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CYTOCHROME *c* DEPENDENT, ANTIMYCIN-A RESISTANT RESPIRATION IN MITOCHONDRIA FROM POTATO TUBER (*SOLANUM TUBEROSUM L.*)

INFLUENCE OF WOUNDING AND STORAGE TIME ON OUTER MEMBRANE NADH-CYTOCHROME-*c*-REDUCTASE

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SUMMARY

Cytochrome *c* has two stimulatory effects on respiration of mitochondria especially those from wounded potato tuber. In the first place a stimulation of succinate- and NADH-consuming, antimycin-A-sensitive respiration, which reaches a maximal value at low cytochrome *c* concentrations, has been found. In the second place, at higher concentrations of cytochrome *c* a stimulation of NADH-consuming respiration occurs, which is antimycin-A-resistant, but KCN-sensitive. This antimycin-A-resistant, NADH-consuming respiration is absent, when no cytochrome *c* is added to the reaction medium. It is insensitive to metal chelators, to which the antimycin-A- and KCN-resistant plant mitochondrial alternative oxidase is sensitive.

By measurements of NADH-cytochrome *c* reductase activities a corresponding antimycin-A-resistant NADH-cytochrome *c* reductase has been found, which is insensitive to osmotic shock treatment. A localization of this antimycin-A-resistant electron transport with NADH as the electron donor in the outer mitochondrial membrane is likely. In the mitochondrial preparations cytochrome *c* might stimulate by acting as an electron-carrier between the outer membrane reductase and the inner membrane cytochrome oxidase.

A big increase of the outer membrane mediated electron transport in the mitochondria has been observed after wounding of potato tuber tissue. The ability of the tissue to produce this electron transport pathway after wounding disappeared after prolonged storage of the tubers.

A possible function of this electron transport pathway in fatty acid desaturation during the wound-reaction is suggested.

INTRODUCTION

Mitochondria from potato tuber contain an electron transport chain, comparable with that in mitochondria from other plant sources [1]. Present is a cytochrome chain with an antimycin-A sensitive cytochrome *b* and with cytochrome aa_3 as

terminal oxidase [2–4]. Two phosphorylating sites are coupled to this chain. Electrons are transported to these cytochromes via a phosphorylating (from internal NADH) or a non-phosphorylating (from succinate) complex. In addition electron transfer to cytochrome *b* is also possible from external NADH via a non-phosphorylating NADH-dehydrogenase [5–8]. All these components are located on the mitochondrial inner membrane [5, 6]. The phosphorylating NADH-dehydrogenase is located on the inside, the non-phosphorylating one on the outside of the inner membrane. The latter can receive its electrons from external (cytoplasmatic) NADH because the outer membrane is permeable to NADH in contrast to the inner membrane [8–11].

After wounding, quantitative and qualitative changes in respiration of potato tuber tissue and isolated mitochondria have been observed. Generally respiration increases after wounding [12–14]. Induction of a CN-resistant terminal oxidase can occur [15] (dependent on the stage of growth and storage time of the tuber), which connects with the main chain between the flavoproteins of the complexes I and II and cytochrome *b*, as demonstrated by the corresponding antimycin-A resistance (L. H. W. v. d. Plas, submitted for publication). This CN-insensitive alternative terminal oxidase, which is metal chelator-sensitive [17, 18], cannot receive electrons from external NADH. So the joining point of this terminal oxidase and the main chain (see Fig. 1) is situated before the point where external NADH injects electrons into this chain [16].

The succinate-consuming respiration of potato mitochondria from tissue before wounding is CN- and antimycin-A-sensitive and can become (partly) CN- and antimycin-A-resistant after wounding, while external-NADH-consuming respiration remains sensitive to KCN and to antimycin-A after wounding.

In the course of a study of changes in respiration of potato tuber tissue after various periods of storage the experiments to be described in this paper showed that cytochrome *c* affects the antimycin-sensitivity of NADH-consuming respiration of mitochondria. This effect varied with the length of the storage period.

