Promotion of Seed Germination by Nitrate, Nitrite, Hydroxylamine, and Ammonium Salts

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ABSTRACT

Action and uptake of azides, nitrates, nitrites, hydroxylamines, and ammonium salts were measured on germination of Amaranthus albus, Lactuca sativa, Phleum pratense, Barbarea vulgaris, B. verna, and Setaria glauca seeds. Nitrate and nitrite reductase activities were measured in vitro for each of these kinds of seeds. Activities were measured in vitro for catalase, peroxidase, glycolate oxidase, and pyridine nucleotide quinone reductase on extracts of A. albus and L. sativa seeds before and after germination. The enzymatic activities measured and the responsiveness of the haemproteins to inhibition by the several compounds indicate that nitrites, azides, and hydroxylamines promote seed germination by inhibition of H2O2 decomposition by catalase. Ammonium salts showed pronounced promotive activity only for B. verna and B. vulgaris seeds, for which they served as metabolic substrates.

The promotion of germination is thought to depend on coupling of peroxidase action to NADPH oxidation, which can regulate the pentose pathway of D-glucose 6-phosphate use. Pyridine nucleotide quinone reductase is the possible coupling enzyme. This enzyme and others required for the action are present in the seeds before imbibition of water.

MATERIALS AND METHODS

Seed Germination. Seeds showing low germination in darkness on water at constant temperatures, but still responsive to some substrate, were available from lots collected over a period of 3 to 6 years and held in tight containers at -20 C. Seeds tested at the indicated temperatures, which were previously found to be optimum for germination, were those of tumble pigweed (Amaranthus albus L. [30 C]); lettuce (Lactuca sativa L. var. Grand Rapids [23 and 30 C]); yellow rocket (Barbarea vulgaris R. Br. [20 C]); winter cress (Barbarea verna [Mill.] Aschers [20 C]); yellow foxtail (Setaria glauca [L.] Beauv. [25 C]); timothy (Phleum pratense L. [25 C]); and barnyardgrass (Echinochloa crusgalli [L.] Beauv. [25 C]). Germination tests were made by placing duplicate lots of 100 seeds on single Whatman No. 3 filter papers, moistened to somewhat more than glistening with the appropriate solution, or about 5 ml per 100 seeds. The covered Petri dishes were held in darkness in a water-saturated atmosphere. Germination, assessed by emergence of the radicle, was determined after 3 to 7 days, with note of any distortion of seedling growth. All tests were repeated two or more times.

Assays. Nitrate was assayed, after the method of Wooley et al. (35), by reduction to nitrite with zinc. The nitrite produced was measured spectrophotometrically after conversion to an azo compound. Nitrite was similarly determined without reduction (29). Cyanide was determined by treatment of solutions with chloramine-T to form CNCl, which was coupled with a pyridine-pyrazolone reagent, and the absorbance was determined at 630 nm (30). Hydroxylamine was assayed by oxidation with iodine to nitrite after the method of Czaky (5). Ammonia was determined by the Conway microdiffusion method (4).

Enzymic Activities. Nitrate reductase activity of seeds was assayed in vivo by the method of Ferrari and Varner (6). This method involved cell leakage of nitrate and nitrite in the presence of 1% (v/v) propanol under anaerobic conditions. Nitrite reductase was measured in vivo by disappearance from the solution ambient to the seed, followed by determination of nitrite in the seeds by leakage under anaerobiosis. An attempt to measure hydroxylamine reductase by aerobic in vivo uptake failed because of nonenzymatic reaction of hydroxylamine with seeds.

