As the present results support the view that liver choline can be rapidly phosphorylated *in vivo*, it seems likely that the level of phosphorylcholine will to some extent be a reflexion of the relative rates of formation and further metabolism of free choline. On the other hand, the GPC content can be expected to depend on the rate of lecithin breakdown occurring in the tissue, as this compound has now been identified as an intermediate in the catabolism of liver lecithin (Dawson, 1955*b*). Any GPC liberated from liver lecithin would be subsequently hydrolysed into free choline and glycerophosphoric acid under the action of the enzyme GPC diesterase present in liver tissue (Dawson, 1956).

Thus in the present investigation the injection of choline into the rat was associated with an expected increase in the phosphorylcholine level without alteration of the GPC content. Starvation, which in the mammal produces a rapid loss of phosphoglycerides from the liver (Kosterlitz, 1947; Hodge *et al.* 1948), causes a marked rise in the GPC content, presumably because of the extra lecithin catabolism, and it might therefore be expected that the phosphorylcholine level would also rise. That such an increase does not occur during starvation may be related to the absence of dietary choline and methionine intake, and the requirements of the animal for these substances for transmethylation reactions. In insulin hypoglycaemia the level of GPC in the tissue is markedly increased, and this, as is to be expected, is associated with an elevation of the phosphorylcholine content.

SUMMARY

1. Specific radioactivities of liver phosphorylcholine and lecithin have been measured at various times after the injection of radioactive phosphate into the rat.

2. The results indicate that phosphorylcholine is not formed in the liver by the breakdown of lecithin, nor does it appear to obey the Zilversmit *et al.* (1943) radioactive criteria for the sole immediate phosphorus precursor of liver lecithin.

3. Chromatographic recovery of phosphorylcholine in the liver is increased after the injection of choline but not after starvation.

4. Conversely, the recovery of liver glycerylphosphorylcholine is increased after starvation but is unaltered by choline administration.

5. Chromatographic recoveries of both liver phosphorylcholine and glycerylphosphorylcholine are markedly increased during insulin hypoglycaemia.

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Enzymic Properties of Microsomes and Mitochondria from Silver Beet

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Extensive studies on cells of animal origin from a wide variety of tissues have already established the localization of enzymic activity in cytoplasmic particles. The enzymic activities of mitochondria from animal tissues indicate that these structural units are sites of respiratory activity (see Schneider, 1953). A more limited series of studies have shown

that the mitochondria from plant cells have a similar function (Miller & Bonner, 1953; Goddard & Stafford, 1954). However, in neither plant nor animal cells is there as yet any clear indication of the metabolic function of the submicroscopic cytoplasmic particles (so-called 'microsomes'), although a number of enzymic activities appear to

be associated with them (see Hogeboom, Schneider & Striebich, 1953; Morton, 1954*a, b*; Bailie & Morton, 1955; Martin & Morton, 1955).

Stafford (1951) studied the intracellular localization of a selected number of enzymes in pea seedlings, but did not establish the occurrence of any of these enzymes in the small particles. However, the chemical analyses showed certain differences between the large- and small-granule fractions. Brown, Jackson & Chayen (1953) and Robinson & Brown (1953) established that small particles (20–37 m μ . diameter) could be isolated from disrupted bean-root cells. They also showed an increase in ribonucleic acid (RNA) content with decrease of particle size. Other workers (Newcomb & Stumpf, 1952; Bokman, Newcomb, Castelfranco & Stumpf, 1953) have described a fatty acid oxidase system which they believe to be localized in the small 'yellow particles' isolated from peanut. Webster (1953) noted that the enzymic breakdown of glutathione by hypocotyls from bean seedlings was partly associated with 'microsomes'.

This and subsequent papers are concerned with the characterization of plant microsomes by investigation of their enzymic activities, chemical composition, size and structure. It was hoped that such studies would provide a basis for determining whether or not microsomes have any metabolic function in the plant cell. It should be stressed that the term 'microsome' is used to designate a particle as isolated from disrupted tissues. This does not necessarily imply that a particle as such exists in the intact cell. Studies relating to the origin of microsomal particles will be reported in a later paper.