MATERIALS AND METHODS

Plant material. Potato tubers (*Solanum tuberosum L.* var. Bintje) were obtained from the "Proefbedrijf Nederlandse Aardappelkeuringsdienst" at Slootdorp and stored since harvest at 7 ± 1 °C in a ventilated room. Anti-sprouting agents were not applied. Tubers were cut into 3-mm thick slices and were incubated in air at 25 °C in a moist chamber. After 1 or 2 days of incubation, the cortex and the tissue containing the vascular bundles were removed and the remaining central tissue was used as wounded tissue. Freshly cut, non-incubated central tissue was used as non-wounded tissue.

Isolation of mitochondria. Mitochondria were isolated by a modification of the procedure described by Verleur [19]. Tissue samples of 50–70 g were homogenized in a Braun multipress MP 50 with 250 ml ice-cold medium containing 0.7 M mannitol, 10 mM potassium phosphate buffer, 1 mM EDTA, 2 mM cysteine and 0.1 % bovine serum albumin, (pH 7.2), pressed through a double layer of perlon gauze and centrifuged for 10 min at $1000 \times g$. The supernatant fluid was centrifuged for 10 min at $10\,000 \times g$. The precipitated mitochondria were washed twice, first in a medium containing 0.7 M mannitol, 10 mM potassium phosphate buffer, 2 mM cysteine and 0.1 % bovine serum albumin (pH 6.9) and after centrifugation (10 min, $10\,000 \times g$) in

a medium containing 0.7 M mannitol, 10 mM potassium phosphate buffer and 0.1 % bovine serum albumin (pH 6.8). The resulting 10 000 $\times g$ pellet was suspended in 2 ml of the second washing medium and this "crude"suspension was used in the experiments. Mitochondrial preparations purified by sucrose gradient centrifugation essentially gave the same results.

Respiration of mitochondria. The O_2-uptake of mitochondria was recorded at 25 °C using a YSI oxygen monitor, model 53. Reactions were carried out in a medium containing 0.7 M mannitol, 10 mM potassium phosphate buffer, 10 mM Tris · HCl buffer, 5 mM EDTA and 0.1 % bovine serum albumin (pH 6.8). To a reaction vessel containing 1.0–1.4 ml of this medium, 0.2 ml mitochondrial suspension was supplied containing about 1.0–2.0 mg mitochondrial protein in the case of non-wounded and wounded tissue, respectively. Depending on the type of experiment, respiration was measured after addition of different combinations of the following substances: 20 μmol succinate, 1.8 μmol NADH, 0.1–0.15 μmol ADP, 5 μmol $MgCl_2$, 6 nmol cytochrome *c*, 0.1 μmol KCN, 3.6 nmol antimycin-A, 0.7 μmol 8-hydroxyquinoline, or 10 nmol rotenone.

Oxygen uptake was generally calculated as ng mol O_2-uptake/min per mitochondria from 1 g tuber tissue (freshweight).

Measurement of cytochrome c reductase activity. The reduction of cytochrome *c* at 550 nm was followed, using a Zeiss PMQ II spectrophotometer.

Reactions were carried out in 1.0 cm cuvettes containing 3.0 lm of a medium with 5 mM potassium phosphate buffer (pH 7.2), 1 mM KCN, 50 μM cytochrome *c* and about 0.1 mg mitochondrial protein. Osmolarity of the buffer was adjusted to the desired value with mannitol. The reaction was started by addition of 1.2 μmol NADH or 30 μmol succinate. Dependent on the type of experiment 12.5 nmol rotenone or 3.5 nmol antimycin-A was added.

Reductase activity was calculated as ng mol cytochrome *c* reduced/minute per mitochondria from 1 g tissue (freshweight), using a millimolar extinction coefficient ε of 21.0 · mM^{-1} · cm^{-1} [20].

RESULTS

1. Influence of cytochrome c on antimycin-A- and CN-resistance

Cytochrome *c*, injected into the reaction medium of respiring mitochondria often has a stimulating effect on oxygen uptake [21–23]. Dependent on the pretreatment of the tissue (wounding or not) mitochondrial respiration of potato mitochondria increases by 20–70 % (Table II). This effect of cytochrome *c* has often been attributed to replacement of cytochrome *c* that has leaked from the mitochondria during the isolation procedure, especially when phosphate-containing buffers have been used [24].