Peroxidase, catalase, glycolate oxidase, and pyridine nucleotide quinone reductase activities were measured at 25 C in aliquots from extracts of imbibed, washed seeds, ground with 100 mesh ALO, at ice-bucket temperature in a Ten-Broeck²

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unit, using 6 ml/0.25 g seeds of 10 mM K phosphate buffer at pH 6.8. Extracts were centrifuged at 12,000g at 3 C for 15 min, followed, in some instances, by millipore filtration to lower the starch content of supernatants. Catalase activity of aliquots was measured by iodometric assay of added H2O2 remaining after various times (10). Peroxidase activity was measured by the rate of loss of H2O2 in the presence of O-anisidine as hydrogen donor, with appearance of absorbance at 460 nm (36). Both activities were expressed in units of µmoles of H2O2 decomposed/min at 25 C/g of seed extracted. Glycolate oxidase was assayed by following the rate of reduction of 2,6-dichlorophenolindophenol with glycolate as a substrate, in the presence of FMN and the presence or absence of O2 (23). Pyridine nucleotide quinone reductase was measured by following the rate of oxidation of NADH or NADPH by p-benzochinone, with correction for the nonenzymic rate (37). Protein in the extracts was measured by the method of Lowry et al. (20) and in seeds by microkjeldahl methods.

Inhibitor Actions. Effects at various concentrations of KNO3, NaNO2, KNO2, NO3, CH3NHOCl, and [(C2H5)2N]2[COOH]2 on catalase and peroxidase activities were measured on the seed extracts, as well as on commercial lyophilized liver catalase and horseradish peroxidase.

Uptake of Substrates. Four to 20 ml of the required salt solutions of known concentrations were placed on 0.25 or 0.5 g of seed in 5-cm diameter Petri dishes and held at the appropriate constant temperatures in darkness in a water-saturated atmosphere for 20 to 72 hr. The seeds thus were covered with solution to a depth of several mm. Some of the indicated tests were made in the presence of 1 mM streptomycin sulfate and 0.5 mM mycostatin to suppress growth of microorganisms. The initial pH values were 7.0, except for KCl solutions, which were >9.0. Initial and final assays were made on aliquots of the solutions. Salts used were commercial ones or were prepared from the appropriate commercial base.

Oxygen consumption. A Clark electrode was used to measure O2 consumption of 0.3 g of seed, which had been maintained in 4 ml of the appropriate solution at 30 C for the indicated periods. Initial values were obtained from the slopes of the O2 consumption versus time curves.

RESULTS

Seed Germination. Percentage of germination for the various kinds of seeds incubated with the several substrates are shown in Table 1. Concentrations listed are near optimum values for maximum germination, as shown by tests over concentration ranges. Variation of A. albus seed germination with concentration of the several substrates are shown for a typical lot (Fig. 1). Some lots of A. albus seeds were somewhat promoted in germination by 10 mM NH4Cl (Table 1).

All lots of B. verna and B. vulgaris seeds tested were most responsive to NH4Cl among the substrates used (Table I, Fig. 2). Germination of P. pratense seeds was inhibited by 10 mM NH4Cl at 30 C, relative to values with water (Fig. 3). The effect was negligible at 1 mM NH4Cl. Germinations of E. crusgalli and A. albus seeds were less responsive to [(C2H5)2N]2[COOH]2 than to NH4OH-HCl (Fig. 4).

Damage to emergent seedlings was noted at substrate concentrations corresponding to the dotted portions of the curves in the figures. Seedlings of A. albus and E. crusgalli were damaged by hydrazine and its 1-methyl, 1,1-methyl, and 1,2-methyl derivatives (results not shown), when used at >0.32

Table 1. Germination in Darkness of Several Kinds of Seeds on Various Salt Substrates and Water