The isolation and enzymic characterization of microsomes from the white petiole of silver beet (*Beta vulgaris*) is described in this paper, together with observations on the comparative enzymic properties of beet mitochondria.

This work was described at the 31st meeting of the Australian and New Zealand Association for the Advancement of Science, Melbourne, in August 1955.

MATERIALS AND METHODS

Diphosphopyridine nucleotide (DPN). This was a commercial preparation (cozymase 90, Sigma Chemical Co.). It was reduced enzymically, with crystalline ethanol dehydrogenase prepared from yeast according to Racker (1950). Reduced DPN (DPNH) as supplied by C. F. Boehringer and Soehne, Mannheim, Germany, was also used.

Triphosphopyridine nucleotide (TPN). This product was 80% pure and it was reduced enzymically with glucose 6-phosphate dehydrogenase. (Both were obtained from Sigma Chemical Co.)

Cytochrome c. This was prepared from ox heart by the procedure of Keilin & Hartree (1945). Reduced cytochrome c was prepared as described by Margoliash (1952).

Other substrates. Commercial A.R. grade chemicals were used.

Antimycin A. This was a gift from Dr F. M. Strong and Dr G. M. Tener, University of Wisconsin. The antimycin A was dissolved in 95% ethanol, to give a stock solution (500 μ g./ml.), from which suitable dilutions for the inhibition studies were prepared in distilled water. Similar dilutions of 95% (v/v) ethanol were used in the controls.

Phosphate buffer. A stock solution containing 42.4 g. of $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ and 238.4 g. of K_2HPO_4 /l. (0.8M, pH 7.4) was prepared and suitably diluted for use. Unless otherwise specified, the term 'phosphate buffer' refers to this preparation.

Determination of enzymic activities

Dehydrogenases. Activities were determined by measuring the rate of reduction of 2,6-dichlorophenolindophenol at 600 m μ ., using a Beckman spectrophotometer (Model DU). Measurements were made in 1 cm. cuvettes at room temp. (approx. 18–22°) in phosphate buffer (pH 7.4; final concn. 0.2M for succinic dehydrogenase and 0.066M for all other dehydrogenases). The final volume in each cuvette was 3.0 ml. and the final concn. of dye was 7×10^{-5} M. The concns. of substrates for the various activities were: for succinic dehydrogenase, 0.06M sodium succinate; for DPNH- or TPNH-diaphorases, 1×10^{-4} M DPNH or reduced TPN (TPNH); for choline dehydrogenase, 0.02M choline hydrochloride; for lactic dehydrogenase, 0.05M sodium DL-lactate; for hypoxanthine dehydrogenase, approx. 0.01M hypoxanthine.

Cytochrome c reductases. The rate of reduction of cytochrome c at 550 m μ . was measured essentially as described by Slater (1950). The final concns. of reactants were: 0.066M phosphate buffer (pH 7.4); 0.001M potassium cyanide; 1×10^{-4} M oxidized cytochrome c; for DPNH- and TPNH-cytochrome c reductases, 1×10^{-4} M DPNH or TPNH; for succinate-cytochrome c reductase, 0.06M sodium succinate.

Cytochrome c oxidase. The rate of oxidation of reduced cytochrome c (1×10^{-4} M) in phosphate buffer (0.066M, pH 7.4) was measured spectrophotometrically at 550 m μ . The solutions used were equilibrated with air.

Fumaric hydrogenase. The method used was a modification of that of Fischer, Roedig & Rauch (1942). The assay system comprised: phosphate buffer (pH 7.4, 0.1M), Janus Green B (5×10^{-4} M), sodium fumarate (0.066M), and sodium dithionite ($\text{Na}_2\text{S}_2\text{O}_4$, 1.0 to 1.2×10^{-3} M). The substrate, buffer, dye and enzyme were mixed in the main compartment of a Thunberg tube. After evacuation and refilling with oxygen-free nitrogen, the sodium dithionite (in phosphate buffer) was tipped from the side arm and the time for reoxidation of the reduced dye to safranin was measured. The amount of enzyme and of dithionite was adjusted so that the time for reoxidation was between 2 and 3 min. A control tube (without fumarate) was handled simultaneously, a T-joint being used to ensure the same gas phase. The time for reoxidation in the control tube was always greater than 20 min. The results were expressed as μ moles of sodium dithionite oxidized/hr./mg. of N. All measurements were made at 38°. In order to use a slight excess of dithionite in the reaction, it was anaerobically titrated against the amount of Janus Green B added. The amount of dithionite/tube was determined by standardization against potassium ferricyanide.

