SPECTROPHOTOMETRIC INVESTIGATIONS ON ENZYME SYSTEMS IN LIVING OBJECTS

III. RESPIRATORY ENZYMES IN HOMOGENATES OF WHEAT ROOTS

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Spectrophotometric investigations of respiratory enzymes of intact wheat roots have revealed the presence of a complete cytochrome system, co-operating with flavoprotein, peroxidase, and DPN-linked enzymes[1-6]. Because homogenates offer certain possibilities of studying the degree of confinement of the single enzymes to the structure of the protoplasm and of alternate pathways of electron transfer the spectrophotometric analysis was extended to different fractions of sediments collected from homogenized wheat roots. The results were compared with those from whole roots.

MATERIAL AND METHODS

Preliminary experiments showed that disintegration of the root tissue by means of mechanical homogenizers (knives rotating at a maximum of 40,000 r.p.m.) is inferior to grinding. Spring wheat was sown on nets of stainless steel suspended above the surface of 4 l nutrient solution in 20 × 15 × 20 cm glass troughs. The seedlings were illuminated by strip lamps and maintained at 15°C. In 2 weeks the roots were about 10 cm long. They were cut at a distance of about 5 cm from the tip, one trough yielding 12–15 g root substance.

The roots were ground by hand in a porcelain mortar at 0°C together with 25 ml 0.3–0.5*M* glucose + 0,02*M* phosphate at pH 6. The brei was spun at 1000 *g* for 1–2 min in order to remove cell walls, etc. (fraction 0). The fractionation of the homogenate was performed according to the following scheme:

I	II	III	IV
3800 *g*, 0–3 min	3800 *g*, 3–13 min	3800 *g*, 13–45 min	20,000 *g*, 30–300 min

The weight of each of the residues I to III amounted to 120–180 mg (40–60 mg dry wt.); fraction IV weighed somewhat less. The supernatant from the last fraction has a yellow color, which turns brownish after a considerable period in air.

The pellet was resuspended in a small quantity (2–5 ml) of the glucose solution and the suspension was transferred to 10 mm deep Beckman quartz cuvettes. The absorption spectrum was recorded in the author's automatic recording spectrophotometer[1,2,4]. *Direct spectrograms*, with a strip of filter paper as reference, were recorded for identification of bands of the oxidized enzymes, *difference spectrograms, e.g.* ε (sample + dithionite) — ε (untreated sample), for measuring bands of the reduced enzymes. Spectrograms were sometimes recorded directly from the pellet or from microcuvettes containing 0.6 ml. These records were run in the author's recording quartz spectrophotometer[1,2].

Besides, as well as simultaneously with, gravitational fractionation, *fractional precipitation* with ammonium sulphate also served as a method for separating enzymes. This method yielded comparatively pure preparations of cytochrome c_1 (see Fig. 7), peroxidase, and flavoprotein. The start was made from the supernatant of fraction III.

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References p. 365.

RESULTS

(a) *Optical analysis of recorded spectra*

The resuspended, aerated sediment from fractions II–III, commonly called the "mitochondrial fraction", shows bands or shoulders at 406–410, and 418–420 mμ. That these bands correspond to cytochromes c, c_1, and b is shown from the spectrum of a suspension to which some dithionite was added. The difference spectrum (ε reduced—ε oxidized) is shown in Fig. 1.

