

undecic 4.6, lauric (dodecic) 4.4, myristic (tetradecic) 4.1, and palmitic (hexadecic) acid 3.9 mm/h.

*Experimental: Separation of the acids from acetic to pelargonic acid.* The paper was used as  $10 \times 50$  cm strips, each for the investigation of three samples. The distance between electrode vessels was 40 cm from liquid to liquid. The acids were placed on the paper as free acids in ethanol as close to the buffer solution in the cathode vessel as possible. In order to avoid the volatilization of the acids they were neutralized on the paper with ammonia vapour. After the solvent had evaporated the whole paper was sprayed with buffer solution the excess of which was removed by two dry filter papers. The paper was then immediately placed between the electrode vessels and kept there horizontally by means of glass rods fastened to the ends of the paper. The whole apparatus was surrounded by saturated water vapour. After ionophoresis the paper was dried. The acids were located with methyl red. The acids from valeric to pelargonic acid can also be located <sup>3</sup> with a solution of rhodamin B.

*Separation of the acids from caproic to palmitic acid.* The paper strips were of the same size as above, but in some cases it was advantageous to use shorter strips than 50 cm in order to speed up the analysis without increasing the electric potential. The paper was immersed in 0.2 N glycerolic sodium hydroxide. The excess of glycerol was removed with the edge of a glass plate. The ethanol solutions of the acids were placed on the paper and the paper laid horizontally between the electrode vessels. After the paper had reached the temperature of the chamber (90°C) the current was put on. The acids were located by spraying the paper first with 2% aqueous copper acetate, removing the glycerol after 10 min. with water, drying the paper, and spraying it finally with a 0.5% solution of rhodamin B in water or in 0.05 N hydrochloric acid. On the dried paper the acids were located conveniently in ultraviolet light.

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## The Effect of Nitrous Oxide on Nitrate Utilization by *Azotobacter vinelandii*

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Nitrous oxide competitively inhibits nitrogen fixation by *Azotobacter vinelandii* but exerts no effect on ammonia utilization <sup>1-3</sup>. Virtanen and Lundbom <sup>4</sup> have found in their long time growth experiments that  $N_2O$  inhibits not only the fixation process but also nitrate reduction by this organism. Because of the importance of this finding for the understanding of the mechanism of biological nitrogen fixation, the effect of  $N_2O$  on nitrate utilization by *A. vinelandii* was reinvestigated in short time respiration experiments.

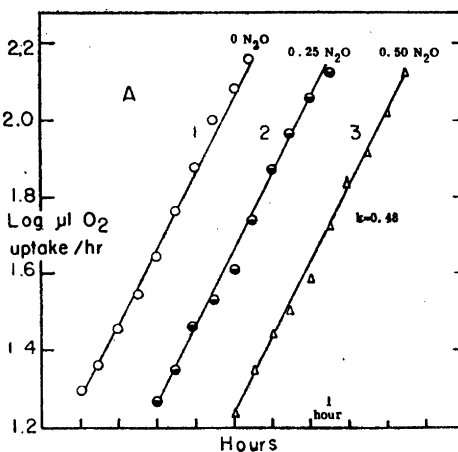


Fig. 1. Lack of inhibition by  $N_2O$  of growth of *A. vinelandii* (strain O) supplied nitrate. Growth was measured by following manometrically the increasing rate of oxygen uptake by the culture.

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Initial trials to demonstrate  $N_2O$  inhibition of nitrate assimilation were performed with *A. vinelandii* (Wisconsin strain O) which had been adapted by growth on nitrate; the growth of aliquots of the diluted culture was followed by measuring their increasing rates of respiration in Warburg respirometers. The respiration of cells incubated in Burk's medium containing 5 ppm  $NO_3^-N$  at  $30^\circ$  under 0.2 atm.  $O_2$ , and 0.0, 0.25 or 0.5 atm.  $N_2O$  (the diluent gas was He) is indicated in Fig. 1. The monomolecular rate constant for nitrogen assimilation,  $k$ , was about 0.48 for all the cultures; this is equivalent to a generation time of 1.6 hours. The effect of an inhibitor on such a vigorous culture should be detected readily; however, there was no significant inhibition of nitrate utilization even with 0.5 atm.  $N_2O$ .

Further experiments were conducted to test whether  $N_2O$  would affect the incorporation of  $^{15}N$  from  $^{15}NO_3^-$  into cells of *A. vinelandii*. Cells adapted to nitrate were supplied  $^{15}NO_3^-$  (200 ppm N) and an atmosphere of 0.2  $O_2$  and either 0.8  $N_2O$  or He. After the  $^{15}NO_3^-$  had equilibrated with the normal nitrate of the medium, the isotope concentration in the nitrate was 5.11 atom per cent  $^{15}N$  excess. The effect of  $N_2O$  on  $^{15}N_2$  fixation also was examined to serve as a control and to confirm by an independent method the previously reported inhibition<sup>1,2</sup>. Cells which had been fixing  $N_2$  were provided 0.1 atm.  $^{15}N_2$  (6.95 atom per cent  $^{15}N$  excess), 0.2

Table 1. Uptake of  $^{15}N$  by *A. vinelandii* (strain O) from labeled  $N_2$  and nitrate as influenced by nitrous oxide.

