21. SWANSON, C. A. and WHITNEY, J. B. The translocation of foliar-applied P^{32} and other radioisotopes in bean plants. Amer. Jour. Bot. 40: 816–823. 1953.
22. VAN OVERBEEK, J. Absorption and translocation of plant regulators. Ann. Rev. Plant Physiol. 7: 355–372. 1956.
23. VERNON, L. P. and ARONOFF, S. Metabolism of soybean leaves. IV. Translocation from soybean leaves. Arch. Biochem. Biophys. 36: 383–398. 1952.
24. WOLFGANG, R. L. and LIBBY, W. F. Absolute excitation function of the Be^9 (d,t) reaction. Phys. Rev. 85: 437–440. 1952.

ELECTRON TRANSPORT AND CYTOCHROMES OF SUB-CELLULAR PARTICLES FROM CAULIFLOWER BUDS[1]

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The fact that substrates of the tricarboxylic acid cycle and reduced diphosphopyridine nucleotide (DPNH), one of the coenzymes involved in the oxidation of these substrates, are oxidized by particulate structures of plant cells is well established (1, 3, 8, 10, 13, 14, 18, 20). Laties (15) has shown that mitochondrial particles from cauliflower oxidize α-ketoglutarate, malate, pyruvate, succinate and oxalacetate with accompanying phosphorylation. The purpose of this paper is to determine the type of particles in a homogenate of cauliflower buds, examine some of the components of these particles, and determine their enzymatic characteristics with respect to the oxidation of DPNH and succinate.

In order to localize the particles which oxidize DPNH and succinate an homogenate of cauliflower tissue was separated into several particulate fractions by differential centrifugation. Each of the fractions was washed thoroughly by repeated sedimentation in fresh sucrose and any obvious contamination by lighter or heavier fractions removed.

MATERIALS AND METHODS

PREPARATION OF PARTICULATE FRACTIONS AND DESCRIPTION OF THE PARTICLES: Preparation of the particles was based on the procedure originally described by Laties (15). Homogenization was carried out in a Waring blendor operated at two-thirds full speed for 20 seconds on batches of 80 gm of cauliflower buds suspended in 100 ml of 0.5 M sucrose containing 0.001 M sodium versenate at pH 7.0. All operations were at 0 to 4° C. This procedure was found to yield particles with DPNH and succinoxidase activities equal to those of particles isolated from a homogenate prepared by grinding in a mortar and pestle with fine sand. Addition of 0.001 M versene was found to stabilize the activity of the particles for storage at − 20° C. The pH of the homogenate varied from 6.3 to 6.6.

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The use of 0.05 M phosphate buffer or adjustment of the pH of the final suspension to pH 7.0 by addition of KOH during the grinding was found to produce less active particles, and to cause difficulty in the separation of fractions. The homogenate obtained from the blendor was squeezed through three layers of cheesecloth to separate most of the cell debris and subjected to a series of centrifugations at increasing velocity after removal of the pellet at each stage. The pellets were resuspended in 0.5 M sucrose and 0.001 M versene in a Potter-Elvehjem homogenizer. Starch granules were left on the bottom of the tube. The centrifugation was in the following order: three minutes at one-half speed in the one-liter angle head of the International PR-1, three minutes at full speed in the PR-1, 10 minutes at 8,000 rpm in the number 30 head of the Spinco model L, 10 minutes at 15,000 rpm in the Spinco, 15 minutes at 40,000 rpm in the number 40 head of the Spinco and 1 hour at 40,000 rpm in the same head. The final supernate was clear with a slight fatty layer on the surface.

Table I shows a list of the fractions with the approximate centrifugal force and time of centrifugation, a description of their appearance, and the designation under which they will be discussed.

TABLE I

CONDITIONS FOR SEPARATION OF PARTICLES

FRACTION	CENTRIFUGAL FORCE × G	TIME OF CENTRIFUGATION, MIN	DESCRIPTION OF PELLET	DESIGNATION
1	600	3	Sticky green grey	Cell debris
2	2,000	3	Fibrous grey	Fibers
3	5,000	10	Smooth brown yellow	Medium
4	25,000	10	Smooth pale yellow	Medium light
5a	105,000	15	Smooth pale yellow	Packed light
5b	105,000	60	Translucent bright yellow	Fluffy light

