Effects of Metabolic Poisons on Rice: The Comparative Sensitivity of Aerobic and Anaerobic Modes of Germination

S. M. Siegel, Muriel Lederman, Olive Daly, and Karen Roberts
Union Carbide Research Institute, Tarrytown, New York 10591

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Summary. Rice germinates equally well when incubated in air and in nitrogen. It was therefore chosen for a comparative study of the effect of oxygen status in a single organism upon the activity of conventional metabolic poisons. Inhibitor activity was based upon the concentration required for 50% inhibition of germination. The inhibitors were: AgNO₃, HgCl₂, phenylmercuric acetate, iodoacetamide, KCN, NaNO₃, NaF, fluoracetate, 2,4-dinitrophenol, Na₂HAsO₄ and CO. Only 5 inhibitors out of 11 differed significantly in their effects on aerobic and anaerobic germination. These included phenylmercuric acetate (N₂), sodium fluoracetate (air), NaNO₃ (air), and 2,4-dinitrophenol (N₂). CO activity was manifest only in air and it was photo-reversible. The effects of CO, NaNO₃, and fluoracetate were consistent with their conventional role in aerobic metabolism. The failure of KCN to discriminate was attributed to its relative non-specificity. 2,4-Dinitrophenol behaved anomalously, requiring a 20-fold lower concentration for inhibition under nitrogen than under air. Among sulphydryl poisons, only phenylmercuric acetate differentiated between germination in air and nitrogen and was nearly 8 times more active under nitrogen. Uptake measurements using phenylmercuric acetate and arsenate showed both compounds to be in greater concentration under aerobic conditions, thereby rendering unlikely any explanation based upon greater permeability under nitrogen. In addition to other anomalies, the fact that NaNO₃, fluoracetate and CO inhibited anaerobic germination at any concentration requires explanation. It was concluded that the general significance and utility of metabolic inhibitor studies in vivo required further evaluation.

The capacity for submerged and anaerobic germination has long been known (7,8,12) and it has recently been recognized to be relatively common among angiosperms (13,14). Of all the species examined for such abilities, none has received the attention given to cereals. Comparisons between rice and other cereals, barley or wheat, for example, have been made with respect to germination, seedling growth, respiration, glycolysis, and specific enzymes as functions of ambient oxygen level (3,6,7,8,10,11,15).

Among higher organisms which generally seem to be obligate aerobes, rice offers an unusual opportunity for the study of the influence that oxygen has upon the properties of a system in which growth and development can be inaugurated equally well with or without it.

The present study is concerned primarily with the influence of oxygen status during germination on the toxicity of known metabolic poisons.

Initially, it was assumed that metabolic poisons would differentiate aerobic from anaerobic modes in seed germination; and that distinctions would be largely consistent with their defined activities as enzyme inhibitors. It is obvious from the data presented below that neither of these assumptions has been well supported.

Material and Methods

Oryza sativa, “Calora”, of the 1964 commercial crop was used in 1965. The grains had not been treated with fungicidal dust.

Germination was carried out at 25° in darkness in petri dishes containing 5 ml of 0.01 m acetate buffer, (pH 6.0). Grains were incubated for 72 hours, yielding in that time 77 ± 6% germination as defined by the presence of a coleoptile 3 mm or more in length. Experiments were carried out in triplicate (33-40 seeds per replicate) and repeated at least once.

Atmospheric control was achieved by incubating seeds in sealed 4 liter anaerobic jars purged continuously at 4 liter per minute with air or N₂ saturated with water. The O₂ content of the nitrogen was...
2,4-Dinitrophenol

Iodoacetallide

NaF

NaAsO₄

Results

Among the 10 metabolic poisons applied in aqueous solution, only 4 showed significant differences in ID₅₀ values (table 1). These were phenylmercuric acetate, which was required in nearly 2-fold greater concentration in air than in nitrogen; sodium fluorooacetate, which required approximately a 7-fold lower concentration to yield a 50% inhibition in air; NaNO₃, which exhibited twice the activity in air that it did in N₂; and 2,4-dinitrophenol which was 20-fold more active in the absence of oxygen. Although some of the remaining compounds exhibit differences of as much as 50% between aerobic and anaerobic cultures, no statistical significance could be attached to them. In darkness, germination was reduced by about one-half by 95% CO, a significant decrease (table II). In the absence of oxygen, the effect of CO was without significance. In light, differences observed with or without CO and with or without O₂ were not significant.