Preliminary experience with different tissues (sugar-beet root, soya-bean seedlings, carrot root, cauliflower inflorescence, radish root, celery stem and white petiole of silver-beet) showed that a satisfactory level of succinic-dehydrogenase activity was to be found in the tissue dispersions of celery, beet petiole and soya-bean seedlings. Initially, celery was chosen for these studies since it had the highest succinic-dehydrogenase activity of the more readily available tissues. Unfortunately, this high activity was found to vary considerably during the short growing season, and celery stem was abandoned in favour of silver-beet petiole, which showed less seasonal variation.

Disruptive procedure. The fragility of plant chloroplasts is well established. Damage results in the appearance of chlorophyll in the supernatant and concomitant reduction in the size of the sedimented-chloroplast layer. Using this criterion, supported by microscopic examination of the isolated fractions, a number of different disruptive techniques were studied. With the green leaf and green petiole of silver beet, it was found that hand grinding of the tissue in a mortar, without the addition of an abrasive, gave most satisfactory cell breakage without appearance of chlorophyll in the supernatant. That disruption of the cell occurred without accompanying damage to the cytoplasmic particle was also confirmed by finding that the succinate dehydrogenase activity sedimented almost entirely in the fraction obtained at 10 000 *g* for 15 min. It should be stressed, however, that cell breakage cannot be considered as quantitative.

Dispersion medium. The hypertonic (0.88M) sucrose solution of Hogeboom, Schneider & Pallade (1947) was initially chosen for isolation of enzymically active particulate components. However, this was abandoned in favour of 0.2M sucrose, since the latter gave higher succinic-dehydrogenase activities in isolated mitochondria. The effect of various protective agents (egg albumin, bovine serum albumin, lactoglobulin, starch and peptone) on the succinic-dehydrogenase activity of the final particulate preparation was investigated. Slight enhancement of activity was obtained with media containing both egg albumin and peptone. Since the tissue dispersion in sucrose solution alone was distinctly acid, potassium phosphate buffer (0.2M) at pH 7.4 was included in the medium. For simplicity and lack of interference with later studies, nitrogenous protective agents were omitted and a medium containing 0.2M sucrose and 0.2M potassium phosphate, adjusted to pH 7.4, was used.

Isolation of particles. Leaves and stem from young silver-beet plants were harvested on the morning of the preparation. The white petiole was dissected free of all leaf tissue and washed thoroughly with cold water. All ensuing operations were carried out in a cold room at about 2°. The petioles were chopped by hand into pieces approx. 0.5 cm. $\times$ 0.5 cm. $\times$ 0.1 cm. About 100 g. of chopped pieces was ground for approx. 2 min. with 150 ml. of the above 0.2M sucrose and phosphate medium, in a large earthenware mortar and pestle previously cooled to about 0°. The dispersion was strained through four thicknesses of muslin cheese cloth. Usually about 400 g. of tissue was used for each preparation, the final volume of the dispersion being about 750–800 ml.

It was observed in preliminary studies that the succinic dehydrogenase activity, used as a guide to the localization of mitochondria, accompanied the particles sedimented at 10000 g for 15 min. Fractionation of the tissue dispersion was therefore carried out as shown in Fig. 1. In order to reduce contamination of the microsomal fraction with mitochondria, an intermediate fraction (mixed particles) was isolated by centrifuging at 20000 g for 15 min.

The first fraction (1500 g, 15 min.) was obtained at 0° with an International refrigerated centrifuge fitted with an angle head. All other fractions were obtained with a Serrall vacuum-type centrifuge (Model SS2), the angle head being cooled to about 0° before use. The temp. rose to about 5° during isolation of the 10000 g fraction, and to about 10° during isolation of the other fractions.