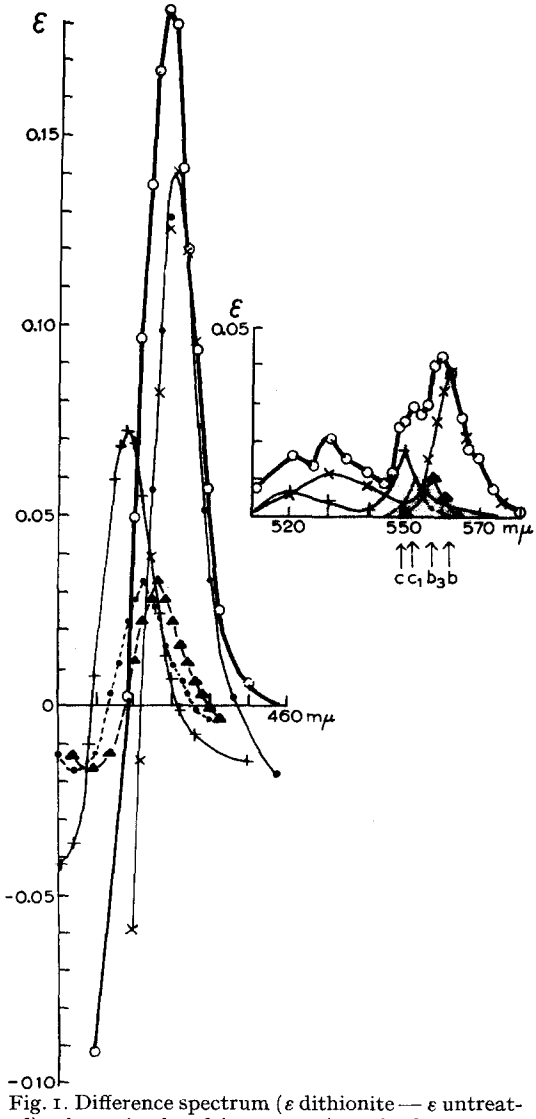

Fig. 1. Difference spectrum (ε dithionite — ε untreated) of a mitochondria suspension of wheat roots. O——O observed spectrum. Calculated participation of +——+ cytochrome c; ×——× cytochrome b (calculated from the height of the α-band, and from the observed γ-band); ●——● cytochrome c_1; ▲——▲ cytochrome b_3.

A high band appears in the γ-region with its peak at 428–430 mμ (the records were made in intervals of 2 mμ). Of twenty spectra, 15 showed the peak at 428, and 5 at 430 mμ, The α-region of the spectrum shows peaks at 550–552 and 560–562 mμ. Because both cytochromes c and c_1 may be isolated from the supernatant fluid of the fractions III and IV, the bands at 552 mμ must be interpreted as a combination of the bands of c at 550 and of c_1 at 552–554 mμ (*cf.* spectra of yeast, ref.[4]).

The position of the α-peak of cytochrome b has been determined in a large series of spectra of whole roots and homogenates. The average value is 562.6 mμ. A detailed analysis of the recorded spectra of homogenates, performed according to the procedure outlined in previous communications[1–4], indicates the probable presence of a second cytochrome b, here called b_3, the band of which (at 556–558 mμ) partly fills the space between cytochromes c_1 and b. This enzyme is probably identical with that cytochrome b_3 which MARTIN AND MORTON[7] have described for the "microsome" fraction of wheat roots. The presence of a second cytochrome b can also be traced in the spectrum of whole roots but its response to anaerobiosis, reoxidation, and common inhibitors is less clear than that of cytochrome c_1.

In homogenates the invariable presence of cytochrome b_3 and cytochrome c_1 explains the fact that the peak of the observed γ-band is moved one or two mμ toward

References p. 365.

the violet. The effect of *combination of adjacent bands* has been repeatedly emphasized in previous communications[1,4]. The combination of only cytochromes c_{550} and b_{562} would give a band with two definite peaks at the expected wavelengths. Similarly, the combination of c_{418} and b_{430} in the difference spectrum would result in a band with two separate peaks. The fact that the observed γ-band of the mitochondrial suspension is smoothly one-peaked (see Fig. 1), in spite of the obvious presence of both cytochrome *c* and cytochrome *b*, thus indicates the existence of further bands between *c* and *b*.

The relation γ/α of pure preparations of cytochromes *c* and *b*, measured in the difference spectrum, amounts to values between 3.8 and 4.5. A pure preparation of cytochrome *c* showed the value 3.9. A calculation of the γ-bands from the α-bands (Fig. 1), assuming a relation $\gamma/\alpha = 4$, results in a combined band which approximately coincides with the observed one. Fig. 1 shows that a band in the difference spectrum, which is composed of four individual bands, may be narrower than the space occupied by the individual bands. This fact depends upon the depressions below the isosbestic line (see Fig. 1), which appear in the difference spectrum. The observed combined band is completely devoid of extra peaks or shoulders and may be easily mistaken for a band of a single cytochrome, if the α-region did not clearly show its complex nature.

The results summarized in Fig. 1 show that all the cytochromes that may be distinguished in whole roots are present in the mitochondria fraction.

The *molar relation* between the cytochromes *b* and *c* in intact roots is close to $b/c = 2$ (this figure is calculated from a large number of new records; earlier calculations yielded somewhat lower values; *cf.* ref.[1,4]). The value 2 corresponds to a relation between the molar extinction values of $b/c = 1.6$ (on the molar extinctions of *b* and *c*; see ref. [4]).

The *mitochondria fraction* shows a ratio between the molar extinctions of $b/c = 2.2$ (average of 21 spectra). This means that the particles have lost about 40% of cytochrome *c* and corresponding quantities of cytochrome c_1. The lost quantities may be recovered from the supernatant of the "microsome" fraction. The calculations from whole roots and homogenates were made from the aerated stage as the oxidized one. The quantities which remain reduced in the aerated specimens (see below) are not included in the calculation.

