TABLEAU I

DOSAGE DE B_{12} COMBINÉ PAR LA MÉTHODE DIFFÉRENTIELLE [(b), (c), (b)–(c)] ET PAR LA MÉTHODE DIRECTE APRÈS FIXATION DE B_{12} LIBRE SUR *Lactobacillus leichmannii* [(d), (e)]

Échantillon	B_{12} *Total* γ/g	B_{12} *libre* γ/g	B_{12} *combiné calculé* γ/g	B_{12} *libre après traitement* % *de* B_{12} *libre*	B_{12} *combiné trouvé* γ/g	*Différence* %
(a)	*(b)*	*(c)*	*(b)–(c)*	*(d)*	*(e)*	*(f)*
31	256	50.4	205.6	0	201	— 2.2
25	339	96	243	2	266	+ 9.5
18	347	15	332	3	336	+ 1.2
15a	281	107	174	0	158	—10
369	359	3	356	2	366	+ 2.8
37	313	14.5	298.5	2	274	—12.1
38	367	9	358	1	346	— 2.9
39	352	0	352	3	352	0
40	198	0	198	0	187	— 1.8

Ces résultats permettent de conclure que le dosage de la vitamine B_{12} combinée peut être effectué dans un mélange où sont présentes les deux formes, libre et combinée de cette vitamine, après élimination de la forme libre par un traitement avec *Lactobacillus leichmannii*.

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THE INCORPORATION OF ^{15}N-LABELLED NITROUS OXIDE BY NITROGEN FIXING AGENTS*

by

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While studying the effects of various gases on the nitrogen fixing system of *Azotobacter vinelandii*, MOLNAR *et al.*[1] found N_2O to be a specific inhibitor of nitrogen fixation. Independent studies by REPASKE AND WILSON[2], and by WILSON AND ROBERTS[3] indicated that this inhibition is competitive. With manometric tests the latter investigators[4] could demonstrate no assimilation of N_2O by the *Azotobacter*, nor could they show such assimilation by supplying N_2O and testing for isotopic dilution in *Azotobacter* cells which had previously been grown on $^{15}N_2$. The most sensitive way to detect incorporation of N_2O by a nitrogen fixing organism is to supply the organism with ^{15}N labelled N_2O and then to test it for its ^{15}N excess. We wish to report here the results of such tests.

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Labelled N_2O containing 21.3 atom % ^{15}N was prepared by the heat controlled decomposition of $^{15}NH_4NO_3$ as described by FRIEDMAN AND BIGELEISEN[5]. This gas was supplied, at 0.1 atm. with 0.2 atm.O_2 and 0.7 atm.He, to a vigorously growing culture of *Azotobacter vinelandii* in Warburg vessels at 30° C. Following the desired time of exposure, the cells were recovered by centrifugation and analyzed for their ^{15}N content. The data from one such experiment are shown in Table I. A final level of 0.2 atom % ^{15}N excess represents an increase in cellular nitrogen of approximately one per cent. Cultures of this aerobic organism incorporated no ^{15}N from $^{15}N_2O$ under anaerobic conditions. The rate of oxygen uptake remained constant throughout the experiment indicating that there was no appreciable proliferation of cells. This steady rate of oxygen absorption was significantly below the level at which the rate of diffusion of gases into the medium becomes limiting.

TABLE I

ATOM % ^{15}N EXCESS IN NITROGEN FIXING AGENTS AFTER EXPOSURE TO ^{15}N LABELLED N_2O

Biological agent	*Time of exposure, minutes*					
	15	*30*	*60*	*120*	*240*	*480*
Azotobacter vinelandii	0.027	0.081	0.118	0.289	0.336	1.226
		0.078	0.145	0.303		1.192
Slices of soybean root nodules	0.054	0.130	0.137	0.134		
	0.111	0.093	0.202	0.123		
	0.057	0.042	0.204	0.170		
	0.040	0.082	0.137	0.164		

Figures are from individual determinations expressed as atom % ^{15}N excess over control cells. The N_2O supplied had 21.3 atom % ^{15}N. The mass spectrometer readily detects 0.01 atom % ^{15}N excess.

Sliced, excised root nodules of field grown Lincoln variety soybeans were exposed at 25° C to a $^{15}N_2O$ containing atmosphere of the composition described earlier, and the ^{15}N content of the acid soluble portion of the nodule slices was determined by the method of APRISON *et al.*[6]. As indicated in Table I the rate of ^{15}N incorporation from N_2O decreases with time; this is comparable to the previously reported rapid loss of nitrogen fixing capacity by excised nodules. The levels of ^{15}N in nodule slices exposed for one hour to $^{15}N_2O$ are similar to the ^{15}N levels of nodule slices from parallel experiments in which $^{15}N_2$ was supplied containing 31 atom % ^{15}N. The ^{15}N levels are very nearly the same despite the higher concentration of ^{15}N in the N_2 than in the N_2O.

Several determinations were made to ascertain whether the stored N_2O decomposed to N_2; any ^{15}N-labelled N_2 thus formed could be assimilated by a nitrogen fixing agent. Our failure to detect labelled N_2 in a sample of labelled N_2O indicated that the N_2O did not decompose to N_2. It was necessary to freeze out the N_2O in liquid air before testing for the presence of N_2 by analysis with the mass spectrometer, for N_2O is cleaved in the electron beam of the mass spectrometer to yield N_2 as one of the products.

As yet the significance of N_2O incorporation by the *Azotobacter* and the soybean nodule slices cannot be evaluated in terms of the overall mechanism of nitrogen fixation. However it is of interest to note that N_2O has been reported to be an abundant constituent of soil air[8], and ADEL[9] suggests that atmospheric N_2O is important in the nitrogen cycle.

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