The Terminal Oxidases of the Potato Tuber

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(Received 29 May 1961)

The affinity for oxygen of the terminal oxidases in plant tissues is perhaps one of the best criteria for determining the nature of these enzymes. Identifications based on such characteristics are not open to the criticism that may be advanced against those based on a study of the action of inhibitors on respiration. Here the inhibition may be the result of an effect upon enzymes other than those under investigation, or the respiratory channel may be changed as a result of the action of the inhibitor or of the conditions under which it is studied (cf. Honda, 1955).

Most of the oxidases that have been suggested as functioning as terminal oxidases in plants have very different affinities for oxygen. Hence the relation between the rate of respiration and the partial pressure of oxygen in the tissue, when compared with the known affinities of the separate oxidase enzymes, would appear to offer a simple means of indicating the enzymes that cannot be functioning and of suggesting those that may possibly be mediating the oxidation.

There is now general agreement that one of the terminal oxidases of the potato is cytochrome *c* oxidase (Levy & Schade, 1948; Goddard & Holden, 1950; James, 1953*a*). Thimann, Yocum & Hackett (1954) studied the relation between the rate of respiration of freshly cut potato slices and the partial pressure of oxygen in the external atmosphere. They reported that the affinity of the terminal oxidase for oxygen was high, and provisionally identified this enzyme with cytochrome *c* oxidase. With more aged slices there was evidence to indicate a change in the nature of the oxidase, but the oxygen affinity was decreased only slightly. The authors concluded that there was no evidence for the participation as a terminal oxidase of oxidases, like ascorbic acid oxidase, which have a relatively low affinity for oxygen.

Although we do not dispute these conclusions so far as they apply to potato slices, we suggest that conclusions reached on the basis of experiments with slices or with homogenates may not necessarily be applicable to the metabolism of the intact tuber. Thus the respiration of potato slices is seven to ten times that of an equal weight of intact tuber tissue, and this fact alone makes it difficult to argue from the results obtained with slices.

We have studied the oxygen uptake of the potato tuber in relation to the partial pressure of oxygen in the external atmosphere and conclude that there is evidence for the participation of at least two terminal oxidases in the respiration of the tuber: one with a high affinity for oxygen, which is probably identical with cytochrome *c* oxidase, and a second with a relatively low affinity, which, on this basis, cannot be identified with either phenolase or ascorbic acid oxidase.

MATERIALS AND METHODS

Potatoes. Three batches of potatoes (var. King Edward) were used in these experiments, two obtained from Teneriffe in 1959 and 1960, and one variety grown at Stretham, Cambs., in 1959. There was no marked difference in the results obtained from these stocks.

Small potatoes (15–20 g.) were selected for two reasons: first, so that a reasonable number (10–15) could be accommodated in the chamber of the respirometer for determining the respiratory activity; secondly, to minimize the length of diffusion pathways within the tuber.

Respirometer. The respirometer used in these experiments was developed at the Low Temperature Research Station by Wager & Porter (1961). In principle the O_2 consumed in respiration is replaced by O_2 generated electrolytically. When the partial pressure of O_2 in the respirometer falls, the required amount of O_2 is produced electrolytically to restore the partial pressure, the time intervals between the production of these increments of O_2 being recorded.

Measurement of respiration. All experiments were conducted at 20°. Potato tubers (approximately 150 g.) were placed in the respirometer chamber at 20° and their rate of O_2 consumption was determined over a period of 24 hr. The atmosphere in the chamber was then changed by flushing with an appropriate gas mixture; a volume of gas of at least 30 times the volume of the chamber was passed through for this purpose. The sample was then left to equilibrate for approximately 1 hr. In these experiments the change from air to a different partial pressure of O_2 led to a rapid adjustment of the respiration to a new and steady level (Fig. 1). The level of the respiration was therefore determined 1 hr. after the change-over and estimated from readings every 3 hr. over the next 12–18 hr. The respiratory changes described are thus the immediate response to changes in the partial pressure of O_2 , and are not due either to adjustments taking some 40 hr. to reach a new steady state (cf. Blackman, 1928) or to the result of long-term effects of alteration in the partial pressure of O_2 such as have been described by Barker & Mapson (1955).