Unlike the cytochromes *c* and c_1 cytochrome a_3 (γ-band at 444 mμ) maintains the same molar relation to cytochrome *b* as in the intact root, amounting to $b/a_3 = 4.1$ to 4.2, or approximately four molecules of *b* for one molecule of a_3. The presence of a_3 was noticed as an elevation or sometimes a shoulder of the combined γ-band in the region 440–448 mμ (Figs. 5 and 6). Its presence was also shown by determination of the disappearance of the respiratory activity of the mitochondria fraction in the presence of cyanide and azide. Peroxidase, which also responds to these inhibitors, is not present in the mitochondria fraction.

The *peroxidase* in wheat roots, which amounts to quantities considerably exceeding the sum of all other hemins[2,6], escapes nearly quantitatively into the supernatant of the mitochondria fraction. No trace of the band at 436 mμ appearing after treatment of peroxidase with dithionite is noticed in the reduced mitochondria fraction. In whole roots peroxidase forms complexes with certain pigments, etc., which remain in solution after homogenizing the tissue. In wheat roots that are exposed to complete anaerobiosis a high band appears at about 571 mμ. The band at 404 mμ of peroxidase disappears simultaneously with the formation of the band at 571 mμ. This band,

References p. 365.

together with a corresponding β-band at 540 mμ, was once believed to represent a new cytochrome called *dh* of *b*-type[3]. Recent investigations[8] have shown that *dh* is most probably a complex between peroxidase and soluble pigments and/or certain C_4-acids. The peroxidase–*dh* complex is extremely rapidly oxidized and may be spectrophotometrically observed only under complete anaerobiosis.

The total quantity of *flavoprotein* in wheat roots exceeds that of the total quantity of cytochromes by a factor 3–4. The concentration of flavoprotein may be calculated from the depression at 460 mμ, following a treatment with dithionite, because reduced flavoprotein is decolorized. In these measurements, however, due attention must be paid to the depressions of the bands of cytochromes *b* and a_3 at 460 mμ[4,5]. At certain concentrations of ammonium sulphate about equimolar quantities of cytochrome *b* and flavoprotein may be precipitated from a homogenate containing mitochondria plus microsomes and dissolved enzymes. The sedimented fractions II–III are never completely devoid of flavoprotein, but the quantities are considerably smaller than those in the supernatant. Recent observations on baker's yeast [4] have shown the close co-operation between cytochrome *b* and an equimolar quantity of flavoprotein. The behaviour of the homogenates, however, shows that flavoprotein is more easily detached from the mitochondria than are the cytochromes. It is doubtful if the bond between cytochrome *b* and flavoprotein is as strong as that between flavoprotein and cytochrome b_2 in yeast[9,10]. The fact that the cytochrome system of the mitochondrial fraction is reduced upon addition of KCN is an argument in support of the assumption that a flavoprotein enzyme, at least in limited quantities, is present in the mitochondria.

The enzymes that are detached from particles in the mitochondria fractions II and III are probably only to a minor extent brought down to molecular dispersion. More easily soluble enzymes are peroxidase and cytochrome c_1 but solutions of these are not precipitated in the centrifuge. Small quantities of peroxidase and of the complex peroxidase–*dh* may be found in the fraction IV (20,000 *g*). This fraction contains more small particles ("microsomes") than do the mitochondria fractions, and it also contains more of the cytochromes *c* and c_1 and possibly of b_3.

(*b*) *Cytochromes* b *and* b_3

Cytochrome *b* remains in more or less constant concentration in all fractions except fraction I, which probably contains more debris from cellulose walls (Table I and Fig. 4). This means that cytochrome *b* is also intimately attached to particles of smaller size than the "mitochondria", because "microsomes" are relatively more frequent in the high speed fraction. The average content of cytochrome *b* per unit dry weight of particles is 2.3–2.7%. This is 5–18 times greater than in dried roots.

TABLE I

RELATIVE QUANTITY OF CYTOCHROME *b* PER UNIT WEIGHT OF THE PELLET

Fraction	*I*	*II*	*III*	*IV*
Cytochrome *b*	31	100	86	90

It has already been mentioned that, whereas the percentage of cytochromes *c* and c_1 and probably also of b_3 rises in the later fractions, the ratio between *b* and a_3

References p. 365.

remains the same. This fact indicates that *b* and a_3 are firmly anchored to structural elements of the mitochondria, which may appear as sub-particles or "microsomes" when the mitochondria are exposed to mechanical disintegration.

