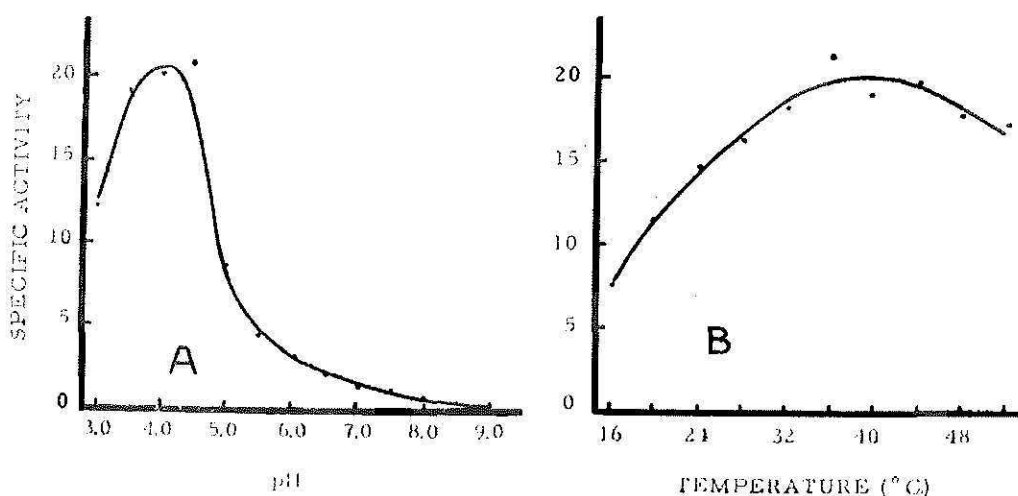

FIG. 3.—A, Effects of variable pH on cytochrome C oxidase from sugarcane roots; B, effects of variable temperature. The standard assay was used in conjunction with indicated treatments.

before measuring optical density. Nevertheless the results are relative and indicate that optimum temperature for the enzyme is about 36 to 40°C. (fig. 3,B).

Cane cytochrome C oxidase activity could be plotted as a first-order reaction both with reference to enzyme concentration (fig. 4,A) and time (fig. 4,B). The reaction rate is constantly decreasing, or, in other words, the reaction proceeds as if there were insufficient substrate to saturate the enzyme. This behavior appears to have been due to a slight "deficiency"
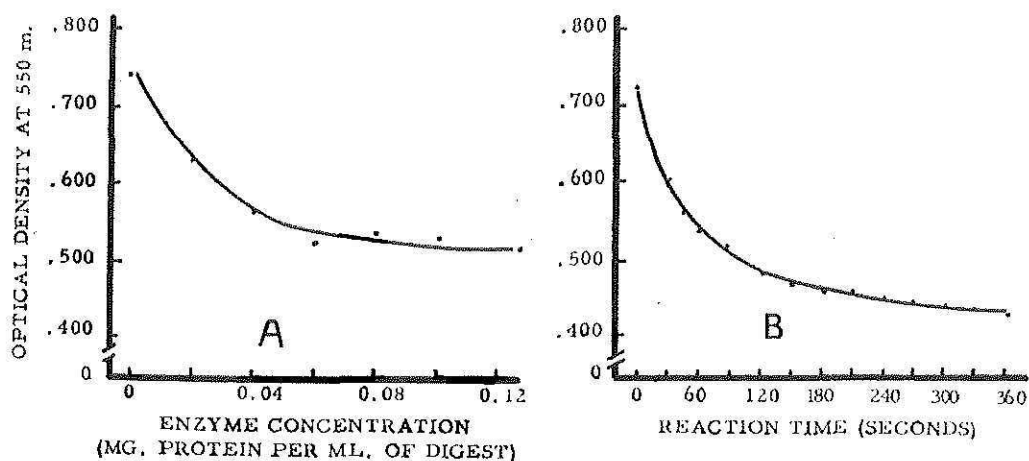
FIG. 4.—Optical-density decline of reduced cytochrome C in the presence of cane cytochrome C oxidase: A, proportionality of the reaction with enzyme concentration; B, proportionality with time. The standard cytochrome C oxidase assay was used throughout in conjunction with the variables indicated above.
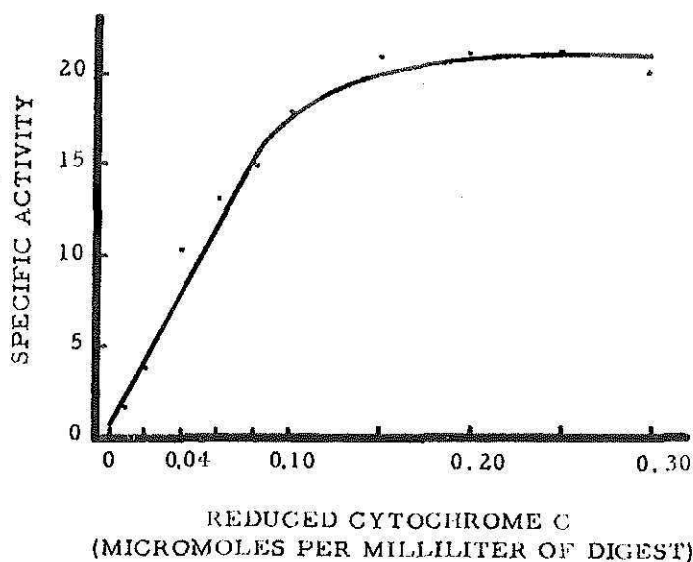