Incubation time, hours	Atom per cent $^{15}N$ excess in cells			
	$^{15}NO_3^-$ supplied		$^{15}N_2$ supplied	
	0.8 $N_2O$	0.8 He	0.7 $N_2O$	0.7 He
1			0.074 0.068	0.442 0.597
2	0.655 0.494	0.591 0.760		
3			0.287 0.202	1.463 1.653
4	1.565 1.827	1.867 1.230		
5			0.475 0.471	2.073

atm.  $O_2$ , and either 0.7 atm.  $N_2O$  or He. At intervals after initiation of incubation at  $30^\circ$ , the cells were sampled for  $^{15}N$  analysis. The data in Table 1 show no inhibition of nitrate utilization by  $N_2O$  beyond the error of measurement, whereas over 80 % inhibition of  $N_2$  fixation by  $N_2O$  is indicated.

Table 2. The effect of nitrous oxide on nitrogen assimilation by *A. vinelandii* (Jensen culture K).

Incubation time, hours	Mg N in cells		Atom per cent $^{15}N$ excess in cells			
	$N_2$ supplied		$^{15}NH_4^+$ supplied		$^{15}NO_3^-$ supplied	
	0.6 $N_2O$	0.6 vac.	0.6 $N_2O$	0.6 vac.	0.6 $N_2O$	0.6 vac.
3	0.124	0.185	1.39	0.816	0.131	0.113
	0.128	0.151	0.871	0.841	0.144	0.121
8	0.197	0.182	1.29	1.22	0.150	0.243
	0.197	0.144	1.28	1.85	0.183	0.483
19	0.239	0.422	2.39	2.78	0.433	0.391
	0.207	0.290	2.87	2.64	0.417	0.468
24	0.233	0.533	3.73	3.70	0.858	0.877
		0.412	4.08	3.94	1.01	0.842

Confirmation of these results was provided by experiments conducted in Helsinki with Jensen's culture K of *A. vinelandii*, the same strain of bacteria employed in the original study by Virtanen and Lundbom<sup>4</sup>. Cultures were supplied nitrogen either as 0.2 atm. normal N<sub>2</sub>, 50 ppm ammonium nitrogen (7 atom per cent <sup>15</sup>N excess), or 500 ppm nitrate nitrogen (7 atom per cent <sup>15</sup>N excess). The test flasks were incubated in desiccators at 30° under 0.2 atm. N<sub>2</sub>, 0.2 atm. O<sub>2</sub>, and either 0.6 atm. N<sub>2</sub>O or 0.6 atm. vacuum. The cells were harvested at 3, 8, 19, and 24 hours after inoculation. Total cellular nitrogen was determined, and the cells exposed to <sup>15</sup>NH<sub>4</sub><sup>+</sup> or <sup>15</sup>NO<sub>3</sub><sup>-</sup> also were analyzed for <sup>15</sup>N. The results of these experiments are summarized in Table 2; the results for total nitrogen on cultures furnished <sup>15</sup>NH<sub>4</sub><sup>+</sup> and <sup>15</sup>NO<sub>3</sub><sup>-</sup> are not recorded in the table, but they gave a picture entirely comparable to the <sup>15</sup>N analyses listed. These data further substantiate the specificity of N<sub>2</sub>O inhibition for nitrogen fixation. It is clear that *A. vinelandii* using combined nitrogen either as ammonia or nitrate, is not inhibited by nitrous oxide.

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**Studies on the Chemistry of Lichens. I. D-Arabitol from *Alectoria jubata* Ach., var. *chalybeiformis* Th.Fr.**

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In the course of a series of investigations on lichen substances in the genus *Alectoria*, the *Alectoria jubata* var. *chalybeiformis* has been studied.

Only one lichen substance, D-arabitol, was obtained by extraction of the lichen with acetone. It crystallized very slowly from saturated solutions. Occasionally the substance precipitated as an oily product from acetone or alcohol and solidified to a crystalline mass on standing or by scratching.

An authentic sample of D-arabitol was not available. The specific rotation from saturated, aqueous boric acid solution was +7.66°, in fair agreement with the value of +7.82° reported by Asahina<sup>1</sup>.

X-ray diffraction patterns of the substance and of L-arabitol were identical.

*Experimental.* Air-dried, ground *Alectoria jubata* var. *chalybeiformis* (208 g) (collected by cand. real. Eilif Dahl, on birch in Rondane, Norway) was continuously extracted with acetone for twenty hours.

The greater part of the solvent was removed by distillation, and on standing in a refrigerator the solution deposited colourless crystals (A). These were separated, washed with ether and dried. The dark green filtrate was concentrated to a small volume and the viscous residue repeatedly treated with cold alcohol. Undissolved material was removed by filtration (B). A and B both appeared to be the same substance and were purified together by recrystallization from acetone. After having been kept at -22° for some ten days 0.32 g of

Table 1. Spacing values in Å. (*w* = weak, *m* = medium, *s* = strong, *vs* = very strong.)

Substance from <i>Alectoria jubata</i> var. <i>chalybeiformis</i>	L-arabitol
7.37 s	7.37 s
5.48 »	5.48 »
4.83 vs	4.83 vs
4.63 »	4.60 »
4.33 »	4.33 »
3.96 w	3.95 w
3.60 vs	3.61 vs
3.42 s	3.42 s
3.23 »	3.23 »
3.12 »	3.11 »
2.83 m	2.83 m
2.52 s	2.52 s
2.41 w	2.42 w
2.37 »	2.38 »
2.15 s	2.16 s
2.08 »	2.09 »
2.03 w	2.03 w