
SUCINOXIDASE AND CYTOCHROME OXIDASE IN BARLEY ROOTS 1, 2

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It has been suggested that the terminal oxidase concerned with the normal respiration in most plants is cytochrome oxidase (7, 8). Evidence from reconstructed systems suggests that other enzymes can also operate as terminal oxidase systems (2, 15, 21, 23, 25, 28, 30). Although tyrosinase and ascorbic oxidase may function there is no experimental evidence that they make energy available for endergonic processes. There is evidence to associate salt accumulation and salt respiration with the cytochrome system (19, 29). If no cytochrome oxidase was present in tissues which accumulate salt some revision of the Lunde- gärdh hypothesis (20, 24) of salt accumulation would be required. Consequently, it is especially interesting that James presents evidence that ascorbic oxidase is a terminal oxidase of barley root tips (10, 12) and implies that this oxidase is concerned in salt accumulation (11). James has been unable to extract cytochrome oxidase or detect cytochrome absorption bands in barley roots more than 5 to 6 days old.

The direct demonstration of cytochrome oxidase in older wheat roots has also failed (4, 28), although the characteristic absorption bands of the cytochromes have been observed in the intact roots (20); thus suitable procedures for extraction and assay may be difficult to find.

In barley as in wheat roots the negative results (10, 12) might mean only that cytochrome oxidase is difficult to extract. Since it has been generally accepted that oxidation of succinate proceeds through the classical cytochrome system (8), the demonstration of succinate oxidation and formation in barley roots (16, 17) suggests that succinoxidase and, thus, cytochrome oxidase function. Therefore, an attempt was made to isolate cytochrome oxidase and, as a confirmatory test, the succinoxidase system.

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METHODS AND MATERIALS

Preparation of Barley: Cape barley (Hordeum vulgare) was used as the source of roots. The grains were soaked in distilled water for 3 to 7 hours at 25°C with 2 or 3 changes of water. In some experiments, an initial soak of one hour in 0.6% H₂O₂ was used to reduce fungal contamination. The soaked grains were allowed to germinate on wet filter paper in a saturated atmosphere and the seedlings were held thus at 25°C for 2 days. For experiments in which longer growth periods were required, seedlings were transferred after 3 days to waxed boards, with holes through which the roots grew in aerated half-saturated CaSO₄ solutions for periods up to 7 days in a room at 25°C. After this treatment the roots were profusely covered with root hairs and often had a curled appearance. The maximum amount of root material that could be grown conveniently for an experiment was about 10 grams.

Preparation of the Enzyme Extract: The root system of the 2-day-old seedlings was excised at the seed and that of 10-day-old seedlings at about 5 cm from the seed. In some experiments the apical cm was removed but no differences between the root segments were found. After being thoroughly rinsed with distilled water the roots were chilled to 5°C. The following operations were carried out in a cold room at 0 to 5°C. The roots were washed in the homogenizing medium at 2°C and cut into about 0.5-cm segments. The segments were blotted to remove excess fluid and homogenized in fresh medium, 1.5 g of roots to 5 ml of medium. Maceration was carried out by hand with a conical Pyrex glass homogenizer, consisting of an outer tube which was immersed in a water-ice bath and a hollow pestle which contained a water-ice mixture. Cooling was important since homogenization of roots required a vigorous action. Cell debris from the unstrained homogenate was removed by centrifugation for 10 minutes at 1000 x g. The resulting supernatant was centrifuged at about 10,000 x g for 25 to 30 minutes at -2 to 0°C.

in a refrigerated centrifuge. The residue thus obtained was resuspended in the homogenizing medium and again sedimented at about 10,000 x g for 25 to 30 minutes. After the supernatant was decanted, the excess liquid in the centrifuge tube was removed with filter paper, the residue was weighed and resuspended with the homogenizing medium. The residue weights averaged about 3% of the fresh root weights for 2-day-old seedlings and 2% for the 10-day-old seedlings.

