

This Week's Citation Classic

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Bergersen F J. Biochemistry of symbiotic nitrogen fixation in legumes. *Annu. Rev. Plant Physiol.* **22**:121-40, 1971. [Division of Plant Industry, CSIRO, Canberra, Australia]

This review covered the nitrogen-fixing properties of intact nodules, summarized experiments which located the site of the primary reactions in the bacteroids, and reviewed their metabolism and the characterization of nitrogenase. The location and role of leghaemoglobin in nodule tissue was discussed. [The *SCI*[®] indicates that this paper has been cited over 105 times since 1971.]

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"*Annual Reviews of Plant Physiology* has included the topic of nitrogen fixation regularly. One reason that the editors asked me to undertake this assignment in 1970 was certainly that other potential authors were already busy with other reviews and books. However, it gave me the opportunity to write a review confined to the symbiotic system of legume root nodules. The review encompassed 129 references: were such a review to be written today covering a similar period of time, perhaps 500 papers or even more might need to be cited. The growth in the scientific work force engaged in this research topic has been largely responsible for the moderately frequent citation of this review, which appeared following a period in which research on the biochemistry of root nodule nitrogen fixation had substantially caught up with the faster-developing understanding of free-living bacterial systems. Later reviews in the series have again dealt broadly with the subject of nitrogen fixation,¹² or have been confined to particular aspects.³

"The 1971 review began by considering the properties of intact, N₂-fixing nodules, including the roles of inorganic plant

nutrients, relationships with photosynthesis by the host plant, and roles for O₂ and H₂ in relation to energetic efficiency. The evidence for the symbiotic forms of *Rhizobium*, the bacteroids, being the site of all of the enzymes concerned in the production of NH₄⁺ from N₂ was then summarized, followed by an account of experiments in which new information was gathered from experiments with bacteroid suspensions. The preparation of cell-free extracts containing active nitrogenase and the purification and properties of the nitrogenase proteins were then reviewed. The small section about the induction of nitrogenase in the bacteroids has been superseded by subsequent research which showed that some strains could produce nitrogenase in culture. The final section of the review about the properties and roles of leghaemoglobin in nodules, although greatly amplified by subsequent research, contains most of the elements of our present understanding.⁴

"The review concluded with a diagram expressing the metabolic roles of various components of nodule tissue. Some of the pathways have been elucidated or modified subsequently; for example, the identification of a role for bacteroid ferredoxin in electron transport to nitrogenase and the roles for uptake hydrogenases elucidated by the group at Oregon State University, or the details of routes for assimilation of fixed NH₄⁺ in the host cytoplasm rather than in the bacteroids, from work by several laboratories. Nevertheless, the broad picture of integrated metabolism constructed nine to ten years ago remains substantially the framework upon which present understanding is based. Currently, research is pointing toward greater understanding of the genetics of the various components of pathways and the mechanisms of their regulation. Such work is challenging enough in free-living bacteria grown in culture, but is daunting in a symbiotic tissue. Future reviewers will face a more difficult task than I did in 1970."

1. **Dilworth M J.** Dinitrogen fixation. *Annu. Rev. Plant Physiol.* **25**:81-114, 1974.
2. **Shanmugam K T, O'Gara F, Andenen K & Valentine R C.** Biological nitrogen fixation. *Annu. Rev. Plant Physiol.* **29**:263-76, 1978.
3. **Phillips D A.** Efficiency of symbiotic nitrogen fixation in legumes. *Annu. Rev. Plant Physiol.* **31**:29-49, 1980.
4. **Bergersen F J.** Leghaemoglobin, oxygen supply and nitrogen fixation: studies with soybean nodules. (Stewart W D P & Gallon J R, eds.) *Nitrogen fixation*. London: Academic Press, 1980. p. 139-60.