ASSAY OF ENZYMATIC ACTIVITY: DPNH oxidase activity was determined spectrophotometrically by following the oxidation of DPNH by decrease in optical density at 340 mμ after addition of enzyme or DPNH to buffer mixtures described below. The buffer solutions and cell compartment of the spectrophotometer were maintained at 37° C. The reaction was started by addition of 0.01–0.05 mg enzyme protein or 0.04 ml of 0.2 % DPNH (reduced diphosphopyridine nucleotide) to the reaction mixture contained in 1-ml cuvettes with 1 cm optical path length. The total volume was one ml. No oxidation occurred in the presence of 3×10^{-5} M KCN, 0.02 μgm of antimycin A (22), 0.1 μgm 2,heptyl,4,hydroxyquinoline-N-oxide (4), or in the case of the medium particles under anaerobic conditions after flushing the solutions with helium. The influence of cytochrome c was determined by adding 0.01 ml of a 1 % solution of Sigma cytochrome c to the reaction mixture. 40 micromoles TRIS [tris(hydroxymethyl)aminomethane] chloride at pH 7.4 and potassium phosphate (40 micromoles) at pH 7.4 were used as buffers in routine assay procedures. At this pH in TRIS buffer maximum oxidation rate was achieved without addition of cytochrome c, whereas in phosphate buffer maximum rate was achieved only by addition of cytochrome c. The requirement for cytochrome c was directly dependent on phosphate concentration in the assay medium, but at higher levels of phosphate addition of cytochrome c did not completely restore activity. The affect of phosphate concentration of DPNH oxidase is shown in table II, and the effect of pH in the two buffer systems is shown in figure 1. The standard assay used in later work was based on these relationships. The extinction coefficient of DPNH was taken as $6.22 \times 10^6 \times \text{cm}^2 \times \text{mole}^{-1}$.

TABLE II

EFFECT OF PHOSPHATE CONCENTRATION ON DPNH OXIDASE ACTIVITY OF MEDIUM PARTICLE

PHOSPHATE, MICROMOLES	DPNH OXIDASE RATE *	
	NO CYT. C	CYT. C ADDED
None **	0.095	0.097
20	0.080	0.085
40	0.042	0.080
80	0.020	0.048

* Assayed at 37° C and pH 7.4 with or without the addition of 0.1 mg cytochrome c in a total volume of 1.0 ml. Specific activity in micromoles DPNH/min × mg.

** Forty micromoles TRIS chloride pH 7.4 as buffer.

The rate of oxidation of DPNH by cytochrome c (DPNH–cytochrome c reductase) was determined by following reduction of cytochrome c by increase in absorbance at 550 mμ at 37° C in a reaction mixture which contained 100 micromoles potassium phosphate pH 7.4, 0.6 ml of 0.2 % DPNH, 0.1 ml of 1 % cyto-

