Cyanide-resistant Respiration in Fresh and Aged Sweet Potato Slices

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ABSTRACT

The respiration of fresh sweet potato (Ipomoea batatas) slices is resistant to, and often stimulated by, cyanide and antimycin A. m-Chlorobenzydroxamic acid (CLAM), a selective inhibitor of the alternate path, inhibits respiration in the presence of cyanide and has a limited inhibitory effect in the presence of antimycin A. Thus, a partial bypass of the antimycin-sensitive site is indicated. Respiration rises 2-fold at best with slice aging, the increment being cytochrome-mediated. The cyanide-resistant pathway contributes neither to coupled fresh slice respiration nor to the induced respiration in the absence of inhibitors of the cytochrome path. In the presence of uncoupler, however, the alternate path is engaged both in fresh and aged slices. \( V_{cyt} \), the maximal capacity of the cytochrome path, remains essentially the same with slice aging, whereas \( V_{all} \) decreases from 20 to 60 per cent. The induced respiration is readily accommodated by the potential cytochrome path capacity of fresh slices, which is realized on aging. Accordingly, there is no need to invoke mitochondrial proliferation in explanation of the development of the induced respiration. The engagement of the alternate path in response to uncoupler reflects substrate mobilization to a degree that substrate oxidation exceeds the electron transport capacity of the cytochrome path.

Fresh slices do not utilize exogenous substrates, whereas aged slices do so readily. Cerulein, a specific inhibitor of fatty acid synthesis, prevents the development of the induced respiration as well as the capacity to oxidize exogenous substrates. It is suggested that lipid, and ultimately membrane, biosynthesis is central to the development of the induced respiration and the ability to use exogenous substrates, much as in potato.

When bulky plant storage organs are cut into thin slices there is an immediate rise in respiration. In the 24 hr following cutting the respiration rises another 2- to 5-fold, depending on the tissue, to yield the wound-induced, or induced, respiration (8, 24). We have found storage organs to fall into two groups. In one group, of which potato is typical, the respiration of fresh slices is predominantly CN-sensitive, and slicing elicits a spate of phospholipid degradation (20). In the other group, of which sweet potato is an example, fresh slice respiration is largely CN-insensitive, and cutting evokes no perceptible phospholipid breakdown.

When white potato slices are aged, the development of the induced respiration goes hand in hand with the development of CN resistance. Although the capacity of the CN-resistant, or alternate, path is sufficient to accommodate the induced respiration, the respiration of coupled aged potato slices has been found to be mediated entirely by the Cyt path (21). Further, the maximal potential Cyt path activity in potato has been found to be essen-

3 Abbreviations: CLAM: m-chlorobenzydroxamic acid; CCCP: carbonyl-cyanide m-chlorophe nyl hydrazone; cerulein: (2S) (3R) 2,3 epoxy-4-oxo-7,10 dodecadienoylamlde; TCAC: tricarboxylic acid cycle; SHAM: 

MATERIALS AND METHODS

Plant Material. Red sweet potato roots (Ipomoea batatas) were obtained from a local market and stored at 7 C and 90% RH.

Slice Preparation—Respiratory Measurements. Sweet potato slices 1 mm thick and 9 mm in diameter were prepared as previously described (21). Slices were aged by incubating discs in 0.1 mM CaSO4 with frequent changes for 24 hr at 25 C on a rotary shaker. Respiratory rates were determined by conventional manometry. Cerulein was dissolved in a minimum amount of alcohol (1 mg of cerulein/75 \( \mu \)l of alcohol) and diluted with water to give a stock solution of 1 mg/ml. Slices were aged in cerulein at a concentration of 0.1 mg/ml 0.1 mM CaSO4. CCCP was 10 \( \mu \)M in 0.01 mM phosphate buffer—0.1 mM CaSO4 (pH 7.3) where indicated.