As to the existence of two cytochromes of *b*-type in wheat roots (see above), attention is called to communications by HILL AND SCARISBRICK and HILL AND WITTINGHAM[12]. These authors describe a "new soluble component", called b_3, with bands in the reduced state at 529 and 559.7 mμ. The pigment is claimed to be widespread in plant tissues, and to show a two-banded spectrum in the oxidized state, too.

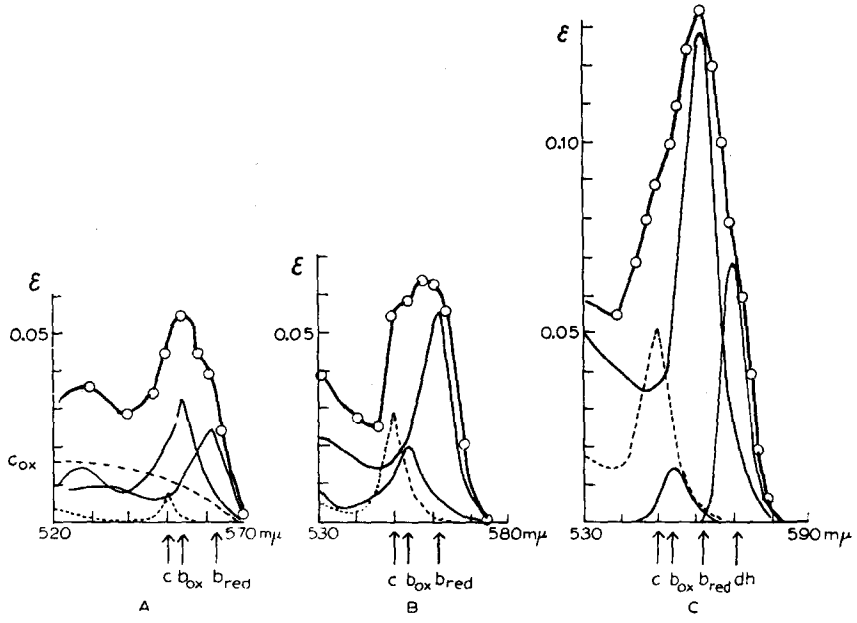

Fig. 2. Direct spectrograms of an 18 mm thick bundle of wheat roots, enclosed in a quartz tube and surrounded by distilled water. (A) Spectrum of the α-β-region at the start (aerated). (B) 5 min from the start (beginning reduction at O_2 deficiency). (C) 20 min from the start (continued reduction). Calculated participation of ------ cytochrome *c*, ——— cytochrome *b* and the peroxidase complex *dh*. During the process of reduction the bands of b_{ox} decrease and those of b_{red} increase. The starting times are in the order $c < b < dh$.

The relation between the two states is not quite clear from the facts yet published. The following observations may contribute to the explanation of the bands in the region 520–570 mμ that are observed in aerated, whole roots.

Roots of wheat and other cereals invariably show a two-banded spectrum in this region, which remains unchanged even at an increased supply of oxygen. A lower β-band is observed at about 535 mμ and a higher α-band at 556–560 mμ (Fig. 2). The bands are broader than those of single cytochromes, with more rounded peaks, the position of which may vary a few mμ. The shape of the bands is reminiscent of those of the difference spectrum between peroxidase–H_2O_2 and peroxidase, but has no relation to this compound. This is shown by the fact that the two-banded spectrum in aerated roots is not changed in the presence of 0.05–0.1 *M* NaF (pH 3.4) which combines with peroxidase as peroxidase–F[3].

If the roots are enclosed in a small quantity of distilled water or dilute salt solution, the oxygen dissolved in the solution soon disappears, and the stages of increasing

References p. 365.

reduction of the enzymes may be closely followed if spectrophotometric records are taken at suitable time intervals (see Fig. 2). As reduction increases the bands are changed in a way which does not support the idea of HILL AND SCARISBRICK that they represent a single cytochrome. The two-banded spectrum of aerated roots may be explained as a combination of oxidized and reduced bands of cytochromes *c*, c_1, and *b*, plus a small quantity of a hemin pigment to which the term b_3 may be applied.

It has been shown[1] that in normally respiring roots cytochrome *b* is still about 15–20% reduced and cytochrome *c* somewhat more. This is the normal steady state of the operating cytochrome system, and it reflects the balance between the dehydrogenase systems and the cytochrome oxidase. Oxidized cytochrome *b* has low bands at 525 and 554[13,4,5] and reduced cytochrome *b* high bands at 530 and 562–563 mμ. A combination of these sets of oxidized and reduced bands in normally respiring roots results in broad combined bands at 525–530 and 556–563 mμ, as shown in Fig. 2, A and B. The bands of partly reduced cytochromes *c* and c_1 complete the picture.