When the resistance of mitochondrial respiration to KCN and antimycin-A is determined either in the presence or absence of cytochrome *c*, some striking differences in sensitivity to these inhibitors with and without added cytochrome *c* have been observed (Table 1, Fig. 2). Without cytochrome *c* the activities are as expected according to the scheme for the electron transport described in the introduction (Fig. 1). In mitochondria from wounded tissue with succinate there is some resistance to KCN and antimycin-A, caused by the action of the alternative oxidase pathway,

TABLE I

OXYGEN UPTAKE WITH SUCCINATE OR NADH AS THE SUBSTRATE, OF POTATO TUBER MITOCHONDRIA WITH AND WITHOUT ADDITION OF CYTOCHROME *c* AND DIFFERENT COMBINATIONS OF INHIBITORS

Potatoes harvested in 1974, stored at 7 °C or at 16 °C; experiments carried out between July 1974 and February 1975. Oxygen uptake as ng mol O_2/min per mitochondria from 1 g tissue (freshweight). Concentrations of substrates, cytochrome *c* and inhibitors as in Material and Methods. Uninhibited respiration measured after addition of 0.1 μmol ADP and 5 μmol $MgCl_2$ to the reaction medium. Respiration in the presence of inhibitors has been measured without ADP; ADP-addition has no effect except a slight inhibition of KCN- or antimycin-A-inhibited respiration with succinate. Numbers between brackets indicate the number of separate isolations upon which the data are based. AA, antimycin A; 8-OHQ, 8-hydroxyquinoline.

	Mitochondria from non-wounded tissue				Mitochondria from 1 day wounded tissue			
	succinate		NADH		succinate		NADH	
	without cytochrome *c*	+ cytochrome *c*	without cytochrome *c*	+ cytochrome *c*	without cytochrome *c*	+ cytochrome *c*	without cytochrome *c*	+ cytochrome *c*
a. uninhibited respiration	12.48 (9)	15.45 (9)	11.26 (9)	13.59 (9)	15.49 (21)	19.89 (21)	14.79 (21)	27.85 (18)
b. +KCN	1.26 (8)	1.28 (8)	1.14 (4)	1.55 (5)	2.34 (22)	2.78 (16)	1.26 (6)	2.56 (6)
c. +antimycin-A	0.25 (4)	0.56 (8)	0.38 (4)	1.74 (6)	1.36 (5)	2.23 (22)	0.98 (6)	6.56 (19)
d. +KCN+8-OHQ	1.01 (9)	1.12 (8)	1.08 (5)	1.59 (5)	0.79 (22)	1.44 (17)	1.34 (6)	2.66 (6)
e. +AA+8-OHQ	0.27 (4)	0.59 (8)	0.63 (4)	1.62 (8)	0.72 (5)	0.93 (4)	1.33 (5)	5.80 (20)
f. +AA+KCN+-OHQ	–	–	–	0.75 (6)	–	0.93 (4)	–	1.03 (20)
e–f	–	–	–	0.87	–	0.0		4.77

TABLE II

STIMULATION OF STATE 3 RESPIRATION BY CYTOCHROME *c* OF MITOCHONDRIA FROM POTATO TUBER

Mitochondria from potatoes, harvested in 1974, stored at 7 °C or at 16 °C; experiments carried out between July 1974 and February 1975. Percentages increase relative to preceding state 3. Between brackets the number of separate isolations upon which the data are based.

	Mitochondria from	
	non-wounded tissue	1 day wounded tissue
NADH as substrate (a)	53.2 % (7)	72.9 % (17)
Succinate as substrate (b)	21.6 % (10)	38.4 % (22)
Extra stimulation of NADH-respiration (a-b)		
in % state 3 respiration	31.6 %	34.5 %
in ng mol O_2/min per g	0.87	4.11

which is reduced by metal chelator (i.e. 8-hydroxyquinoline). With NADH as the substrate and in mitochondria from non-wounded tissue with NADH or with succinate as the substrate, resistance to KCN and to AA is less and subsequent addition of metal chelator does not reduce this resistant respiration.

Alternative oxidase activity (metal chelator sensitive) is only present in wounded tissue and does not connect with the external-NADH dehydrogenase complex.

