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The production of heterocysts by the nitrogen-fixing cyanobacterium *Anabaena cylindrica* is promoted by supply of carbon sources, such as glucose or succinate, and decreased by combined nitrogen sources, such as nitrate or amino acids. Ammonium salts produce a complete inhibition of heterocyst formation. [The *SCI*® indicates that this paper has been cited in over 180 publications.]

Heterocysts and Nitrogen Fixation in Cyanobacteria

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Working on nitrogen fixation by the blue-green alga (or cyanobacterium) *Anabaena cylindrica*, I could not help being intrigued by the empty-looking thick-walled cells, heterocysts, occurring at intervals along its simple filaments. Professor F.E. Fritsch, my teacher and the greatest authority on these organisms at the time, described heterocysts as "botanical enigmas" and could do no better than surmise that they might be archaic and now functionless reproductive organs. Thus heterocysts did not seem to be particularly profitable to study but they were easy to count, and in the Department of Botany of University College London—housed in delapidated attic rooms in premises shattered by the bombing of World War II and with a minimum of equipment and funds—simple research on the effect of different conditions on their production was feasible.

The feature of this research with which I was most pleased at the time was the use of allometry, the logarithmic plotting technique for study of relative growth introduced by the zoologist Julian Huxley, for following the shifting rates of production of heterocysts during the growth of batch cultures. Only some time after the publication of my paper did I realize that the head of my department, Professor W.H. Pearsall, had already used allometry in plant physiological studies.[1] With characteristic tolerance he forbore to point this out, and perhaps I may now make my apologies for having overlooked his prior claim to this innovation to the shade of that great and generous man.

Actually the important result to emerge was that production of heterocysts was partially suppressed when nitrate was provided as nitrogen source in addition to the free nitrogen of the air and was completely inhibited by ammonium salts. Previous workers had grown *Anabaena* spp. in the presence of nitrate or ammonium salts but had missed this effect because they left microscopical examination until the cultures had exhausted the combined nitrogen supplied. It may well be asked why it took nearly 20 years for the significance of this observation in relation to nitrogen fixation to be realized. The answer is first that heterocysts are of wide occurrence in cyanobacterial genera, which were not at that time thought to fix nitrogen, and second that there was no reason to suppose that fixation needed to be separated from other cell processes. The discovery of more genera fixing nitrogen and having heterocysts raised the thought that these structures might be involved in nitrogen fixation, but when a preparation of intact isolated heterocysts failed to show any evidence of being able to utilize molecular nitrogen,[2] the idea was shelved.

The resolution of the enigma came by serendipity. My colleague W.D.P. Stewart had just returned from Wisconsin, where he had learned of the oxygen-inactivation of nitrogenase. I, having just completed an elementary text on photosynthesis, had the different rôles of the photosystems well in mind, and another colleague, P. Fay, had been looking at the pigments of heterocysts and had found that they lacked those belonging to the oxygen-evolving photosystem II. In an informal conversation everything suddenly clicked into place: heterocysts provide the anaerobic environment necessary for nitrogen fixation. Lacking photosystem II they do not produce oxygen but can still generate ATP by photosystem I and derive the necessary hydrogen donor for fixation from adjacent cells carrying out oxygenic photosynthesis.[3] Isolated heterocysts supplied with a hydrogen donor and ATP under anaerobic conditions were later found to fix nitrogen.[4] When readily available nitrogen in the form of an ammonium salt is supplied, nitrogenase and the heterocysts to contain it are not needed and their formation is suppressed. From this key observation has sprung a productive line of investigation into the biochemistry and molecular biology of nitrogen fixation[5] besides interesting work on cell differentiation.[6]

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