**Homogenizing and Assay Medium:** Various homogenizing and assay media were tested. The final media which consistently allowed the demonstration of succinoxidase activity contained the reagents listed in table I. The homogenizing medium was hypertonic to the barley roots.

Preparations without cysteine in the homogenizing medium did not show endogenous oxygen uptake or succinoxidase activity although cytochrome oxidase was demonstrated. Variation of the cysteine concentration over the range 10^{-4} to 10^{-2} M was not found to influence the succinoxidase activity. Cysteine was not required in the assay medium.

The presence of EDTA was especially effective in decreasing "autoxidation" of ascorbate. Presumably EDTA chelated contaminating metal ions. The routine addition of crystalline insulin as a source of pure protein presumably also served to bind metal ions. No consistent effect in stabilizing enzymatic activity was noted although some reduction of ascorbate autoxidation was found. The low solubility of insulin, however, may have prevented the introduction of effective amounts. Provided that EDTA were present, the autoxidation of ascorbic acid was low so no corrections were applied. Ascorbic acid to be added was usually neutralized by TRIS rather than by an inorganic base in order to reduce non-enzymatic oxidation of ascorbate.

Maintenance of the homogenate near pH 7 was essential for consistent demonstration of succinoxidase and cytochrome oxidase. A relatively high concentration of phosphate buffer (0.1 M) was used in the homogenization and assay media. The homogenates otherwise would have been acidic owing to the presence of EDTA, cysteine and vacuolar contents. Final pH values of the assay medium were about 7.

Results reported are not necessarily from experiments employing the formulations given in table I. Details are given as required.

**Assay for Enzymatic Activity:** Conventional Warburg manometric techniques were used to assay for enzymatic activity at 25°C.

In general, as the amount of enzyme preparation was limited, cytochrome oxidase assays were carried out in the same vessels and with the same preparations as used for the succinoxidase assay. One ml of enzyme extract was used in the initial 4.00 ml reaction mixture for the succinoxidase assay. After a reaction period, 0.5 ml of TRIS-neutralized ascorbic

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### Table I

**Composition of the Homogenizing Medium and Assay Media for Succinoxidase and Cytochrome Oxidase to Show Consistent Activity**

<table>
<thead>
<tr>
<th>Component</th>
<th>Homogenizing</th>
<th>Succinoxidase</th>
<th>Cytochrome Oxfordase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(M)</td>
<td>(ASSAY)</td>
<td>(ASSAY)</td>
</tr>
<tr>
<td>Cysteine</td>
<td>5.0 x 10^{-4}</td>
<td>1.25 x 10^{-4}</td>
<td>1.11 x 10^{-4}</td>
</tr>
<tr>
<td>Insulin</td>
<td>50.0 mg/l</td>
<td>12.5 mg/l</td>
<td>11.1 mg/l</td>
</tr>
<tr>
<td>EDTA</td>
<td>1.0 x 10^{-4}</td>
<td>2.50 x 10^{-4}</td>
<td>2.22 x 10^{-4}</td>
</tr>
<tr>
<td>pH 7.5</td>
<td>1.00 x 10^{-3}</td>
<td>1.00 x 10^{-2}</td>
<td>8.90 x 10^{-2}</td>
</tr>
<tr>
<td>K-succ, pH 7</td>
<td>2.00 x 10^{-2}</td>
<td>1.78 x 10^{-4}</td>
<td>1.00 x 10^{-4}</td>
</tr>
<tr>
<td>Cyt c</td>
<td>6.40 x 10^{-4}</td>
<td>5.70 x 10^{-4}</td>
<td>4.40 x 10^{-4}</td>
</tr>
<tr>
<td>TRIS</td>
<td></td>
<td>3.95 x 10^{-4}</td>
<td></td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>1.80 x 10^{-3}</td>
<td>1.43 to 1.27</td>
<td>2.40 x 10^{-1}</td>
</tr>
<tr>
<td>Sucrose</td>
<td></td>
<td>2.13 x 10^{-1}</td>
<td></td>
</tr>
<tr>
<td>Calculated tonicity</td>
<td>0.5</td>
<td>0.5</td>
<td>0.4</td>
</tr>
</tbody>
</table>