Addition of cytochrome *c* to succinate-consuming mitochondria from non-wounded or wounded tissue does not markedly change the CN-resistant or antimycin-A-resistant respiration nor the effect of subsequent addition of metal chelator. Addition of cytochrome *c* to NADH-consuming mitochondria from wounded tissue, however, produces a very high resistance to antimycin-A which has not been found with KCN (Table I, last column). Addition of chelator has no appreciable effect on this antimycin-A resistance while simultaneous addition of KCN and antimycin-A reduces respiration to the same level as observed in the presence of either KCN or antimycin-A without addition of cytochrome *c*. Addition of KCN subsequent to

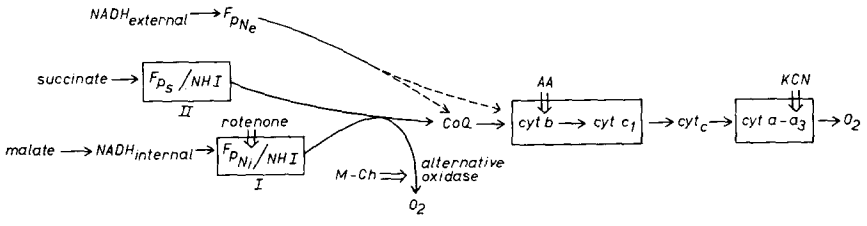

Fig. 1. Possible scheme for the electron transport chain in the inner membrane of potato mitochondria after wounding. Abbreviations: fp_s : flavoprotein associated with complex II; fp_{ni}: flavoprotein associated with complex I; fp_{ne}: flavoprotein associated with the dehydrogenation of external NADH; NHI: non-haem iron; CoQ: coenzyme Q; cyt *a*, *b*, *c*: cytochrome *a*, *b*, *c*. I, II, III, IV: complexes I, II, III, IV. AA: site of action of antimycin-A; KCN: site of action of KCN; MCh: site of action of metal chelators.

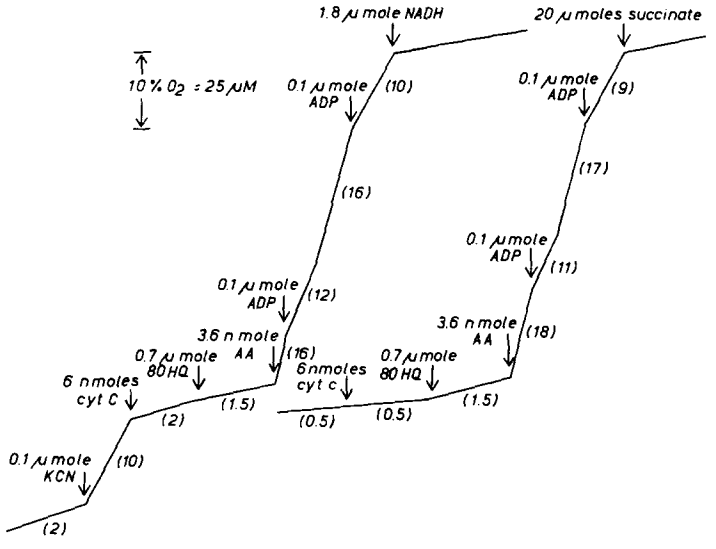

Fig. 2. The effect of cytochrome *c* on inhibitor-sensitivity of respiration of potato tuber mitochondria isolated 1 day after wounding. Mitochondria from 4 g tissue (freshweight); additions as indicated. Numbers in parentheses refer to ng mol O_2 uptake/min per mitochondria from 1 g tissue (freshweight).

antimycin-A to succinate respiring mitochondria has no effect under the same circumstances.

So this extra cytochrome *c*-dependent antimycin-A-resistant respiration with NADH as the substrate is KCN-sensitive and can be calculated as the difference between respiration in the presence of antimycin-A and metal chelator, and respiration with antimycin-A, KCN and metal chelator in the reaction mixture (respectively, e and f in Table I). Calculated in this way the extra antimycin-A resistant respiration after wounding amounts to 4.77 ng mol O_2 min per mitochondria from 1 g tissue (freshweight), while for the corresponding mitochondria from non-wounded tissue a value of only 0.87 ng mol O_2/min per g has been found.

