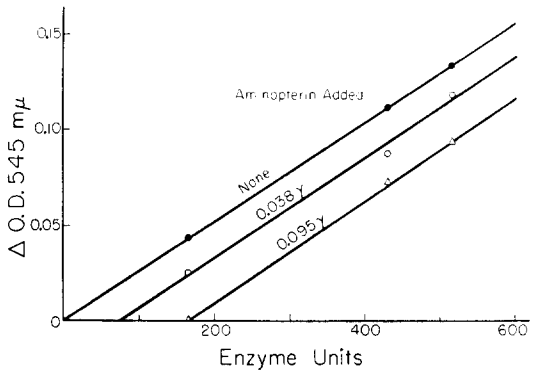

Fig. 1. Titration of folic acid reductase with aminopterin. The conditions were the same as those given in Table I except that the enzyme preparation was an aqueous extract of sheep-liver acetone powder and TPNH was generated *in situ* from 0.5 mg TPN and 10 μmoles sodium citrate. Extrapolation of the curves to zero Δ O.D. yield the number of enzyme units which would be completely inhibited by the given amount of aminopterin.

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Nitrogen fixation by nodules formed on isolated bean roots*

It has been shown[1] that nodules will form on isolated bean roots inoculated with the appropriate rhizobia if a modified technique of aseptic root culture[2] is used. Furthermore, such nodules are histologically similar to those formed on the roots of intact plants. The present paper reports work undertaken to find whether such nodules also will function in nitrogen fixation.

Isolated roots of *Phaseolus vulgaris* L., var. "Pencil Pod" black wax bean, were grown and inoculated as described earlier[1], except that three roots, instead of one, were grown per Petri dish. Each dish contained 50 g of washed silica sand moistened with 10 ml of the inorganic salts of medium "O"[1]. The vials contained 10 % sucrose, and glycine, thiamin, niacin and pyridoxine at the levels indicated for medium "O" or

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20-times higher. Various substances (nitrate, phosphate, kinetin, adenine, several vitamins) were added to the basal medium in the vials in different experiments. The effects of these additions on nodulation *per se* will be reported elsewhere. 15 to 20 days after inoculation, the roots bearing nodules were exposed to a gas mixture consisting of 0.05 atm. N_2 (containing 94.6 atom % $^{15}N_2$ excess), 0.45 atm. O_2 and 0.5 atm. He. After exposure, the nodules were detached from the roots, and both the roots and the nodules were subjected separately to Kjeldahl digestion. The ammonia was distilled from the digested sample, converted to N_2 with alkaline hypobromite, and analyzed for ^{15}N concentration with a Consolidated-Nier mass spectrometer.

As the same roots generally were used for counting the nodules formed and for exposing to $^{15}N_2$, 2 to 3 h elapsed between the removal of the roots from the dishes and exposure. Detaching the nodules consumed another 1 to 2 h between the end of the exposure to $^{15}N_2$ and Kjeldahl digestion. Furthermore, the limited amount of material available necessitated the analysis of the entire nodules rather than the acid-soluble fraction, which carries a higher percentage of ^{15}N.

In spite of these limitations, significant fixation of the isotope was detected in all samples of nodules (but not in the roots). Treatments were not replicated within experiments, since the objective pursued in this preliminary phase of the work was primarily the detection of fixation and not a comparison of the effects of different treatments. The results recorded here are means of the values obtained in various runs with the data grouped according to the length of exposure of the nodulated roots to $^{15}N_2$. Even with exposures as short as 60 min, an average of 0.035 atom % ^{15}N excess was obtained in 3 runs. With longer periods of exposure, the following averages were obtained: 3 h, 0.085 (10 runs); 18 h, 0.116 (8 runs); 20 h, 0.190 (4 runs). In one experiment the nodulated roots were exposed immediately after removal from the dishes; this gave the highest value observed, 0.303 atom % ^{15}N excess after exposure to the isotope for 24 h.

It also is interesting that when nodules formed on isolated bean roots are crushed in water at 5°, the clear reddish supernatant shows absorption maxima at about 537 and 573 mμ; these bands correspond closely to the values reported for the hemoglobin from soybean nodules[3]. A correlation between the hemoglobin content of nodules and their vigor of N_2 fixation has been reported by several investigators.

The demonstration that nodules formed on isolated bean roots are morphologically and functionally similar to those formed on intact plants may provide a basis for a new approach to some of the problems connected with symbiotic nitrogen fixation.

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