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**Abbreviations employed in this paper will be as follows:** EDTA, ethylenediamine-tetraacetic acid; TRIS, tri-(hydroxy-methyl)-amino-methane; Succ, succinate; cyt c, cytochrome c; DDC, diithionite-diithiocarbamate; Cyst, cysteine.
The succinoxidase system is presumed to have been present when the oxygen uptake of reaction mixtures containing exogenous succinate was increased over controls without succinate (fig 2). Additional evidence that the system studied was succinoxidase came from a malonate inhibition of oxygen uptake (fig 1). The initial activity of the heated control in the experiment in figure 1 can be explained by insufficient heating because the particle suspension in a chilled tube was heated only for one minute in boiling water. In other experiments complete lack of succinoxidase activity was found for suspensions heated for longer periods.

Cytochrome oxidase is presumed to have been present when the oxygen uptake of reaction mixtures containing cytochrome c was greater than that of mixtures without cytochrome c (see fig 2 after ascorbate addition). Lack of cytochrome oxidase is not necessarily shown if cytochrome c did not stimulate activity. A more detailed discussion will follow.

Specific activities are reported on the basis of microliters O₂ uptake per hour per mg residue fresh weight, here designated Q₀₂. Various types of packing of spherical particles in the residue would allow 50 to 30% of the packed volume to have been liquid. Consequently, the Q₀₂ is an underestimate of activity per particle weight. Since the homogenizing medium contained nitrogenous compounds, specific activities based on nitrogen were approximate. When corrected for the nitrogen content of the medium activity per hour per mg particle nitrogen, (Q₀₂(N)) is about 100 × Q₀₂. All values reported are computed from total oxygen uptakes, i.e., uncorrected for autoxidation and endogenous activity.

### Results

**Succinoxidase:** In early experiments succinoxidase was demonstrated only rarely in the particles isolated from barley root homogenates when the media used for extraction were composed of sucrose, sucrose plus phosphate, glucose plus phosphate, or variation of these with EDTA and protein. On the other hand, cytochrome oxidase was demonstrated. A report concerning an apparent functional succinic dehydrogenase in intact tissue but not in extracts (3) illustrates the difficulty in its demonstration in vitro in some plant extracts. The functioning in vivo of succinic dehydrogenase was shown in barley roots (16, 17).

Thus, the part of the succinoxidase system sensitive to damage was considered to be succinic dehydrogenase which is known to be an enzyme with essential sulfhydryl groups inactivated by RSSR compounds. Reactivation can be achieved with RSH compounds (9). Incorporation of the RSH compound cysteine into the homogenizing medium resulted in consistent demonstration of succinoxidase and cytochrome oxidase. Typical activities for succinoxidase were Q₀₂(N) = 192, and for cytochrome oxidase Q₀₂(N) = 379.

A marked oxidation of cysteine by the cytochrome system may have been indicated by the additional oxygen uptake of reaction mixtures with cytochrome c but without succinate. This was undesirable as cysteine oxidized by cytochrome c to cystine, or the free radical, inhibits succinic dehydrogenase (1). Incorporation of EDTA into the medium was accompanied by a lowering to the control level, of oxygen uptake of reaction mixtures with cytochrome c. The catalytic effect of cytochrome c in the cytochrome oxidase assay was unaffected (fig 2). From all experi-