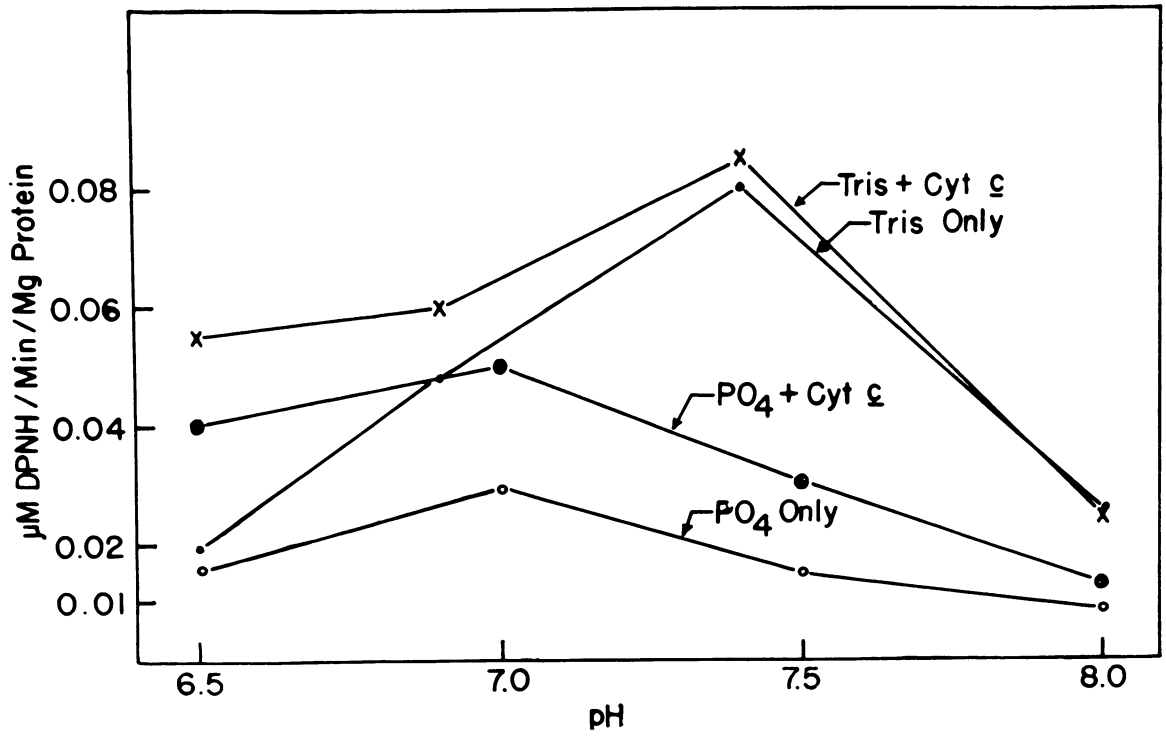

FIG. 1. The effect of pH and buffer on the requirement for cytochrome c in the DPNH oxidase activity of the medium particle. Assayed with 40 micromoles TRIS chloride or 80 micromoles of potassium phosphate buffer with 0.1 mg cytochrome c in a total volume of 1.0 ml.

chrome c and 0.3 micromoles potassium cyanide in a total volume of 1.0 ml. The oxidation rate was the same when phosphate was replaced by TRIS chloride at the same concentration and at the same pH. The difference between the extinction coefficient for oxidized and reduced cytochrome c was taken as $19.7 \times 10^6 \times cm^2 \times mole^{-1}$.

Diaphorase activity was determined at 37° C by following the reduction of 2,6-dichlorophenolindophenol by decrease in absorbance at 600 mμ after addition of enzyme or DPNH to a reaction mixture containing the same components as were used in the cytochrome c reductase assay, except that 0.1 ml of 0.2 % indophenol was substituted for cytochrome c. In both the diaphorase and cytochrome c reductase assays there is considerable reduction of indophenol and cytochrome c by the homogenate and supernate without addition of DPNH. All rates given are corrected for this endogenous rate. The extinction coefficient for indophenol was determined to be 16.1×10^6 $cm^2 \times mole^{-1}$ at this pH.

Succinic oxidase (5), succinic cytochrome c reductase (9) and cytochrome oxidase (16) were determined as previously described.

Protein was determined by biuret reaction in presence of deoxycholate (9). Beef serum albumin (Armour and Co.) was dialyzed 25 hours against distilled water before use. Absorption spectra were recorded on the Beckman DK-1 spectrophotometer. Enzymatically reduced DPNH and cytochrome c were obtained from Sigma Chemical Co., St. Louis and antimycin A from Wisconsin Alumni Research Foundation, Madison.

TABLE III

ENZYMATIC ACTIVITY OF PARTICLES

FRACTION	SPECIFIC ACTIVITY *				
	DPNH OXIDASE	DPNH CYT. C REDUCTASE		DIAPHORASE	CYTOCHROME OXIDASE
		NO ANTIMYCIN	ADDED ANTIMYCIN		
Orig. homogenate	0.028	0.008	0.008	0.029	0.070
Cell debris (1)	0.003	0.001	....	0.016	0.019
Fibrous (2)	0.060	0.014	0.019	0.072	0.042
Medium (3)	0.137	0.040	0.025	0.156	0.250
Medium light (4)	0.063	0.039	0.030	0.102	0.070
Light (5a)	0.033	0.035	0.050	0.081	0.033
Light (5b)	0.015	0.050	0.055	0.052	0.007
Final supernate	0.014	0.003	0.003	0.006	0.002

* All rates are expressed as μeq/min × mg protein for a two-electron transfer. Thus the observed rate of oxidation of reduced cytochrome c in micromoles is two times that given here. DPNH oxidase assayed in TRIS buffer.