Analysis of Titration Data. Titration of sweet potato slice respiration with CLAM (21) and CN or antimycin A was conducted by the method of Bahr and Bonner (2). The equation describing the total respiration of sweet potato slices is:

\[ V_T = \rho \cdot g(i) + V_{cyt} + V_{res} \] (1)

where \( V_T \) is the total respiration rate, \( V_{cyt} \) is the contribution by the Cyt path, \( V_{res} \) is the rate of the residual respiration which is uninhibited by both KCN and CLAM together, and \( g(i) \) is the maximal contribution by the alternate path at given concentrations of alternate path inhibitor, e.g. CLAM. \( \rho \), a number between 0 and 1, defines the fraction of the full alternate path which is operating. \( V_{all} \) is the maximal capacity of the alternate path, and \( \rho \times V_{all} \) is the actual contribution of the alternate path in the absence of inhibitor. When \( V_{res} \), which is constant, is subtracted,
we have equation 2, in accordance with the expression developed for mitochondrial respiration (2, 21):

\[ V_r' = \rho \cdot g(i) + V_cyt \]  

(2)

**Oxidation of Exogenous Labeled Substrates.** Forty-two slices (about 3 g fresh wt) were placed in 10 or 15 ml of solution in a 125-ml Erlenmeyer flask. Labeled compounds were added as follows: 10 μCi of [1,5-14C]citrate (4 mCi/mmol), or 10 μCi of uniformly 14C-labeled glucose (200 mCi/mmol), or 10 μCi of [1,2-14C]acetate (56.7 mCi/mmol). Samples were incubated in a water bath shaker rotation at 25°C. Respiratory CO2 was absorbed in 0.2 ml of 10% NaOH dispersed on a strip of Whatman GT/A glass paper (1 x 8 cm) bent into a loop and suspended from a hook fixed in the center of a rubber stopper tightly held in the top of the Erlenmeyer flask (7). Slices were incubated for 2 hr. The NaOH loops were changed every 20 min. The loops were dried in an oven at 80°C, and then added directly to a vial with 15 ml of toluene containing 4 g of PPO and 0.05 g of POPP/liter. Radioactivity was determined with a Beckman scintillation counter model LS-100-C. Duplicate samples were counted for 10 min each. 14CO2 evolution is expressed as dpm/3 g fresh wt hr.

**Biochemicals.** CCCP and antimycin A were obtained from Sigma. UL-[14C]Glucose and [1,2-14C]acetate were from ICN, [1,5-14C]Citrate was purchased from New England Nuclear. CLAM was synthesized as previously described (21). PPO was obtained from Amersham/Searle, and POPP was purchased from Nuclear-Chicago.

**RESULTS**

**EFFECT OF KCN, ANTIMYCIN A, AND CLAM ON FRESH SLICE RESPIRATION**

Figure 1 shows the effect of CN and antimycin A on the respiration of fresh sweet potato slices in the presence and absence of CLAM (17). Conventional concentrations of CN (viz. 0.5 mM) or antimycin (10 μM) stimulate the respiration of coupled fresh sweet potato slices 24 and 34%, respectively, whereas the same inhibitor concentrations resulted in 80% inhibition in fresh potato slices (21). At high CN concentration in the presence of 1 mM CLAM, fresh slice respiration is inhibited 86% (Fig. 1A). The residual respiration (14%) is resistant to CN and CLAM together. On the other hand, at high antimycin concentration in the presence of 1 mM CLAM respiration is inhibited only 56% (Fig. 1B). The weak synergistic effect of antimycin and CLAM is reminiscent of that observed in aged potato slices, where in the presence of CLAM a significant fraction of the residual respiration is mediated via an antimycin-resistant branch of the Cyt path (22).

**CONTRIBUTION OF ALTERNATE PATH IN COUPLED AND UNCOUPLED FRESH SLICES**

Values of ρ in Coupled and Uncoupled Fresh Slices as Measured with CN and CLAM. The synergistic effect of KCN and CLAM on fresh slice respiration (Fig. 1A) establishes the presence of the alternate path in fresh sweet potato slices. Titrations of respiration in coupled and uncoupled fresh slices with CLAM in the presence and absence of CN are shown in Figure 2. Figure 2A shows that in coupled slices CLAM at concentrations as high as 4 mM does not inhibit the respiration in the absence of CN. However, in the presence of 0.1 mM KCN, CLAM results in 85% inhibition. The data in Figure 2A were replotted according to equation 2, and the results are shown in Figure 2B. A horizontal line is obtained, with slope (ρ) of 0, indicating that the alternate path does not contribute to the respiration in the absence of CN. Experiments similar to those shown in Figure 2A were carried out in the presence of CCCP, and the results are presented in Figure 2C. In the absence of CN, CLAM partially inhibits respiration, the respiratory rate reaching a plateau at 4 mM CLAM. In the presence of CLAM and 0.1 mM KCN almost complete inhibition is obtained (Fig. 2C). When titration with CLAM is carried out with low CN (0.01 mM KCN), the inhibitory pattern of CLAM remains the same, although inhibition is less severe.