### Table II

**Correlations between Initial Q₀₂ and Particle Concentration in the Succinoxidase and Cytochrome Oxidase Assays**

<table>
<thead>
<tr>
<th>ASSAY</th>
<th>MEASUREMENTS *</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Cyst c</td>
</tr>
<tr>
<td>Succinoxidase</td>
<td>Coef of correlation ...</td>
<td>-0.04 ± 0.22 **</td>
</tr>
<tr>
<td></td>
<td>Coef of regression, Q₀₂/mg × ml ...</td>
<td>-0.02</td>
</tr>
<tr>
<td></td>
<td>Extrapolated Q₀₂ ...</td>
<td>1.12</td>
</tr>
<tr>
<td></td>
<td>Mean Q₀₂ ...</td>
<td>0.80 ††</td>
</tr>
<tr>
<td></td>
<td>Standard error of estimate, Q₀₂ ...</td>
<td>0.65</td>
</tr>
<tr>
<td></td>
<td>No. of cases ...</td>
<td>21</td>
</tr>
<tr>
<td>Cyt oxidase</td>
<td>Coef of correlation ...</td>
<td>-0.28 ± 0.20 **</td>
</tr>
<tr>
<td></td>
<td>Coef of regression, Q₀₂/mg × ml ...</td>
<td>-0.03</td>
</tr>
<tr>
<td></td>
<td>Extrapolated Q₀₂ ...</td>
<td>1.93 ††</td>
</tr>
<tr>
<td></td>
<td>Standard error of estimate, Q₀₂ ...</td>
<td>0.60</td>
</tr>
<tr>
<td></td>
<td>No. of cases ...</td>
<td>22</td>
</tr>
</tbody>
</table>

Assay mixtures contained EDTA. Treatments in the cytochrome oxidase assay all contained ascorbic acid.

* All values corrected for number of cases.
** Not significant at P = 0.05.
† Significant at P < 0.01.
††, ‡‡, ††, * Pairs of figures with the same symbol significantly different from each other at P ≤ 0.002.
Fig. 2. Assay for succinoxidase and cytochrome oxidase with the same particle preparation from roots of 2-day-old seedlings. Conc of addenda in 2.00 ml for succinoxidase assay and corresponding conc in the homogenizing medium in brackets: particles 17.0 mg/ml (35.1); K-succ 2.00 x 10^{-6} M; cyt c 2.6 x 10^{-5} M; pH 7.5 phosphate buffer 6.00 x 10^{-2} M (1.00 x 10^{-4}); cyst-HCl 1.00 x 10^{-4} M (2.00 x 10^{-6}); EDTA 5.00 x 10^{-4} M (1.00 x 10^{-6}); and sucrose to adjust medium to 0.5 osmolar (0.6). Conc of K-ascorbate in 2.30 ml for cytochrome oxidase assay were 1.16 x 10^{-2} M.

ments with EDTA and without succinate, the mean \( Q_o \) with cytochrome c (table II, column 2) was not significantly different (\( P = 0.05 \)) from treatments without cytochrome c (column 1) in the succinoxidase assay.

Sucinoxidase activity decreased with time, especially in experiments employing a low concentration of particles per reaction volume (below 10 mg/ml). In experiments with dilute suspensions usually only the reaction mixtures containing succinate plus cyto-
chrome c showed initial activity. For all experiments with cysteine and succinate present (table II, columns 3 and 4), the effect of cytochrome in stimulating initial succinoxidase activity was significant (P = 0.002). Sustained succinoxidase activity was achieved by increasing the particle concentration. Although cytochrome c at the concentrations applied stimulated the activity of dilute suspensions, no sustained activity resulted.

**Cytochrome Oxidase:** Although cytochrome oxidase was consistently demonstrated, reproducible specific activities were difficult to obtain. A dilution test was desirable to determine whether the variations in activity were a result of dilution (loss of a factor) or of inhibitor action. Since a limited amount of particles could be prepared for any one experiment, usually no preparation was available for dilution tests after samples were allotted for the minimum number of controls. Consequently, the relation of specific activity to concentration of particles was determined from the correlations of results of several experiments. Data for correlations and regression lines are in table II and figure 3. Cytochrome oxidase activity (assayed with cytochrome c) was negatively correlated with particle concentration. Succinoxidase activity was not significantly correlated. For cytochrome oxidase a decrease of 0.12 to 0.23 Q_{02} per mg/ml increase in particle concentration was found (table II). It is inferred that some inactivating substance remained with the particle even after a wash treatment in the cold.