The occurrence of such a cytochrome *c* induced increase in antimycin-A resistant respiration might also explain why the stimulation of respiration by cytochrome *c* (Table II) is always greater with NADH-consuming mitochondria than with succinate-consuming mitochondria under the same circumstances. The extent of the extra stimulation by cytochrome *c* of NADH-consuming respiration in ng mol O_2 (see Table II) fits pretty well with the values calculated above for the extra antimycin-A resistant, KCN-sensitive respiration (Table I).

Apparently cytochrome *c* has two stimulatory effects on mitochondrial respiration. This can also be concluded from experiments on the stimulation of respiration by different concentrations of cytochrome *c* (Fig. 3). One effect is the stimulation in NADH-consuming mitochondria of the antimycin-A-sensitive respiration which is maximal at very low cytochrome *c* concentrations (V reached at about 2 μM, K_m about 0.16 μM). This stimulation is also found with succinate as the substrate. The other effect is the stimulation of the antimycin-A resistant NADH-consuming respiration which is very low without cytochrome *c* and reaches its maxi-

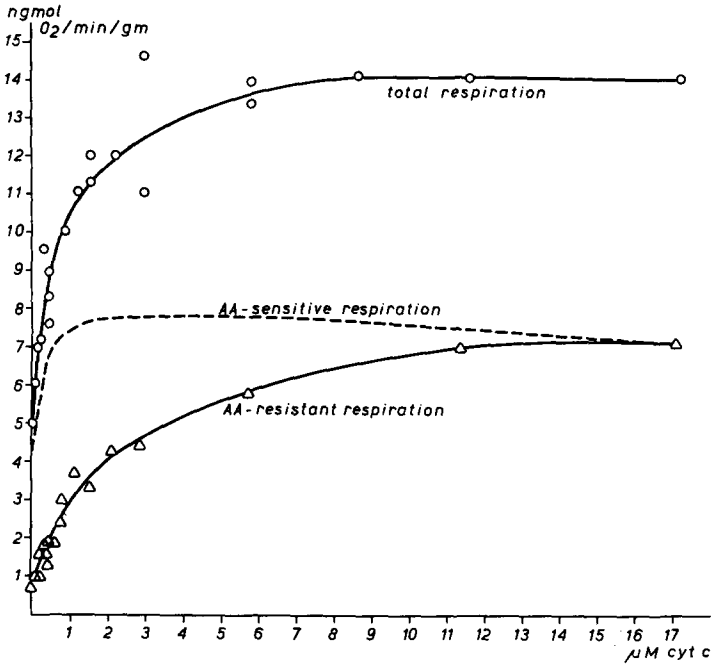

Fig. 3. The influence of different concentrations of cytochrome c on NADH-consuming respiration. Mitochondria from 1 day wounded tissue. Respiration measured after addition of 0.1 μmol ADP. No $MgCl_2$ added to the reaction mixture; △–△, antimycin-A-resistant respiration (a); ○–○, respiration without antimycin-A-addition (b); – – – –, antimycin-A-sensitive respiration (difference between a and b).

mum at cytochrome *c* concentrations about 10 times as high (V reached at about 17 μM, K_m about 1.9 μM).

2. *Nature of the antimycin-A-resistant, cytochrome c-dependent respiration*

This respiratory pathway has been investigated further by determining NADH-cytochrome *c*-reductase activities and succinate-cytochrome *c*-reductase activity.

From Fig. 4, three NADH-cytochrome c-reductase activities can be distinguished in mitochondria from potato, which is in accordance with the results of Douce et al. [5, 25] and Day and Wiskich [8]. One is antimycin-A-resistant, two are antimycin-A sensitive of which one is also rotenone sensitive while the other is rotenone resistant.

