The Effect of β-Aminopropionitrile (BAPN) on Bacteria[1]

When osteolathyrogenic agents such as BAPN are administered to rats, they appear to affect specifically the connective tissues (skeleton, blood vessels, skin). It is now well established that soluble collagen fractions are markedly affected[2]. However, changes in mucopolysaccharide components have also been repeatedly observed[3]. It is not certain whether these changes are primary or secondary in nature. It was felt that microorganisms might serve as useful tools for the study of the effects of lathyrogenic agents on polysaccharides, if BAPN susceptible strains could be found.

A selection of bacteria, including known mucopolysaccharide producers, were obtained from our Microbiology Department and from the American Type Culture Collection (Table). These were maintained on media appropriate to insure growth, and 24 h cultures were used to inoculate BAPN test media. Optical densities at 660 mμ were read over a 20 h period on a Coleman Universal Spectrophotometer Model 14, the 20 h increase being found satisfactory as a criterion of growth. The BAPN was added sometimes before and at other times after autoclaving the basal media. Results are shown in the Table.

The data seem to indicate that these bacteria show little promise as tools for the study of lathyrogenic agents. At concentrations ranging from 0.0001% to 10% BAPN fumarate, no effects except growth inhibition at the 1% or 10% levels were observed, with the single exception of *E. coli* which was stimulated at about the 1% level in the richer, more alkaline media. Since monopotassium fumarate showed a similar effect, this finding was not pursued further.

It is possible that motile rods such as *Bacillus subtilis* would be more satisfactory for this type of study. They are reported to be lysed by cystamine (another known lathyrogen[4]) and related compounds[5] and have been shown to use nitriles as nitrogen sources[6]. However, since an unidentified rod-shaped motile bacterium isolated by us in the course of this work seemed to use propionitrile as well as BAPN for growth, we did not consider this approach promising.

Since the cell walls of both gram-positive and gram-negative bacteria are reported to contain an insoluble ground substance consisting of hexosamine polymers derived in part from N-acetylglucosamine and galactosamine[7], and since the hyaluronic acid of *Streptococci* is identical to mammalian hyaluronic acid[8], the lack of a BAPN effect in the above experiments does not support

20 h optical density readings at 660 mμ for bacteria growing in various media supplemented with BAPN fumarate

Bacteria[a]	% BAPN fumarate						
	10	1	0.1	0.01	0.001	0.0001	0
β-Hemolytic Streptococcus[b]	0.02	0.16	0.17	0.17	0.18	0.18	0.17
	0.02	0.22	0.20	0.20	0.19	0.18	0.17
Staphylococcus aureus[b]	0.28	0.28	0.27	0.27	0.27	0.29	0.35
	0.15	0.52	0.53	0.46	0.45	0.46	0.47
Staphylococcus aureus[c]	0.19	0.36	0.45	0.46	0.44	0.30	0.46
	0.18	0.39	0.46	0.43	0.44	0.45	0.45
Staphylococcus aureus[d]	0.39	1.24	1.14	1.10	1.10	1.05	1.05
	0.18	1.07	1.05	0.98	0.82	1.05	1.08
Non-hemolytic Streptococcus[b]	0.04	0.06	0.09	0.09	0.10	0.11	0.10
	0.07	0.29	0.35	0.24	0.23	0.22	0.22
Streptococcus fecalis[b]	0.03	0.25	0.26	0.21	0.22	0.22	0.22
	0.05	0.28	0.32	0.25	0.25	0.25	0.26
Diplococcus pneumoniae No. 6303 (Pneumococcus, type 3)[e]	0.01	0.01	0.09	0.09	0.13	0.12	0.12
	0.003	0.01	0.09	0.15	0.17	0.13	0.12
Diplococcus pneumoniae No. 6305 (Pneumococcus, type 5)[e]	0.01	0.02	0.16	0.13	0.11	0.11	0.13
	0.02	0.01	0.12	0.11	0.10	0.12	0.12
Escherichia coli[b]	0.65	1.18	0.77	0.76	0.77	0.84	0.98
	0.65	1.18	0.73	0.70	0.68	0.75	0.87
Escherichia coli[e]	0.23	1.33	0.88	0.85	0.93	0.84	0.89
	0.17	1.40	0.95	0.89	0.94	0.96	0.95
Escherichia coli[c]	0.43	0.93	1.00	1.06	0.95	1.07	1.08
Escherichia coli[d]	0.05	2.0+	1.30	1.33	1.40	1.45	1.40
	0.05	2.0+	1.38	1.33	1.26	1.37	1.40
Escherichia coli[f]	–	0	1.15	0.60	0.47	0.52	0.59
	–	0	0.69	0.55	0.53	0.53	0.54
Escherichia coli[g]	0.03	0.48	0.65	0.56	0.55	0.55	0.60
	0.03	0.44	0.66	0.57	0.54	0.53	0.56

