Cytochrome Components & Electron Transfer in Sweet Potato Mitochondria [1]

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Studies of sweet potato (*Ipomoea batatas*, Poir) mitochondria were extended, in our present investigation, to include spectrophotometric observation of cytochromes in response to substrates and inhibitors. These studies were facilitated by the use of a white variety of sweet potato (var. Pelican Processor) which is low in carotenoids, and by the use of a modified Perkin-Elmer Spectracord. Cytochrome spectra were also obtained at liquid nitrogen temperature.

We used a preparation showing good P/O ratios, no cytochrome *c* requirement, and a degree of respiratory control by ADP[2] (2-fold stimulation by ADP). Observations on the mitochondrial respiratory chain, made by manometric and spectrophotometric methods, were integrated. Electron transfer in this system was relatively insensitive to respiratory-chain inhibitors. This phenomenon has been the subject of intensive investigations in a number of plant systems (2, 3, 5, 8, 10, 13, 14, 15, 16, 17, 22, 25, 27, 28). It is hoped that our results will add some insight into the problem of inhibitor-resistant respiration, and increase our understanding of the cytochrome components in plant mitochondria.

Materials & Methods

► Preparation of Mitochondria: Cured sweet potato roots were stored at 15.5 C and used as needed. Roots were transferred to a cold room (0–2 C) about one hour preceding the isolation of mitochondria. Tissue (200 g) from two or three chilled roots was grated rapidly into a cold medium containing tris buffer, 0.15 M; sucrose, 0.25 M; EDTA, 0.01 M; ascorbate, 0.01 M. The ascorbate was added to a solution of the other components which were at pH 8.0. The final pH of the tissue homogenate was 7.7. The method used to isolate the mitochondria was similar to that used by Lieberman and Biale (21). Sucrose at 0.4 M was used to wash and resuspend the mitochondria which were collected at 12,000 × *g* for 15 minutes in early experiments and at 8,000 × *g* for 15 minutes in later experiments.

► Spectrophotometric Studies: Difference spectra of mitochondrial pigments were recorded with a Model 4000-A Perkin-Elmer Spectracord which was modified to provide an expanded scale. These recordings represented differences in light absorption between samples with and without substrate. The band widths of the monochromatic beams were about 1 mμ in the 550 to 560 mμ region of the spectrum. Scans were made from 620 mμ to 400 mμ and the time required was 1½ minutes.

Low-temperature spectra of the mitochondrial cytochromes were obtained with a single-beam spectrophotometer described by Butler and Norris (9). A mitochondrial suspension (4 ml) containing about 0.5 mg N, placed in the cell, produced a sample of 4 or 5 mm depth. The system response of the single-beam instrument was adjusted to give a flat baseline with a non-absorbing, light-scattering sample of $CaCO_3$. This approximately compensated for the scatter losses of the mitochondrial suspension. Two to four minutes after adding succinate to the suspension, the sample was cooled to −196 C in a special Dewar flask, and maintained at this temperature throughout the scanning period by liquid nitrogen around the cell (23).

► Manometric Experiments: Oxygen utilization by the mitochondria, in the presence of various substrates, was measured manometrically. Concomitant esterification of inorganic phosphate was followed by measuring its disappearance, using the method of Bernhart and Wreath (4). Robbie's method (24) was used for controlling the concentration of HCN in reaction mixtures. Nitrogen contents of the mitochondrial suspensions were determined by digestion of samples and Nesslerization as described by Thompson and Morrison (26).

Results

► Manometric Studies: Values for oxidative phosphorylation by the sweet potato mitochondria in the presence of various substrates are recorded in table I. No values for oxygen uptake in vessels without substrate are given since they were always negligible, i.e., they rarely differed from the thermobarometer reading. Succinate served particularly well as a substrate, yielding much higher rates of oxygen uptake than either α-ketoglutarate, citrate, or any other Krebs-cycle intermediate. It appears possible that most of the oxygen uptake in the presence of succinate was due to the one-step oxidation to fumarate, since

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[2] Abbreviations used are as follows: ADP, adenosine diphosphate; ATP, adenosine triphosphate; DPT, diphosphothiamine; CoA, coenzyme A; DPN, diphosphopyridine nucleotide; HOQNO, 2-*n*-heptyl-4-hydroxyquinoline-N-oxide; tris, tris(hydroxymethyl)-aminomethane; EDTA, ethylenediaminetetraacetate; P_i, inorganic phosphate; BSA, bovine serum albumin.