Fig. 1. Response of the respiration of potato tubers to change in partial pressure of oxygen. For details see text.

Preparation of phenolase and estimation of its activity. Potato peelings (200 g.) were extracted with 250 ml. of sucrose (0.25M), phosphate (0.05M; pH 6.3) and KCl (0.1M) at -3° . The extract was treated with $(\text{NH}_4)_2\text{SO}_4$ to give 35% saturation, the precipitate discarded, and $(\text{NH}_4)_2\text{SO}_4$ added to 55% saturation. The precipitate so obtained was dissolved in 30 ml. of 0.05M-phosphate buffer, pH 6.3, and dialysed against 0.01M-phosphate buffer, pH 6.3, to remove excess of $(\text{NH}_4)_2\text{SO}_4$.

The activity of the phenolase was measured by following the rate of oxidation of ascorbic acid, catalysed by the enzyme and a phenolic substrate, at different partial pressures of O_2 between 0.025 and 1 atm., which were obtained by bubbling the appropriate gas mixture through the solution. The assay mixture contained, in a total volume of 6 ml., 2 ml. of a buffer consisting of 0.025M-tris with maleic acid (0.025M) adjusted to pH 6.3 with NaOH. The final solution contained, in addition, 0.085M-ascorbic acid, 10 mM-phenolic substrate, enzyme and mM-EDTA. The rate of oxidation of ascorbic acid was determined by removing portions into metaphosphoric acid and determining the ascorbic acid by titration against indophenol dye (Harris & Oliver, 1942).

Some measurements of enzyme activity were also carried out by the colorimetric method of Baruah & Swain (1959) by using the same assay mixture with the addition of 0.1 mM CuSO_4 but omitting ascorbic acid and EDTA.

Test for the presence of ascorbic acid oxidase. Fresh potato tubers (100 g.) were sliced and extracted with 200 ml. of ice-cold $\text{Na}_2\text{HPO}_4\text{-KH}_2\text{PO}_4$ buffer (0.05M; pH 6.2) containing EDTA (mM) and L-ascorbic acid (0.05M) in a blender in which air had been replaced by N_2 . The extract was centrifuged at 1600g for 5 min. and the supernatant solution decanted. Solid $(\text{NH}_4)_2\text{SO}_4$ was added to give 90% saturation, and after 15 min. at -3° the precipitate was separated by centrifuging and dissolved in 10 ml. of the phosphate buffer containing EDTA. The enzyme solution was then dialysed for 24 hr. against several changes of the same buffer solutions to remove phenols. The ability of the solution to oxidize added ascorbic acid at pH 6.2 and 25° was then determined by the method described above.

Estimation of the partial pressure of oxygen in the intercellular space. The relation between the O_2 deficit in a

tuber and the rate of its uptake of O_2 is given (Burton, 1950) by the equation

$$p_e - p_i = \frac{qL}{AK_1},$$

where p_e and p_i are the partial pressures of O_2 (atm.) outside the tuber and in the peripheral intercellular space respectively; q is the volume of O_2 diffusing into the tuber (ml./cm.²/sec.); L is the length of the diffusion path, assumed to be the thickness of the periderm (cm.); A is the proportion of the periderm which is permeable to O_2 ; and K_1 is the coefficient of diffusion (ml./cm.² of permeable surface/sec.) through the periderm.