The data in Figure 2C were replotted according to equation 2 and the results are shown in Figure 2D. In the absence of CN, a straight line is obtained with slope (ρ) of 0.9, indicating 90% engagement of the alternate path. On the other hand, in the presence of 0.01 mM KCN, a straight line with slope (ρ) of 1 is obtained, with its intercept on the y axis lowered, indicating that the alternate path operates at its maximum capacity while the rate of the Cyt path (Vcyt) has been reduced.

**Quantitative Relations of Vcyr, VaII, and Vres in Coupled and Uncoupled Fresh Slices Estimated with CN and CLAM.** The respiratory fluxes of coupled and uncoupled fresh sweet potato slices determined from Figure 2, B and D, are summarized in Table I. Data from another set of experiments are also presented (Table I, experiments 4–6). The main difference between the two sets of experiments is that in the first set (experiments 1–3) CN stimulates the respiration, and VaII is thus higher than Vcyt, whereas in the second set (experiments 4–6) CN slightly inhibits the

![Fig. 1. Effect of CN and antimycin A with and without CLAM on the respiration of coupled fresh sweet potato slices. A: KCN; B: antimycin A. CLAM concentration: 1 mM.](image-url)
respiration and $V_{alt}$ is accordingly less than $V_{cvt}$. The above discrepancy depends on the storage history of the root. Freshly dug roots (purchased in September) have a smaller alternate path capacity than do roots stored for long periods (purchased in April). In both cases in coupled slices in the absence of CN (experiments 1 and 4) $V_T$ is the sum of $V_{cvt}$ and $V_{res}$. The contribution of the alternate path is zero, since $p$ is 0. When the activity of the Cyt path of coupled slices is decreased by a low concentration of CN (experiment 5) $p$ is shifted to 0.5, and the contribution of the Cyt path decreases, without any change in $V_T$ compared with experiment 4.

Uncouplers of oxidative phosphorylation stimulate fresh sweet potato slice respiration. Accordingly, $V_T$ increases (compare experiment 1 with experiment 2, or experiment 4 with 6) and the alternate path is engaged, since $p$ is equal to 1. The activity of the Cyt path increases 16 to 27% with CCCP (compare the $V_{cvt}$ values of experiment 1 and 2 or 4 and 6), indicating that in coupled slices the Cyt path operates below its full potential capacity. Thus, the bulk of the uncoupler-evoked increment is alternate path-mediated. The decrease of $V_T$ in the presence of 0.01 mM KCN in uncoupled slices is due to a decrease in $V_{alt}$, the contribution of the alternate path remaining the same ($p = 1$).

The engagement of the alternate path in the presence of uncoupler is not always observed. Table II shows the values of $p$, $V_{cvt}$, $V_{alt}$, and $V_{res}$ estimated in the presence and absence of CCCP in fresh slices prepared from two different root purchases in April (experiments 1 and 2) and September (experiments 3 and 4), respectively.

$V_T$ increases 65% in response to uncoupler (compare $V_T$ values of experiment 1 with 2 or experiment 3 with 4), but the alternate path remains disengaged as indicated by the value of $p$ equal to 0. Whereas $V_{cvt}$ values increased 16 to 27% in response to CCCP in Table I, $V_{cvt}$ increased 65 to 83% in the experiments of Table II, indicating that the fresh slices described in Table II have a much higher potential $V_{cvt}$ than those in Table I. The above observation implies that since $V_{cvt}$ in Table II is much higher than that in Table I, substrate mobilization caused by uncouplers is not enough to saturate both pathways. The engagement of the alternate path is seemingly regulated by the traffic delivered to the electron transport chain.

Values of $p$, $V_{cvt}$, $V_{alt}$, and $V_{res}$ Estimated with Antimycin A and CLAM in Fresh Coupled and Uncoupled Slices

<table>
<thead>
<tr>
<th>Experiment</th>
<th>CCCP</th>
<th>$V_T$</th>
<th>$V_{alt}$</th>
<th>$p$</th>
<th>$V_{cvt}$</th>
<th>$V_{res}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-</td>
<td>135</td>
<td>140</td>
<td>0</td>
<td>107</td>
<td>28</td>
</tr>
<tr>
<td>2</td>
<td>+24</td>
<td>96</td>
<td>90</td>
<td>0</td>
<td>196</td>
<td>25</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>94</td>
<td>90</td>
<td>0</td>
<td>80</td>
<td>14</td>
</tr>
<tr>
<td>4</td>
<td>156</td>
<td>35</td>
<td>30</td>
<td>0</td>
<td>133</td>
<td>21</td>
</tr>
</tbody>
</table>

with 85% inhibition in the presence of 0.1 mM KCN (Fig. 2A). In Figure 3B, $V_T$ is plotted as a function of $g(i)$, the data deriving from Figure 3A. A straight line with slope $p = 0$ is obtained, indicating that the alternate path does not contribute to $V_T$.