The time elapsing from the dispersion of the tissue to the sedimentation of the microsomal fraction (50000 g, 90 min.) was normally about 6-7 hr. Washing and refractionating required an additional 5 hr. In order to minimize loss of enzymic activity, samples were normally removed as soon as sedimented and used for enzymic determinations (see Fig. 1). The remainder was then washed as shown in Fig. 1 (to remove contaminating soluble constituents and dispersion medium) and used for chemical analyses, which will be described in a later paper.

RESULTS

Enzymic activities of fractions isolated from beet petiole

The succinic-dehydrogenase, DPNH-cytochrome *c* reductase, and DPNH-diaphorase activities were estimated for each of the isolated fractions. These

Table 1. *Enzymic activities of fractions from beet-petiole dispersion*

The fractions were isolated by differential centrifuging of tissue dispersions from beet petiole and activities were determined as described in the text. Mean activities and ranges are given for four different preparations. Activities are expressed as μ moles of acceptor reduced/hr./mg. of N.

Fraction	Enzymic activities								
	Succinic dehydrogenase		DPNH-cytochrome <i>c</i> reductase		DPNH-diaphorase		Ratio of activities		
	Mean	Range	Mean	Range	Mean	Range	<i>a</i> *	<i>b</i> †	<i>c</i> ‡
1500 g, 15 min. (unbroken cells, etc.)	1.1	0.0-2.9	8.0	3.4-14.0	40.0	33.0-47.0	7	37	5
10000 g, 15 min. (mitochondria)	1.2	0.9-1.6	16.2	12.0-21.0	55.0	51.0-59.0	14	47	3
20000 g, 15 min. (mixed particles)	0.4	0.0-0.6	23.3	12.0-33.6	22.6	22.0-24.0	58	57	1
50000 g, 90 min. (microsomes)	0.1	0.0-0.2	39	27.0-55.0	49.0	43.0-55.0	390	490	1

* $\frac{\text{DPNH-cytochrome } c \text{ reductase}}{\text{Succinic dehydrogenase}}$ † $\frac{\text{DPNH-diaphorase}}{\text{Succinic dehydrogenase}}$ ‡ $\frac{\text{DPNH-diaphorase}}{\text{DPNH-cytochrome } c \text{ reductase}}$

activities have been determined on a large number of different preparations over a period of about 18 months, during which time a considerable variation in total activity of the petiole dispersions has been observed. However, the relative activities of the different fractions have always been essentially the same. Table 1 summarizes the results of four representative experiments which were carried out over a period of 10 days. It can be seen that the ratios of the various enzymic activities of the isolated fractions show a distinct change in enzymic pattern with decrease of particle size.

Distribution of enzymes in dispersions of beet petiole

Table 2 shows the distribution of the total nitrogen and of the succinic-dehydrogenase, DPNH-cytochrome *c* reductase and DPNH-dia-phorase activities in the various fractions isolated from beet petiole. Results are expressed as a percentage of the total activity of the original dispersion. The results shown in Table 2 are representative of results obtained in two different experiments. Good recoveries of activities were obtained except in the case of succinic dehydrogenase, for which several repetitions gave no greater recovery than 20 % of the activity of the dispersion.

Effect of antimycin A on enzymic activities of beet petiole

Ahmad, Schneider & Strong (1950) and Potter & Reif (1952) showed that the antibiotic antimycin A is a powerful inhibitor of succinic dehydrogenase and of DPNH-cytochrome *c* reductase of rat liver. However, as yet there have been no comparable studies with plant tissues.

Experiments were carried out with mitochondria and microsomes isolated from beet petiole by the following modification of the standard procedure:

Small quantities (50–100 g.) of petiole were dispersed in the medium as previously described. The dispersion was centrifuged at 1500 *g* for 15 min. at 0°. The supernatant was recentrifuged at approx. 10000 *g* for 20 min. at 0° with the high-speed attachment of the International refrigerated centrifuge. The sediment (mitochondria) was resuspended in 0.15M-NaCl, pH 7.4, at 0°. The supernatant was recentrifuged at 60000 *g* for 1 hr. in a Spinco centrifuge, Model L, at 0°. The precipitate (microsomes) was suspended in 0.15M-NaCl, pH 7.4, at 0°.