The antimycin-A sensitive reductase activities are affected by osmotic shock: the activities increase when osmolarity is decreased (Fig. 4, Table III). As the outer mitochondrial membrane is not permeable to cytochrome *c* [9, 10] this increase in reductase activity can be attributed to localization of these reductases on the inner mitochondrial membrane and increase of permeability of the outer membrane due to osmotic shock. The rotenone resistant, antimycin-A sensitive reductase activity reaches a maximal value with a smaller drop in osmolarity (700 to about 200 mOsm) than the rotenone-sensitive, antimycin-A-sensitive reductase activity (maximal value at about 20 mOsm). In osmotic shock experiments succinate-cytochrome *c* reductase

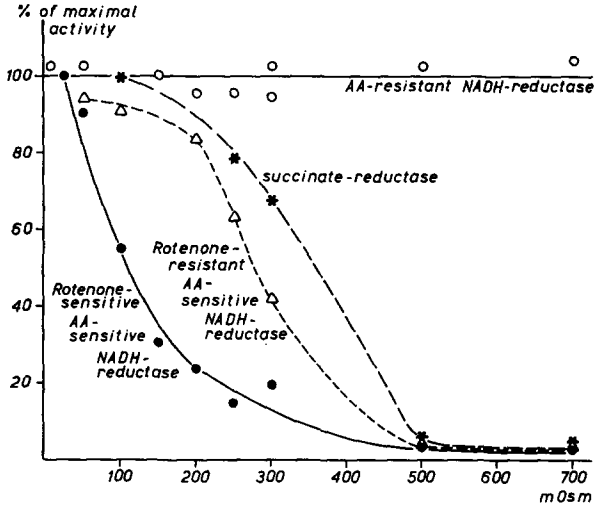

Fig. 4. Influence of osmolarity of the reaction medium on succinate- or NADH-cytochrome *c* reductase activities of mitochondria from non-wounded tissue. ○–○, antimycin-A-resistant, NADH-cytochrome *c* reductase activity; △–△, antimycin-A-sensitive, rotenone-resistant, NADH-cytochrome *c* reductase activity; ●–●, antimycin-A-sensitive, rotenone-sensitive, NADH-cytochrome *c* reductase activity; ×–×, succinate cytochrome *c* reductase activity.

activity behaves as the rotenone-resistant, antimycin-A sensitive NADH-cytochrome *c*-reductase activity.

As the mitochondrial inner membrane is impermeable to NADH [11] the rotenone-resistant respiration of mitochondria with added NADH (external NADH, Fig. 1) should be mediated by a NADH-dehydrogenase located outside the inner membrane barrier. Therefore, the rotenone-resistant NADH-cytochrome *c*-reductase activity most likely operates through this external-NADH dehydrogenase. The rotenone-resistant NADH-cytochrome *c*-reductase activity needs a smaller drop in osmolarity for maximal activity than the rotenone-sensitive one. Again, this suggests a localization of the rotenone-resistant NADH-dehydrogenase on the outside of the inner membrane [5, 25]. The rotenone-sensitive NADH-cytochrome *c*-reductase activity then corresponds with the internal-NADH oxidizing dehydrogenase on the inside of the mitochondrial inner membrane. The latter can only function with NADH from the reaction medium when both outer and inner membrane have been disrupted by osmotic shock, while the former needs only disruption of the outer membrane.

In contrast to the antimycin-A sensitive reductases the single antimycin-A resistant NADH-cytochrome *c*-reductase activity at 700 mOsm is roughly the same as at lower osmolarities (Table III, Fig. 4), so there is no effect of osmotic shock. As the outer membrane of mitochondria is impermeable to cytochrome *c* [9] it is likely that this reductase activity is located on the outside of the outer membrane of the mitochondria.

This outer membrane NADH-cytochrome *c*-reductase activity is the only one which is antimycin-A-resistant and therefore should function in the antimycin-A-resistant cytochrome *c* dependent NADH-consuming respiration described in the

TABLE III

NADH-CYTOCHROME *c* REDUCTASE ACTIVITIES OF MITOCHONDRIA FROM POTATO TUBER AT DIFFERENT OSMOLARITIES

Potatoes harvested in 1974, stored at 7 °C or at 16 °C; experiments carried out between July 1974 and February 1975. Between brackets the number of separate isolations upon which the data are based.