In a reasonably uniform sample of tubers L/AK_1 is approximately constant and there is thus direct proportionality between $p_e - p_i$ and q [see, for example, the results of Burton (1950) on the variety Arran Consul]. If therefore the value of L/AK_1 is calculated for a representative sample of experimental tubers it may then be used to derive the figures for p_i which correspond to any measured values of p_e and q . In the present work L/AK_1 was determined by measuring the composition of the intercellular gas and the rate of O_2 uptake, in air, in an apparatus devised by W. H. Smith & A. J. Topping [unpublished work, but developed from that of Smith (1947)] to give simultaneous estimations of O_2 uptake, CO_2 output and the composition of the gas in the intercellular space. A 1 mm. diameter hole was drilled through the tuber and the ends were sealed with wax. The atmosphere in the cavity was allowed to equilibrate with that in a glass tube attached to a hypodermic needle inserted into the cavity to a position corresponding to the centre of the tuber and sealed in position. The tuber was in a sealed chamber containing 0.1N-NaOH as CO_2 absorbent, the daily titration of which gave a measure of the CO_2 output. Sufficient O_2 to maintain the pressure in the chamber was automatically metered in, this volume of O_2 giving a measure of the O_2 uptake. A sample of the gas in the tube in connexion with the internal atmosphere was ejected and analysed once every 24 hr. This analysis gave an estimate of the average composition of the internal atmosphere, in that the cavity extended from the centre to the periphery. In the intact tuber there would be a drop in O_2 concentration between the periphery of the flesh and the centre, the magnitude of the drop being $ar^2/6D$ atm. (Goddard, 1947; Burton, 1950), where a represents O_2 uptake in ml./sec./ml. of tissue, r the radius of the tuber in cm. and D the diffusion coefficient of O_2 in the tissue (ml./cm.² of tissue area/sec.). Burton (1950) calculated that D for potato tissue, over the temperature range $1\text{--}25^{\circ}$, was about 2.9×10^{-4} ml./cm.²/sec. With the sizes of tubers used and the rates of O_2 uptake encountered in the present work we should thus expect the partial pressures of O_2 in the intercellular space at the periphery and at the centre to differ by about $4.6R \times 10^{-3}$ (where R is respiration in ml./100 g./hr.): i.e. by amounts varying from 3.7×10^{-3} atm. in air ($R = 0.80$) to 1.1×10^{-3} atm. in 1.45% of O_2 ($R = 0.24$). We may reasonably assume that the O_2 concentrations immediately under the skin would differ from the mean values obtained in the apparatus described above by only small amounts, probably of the order of 2×10^{-3} atm. in air and 5×10^{-4} atm. in 1.45% of O_2 . The value of L/AK_1 for the potatoes used in the present work was calculated to be 1.96×10^4 . For the reason given the value for p_i in air was taken as 2×10^{-3} atm. less than the mean experimental value.

Spectroscopic examinations. In these experiments the skin of small tubers was rubbed or scraped off two diametrically opposite areas, and the tubers were placed in small glass containers through which gas mixtures of different composition could be passed. The tubers were equilibrated with the gas mixture for 1-3 hr. before the spectra were examined. Because of the two 'windows' without skin it is difficult to assess what would have been the composition of the internal atmosphere of the tubers, except that it would be between that to be expected in intact tubers and that of the surrounding atmosphere. Absorption bands were examined by means of the spectrocoulometer described by Hill (1936). In some experiments the intensities of the bands of the cytochrome *a* and *b* components were determined by a comparison with the absorption band of a solution of denatured ox-globin haemochromogen in NaOH, and from this comparison the percentage reduction of the *a* and *b* components could be approximately estimated.

RESULTS

Relation between oxygen concentration and respiration of potato tubers

The results are tabulated in Table 1, and the response of the respiration of the tubers to altera-

tion in the partial pressure of oxygen in the external atmosphere from 0.014 to 1.0 atm. oxygen is illustrated in Fig. 2 (*a*). In Fig. 2 (*b*) the same results are related to the calculated partial pressure of oxygen in the intercellular space of the tubers. They show that an increase in the internal oxygen partial pressure from zero to about 0.01 atm. caused a rapid rise in the rate of oxygen uptake to about 50% of the rate in pure oxygen. Thereafter the rate of increase in the respiration with increasing partial pressure of oxygen, up to 1.0 atm., was relatively slow.