In this way, cytoplasmic particles were prepared within 3 hr. of disruption of the tissue, as compared with 8 hr. by the standard procedure.

Table 3 shows that mitochondrial succinate- and DPNH-cytochrome *c* reductase activities are inhibited by antimycin A, whereas the microsomal DPNH-cytochrome *c* reductase activity is unaffected. The higher activities of both the mitochondria and microsomes as shown in Table 3, as

Table 2. *Distribution of nitrogen and enzymic activities among fractions from beet-petiole dispersion*

Fraction	Nitrogen mg./100 g. fresh weight	Succinic dehydrogenase		Fumaric hydrogenase		DPNH-cytochrome <i>c</i> reductase		DPNH-dia-phorase	
		Activity	% whole dispersion	Activity	% whole dispersion	Activity	% whole dispersion	Activity	% whole dispersion
Whole dispersion	43.8	0.22	100	0.17	100	0.76	100	9.3	100
1500 g, 15 min. (unbroken cells, etc.)	3.55	0.19	7.0	0.17	8.1	1.63	17.4	20.2	17.6
10000 g, 15 min. (mitochondria)	3.00	0.34	10.6	0.26	10.5	3.55	32.3	18.8	13.6
20000 g, 15 min. (mixed particles)	1.04	0	0	0	0	6.8	21.1	15.5	3.9
50000 g, 90 min. (microsomes)	1.57	0	0	0	0	7.1	33.7	22.9	8.8
Supernatant	31.6	0	0	0	0	0	0	8.1	62.0
Total	—	—	18	—	19	—	105	—	106

The fractions were isolated by differential centrifuging of tissue dispersions from beet petiole. Each fraction was suspended in a known volume of 0.2M sucrose solution for chemical and enzymic estimations, carried out as described under Methods. Activities are expressed as for Table 1, except for fumaric hydrogenase. This activity is expressed as μ moles of $\text{Na}_2\text{S}_2\text{O}_4$ oxidized/hr./mg. of N.

Table 3. *Effect of antimycin A on succinate- and DPNH-cytochrome c reductases of beet-petiole mitochondria and microsomes*

Fractions were isolated from beet-petiole dispersion by a modification of the standard procedure, designed to preserve enzymic activities by reducing the preparative time from about 8 to 3 hr. (see text, p. 700). The reactions were started by the addition of substrate. Activities were measured as described under Methods and are expressed as μ moles of cytochrome *c* reduced/hr./mg. of N.

Fraction	Substrate	Antimycin A* concn. μ g./mg. of nitrogen	Enzymic activities		% inhibition
			Control	With antimycin A	
10 000 g, 20 min. (mitochondria)	Succinate	5	11.2	0.86	93
		250	12.9	0.13	99
	Reduced DPN	0.25	13.8	13.8	0
		0.5	13.3	11.6	13
		1.0	16.3	12.1	26
		2.5	13.3	9.0	32
		5.0	15.0	9.9	34
		25	14.2	9.2	35
		250	16.0	10.2	36
60 000 g, 60 min. (microsomes)	Reduced DPN	60	70.0	70.0	0
		300	68.0	68.0	0

* The highest concn. of antimycin A used in each case (mitochondria and microsomes) corresponded to 3μ M in the cuvette.

Table 4. *Other enzymic activities of beet mitochondria and microsomes*

Mitochondria (10 000 g, 15 min. fraction) and microsomes (50 000 g, 90 min. fraction) were prepared as described in the text. Enzymic activities were determined as described under Methods, and are expressed as μ moles of acceptor reduced/hr./mg. of N. The results are representative activities from different preparations.

Enzymic activity	Mito- chondria	Microsomes
TPNH-cytochrome <i>c</i> reductase	5.4	25
TPNH-diaphorase	21.7	12.4
Hypoxanthine dehydrogenase	0.6	2.0
Choline dehydrogenase	0.2	0.7
Cytochrome <i>c</i> oxidase	2.6	0.2
Lactic dehydrogenase	0.7	Nil

compared with the activities shown in Tables 1 and 2, are undoubtedly due to the more rapid preparative procedures used in this experiment.