TABLE IV

RECOVERY OF ENZYMATIC ACTIVITY IN THE PARTICULATE FRACTIONS

FRACTION	UNITS OF ACTIVITY **					
	PROTEIN, MG	DPNH OXIDASE	DPNH CYT. C REDUCTASE		DIAPHORASE	CYT. OXIDASE
			NO ANTIMYCIN	ADDED ANTIMYCIN		
Orig. homogenate*	4560	128.0	45.0	45.0	129.0	320
Cell debris (1)	69	0.2	0.1	...	1.1	1
Fibrous (2)	56	3.4	0.8	1.3	3.2	2
Medium (3)	307	42.2	12.3	7.7	48.0	77
Medium light (4)	124	7.8	4.9	3.7	12.7	9
Light (5a)	112	3.7	3.9	5.6	9.1	4
Light (5b)	81	1.2	4.1	4.4	4.2	1
Final supernate	3460	49.0	10.4	10.4	21.0	7
Recovery	4189	107.5	36.5	33.1	99.3	101

* The original homogenate represents the filtered juice from 540 gm wet wt of cauliflower buds.

** Units of activity refers to μeq of DPNH which would be oxidized per minute by the total fraction and represents the specific activity and the total protein in each fraction.

RESULTS

DISTRIBUTION OF ENZYMATIC ACTIVITIES: The specific activities of each of the particles for oxidation of DPNH by oxygen, cytochrome c and indophenol and for oxidation of reduced cytochrome c by molecular oxygen are given in table III as well as some data on the antimycin sensitivity of the cytochrome c reductase reaction. DPNH oxidase activity is concentrated in the medium particles, whereas the DPNH cytochrome c reductase activity is relatively high in the lighter particles and is not inhibited by antimycin A, except for a small inhibition in the medium particles. The diaphorase activity is in each case closely approximate to a summation of the DPNH oxidase and DPNH cytochrome c reductase activities. The ability to oxidize reduced cytochrome c by molecular oxygen is concentrated almost exclusively in the medium particle, and in other particles is present in proportion to the DPNH oxidase activity except in the cell debris where the activity, although low, is five times the DPNH oxidase activity. Since this cell debris material presents many technical problems a further investigation of the nature of this cytochrome oxidase activity has not been attempted at the present, although this activity could be of considerable significance in view of the large mass of the cell debris fraction which is eliminated from the fractionation by the preliminary filtration step.

In order to determine that the isolated particles account for the activities in the original filtrate the

TABLE V

SUCCINATE OXIDATION BY PARTICULATE FRACTIONS *

FRACTION	SUCCINOXIDASE		SUCCINIC CYT. C REDUCTASE
	NO CYT. C	ADDED CYT. C	
Fibrous (2)	0.006	0.010	0.004
Medium (3)	0.008	0.015	0.006
Medium light (4)	0.006	0.010	0.005
Light (5a)	0.001	0.002	0.002

* Activity expressed as micromoles succinate oxidized per minute per mg protein.

recovery of protein and enzymatic units has been determined (cf. table IV). The recovery in each instance is good in view of the losses which can be expected from the several handlings except for unexplained loss of cytochrome oxidase.

The DPNH oxidase activity of each particle has also been determined in the presence of an equivalent amount of supernate, and the light and medium particles have been mixed in equal proportions for assay, with no evidence of mutual stimulation or inhibition.

OXIDATION OF SUCCINATE: The ability to catalyze the oxidation of succinate by oxygen or cytochrome c is restricted primarily to the medium particles. The succinoxidase rate is stimulated about twofold by addition of cytochrome c. This effect may be related to the use of phosphate buffer in the assay. No attempt has been made to introduce tris buffer into the assay. The rates of succinate oxidation by the particles are shown in table V. It has not been possible to get sufficiently accurate rates on the original homogenate because of a large endogenous rate which renders the balance of units somewhat untrustworthy.

CYTOCHROME COMPONENTS: The presence of cytochromes has been demonstrated in all particulate units of animal and plant cells involved in electron transport which have been examined in this respect. In the case of animal mitochondria it has been possible to clarify the suspension by the use of deoxycholate and obtain spectra showing the absolute absorbance of these cytochromes. When cauliflower particles are treated in this manner the suspension is partially clarified, but the spectrum still shows some end absorption from turbidity with large peaks at 475 and 450 mμ which appear to represent carotenes in the plant particles. After addition of dithionite to this suspension two slight shoulders appear in the region of 600 and 560 mμ. It is of interest that there is no peak at 665 mμ in any of the particles, except for the cell debris which may indicate the presence of a small amount of chlorophyll in this material. It has been found, however, that excellent spectra of the cytochromes can be obtained if the spectrum of a clarified suspension reduced by dithionite is measured against a suspension which has not been reduced, as a reference, so that the spectrum obtained represents the reduced – oxidized difference spectrum. Difference spectra of the particles obtained in this manner are of two general categories. The first type is found in the heavier particles, whereas, the second is found in the lighter material. The medium particles show peaks on reduction at 603, 525 and 428, with a broader band from 560 to 550 mμ, a small shoulder around 440 mμ and a drastic decrease in absorption in the region around 400 mμ. The light particles show peaks at 559, 525 and 428 mμ with very little absorption at 603 mμ, and do not show the drastic decrease in absorption at 400 mμ which is characteristic of the heavy material. These spectra are shown in figures 2 and 3.