The relation between respiration and oxygen concentration is more clearly shown in Fig. 3 where the reciprocal of the rate of respiration ($1/R$) is plotted against the reciprocal of the intercellular oxygen partial pressure ($1/p_i$) (see Lineweaver & Burk, 1934). These plots relate only to the potatoes grown at Stretham, as it was on these that the permeability of the skin was determined, and the calculated values for internal oxygen concentration are thus likely to be more reliable than with the Teneriffe potatoes. Within the range of oxygen

Table 1. *Oxygen uptake and intercellular partial pressure of oxygen of potato tubers exposed to various partial pressures of oxygen*

Potatoes, var. King Edward, were grown at Stretham, Cambs., in 1959. Figures in parentheses are % of rate in air.

Atmosphere	Respiration		$p_a - p_i$ (atm.) at periphery	p_i (atm.) (calculated range from centre to periphery)
	(ml. of O_2 / 100 g./hr.) (<i>R</i>)	(μ ml. of O_2 / cm. ² /sec.) (<i>g</i>)		
Air	0.71	1.16	0.023	0.182-0.185
7% O_2	0.515 (73)	0.84	0.016	0.052-0.054
Air	0.79	1.30	0.025	0.179-0.183
5% O_2	0.56 (71)	0.92	0.018	0.029-0.032
Air	0.64	1.05	0.021	0.184-0.187
5% O_2	0.44 (69)	0.72	0.014	0.034-0.036
Air	0.80	1.31	0.025	0.179-0.183
5% O_2	0.565 (71)	0.93	0.018	0.029-0.032
Air	0.66	1.08	0.022	0.183-0.186
2.5% O_2	0.385 (58)	0.63	0.012	0.011-0.013
Air	0.63	1.03	0.020	0.185-0.188
100% O_2	0.73 (116)	1.20	0.024	0.973-0.976
Air	0.65	1.06	0.020	0.185-0.188
1.35% O_2	0.345 (53)	0.56	0.010	0.002-0.004
Air	0.535	0.88	0.017	0.188-0.191
1.35% O_2	0.305 (57)	0.50	0.009	0.003-0.005
Air	0.50	0.82	0.016	0.190-0.192
1.35% O_2	0.30 (60)	0.49	0.009	0.003-0.005
Air	0.755	1.24	0.025	0.179-0.183
1.35% O_2	0.36 (48)	0.59	0.011	0.001-0.003
Air	0.63	1.03	0.020	0.185-0.188
14.7% O_2	0.58 (92)	0.95	0.019	0.125-0.128
Air	0.65	1.07	0.021	0.184-0.187
2.45% O_2	0.38 (59)	0.62	0.012	0.011-0.013
Air	0.685	1.12	0.022	0.183-0.186
9.75% O_2	0.55 (80)	0.90	0.018	0.077-0.080
Air	0.645	1.06	0.021	0.184-0.187
9.75% O_2	0.525 (82)	0.86	0.017	0.079-0.081

Fig. 2. (a), Relation between external partial pressure of oxygen and respiration (oxygen consumption) of potato tubers. Potatoes used were King Edward, Stretham 1959, King Edward, Tenerife 1959, and King Edward, Tenerife 1960. (b), Relation between respiration and intercellular partial pressure of oxygen of potato tubers. $p(\text{O}_2)$, Partial pressure of oxygen. $\circ$, King Edward, Stretham 1959; $\triangle$, King Edward, Tenerife 1959; $\times$, King Edward, Tenerife 1960.