Table I

Oxidative Phosphorylation of Sweet Potato Mitochondria with Various Substrates*

Expt.	Duration of expt. Min	Substrate	Oxygen uptake μatoms	Orthophosphate esterified μmoles	P/O
1	50	α-Ketoglutarate	12.7	26.4	2.1
		α-Ketoglutarate + malonate	3.06	9.3	3.0
2	50	Succinate	20.0	38.4	1.9
3		Citrate	10.5	23.0	2.2
4	60	DPNH	14.6	20.6	1.4

* The reaction mixture contained in micromoles: sucrose, 1200; CoA, 3.9×10^{-2}; DPT, 6.6×10^{-2}; DPN, 3.3×10^{-1}; glucose, 60; ATP, 2.0; PO_4, 60; $MgSO_4$, 18; substrate, 30; malonate, where indicated, 30. The total volume was 3.0 ml and also contained 1.0 mg hexokinase, 0.03 % BSA, and mitochondrial suspension containing approximately 0.5 mg N. The temperature of the reaction mixture, under an air gas phase, was maintained at 25 C.

Table II

Effects of ADP & Freezing on Respiration & Oxidative Phosphorylation of Sweet Potato Mitochondria*

Expt.	Duration of expt.	Substrate	Treatment	Oxygen uptake μatoms	Orthophosphate esterified μmoles	P/O
1	40 min	α-Ketoglutarate	Minus ADP	7.06	0	
			ADP, 16.6×10^{-5} M	11.8	15.9	1.35
			ADP, 6.6×10^{-4} M	15.5	28.4	1.83
2	40 min	Succinate	Control	19.5	29.8	1.53
			Frozen overnight & thawed	18.4	24.0	1.30
		Citrate	Control	7.0	14.0	2.0
			Frozen overnight & thawed	3.5	4.4	1.25

* N content in the reaction mixture was 0.5 mg.

Table III

Effect of Various Inhibitors on Oxidative Phosphorylation by Sweet Potato Mitochondria*

Expt.	Duration of expt. Min	Substrate	Inhibitor	Oxygen uptake μatoms	Orthophosphate esterified μmoles	P/O
1	30	Succinate	None	10.3	15.9	1.55
			0.5 μg antimycin A	6.03	0	0
			1.0 " " "	5.6	0	0
			5.0 " " "	5.3	0	0
2	50	Succinate	None	16.4	32.7	2.00
			3.3×10^{-4} M NaN_3	9.9	0	0
3	50	Succinate	None	14.6	26.3	1.80
			1×10^{-4} M HCN	11.0	4.4	0.40
			4.6×10^{-4} M HCN	10.0	0	0
4	50	Succinate	None	22.5	33.6	1.5
			3 μM HOQNO	17.0	0	0
		Citrate	None	12.0	20.9	1.8
			3 μM HOQNO	6.6	0	0

* Reaction mixtures and conditions were as described in table I.

the rate of fumarate oxidation was less than one-eighth that for succinate. Oxidative phosphorylation in the succinoxidase system was more resistant to freezing than that associated with citrate oxidation, as shown in table II.

The stimulatory effect of ADP on oxidation of α-ketoglutarate is shown in table II. In other experiments various concentrations of ADP gave the same magnitude of response over the first 10 minutes. Values in table II were taken at the end of the experiment, and, therefore, reflect a continuing requirement for phosphate acceptor by the oxidative process.