Other enzymic activities

In order to investigate whether particulate cytoplasmic components of beet petiole may have associated enzymic activities other than the three enzymes mentioned in Table 1, a survey was carried out with substrates other than succinate or reduced DPN in the dehydrogenase-assay system. Table 4 lists enzymes found in the mitochondrial or microsomal fractions with definite detectable activities. In addition, the following enzymes were shown to be absent or of an activity too low to be estimated accurately: adenosine triphosphatase, inorganic pyrophosphatase, glycylglycine dipeptidase, glycylleucine dipeptidase and methyl butyrate esterase.

DISCUSSION

Claude (1938, 1944) described 'microsomes' as submicroscopic particles isolated from liver by differential centrifuging of the dispersed tissue. Subsequently, Kabat (1941) demonstrated that alkaline phosphatase was associated with submicroscopic particles from kidney which sedimented only at high centrifugal force. A considerable amount of evidence has since been obtained by various authors which permits characterization of microsomes from animal tissues on the basis of their size, characteristic enzymic activities and unique chemical composition (see Hogeboom *et al.* 1953; Morton, 1954*a, b*).

Isolation of submicroscopic particulate material alone in no way suffices to characterize microsomes. It has been repeatedly demonstrated that submicroscopic lipoprotein particles, comparable to microsomes in size and sedimentation characteristics, may be isolated by disruption of mitochondria from animal tissues (Green, 1952; Cleland & Slater, 1953). While the observations of a number of authors, as noted in the introduction, suggest that microsomes may occur in plant tissues, in no case hitherto has the characterization been sufficient to justify the use of this term.

It seems likely that most of the cytoplasmic material obtained in this work originated from the cortical cells of the beet petiole, with some contribution from the phloem and other cells.

Occurrence of microsomes in plant cells

The evidence presented establishes that the particles sedimented between 20 000 and 50 000 g (see Fig. 1) correspond essentially to the 'micro-

some' fraction already well-characterized for animal cells.

The sedimentation characteristics, supported by electron-microscope observations (Hodge, Martin & Morton, unpublished work) show that the particles fall in the size range 30–300 m μ . Moreover, they are quite distinct from plant mitochondria both in size and structure. As indicated above, such submicroscopic particles quite possibly could be obtained as artifacts produced by disruption of the plant cell. However, the preparative procedure used caused no substantial damage to fragile cytoplasmic components such as chloroplasts or mitochondria. Accordingly, these may be disregarded as sources of the submicroscopic particles in this investigation. The evidence (Hodge *et al.* unpublished work) for the origin of the plant microsomes from a primitive type of endoplasmic reticulum will be presented in a subsequent paper.

Particularly striking is the unique enzymic constitution of the submicroscopic particles, as compared with that of mitochondria. This is very clearly indicated by consideration of the ratios of enzymic activities shown in Table 1. Whereas the ratio of DPNH-cytochrome *c* reductase to succinic dehydrogenase is about 14 for mitochondria, it is about 390 for the microsomes. Corresponding differences are seen in the ratios of the other enzymic activities. Although the recovery of succinic-dehydrogenase activity is low (Table 2) there is no reason to believe that the loss of activity preferentially occurred from any one isolated fraction.

The results shown in Table 2 suggest that no enzymic activity is exclusively localized in the microsome fraction. This is misleading, however, since the DPNH-cytochrome *c* reductase activities of mitochondria and of microsomes clearly belong to different enzymic systems. They are differentiated on the basis of sensitivity to antimycin A (Table 3). Whereas the microsomal activity is completely insensitive to antimycin A, even at very high concentrations (3 μ M, 300 μ g. of antimycin A/mg. of nitrogen), the mitochondrial DPNH-cytochrome *c* reductase activity is inhibited by very low levels (0.006 μ M, 0.5 μ g. of antimycin A/mg. of nitrogen) of antimycin A.

