Summary

An analysis of the intracellular distribution of the enzyme that converts tryptophan to indoleacetic acid has been made for the mung bean seedling. Cellular fractions were separated by differential centrifugation and their indoleacetate-forming activities were determined by chromatographic resolution of the auxin. It is concluded that the tryptophan-to-indoleacetate enzyme system occurs as a soluble component of the cytoplasm, and that it is in the group of heterogeneous macromolecules with sedimentation coefficients between zero and four Svedberg units.

The participation of Dr. Clifford Sato at the start of this investigation is gratefully acknowledged. I also wish to thank Dr. Arthur L. Koch and Mr. William A. Lamont for their help in determining the sedimentation patterns, and Mrs. Marcia Eib for her technical assistance. A preliminary summary of this work has appeared (3).

Literature Cited

1. Gordon, S. A. Occurrence, formation, and inactivation of auxins. Ann. Rev. Plant Physiol. 5: 341–378. 1954.
2. Gordon, S. A. The biogenesis of natural auxins. In: The Chemistry and Mode of Action of Plant Growth Substances, R. L. Wain and F. Wightman, eds. Pp. 65–75. Butterworth Scientific Publications, London 1956.
3. Gordon, S. A. Auxin biosynthesis: a cytoplasmic locus of radiation damage. In: Proceedings of the International Conference on Radiology. Pp. 44–51. Cambridge University Press, Cambridge 1956.
4. Gordon, S. A. The effects of ionizing radiation on plants: biochemical and physiological aspects. Quart. Rev. Biol. 32: 3–14. 1957.
5. Gordon, S. A. and Paleg, L. G. Observations on the quantitative determination of indoleacetic acid. Physiol. Plantarum 10: 39–47. 1957.
6. Larsen, P. Growth substances in higher plants. In: Modern Methods of Plant Analysis, Vol. III, K. Paech and M. V. Tracy, eds. Pp. 565–625. Springer-Verlag, Berlin 1955.
7. Maclendon, J. H. and Blinks, L. R. The use of high molecular weight solutes in the study of isolated intracellular structures. Nature 170: 577–579. 1952.
8. Nason, A., Oldewurtel, H. A. and Probst, L. M. Role of micronutrient elements in the metabolism of higher plants. I. Arch. Biochem. Biophys. 38: 1–13. 1952.
9. Sorof, S. and Cohen, P. P. Electrophoretic and ultracentrifugal studies of soluble proteins of rat liver. Jour. Biol. Chem. 190: 311–316. 1951.
10. Svedberg, T. and Pedersen, K. O. The Ultracentrifuge. Oxford University Press, Oxford 1940.
11. Wildman, S. G. and Bonner, J. The proteins of green leaves. I. Isolation, enzymatic properties and auxin content of spinach cytoplasmic proteins. Arch. Biochem. 14: 381–413. 1947.
12. Wildman, S. G., Ferri, M. G. and Bonner, J. The enzymatic conversion of tryptophan to auxin by spinach leaves. Arch. Biochem. 13: 131–144. 1947.
13. Wildman, S. G. and Jagendorf, A. Leaf proteins. Ann. Rev. Plant Physiol. 3: 131–148. 1952.

OXIDATIVE PHOSPHORYLATION AND FUNCTIONAL CYTOCHROMES IN SKUNK CABBAGE MITOCHONDRIA[1,2]

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The extremely rapid respiration of the spadix of certain members of the Araceae has attracted special attention because of its insensitivity to such classical inhibitors as cyanide and carbon monoxide. Although it was concluded that a flavoprotein serves as the terminal oxidase in such tissues (15), it is now clear that they contain high concentrations of cytochromes which probably function in respiration (2, 4, 12, 20, 22, 25). Following the demonstration that isolated spadix mitochondria oxidize Krebs cycle acids very rapidly (13), it was shown that most of the cytochrome components are also localized on these particles (2, 4, 12). The mitochondria contain enough cytochrome c oxidase (22) and diphosphopyridine nucleotide (DPNH) oxidase (12) to account for essentially all of the tissue respiration. The conclusion that terminal respiration in the Aroid spadix, like that in other plant tissues (11), is mediated by the particle-bound cytochrome system is supported by spectrophotometric observations of the intact tissue (25).