Effects of various inhibitors on oxygen uptake and phosphorylation by the mitochondria are summarized

Table IV

Effects of Antimycin A & HOQNO on Oxidation of DPNH & Reduction of Cytochrome *c* by Sweet Potato Mitochondria

Expt.	Reaction* mixture	−Δ O.D. 340/min.	Δ O.D. 550/min	% Inhibition
	DPNH oxidation			
1	+ DPNH	0.328		
	+ DPNH & 0.16 μg antimycin A/ml	0.020		94
2	+ DPNH	0.300		
	+ DPNH & 3 μM HOQNO	0.007		98
	Cytochrome *c* reductase			
3	+ DPNH		0.076	
	+ DPNH & 3 μM HOQNO		0.004	95
4	+ Succinate		0.040	
	+ Succinate & 3 μM HOQNO		0.000	100
5**	Control		0.400	
	+ 1.7 μg antimycin A/ml		0.020	95

* In experiments 1 and 2, the reaction mixtures contained 1 mg hexokinase, mitochondrial suspension containing approximately 25.0 μg N, and the following in micromoles: DPNH, 0.06; PO_4, 20; ATP, 1.0; Mg, 6.0; glucose, 60; sucrose, 800; tris buffer, pH 7.5, 100. Alcohol or an alcoholic solution of antimycin A or HOQNO was added, depending on whether a control or inhibition assay was run.
In experiments 3 and 4, the basic additions were the same as in 1 and 2, except that 0.0015 μ mole cytochrome *c* and 12 μ moles succinate or 0.06 μ mole DPNH were used.

** In experiment 5, mitochondrial suspension containing approximately 250 μg N was incubated with the reaction mixtures for 1 hour at room temperature before starting the reaction with succinate. Rates of cytochrome *c* reduction were measured from difference spectra recorded with the Spectracord.
All values represent single assays but were reproducible.

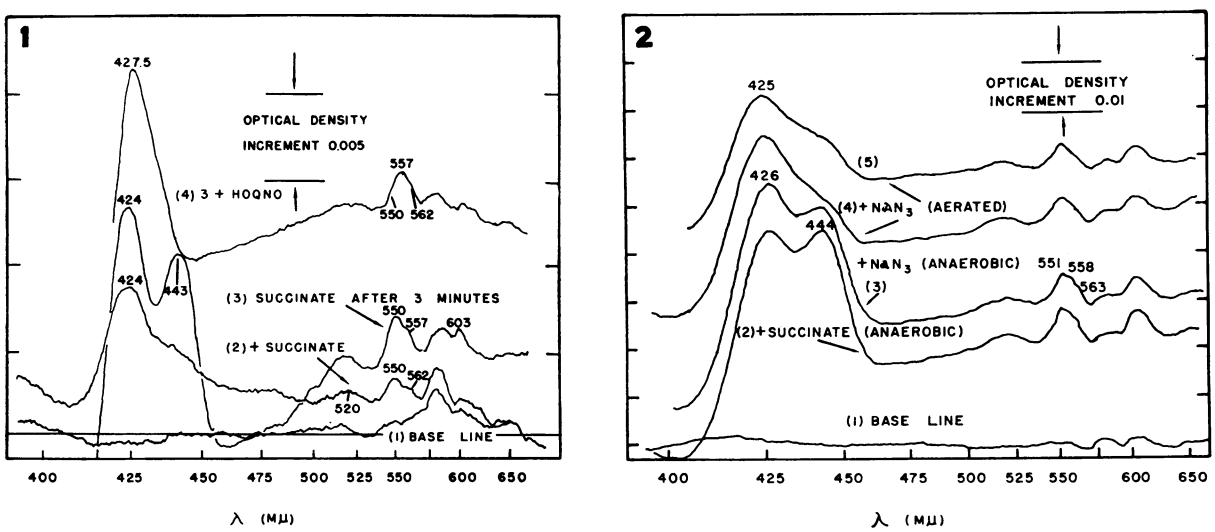

Fig. 1. Difference spectrum of a suspension of mitochondria supplied succinate (0.005 M) and an aerobic suspension without succinate.

Curve 1. Base line obtained with aerated suspensions of mitochondria without substrate in both light paths. *Curve* 2. Succinate added to the sample cuvette and a recording of the difference spectrum begun immediately. The time required to make the scan from 650 to 400 mμ was 2.0 minutes. *Curve* 3. A second difference spectrum recorded 3 minutes after curve 2 was started. *Curve* 4. Difference spectrum after HOQNO (3 μM) was added to the succinate-reduced suspension with concomitant aeration.

Fig. 2. Effect of azide on the difference spectrum of a system similar to that described for figure 1.