The contention that the DPNH-cytochrome *c* reductase activity of the microsomal fraction is distinct from that of mitochondria is supported by the finding that the reductase activity of the microsome fraction is intimately associated with cytochrome *b*₃ (Martin & Morton, 1955). Studies of the distribution of haem proteins in the isolated fractions have shown that cytochrome *b*₃ is exclusively localized in the microsomes (Martin & Morton, unpublished). TPNH-cytochrome *c* reductase activity is greater in beet microsomes than

in mitochondria (Table 4). Like DPNH-cytochrome *c* reductase activity, it is also associated with cytochrome *b*₃ in the microsomes and is not inhibited by antimycin A (Martin & Morton, 1955). It should be noted that cytochrome *c* is used merely as a convenient acceptor for hydrogen atoms from reduced DPN (and reduced TPN), in order to differentiate the activity from that of DPNH- (and TPNH-) diaphorase. While cytochrome *c* is probably the natural acceptor for the mitochondrial DPNH- (and TPNH-) cytochrome *c* reductases, it may be excluded as a component of the microsomal system (Martin & Morton, 1955).

The enzymic evidence thus leaves no doubt that the isolated submicroscopic particles have associated metabolic activities distinct from those of mitochondria or other cytoplasmic fractions. Chemical analyses, which will be reported in a subsequent paper, also reveal differences between the mitochondrial and microsomal fractions.

Enzymic activities of mitochondria from beet petiole

The mitochondria obtained from the beet petiole closely resemble those isolated from other plant tissues, such as mung bean, avocado pear and cauliflower inflorescence (see Bonner & Millerd, 1953; Goddard & Stafford, 1954). As found by other workers with plant tissues (Price & Thimann, 1951; Laties, 1953, 1954) the succinic dehydrogenase of beet mitochondria is particularly labile. The low recovery of activity (Table 2) appears to be largely attributable to a continual decline of activity during the prolonged isolation procedure. This procedure was necessary in order to isolate sufficient material to carry out both enzymic and chemical studies. In other experiments, such as that reported in Table 3, in which the preparative period was greatly shortened, the succinic-dehydrogenase activity of the isolated mitochondria was considerably higher.

The components of the succinic oxidase system in plants have not, as yet, been isolated or identified. Like mitochondria from animal tissues, those from silver beet petiole contain considerable amounts of cytochrome *b*, which has been implicated in the succinate-cytochrome *c* reductase system (Keilin & Hartree, 1949; Slater, 1949). However, the succinic dehydrogenase of heart muscle is not identical with cytochrome *b* (Morton, 1950, and unpublished work; see Chance & Smith, 1952). Slater (1949) has suggested that a labile factor mediates between the cytochrome *b* and cytochrome *c* components of the succinate-cytochrome *c* reductase system of animal tissues. This component appears to be similar to a factor specifically inhibited by antimycin A (Ahmad *et al.* 1950; Potter & Reif, 1952; Chance, 1952). The very

strong inhibition of the succinate-cytochrome *c* reductase activity by low concentrations of antimycin A (Table 3) suggests that a component similar to the Slater factor is involved in the reduction of cytochrome *c* by succinic dehydrogenase of plant tissues.

It was shown by Fischer and co-workers (Fischer & Eysenbach, 1937; Fischer *et al.* 1942) that yeast contains an enzyme which catalyses reduction of sodium fumarate in the presence of sodium dithionite and suitable dyes. The enzyme, called 'fumaric hydrogenase', was partially purified and shown to be a flavoprotein. The relationship of fumaric hydrogenase to succinic dehydrogenase of heart muscle was investigated by Harrison & Morton (unpublished work), who showed that the soluble, partially purified succinic dehydrogenase (Morton, 1950) also had associated hydrogenase activity. Subsequent studies (Morton, unpublished work) have confirmed that fumaric-hydrogenase activity is manifested by a component of the heart-muscle succinic-dehydrogenase system. It is therefore of considerable interest that the distribution of fumaric-hydrogenase activity of beet petiole closely followed the distribution of succinic dehydrogenase (Table 2). As in the case of succinic dehydrogenase, the total recovery of activity was low (about 20% of that of the original dispersion). These findings confirm the intimate relationship of fumaric-hydrogenase and succinic-dehydrogenase activities.