Fig. 3. Lineweaver-Burk plots of the relation between respiration and the intercellular partial pressure of oxygen in potato tubers. Curve 1, complete plot of $1/R$ against $1/p(\text{O}_2)$; curve 2, the same plot for values of $1/p$ between 1 and 83. K_m for the high-affinity oxidase (curve 1) was determined from the intercept of slope with the base-line ($1/K_m$). K_m is thus 3.1×10^{-4} atm. or 4×10^{-7} M.

pressures 0.014–0.025 atm. ($1/p = 83$ –500) the reciprocal plot approximates to a straight line (curve 1, Fig. 3). The slope of the line gives a value of $0.4 \mu\text{M}$ for the Michaelis constant (K_m) of the high-affinity enzyme in respect of oxygen, or an affinity ($1/K_m$) of 2.5×10^6 . It should be pointed out, however, that no great degree of reliability can be claimed for this value for oxygen affinity. The internal oxygen partial pressures involved are so low that quite small variations are of relatively great importance, and the values for $1/p_i$ are therefore of doubtful validity. All that can safely be said is that the slope of the line relating to this first terminal oxidase differs very considerably from that relating to a second terminal oxidase operative at oxygen partial pressures between 0.05 and 1.0 atm. ($1/p, 1$ –32).

The affinity for oxygen of this second low-affinity terminal oxidase cannot be deduced from the initial slope of curve 2 (Fig. 3) since this curve was constructed by plotting the reciprocals of the total respiration against the reciprocals of p_i , and as such includes the effect of oxygen on both oxidases.

The value of R_{max} for the high-affinity oxidase can, however, be determined from curve 1, and this was found to account for 50 % of the total respiration rate of the tubers in oxygen. If we make the reasonable assumption that this enzyme will operate at a maximum rate throughout the whole range of the higher oxygen partial pressures, we may obtain by subtraction the rate of the residual respiration, as affected by oxygen concentration which is mediated by the oxidase or oxidases of lower affinity. A reciprocal plot based on these corrected rates permits an estimate of the affinity for oxygen of the second oxidase (Fig. 4). This affinity is calculated to be 6.5×10^3 (K_m 1.5×10^{-4}), which is less than that of the other oxidase by a factor of about 2×10^3 .

Fig. 4. Reciprocal plots of the residual respiration mediated by the oxidase of low affinity and intercellular oxygen concentration. K_m , 1.5×10^{-4} .

Effect of oxygen concentration upon the activity of phenolase

Bonner (1957), in his review of the functions of phenolase, points out that apart from some results of Kubowitz (1939) there have been no investigations on the affinity of this enzyme for oxygen. We have determined this for a preparation of phenolase, extracted as described above, from the same batches of potatoes as were used in the respiration experiments. Four phenolic substrates were used, homocatechol, catechol, caffeic acid and chlorogenic acid. Many methods have been proposed for measuring the activity of the phenolase enzyme. We considered it advisable in the first place to use a method that registers only the oxidative change from a diphenol to the quinone, for if phenolase is acting as a terminal oxidase this is the reaction we might expect to be involved.

The values of K_m , with respect to oxygen, calculated by the Lineweaver-Burk method ranged from 4.3×10^{-4} to $5.8 \times 10^{-4}M$ (Table 2) for all the substrates studied. This gives an affinity of the enzyme for oxygen of between 1.7×10^3 and 2.3×10^3 , a figure that is lower than that of 3.3×10^4 reported by Kubowitz (1939). He, however, determined the oxygen affinity by measuring the rate of oxygen uptake of enzyme and substrate, a method which measures in addition the oxygen uptake in non-enzymic reactions subsequent to the oxidation of the phenol.

Since ascorbic acid has been stated to have an inhibitory effect on phenolase (Kertesz, 1951, 1952; Baruah & Swain, 1959), and because of the difference between our results and those of Kubowitz, we performed parallel experiments with the colorimetric assay of Baruah & Swain (1959), in which ascorbic acid is absent. The affinity for oxygen with the substrates, homocatechol, catechol and chlorogenic acid was between 3×10^3 and 6×10^3 (Table 2). Although these values are different from those obtained by the first method, they differ yet more from that reported by Kubowitz. They are not dissimilar to the value given above for the low-affinity terminal oxidase of the potato and we cannot therefore exclude the possibility that this oxidase is phenolase. Nor would the same evidence exclude the possibility that the oxidase was ascorbic acid oxidase, which