The above findings do not indicate whether the rapid consumption of oxygen by spadix tissue is associated with the supply of utilizable energy to the cells. Is the respiration normally linked with phosphorylation and synthesis, or is it "uncoupled" and used for some other purpose, such as the generation of heat? To answer this question, it is first necessary to know whether the mitochondria are able to couple substrate oxidations to phosphorylations. Preliminary determinations indicated a very low efficiency of oxidative phosphorylation (13) and the present study was undertaken to obtain further evidence on this point. An attempt has also been made to examine quantitatively some of the cytochromes involved in

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the reaction of the mitochondria with oxygen. The effects of inhibitors were studied in order to explore the mechanism of the cyanide-resistant respiration.

Materials and Methods

Young skunk cabbage (*Symplocarpus foetidus*) spadices were collected either before the stamens had emerged from between the sepals or shortly thereafter and stored in the refrigerator (4° C). Only the flowers, cut away from the central axis, were used in the experiments. Cell fractions were isolated by differential centrifugation of tissue homogenates, prepared by grinding in a glass mortar with sand, and all operations were carried out at roughly 0° C. The grinding medium contained 0.5 M sucrose, 0.05 M TRIS (tris hydroxymethylaminomethane) buffer (pH 7.0), and 0.01 M neutralized EDTA (ethylenediamine tetraacetate) when particles were to be used in the manometric studies; otherwise, it contained 0.5 M sucrose and 0.05 M phosphate buffer (pH 7.0). The homogenate was strained through cheesecloth and centrifuged at 1,000 × G for 5 minutes to remove sand and cell debris. The mitochondrial fraction was isolated by centrifuging at 10,000 × G for 15 or 20 minutes, and washed by resuspending in the isolation medium and recentrifuging. Particles to be used in the spectrophotometric studies were washed twice in this manner. The washed pellet was suspended in a small volume of 0.5 M sucrose (for manometric studies) or 0.5 M sucrose–0.05 M phosphate buffer.

Oxygen uptake was measured at 30° C using conventional Warburg manometric techniques. The substrate, usually α-ketoglutarate, was tipped in after 10 minutes of equilibration. Phosphate esterification was measured by determining the disappearance of inorganic phosphate from the reaction mixture, to which glucose and hexokinase had been added. Fluoride was included to prevent hydrolysis of ATP (adenosine triphosphate) and malonate to block oxidation at the level of succinate. The cofactors added were: DPN, ADP (adenosine diphosphate), DPT (diphosphothiamine), and CoA (coenzyme A). The reaction was stopped after the appropriate interval by tipping in 0.5 ml of 20 % trichloracetic acid. Inorganic phosphorus was determined by the method of Bernhart and Wreath (3).

For the spectrophotometric measurements, an appropriate volume of mitochondrial suspension (equivalent to roughly 5 g of initial tissue) was transferred to a Lazarow and Cooperstein cell (17); buffer was added to give a final concentration of 0.025 M phosphate and 0.25 M sucrose in 3 to 4 ml. Substrate and/or inhibitor were added as the solid sodium salts of citrate, cyanide, or azide. The cell was then gassed for 10 minutes with nitrogen and the stopcocks closed. The absorption spectrum of the turbid suspension was determined in a Beckman DU spectrophotometer, equipped with photomultiplier attachment, using filter paper as the blank. Typical slit-widths were 0.05 mm for 400 to 500 mμ, 0.02 mm for 500 to 600 mμ, and 0.03 mm for 600 to 630 mμ; these values would give representative band widths of 1.5 mμ at 450 mμ, and 1 mμ at 550 mμ. The spectrophotometer was calibrated against mercury emission lines (Beckman mercury lamp). Following a determination of the spectrum either above or below 500 mμ, the cell was gassed for 10 minutes with oxygen, and another spectrum recorded. Difference spectra were obtained by subtracting the absorbance in oxygen from that in nitrogen. During the measurement of the spectra there was a slight decrease in absorbance at all wavelengths, possibly due to swelling or aggregation of particles; since the time of measurement (roughly one half hour) after gassing with N_2 or O_2 was essentially constant, this factor probably does not affect the difference spectra greatly.