FIG. 5.—Effects of variable substrate concentration on the activity of sugarcane cytochrome C oxidase.

of iron (Fe), since in each instance the incorporation of additional Fe, rather than increased cytochrome, brought about the normal diphasic activity response.

Substrate Concentration, Km

Cytochrome C oxidase was measured with substrate concentrations ranging from 0.01 to 0.30 μmol/ml. of digest (fig. 5). Maximum velocity

was recorded at 0.09 μmol/ml. Km is therefore about 4.5 $\times$ 10^{-5} mol of reduced cytochrome C per liter.

Activation and Inhibition

Mercury and cysteine-HCl markedly stimulated cytochrome C oxidase at the concentration 2.0 μmols/ml. of digest (table 3). Boron and nitrate slightly stimulated the reaction at the same concentration, while CN caused total inhibition. Subsequent trials showed that CN inhibition was easily measurable with only 0.02 μmol of CN present per milliliter (fig. 6).

Carbon monoxide (CO) was tested as a possible inhibitor by bubbling the gas through a standard cytochrome C oxidase preparation for 30 min-

TABLE 3.—*Effects of additives on cytochrome C oxidase from roots of sugarcane*[1]

Additive	Specific activity	Additive	Specific activity	Additive	Specific activity
Control (+ H$_2$O)	18.0	F	18	CN	0
Hg	29.0	B	23	Thiourea	21
Mo	[2]	Ca	17	Hydroquinone	18
Zn	16.8	Mg	20	Cysteine-HCl	33
As	21.0	Cu	[3]	β-glycerophosphate	17
Br	18.0	I	19	Nitrate	24

[1] The standard digest was employed throughout in conjunction with a final additive concentration of 2 μmol/ml. of digest. The following additive sources were used: Calcium chloride, magnesium chloride, boric acid, sodium fluoride, sodium bromide, potassium iodide, cupric sulfate, sodium arsenate, zinc sulfate, potassium cyanide, sodium β-glycerophosphate, and sodium nitrate.

[2] The presence of Mo caused a precipitate to form upon incorporation of cytochrome C.

[3] Copper induced a rapid but nonenzymatic decline of optical density readings.

utes. Carbon monoxide was generated by passing concentrated H$_2$SO$_4$ into a 2 N solution of formic acid. No effect was noted upon oxidase activity, although the CO treatment was carried out under normal light conditions of the laboratory. It has long been known that light reverses the CO-inhibition of respiratory enzymes (7,15), and it is possible that any CO effect upon the cane enzyme was reversed before an assay could be carried out.[8] Isolation of cane cytochrome C oxidase in roots would normally minimize any potential light effects.