<table>
<thead>
<tr>
<th>Salt</th>
<th>Conc</th>
<th>A. albus</th>
<th>L. sativa</th>
<th>B. verna</th>
<th>B. vulgaris</th>
<th>S. glanca</th>
<th>P. pratense</th>
<th>E. crusgalli</th>
</tr>
</thead>
<tbody>
<tr>
<td>KNO3</td>
<td>10.0</td>
<td>77*</td>
<td>34*</td>
<td>14*</td>
<td>31*</td>
<td>46*</td>
<td>82*</td>
<td>65*</td>
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<tr>
<td></td>
<td></td>
<td>(44)</td>
<td>(30)</td>
<td>(1)</td>
<td>(30)</td>
<td>(26)</td>
<td>(56)</td>
<td>(46)</td>
</tr>
<tr>
<td>NaNO2</td>
<td>1.0</td>
<td>92*</td>
<td>30*</td>
<td>6*</td>
<td>26*</td>
<td>58*</td>
<td>68*</td>
<td>66*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(56)</td>
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<td>(30)</td>
<td>(35)</td>
<td>(31)</td>
<td>(46)</td>
</tr>
<tr>
<td>NH4OH·HCl</td>
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<td>99</td>
<td>34*</td>
<td>2*</td>
<td>1*</td>
<td>39</td>
<td>88</td>
<td>68</td>
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<td>(44)</td>
<td>(30)</td>
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<td>(1)</td>
<td>(20)</td>
<td>(77)</td>
<td>(46)</td>
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<td>65</td>
<td>28*</td>
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<td>30*</td>
<td>34</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(44)</td>
<td>(30)</td>
<td>(1)</td>
<td>(30)</td>
<td>(26)</td>
<td>(56)</td>
<td>(46)</td>
</tr>
<tr>
<td>KCN</td>
<td>0.7</td>
<td>81</td>
<td>74</td>
<td>24</td>
<td>54*</td>
<td>90*</td>
<td>81</td>
<td>81</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(32)</td>
<td>(15)</td>
<td>(1)</td>
<td>(1)</td>
<td>(58)</td>
<td>(92)</td>
<td>(68)</td>
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<td>KN3</td>
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<td>86</td>
<td>21</td>
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<td>57</td>
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<td>(0)</td>
<td>(47)</td>
<td>(47)</td>
<td>(68)</td>
<td>(68)</td>
</tr>
</tbody>
</table>

1 Values shown in parentheses are for germination in water.
2 Concentration was 10 mM.
* Only results so indicated fail to differ from the water controls at the 95% level of significance.

FIG. 1. Germination of A. albus seeds after 3 days at 30 C, as a function of concentrations of various salts. Dotted portions of curves indicate concentrations causing seedling injury.

FIG. 2. Germination of B. verna seeds after 6 days at 20 C, as a function of concentration of NH4Cl. Results for seeds in the presence of 0.01 mM NaNO3 or NH4NO3 are indicated. The dotted portion indicates concentrations causing seedling injury.
from the several integrations over time and the various conditions of measurement.

**Catalase and Peroxidase Activities.** Seeds of *A. albus* at 30°C and *L. sativa* at 23°C (>600 nm irradiated) are just beginning to germinate after 20 hr imbibition. After 44 hr under these conditions, germination is >60%, and the first internodes of the seedlings are about 1 cm in length. Peroxidase activity increases concomitantly (Table IV) with a smaller increase in catalase activity. It is important to note that *L. sativa* seeds do not germinate significantly at 0.1 mM.

**Uptake of Salts.** Salt disappearance from ambient solutions with all the seeds tested indicated that values for nitrate and nitrite were of similar magnitude, but much less than those for ammonium salts (Table II). A nonmetabolic component of uptake was probably predominant with NH₄OH·HCl, as evidenced by reaction with dead seeds. *A. albus* seeds changed from deep purple to red, and *B. vulgaris* seeds turned red after 24 hr in NH₄OH·HCl solutions >1 mM at pH 7.

**Nitrate and Nitrite Reductase Activities.** Both reductases were present in all the seed lots tested. Induction of nitrate reductase activity was evident in *A. albus, L. sativa,* and *P. pratense* seeds, but not in *B. verna, B. vulgaris,* and *S. glauca* seeds (Table III). The activity of nitrite reductase was adequate to use the nitrite formed by nitrate reductase action. Measured reductase activities were compatible with the values for disappearance of the salts from solutions (Table II) on *A. albus, L. sativa,* and *P. pratense* seeds. They were low by a factor of three for *B. verna* and *S. glauca,* which could well have arisen