[a] All bacteria were obtained from the Department of Microbiology, The Chicago Medical School, except *D. pneumoniae* types 3 and 5, which were obtained from the American Type Culture Collection.
[b] Heart Infusion Broth, Difco 0038-01, pH 7.4, autoclaved with BAPN fumarate.
[c] Nutrient Broth, Difco 0003-02, pH 6.8, autoclaved; BAPN fumarate Seitz-filtered and added aseptically.
[d] Todd Hewitt Broth, Difco 0492-02, pH 7.8, autoclaved, BAPN fumarate Seitz-filtered and added aseptically.
[e] As in [b], but BAPN fumarate Seitz-filtered and added aseptically.
[f] As in [e], but with potassium hydrogen fumarate replacing BAPN fumarate.
[g] As in [b], but with ammonium chloride replacing BAPN fumarate.

[1] This work was supported by Research Grant A-4002 from the National Institute of Arthritis and Metabolic Diseases, U.S. Public Health Service. We are also grateful to Abbott Laboratories, North Chicago (Illinois) for generous gifts of mono-β-aminopropionitrile (BAPN) fumarate.

[2] See for example: J. GROSS and C. I. LEVENE, J. exp. Med. *110*, 771 (1959). – L. MIKKONEN, T. TUOMINEN, and E. KULONEN, Biochem. Pharmacol. *3*, 181 (1960). – W. DASLER, R. E. STONER, and R. V. MILLISER, Metabolism *10*, 883 (1961). – D. J. SMITH and R. C. SHUSTER, Arch. Biochem. Biophys. *98*, 498 (1962).

[3] See for example: K. PYORALA, S. PUNSAR, T. SEPPALA, and K. KARLSSON, Acta path. microbiol. scand. *41*, 497 (1957). – C. J. SCHWARTZ, Brit. J. exp. Path. *40*, 44 (1959). – A. A. CASTELLANI, C. CASTELLANI-BISI, and E. R. FRIGERIO, Arch. Biochem. Biophys. *80*, 57 (1959). – M. J. KARNOVSKY and M. L. KARNOVSKY, J. exp. Med. *113*, 381 (1961).

[4] W. DASLER and R. V. MILLISER, Proc. Soc. exp. Biol. Med. *98*, 759 (1958).

[5] E. D. WEINBERG, Exp. Cell Res. *13*, 175 (1957). – E. D. WEINBERG, A. K. SAZ, and E. Y. PILGREN, J. gen. Microbiol. *19*, 419 (1958).

[6] G. S. TRELAWNY, V. SCHATZ, K. BARTH, and A. SCHATZ, Proc. Penn. Acad. Sci. *30*, 44 (1956).

[7] S. M. SIEGEL, *The Plant Cell Wall* (Pergamon Press Ltd., New York 1962), p. 79.

[8] A. DORFMAN and J. A. CIFONELLI, in Ciba Foundation Symposium, *Chemistry and Biology of Mucopolysaccharides* (Ed. by G. E. W. WOLSTENHOLME and M. O'CONNER, Little, Brown and Co., Boston, Mass. 1958), p. 64, 81.

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.

T. B. NORTON,
S. F. HORWITZ, and W. DASLER

Department of Biochemistry, The Chicago Medical School, Chicago (Illinois, U.S.A.), July 23, 1963.

Inhibition of Germination of *Aspergillus niger* Conidia by β-Aminopropionitrile and its Reversal by Certain Aldoses[1]

LEVENE[2] has presented evidence supporting the hypothesis that lathyrogens such as β-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 lathyritic chick embryo bones. Since BAPN has been found by us to inhibit germination of *A. niger* conidia[3], 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*[4] grown for 2 to 3 days[5] were inoculated onto slides[6] 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[7] 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[8], and since clear-cut inhibition of conidia germination occurred at pH's around 7.7, but not at neutrality[8], 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[a]

BAPN[b]	Aldose[c]	% Germination		
		Fresh media[d]	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[e]	92.7	91.0
+	sucrose	0[e]	0	0

[a] 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).

[b] 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*.

[c] 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.

[d] 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.

[e] Temperature during growth and germination reached 29–30°C.

Zusammenfassung. Die Keimung der Conidien von *Aspergillus niger* wurde durch β-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.

T. B. NORTON and W. DASLER

Department of Biochemistry, The Chicago Medical School, Chicago (Illinois, U.S.A.), September 13, 1963.

[1] This investigation was supported by Research Grants A-4002 and AM-01427 from the National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, U.S. Public Health Service.

[2] C. I. LEVENE, J. exp. Med. *116*, 119 (1962).

[3] T. NORTON and W. DASLER, Fed. Proc. *22*, 611 (1963).

[4] Strain AN-6275 from American Type Culture Collection.

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

[6] 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.

[7] V. L. YEAGER and A. R. SEVERSON, Proc. Soc. exp. Biol. Med. *108*, 572 (1961).

[8] T. NORTON, unpublished observations.