Slater (1950) proposed that the DPNH-cytochrome *c* reductase system of heart muscle comprised several components and could be represented by the following scheme:

where the arrows represent the direction of hydrogen transfer. This scheme involves the same factor (sensitive to antimycin A) as that implicated in the succinate-cytochrome *c* reductase system (see p. 702). Whereas the mitochondrial succinate-cytochrome *c* reductase is almost completely inhibited by antimycin A (Table 3), the mitochondrial DPNH-cytochrome *c* reductase, while sensitive to low levels of antimycin A, is only partially inhibited (Table 3). It seems very unlikely that the finding of an inhibition of only 36% at high concentrations of antimycin A is due to the occurrence in mitochondria of the antimycin A-resistant DPNH-cytochrome *c* reductase of microsomes. Rather, the results suggest that the coupling of reduced DPN to cytochrome *c* via the Slater factor is not as complete in plant mitochondria as it is in animal mitochondria, in which the system is more strongly inhibited by antimycin A (Chance, 1952; Pappenheimer & Williams, 1954).

DPNH-diaphorase activity of plant tissues

Considerable interest still attaches to the relationship between DPNH-diaphorase and DPNH-cytochrome *c* reductase. As indicated above, Slater (1950) proposed that diaphorase and an additional factor were components of the DPNH-cytochrome *c* reductase system in heart muscle. Loss of the factor linking the flavoprotein to cytochrome *c* would account for diaphorase activity without associated DPNH-cytochrome *c* reductase activity.

The results in Table 2 show that in plants the DPNH-diaphorase and DPNH-cytochrome *c* reductase activities are not necessarily related. While all the DPNH-cytochrome *c* reductase activity of the beet petiole is associated with particulate material, most of the DPNH-diaphorase activity (62%) remains in the final supernatant fraction. In view of the good recovery of activity, it seems unlikely that the DPNH-diaphorase activity of the supernatant is due to damage to the particulate components with consequent release of enzyme to the supernatant. The evidence suggests that the total DPNH-diaphorase activity of the petiole dispersion is the resultant of at least three different systems. Mitochondrial DPNH-cytochrome *c* reductase and microsomal DPNH-cytochrome *c* reductase may both exhibit some dye reductase (diaphorase) activity, while a third and distinct DPNH-diaphorase, not associated with cytochrome *c* reductase activity, occurs in the supernatant fraction. The significance of this latter enzyme in plant metabolism is quite obscure at the present time.

Function of plant microsomes

Recent evidence indicates that the microsome fraction, derived from the endoplasmic reticulum of liver, is directly concerned in the uptake of amino acids and probably in protein synthesis in liver (Keller, Zamecnik & Loftfield, 1954). The findings presented here and elsewhere (Martin & Morton, 1955; Bailie & Morton, 1955) leave little doubt that microsomes from plants contain enzymic systems analogous to those of microsomes from animal tissues. It therefore seems probable that the microsome fraction of plants, like that of animal tissues, is also concerned in protein synthesis.

SUMMARY

1. White petiole of silver beet (*Beta vulgaris*) was used to obtain cytoplasmic components with minimal disruption of mitochondria or plastids. The particulate components were isolated by differential centrifuging of the tissue dispersion.

2. Succinic-dehydrogenase, DPNH-cytochrome *c* reductase and DPNH-diaphorase activities were

determined on all fractions. The relative enzymic activities in the microsomal fraction were different from those in the mitochondrial fraction.

3. The DPNH-cytochrome *c* reductase activity of beet microsomes is not inhibited by antimycin A, while the similar activity of the mitochondria is sensitive to very low concentrations of this antibiotic.

4. The succinate-cytochrome *c* reductase activity of beet mitochondria is almost completely inhibited by low concentrations of antimycin A. This suggests that a factor, similar to the Slater factor, is concerned in the succinate-cytochrome *c* reductase system of plants.

5. Fumaric-hydrogenase activity closely parallels that of succinic dehydrogenase, both in cytoplasmic localization and in lability.

6. At least three different systems appear to contribute to the total DPNH-diaphorase activity of beet-petiole dispersions. Over 60% of the diaphorase activity occurs in the 'soluble' supernatant fraction, and all the DPNH-cytochrome *c* reductase is associated with particulate material.

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