vertical direction. To minimize the effect of electroosmotic flow the surface of the buffer solution on the cathodic side was held about 20 cm above that on the anodic side.

The run can be followed by mixing with serum a quantity of stain which is bound to albumin (*e. g.* bilirubin). As coloured fractions are often undesirable, however, inspection of the block in ultraviolet light is to be preferred. Here a green fluorescence shows the exact position of the advancing albumin band (and often also of β-globulin). It seems possible that also other proteins can be made visible by adding some fluorescent substance.

After the separation of the fractions has been effected (usually about 10 hours under the conditions used by us), and provided no exact data on their quantitative distribution are desired, the localization of each protein fraction can be carried out simply and rapidly by pressing the edge of a narrow strip of filter paper (*e. g.* Munktell 20) lengthwise against the still moist surface of the medium so that the liquid (containing the proteins) is absorbed into it. When this strip is then stained with bromophenol blue (for 3—4 minutes) and washed in running tap water the exact position of the fractions becomes visible just as in a paper electrophoresis strip.

The eluation of the proteins from the starch portions, each containing one major fraction, is best done with a slightly alcaline buffer which contains glycine (0.5 *M*). The yield naturally increases with the buffer/starch ratio. The eluates can be concentrated by dialyzing them against concentrated dextran solution or by lyophilization. The purity of serum protein fractions obtained by this method has been controlled by free electrophoresis with an optical device.

1. Gordon, A. H., Keil, B., and Sebesta, K. *Nature* **164** (1949) 498.
2. Svensson, H., and Brattsten, I. *Arkiv Kemi* **1** (1950) 401.
3. Grassman, W., and Hannig, K. *Naturwissenschaften* **37** (1950) 397.
4. Kunkel, H. G., and Slater, R. J. *Proc. Soc. Exp. Biol. Med.* **80** (1952) 42.

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Inhibition by Nitrous Oxide of Biological Nitrogen Fixation and the Uptake of Combined Nitrogen

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Molnar, Burris and Wilson [1], Repaske and Wilson [2] have found that N_2O inhibits N_2-fixation with *Azotobacter*, but not the uptake of combined nitrogen. N_2O thus seemed to influence nitrogen fixation specifically. These authors used only ammonium nitrogen as the source of combined nitrogen.

Virtanen [3] has suggested that in N_2-fixation may be formed as an intermediate a nitrogenoxygen compound which would be identical with an intermediate formed in nitrate reduction too. Both processes would thus join on this stage. If this suggestion is correct, N_2O may inhibit not only N_2-fixation but also the utilization of nitrate. It was therefore interesting to compare the influence of N_2O on N_2-fixation, and on the utilization of NO_3-N and NH_4-N with *Azotobacter*. Anaerobic *Clostridium butyricum* was also included in the experiments, as there is no previous information on the influence of N_2O on anaerobic nitrogen fixation.

In experiments both with *Azotobacter* and *Clostridium* the atmosphere of the test-flask contained 0, 5, 25 and 50 % N_2O. Primary atmosphere in *Azotobacter*-cultures was air, and in *Clostridium*-cultures N_2. Also *Clostridium butyricum* could be made to grow in synthetic nutrient solution by adding different vitamins to the solution.

The results of our experiments will appear from Figs. 1 and 2. From these can be seen that:

1) N_2O inhibits both N_2-fixation and utilization of nitrate in about the same concentrations.

2) N_2O does not influence the utilization of ammonium-N even in the maximal concentration used (50 % N_2O).

3) N_2O has the same influence both on aerobic *Azotobacter* and anaerobic *Clostridium*. N_2O inhibits N_2-fixation and nitrate

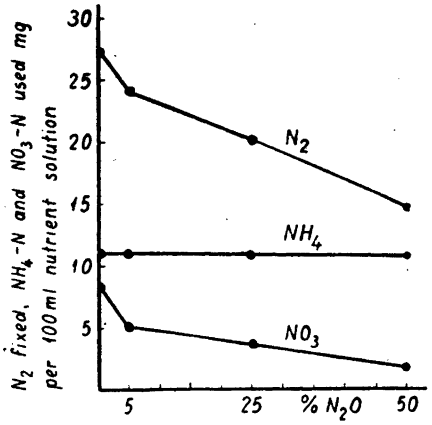

Fig. 1. Influence of N_2O *on* N_2*-fixation and on the use of* NH_4-N *and* NO_3-N *with A z o t o b a c t e r v i n e l a n d i i.*

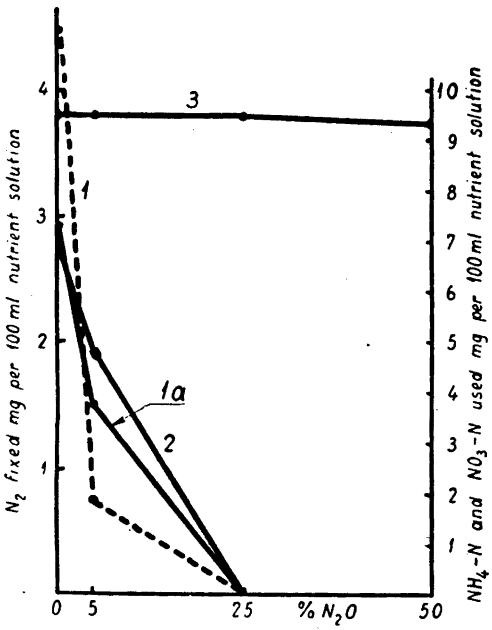

Fig. 2. Influence of N_2O *on* N_2*-fixation and on the use of* NH_4-N *and* NO_3-N *with C l o s t r i d i u m b u t y r i c u m.*
1 and 1a: N_2*-fixation, 2: use of* NO_3-N, *3: use of* NH_4-N.

uptake also with *Clostridium butyricum.* The anaerobic N_2-fixation is more sensible to N_2O than the aerobic one.

The results may be regarded as a corroboration of the conception that N_2-fixation leads to an intermediate which appears also as an intermediate in nitrate reduction. As to the mechanism of aerobic and anaerobic N_2-fixation the results may corroborate the assumption of a similar mechanism in both cases. It would, however, be an exaggeration to state that the results prove these conclusions, because it is possible that N_2O competes with N_2 for the enzyme which causes the activation of N_2, as well as for the enzyme which acts in some phase of nitrate reduction. The possible similarity of the arrangement of electrons in the nitrogen molecule, and in the intermediate of nitrate reduction would thus be the reason for the similar action of N_2O in N_2-fixation and in nitrate reduction. Concerning the mechanism of N_2-fixation, results in this case give no indications.

1. Molnar, D. M., Burris, R. H., and Wilson, P. W. *J. Am. Chem. Soc.* **70** (1948) 1713.
2. Repaske, R., and Wilson, P. W. *J. Am. Chem. Soc.* **74** (1952) 3101.
3. *cf.* Virtanen, A. I. *Ann. Acad. Sci. Fennicae,* **Ser. A. II. No. 43** (1952); *Angew. Chem.* **65** (1953) 1.

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