Results

Oxidative Phosphorylation: Washed skunk cabbage mitochondria show essentially no endogenous respiratory activity, but they take up oxygen rapidly in the presence of Krebs cycle acids; with α-ketoglutarate, like succinate (12), the rate tends to fall off after the first 15 minutes. Table I shows the results of four experiments in which the oxygen uptake and phosphate esterification were determined in the presence of α-ketoglutarate. Although there is some variation in the results, phosphate/oxygen (P/O) ratios of greater than three were obtained in each experiment. In two other experiments, all the P/O values were between two and three. In general, the lower P/O ratios were correlated with higher oxygen uptake, suggesting that these preparations may have

Table I

Oxidative Phosphorylation with α-Ketoglutarate

Experiment	Oxygen uptake μ atoms/hr × vessel	Phosphate esterified μ atoms/hr × vessel	P/O
1	3.84	13.55	3.53
	4.55	15.29	3.37
	2.23	6.88	3.08
2	3.98	15.00	3.78
	3.58	13.25	3.70
	5.14	13.65	2.65
	5.75	14.81	2.59
3	1.43	5.03	3.52
	2.14	7.16	3.35
4	2.58	9.65	3.74
	3.12	11.42	3.66
	2.58	9.28	3.60

Contents of complete reaction mixture (in micromoles): substrate—60, phosphate—30, $MgSO_4$—1.8, ADP—3, DPN—1.2, DPT—0.2, CoA—0.1, NaF—30, malonate—6, glucose—60, and hexokinase—1 mg in a total of 3 ml. pH = 7.0. Each vessel contained mitochondria from approximately 3.3 g of tissue. Temp—30° C; gas phase—air; duration of experiment—30 minutes for 1 and 2, 20 minutes for 3 and 4.

TABLE II

EFFECTS OF DNP, AZIDE, AND CYANIDE ON OXIDATIVE PHOSPHORYLATION

EXPERIMENT	NUMBER OF VESSELS	INHIBITOR M CONC	O_2 UPTAKE μ ATOMS *	P_i ESTERIFIED μ ATOMS *	P/O
1	3	0	9.3	21.00	2.19
	2	10^{-5} DNP	7.4	3.78	0.53
	2	10^{-4} DNP	6.5	2.08	0.33
2	3	0	2.76	10.12	3.67
	2	10^{-3} Azide	1.9	4.84	2.54
	2	10^{-2} Azide	1.0	2.52	2.52
3	3	0	5.63	14.51	2.58
	2	10^{-3} Azide	3.91	7.36	1.89
	1	10^{-2} Azide	3.31	5.61	1.69
4	2	0	1.73	6.10	3.44
	3	4.6×10^{-4} Cyanide **	2.15	3.39	1.58

For contents of reaction mixture, see table I.
* Average value per vessel.
** KCN in main compartment and appropriate KCN-KOH mixture in center well.

been partially uncoupled. Omission of malonate from the medium in one experiment did not significantly alter the ratio. The average value for the P/O in all the experiments was 3.17, with a maximum of 3.78.

In order to verify the fact that the measured changes are due to oxidative phosphorylation, the effect of the uncoupling agent 2,4-dinitrophenol (DNP) was tested. Table II shows that low concentrations of DNP cause a drastic inhibition of phosphorylation, even though oxygen uptake is only slightly reduced. The small amount of phosphorylation remaining may be linked to substrate oxidation, since this step is not sensitive to DNP. This inhibitor is known to uncouple only the three phosphorylations associated with the oxidation of DPNH by O_2 (14).

The effects of two agents, azide and cyanide, which are used to block electron transport in the cytochrome system were determined. It has been shown that organic acid oxidations by spadix mitochondria are relatively insensitive to cyanide (12, 16, 22). Similarly, citrate and α-ketoglutarate oxidation by skunk cabbage particles are only inhibited approximately 30 % by 10^{-3} M azide. Table II shows that azide, at 10^{-3} and 10^{-2} M, inhibits phosphate esterification considerably more than O_2 uptake, so that the P/O value is lowered by roughly one unit. This was true whether the control value was less than or greater than three. A concentration of cyanide which had no effect on the oxygen uptake markedly inhibited the phosphorylation (table II); the decrease in P/O ratio suggests that two phosphorylative steps have been uncoupled. Although the cyanide results represent a single experiment, the replication was very good.

SPECTROPHOTOMETRIC OBSERVATIONS: The absolute absorption spectrum of a mitochondrial suspension shows a high absorbance between 400 and 500 mμ and relatively little at longer wavelengths. There are three major absorption bands, at 420 to 430, 440 to 450, and 475 to 480 mμ; the position and height of the first two bands are altered on going from the reduced to the oxidized state. A slight elevation in the absorbance at 550 to 560 mμ can be detected when the particles are in nitrogen. These spectra are very similar to those obtained with deoxycholate-solubilized skunk cabbage mitochondria (12).