Similar titration in the presence of CCCP is shown in Figure 3C. In the absence of antimycin, CLAM partially inhibits respiration, the respiratory rate reaching a plateau at high CLAM concentrations. In the presence of antimycin inhibition is less than with CN, and $V_{res}$ is accordingly unduly high. In Figure 3D, $V_T$ is plotted against $g(i)$ with data from Figure 3C. A straight line with slope $p = 0.8$ is obtained, indicating 80% engagement of the alternate path.

The respiratory fluxes of coupled and uncoupled fresh sweet potato slices determined from Figure 3, A, B, C, and D, are summarized in Table III. The weak synergistic effect of antimycin and CLAM results in an overestimation of $V_{res}$ (compare $V_{res}$ values of experiment 1 in Table III with those of experiment 1 in Table I), with the result that $V_{cvt}$ and $V_{alt}$ are underestimated (compare $V_{alt}$ and $V_{alt}$ values of experiment 1 in Table III with those of experiment 1 in Table I). Antimycin is seen to be an unsuitable inhibitor in studies designed to determine the magnitude of the Cyt and alternate paths in fresh sweet potato slices, since it fails to inhibit the Cyt path completely. Antimycin is even less effective in aged slices (see Table V).

Effect of Uncouplers on Activity of Alternate Path

Figure 4 shows $g(i)$ values in the presence of uncoupler plotted against $g(i)$ values in the absence of uncoupler with $g(i)$ values determined with KCN (plot A) and antimycin (plot B), respectively. In both instances straight lines are obtained, but the slope derived with KCN is 0.6, whereas the slope derived with antimycin is 0.9 to 1. A slope less than 1 indicates that the activity of the alternate path has been decreased by uncoupler.

Although the effect of uncoupler on $g(i)$ as determined with CN suggests that the energy state regulates the activity of the alternate path, the absence of a similar effect of uncoupler on $g(i)$ as

![Figure 3](https://example.com/figure3.png)

**Figure 3.** Effect of CLAM in presence and absence of antimycin A on coupled and uncoupled fresh sweet potato slices. A and B: coupled; C and D: uncoupled. $g(i)$ in B and D derived from A and C, respectively. $V_{res}$ is subtracted in calculating $g(i)$. $V_T$ and $g(i)$ are in μl of O₂/g fresh wt hr.

| Table II. Respiratory Rates of Coupled and Uncoupled Fresh Sweet Potato Slices. Data were obtained from analyses similar to those of Figure 2. Expts. 1 and 2 were from the same root purchased in April; Expts. 3 and 4 were from the same root purchased in September. |
|-----------------|--------|---------|--------|--------|--------|--------|
| Experiment | CCCP | $V_T$ | $V_{alt}$ | $p$ | $V_{cvt}$ | $V_{res}$ |
| 1          | -    | 135  | 140     | 0  | 107     | 28      |
| 2          | +24  | 96   | 90      | 0  | 196     | 25      |
| 3          | -    | 94   | 90      | 0  | 80      | 14      |
| 4          | 156  | 35   | 30      | 0  | 133     | 21      |

| Table III. Respiratory Rates of Coupled and Uncoupled Fresh Sweet Potato Slices. The data were obtained from Figure 3A, B, and C. Expts. 1 and 2 were from the same root. |
|-----------------|--------|---------|--------|--------|--------|--------|
| Experiment | CCCP | $V_T$ | $V_{alt}$ | $p$ | $V_{cvt}$ | $V_{res}$ |
| 1          | -    | 135  | 140     | 0  | 107     | 28      |
| 2          | +24  | 96   | 90      | 0  | 196     | 25      |
| 3          | -    | 94   | 90      | 0  | 80      | 14      |
| 4          | 156  | 35   | 30      | 0  | 133     | 21      |
determined with antimycin argues against this view. Seemingly, CN decreases $V_{atm}$ in uncoupled slices in a nonspecific way. The underestimation of $g$ in uncoupled slices resulting from the nonspecific effect of CN leads in an overestimation of $p$, which has been found to be 0.9 to 1 in uncoupled slices. The true value of $p$ in this case is about 0.5 to 0.6. Furthermore, the value $p = 0.8$ obtained with antimycin in uncoupled slices (Table III) is also overestimated, since the unduly high values of $V_{res}$ result in an underestimation of $g$ in the tissue model. A $p$ equal to 0.5 is closer to reality.