**Table II. Disappearance of Several Salts from Solutions on Various Kinds of Seeds in Darkness**

<table>
<thead>
<tr>
<th>Salt</th>
<th>Conc</th>
<th>Amount Applied</th>
<th>A. albus (0 hr)</th>
<th>L. sativa (0 hr)</th>
<th>S. glauca (2 hr)</th>
<th>B. verna (2 hr)</th>
<th>P. pratense (2 hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mM</td>
<td>µmoles/g seed</td>
<td>µmoles/g seed</td>
<td>µmoles/g seed</td>
<td>µmoles/g seed</td>
<td>µmoles/g seed</td>
<td>µmoles/g seed</td>
</tr>
<tr>
<td>KNO₃</td>
<td>10.0</td>
<td>40</td>
<td>1² &lt; 1²</td>
<td>5°</td>
<td>6°</td>
<td>1³</td>
<td></td>
</tr>
<tr>
<td>NaNO₂</td>
<td>1.0</td>
<td>10</td>
<td>0.3</td>
<td>3.0</td>
<td>1.4</td>
<td>5°</td>
<td>2°</td>
</tr>
<tr>
<td>NH₂OH·HCl</td>
<td>0.1</td>
<td>1</td>
<td>2</td>
<td>1.6</td>
<td>1.6</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>NH₄Cl</td>
<td>10.0</td>
<td>40</td>
<td>14</td>
<td>10°</td>
<td>14°</td>
<td>33°</td>
<td>11</td>
</tr>
<tr>
<td>KCN</td>
<td>0.1</td>
<td>8</td>
<td>1.6</td>
<td>2.7</td>
<td>0.4</td>
<td>0.6</td>
<td>0</td>
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</tbody>
</table>

1 Ion measured.
2 In the presence of 1.2 mM streptomycin sulfate and 2 mM mycostatin.

**Table III. Nitrate and Nitrite Reductase Activities Assayed in Vivo for Several Kinds of Seeds**

Nitrate reductase was measured in 1 mM KNO₃ after the indicated hours imbibition in H₂O or 10 mM KNO₃ by the method of Ferrari and Varner (6). Nitrite reductase activities were taken as the nmoles NaNO₂/g seed-hr disappearing from 1 mM NaNO₂, averaged over the indicated imbibition time.

**Table IV. Enzymic Activities of Catalase and Peroxidase at 25°C in Extracts of *A. albus* and *L. sativa* Seeds Imbied in Water**

<table>
<thead>
<tr>
<th>Seed</th>
<th>Imbibition Temperature</th>
<th>Germination (in hr)</th>
<th>Catalase Activity¹</th>
<th>Peroxidase Activity¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>µmoles H₂O₂ g seed/hr</td>
<td>µmoles H₂O₂ g seed/hr</td>
</tr>
<tr>
<td>A. albus</td>
<td>30</td>
<td>65</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>L. sativa</td>
<td>23</td>
<td>99</td>
<td>20</td>
<td>23</td>
</tr>
<tr>
<td>L. sativa</td>
<td>30</td>
<td>0</td>
<td>20</td>
<td>9</td>
</tr>
</tbody>
</table>

¹ 20 to 28% of seed protein was present in the extracts.

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**Fig. 3.** Germination of *P. pratense* seeds after 6 days on H₂O, 10 mM NH₄Cl, or 10 mM KNO₃ at various temperatures (a), and at 30°C as a function of concentration for NH₄Cl and KNO₃ (b).

**Fig. 4.** Germination of *E. crusgalli* and *A. albus* on NH₂OH·HCl and [(CH₃)₂ NOH]₂·[COOH]₂ solutions of various concentrations. Dotted portions of curves indicate concentrations causing seedling injury.
not germinate at 30 C. Catalase and peroxidase activities in extracts from these seeds increased 2- to 3-fold between the 20th and 44th hr for seeds imbibed in water. The measured extracted activities were not significantly changed by imbibition in 1 mM NH$_4$OH or 0.1 mM KN$_6$ (results not detailed).

**Inhibitor Actions.** Catalase activities in lettuce seed extracts were inhibited 50% by 1.7 $\mu$M KN$_6$ and by 10 $\mu$M NH$_2$OH·HCl. Activities of lyophilized liver catalase in pH 6.8 K-orthophosphate buffer was inhibited 50% by 0.3 $\mu$M KN$_6$ or by 1.0 $\mu$M NH$_4$OH. The inhibition was 30% in 1 mM CH$_3$NH$_2$OHCl, 15% in 1 mM [(CH$_3$)$_2$NOH]·[COOH]$_2$, and >10% in 10 mM KNO$_3$.

