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Growth Stimulation of Neurospora Cholineless Mutant by Dimethylaminoethanol.

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It was recently noted¹ that dimethylaminoethanol had a choline-like effect in promoting growth and preventing perosis in chicks, and it was thought that this might be due to the addition of a "labile" methyl group to dimethylaminoethanol to form choline. It has been observed that the chick,² in contrast to the rat, is unable to use methionine or betaine

as a substitute for choline when fed with purified diets. The "cholineless" mutant of *Neurospora crassa* No. 34486 appears to resemble the chick rather than the rat, for this mutant is unable to use betaine as a substitute for choline, and methionine has very little effect.³ It was thought that a combination of betaine or methionine with dimethylamino-

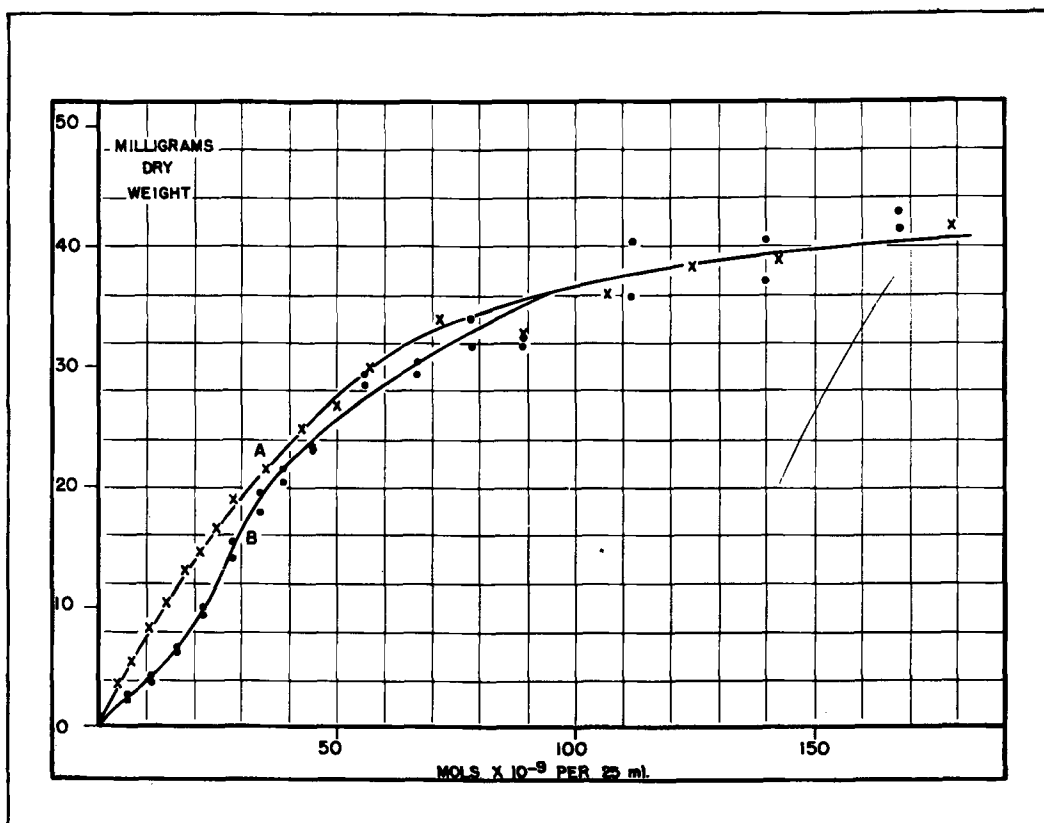


FIG. 1.

Growth of cholineless mutant No. 34486 on choline chloride (curve A) and dimethylaminoethanol (curve B) after 3 days. Choline chloride = X; dimethylaminoethanol = ●.

¹ Jukes, T. H., and Oleson, J. J., *J. Biol. Chem.*, 1945, **157**, 419.

² Jukes, T. H., *J. Nutrition*, 1941, **22**, 315; Almquist, W. J., and Grau, C. R., *Ibid.*, 1944, **27**, 263;

McGinnis, J., Norris, L. C., and Heuser, G. F., *Proc. Soc. Exp. Biol. and Med.*, 1944, **56**, 197.

³ Horowitz, N. H., and Beadle, C. W., *J. Biol. Chem.*, 1943, **150**, 325.

ethanol might stimulate the growth of the mutant.

The assay with the "cholineless" mutant* was carried out by the method of Horowitz and Beadle.³ Aminoethanol and betaine were inactive and methionine had a very slight activity, which confirms a previous report.³ It was found that dimethylaminoethanol alone or in combination with betaine or methionine had a marked effect in stimulating growth. A comparative assay showed that dimethylaminoethanol alone was approximately 1.5 times as active as choline chloride. When calculated to a molar basis this result indicates that the two compounds show about the same activity in the upper part of the response curve, but in the lower portion of the curve the response to choline was greater than the response to dimethylaminoethanol. This is illustrated in Fig. 1. Diethylaminoethanol was found to have less than 0.1% of the activity of dimethylaminoethanol.

A solution of dimethylaminoethanol, 0.5

* Cultures of the mutant were kindly supplied by Dr. N. H. Horowitz and by Dr. Earle Arnow.

mg per cc, was submitted to permutit adsorption and elution³ and the eluate and filtrate were assayed with the mutant. The biological activity was found to pass quantitatively into the eluate. The filtrate was inactive.

A solution of choline chloride was submitted to reineckate precipitation.⁴ The reineckate precipitate was measured colorimetrically and the filtrate was assayed with the mutant. When dimethylaminoethanol was added to the choline chloride solution, the dimethylaminoethanol appeared to be partially precipitated, as indicated by the increased colorimetric value of the precipitate and the increased assay value of the filtrate. Dimethylaminoethanol in pure solution, however, was not precipitated with reineckate under the conditions used.

The results with the mutant might indicate that it is able to synthesize a "methyl donor," such as, perhaps, methionine, but is defective in being unable to synthesize the postulated "methyl acceptor," dimethylaminoethanol, in the formation of choline.

⁴ Glick, D., *J. Biol. Chem.*, 1944, **156**, 643.

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A Method for Production of a Localized Gonococcal Infection in the Rabbit's Eye.*

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Investigation of certain problems related to gonococcal infection have been neglected because of lack of a satisfactory method of producing a localized infection experimentally in some laboratory animal. Among the many

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¹ For a comprehensive bibliography see the excellent review of the literature by Justina Hill, *Am. J. Syph., Gon. and Ven. Dis.*, 1944, **28**, 334 and 471.

attempts reported in the literature¹ only that by Cohn² warrants mention here. He injected gonococci into the anterior chamber of rabbits' eyes and occasionally succeeded in recovering them from that site several days thereafter and on 2 occasions 28 days after inoculation with 2 particularly virulent strains. He, nevertheless, concluded that the longevity of the microorganisms was "variable and irregular" and that the anterior chamber of the rabbit was unsuitable for his purposes.

The method described below was developed

² Cohn, Alfred, *Derm. Z.*, 1930, **60**, 35.