**Inhibitor Constant of CLAM.** The inhibitor constant ($K_i$) was determined from Dixon plots of the reciprocal of the respiratory rate in the presence of 0.1 mM CN, against CLAM concentration (4). Table IV compares $K_i$ values of CLAM in fresh sweet potato slices with those reported in other tissue slices. The values are half those reported for *Arum* and avocado slices, and are very similar to those reported for aged potato and fresh preclamatic banana slices. The similarity in $K_i$ values suggests that the hydroxamate-sensitive component may well be of a similar nature among the various tissues, with the possible exception of Philodendron spadix slices.

**Quantitative Relations of $V_{vct}$, $V_{atm}$, and $V_{res}$ in Fresh and Aged Slices.** The respiratory fluxes of coupled and uncoupled fresh and aged sweet potato slices determined from titrations with CN in the presence of CN are summarized in Table V. Slicing an intact sweet potato root results in an 8- to 10-fold increase in respiration. The wound respiration is the sum of $V_{vct}$ and $V_{res}$, the contribution of the alternate path to the fresh slice respiration being zero ($p = 0$; Table V, experiment 1). Uncouplers stimulate fresh slice respiration and partially preclude the alternate path ($p = 0.25$; Table V, experiment 2). Thus, the bulk of the uncoupled respiratory rate (90%) is Cyt path-mediated. The above observation indicates an excess of unexpressed Cyt path capacity in fresh slices (compare the $V_{vct}$ values of experiment 1 with experiment 2).

The induced respiration in aged sweet potato slices represents a modest 30% increase over the fresh slice rate as contrasted with white potato slices where aging results in a 4- to 5-fold increase of respiration (5, 21). The maximum increase in $V_T$ during aging of sweet potato slices has never been observed to be more than 2-fold. Much as in white potato, however, the maximal potential capacity of the Cyt path, i.e., that realized in the presence of uncoupler, is much the same in fresh and aged slices. In sweet potato slices, however, $V_{atm}$, the maximal capacity of the alternate path, decreases with aging, while the actual Cyt path contribution in coupled slices increases (Table VA). By contrast, the alternate path is absent in fresh white potato slices and well developed in aged. In Table VB, we see that antimycin inhibition in aged slices is incomplete (22), with the result that $V_{vct}$ is grossly underestimated and $V_{res}$ is grossly overestimated.

The data of Table V indicate that substrate mobilization caused by uncouplers in fresh slices is not enough to saturate both the Cyt and alternate paths, with the result that $p = 0.25$. On the other hand, in aged slices substrate mobilization by uncoupler saturates both pathways despite the quantitative similarity between uncoupled $V_T$ in fresh and aged slices. The above discrepancy is attributable to the reduction of $V_{atm}$ in aged slices.

In sum, the potential Cyt path capacity of fresh sweet potato slices is enough to sustain the respiration of aged slices, and the CN-resistant pathway does not contribute to the development of the induced respiration. In consequence, there is no need to invoke the proliferation of mitochondria in explanation of the development of the induced respiration in sweet potato slices (1, 16).

**Utilization of Exogenous Substrates by Fresh and Aged Slices.** Table VI shows the 14CO2 evolution from uniformly labeled [14C]glucose, [1,5-14C]citrate, and [1,2-14C]acetate by fresh and aged sweet potato slices. Aged slices readily oxidize each of the substrates to yield copious quantities of 14CO2, whereas fresh slices fail to release significant quantities of radioactive CO2 from any of the substrates in question. Although glucose, citrate, and acetate uptake increase with aging, absorption by fresh tissue is nevertheless significant, especially where glucose and acetate are concerned. It is evident from Table VI that the enhancement of utilization with aging far exceeds the augmentation of absorption capacity. Further, a restraint on glycolytic and TCAC activity is seemingly lifted with aging in sweet potato slices much as in white potato slices (7) albeit there is no measurable lipid breakdown on slicing in sweet potato (Theologis and Laties, unpublished). Fatty acids arising from lipid breakdown have been considered as the inhibitors of glycolysis and TCAC activity in fresh potato slices (6, 9).