To reveal the nature of the components which are involved in the reaction with molecular oxygen, difference spectra (N_2-O_2) were calculated and a typical curve from 400 to 500 mμ is shown in figure 1. In this region there are two major peaks, at 426 to 428 and 443 to 445 mμ; in both cases, the slight asymmetry suggests that more than one component may be

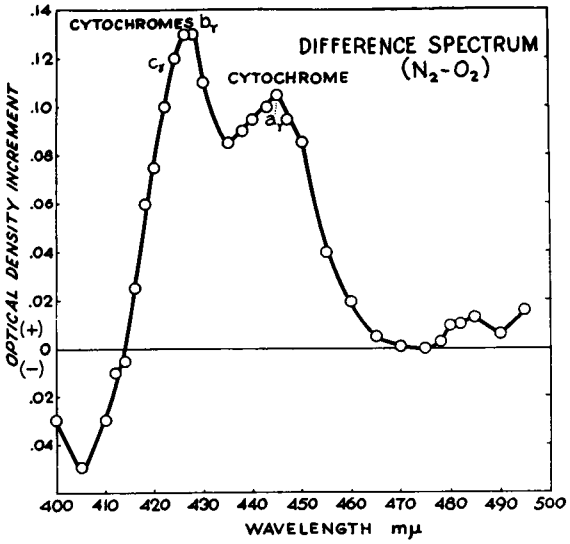

FIG. 1. The difference spectrum of a mitochondrial suspension gassed initially with N_2 and then with O_2.

represented. The first peak probably represents the combined bands of cytochromes of the b and c type, whereas the second corresponds well with the cytochrome oxidase (a_3) maximum. A smaller band at 480 mμ, with two apparent peaks, is also present in the difference spectrum. Although its nature has not been established, the fact that carotenoids absorb strongly in this region suggests that they may be altered when O_2 is added.

Figure 2 shows a difference spectrum from 500 to 630 mμ plotted on a wider optical density scale. The band centered around a peak at 525 mμ, with shoulders on each side, can be attributed to the combined β-bands of cytochromes c and b. There is a broad band from 543 to 565 mμ, with a maximum at 553; this band shows marked asymmetry towards the longer wavelengths, including humps at 558 and 562 mμ. These peaks represent the α-bands of cytochromes of the c and b type, with at least two b's suggested. The third band, with a peak at 602 to 605 mμ, corresponds well with the α maximum of cytochrome oxidase (a-a_3). Using values for the absorption maxima and isobestic points (reference wavelengths) determined for similar animal cytochromes (9), the amounts of the various cytochromes involved in the reaction with oxygen were computed (table III). The concentrations of functional cytochromes are of the order of 10^{-6} M; since the volume of suspension employed corresponded roughly to the volume of tissue from which the particles were obtained, similar intracellular concentrations are indicated. Bendall and Hill (2) have described a cytochrome b_7, with absorption maximum at 560 mμ, in mitochondria isolated from the sterile portion of *Arum maculatum* spadices. If this value of 560 mμ is substituted for the 564 mμ maximum of b, the calculated cytochrome concentration is 1.4 micromoles; this suggests that there may be considerably more b_7 than b in the particles. If the wavelength maximum at 553 mμ is used for the calculation of the amount of c-type cytochrome, a value of 1.42 micromoles is obtained. This may give an indication of the content of cytochrome c_1, which has an absorption maximum at 554 mμ (24), assuming it shows the same molecular extinction change.

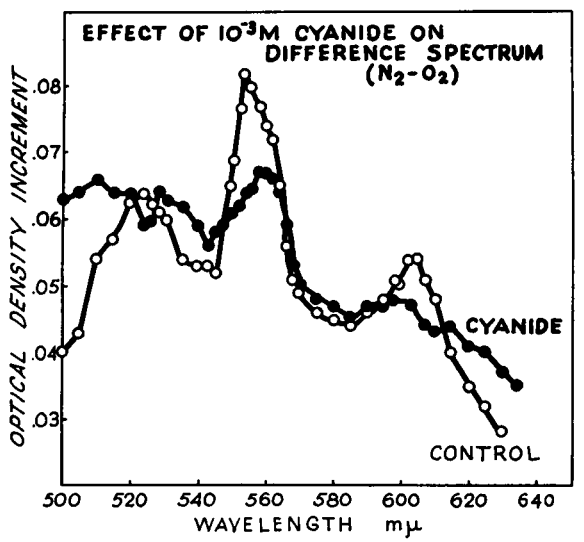

FIG. 2. The effect of cyanide on the difference spectrum of a mitochondrial suspension. The absolute spectra for the control in N_2 and O_2 were recorded, after which NaCN was added and the recordings repeated.