STABILITY: The DPNH and succinoxidase activities of these particles decline only slightly when the particles are stored in 0.5 M sucrose containing 0.001 M versene at – 20° C for two or three days. After seven days of storage there is a considerable decline in DPNH oxidase activity when assayed either in TRIS chloride buffer or in phosphate buffer in the presence of cytochrome c. A good part of this loss in activity can be restored by the addition of 0.1 ml of 10 % solution of dialyzed beef serum albumin to the assay mixture. In many cases a complete restoration of activity has been achieved. On continued storage there is a further slow irreversible decline in activity under all assay conditions which have been tested. The DPNH oxidase activity of fresh and seven-day-old material is shown in table VI.

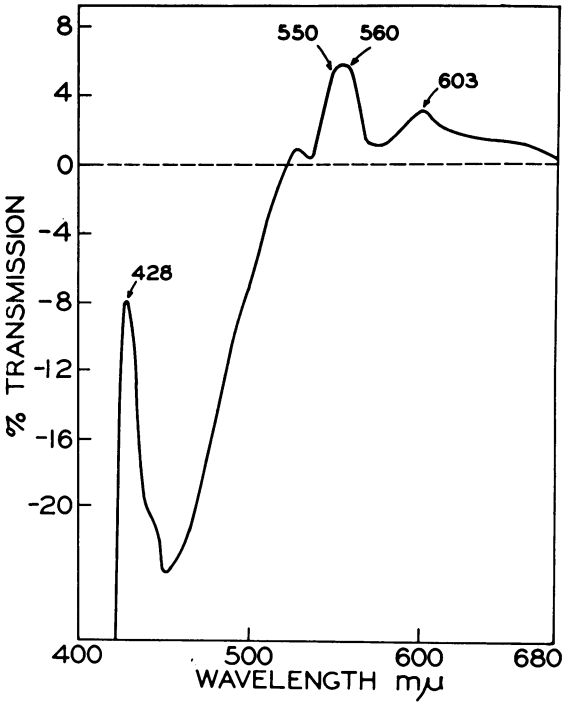

FIG. 2. Reduced – oxidized difference spectrum of the medium particle. Three and seven-tenths mg protein, 0.01 mg potassium deoxycholate and 40 micromoles potassium phosphate in a total volume of 1.0 ml reduced with a few grains dithionite. One cm light path.

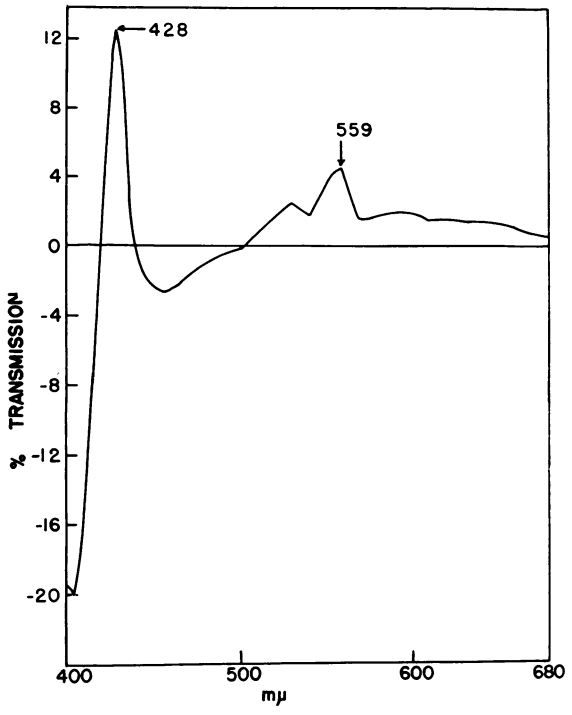

FIG. 3. Reduced – oxidized difference spectrum of the fluffy light particle. Three and one-half mg protein under the conditions described for figure 2.

The loss of DPNH cytochrome c reductase is much less than that of the DPNH oxidase, with 50 % of the original activity present after two to three weeks of storage. Beef serum albumin does not restore reductase activity under the assay conditions described.