**Effect of Cerulenin on Development of Induced Respiration and Glucose Utilization Capacity by Sweet Potato Slices.** Cerulenin, an antibiotic which curtails *de novo* fatty acid synthesis by irreversible covalent attachment to β-ketoacyl-acyl carrier protein synthetase (14), inhibits the development of the induced respiration. Table VII shows the effect of cerulenin both on the development of the induced respiration and on the capacity of aged sweet potato slices to utilize exogenous glucose. The respiration of cerulenin-aged slices remains at levels only slightly higher than those observed for freshly cut material. Furthermore, $V_{vct}$ does not rise in slices aged in cerulenin. Cerulenin inhibits the enhanced utilization of exogenous glucose as well as the increase in glucose absorption by aged (Table VIIB).
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CN
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(21),

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appearance
from
not
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extensive
An
unpublished).

slices
fresh


Plant
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Resistance
Cyanide
An


Table VI. Oxidation of Exogenous Labeled Substrates by Fresh and Aged
Sweet Potato Slices.
The radioactivity in 15 ml experimental solution was glucose, ([14C]
18.5 \times 10^4 dpm; citrate (1.5 \times 10^4 C), 20 \times 10^4 dpm and acetate (1.2 \times 14 C),
20.2 \times 10^4 dpm respectively.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>1^4C CO_2 Release</th>
<th>Uptake</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glucose Citrate Acetate</td>
<td>Glucose Citrate Acetate</td>
</tr>
<tr>
<td></td>
<td>dpm \times 10^4/ g fresh wt*2 hr</td>
<td>Percent Initial radioactivity</td>
</tr>
<tr>
<td>Fresh</td>
<td>0.5% &lt; 1 3.4</td>
<td>9 2.5 21</td>
</tr>
<tr>
<td>Aged</td>
<td>165 120 136</td>
<td>96 40 80</td>
</tr>
<tr>
<td>Ratio</td>
<td>Aged/Fresh 196 &gt; 120 40</td>
<td>11 16 3.8</td>
</tr>
</tbody>
</table>

Table VII. Effect of cerulenin on the development of the Induced
Respiration and on glucose oxidation in sweet potato slices.
Slices were aged 24 hr where indicated. Initial ([14C]) glucose
radioactivity in 10 ml experimental solution was 18.3 \times 10^4 dpm.

A. Respiration

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Fresh</th>
<th>Control</th>
<th>Cerulenin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>99</td>
<td>192</td>
<td>114</td>
</tr>
<tr>
<td>KCN 0.1 m</td>
<td>152</td>
<td>194</td>
<td>194</td>
</tr>
<tr>
<td>CLAM 1 m</td>
<td>98</td>
<td>188</td>
<td>109</td>
</tr>
<tr>
<td>KCN + CLAM</td>
<td>39</td>
<td>55</td>
<td>60</td>
</tr>
<tr>
<td>CCCP, 10 \mu M</td>
<td>182</td>
<td>256</td>
<td>178</td>
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</table>

B. Glucose oxidation

<table>
<thead>
<tr>
<th>Tissue</th>
<th>V_T</th>
<th>V_max</th>
<th>V_Cyt</th>
<th>V resale</th>
<th>V_T uncoupled</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>dpm \times 10^4/g fresh wt*2 hr</td>
<td>Relative rate</td>
<td>Percent Initial radioactivity</td>
<td>Relative rate</td>
<td></td>
</tr>
<tr>
<td>Fresh</td>
<td>99</td>
<td>113</td>
<td>59</td>
<td>39</td>
<td>182</td>
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<tr>
<td>Aged</td>
<td>290</td>
<td>131</td>
<td>55</td>
<td>256</td>
<td>178</td>
</tr>
<tr>
<td>Aged + Cerulenin</td>
<td>114</td>
<td>94</td>
<td>60</td>
<td>178</td>
<td></td>
</tr>
</tbody>
</table>

The above data indicate that fatty acid biosynthesis—and by
extension the biosynthesis of membrane-lipid components—is a
requirement for the development of the induced respiration and
the enhancement of glucose utilization which accompanies aging
of sweet potato slices.

DISCUSSION

Cyanide Resistance in Fresh and Aged Slices. It has been
axiomatic through the years that the respiration of fresh slices
from various storage organs is CN-sensitive and that the develop-
ment of respiration with slice aging is concurrent with the
appearance of the CN-resistant pathway (9, 24). The behavior of
potato slices is largely responsible for the erroneous generalization.
An extensive comparative study of 20 storage tissues has shown
that fresh slices fall into two main categories. The first group
includes tissue slices which are initially CN-sensitive and which
develop CN resistance with aging. In the second group, fresh slices
are resistant and often stimulated by CN, and aging results in
some diminution of alternate path activity (Theologis and Laties,
unpublished). In both groups slice aging results in the development
of a wound-induced respiration, indicating that the enhancement
of respiration with aging is independent of whether the alternate
path exists in fresh slices.