In general, rice germination appeared to require unusually high concentrations of inhibitors whether aerobically or anaerobically supported. As illustrations, the ID₅₀ for KCN was 0.045 to 0.049 μM; for NaF, 0.006 to 0.011 μM; and for iodoacetamide was 0.001 to 0.0015 μM. The greater resistance of air incubated rice was shown when it was compared with lettuce and pea, both obligate aerobes (table III). With only 1 exception, iodoacetamide, out of 7 metabolic inhibitors compared, appreciably higher concentrations of compounds were necessary to attain the ID₅₀ level in rice.

When exogenous regulators, including metabolic poisons, are studied in connection with some other variable, it is desirable to gain some information about the uptake of the regulator or inhibitor. In the present study, the oxygen status of germination is such a variable. Preliminary determinations of inhibitor uptake under air and nitrogen were carried out using phenylmercuric acetate, which has a pronounced differential effect, and sodium arsenate, which does not. Nevertheless, in both cases, aerobic conditions favored uptake of the metal compound (table IV).

Table I. Effect of Atmosphere During Germination Upon the Concentration Required for 50% Inhibition (ID₅₀) by Metabolic Poisons

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Air</th>
<th>N₂</th>
<th>Air: N₂ ID₅₀, in μmoles/liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>AgNO₃</td>
<td>6.5 ± 0.5</td>
<td>7.5 ± 0.5</td>
<td>0.87</td>
</tr>
<tr>
<td>HgCl₂</td>
<td>9.4 ± 0.5</td>
<td>8.3 ± 0.5</td>
<td>1.13</td>
</tr>
<tr>
<td>Phenylmercuric acetate</td>
<td>5.4 ± 0.4</td>
<td>0.7 ± 0.4</td>
<td>7.71**</td>
</tr>
<tr>
<td>Iodoacetamide</td>
<td>1.5 ± 0.7</td>
<td>1.0 ± 0.4</td>
<td>1.50</td>
</tr>
<tr>
<td>KCN</td>
<td>45.0 ± 2.0</td>
<td>49.0 ± 1.7</td>
<td>0.91</td>
</tr>
<tr>
<td>NaF</td>
<td>0.3 ± 0.2</td>
<td>0.7 ± 0.3</td>
<td>0.43**</td>
</tr>
<tr>
<td>NaNO₃</td>
<td>11.0 ± 1.0</td>
<td>6.0 ± 0.5</td>
<td>1.07</td>
</tr>
<tr>
<td>Fluoroacetate</td>
<td>6.0 ± 0.6</td>
<td>40.0 ± 1.7</td>
<td>0.15**</td>
</tr>
<tr>
<td>2,4-Dinitrophenol</td>
<td>8.0 ± 0.6</td>
<td>0.4 ± 1.3</td>
<td>20.00**</td>
</tr>
<tr>
<td>Na₃HAsO₄</td>
<td>1.1 ± 0.3</td>
<td>0.8 ± 0.5</td>
<td>1.37</td>
</tr>
</tbody>
</table>

* Mean ± standard error.
** Highly significant difference (P ≤ 0.01) air vs N₂.