TABLE III

FUNCTIONAL AMOUNTS OF CYTOCHROMES DETERMINED FROM DIFFERENCE SPECTRUM

Cytochrome	a	b	c
Maximum λ	605	564	550
Reference λ	630	575	540
O.D. (cm^{-1})	.026	.019	.016
Conc (micromoles) *	1.62	.95	.84

* Computed from $\Delta\Sigma$(cm^{-1} × millimoles^{-1}) values of 16, 20, and 19 for cytochromes a, b, and c respectively (9).

The effect of 10^{-3} M cyanide on the difference spectrum (N_2-O_2) is also shown in figure 2. The change in the α-band of cytochrome oxidase indicates that cyanide has largely blocked its reaction with O_2; the inhibition averaged about 80 %. The shape of the 550- to 565-mμ band is markedly altered, the major peak now being at 560 to 562, rather than at 553 mμ. This means that although oxidation of the c-type cytochrome is largely inhibited (80 %), some cytochrome b is relatively unaffected (oxidation only about 20 % inhibited). Similar results were obtained with three separate preparations. This suggests that when the cytochrome c–oxidase system is largely inhibited, most of the cytochrome b can still be oxidized by O_2. The 3rd absorption band in this region also shows a slight shift towards a longer wavelength, suggesting a relatively greater participation of b, with its peak around 530 mμ. The cause of the change in the difference spectrum around 500 mμ is not known, although flavoproteins do absorb in this region. In the Soret region, cyanide caused a marked reduction of the 445-mμ peak (a_3) and changes in the 410- to 430-mμ peak which suggest less inhibition of cytochrome b than c; this evidence was limited to a single difference spectrum.

Preliminary experiments were carried out with other inhibitors. In two cases, 10^{-2} M azide reduced the functional amount of cytochrome oxidase by 55 and 72 %, as calculated from the absorbance at 445 minus 455 mμ. Carbon monoxide was bubbled through an anaerobic suspension of mitochondria and the absorption determined. The difference spectrum (N_2 + CO) – (N_2) showed a peak around 428 mμ, which is close to the correct position for the cytochrome a_3-CO complex (6).

DISCUSSION

The maximum P/O values that have been obtained for α-ketoglutarate oxidation by animal mitochondria approach four (14). In recent work, P/O ratios greater than three have been reported for mitochon-

dria isolated from etiolated peas (23), sweet potatoes (18), and castor bean endosperm (1), although the values for plant particles are generally lower (11). The unusually high values reported here (average 3.17, maximum 3.78) suggest that the phosphorylation mechanisms in skunk cabbage mitochondria are as efficient as those in other comparable plant and animal particles. The very low values obtained previously with *Arum maculatum* (13) probably resulted from the use of inadequate isolation methods. The demonstration that isolated particles possess a high capacity for phosphorylation does not prove that they operate with maximum efficiency in vivo, although this is a reasonable hypothesis. Assuming that the mitochondria are the normal respiratory centers, the results suggest that the very rapid respiration of the Aroid spadix can be coupled to energy-requiring processes.

The difference spectra show clearly the participation of cytochromes of the a, b, and c types in the reaction of skunk cabbage mitochondria with oxygen. The positions of the peaks in the α and Soret regions, the reaction with CO, and the previous demonstrations of cytochrome oxidase activity (12, 22, 25) all suggest that a typical cytochrome a-a_3 complex is functioning in these particles. As yet, no other a-type oxidase has been found. The difference spectrum in the 560 mμ region suggests that more than one cytochrome b is involved, in agreement with the spectroscopic observations of Bendall and Hill (2). The fact that the major peak in the α-region is at 553 mμ, rather than at 550 mμ, suggests that the particles may contain both cytochromes c and c_1, with a preponderance of the latter. A recent report indicates that this may also be the case in other plant mitochondria (20). Like the comparable animal particles (9), skunk cabbage mitochondria contain more cytochrome a than b and c (table III). However, they apparently differ by having relatively high concentrations of other components, described here as cytochromes b_7 and c_1; the total concentration of b's is probably greater than a. Although the exact roles of the various components have not been defined, they are almost certainly involved in the oxidative phosphorylations. The fact that the P/O ratio can approach four suggests that the normal electron transport proceeds from the level of cytochrome b to c to a to oxygen, as in animal mitochondria (8).