Dialysis Effects; Essentiality of Fe

An almost immediate activity decline was observed when cytochrome C oxidase preparations were dialyzed against distilled water (fig. 7).

[8] Similar CO treatment of cane tyrosinase preparations caused almost 100-percent inhibition of that enzyme.

Over 70 percent of the activity was lost within 2 hours, and only a trace of the reaction could be detected after 44 hours.

Subsequent attempts to reactivate dialyzed oxidase were successful with cysteine-HCl, and particularly so with Fe (table 4). With added Fe the dialyzed preparation was far more active than the undialyzed control. As little as 2×10^{-5} μmols of Fe per milliliter of digest caused partial reactivation (table 5), while 2×10^{-2} μmols of Fe restored activity to the predialysis level.

Iron, of course, is a known essential component of the cytochrome C oxidase system. However, the dialysis studies have shown, as evidenced by the much greater activity of "cleaned-up" preparations, that this en-
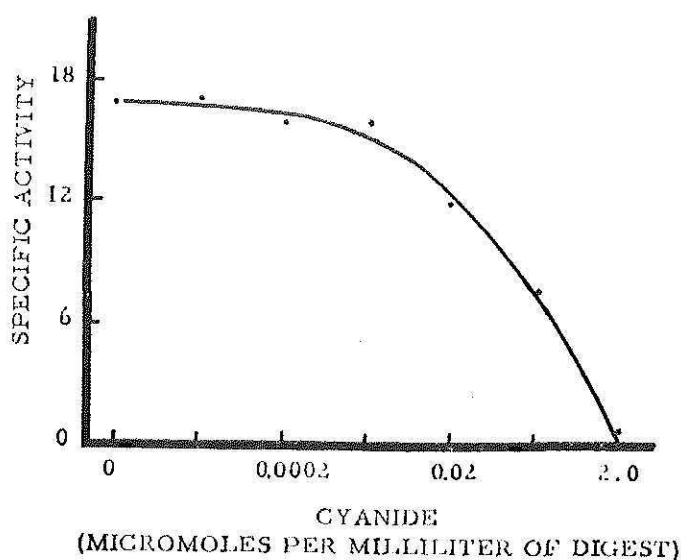

FIG. 6.--Cyanide inhibition of sugarcane cytochrome C oxidase.

zyme must be bound with other ions which retard the effect of Fe without themselves serving to activate the catalyst. This might also be true of the enzyme *in vivo*, which would help explain the apparent effects of certain nutrients and nonessential factors upon the cytochrome oxidases of plants grown under experimental conditions.

SIGNIFICANCE OF CYTOCHROME C OXIDASE IN SUGARCANE

Cytochrome C oxidase undoubtedly plays an important role in animal respiration, yet its importance in higher plants is clouded by the presence of other enzymes capable of performing the tasks of a terminal oxidase. Sugarcane certainly is no exception, for we can obtain peroxidase and tyrosinase far more readily from cane than cytochrome C oxidase. The tissues themselves seem to determine a predominant oxidase. High tyro-

sinase levels in meristem contrast markedly with cytochrome C oxidase localization in the roots, especially so since the former has a pH preference of 7.4 (4), and the latter a pH of 4.5. Peroxidase is distributed abundantly among leaf, sheath, and meristem tissues (3).
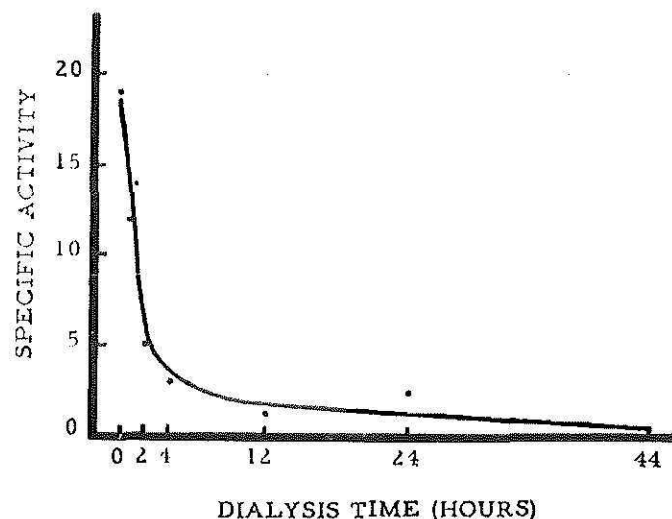