Table II. Differential Effect of 95% CO in Aerobic and Anaerobic Germination of Rice

<table>
<thead>
<tr>
<th>Atmosphere</th>
<th>Dark Germination</th>
<th>Inhibition</th>
<th>Light Germination</th>
<th>Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>95% N₂ + 5% O₂</td>
<td>74 ± 7%</td>
<td>54 ± 4%</td>
<td>86 ± 8%</td>
<td>92 ± 9%</td>
</tr>
<tr>
<td>95% CO + 5% O₂</td>
<td>34 ± 4%</td>
<td>17 ± 7%</td>
<td>92 ± 8%</td>
<td>89 ± 10%</td>
</tr>
<tr>
<td>95% CO + 5% N₂</td>
<td>61 ± 6%</td>
<td>17 ± 7%</td>
<td>92 ± 9%</td>
<td>90 ± 10%</td>
</tr>
</tbody>
</table>
Discussion

When compared with the germination of obligate aerobes such as lettuce and pea, rice in air was less sensitive toward several metabolic inhibitors. This may in part reflect the presence of an exceptionally developed glycolytic system. Evidence for glycolysis in rice grains has been presented (11,15), but it is obvious that anaerobic processes, glycolytic or otherwise support germination as well as do aerobic processes. The non-selective activity of NaF suggests that glycolysis is equally limiting in germination-linked metabolism whether in N₂ or air. Among the 4 inhibitors conventionally active in aerobic metabolism 3 exhibit the expected selectivity, namely CO₂, NaN₃ and fluoroacetate. The fact that these compounds are among the most specific terminal oxidase and Krebs cycle inhibitors respectively, suggests that more weight is given to their effects than to KCN, which failed to differentiate aerobic and anaerobic. Cyanide ion is far from reactive and, when active only at high concentrations, may be non-specific.

The response to dinitrophenol was highly unexpected. Its selective inhibition of germination under N₂ is wholly inconsistent with the established uncoupling activity of dinitrophenol in oxidative phosphorylation. It does not follow that aerobic germination is independent of oxidative phosphorylation or that oxidative phosphorylation in rice is dinitrophenol-resistant. It is more likely that the anomaly involves a system unique in aerobic rice and not necessarily related to energy metabolism. Finally, rice germinating anaerobically may be more permeable to dinitrophenol than its counterpart in air. This alternative cannot be judged without a direct test, but evidence gained from measurements of phenylmercuric acetate and arsenate uptake consistently show greater uptake under aerobic conditions. There is no reason to suppose that dinitrophenol behaves differently.

Although the reactivity of protein SH-groups may be influenced by neighboring groups and local environment, they are ubiquitous among the enzymes of carbohydrate metabolism. The similar responses of rice under air and N₂ to AgNO₃, HgCl₂ and iodoacetamide is consistent with their presumed activity as non-selective thiol reagents. Accordingly, the differential activity of phenylmercuric acetate requires further consideration. In air, the organo-mercuro compound is significantly more active than the chloride (1>6), but the difference under N₂ is far greater. The heightened anaerobic sensitivity toward phenylmercuric acetate is even more striking when uptake is considered. In air, 12 mg/gm of Hg was present in completely uninhibited seeds, whereas under N₂, only 5 mg/g of Hg were present at about 50% inhibition. These data do not permit any conclusion as to the target for phenylmercuric acetate inhibition, but they suggest that it is not the presence of an Hg compound per se but, rather, a condition or state of the anaerobic system that determines inhibition. It
is interesting, although perhaps coincidental, that the
2 inhibitors yielding the most anomalous results are
both aromatic.

From a purely comparative viewpoint, we have
seen that certain inhibitors in aerobic systems namely,
NaNO3, fluoroacetate, and CO behave in a proper, that
is, expected manner. From a more absolute viewpoint, however, a question of major importance has
been left unanswered. If the conventional assignment
for target of inhibition of these compounds is at all
meaningful, then why should any inhibitory activity
at all be manifest under nitrogen? Even if it were
assumed that some form of aconitate dependent or-
ganic acid cycle were operative under nitrogen as an
explanation for fluoroacetate inhibition, no such
alternative can be advanced for the existence of azide
and carbon monoxide inhibitors under anoxia.

On the basis of these results it appears that exten-
sive study is required of the diagnostic significance
of conventional metabolic poisons when they are applied
in vivo.

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