Curve 1. Base line obtained as in figure 1. *Curve* 2. Succinate was added to the sample cuvette and this recording was made after anaerobic conditions were reached. *Curve* 3. Recording made after NaN_3 (0.002 M) was added to the succinate-reduced suspension with minimum aeration. *Curve* 4. Recording made immediately after vigorous aeration. *Curve* 5. Same as curve 4, but made 3 minutes later.

in table III. These inhibitors were only moderately effective in inhibiting oxygen utilization by these particles. The inhibition decreased somewhat with time in the case of antimycin A, which is similar to the observation of Lieberman (20) in the study of apple particles. However, the same concentrations of inhibitors (cyanide, azide, antimycin A, & HOQNO) produced drastic effects on the ability of the particles to esterify inorganic phosphate. The results in table III show stronger inhibitory effects on phosphorylation by the compounds than reported by Hackett et al. (16).

Succinic-cytochrome *c* reductase and DPNH-cytochrome *c* reductase activities of the mitochondria were almost completely inhibited by antimycin A and HOQNO (table IV). Succinoxidase activity, however, was only moderately inhibited by similar concentrations of these inhibitors (table III). Interpretation of these data with respect to an alternate pathway will be considered in the Discussion.

The possibility that the antimycin A-resistant oxidation might be mediated by a polyphenol oxidase system or by the formation of peroxide was evaluated by adding catalase and phenylthiourea to the antimycin-inhibited system. No substantial inhibitory effects by these inhibitors were observed (table V).

► Spectrophotometric Studies: Difference spectra between mitochondria in media with and without substrate, were recorded at 2-minute intervals after the addition of substrate. In order to include more than one trace in figures 1 and 2, the recorder pen was moved up along the y axis for each recording. One should imagine each curve in the position of the base line and mentally subtract the base line from each to determine the absorption due to components being reduced or oxidized. Immediately after the addition of succinate (fig 1), observations were made of components which absorbed at 560 mμ, 551 mμ, and which produced a broad absorption band centering at 424 mμ, in the Soret region. As the medium approached