OPENING EFFECT: Exposure of the electron transport particle isolated from beef heart mitochondria to deoxycholate has been shown to produce a requirement for cytochrome c in the oxidation of DPNH and succinate, with the corresponding appearance of DPNH and succinate cytochrome c reductase activity (6, 16). Treatment of the medium cauliflower particles with deoxycholate under the same conditions causes complete loss of DPNH oxidase activity which cannot be restored by addition of cytochrome c, as well as almost complete loss of cytochrome c reductase activity. An effect which is somewhat similar to this opening phenomenon has been observed, however, when the cauliflower medium particles are exposed to 0.1 M phosphate in the presence of 10 % ethanol. A suspension of medium particles in 0.5 M sucrose was mixed with an equal volume of 0.2 M potassium phosphate and ethanol was added slowly to a final concentration of 10 %. The suspension was centrifuged for 15 minutes at 105,000 × g in the No. 40 Spinco head. The pellet was washed once by resuspending in 0.5 M sucrose followed by centrifugation and finally taken up in 0.5 M sucrose.

The treated particles show twice as much DPNH and succinic cytochrome c reductase activity as the original particles. This increase represents new enzymatic units brought into action by the treatments employed and is not a purification of the pre-existing activity. In respect to cytochrome c reductase activity this phenomenon corresponds to the "opening" of beef heart ETP. The results differ however in that there is no proportional decrease in DPNH oxidase units, or corresponding increase in reduced cytochrome oxidase units. It also differs in that there is no increased requirement for cytochrome c in the DPNH oxidase activity of the treated particles either in TRIS or phosphate buffer. A comparison of the units of activity in the original and treated particles is shown in table VII. The additional DPNH cytochrome c reductase activity produced by the phosphate-ethanol treatment is inhibited at least 50 %, but never more than 80 % by 0.02 μgm antimycin A in all preparations tested.

TABLE VI

EFFECT OF AGEING ON DPNH OXIDASE ACTIVITY UNDER VARIOUS ASSAY CONDITIONS *

AGE OF PREPARATION, DAYS	BUFFER	DPNH OXIDASE SPECIFIC ACTIVITY			
		WITHOUT BSA **		WITH BSA **	
		No CYT. C	CYT. C ADDED	No CYT. C	CYT. C ADDED
1	TRIS	0.137	0.130	0.125	0.130
1	Phosphate	0.018	0.120	0.016	0.130
7	TRIS	0.019	0.022	0.056	0.056
7	Phosphate	0.014	0.028	0.014	0.056

* Particles were stored under conditions described in the text.
** BSA indicates beef serum albumin.

TABLE VII

EFFECT OF PHOSPHATE-ETHANOL TREATMENT ON ENZYME ACTIVITY OF MEDIUM PARTICLES

FRACTION	UNITS OF ACTIVITY *					
	PROTEIN, MG	DPNH OXIDASE		CYTOCHROME OXIDASE	DPNH-CYT. C REDUCTASE	SUCCINIC-CYT. C REDUCTASE
		No CYT. C	CYT. C ADDED			
Original	66.0	3.0	11.1	4.6	6.8	0.9
Treated	44.4	2.1	7.5	3.9	10.0	2.5
Supernate	10.2	0.0	0.0	0.0	0.0	0.0

* Units of activity as defined in table IV. DPNH oxidase assayed in phosphate buffer.

DISCUSSION

On the basis of physical appearance there are four predominant particle types in the filtrate from a cauliflower bud homogenate as well as a large amount of soluble protein. The heavy fibrous material may be cell wall debris with a certain amount of lighter particles attached to it or enmeshed in it. The medium

particle seems to correspond to mitochondria on the basis of its enzymatic properties and the lighter fractions correspond to microsomes. The striking difference in physical appearance between the two light fractions should be noted, although they do not differ greatly in the enzymatic properties studied.