The results in table II also show that, in the cytochrome oxidase assay, the activity with cysteine and succinate (column 3) was significantly correlated, negatively, with particle concentration. However, the importance of the correlation is small since the coefficient of regression was low; i.e., the degree of inhibition by increased particle concentration, although significant, was small for the relatively low activity. Moreover, the Q_{02} value extrapolated to infinite dilution for the cysteine plus succinate treatment was not significantly different (P = 0.05) from the cysteine treatment. It may be concluded that the particle concentration was of importance only for the cytochrome oxidase stimulated by cytochrome c.

The addition of ascorbic acid to the particles resulted in real increases of oxygen uptake even in suspensions without cytochrome c in the cytochrome oxidase assay (figs 2 and 4). The extrapolated Q_{02} values in the cytochrome oxidase assay were significantly greater (P ≤ 0.0001) than those in succinoxidase assay (table II, columns 1 and 3). Since the oxygen uptakes were greater than could be accounted for by autoxidation of ascorbic acid, the activity may have been due to ascorbic oxidase or cytochrome oxidase through endogenous cytochrome c. Thus, lack of cytochrome c stimulation is not necessarily evidence for lack of cytochrome oxidase in these assays. Since cytochrome c had significant stimulating effects (table II) the ambiguity was not of concern.

**Succinoxidase and Cytochrome Oxidase from Older Roots:** The methods used for 2-day-old seedlings were applied to roots of 10-day-old seedlings. Again both succinoxidase and cytochrome oxidase were found with particles isolated from the roots (fig 4). Rapid inactivation of the succinoxidase system occurred in this experiment, presumably owing to the low particle concentration used. This was also observed for preparations from 2-day-old seedlings and thus was not peculiar to the older tissue extracts. The small amount of succinoxidase activity was always observed when cytochrome and succinate were added to dilute particle suspensions. Moreover, the initial specific activity was substantially the same as that in experiments with higher particle concentrations which sustained the activity. Specific activity of cytochrome oxidase was as high as that of particles isolated from 2-day-old roots. In general, there was no detectable enzymatic difference between the particles from roots of 2-day-old seedlings and particles from roots of 10-day-old seedlings.

**Discussion**

It was found that both succinoxidase and cytochrome oxidase are extractable from roots of barley seedlings as old as 10 days. Since these findings are contrary to those previously reported (10, 12), the method of demonstration will be discussed.

**Succinoxidase:** It was considered necessary to stabilize the labile succinic dehydrogenase of the succinoxidase system. Since cysteine treatment allowed activity to be demonstrated, it is inferred that RSH inactivators are released upon homogenization of the barley roots. Evidently only if appropriate steps to
**Figure 4.** Assay for succinoxidase and cytochrome oxidase of particles from roots of 10-day-old seedlings. Conc of addenda in 4.00 ml for succinoxidase assay and corresponding conc in the homogenizing medium in brackets: particles 6.0 mg/ml (24.1); K-suec 2.00 x 10^-2 M; cyt c 6.4 x 10^-4 M; pH 7.5 phosphate buffer 1.00 x 10^-2 M (1.00 x 10^-2); cyst-HCl 2.50 x 10^-4 M (1.00 x 10^-4); EDTA 2.50 x 10^-4 M (1.00 x 10^-4); and sucrose to adjust medium to 0.5 osmolar (0.5). Conc of TRIS-ascorbate in 4.50 ml for cytochrome oxidase assay was TRIS 4.44 x 10^-4 M and ascorbic acid 3.98 x 10^-4 M.
counteract the inactivation were taken, could succinoxidase be demonstrated consistently. Either the barley root succinic dehydrogenase may be more sensitive or there may be more RSH inactivator in barley roots than in potato tuber or cauliflower buds where the dehydrogenase is more easily demonstrated. For extracts from these latter two tissues appropriate steps to counteract apparent RSH inactivators increased oxidative activity but were not necessary for activity (18, 26).