Table 2. Affinity for oxygen of potato phenolase

Substrate	$10^4 K_m$ (measured with ascorbic acid)	$10^{-3} \times$ Affinity	$10^4 K_m$ (measured by colorimetric method)	$10^{-3} \times$ Affinity
Catechol	4.4	2.2	1.5	6.6
Homocatechol	5.8	1.7	3	3.3
Caffeic acid	5.8	1.7	—	—
Chlorogenic acid	4.3	2.3	1.5	6.6

Table 3. *Changes in the cytochrome spectrum of potato tubers in different partial pressures of oxygen*

Date	Atmosphere (atm. O ₂)		Cytochrome components	
	External	Internal (approx.)	<i>a</i> (603 mμ)	<i>b</i> (560 mμ)
29. vi. 59	0	0	Slight band	Very strong band
	0.025	0.01-0.025	Doubtful	Very strong band
	0.05	0.03-0.05	No band	Strong band
	0.208	0.18-0.208	No band	Weaker band
30. vi. 59	0	0	Strong band, fully reduced	Strong band
	0.025	0.01-0.025	Band, 40% reduced	Strong band
	0.05	0.03-0.05	No band, completely oxidized	Strong band
	0.208	0.18-0.208	No band, completely oxidized	Weak band
3. vii. 59	0	0	Band	Strong band
	0.025	0.01-0.025	Weak band	Strong band
	0.05	0.03-0.05	Doubtful	Strong band
	0.208	0.18-0.208	No band	Weak band
15. x. 59	0.0135	0.001-0.0135	Slight band	Strong band, fully reduced
	0.0135	0.001-0.0135	Slight band	Strong band, fully reduced
	0.208	0.18-0.208	No band	Band, 50% reduced
	0.208	0.18-0.208	No band	Band, 50% reduced
	1.0	0.97-1.0	No band	Slight band, 30% reduced

has an oxygen affinity of 6×10^3 (Thimann *et al.* 1954; Butt & Halloway, 1958), and which has frequently been suggested as a terminal oxidase (cf. James, 1953b). We could not, however, detect this enzyme in potato tubers. Extracts in which the phenolic substrates were removed by dialysis did not catalyse the oxidation of added ascorbic acid, although on the addition of a phenol (homocatechol) a rapid oxidation occurred.

Cytochrome components

Spectroscopic examination of changes in the spectrum of the cytochrome components resultant upon changes in the partial pressure of oxygen were also carried out, special attention being paid to the absorption bands at 560 and 603 mμ. We are indebted to Dr R. Hill for this examination, the results of which are shown in Table 3.

As the external partial pressure was increased, from 0.05 to 1.0 atm. oxygen, there was a gradual reduction in the intensity of the cytochrome *b* band. Even in oxygen, however, the *b* component was not completely oxidized, and in air we estimate that it was approximately half reduced in the tuber. In gas mixtures in which the intercellular oxygen pressure was greater than 0.03-0.05 atm., no absorption bands at either 603 or 550 mμ were observed, indicating that types *a* and *c* cytochromes were fully oxidized, whereas a strong band was visible at 560 mμ. At low intercellular oxygen pressure (0.0-0.025 atm.) a band became visible at 603 mμ and was accompanied by the appearance of a type *c* cytochrome band at 550 mμ. It was impossible to be certain that no further intensification of the band at 560 mμ occurred as a result of

the decrease of oxygen pressure from 0.025 atm. to zero since the bands *b* and *c* tended to fuse into a single broad band under anaerobic conditions.