As reduction slowly increases (see Figs. 2 and 3) the bands are changed in the following way. The left side of the combined α-band is rapidly (in 1–2 min) raised at 550 mμ, and later at 552 mμ, owing to the rapid reduction of cytochromes $c + c_1$ (*cf.* ref.[6]). In a few more minutes a similar rise is observed at the right side of the combined band owing to the reduction of cytochrome *b*, which is then becoming appreciable. Ten to fifteen minutes after the start of reduction of cytochrome *c* a band is rising at about 570 mμ, corresponding to the appearance of the reduced peroxidase–*dh* complex (see above, and ref.[8]).

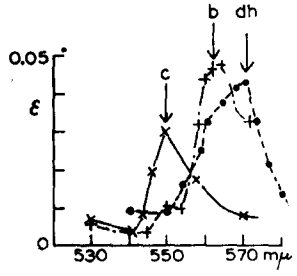

Fig. 3

Fig. 3. Difference spectra of an 18 mm thick bundle of wheat roots, exposed to increasing reduction at lack of O_2. ×——× difference between 1 min from the start and aerated; +—·—+ difference between 5 and 1 min from the start, ●----● difference between 10 and 5 min from the start. Time order of reduction as in Fig. 2. Top of the *b* α-band at 562–563 mμ.

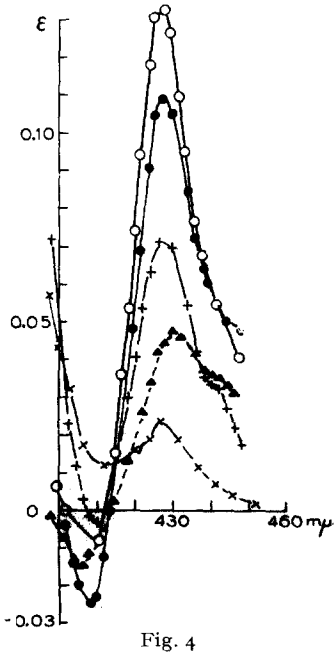

Fig. 4

Fig. 4. Difference spectra (ε dithionite − ε untreated) of fraction I (×——×); fraction II (+——+); fraction III (O——O); fraction IV, 30 min (●——●), and 300 min (▲——▲). The fractions resuspended in approximately the same quantity of glucose solution.

Reduction of whole roots in dithionite + PO_4, pH 6, is a less suitable procedure for incisive studies of the kinetics of the cytochrome system because of the high percentage of peroxidase, which develops a high band at 556 mμ[12,13]. This band covers a possible α-band of cytochrome b_3, a fact to be considered in studies of the "microsome"

References p. 365.

fraction in which some peroxidase is always present. Dithionite, too, has a tendency to disorganize the living structure. The reduction and reoxidation of cytochromes *b* and *c* may, however, be studied in whole roots if 0.02 *M* dithionite is combined with 0.001 *M* cyanide and 0.02 *M* phosphate, pH 6, and the reoxidation is performed in cyanide solutions without dithionite. Both *b* and *c* are in fact fairly insensitive to CN, but the peroxidase is blocked as peroxidase–CN[3,5]. Cyanide also inactivates the cytochrome oxidase. As previously shown[3], cytochrome *b* of wheat roots is, however, autoxidizable, this enzyme thus forming a shunt to oxygen, bypassing cytochrome a_3. The reoxidation of whole roots in cyanide solutions, following a reduction in dithionite + cyanide, now shows a negative band of cytochrome *b*, which is slightly extended toward 558 mμ, obviously because of the presence of a small quantity of cytochrome b_3.

Comparative studies of intact roots thus confirm the probable existence of a cytochrome b_3, the quantity of which, however, attains only about 1/4 of that of cytochrome *b*. New measurements on whole roots give the following approximative molar relations between the involved cytochromes:

Cytochromes	*c*	c_1	*b*	b_3	$a + a_3$
Molar relation	2	1	4	1	1

Cytochrome b_3 thus amounts to 10–12% of the total cytochrome content of wheat roots, a figure in agreement with the calculations of MARTIN AND MORTON[14]. These authors find bands of b_3 at 425, 525, and 560 mμ. My observations point to a somewhat lower position of the α-band, *viz.* 556–558 mμ. MARTIN AND MORTON show, too, that b_3 is not confined to the microsome fraction, although its relative concentration increases in the high speed fractions. In my experiments an extension of the centrifugation at 20,000 *g* to 9 h at 15°C added only very little further sediment to that obtained at 30 min centrifugation, and this final sediment showed no trace of cytochromes.

