the three sulphur atoms may be partly or completely substituted by selenium or tellurium. Thus, the arguments apply to compounds of the trisulphide and trisele-nide type, as has been discussed by other authors¹⁴⁻¹⁶ on the basis of dipole moments in solutions, and with reference to Raman spectra in the case of hydrogen trisulphide¹⁷. The configurations of the following compounds are known in crystals.

Bis(2-iodoethyl) trisulphide^{18,19} has a *trans* form and a twofold axis of symmetry. The space group¹² is the enantiomorphous one, $D_4^4-P4_12_12$ with $Z = 4$. Selenium diselenocyanate²⁰, $\text{Se}(\text{SeCN})_2$, has the space group $D_{2h}^{16}-Pnma$ with $Z = 4$. The molecule occurs in a *cis* form, and possesses a mirror plane of symmetry²¹.

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On the Technique of Preparative Electrophoresis of Proteins in Supporting Medium (Starch)

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In efforts to adapt the principle of electrophoresis to preparative purposes, particularly in the field of protein chemistry, several methods have been suggested¹⁻³. In their practical application, however, many unexpected factors often prevent a clear-cut and reproducible separation and/or satisfactory recovery of individual fractions. Starch as a supporting medium was recently introduced by Kunkel and Slater⁴ and it seems to have many advantages compared with other media. Nevertheless this method also is rather sensitive to various influences and, when performed as presented by them, gives reproducible and otherwise ideal results only accidentally. The following reports briefly on some modifications of the technique which seem to have improved the usefulness of preparative zone electrophoresis.

The experiments were made with human blood serum using a rectangular perspex vessel packed with buffer-moistened potato starch. A point of primary importance is completely to prevent evaporation from the surface of the medium. To this end melted paraffin was poured on the starch block and allowed to solidify, forming quite a tight cover. Serum could then be applied to the starch through a narrow window cut into this cover. The contact from the ends of the block to the buffer vessels was mediated by strips of heavy filter paper.

It appeared that the apparently poor separation of fractions was often due to gravity causing an accumulation of serum in the lower starch layers when the run was made in a horizontal position, and this led to an uneven migration velocity at different levels of the block. This effect was avoided by making the run in the

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 elution of the proteins from the starch portions, each containing one major fraction, is best done with a slightly alkaline 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.

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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 Wilson¹, Repaske and Wilson² 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.

Virtanen³ 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