Although a succinoxidase system was assumed to be evidence for the classical cytochrome oxidase (8) this is not necessarily so. Succinate oxidation mediated by a system presumably not involving cytochrome c oxidation has been observed (6). The system from barley roots when highly diluted showed activity, although short lived, only in the presence of cytochrome c. Therefore it appears that the succinoxidase system of barley roots includes cytochrome oxidase.

**Cytochrome Oxidase:** Cytochrome oxidase itself was demonstrated using ascorbic acid as reducing agent. Since ascorbic oxidase is present in barley roots a small amount of contamination could account for ascorbate oxidation by particles from barley roots. However, the threefold stimulation of oxygen uptake by addition of cytochrome c showed that a large portion of the activity was mediated by cytochrome oxidase. In addition, the presence of cytochrome oxidase was confirmed with hydroquinone as reducing agent. The QO2 of particles assayed with the medium in table I (hydroquinone replacing ascorbate and without cysteine, insulin, and succinate) was 4.34 and 0.00 with and without cytochrome c, respectively.

Slater showed that non-enzymatic oxygen uptake was inhibited by cytochrome c when ascorbate was the reducing agent in a manometric assay for cytochrome oxidase. In addition, the presence of enzyme preparation lowered the “blank” correction (27). These data presumably indicate that non-enzymatic catalysis of ascorbic acid oxidation by metal ions is reduced by non-specific binding of the metal ions by protein. Oxygen uptake without barley root particles was zero with EDTA or, if not zero, was much lower than oxygen uptake by particles with no added cytochrome c and succinate. Particles, in contrast to Slater’s results, therefore produced greater oxygen uptake than the non-enzymatic oxygen uptake. Further, both heating and malonate treatment lowered oxygen uptake of particles in the succinoxidase assay. Consequently, it is inferred that the oxygen uptake without exogenous succinate and cytochrome c was due to particle oxidation of endogenous substrate and cysteine. It is further inferred that this oxidation proceeded through a particle-bound cytochrome c system since the requirement for cysteine for endogenous activity paralleled its requirement for succinoxidase activity.

The inhibitory effect of high particle concentration was apparently specific to cytochrome oxidase and affected the succinoxidase system only slightly. The succinoxidase system may have been already rate limited, possibly by succinic dehydrogenase, as might be inferred from figure 2 (cf. Chance and Smith, 5). Consequently partial inhibition of excess cytochrome oxidase may not have markedly affected the succinoxidase system. Since succinoxidase was inactivated during the course of the reaction and cytochrome oxidase was not, even though its activity was assayed as much as one hour later, and since increased particle concentration sustained succinoxidase activity while it depressed cytochrome oxidase activity, it is obvious that a full explanation is complex. The relationship of high particle concentration to low specific activity may account partially for previous failures in isolating cytochrome oxidase from barley roots.

**Cytochrome Oxidase from Older Roots:** It is known that barley roots exhibit a salt respiration (22). From the close correlation between cytochrome oxidase and salt accumulation and salt respiration it might be expected that cytochrome oxidase is active in barley roots. It was demonstrated here that succinoxidase and cytochrome oxidase are extractable from roots of both 2- and 10-day-old seedlings. The presence of cytochrome oxidase is consistent with but not sufficient to prove its activity in intact tissue.