(*c*) *The influence of various agents on the homogenates*

Fraction IV (20,000 *g*) invariably shows the presence of the cytochromes *b* and a_3. a_3 was calculated from the difference between the observed slope of the band from 430 to 450 mμ and the calculated slope of the *b*-band; sometimes the presence of a_3 appears as a separate shoulder (see Figs. 4, 5, 6), whereas the calculated quantities of *c* and c_1 are lower than those observed in the intact roots, and also lower than in the mitochondria fraction (see above). Because the mitochondria thus appear having a less damaged respiratory system the experiments with inhibitors were performed with them.

Mitochondria reduce added cytochrome *c* in the absence of O_2. The effect of succinate on aerated whole roots is weak, whereas the response to fumarate and malonate[1,2] is evident. *Succinate* added to an aerated suspension of mitochondria reduces predominantly the cytochrome *c* which is attached to the system, whereas cytochrome *b* is little affected. A similar, but reversed effect is observed with *malonate*. Addition of malonate to the mitochondria suspension results in a strong oxidation of especially cytochromes *c* and c_1. This result differs from similar experiments with intact roots, in which cytochrome *b* is strongly oxidized in the presence of malonate. The strong negative band at 418–420 mμ appearing in aerated mitochondria following an addition of malonate shows that the cytochromes *c* and c_1 are partially reduced from the start. As mentioned above cytochrome *c* is more reduced than cytochrome *b* in the steady state of normally respiring roots, too. The electron transference through the cytochrome system of mitochondria is, however, considerably slowed down as compared

References p. 365.

with whole roots (see below), a fact which may contribute to the observed differences.

Ascorbate (pH 6) induced about 50% reduction of the cytochromes *b* and a_3.

Reduced *diphosphopyridine nucleotide* (DPNH) promoted reduction of the cytochrome system both in air and in N_2. Here again the bands in N_2 attained only about 50% of the height of the bands in dithionite. Addition of DPNH to an aerated suspension of fractions III and IV developed a band at 424–426 mμ, which increased in N_2. Because these fractions still contain cytochrome *b* this result indicates a preference of DPNH to cytochrome b_3 (γ-band of b_3 at 425, of *b* at 430 mμ) (see Figs. 5 and 6).

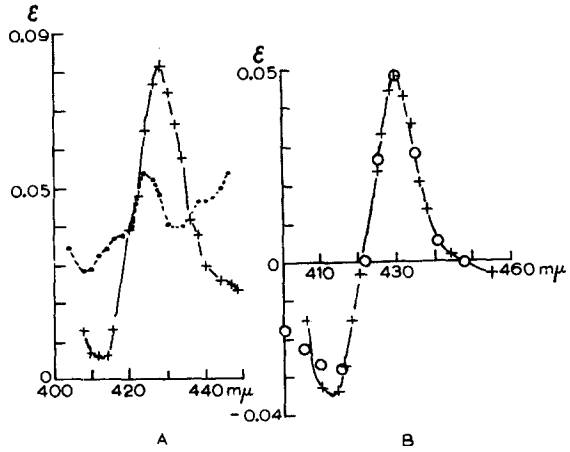

Fig. 5. Difference spectra (ε treated — ε untreated) of a resuspended mitochondria fraction. (A) +——+ spectrum of reduced cytochromes (dithionite) and ●——● of the response to aerated DPNH, showing partially reduced cytochromes c_1 and b_3. (B) Difference between the two curves in (A), showing a pure spectrum cf cytochrome *b* (o are values calculated from a pure preparation of *b*).

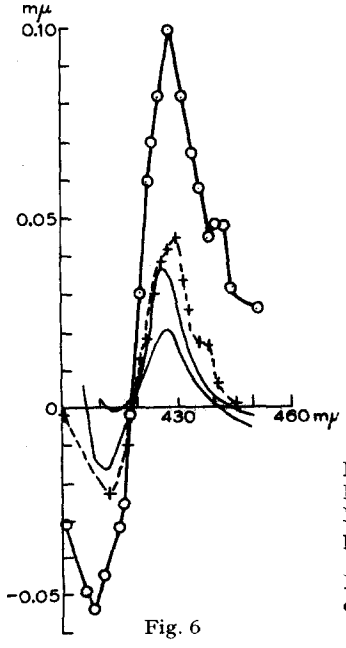

Fig. 6

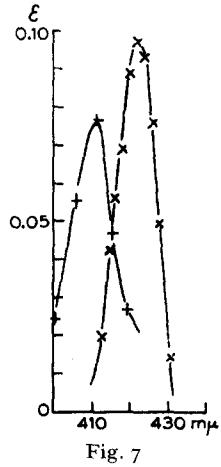

Fig. 7

Fig. 6. Difference spectra (ε treated — ε untreated) of fraction IV. ——— samples treated with DPNH in air (lower curve) and N_2 (higher curve). Top of the γ-band at 425 mμ. +——+ sample treated with DPNH + antimycin A. Top of the band at 430 mμ. o——o dithionite.

