Meetings

Extending Symbiotic Nitrogen Fixation to Increase Man's Food Supply

At present the world's food supply depends heavily on crops sustained by chemical fertilizers. More direct utilization of the vast reservoir of molecular nitrogen in the atmosphere would partially circumvent the costs of chemical fertilizer production, the logistical problems of its distribution and local application, and the potential hazards of overloading groundwaters with excessive soluble inorganic nutrients. Although the possibility of establishing the leguminous type of *Rhizobium*-induced root nodules on monocots seems remote at this stage of our knowledge, optimism was expressed for increasing and exploiting the intimate protocooperative relationships which exist under some circumstances in the rhizosphere between certain free-living bacteria, either aerobic or anaerobic, blue-green algae, certain fungi, and the extensive root systems of the monocotyledonous plants.

The enzyme nitrogenase, which catalyzes the conversion of nitrogen to ammonia, has remarkably similar characteristics and properties in all biological systems where it has been studied. For activity the system requires the following components: a two-protein complex (an iron-molybdenum protein and an iron protein which together form the enzyme), a high energy phosphate source as adenosine triphosphate (ATP), Mg^{2+}, and a strong electron donor such as reduced ferredoxin. These essential components have been found in all nitrogenase systems studied to date and are presumed to be the minimum elements of an effective nitrogen-fixing system. Effective organisms probably achieve an essentially oxygen-free intracellular environment for the enzyme, either by cellular compartmentalization or by elaborate structural barriers in a multicellular nodular structure. In all known cases, prokaryotic organisms are involved in the nitrogen fixation systems. In legumes, the nitrogenase is found associated with *Rhizobium* bacteroids as they occur within membrane-bound packets within the central tissues of the root nodule.

On 5 and 6 April 1971, a group of scientists (*1*) met in New York City to discuss the possibilities of extending symbiotic nitrogen fixation to important food crops, particularly the grasses and cereal grains. The meeting served as an intensive workshop for specialists in nitrogen fixation.

Discussions centered on the possibilities and probabilities for extending the complex root nodule system to plants like corn and wheat. Three major areas were explored in depth: the nature of the infection process and nodule formation in legumes and nonleguminous plants; the generation of enzymes and other proteins, notably leghemoglobin, in root nodules; and the application of genetic techniques to both the host and the bacterial symbiont in an effort to broaden the range of effective nitrogen fixation systems.

Reasons for believing in the possibility of some extension of the present limits of nitrogen fixation to other systems included (i) the increased evidence for the occurrence of nitrogen fixation in nonleguminous vascular plants, such as *Alnus, Casuarina,* or *Myrica* distributed across several unrelated plant families; (ii) increasing evidence for promiscuity in *Rhizobium* infectiveness among the legumes; (iii) the possibility of applying modern molecular genetic methods to bacterial symbionts; (iv) the recent experimental establishment of an effective nitrogenase activity in vitro by combining effective bacteria and host tissues by tissue culture techniques; and (v) increased accessibility of the problem by the recent development and exploitation of a rapid and sensitive assay with gas chromatographic monitoring of acetylene reduction by the fixing system.

Attention focused on an analysis of the effective symbiosis in the *Rhizobium*-legume complex where in nature a delicately balanced relationship has evolved. Nitrogen fixation could be increased in this system if effective nodulation were to begin earlier, end later, or occur at a more rapid rate. The initial infection process itself is poorly understood. Although the root hair infection has been described in some detail, even filmed, the biochemical events have escaped definition and remarkably little is known about the chemical basis for cross-inoculation specificities between bacteria and host or their mutual biochemical interactions as the bacterial thread penetrates the root cortical cells. Circumstantial evidence for a bacterial stimulus to cell division of cortical cells initiating nodule primordia suggests the involvement of cytokinin production by the bacteria and a complementary hormonal stimulus originating in the host root tissues.

In tissue culture, infection thread formation of root hairs is bypassed, and *Rhizobium* infection involves direct penetration of cultured multicellular tissue masses. Evidence for bacterial infection of cultured cells of soybean includes electron micrographic proof of bacteria within pseudovesicles in the cells. Tissues infected in this way show acetylene reduction activity, diagnostic of nitrogenase activity.

Within effective nodules, the bacteroids are the site of the nitrogenase activity. Cell-free extracts are very sensitive to oxygen and must be prepared and assayed anaerobically. The nitrogenase enzyme of such cell-free extracts is similar to that from free-living nitrogen fixers such as *Azotobacter, Clostridium,* or blue-green algae. Of particular interest is the evidence that a molybdenum-containing subunit of nitrogenase can substitute for a similar subunit of nitrate reductase in *Neurospora crassa.* The pigment leghemoglobin occurs in effective nodules but is not required for activity of nitrogenase in vitro. Its location, whether within the bacteroidal cells, the envelope surrounding the bacteroids, or in the cytoplasm of the nodule cells is still debated, as is its possible role in the facilitated diffusion of oxygen which is essential for nitrogen fixation within nodules. From experiments with infections of different plant hosts by a common bacterial strain, it is clear that the protein component of the pigment is coded for by the host plant genome. The location of the genetic information for the nitrogenase components is not known.