	Non-wounded tissue		1-day wounded tissue	
	Assay at 15 mOsm	Assay at 700 mOsm	Assay at 15 mOsm	Assay at 700 mOsm
Antimycin-A-sensitive NADH-cytochrome *c* reductase activity				
in ng mol cytochrome *c* reduced/min per g	46.8 (6)	6.2 (6)	125.3 (10)	32.9 (10)
relative to activity at 15 mOsm	100 %	13.2 %	100 %	26.3 %
Antimycin-A-resistant NADH-cytochrome *c* reductase activity				
in ng mol cytochrome *c* reduced/min per g	4.4 (6)	4.6 (6)	48.2 (10)	51.8 (10)
relative to activity at 15 mOsm	100 %	104.5 %	100 %	107.5 %

previous section. Indeed both greatly increase after wounding of the tissue (Tables I and III).

DISCUSSION

After wounding of potato tuber tissue an antimycin-A resistant, cyanide-sensitive, cytochrome-*c*-dependent, NADH-consuming respiration is induced in the mitochondria.

From experiments with osmotic shock treatment it can be concluded that the antimycin-A-resistant, NADH-cytochrome *c*-reductase activity is located on the outer membrane of the mitochondria. A corresponding outer membrane NADH-cytochrome *c* (cytochrome b_5) reductase has been described for other plant and animal sources [5, 8, 23, 26, 27].

Also in the purified outer membranes of potato mitochondria a cytochrome b_{558} has been found [28]. The cyanide-sensitivity suggests that cytochrome oxidase functions as the terminal oxidase, which is known to be located on the inner membrane of the mitochondria [11]. So in the antimycin-A-resistant NADH-consuming respiration there is a need for a carrier to transfer electrons from the outer membrane to the inner membrane. Cytochrome *c*, when present in rather high concentrations (Fig. 3), might function as such. The antimycin-A-sensitive stimulatory action due to much lower concentrations of cytochrome *c* is perhaps the result of a replacement of some cytochrome *c* which has leaked from mitochondria during the isolation procedure.

A function of cytochrome *c* as a carrier of electrons from the outer to the inner membrane is in contradiction with the impermeability of the outer membrane for cytochrome *c* at the osmolarity used (see Wojtczak [9]; but compare with Palmer and Kirk [29]), which is also demonstrated by the extremely low inner membrane reductase activities of mitochondria not osmotically shocked (Fig. 3, 700 mOsm), and so

the question arises whether it is necessary to suppose an involvement of cytochrome oxidase of intact mitochondria.

In several experiments we have tried to obtain indications for permeation of cytochrome *c* (either oxidized or reduced) through the outer membrane into isolated mitochondria under conditions used in the respiration experiments. The results did not provide positive indications.

All these experiments lead to the conclusion that a penetration of cytochrome *c* through the outer membrane does not take place. Yet, transfer of electrons to cytochrome oxidase occurs, as indicated by the CN-sensitivity of outer membrane-mediated electron transport from NADH to oxygen. This might be explained on the assumption that in the mitochondrial preparation so many mitochondria have been disrupted, that enough free accessible cytochrome oxidase is present to oxidize the reduced cytochrome *c* delivered into the medium by the outer membrane reductase of both disrupted and non-disrupted mitochondria. Then, this cytochrome oxidase action is responsible for the oxygen uptake observed.

Cytochrome oxidase in potato tuber mitochondria is present in great excess [2, 30, 31]. So the rupture of only a small part of the mitochondria can provide enough free accessible cytochrome oxidase. Assuming that the activity of inner membrane (antimycin-A-sensitive) reductase in not osmotically shocked mitochondria (700 mOsm, Table III) reflects the quantity of mitochondria in the preparation which were not intact, about 10 % (before wounding) to 25 % (after wounding) of the mitochondria is disrupted in the preparations. As compared to the observed state 3 respiration cytochrome oxidase activity in the mitochondrial preparation is 3–4 times as high (50–60 ng mol O_2/min per mitochondria from 1 g freshweight (unpublished observations)). So 10–25 % of this activity (corresponding to 5–15 ng mol O_2/min per g) is more than enough to provide for the electron transport from outer membrane reductase to oxygen which resulted in 5 ng mol O_2 uptake/min per g after 1 day of wounding (Table I). We tested, whether there is always enough free accessible cytochrome oxidase in the mitochondrial preparations by adding extra cytochrome oxidase (sonicated mitochondria of non-wounded tissue which have no outer membrane mediated electron transport) and by measuring activity of mitochondrial preparations after sonication or treatment with osmotic shock, which increase the quantity of free accessible cytochrome oxidase. In both cases the activity of the outer membrane mediated electron transport to oxygen remains the same as in the original preparation. Although we could not prove that cytochrome oxidase of intact mitochondria is not involved in the reaction, our experiments also do not necessarily lead to the conclusion that it is involved. An explanation based on the accessibility of cytochrome oxidase of those mitochondria which are damaged is much more likely. The complete lack of effect of ADP-addition on the outer membrane reductase mediated electron transport to oxygen despite the fact that phosphorylation site III is part of the system involved, is another argument for the functioning of cytochrome oxidase only from disrupted mitochondria.