Fig. 7. γ-bands of oxidized (+——+) and reduced (×——×) cytochrome c_1 from a fractionated precipitation of the supernatant with ammonium sulphate.

References p. 365.

Antimycin A added to DPNH in an aerated sample developed a peak at 430 mμ (Fig. 6). Antimycin A is known as a specific inhibitor of the oxidation of cytochrome *b*. The experiments with DPNH and Antimycin A indicate different paths of reduction of the cytochromes *b* and b_3.

The *peroxidase complex dh* (see above) was never observed in the mitochondrial fraction and is only occasionally seen in fraction IV. Small quantities of peroxidase are present in the high speed fraction together with traces of non-hemin pigments which are combined with peroxidase to form the complex *dh*. MARTIN AND MORTON (see ref. [14], p. 405) have not observed *dh* in living roots. This is not surprising because they studied the roots in the presence of cyanide which inactivates the peroxidase, because peroxidase–CN is formed. The complex *dh* formed anaerobically disappears if cyanide is added. Dithionite has a similar effect, but weaker, as a consequence of which traces of *dh* may appear after treatment of fraction IV with dithionite, if the right components are present.

(d) O_2-consumption of homogenates

The O_2-consumption of the fractions I–IV, resuspended in 0.3–0.5 *M* glucose + 0.002 *M* phosphate pH 6, was studied in the Warburg respirometer at 25°C.

Fraction O respires only about 30% as fast as whole roots (the values are referred to unit dry weight). Because all enzymes and co-factors are present in the crude preparation the strong retardation of the respiration must depend on the partial disorganization of the systems. Some loss of cytochromes *c* and c_1 was observed in the mitochondria but a more important change is probably the separation between the cytochrome system on one hand, and peroxidase, quantities of flavoprotein, and DPN-linked enzymes on the other hand. It was observed that those quantitities of cytochromes *c* and c_1 which are going over into the supernatant are not reduced by added DPNH.

TABLE II

OXYGEN CONSUMPTION OF THE FRACTIONS O–III OF THE HOMOGENATES

Fraction	*O*	*I*	*II*	*III*
O_2-uptake, relative	100	6.1	7.3	2.3

Fractions I–III, comprising about 80% of the total sediment, together display only 16% of the original respiration of the crude homogenate, which in its turn respires at only 30% of the intensity of whole roots (see Table II).

TABLE III

EFFECT OF ADDITIONS OF VARIOUS SUBSTANCES ON THE RESPIRATION OF THE MITOCHONDRIA FRACTION

No effect = o. Stimulation = +.

Added substance	*Cytochrome c*	*Succinate pH 4*	*Adenosine triphosphate*	*Ascorbate pH 6*	*DPN + nicotinamide*	*Malonate pH 4*	*NaN_3 0.001 M*
Effect	o — (+)	o — (+)	o — (+)	+	++	—	—

References p. 365.

As shown by Table III additions of DPNH, DPNH + nicotineamide and ascorbate stimulate the respiration of the mitochondria. These substances, as also peroxidase and most of the flavoprotein, are absent from this fraction. The low flavoprotein content of the mitochondria probably explains the before-named incomplete reduction of the mitochondria cytochromes in N_2 and DPNH. The missing or weak effect of added cytochrome *c* obviously depends upon the presence of this enzyme in the mitochondria. The presence of DPN in living roots has been shown by spectrophotometric measurements at 340 mμ during reduction and reoxidation of the cytochrome system. Earlier measurements pointed to values of DPN up to five times higher than those of cytochrome *b*. Recent measurements yielded considerably higher values, up to 30 times those for cytochrome *b*.

DISCUSSION

One result of the investigation is the conclusion that fractionation by means of centrifugation of homogenates does not effectively separate "mitochondria" and "microsomes". KMETEC AND NEWCOMB[15], who studied particulate fractions from peanut cotyledons, arrived at the conclusion that all sorts of particles are present in the mitochondria fraction. The larger particles of wheat root homogenates, which are of course dominant in the fractions II–III, may, at least to a certain extent, be identical with observed microscopic particles in the living protoplasm. Whether smaller particles, down to microsomes, really correspond to identical items in the living protoplasm is still open to discussion.