The presence of effective nitrogen-fixing root nodules in nonlegumes, including some 12 genera of woody dicots scattered among a number of families, involves symbioses between unidentified actinomycete-like microorganisms and the roots of the host plants. Analogies to the legume symbiosis are striking: specialized root structures are involved, nitrate inhibits nodule development, and ineffective nodules have been described. In *Alnus*, which is the most studied nonlegume nodular symbiosis, the infective microorganism is still unknown, cannot be grown in pure culture, yet can be transferred by inoculation with crushed nodules. Infection is apparently via root hairs and involves proliferation of root cortical tissues and the formation of a thick periderm.

Although nodule-like growths have been reported on roots of monocots, including some grasses and sedges, and an occasional report has appeared on nitrogen fixation by grasses, no carefully documented occurrence of symbiotic nitrogen fixation by root nodules in cereals or monocots is known. Rather, evidence is increasing that grasses and cereals establish a close relationship with free-living microorganisms in the soil around the roots and on the mucilaginous covering of the roots themselves, where they are effective in fixing atmospheric nitrogen in respectable amounts. These organisms include, under different circumstances, anaerobic bacteria such as *Clostridium*, aerobes such as *Azotobacter*, many different species of blue-green algae, and facultative anaerobes such as *Klebsiella*. That these intimate interdependencies are important in the overall nitrogen economy of the monocots seems well established and might well be exploited by means which foster the relationship. The importance of maintaining beneficial associations between rhizosphere and plant during plant introduction was emphasized.

Discussions of the possible evolutionary origin of the *Rhizobium*-legume symbiotic association raised questions concerning the existence of other still unidentified symbioses or of the potentialities of genetic manipulation of both the host and the bacterial symbiont to increase the occurrence of the nitrogen-fixing capacity. Little optimism was expressed for success on this front until more basic knowledge is achieved about the steps involved in the infection process, nodule formation, and bacteroid formation. The need for more basic research in these areas was emphasized. Also, a wide-ranging search should be made for monocots that show evidence of nitrogen fixation capacity, by way of either nodulated structures or rhizosphere relationships. Discoveries in this area could open up new avenues for extending the capacity to fix nitrogen to the major food crops.

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Note

1. The conference was organized and supported by the Rockefeller Foundation. The conferees from nine countries included university scientists in plant physiology and biochemistry, plant breeders, bacterial geneticists, and agricultural specialists in soil and agronomy. Participants, in addition to the Rockefeller staff, included: C. A. Appleby, New York; F. J. Bergersen, Australia; G. Bond, Scotland; R. H. Burris (chairman of the conference), Wisconsin; G. W. Burton, Georgia; J. C. Burton, Wisconsin; P. J. Dart, England; C. C. Delwiche, California; M. J. Dilworth, Western Australia; H. J. Evans, Oregon; M. Fried, Austria; B. O. Gillberg, Sweden; R. W. Hardy, Delaware; J. D. Menzies, Maryland; F. N. Ponnamperuma, Philippines; A. Quispel, Netherlands; J. G. Torrey, Massachusetts; J. Totter, Maryland. The recorder of the meeting was D. A. Phillips, Massachusetts.

Forthcoming Events

October

18–20. **Soil Microcommunities** Conf., Syracuse, N.Y. (D. Dindal, Dept. of Forest Zoology, State Univ. College of Forestry at Syracuse Univ., Syracuse 13210)

19–22. **Acoustical** Soc. of America, Denver, Colo. (Miss B. H. Goodfriend, ASA, 335 E. 45 St., New York 10017)

19–22. American Soc. for **Microbiology**, Atlantic City, N.J. (R. W. Sarber, ASM, 1913 Eye St., NW, Washington, D.C. 20006)

20–21. **Chemurgic** Council, 33rd annual, Washington, D.C. (J. W. Ticknor, CC, 350 Fifth Ave., New York 10001)

20–22. **Transplutonium** Symp., 3rd, Argonne, Ill. (D. C. Stewart, Chemistry Div., Argonne National Lab., 9700 Cass Ave., Argonne 60439)

21–23. American Acad. of **Clinical Toxicology**, Philadelphia, Pa. (E. G. Comstock, P.O. Box 2565, Houston, Tex. 77001)

21–24. Society for **Psychophysiological Research**, Clayton, Mo. (K. M. Kleinman, Dept. of Psychology, Southern Illinois Univ., Edwardsville 62025)