Peroxidase activities in *A. albus* seed extracts were inhibited 20% by 0.1 mM KN$_6$, 40% by 0.1 mM NH$_4$OH·HCl, and by <10% by 0.1 mM NaNO$_2$ and 1 mM KNO$_3$. Horseradish peroxidase activity was inhibited 10% by 0.2 mM KN$_6$ or 0.2 mM NH$_4$OHCl.

**Glycolate Oxidase and Pyridine Nucleotide Quinone Reductase Activities.** Glycolate oxidase activity was not detected in seeds of *A. albus* and *L. sativa* after 20 hr imbibition in water at 23 C or 30 C, respectively. *A. albus* seeds were just beginning to germinate. The oxidase was present after 3 days in seedlings exposed to diffuse sunlight for 10 hr each day. Activities, expressed as moles of 2,6-dichlorophenolindophenol reduced/g seed or in the seed extracts were 3 $\times$ 10$^{-1}$ and 1 $\times$ 10$^{-1}$ mole/g seed for *A. albus* and *L. sativa*, respectively. Pyridine nucleotide quinone reductase activities in the seed extracts after 0 or 20 hr imbibion were 1300 or 3200 units/g seed·min for both *A. albus* and *L. sativa* seeds, with NADH as hydrogen donor and 6,400 units/g seed·min with NADPH after 20 hr. These values correspond to 55 and 110 units/mg extracted protein·min, respectively. A unit is $\Delta A = 0.001$ at 340 nm (29). Endogenous rates in the absence of the p-benzoquinone substrate, for which correction was made, were 10 to 20% of these values.

**Oxidation Consumption.** *A. albus* seeds after 20 hr imbibition in solutions gave values of 1.47 to 1.57 $\mu$O$_2$/g seed·min with 3.17 mM KNO$_3$, 0.1 and 1 mM NH$_4$OHCl and H$_2$O. Values for *S. glauca* seeds after 20 hr in the respective solutions were 1.58 to 1.65 $\mu$O$_2$/g seed·min with 10 mM KNO$_3$, 1 mM NaNO$_2$, and H$_2$O and 1.26 $\mu$O$_2$/g seed·min with 0.1 mM NH$_4$OHCl. After 44 hr solution imbibition, *B. vulgaris* seeds consumed 4.2 to 4.5 $\mu$O$_2$/g seed·min on 0.1 or 1 mM NH$_4$OHCl and H$_2$O. This value was reduced by 88% on 10 mM NH$_4$OHCl and by greater than 75% at 1 mM for several seed kinds. Presence of antibiotics did not alter the values, but it should be noted that hydroxylamine forms oximes with both streptomyecin and mycostatin. Cyanide at 10 mM reduced O$_2$ consumption of *A. albus* seeds after 18 hr imbibion to 80% of the value on water. This is suggestive of some cyanide-insensitive O$_2$ consumption.

**DISCUSSION.**

Germination in darkness of *A. albus*, *S. glauca*, *P. pratense*, and *E. crusgalli* seed is enhanced in the order NH$_4$OHX $\ll$ XNO$_2$ $\ll$ NHX (where X is the balancing cation or anion) in terms of reciprocal concentrations for equal effectiveness (Table I). Disappearance of the salts from the ambient solutions is in the reverse order (Table II). Failure of NH$_X$ salts to promote germination despite high uptake (Tables I and II) indicates that the compounds of higher nitrogen oxidation levels are not effective by reduction to NH$_X$. The fact that nitrates are as effective as nitrites, and uptake of both is low, indicates that the process of reduction is not determinative for germination, other than to produce nitrite.

Measured *in vivo* activities of nitrate and nitrite reductases (Table III) are adequate, with concentrations of XNO$_2$ and XNO$_3$ promotive for germination, to produce sufficient NH$_4$OH·HCl for the germination effect, provided reductant is adequate and the hydroxylamine is an intermediate. While the course of nitrite reduction in plant tissue is not well understood, particularly with respect to possible free or bound intermediates at the hydroxylamine level of reduction (12), NH$_4^+$ is generally thought to be the end product (1).

