

the hypothesis that mucopolysaccharide synthesis is the primary chemical site of attack by lathyrogenic agents.

*Zusammenfassung.* Alle untersuchten Bakterien waren gegenüber BAPN entweder ganz unempfindlich oder zeigten nur eine unspezifische Wachstumsverminderung. Diese Bakterien scheinen zum Studium lathyrogenischer Substanzen ungeeignet. Die Annahme, dass BAPN auf

Mucopolysacchariden wirkt, konnte durch diese Untersuchung nicht gestützt werden.

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**Inhibition of Germination of *Aspergillus niger* Conidia by  $\beta$ -Aminopropionitrile and its Reversal by Certain Aldoses<sup>1</sup>**

LEVENE<sup>2</sup> has presented evidence supporting the hypothesis that lathyrogens such as  $\beta$ -aminopropionitrile (BAPN) block carbonyl groups normally present in collagen. One of his findings is that DL-glyceraldehyde can reverse the increased neutral saline extractibility of collagen found in lathyrus chick embryo bones. Since BAPN has been found by us to inhibit germination of *A. niger* conidia<sup>3</sup>, we felt it to be of interest to determine whether this effect might not also be reversed by DL-glyceraldehyde.

Spores from slants of *A. niger*<sup>4</sup> grown for 2 to 3 days<sup>5</sup> were inoculated onto slides<sup>6</sup> in a medium supplemented

with BAPN, DL-glyceraldehyde, and other sugars as desired. They were allowed to germinate overnight at 26°C and were then counted.

The results shown in the Table seem to indicate that the BAPN inhibition of conidia germination can indeed be overcome by DL-glyceraldehyde as well as by D-galactose. On the other hand, sucrose, which by itself stimulates spore germination, appears to potentiate the inhibition. This evidence, along with the fact that media containing DL-glyceraldehyde and BAPN together became an intense orange-amber color, agrees with the assumption that BAPN acts as a carbonyl reagent.

YEAGER and SEVERSON<sup>7</sup> have indicated that BAPN, although lathyrogenic in the tadpole notochord tumor test, shows little blocking action for the histochemical aldehyde-Schiff reaction. However, their pH's were not controlled. Since alkaline conditions seemed to be required to demonstrate a reaction between *p*-nitrobenzaldehyde and BAPN<sup>8</sup>, and since clear-cut inhibition of conidia germination occurred at pH's around 7.7, but not at neutrality<sup>8</sup>, the different results in BAPN-carbonyl reactivity observed by different workers may be reconcilable.

Effect of BAPN and aldoses on germination of *A. niger* conidia<sup>a</sup>

| BAPN <sup>b</sup> | Aldose <sup>c</sup> | % Germination            |                   |                    |
|-------------------|---------------------|--------------------------|-------------------|--------------------|
|                   |                     | Fresh media <sup>d</sup> | Media, 1 week old | Media, 2 weeks old |
| 0                 | 0                   | 49.6                     | 65.8              | 61.6               |
| 0                 | 0                   | 51.2                     | 65.1              | —                  |
| 0                 | D-galactose         | 84.2                     | 94.0              | —                  |
| 0                 | DL-glyceraldehyde   | 50.0                     | 78.7              | —                  |
| +                 | 0                   | <2.1                     | 24.4              | 29.0               |
| +                 | 0                   | 0.5                      | 20.6              | —                  |
| +                 | D-galactose         | 50.1                     | 80.0              | —                  |
| +                 | DL-glyceraldehyde   | 49.7                     | 66.6              | —                  |
| 0                 | sucrose             | 18.9 <sup>e</sup>        | 92.7              | 91.0               |
| +                 | sucrose             | 0 <sup>e</sup>           | 0                 | 0                  |

<sup>a</sup> The basal medium contained 0.01% sucrose, 0.5% agar (Difco 0140-01), and 0.025 M potassium phosphate buffer, pH 7.6. It was not sterilized but was used fresh or refrigerated at 0° to 4°C until used. Bacterial growth was not a problem at the germination temperature (26°C).

<sup>b</sup> The free base, BAPN, was obtained from California Corporation for Biochemical Research, Los Angeles. 0 signifies no BAPN present; + signifies a BAPN concentration of 0.05 M.

<sup>c</sup> D-Galactose and DL-glyceraldehyde were obtained from Nutritional Biochemicals Corp. The sucrose was Fisher Co.'s certified reagent grade. 0 signifies no aldose present; where aldoses are indicated, they were present at 0.05 M concentrations.

<sup>d</sup> Since germination percentages varied from spore batch to spore batch in spite of attempts to control lighting, temperatures, etc. during mold growth and conidia germination, controls *always* were run along with test compounds. BAPN seemed to deteriorate slowly in alkaline solutions as indicated by a decreased inhibitory effect. Therefore results on aged media were considered valid only if in basic agreement with results on fresh media.

<sup>e</sup> Temperature during growth and germination reached 29–30°C.

*Zusammenfassung.* Die Keimung der Conidien von *Aspergillus niger* wurde durch  $\beta$ -Aminopropionitril (BAPN) gehemmt. Anscheinend wird die Hemmung durch DL-Glycerinaldehyd oder durch Galactose aufgehoben, während Saccharose sie verstärkt. Diese Resultate stimmen mit der Annahme überein, dass BAPN mit Carbonylgruppen reagiert.

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<sup>2</sup> C. I. LEVENE, J. exp. Med. 116, 119 (1962).

<sup>3</sup> T. NORTON and W. DASLER, Fed. Proc. 22, 611 (1963).

<sup>4</sup> Strain AN-6275 from American Type Culture Collection.

<sup>5</sup> The mold was grown at 26°C on Neurospora Culture Agar, Difco 0321-15, pH 6.7, made up to 2/3 strength.

<sup>6</sup> The thoroughly washed and rinsed slides were soaked in 0.6 N HCl for several days and rinsed three times with deionized water before use.

<sup>7</sup> V. L. YEAGER and A. R. SEVERSON, Proc. Soc. exp. Biol. Med. 108, 572 (1961).

<sup>8</sup> T. NORTON, unpublished observations.