The medium particle is capable of catalyzing the oxidation of DPNH and succinate by molecular oxygen. Oxidation of DPNH is sensitive to antimycin A, 2,heptyl,4,hydroxyquinoline-N-oxide and cyanide. It is also inhibited by phosphate ion, and activity is restored by addition of cytochrome c. This particle resembles the heavy fraction of beef heart mitochondria, and would appear to contain very little of the phosphate stimulated DPNH oxidase system of the type found in the beef heart (5) or Azotobacter (2) electron transport particles (ETP). The inhibition by phosphate ion can best be explained at present on the basis that fairly concentrated salt solutions may extract cytochrome c from the particle, as has been shown with animal mitochondria (24), and a high exogenous level of cytochrome c causes replacement of this compound. Such a requirement for cytochrome c in the overall oxidation process does not necessarily mean that DPNH cytochrome c reductase activity can be shown in the particle, as is suggested by the fact that the reductase activity is not effected by assay in phosphate buffer, and from studies with deoxycholate treatment of beef heart ETP (6). We feel that the antimycin insensitive DPNH cytochrome c reductase of these particles must be caused by contaminating microsomes, or by an altered DPNH oxidase system indicated by the opening effect, since all of the complete DPNH oxidase activity is antimycin sensitive. This differs from Martin and Morton's interpretation of similar activity found in particles from silver beet (18).

The lighter particles contain an antimycin insensitive DPNH cytochrome c reductase and very little cytochrome oxidase activity and are thus similar to microsomal particles found in liver. Similar particles have been reported in silver beet by Martin and Morton (18) although the ratio of diaphorase to cytochrome c reductase in these particles is much higher under different assay conditions.

There is also a small amount of DPNH oxidizing capacity in the original homogenate which is not accounted for in the diaphorase activity, and is recovered in the final supernate. Such an effect could be expected since reducible substrates and the enzymes to carry out their reduction by DPNH could be expected in the homogenate (17, 1a, 21a). A similar soluble DPNH oxidase of unknown nature has been reported by Romberger (23) in barley root homogenates. On the other hand the diaphorase activity of the fractions is associated almost exclusively with the particulate components and is proportional to the DPNH oxidase or cytochrome c reductase activities. Therefore there is no evidence in this tissue for DPNH diaphorase which is not associated with the particulate enzymes.

The loss of cytochrome oxidase units during the fractionation cannot be explained at this time. A recombination of various fractions would be desirable in approaching this problem since some system other than the usual cytochrome oxidase may be operating here. For example a cytochrome peroxidase separated from a supply of H_2O_2, or polyphenol oxidase separated from phenolic substrates by the fractionation procedure could account for the lost activity.

The cytochrome components of the particles show the differences which would be expected between mitochondria and microsomes. The medium particles contain peaks at 603, 560 and 550 mμ which would indicate the presence of cytochromes similar to cytochrome a, b_3 and c or c_1 (11). The light particles, however, contain one predominant cytochrome which would appear to correspond most closely to cytochrome b_3. It is of interest that the 560 peak in the mitochondria is very close to the 559 peak in the microsomes so there is not evidence as yet for the existence of a separate b heme in the two particles. This 559 mμ cytochrome is also different from any reported in animal material and is apparently very similar to the b_3 of Hill and Scarisbrick (12) or Martin and Morton (19) with peaks reported at 560 and 425.

The nature of the component in the medium particles responsible for the decrease in extinction around 400 mμ is unknown. It cannot be accounted for on the basis of spectral properties of known hemes and flavins.

The ability of material like serum albumin to restore activity indicates that certain structural or functional components are destroyed or disarranged by aging. A similar effect of ageing on particles from rat skeletal muscle has been shown by Nason to be reversed by lipid materials (21). This ageing effect as well as the spectral evidence for other than the usual heme and flavin components in the particles provides new leads to an understanding of their enzymatic properties. The discovery of a quinone in the ETP of beef heart and Azotobacter should lend emphasis to this viewpoint (7).

Summary

The separation of three distinct types of particles from cauliflower bud homogenates is described. They are 1) a grey fibrous material, 2) brown-yellow particles with enzymatic activities and cytochrome components corresponding to those of mitochondria, and 3) light yellow particles occurring in fluffy and packed forms with enzymatic activities and cytochrome components associated with microsomes.

The enzymatic activities investigated were: DPNH oxidation by oxygen, cytochrome c, and indophenol, oxidation of succinate by oxygen, and oxidation of reduced cytochrome c by oxygen. DPNH oxidase is concentrated in the mitochondrial particles and is inhibited by cyanide or antimycin whereas the DPNH cytochrome c reductase is concentrated in the light particles and is not sensitive to antimycin.

The effect of phosphate on the requirement for

cytochrome c for DPNH oxidase is described. DPNH oxidase activity declines on storage and is restored by addition of serum albumin. New DPNH and succinic cytochrome c reductase activity appears in medium particles treated with phosphate and ethanol.