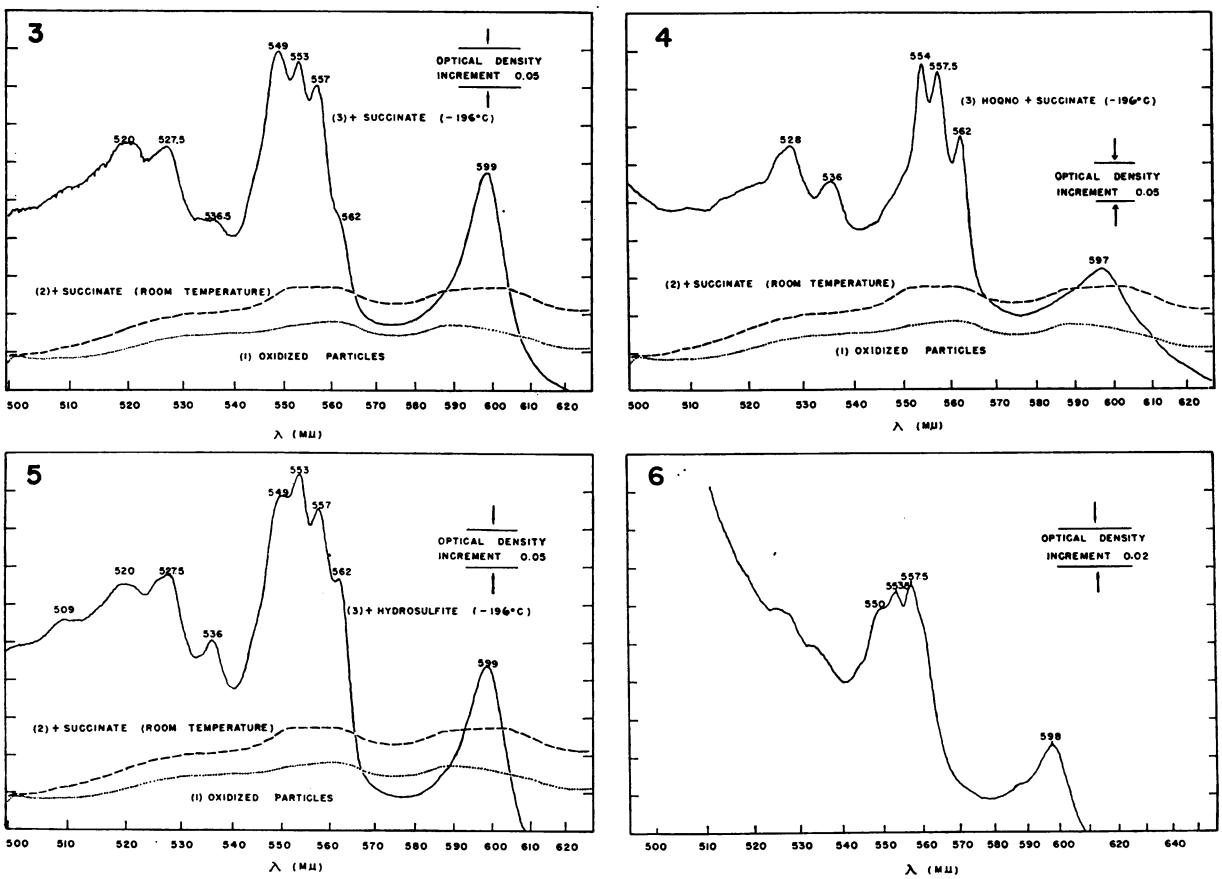

Fig. 3. Low-temperature absolute spectrum of succinate-reduced mitochondria. (1) Aerobic suspension of mitochondria at room temperature; (2) Succinate-reduced, anaerobic suspension of mitochondria at room temperature; (3) Succinate-reduced suspension cooled to the temperature of liquid nitrogen.

Fig. 4. Low-temperature absolute spectrum of mitochondria reduced with succinate after pre-treatment with HOQNO.

Fig. 5. Low-temperature absolute spectrum of mitochondria reduced with hydrosulfite.

Fig. 6. Low-temperature absolute spectrum of a ⅛ inch slice of sweet potato tissue.

anaerobiosis, bands appeared with maxima at 603 and 443 mμ. Addition of antimycin-A with stirring, caused the appearance of strong bands with absorption maxima at 428 and 557 mμ and the disappearance of the 551, 603, and 443 mμ bands. In this particular spectrum, a skewing toward longer wavelengths indicated that a component absorbing between 560 and 565 mμ might be present. These spectra and changes in them due to antimycin-A treatment and anaerobiosis, indicate the participation of cytochrome types *b*, *c*, and *a*. Photoreversibility of carbon-monoxide inhibition of electron transport indicates that the terminal oxidase is cytochrome a_3 (16).

Table V

Oxygen Uptake by Mitochondria in Presence of Antimycin A & Antimycin A plus Catalase or Phenylthiourea*

Reaction mixture	Oxygen uptake after 60 min μl
Complete	178
+ 5 μg Antimycin A	124
+ 10^{-3} M Phenylthiourea	167
+ 5μg Antimycin A & 10^{-3} M Phenylthiourea	105
+ 5 μg Antimycin A & 0.1 ml Catalase	108

* Contents of the reaction mixture and conditions were as described in table I. The nitrogen content of the added mitochondrial suspension was ca. 0.25 mg.

Azide added to an anaerobic, succinate-reduced suspension of mitochondria, with a minimum of aeration, produced no striking change in the difference spectrum (fig 2). Further aeration resulted in a decreased absorption at 443 to 444 mμ and an increased absorption at about 430 mμ. Anaerobiosis again was reached, since azide only partially inhibited oxygen uptake in the system, and as this occurred, the band at 443 mμ reappeared. This could indicate that a portion of the ferri-cytochrome a_3 was uncombined with azide, or that a dissociation of the a_3-azide complex allowed a slow reduction of a_3. It would appear that more than a trace of oxygen is required in this system for the ferro-ferri-cytochrome a_3 shift observed by Keilin and Hartree (19).