The present results are more in accordance with the assumption that the larger particles carry a number of enzymes which are in close molecular contact with one another and together constitute an electron-transporting unit. It seems doubtful, however, if we may speak of an electron-transporting *particle* (ETP, see ref. [8]) of definite construction because a number of enzymes belonging to the complete pathway from dehydrogenases to oxydases are lost or at least reduced in quantity as early as the first fraction. The results show that only the cytochromes *b* and a_3 are so firmly attached to a structural carrier that the bonds successfully resist homogenization. Even a mild treatment removes certain quantities of the cytochromes *c* and c_1 which, according to observations of the kinetics of the complete assembly [6], are indispensable members of the original "electron-transport unit".

The results do not support the idea that "microsomes" may carry enzyme systems which act independently of systems attached to "mitochondria". It is more likely to assume that microsomes carry enzymes which have been mechanically separated from a larger unit. Enzymes may be more or less firmly or losely attached to the respiratory structure, but all experimental evidence points to the existence of a structural unit, composed of cytochromes and flavoprotein, around which DPN-linked enzymes and peroxidase serve as bridges to processes going on in the ground substance of the protoplasm.

A number of investigators claim that the respiratory units are located in the surface layer of the mitochondria. Other possible sites are the outer and inner plasma membranes separating the protoplasm of the plant cell from the medium and the central vacuole, respectively. These questions are by no means yet settled.

References p. 365.

SUMMARY

Particulate fractions of wheat root protoplasm, from mitochondria to microsomes, all carry the cytochromes a_3, c, c_1, b and b_3, and probably some flavoprotein. The mitochondria fractions show more of the cytochromes a_3 and b, the microsome fraction more of the cytochromes c, c_1, and b_3, which are partly detached from the "respiratory particles". Peroxidase, DPN-linked enzymes, and more or less of the flavoprotein and cytochromes c and c_1 go into the supernatant of the fractions. The results are compared with observations on living roots.

REFERENCES

1 H. LUNDEGÅRDH, *Arkiv. Kemi*, 5 (1952) 97.
2 H. LUNDEGÅRDH, *Arkiv. Kemi*, 7 (1954) 451.
3 H. LUNDEGÅRDH, *Physiol. Plantarum*, 8 (1955) 84, 95.
4 H. LUNDEGÅRDH, *Biochim. Biophys. Acta*, 20 (1956) 469.
5 H. LUNDEGÅRDH, *Biochim. Biophys. Acta*, 25 (1957) 1.
6 H. LUNDEGÅRDH, *Acta Chem. Scand.*, 10 (1956) 1083.
7 E. M. MARTIN AND R. K. MORTON, *Biochem. J.*, 62 (1956) 696.
8 H. LUNDEGÅRDH, *Nature*, (in the press).
9 C. A. APPLEBY AND R. K. MORTON, *Nature*, 173 (1954) 749.
10 E. BOERI AND L. TOSI, *Arch. Biochem. Biophys.*, 60 (1956) 463.
11 R. HILL AND R. SCARISBRICK, *New Phytologist*, 50 (1951) 98.
12 R. HILL AND C. P. WITTINGHAM, *Photosynthesis*, Methuen's Monographs, London, 1955.
13 G. HÜSCHER, M. KIESE AND R. NICOLAS, *Biochem. Z.*, 325 (1954) 223.
14 E. M. MARTIN AND R. K. MORTON, *Biochem. J.*, 65 (1957) 404.
15 E. KMETEC AND E. H. NEWCOMB, *Am. J. Botany*, 43 (1956) 333.
16 D. KEILIN AND E. F. HARTREE, *Biochem. J.*, 49 (1951) 88.
17 B. MACKLER AND D. E. GREEN, *Biochim. Biophys. Acta*, 21 (1956) 1.

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METABOLISM OF MALTOSE LABELLED WITH ^{14}C IN THE REDUCING GLUCOSE MOIETY, BY TOBACCO LEAF DISKS

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Metabolism of sugars labelled with ^{14}C by leaf tissues has been the subject of a number of investigations[1–7]. The results are in accordance with the view that the pathway of synthesis of sucrose and starch includes a step in which hexose units, whether supplied free or combined, become equilibrated and are only then available as substrates for respiration and further synthesis. For example when sucrose was supplied to tobacco leaf disks, there was no discrimination between the glucose and fructose moieties as substrates for starch synthesis and respiration, and sucrose was incorporated into the equilibrating system or metabolic "pool" without prior formation of free hexose[4].

A number of disaccharides with different types of linkage between the hexose units has now been tested as possible substrates for sucrose and starch formation

References p. 370.