James (12) suggested that cytochrome oxidase is replaced in older barley roots by ascorbic oxidase as the terminal oxidase. However, the evidence for James’s conclusion was not unequivocal. The nega-
tive evidence of the lack of extractable cytochrome oxidase is now removed. The conclusion of James now depends largely upon the use of diethylthiocarbamate (DDC) as a specific inhibitor of ascorbic oxidase in vivo. James and Garton (13) have shown the differential effect of DDC upon extracted ascorbic oxidase and extracted cytochrome oxidase. Nonetheless, the application of DDC to intact tissues cannot be considered to give conclusive results. It was confirmed that DDC markedly inactivates ascorbic oxidase in vivo.4 However, the partial inhibition of respiration may not depend on the partial inactivation of ascorbic oxidase. Enough active ascorbic oxidase to accommodate the entire uninhibited respiration was extracted from 10-day-old barley root tips treated with DDC.4 DDC can be oxidized to give a product which markedly inhibits succinic dehydrogenase (14). It is known that malonate, an inhibitor of succinic dehydrogenase, can reduce the oxygen uptake of barley root segments by 65% (16), the same level of inhibition by DDC under similar conditions (13).

**Summary**

A method for the isolation and assay for particle-associated succinoxidase and cytochrome oxidase from barley roots is described. Some critical factors for the successful isolation of succinoxidase are the incorporation of cysteine into the homogenizing medium, maintenance of the pH near 7 during extraction and assay, and the avoidance of a dilute particle suspension in the assay. Although cytochrome oxidase is relatively easy to demonstrate, the final concentration of particles in the assay is important. An inhibitor of cytochrome oxidase which is retained even with the washed preparations was found to be associated with the particles.

In contrast to previous reports of unsuccessful attempts to demonstrate cytochrome oxidase in barley roots, cytochrome oxidase and also succinoxidase have been isolated from roots of both 2- and 10-day-old seedlings of Cape barley.

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4 Honda, S. I. Unpublished experiments.

**Literature Cited**

EFFECTS OF ANTIMETABOLITES OF NUCLEIC ACID COMPONENTS ON THE GROWTH OF DIPLODIA NATALENSIS

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Since the discovery that structural analogs of compounds of biological importance can interfere with enzyme systems and with growth, the use of antimetabolites for elucidating metabolic pathways has become widespread (16). In recent years antimetabolites have been used with considerable success in the study of the relation of nucleic acid synthesis to the metabolism and growth of bacteria and tissues of animals and higher plants. These methods, however, have not been employed extensively with fungi.

This paper will report the results of a preliminary survey of the effects of some analogs of purine and pyrimidine constituents of nucleic acids on the growth of Diplodia natalensis and attempts to reverse these inhibitions with the normal bases. Some attention was also paid to the interrelations of folic acid analogs and purine and pyrimidine bases to growth.

MATERIALS AND METHODS

Diplodia natalensis Pole-Evans (U.S.D.A. strain 4725, the asexual stage of the ascomycete, Physalospora rhodina) was used as the test organism. It was grown on the following medium: glucose, 10 gm; asparagine-HCl, 2.0 gm; K₃HPO₄, 1.0 gm; MgSO₄ ·7 H₂O, 0.5 gm; Hoagland’s micronutrient supplement, 1 ml; agar, 20 gm; water to 1 liter; pH 7.0. Inoculated Petri dishes were incubated for 3 days at 24° ± 2° C at which time disks 5 mm in diameter were cut from the advancing edge of the mycelium with a sterile cork borer. In all experiments, a liquid medium was used having the same composition as above minus agar.

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RESULTS AND DISCUSSION

PURINE ANALOGS: The only noteworthy feature of the effects of adenine and guanine was the inhibition of growth caused by 0.01 M adenine (table 1). Neither 2,6-diaminopurine nor benzimidazole, at the concentration tested, were highly inhibitory in contrast to the effect of 8-azaguanine. The fact that 2,6-diaminopurine relieved the inhibition caused by the superoptimal concentration of adenine suggests that it may do so by reducing the physiological level of adenine. The inhibitions of growth caused by the purine antimetabolites were, in all cases, almost completely reversed by high concentrations of either adenine or guanine. The reversals by the normal bases of the inhibitions caused by 8-azaguanine appear to be functions of the concentrations of the metabolites.

In two other ascomycetes previously examined, an