Ammonium salts inhibited germination of *P. pratense* seeds, particularly as the temperature was increased to 30 C (Fig. 3). Inhibition was minor or absent in other cases. An inhibition is an expected possibility because of the considerable uptake of NH$_X$ and the known actions of ammonium salts as inhibitors of L-amino acid oxidase and as uncouplers of oxidative phosphorylation (18). Findings for *B. verna* seed germination, and *B. vulgaris* to a lesser extent, contrast sharply with the response to the nitrogen compounds by the other seeds. Only NH$_X$ is highly effective for promoting germination in darkness (Table I, Fig. 2). It also is avidly taken up (Table II). These are expected results if ammonium ion serves as a reduced nitrogen source (28) for metabolic processes limiting germination in these seeds.

Neither NO$_3^-$ nor NO$_2^-$ can be acting to any considerable extent as substrates to spare oxygen consumption. This is evident from the limited effectiveness of NO$_2$ and NOX taken up (in the concentration ranges promoting germination) are on the order of 1 $\mu$ mole/g seed·day, while oxygen consumptions are about 100 $\mu$ moles O$_2$/g seed·day.

Two extreme classes of germination dependence on nitrogen are thus evident at (a) the nitrite or hydroxylamine or (b) the ammonium oxidation level. *A. albus* seeds possibly have both dependencies, while *L. sativa* seeds are deficient in both respects, but respond to azide. Two cautions are raised about possible actions of hydroxylamine in seed germination. Its disappearance from solution is in part non-metabolic as shown by reaction with dead seed and seedling injury is evident at concentrations exceeding 0.3 mM (Fig. 1). Hydroxylamine salts, when effective, maximally promote germination at 0.1 mM or less and inhibit oxygen consumption only at greater concentrations. The respiration and possible injury effects thus are probably not significant at 0.1 mM where germination responses are pronounced.

Hydroxylamine is a strong reductant and a strong chelating agent. It reacts to form oximes with aldehydes and ketones, or nitrogen ethers with aldehydes, when mono-N substituted (32). In a metabolic sense it can be reduced to NH$_X$ and can probably form an oxime with the formyl group of cytochrome a to limit electron flow to oxygen or associated phosphorylation (18) in respiration. Formation of oximes, followed by reduction, has sometimes been considered in amino acid formation (34, 38). Hydroxylamine is an acceptor with low Km value for the glutamyl radical in transamination reactions (7).

In considering the possibility of oxime involvement in the germination effects, tests were made with a number of substituted hydroxylamines and hydrazines. Results are reported only for NH$_4$OH·HCl, CH$_3$NH$_2$OHX, and (CH$_3$)$_2$NHOOH (Figs. 1 and 4). If the cause for promotive action of these three types of compounds is similar, it cannot be by oxime formation or reduction to the ammonium ion, which cannot directly occur for R,NHOOH. The role of oximes in nitrogen metabolism has recently been reviewed by Mahadevan (21). In the light of his review, we tested for HCN formation through reactions 11 and 12 of Mahadevan's Figure 2, as possible evidence of involvement of oximes. Cyanide was not detected by the exceedingly sensitive pyridine-pyrazolone reagent (30) (data not reported) when *A. albus* were imbibed in NH$_4$OHCl solutions.
The marked chelating capacities of hydroxylamine for the iron atoms of haemoproteins (19) and the definite but lower capacities for N-aliphatic substituted hydroxylamines indicate the presence of this type of action in seeds. The promotion of germination at concentrations that do not significantly affect respiration, however, show that the haemoprotein involved is not a cytochrome component of the respiratory chain. The two other prominent haemoproteins in plant tissues are catalase and peroxidase. Results in Table IV show these enzymatic activities at various germination stages in A. albus and L. sativa seeds and in nongerminating L. sativa seeds. The degree of inhibition of catalase and peroxidase by hydroxylamines and azide in vitro indicate that the primary inhibitory action can be on catalase in the seeds.