DISCUSSION

Our results differ in several respects from those published by Thimann *et al.* (1954) on the respiration, at 25°, of freshly cut potato disks. With an external atmosphere containing only 0.045 atm. oxygen, these authors found that 90% of the respiratory rate in air was achieved and with 0.065 atm. oxygen the rate of respiration was equal to that in air. There was very little evidence of any appreciable participation of a terminal oxidase other than what may have been a cytochrome oxidase with a high affinity for oxygen. The K_m of this oxidase was 3 μM (i.e. affinity 3.3×10^5). This affinity is lower than that of cytochrome oxidase isolated from yeast, given by Strittmatter & Ball (1954) as 4×10^7 . Our value of the affinity for oxygen of the potato cytochrome oxidase lies between these values, i.e. 2.5×10^6 . However, as stated previously, no great accuracy can be claimed for this figure. Clearly our results and those of Thimann *et al.* agree with the concept of the functioning of a terminal oxidase of high oxygen affinity but differ in that our results show that in the intact tuber an appreciable part of the respiration (30% of the total in air and 50% in oxygen) is mediated by an enzyme with a much lower affinity for oxygen. Previous workers have also found evidence that two terminal oxidases may be operating. Thus Schade & Levy (1949) noticed, as did Thimann *et al.* (1954), an increase in the sensi-

tivity of the terminal oxidases of washed potato slices to lowered oxygen pressures on aging. On these grounds, and from the fact that the sensitivity of the oxidases to carbon monoxide also decreased with age, they reached the conclusion that at least two terminal oxidases were operating in the respiration of the cut slice: one with a high affinity for oxygen and a high sensitivity to carbon monoxide, and the other with low affinity for oxygen and insensitive to carbon monoxide. The high-oxygen-affinity oxidase they identified as cytochrome oxidase. The possibility that the low-oxygen-affinity enzyme was operating in the intact tuber they dismissed on the supposition 'that since the intact tuber depends mainly on diffusion for its supply of oxygen, the pO_2 in the tuber is probably of such a low value that any terminal oxidase which is highly sensitive to lowered partial pressures of O_2 would be inactive'. This supposition is contrary to the experimental evidence that in fact the partial pressure of oxygen, even in the centre of a potato tuber, is considerable (Burton, 1950), and a reconsideration of the findings of Schade & Levy (1949), in the light of this evidence, indicates that in fact a low-oxygen-affinity oxidase might be operating. This we have found to be the case.

The results, reported here, of the spectroscopic examination of the cytochromes support the view of earlier workers that the terminal oxidase of high oxygen affinity is cytochrome oxidase. Our results give no clue as to the identity of the terminal oxidase with the lower affinity for oxygen. On the evidence of oxygen affinity alone it might well be phenolase. On the other hand, in view of the carbon monoxide insensitivity of the low-affinity oxidase, reported by Schade & Levy (1949), it is more probable that a *b*-type cytochrome is involved.

Of recent years considerable evidence has accumulated indicating that the *b*-type cytochromes are active as terminal oxidases in both plant and animal tissues. Strittmatter & Ball (1954) described an auto-oxidizable haemochromogen present in liver microsomes, and a similar cytochrome was reported as being concerned in the respiration of silkworm tissues (Pappenheimer & Williams, 1954) and in the microsomes from mammary glands and intestinal mucosae (Baillie & Morton, 1955). Martin & Morton (1956) investigated the microsomes isolated from petioles of *Beta vulgaris*. They found that cytochrome b_5 , which was exclusively localized in these particles, was associated with reductase enzymes catalysing electron transfers from di- and tri-phosphopyridine nucleotides. The work of Bendall & Hill (1956) on the cyanide-insensitive auto-oxidizable cytochrome *b*, in *Arum* spadix also demonstrated that cytochrome *b* components are active in the respiration of plant tissue.

SUMMARY

1. Evidence has been obtained, based on the affinity of the enzymes for oxygen, of the participation of two terminal oxidases in the respiration of potato tubers.

2. One of the oxidases has a high affinity for oxygen and is probably cytochrome *c* oxidase, the second with a lower affinity (6.5×10^3) remains to be identified. This second oxidase could not, however, be ascorbic acid oxidase since this enzyme was not found in potato tubers.

3. In air approximately 70 % of the respiration passes over cytochrome *c* oxidase, and 30 % over the oxidase with the lower affinity for oxygen. In oxygen the proportion of the respiration passing over the second enzyme is increased to approximately 50 %.

We are greatly indebted to Dr R. Hill for the spectroscopic examination and to both Dr R. Hill and Dr J. Barker for their interest and advice.

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