In order to investigate these cytochrome components more closely, low-temperature spectra of the particles were observed after various treatments. A single-beam spectrophotometer was used in these experiments, and the spectra of the reduced mitochondrial cytochromes were recorded rather than difference spectra. The spectrum of a succinate-reduced suspension recorded at room temperature and at −196 C is given in figure 3. At −196 C, the recording showed sharply defined absorption bands with maxima at 549, 553, 557, 562, and 599 mμ. Treatment of the mitochondria with HOQNO (3 μM) before the addition of succinate, produced a spectrum with absorption maxima at 554, 558, and 562 mμ (fig 4). The bands of cytochromes *c* and $a + a_3$ at 549 and 598 to 599 mμ were decreased markedly by this treatment. Figure 5 shows that reduction of the mitochondria with hydrosulfite resulted in somewhat greater intensity in the 553, 557, and 562 mμ peaks, but no additional cytochromes were reduced. Similar absorption bands were observed in low-temperature spectra of sweet potato tissue slices (fig 6). Due to the low concentration of oxygen in the tissues, these components are normally reduced.

Two subcellular fractions sedimenting at higher centrifugal forces than mitochondria gave the low-temperature spectra recorded in figure 7, when reduced with hydrosulfite. The adsorption maxima in these spectra agree closely with those of microsomal fractions of other plant materials (6, 22). The absorption bands in the low-temperature spectrum of whole tissue (fig 6) could very well represent those of cytochrome components in mitochondria and microsomes. The 554 mμ band of microsomes would coincide with the band at 553 to 554 mμ in mitochondria. The 562 mμ band observed in mitochondrial preparations and the 560 mμ band observed in microsomal preparations could produce the poorly defined band at ca. 560 mμ observed in spectra of tissue slices.

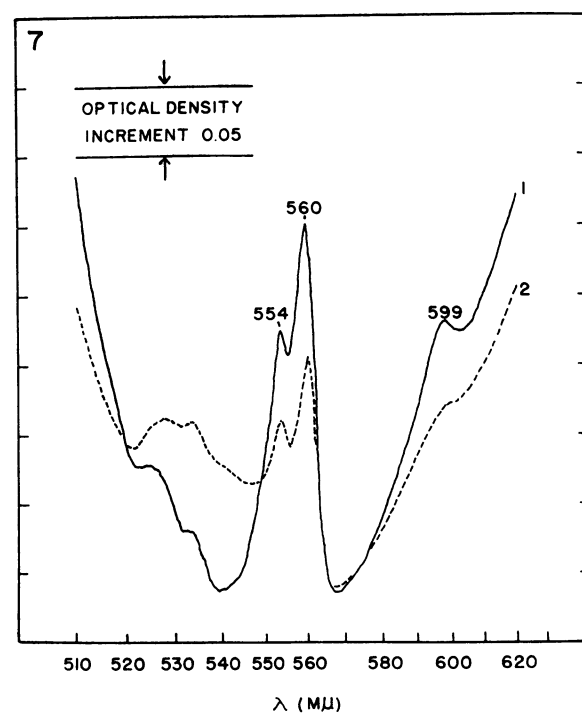

Fig. 7. Low-temperature absolute spectra of particles sedimenting at higher centrifugal speeds than mitochondria (microsomal fractions). (1) Hydrosulfite-reduced particles which sedimented from 36,000 × *g* to 100,000 × *g* for 1 hour. (2) Hydrosulfite-reduced particles which sedimented between 19,000 × *g* and 36,000 × *g* for 1 hour.

Discussion

Responses of cytochromes to substrates and inhibitors indicate that an electron transport system involving cytochromes of the types *b*, *c*, and *a*, operates in respiring sweet potato mitochondria. Prominent absorption bands at 549, 553, 557, 562, and 599 mμ were evident in low-temperature spectra of succinate or hydrosulfite-reduced mitochondria (figs 3 & 5). Treatment of the mitochondria with antimycin A or HOQNO prior to adding succinate resulted in the disappearance of the 549 and 599 mμ bands, which is interpreted to result from the blocking of electron flow to cytochrome *c* by these compounds. Consequently, cytochromes *c* and $a + a_3$ were oxidized and not again reduced. Similar results were observed by Bendall (2), who studied Arum mitochondria and Bonner (6), who studied a number of plant mitochondrial systems. As Bendall (2) noted, the 553 mμ component does not react as cytochrome c_1, since the reduction of c_1 is inhibited by antimycin A. Also of significance, is the persistence of three bands at 554, 558, and 562 mμ after antimycin A or HOQNO treatment, instead of the one band for cytochrome *b* observed with animal mitochondria.