As outer membrane reductase mediated oxygen uptake can only be observed due to the presence of disrupted mitochondria in the preparation, and in addition is dependent on high concentrations of cytochrome *c*, it is not to be expected that the outer membrane reductase mediated electron transport has a function in normal respiration in vivo. In considering what might be the function then, a role in wound

TABLE IV

CHANGES IN THE ANTIMYCIN A-RESISTANT OUTER MEMBRANE MEDIATED ELECTRON TRANSPORT OF POTATO MITOCHONDRIA AT PROLONGED STORAGE OF THE TUBER

Activities expressed in ng mol O_2/min per g freshweight (calculations 1 and 2) and in ng mol cytochrome *c* reduced/min per g freshweight (calculation 3). Between brackets the number of separate isolations, upon which the data are based.

	Mitochondria from		
	Non-wounded tissue	1-Day wounded tissue	2-Days wounded tissue
A. Potatoes, harvested in september 1973, experiments carried out before January 1974.			
1. Activity calculated as in Table II	1.17 (4)	9.46 (5)	17.30 (4)
B. Potatoes as in A, experiments carried out after June 1974.			
1. Activity calculated as in Table II	–	2.72 (4)	3.02 (1)
2. Activity calculated as in Table I	0.63 (2)	0.45 (5)	0.98 (2)
3. Activity calculated as in Table III	–	4.8 (2)	7.1 (1)
C. Potatoes harvested in July, August and September 1974, experiments carried out before January 1975.			
1. Activity calculated as in Table II	0.81 (8)	6.69 (10)	–
2. Activity calculated as in Table I	0.93 (6)	5.84 (14)	10.06 (3)
3. Activity calculated as in Table III	4.6 (4)	50.9 (4)	–

repair seems very likely as the increase in activity after wounding is much greater for this outer membrane reductase than for the inner membrane reductase (table III). In literature a function in the desaturation of fatty acids has been suggested for outer membrane reductase from animal sources [32]. In the desaturation of fatty acids in liver microsomes [33–35], indeed a NADH-cytochrome b_5 functions with generally NADH as the preferred electron donor. Desaturated fatty acids play an important role after wounding of potato tuber [36, 37], as components of the increasing endoplasmatic reticulum [36] and of the suberin which is deposited on the cell walls of the wound periderm [37]. Lange [38] observed during prolonged storage of the tubers a gradual disappearance of the formation of wound periderm and suberin deposition after wounding. As shown in Table IV, the induction after 1 or 2 days of wounding of the activity of antimycin-A resistant, cytochrome *c*-dependent, NADH-consuming respiration (calculation method 2), the extra stimulation by cytochrome *c* of NADH-consuming respiration relative to succinate-consuming respiration (calculation method 1) and the activity of antimycin-A-resistant, NADH-cytochrome *c*-reductase has also largely disappeared after 1 year of storage. These parallel phenomena suggest a functional relation between outer membrane reductase activity and wound periderm formation possibly through fatty acid desaturation.

This seems to be in contradiction with Abdelkader [39] who reports that fatty

acid desaturase activity in mitochondrial preparations from wounded potato tissue is low and due to microsomal contamination. However, the same holds for the antimycin-A-resistant, NADH-cytochrome *c* reductase activity of his preparations. In contrast to this, after sucrose gradient centrifugation of our mitochondrial preparations the main activity of this reductase was found in the mitochondrial band (in the order of 70 %), demonstrating that this reductase is indeed of a mitochondrial nature. The disagreement of results might be caused by differences in storage of the tubers.

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