FIG. 7.—Decline of cytochrome C oxidase activity following dialysis of the enzyme against distilled water. Enzyme samples were dialyzed at 2° C. for the indicated period of time. Afterward they were stored at 2° C. until the 44-hour treatment had been completed. Each sample was then assayed according to the standard procedure.

TABLE 4.—*Reactivating effects of different additives upon dialyzed cytochrome C oxidase preparations*[1]

Data classification	Treatment of cytochrome C oxidase preparations						
	Undialyzed (control)	Dialyzed plus H₂O	Dialyzed plus 1.0 μmol/ml. of—				
			Fe	Hg	B	Mg	Cysteine HCl
Specific activity	19.5	0	32.0	0	9.0	6.0	22.5

[1] The treatment enzyme preparation was dialyzed for 48 hours against 3 changes of distilled water. Both control and treatment samples were refrigerated at 2°C. during the dialysis period. Standard assays were then run with undialyzed oxidase, and with the dialyzed enzyme in the presence of additives.

The relative activities of these two catalysts, as we have thus far studied them, are not sufficient to state which one is of greatest importance. Tyrosinase undoubtedly is able to mask the action of its iron-bearing counterpart and, furthermore, the water extraction of these enzymes does not necessarily give a fair picture of their content or activity. The relative insolubility of cytochrome C oxidase has been pointed out by several investigators (8, p. 220, 23,5), whereas tyrosinase is readily soluble in water. The

most that we can say at the moment is that tyrosinase probably mediates terminal oxidation in meristem, and that cytochrome C oxidase can do so in roots. More information could be gained by comparing gross respiratory activity among different cane tissues. By determining which respiratory inhibitors are most effective (*i.e.*, those retarding the iron-porphyrin system or those acting upon the copper proteins), the predominate terminal oxidase in any tissue would be confirmed.

Yet even if cytochrome C oxidase did not count for much in terminal oxidation, its potential role in other biochemical processes would still be formidable. "Oxidative phosphorylation" [9] is a good example. According to Fruton and Simmonds (*12*, p. 381), numerous workers have shown that aerobic respiration of cells and tissue preparations is associated with in-

TABLE 5.—*Stimulating effects of added iron upon sugarcane cytochrome C oxidase following inactivation by dialysis against distilled water* [1]

Data classification	Undialyzed (control)	Treatment of cytochrome C oxidase preparations						
		Dialyzed enzyme plus Fe at indicated concentrations ($\mu mol/ml.$ of digest)—						
		0	2×10^{-5}	2×10^{-4}	2×10^{-3}	2×10^{-2}	2×10^{-1}	2×10^{-0}
Specific activity	17.0	0	7.5	8.2	10.0	17.2	31.4	28.7

[1] The treatment enzyme preparation was dialyzed for 48 hours against 3 changes of distilled water. Both control and treatment preparation were refrigerated at 2°C. during the dialysis period. Standard assays were then run with undialyzed oxidase, and with the dialyzed enzyme in the presence of iron additive.

organic phosphate uptake. It was found that respiration was responsible for the formation of the pyrophosphate bonds of ATP. Considerable evidence is now available indicating that one equivalent of ATP is formed per atom of oxygen consumed in the oxidation of cytochrome C by O_2 (*17*, *11*). Conn and Stumpf (*8*, p. 223) have used the diagram presented in figure 8 to illustrate the chain of electron transport during a reaction sequence which includes a mitochondrial source of enzymes, inorganic phosphate, FAD (flavin adenine dinucleotide), NAD (nicotinamide adenine dinucleotide), and cytochrome C.