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Literature Cited

1. Beevers, H. and Walker, D. A. The oxidative activity of particulate fractions from germinating castor beans. Biochem. Jour. 62: 174–179. 1956.
1a. Beevers, H. The oxidation of reduced diphosphopyridine nucleotide by an ascorbate system from cucumber. Plant Physiol. 29: 265–269. 1954.
2. Bruemmer, J. H., Wilson, P. W., Glenn, J. L. and Crane, F. L. Electron transporting particle from Azotobacter vinelandii. Jour. Bacteriol. 73: 113–116. 1957.
3. Brummond, D. O. and Burris, R. H. Reactions of the tricarboxylic acid cycle in green leaves. Jour. Biol. Chem. 209: 755–765. 1954.
4. Cornforth, J. W. and James, A. T. Structure of a naturally occurring antagonist of dihydrostreptomycin. Biochem. Jour. 63: 124–130. 1956.
5. Crane, F. L., Glenn, J. L. and Green, D. E. Studies on the electron transfer system. IV. The electron transfer particle. Biochim. Biophys. Acta 22: 475–487. 1956.
6. Crane, F. L. and Glenn, J. L . Studies on the electron transport system. VI. The action of deoxycholate on the electron transfer particle. Biochim. Biophys. Acta 24: 100–107. 1957.
7. Crane, F. L., Hatefi, Y., Lester, R. L. and Widmer, C. Isolation of a quinone from beef heart mitochondria. Biochim. Biophys. Acta 25: 220–221. 1957.
8. Goddard, D. R. and Stafford, H. A. Localization of enzymes in the cells of higher plants. Ann. Rev. Plant Physiol. 5: 115–132. 1954.
9. Green, D. E., Mii, S. and Kohout, P. M. Studies on the terminal electron transport system. I. Succinic dehydrogenase. Jour. Biol. Chem. 217: 551–567. 1955.
10. Hackett, D. P. Pathways of oxidation in cell free potato fractions. Plant Physiol. 31: 111–118. 1956.
11. Hill, R. and Hartree, E. F. Hematin compounds in plants. Ann. Rev. Plant Physiol. 4: 115–150. 1953.
12. Hill, R. and Scarisbrick, R. The hematin compounds of leaves. New Phytologist 50: 98–111. 1951.
13. Humphreys, T. E. and Conn, E. E. The oxidation of reduced diphosphopyridine nucleotide by Lupine mitochondria. Arch. Biochem. Biophys. 60: 226–243. 1956.
14. Kmetec, E. and Newcomb, E. H. Properties of particulate fractions isolated from homogenates of peanut cotyledons. Amer. Jour. Bot. 43: 333–341. 1956.
15. Laties, G. G. The dual role of adenylate in the mitochondrial oxidations of the higher plant. Physiol. Plantarum 6. 199–214. 1953.
16. Mackler, B. and Green, D. E. Studies on the electron transport system. II. On the opening phenomenon. Biochim. Biophys. Acta 21: 1–6. 1956.
17. Mathews, M. B. The oxidation of reduced diphosphopyridine nucleotide in green peas. Jour. Biol. Chem. 189: 695–704. 1951.
18. Martin, E. M. and Morton, R. K. Enzymatic properties of microsomes and mitochondria from silver beet. Biochem. Jour. 62: 696–704. 1956.
19. Martin, E. M. and Morton, R. K. Cytochromes of microsomal particles. Nature 176: 113–114. 1955.
20. Millerd, A. Respiratory oxidation of pyruvate by plant mitochondria. Arch. Biochem. Biophys. 42: 149–193. 1953.
21. Nason, A. and Lehman, I. R. The role of lipids in electron transport. II. Lipide cofactor replaceable by tocopherol for the enzymatic reduction of cytochrome c. Jour. Biol. Chem. 222: 511–530. 1956.
21a. Nason, N., Wosilait, W. D. and Terrell, A. J. The enzymatic oxidation of reduced pyridine nucleotides by an oxidation product of ascorbic acid. Arch. Biochem. Biophys. 48: 233–235. 1954.
22. Reif, A. E. and Potter, V. R. Studies on succinoxidase inhibition. I. Pseudoirreversible inhibition by a naphthoquinone and by antimycin A. Jour. Biol. Chem. 205: 279–290. 1953.
23. Romberger, J. A. Some characteristics of the soluble DPNH oxidase system of barley roots. Plant Physiol. 32 (Suppl): *xxix*. 1957.
24. Tsou, C. L. Exogenous and endogenous cytochrome c. Biochem. Jour. 50: 493–499. 1952.