The oxidation of organic acids by isolated Aroid spadix mitochondria is relatively resistant to cyanide and azide. Although James and Elliott (16) observed little or no inhibition by 10^{-3} M cyanide, subsequent experiments have indicated that this concentration reduces the oxidations between 50 and 60 % (12, 22); in this study, there was no inhibition by 4.6×10^{-4} M HCN (table II). This is in marked contrast to the great sensitivity of the cytochrome oxidase activity, which is essentially completely blocked by 10^{-4} M HCN (12, 22, 24). As suggested by Simon (22), a possible explanation for this discrepancy is that there is an internal cytochrome oxidase, which is inaccessible to added cytochrome c and is not readily inhibited by cyanide; in other words, there is a sufficient excess of the oxidase to account for the cyanide-resistant particle (and tissue) oxidations. Some support for this hypothesis may be gathered from the fact that the difference spectra reveal only an 80 % inhibition of the oxidase by 10^{-3} M HCN; the remaining 20 % might maintain the maximal respiration rate. An alternative explanation is that there is a separate, cyanide-resistant respiratory pathway, which might be functional only in the presence of the inhibitor. This alternate, internal pathway would only be available to added organic acids and not to added cytochrome c. In this connection, the relatively small cyanide inhibition of the functional cytochrome b (fig 2) suggests that it could participate in such a pathway. One of the properties of the mitochondrial cytochrome b_7 is its rapid autoxidation in the presence of cyanide (2). It is not yet known whether this reaction between cytochrome b and O_2, which may involve other components (7), is sufficiently rapid to account for all the cyanide-resistant respiration. Spectrophotometric observations on intact spadix tissues have also shown that cytochrome b can be oxidized in the presence of cyanide, and its possible respiratory role has been considered (25). The striking similarity between the difference spectra obtained with intact tissues and isolated mitochondria supports the assumption that these particles are the normal respiratory centers.

The effects of inhibitors on the oxidative phosphorylation may throw some light on the respiratory pathways. Both azide and cyanide act as "uncoupling agents," reducing phosphorylation more than oxygen uptake (table II). If the relative insensitivity of the respiration to these agents were due solely to an excess of cytochrome oxidase, it is not clear why they should lower the P/O ratio. A possible explanation is that they inhibit the phosphorylation mechanism itself, as well as the terminal oxidase. This is probably the case with azide, which is known to interfere with phosphorylation (19) and the exchange of inorganic phosphate (P^{32}) with ATP (21). No comparable effect on phosphorylation has been reported for cyanide; it causes relatively little inhibition of the P^{32}-ATP exchange (5). An alternative explanation is that the inhibitor diverts the electron transport from the normal pathway to an insensitive, non-phosphorylating pathway, which does not play an appreciable role in the absence of inhibitor. The spectrophotometer evidence suggests that the "switch-over" may take place in the region of cytochrome b. Since there are probably two phosphorylative steps between cytochrome b and O_2 (10), it is suggestive that cyanide apparently uncoupled two of the four phosphorylations associated with α-ketoglutarate oxidation (table II).

Summary

The mitochondrial fraction isolated from skunk cabbage flowers can couple the oxidation of α-keto-

glutarate to the esterification of inorganic phosphate. The high P/O ratios (average 3.17, maximum 3.78) indicate that this oxidative phosphorylation is as efficient as that shown with other plant and animal mitochondria. The phosphorylation can be uncoupled from respiration to a large extent by 2,4-dinitrophenol and to a lesser extent by azide and cyanide. The difference spectrum (N_2-O_2) of a whole mitochondrial suspension shows maxima characteristic of cytochromes a, b, and c, with evidence of relatively high concentrations of b_7 and c_1. In the presence of cyanide, the spectrum shows a large inhibition of c and a, but b is relatively unaffected. The possible role of a b-type cytochrome in the cyanide-resistant respiration is considered.