Phosphorylation is believed to take place as a pair of electrons makes its way along the pathways of electron transport. One such reaction occurs when reduced cytochrome C is oxidized by molecular oxygen. The other

[9] "Oxidative phosphorylation" is associated with reactions of electron transport. This is distinguished from "substrate level phosphorylation," in which phosphorylated or thioester forms of substrate react with ADP to form ATP, as in certain reactions of the glycolytic and Krebs-cycle pathways.

two phosphorylations are accomplished when a pair of electrons makes its way from NADH to cytochrome C. Such reactions have been studied with mitochondria preparations of both plants and animals, and it is presumed that identical sequences would exist in sugarcane.

Finally, one cannot overemphasize the fact that the importance of cytochrome C oxidase lies not in its role as a single catalyst, but as an integral part of an extremely complex series of mitochondrial enzymes.[10] This combination of enzymes includes catalysts of the tricarboxylic acid cycle, of oxidative phosphorylation, of fatty acid oxidation, and enzymes involved in synthesis of specific cell materials. This physiological "machine", of which cytochrome C oxidase is an important component, is an excellent
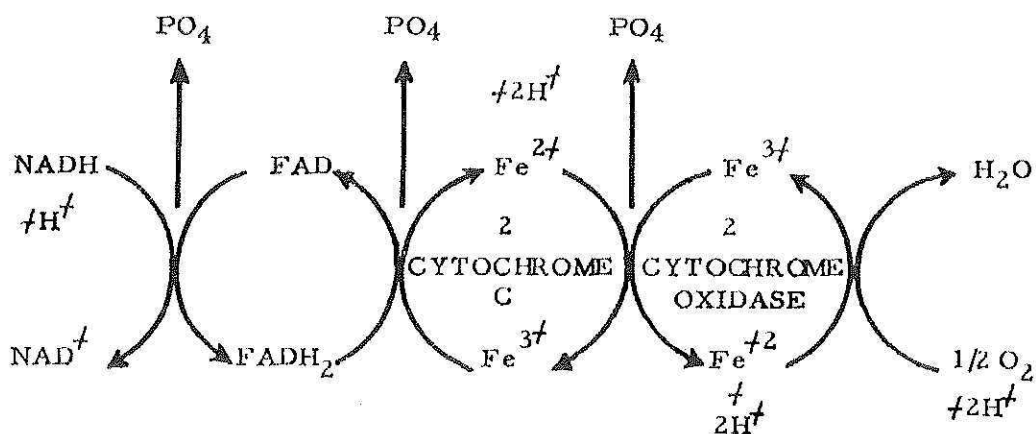

Fig. 8.—Diagramatic summary of oxidative phosphorylation, as illustrated by Conn and Stumpf (8).

example of the integrated multienzyme action which is indispensable for the maintenance of living matter.

SUMMARY

A study was made of the distribution and properties of cytochrome C oxidase in sugarcane. The enzyme was extracted from fresh root tissues of variety P.R. 980 with water and precipitated with ammonium sulfate. Cytochrome C oxidase was assayed spectrophotometrically at 550 mμ by measuring the optical-density decline of a buffered solution of reduced ox-heart cytochrome C and enzyme.

[10] Mitochondria are rod- or spherical-shaped bodies found in nearly all plant and animal cells. Intact mitochondria seem to act as complete biochemical units. Intermediates of the tricarboxylic acid cycle and fatty acid oxidation are usually oxidized without adding cofactors, and, in like manner, complete compliments of cytochromes needed for electron transport are found in the mitochondria. It is interesting to note that extracts of rat-liver mitochondria have been found to contain particles which in themselves perform oxidative phosphorylation (10, 13).

Fractionation of cane extracts with ammonium sulfate showed that cytochrome C oxidase is precipitated primarily between 80- and 95-percent saturation. This corresponds to the range of little or no tyrosinase precipitation, indicating that tyrosinase may act to mask the action of cytochrome C oxidase in crude cane preparations. Richest source of the oxidase was root tissue, although moderate activity was also present in the 80- to 95-percent fraction of meristem extracts. Some activity was likewise obtained with sheath and node preparations.