An extensive study of CN-resistant respiration in potato slices
(21), a member of the first group, showed that the alternate path
does not normally contribute to the induced respiration in potato
slices. In the present study, red sweet potato slices which belonged
to the second group, were investigated to determine the extent to
which the Cyt and CN-resistant paths contribute to the fresh and
induced respiration. As demonstrated, the alternate path exists in
both fresh and aged sweet potato slices. However, the respiratory
rate of coupled fresh and aged slices, corrected for the residual
respiration, has been found to be less than predicted from the sum
of the CN-resistant (V_{CN}) and CLAM-resistant (V_{CLAM}) rates
measured independently, and we have determined that in neither case
does the alternate path contribute to the respiration in the absence
of inhibitors.

The extent of saturation of the Cyt path determines the flux
through the alternate path. When the flux through the Cyt path
is decreased by low CN concentrations, the value of r shifts from
0 to a value greater than 0. Restriction of Cyt path activity leads
to the engagement of the alternate path.

In uncoupled fresh sweet potato slices the alternate path seems
fully engaged. That is, the uncoupled respiratory rate corrected
for the residual respiration equals the sum of V_{CN} + V_{CLAM}. The bulk
of the respiratory increment induced by CCCP is mediated by the
alternate path. However, the somewhat depressing effect of CN
on the alternate path in uncoupled slices (Fig. 4) leads to an
underestimation of i(r) with a corresponding overestimation of r.
Thus, the actual participation of the alternate path ranges between
50 and 60% of its maximum capacity when the underestimation of
i(r) is taken into consideration. The engagement of the alternate path
by uncouplers in aged white potato slices has been attributed to
an increase in substrate mobilization due to the enhancement of
glycolysis (21). We interpret the response to uncouplers in sweet
potato slices in the same way. When substrate oxidation exceeds
the electron transport capacity of the Cyt path, the alternate path
is engaged. The alternate path is not invariably operative in the
presence of uncoupler. When the potential capacity of the Cyt
path is large, uncouplers do not cause the engagement of the CN-
resistant path (Table II). On the other hand, when the capacity
of the alternate path is higher than V_{CLAM}, CN will stimulate respiration
in the face of a significant Pasteur effect.

Our presumption that substrate flux controls the operation of
the alternate path is strengthened by the observation that in
mitochondrial studies neither CN nor uncoupler (in state 3) leads
to respiratory stimulation, since substrate is normally saturating.
In sweet potato mitochondria the value of r in state 3 with
succinate or malate as substrate is always greater than zero (Grover
and Laties, unpublished), whereas in sweet potato slices r is equal
to 0. Furthermore, whereas in mitochondrial studies the Cyt path
is fully saturated (2, 3), in coupled tissue slices the potential
capacity of the Cyt path is not always fully utilized (Tables I and
V). In practice, the switching mechanism of Bahr and Bonner (3)
acts more like an on-off switch than an on-off switch. That is,
the capacity of the Cyt path must seemingly be exceeded for
the alternate path to come into play. The stimulation of
respiration by CN in plant tissues has been ascribed to the
activation of the alternate path by CN (18). Our results do
not support this proposal since antimycin stimulates respiration much
as does CN (Figs. 1 and 3).

The weak synergistic inhibitory effect of antimycin and CLAM
in fresh slices is reminiscent of that observed in aged potato slices
(22). The ineffectiveness of antimycin in inhibiting the Cyt path
completely is attributable neither to the impenetrability of the
inhibitor nor to the impairment of CLAM effectiveness by
antimycin. The data suggest an operational bypass around the
antimycin-sensitive site similar to that found in aged potato slices (22).
The mitochondrial seat of the antimycin-resistant bypass in sweet
potato tissue is implied by the observation of Tomlinson and
Moreland (23) that the respiration in the presence of HOQNO
plus SHAM in sweet potato mitochondria is higher than that in
the presence of KCN plus SHAM, much as in the case of sweet
potato tissue slices.