Keilen and Hartree (15, 16) found that catalase, in the absence of H$_2$O$_2$, chelates with azides. In the presence of H$_2$O$_2$, the azide in the chelated compound is oxidized to nitric oxide, and the iron is reduced to the ferrous form. The resulting NO-ferrocatelase has a lower dissociation constant ($< 10^{-9}$ M) and a lower catalytic effectiveness for decomposition of H$_2$O$_2$. A model system is formation of nitrosylferriyocardiac (nitropriusside) and other nitrosyl-complex salts of transition metals by the classical hydroxylamine method (14). Hydroxylamines, nitrates, and other nitrogenous compounds yielding NO under strong oxidation have actions similar to those of azides. These are the striking promoters for germination of some kinds of seeds as found in this work. N-Diethylhydroxylamine is neither oxidized readily to NO, nor does it chelate avidly with the iron in catalase, as shown by the high concentrations necessary for inhibition of catalase action. Higher concentrations are required than for N-methylhydroxyamine and hydroxylamine for equivalent enhancement of germination (Fig. 4). Nitric oxide gas, at 1 mm if fully absorbed as such, completely inhibited catalase, as also did XNO$_3$ (data not presented). Peroxidase, in contrast to catalase, probably does not oxidize the several compounds to nitric oxide. We find that horseradish peroxidase is less inhibited by KN$_3$ and NH$_2$OH·HCl than is catalase.

Action of nitrate on seed germination thus is ascribed to inhibition of catalase either by nitrite or hydroxylamine formed upon reduction, or by nitric oxide. The central question accordingly is: How does inhibition of catalase serve to promote seed germination? A suggested answer is that inhibition of catalase-spares some H$_2$O$_2$ for peroxidase action. We now consider a probable way in which peroxidase action might promote seed germination.

Roberts (24–26) and his associates have accumulated evidence for control of barley and rice seed germination by a shift from the Embden-Meyerhof-Parnas pathway of glucose utilization to the pentose phosphate pathway. Breaking of dormancy is associated with decrease in the C6/C1 ratio of CO$_2$ evolved from exogenous D-glucose, indicative of the change in metabolic pathway. The pentose phosphate pathway utilizes NADPH as an oxidant, which can be limited by the rate of reoxidation of NADPH. We suggest that nitrate and the several other compounds considered here could have a regulatory function in this reoxidation.

Pyridine nucleotide quinone reductase, which has been concentrated from imbibed nongerminating pea seeds (37), might be the coupling enzyme for NADPH oxidation. We find it in extracts of A. albus and L. sativa seeds at the same order of activity as found in extracts of pea seeds. It has a somewhat lower Km (0.8 $\mu$M) for NADPH than for NADH (2.1 $\mu$M) and is not particularly specific for the quinone substrate (37). Catalase, peroxidase, and pyridine nucleotide quinone reductase are present before imbibition in both A. albus and L. sativa seeds, as well as in seeds of L. sativa held at 30 C (Table IV), which prevents germination. The values of Km for the several catalyzed reactions are low (on the order of $\mu$M), and the values of k$_m$ and k$_c$ in the different steps are probably adequate to maintain reasonable reaction rates with endogenous substrates.

An NADPH-oxidizing enzyme system was observed in water extracts of wheat germ by Conn et al. (3) in 1952. They established that peroxidase was involved and that the oxidation was inhibited by catalase, apparently through H$_2$O$_2$ destruction. They realized that the oxidizing system involved enzymes other than peroxidase and catalase.

Production of H$_2$O$_2$ in the germinating seeds probably involves a flavin-sensitized nonrespiratory chain reduction of oxygen. Two sources to be considered for H$_2$O$_2$ generation are in actions of enzymes of peroxisomes and glyoxysomes. Glycolate oxidase action would be likely if peroxisomes were involved, but these are predominantly associated with chlorophyllous tissue and develop chiefly upon greening of seedlings (33). Action of the glyoxysomal enzymes is the most likely source of the H$_2$O$_2$. The early utilization of fat in germination of seeds having high oil contents, with conversion of fat to carbohydrate, was noted by Murlin in 1933 (22). Gröhne in 1952 (9) reported formation of starch from fat in Digitalis purpurea L. seeds within 3 hr of initiation of germination by light. The discovery of the glyoxysomes (2) and elaboration of knowledge about enzymes of the glyoxylate cycle, as well as knowledge of fat utilization in seeds (13), established the basis for understanding the fat to carbohydrate conversion. It is likely that fat degradation is an early step in the germination of many kinds of seeds, other than those having high fat contents.

LITERATURE CITED