Observations were made in our study (figs 3 & 4) which indicate that these three bands represent different components, and not satellite bands resulting from low temperature. These include: A: increase in the intensity of the 562 mμ band after HOQNO treatment: B: equal intensity of the 553 and 558 mμ bands; C: three distinct β bands which are accentuated by HOQNO treatment. Whether these components are obligatory participants in the electron transport chain has not been proved. There is some evidence that cytochrome *b* is not an obligatory component in the oxidation of succinate by submitochondrial particles from heart tissue (12), but no comparable studies have been made with plant systems. A sequential reduction of cytochromes *b* or *b*'s, *c*, and $a + a_3$ is observed in difference spectra as in figure 1, although the course of reduction in that particular case has progressed to such an extent that one can only see that cytochrome a_3, or the 443 mμ component is reduced last.

Although the cytochrome system of sweet potato mitochondria responds to substrates and respiratory-chain inhibitors much as expected, a considerable amount of oxygen utilization by the mitochondria is observed when inhibitors of cytochrome oxidase and compounds inhibiting at the level of cytochrome *b*, (antimycin A & HOQNO) are included in the assay medium. A number of hypotheses have been considered recently in attempts to elucidate similar results observed with other plant systems. Bendall and Hill (3), on the basis of absorption spectra of respiring Arum mitochondria, concluded that a *b*-type cytochrome remained largely oxidized, while cytochromes *c* and *a* remained largely reduced in the presence of cyanide. They suggested that this cytochrome, which they named cytochrome b_7 might mediate cyanide-resistant respiration by virtue of its autoxidizability. Bendall (2) showed also that antimycin A did not prevent the oxidation of this cytochrome. These results suggested the operation of an alternate pathway which would account for both cyanide- and antimycin A-resistant respiration. However, Bonner (6) and Bonner and Ito (7) found no evidence of a *b* cytochrome peculiar to mitochondria which exhibit inhibitor-resistant respiration. This has also been our experience. The relative amounts of the cytochromes in various systems may have a bearing on this matter, since Hackett et al. (15) observed an increase in concentration of a *b* cytochrome in potato tuber tissue as cyanide-resistant respiration developed.

Chance and Hackett (10) accepted the b_7-alternate pathway hypothesis as a possibility, but pointed out that residual oxidase not complexed with inhibitor might be sufficient to account for cyanide-resistant respiration. Keilin and Hartree (18) originally observed that carbon monoxide had little effect on the activity of heart-muscle cytochrome oxidase, unless substrate and cytochrome *c* were present in excess. This means simply, that an enzyme catalyzing a reaction of a coupled sequence can be partially poisoned without affecting the overall rate, if the reaction it catalyzes is not the rate-limiting one. The same possibility exists with respect to the antimycin A- and HOQNO-sensitive reaction.

A finding by Hackett and Haas (14) which doesn't seem to support the excess enzyme hypothesis is that low concentrations of cyanide inhibit oxidative phosphorylation more severely than respiration in skunk cabbage mitochondria. This effect is not observed with animal systems. Further investigations (12, 15) yielded similar results for potato tuber tissue and mitochondria. Results of Hackett et al. (16) and Baker and Lieberman (1), show that antimycin A and HOQNO also inhibit oxidative phosphorylation more severely than the oxygen utilization of respiring plant mitochondria. This effect is illustrated by the data presented in table III. Hackett et al. (16) pointed out that these results could be produced by the operation of an alternate non-phosphorylative pathway. They realized that other possible reasons for this phenomenon exist, e.g., the rate of oxidative phosphorylation may be more dependent on steady-state oxidation-reduction levels of the respiratory components than is electron transfer. We would like to point out that the reactions involved in the esterification of inorganic phosphate have not been characterized for plant systems, and that possibly one or more of these are inhibited by the compounds in question. The operation of an alternate pathway not involving cytochromes *c*, and $a + a_3$ has not been demonstrated unequivocally.