Cytochrome C oxidase was stable for about 24 hours when maintained in dilute cysteine-HCl solution at 2°C. More than 50-percent of the activity was lost after 9 days. The enzyme was not adversely affected by freezing. It was totally inactivated by boiling for 5 minutes.

Optimum temperature lay in the range of 36° to 40°C. Optimum pH was about 4.3. Cytochrome C oxidase activity was plotted as a first-order reaction with reference both to enzyme concentration and time. Iron appeared to be a limiting factor in the standard root preparations. Km was 4.5×10^{-5} mols of reduced cytochrome C per liter.

Both mercury and cysteine-HCl stimulated the enzyme at the concentration 2.0 μmols/ml. of digest. Boron and nitrate stimulated to a lesser extent at the same concentration, and cyanide caused total inhibition. Carbon monoxide did not inhibit when tested under normal light conditions.

Dialysis against distilled water caused 70-percent activity decline within 2 hours, and almost total inactivation by 44 hours. Cysteine-HCl served to reactivate the enzyme. Iron increased activity of the dialyzed preparation over that of the undialyzed control, indicating that this element is essential for maximum activity.

Significance and potential roles of cytochrome C oxidase in sugarcane were discussed.

RESUMEN

Se hizo un estudio de la distribución y propiedades de la citocroma C oxidasa en la caña de azúcar. La enzima se extrajo con agua, de tejidos frescos de la raíz y se precipitó con sulfato de amonio. La prueba se hizo con un espectrofotómetro a 550 mμ, midiéndose la disminución en densidad óptica de una solución amortiguada y reducida de citocroma C, preparada de corazón de buey, y enzima.

Al separarse por fraccionación los extractos de la caña con sulfato amónico la citocroma C oxidasa se precipitó principalmente cuando alcanzó una saturación de 80 a 95 por ciento. Esto corresponde al nivel donde hay poca o ninguna precipitación en el caso de la tirosinasa, indicando que ésta puede actuar enmascarando la acción de la citocroma C oxidasa en preparaciones crudas de caña. La mejor fuente de oxidasa fue el tejido de

la raíz, aunque también se observó una actividad moderada en una fracción de 80 a 95 por ciento que se separó de los extractos meristemáticos. Asimismo se observó alguna actividad en las preparaciones de la yagua y el nudo.

La citocroma C oxidasa se mantuvo estable alrededor de 24 horas cuando permaneció en una solución diluída de cisteína-HCl a 2°C. Más del 50 por ciento de la actividad se suspendió después de 9 días. La enzima no se afectó adversamente al congelarse, aunque se inactivó totalmente cuando se hirvió por 5 minutos.

La temperatura óptima fue de 36 a 40°C. y el pH óptimo de alrededor de 4.3. La actividad de la citocroma C oxidasa se trazó como una reacción de primer orden con referencia tanto a la concentración enzimática como al factor tiempo. El hierro pareció ser un factor limitativo en las preparaciones standard de la raíz. El Km fue 4.5×10^{-5} mols de citocroma C reducida, por litro.

Tanto el mercurio como la cisteína-HCl estimularon la enzima a una concentración de 2.0 mols por ml. de digesto. El boro y el nitrato la estimularon en menor medida a la misma concentración y el cianuro causó una inhibición total. El monóxido de carbono no causó inhibición alguna cuando se probó bajo condiciones normales de luz.

La diálisis en presencia de agua destilada causó una disminución de 70 por ciento en la actividad, en 2 horas, y la inactivación casi total en 44 horas. La cisteína-HCl sirvió para reactivar la enzima. El hierro aumentó la actividad de la preparación dializada, en contraste con la no dializada, lo cual demuestra que este elemento es esencial para lograr una actividad máxima.

Se discute en este trabajo el significado y el papel potencial que desempeña la citocroma C oxidasa en la caña de azúcar.

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