Development of Induced Respiration. The wound respiration of
fresh sweet potato slices is several fold higher than that of the intact
organ (Fig. 4). The fresh slice wound respiration is resistant to, and often
stimulated by CN (Fig. 2), contrary to that of the fresh potato slice which is CN-sensitive (21). The synergistic effect of CN and CLAM indicates the presence of the alternate path in fresh sweet potato slices, whereas the alternate path in fresh potato tissue is lacking or inactive (21). The loss of the alternate path in potato slices has been attributed to the extensive membrane-lipid degradation indicated by cutting (20). Consistent with this supposition membrane destruction is not observable in fresh sweet potato slices, where the alternate path can be demonstrated.

The respiration of aged sweet potato slices is at best twice that of fresh slices (Tables V and VII). The alternate path, present in fresh slices, persists at a level which is reduced or at most remains the same (Tables V and VII). Thus, the development of the alternate path, a characteristic of white potato slice aging, is not central to the development of the induced respiration in sweet potato slices. Rather, the induced respiration reflects the realization of the latent Cyt path capacity of fresh slices. Since the alternate path does not normally contribute to the respiration of aged white potato slices, however (21), the difference is not as great as it appears. Whereas fresh slice respiration is stimulated by CN in sweet potato, aged slice respiration is partially inhibited, reflecting the drop in \( V_{\text{sat}} \) with aging.

When the similar values of \( V_{\text{cyt}} \), in uncoupled fresh and aged sweet potato slices are taken into account, the premise that mitochondria biogenesis and de novo synthesis of respiratory enzymes are necessary for the increase of respiration in sweet potato slices during aging becomes untenable (1, 16). We have come to the view that the potential Cyt path capacity in fresh sweet potato slices is more than enough to sustain the elevated rates of the induced respiration. Aging simply involves the realization of preexisting mitochondrial respiratory capacity. Whereas increased mitochondrial activity may be due to an intrinsic change in the mitochondria (13, 25, 26), the prospect that respiratory enhancement in vivo is related to substrate mobilization warrants particular attention.

The inability of fresh sweet potato slices to utilize exogenous substrates (Tables VI and VII) and the dramatic enhancement of exogenous substrate utilization with slice aging recall the situation in white potato slices (8). The behavior of freshly cut potato slices has been attributed to the inhibition of glycolysis and the TCAC by free fatty acids released during cutting (20), and to the prevalence of anomalous \( \alpha \) oxidation of fatty acids (6, 10).

The slicing of sweet potato roots does not elicit demonstrable membrane lipid breakdown (Theologis and Laties, unpublished). Furthermore, in contrast to potato, the respiratory increment elicited by uncoupler in fresh sweet potato slices is unaffected by imidazole, a specific inhibitor of fatty acid \( \alpha \) oxidation (12, and Theologis and Laties, unpublished). Thus, the explanation adduced herefore for fresh slice behavior in potato, and its alteration with aging, cannot simply be applied to sweet potato slices. Undoubtedly more is an issue in both cases than has yet been recognized. Nevertheless, the gross transformation of both CN-sensitive and CN-resistant slices with aging must have elements in common, as suggested by the rise in respiration, the burgeoning ability to oxidize substrates, and the repression of the developmental changes in both cases by cerulinin.

Cerulinin, an antibiotic which inhibits de novo fatty acid synthesis by irreversible covalent binding to \( \beta \)-ketoacyl-acyl carrier protein synthetase, has been shown to be an effective inhibitor of fatty acid synthesis in yeast, bacteria, and animals (14). Recently it has been shown that cerulinin inhibits the development of the wound-induced and CN-resistant respiration in potato slices, indicating the crucial role of lipid biosynthesis for respiratory enhancement during potato slice aging (26).

The respiration of cerulinin-aged sweet potato slices is much the same as that of fresh discs (Table VII). Cerulinin not only suppresses the development of the induced respiration but also inhibits the uptake and utilization of exogenous substrate. The effect of cerulinin strongly suggests that fatty acid and presumably phospholipid biosynthesis is a crucial element in the development of the mitochondrial respiratory transport systems in sweet potato slices. The nature of the membrane components synthesized during aging, necessary for the realization of preexisting mitochondrial respiratory capacity as well as for the implementation of substrate absorption, remains to be determined.

**Acknowledgments** A. Theologis is indebted to UCLA for a postdoctoral fellowship and to PhBeta Kappa for a foreign student predoctoral scholarship. The authors thank S. Omura of the Kitasato Institute, School of Pharmaceutical Sciences, Kitasato University, Tokyo, Japan, for the kind gift of cerulinin.

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