With respect to the oxygen uptake by sweet potato mitochondria, in the presence of various inhibitors (table III), three additional points should be considered. First, systems which probably do not contribute to the residual oxygen uptake, are ones involving polyphenol oxidase or formation of hydrogen peroxide. Neither phenylthiourea nor catalase ap-

preciably reduced the antimycin A-insensitive respiration (table V).

Second, cytochrome oxidase activity of sweet potato mitochondria was effectively inhibited by NaN_3 (data not presented). However, it is possible that some of the enzyme is inaccessible to inhibitors such as cyanide and azide, and that activity measured in assays utilizing cytochrome *c* was due to enzyme on the surface of the particles. An observation which might indicate that these mitochondria contain some cytochrome a_3 free to be oxidized and reduced in the presence of azide, is the reappearance of an absorption band at 443 to 445 mμ, after azide treatment (fig 2). No conclusive statement can be made, however, on the basis of these data.

A third point is that DPNH-oxidase and cytochrome *c* reductase activities of the mitochondria are effectively inhibited by antimycin A or HOQNO (table IV). Since resistance to these inhibitors increases with time in the manometric assay, the possibility that they might be inactivated with time had to be considered. This possibility was tested by incubating mitochondria with and without antimycin A for 1 hour before measuring succinic cytochrome *c* reductase activity (table IV). Succinic-cytochrome *c* reductase activity of the particles was still effectively inhibited at the end of 1 hour. Appropriate tests were made in order to insure that this result was not complicated in any way by the presence of microorganisms. All of the data point to the effectiveness of antimycin A in blocking electron transfer to cytochrome *c*. Although the data hint strongly of an alternate pathway or shunt which branches away from the normal pathway at the level of cytochrome *b*, they do not constitute proof. The critical experiment which would actually prove or disprove the existence of such a pathway, is yet to be devised. In the meantime, attempts should be made to clarify the role of the cytochrome components present in plant mitochondria which exhibit certain properties of cytochrome *b*. Are they obligatory participants in the oxidation of succinate and other substrates by mitochondria or do they have other specific functions? These and other questions are now under consideration.

Summary

Our investigation indicates that cytochromes of types *b*, *c*, and *a* are involved in electron transport of respiring sweet potato mitochondria. Adding succinate to an aerobic suspension of mitochondria caused reduction of pigments which absorbed light maximally at 558, 551, 600 to 605, and 443 mμ, in that sequence. These components responded to the inhibitors, antimycin A, HOQNO, and azide, much as expected.

Low-temperature spectra of succinate- or hydrosulfite-reduced mitochondria revealed absorption maxima at 549, 553, 558, 562, and 598 mμ. Treatment of the mitochondria with HOQNO, before adding succinate, produced a three-banded spectrum with peaks at 554, 558, and 562 mμ. Observations indicate that these bands represent three separate cytochrome components. The role of these components in electron transport has not been established. None of the cytochromes present in these mitochondria exhibited properties of cytochrome c_1. Low-temperature spectra of tissue slices revealed absorption bands which could be ascribed to cytochrome components of the mitochondria and microsomes.

Findings by other investigators are corroborated in that, azide, cyanide, antimycin A, and HOQNO affected oxidative phosphorylation, of respiring sweet potato mitochondria, more severely than oxygen uptake. Antimycin A inhibition of mitochondrial respiration decreased with time. Evidence is presented to show that this decrease was not due to inactivation of antimycin A. Indeed, all evidence, such as inhibitory effects of antimycin A on cytochrome *c* reductase activities, indicate that this compound effectively blocks transfer of electrons to cytochrome *c*, by sweet potato mitochondria. All of these results are consistent with the idea that a non-phosphorylative pathway of electrons, or a shunt bypassing the antimycin A-sensitive factor and cytochrome *c*, exists in certain plant mitochondria. However, no proof for such a pathway is offered.

A recent report, Bonner, W. D. and Voss, D. O., Nature 191: 682–684, show absorption bands in low-temperature spectra of sweet-potato mitochondria similar to ones we observed.

Acknowledgments

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