Literature Cited

1. Akazawa, T. and Beevers, H. Oxidative phosphorylation in mitochondria from the developing castor bean seedling. Plant Physiol. 31 Suppl.: *xxiv*. 1956.
2. Bendall, D. S. and Hill, R. Cytochrome components in the spadix of *Arum maculatum*. New Phytol. 55: 206–212. 1956.
3. Bernhart, D. N. and Wreath, A. R. Colorimetric determination of phosphorus by modified phosphomolybdate method. Anal. Chem. 27: 440–441. 1955.
4. Bonner, W. D., Jr. and Yocum, C. S. Spectroscopic and enzymatic observations on the spadix of skunk cabbage. Plant Physiol. 31 Suppl.: *xli*. 1956.
5. Boyer, P. D., Luchsinger, W. W. and Falcone, A. B. O^{18} and P^{32} exchange reactions of mitochondria in relation to oxidative phosphorylation. Jour. Biol. Chem. 223: 405–421. 1956.
6. Chance, B. The carbon monoxide compounds of the cytochrome oxidases. I. Difference spectra. Jour. Biol. Chem. 202: 383–396. 1953.
7. Chance, B. and Pappenheimer, A. M., Jr. Kinetic and spectrophotometric studies of cytochrome b_5 in midgut homogenates of Cecropia. Jour. Biol. Chem. 209: 931–943. 1954.
8. Chance, B. and Williams, G. R. Respiratory enzymes in oxidative phosphorylation. IV. The respiratory chain. Jour. Biol. Chem. 217: 429–438. 1955.
9. Chance, B. and Williams, G. R. The respiratory chain and oxidative phosphorylation. Adv. in Enzymol. 17: 65–134. 1956.
10. Chance, B., Williams, G. R., Holmes, W. F. and Higgins. J. Respiratory enzymes in oxidative phosphorylation. V. A mechanism for oxidative phosphorylation. Jour. Biol. Chem. 217: 439–451. 1955.
11. Hackett, D. P. Recent studies on plant mitochondria. Int. Rev. Cytol. 4: 143–196. 1955.
12. Hackett, D. P. Respiratory mechanisms in the Aroid spadix. Jour. Exptl. Botany 8: 157–171. 1957.
13. Hackett, D. P. and Simon, E. W. Oxidative activity of particles prepared from the spadix of *Arum maculatum*. Nature 173: 162–163. 1954.
14. Hunter, F. E., Jr. Oxidative phosphorylation during electron transport. In: Phosphorus Metabolism, W. D. McElroy and B. Glass, etc. Vol. 1. Pp. 297–330. The Johns Hopkins Press, Baltimore 1951.
15. James, W. O. and Beevers, H. The respiration of Arum spadix. A rapid respiration, resistant to cyanide. New Phytol. 49: 353–374. 1950.
16. James, W. O. and Elliott, D. C. Cyanide-resistant mitochondria from the spadix of an Arum. Nature 175: 89. 1955.
17. Lazarow, A. and Cooperstein, S. J. Versatile anaerobic spectrophotometer cell. Science 120: 674–675. 1954.
18. Lieberman, M. and Biale, J. B. Oxidative phosphorylation by sweet potato mitochondria and its inhibition by polyphenols. Plant Physiol. 31: 420–424. 1956.
19. Loomis, W. F. and Lipmann, F. Inhibition of phosphorylation by azide in kidney homogenate. Jour. Biol. Chem. 179: 503–504. 1949.
20. Martin, E. M. and Morton, R. K. Haem pigments of cyto-plasmic particles from non-photosynthetic plant tissues. Biochem. Jour. 65: 404–413. 1957.
21. Robertson, H. E. and Boyer, P. D. The effect of azide on phosphorylation accompanying electron transport and glycolysis. Jour. Biol. Chem. 214: 295–305. 1955.
22. Simon, E. W. Succinoxidase and cytochrome oxidase in mitochondria from the spadix of Arum. Jour. Exptl. Botany 8: 20–35. 1957.
23. Smillie, R. M. Enzymic activity of particles isolated from various tissues of the pea plant. Australian Jour. Biol. Sci. 8: 186–195. 1955.
24. Stotz, E. H., Morrison, M. and Marinetti, G. Components of the cytochrome system. In: Enzymes: Units of biological structure and function. O. H. Gaebler, ed. Pp. 401–416. Academic Press, New York 1956.
25. Yocum, C. S. and Hackett, D. P. Participation of cytochromes in the respiration of the Aroid spadix. Plant Physiol. 32: 186–191. 1957.