terms, must be used.

What Cooper and co-workers have shown is that the valence bond picture is improved if the atomic orbitals are 'optimized' (each one spread out slightly towards its neighbours). Then the two Kekulé structures alone give very nearly the same result. In other words, as they are progressively refined, the two ways of constructing electronic wavefunctions (one using orbitals localized around individual atoms, the other using orbitals spread over the whole molecule) approach the same end point at very different rates; a few Kekulé structures based on well-chosen localized orbitals may apparently be equivalent to a much longer expansion of the electronic wavefunction in terms of molecular orbitals.

The new approach thus does not in practice lead to a new electronic structure for benzene, for the term 'structure' really means the electron density, and that can be obtained by either method with equal precision. Neither, unfortunately, will the method provide an easy route to similar *ab initio* calculations on large molecules, an arduous task whatever method is used. What it does do is to provide a remarkable vindication of many of the qualitative ideas formulated long before the era of the computer, expounded at length in the books by Pauling and by Wheland; ideas which have long been a cornerstone in chemistry and which will continue to stimulate the imagination of both experimental and theoretical chemists. □

Roy McWeeney is Professor of Theoretical Chemistry in the Department of Chemistry and Industrial Chemistry, University of Pisa, Via Risorgimento 35, 56100 Pisa, Italy.

Nitrogen fixation

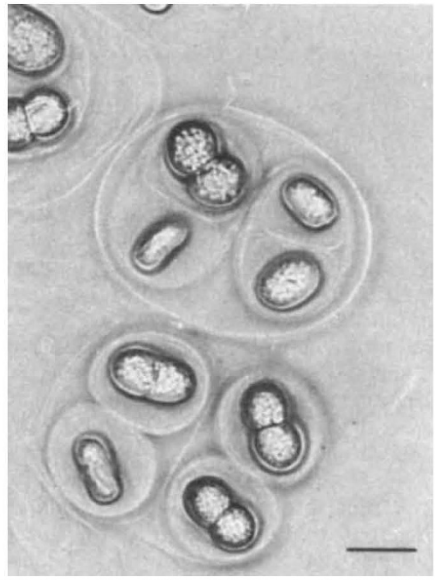
Finding a time and a place

from A.E. Walsby

NITROGEN fixation presents a problem for the cyanobacteria because the enzyme that catalyses the reduction of dinitrogen is inactivated by oxygen and oxygen is generated by photosynthesis. It is essential, therefore, that these two processes are separated from one another. In many filamentous cyanobacteria they are separated spatially, photosynthesis into vegatative cells and nitrogen fixation into heterocysts (Minchin, F. *Nature News and Views* **320,** 483; 1986). A. Mitsui and his collaborators now describe on page 720 of this issue how in a unicellular cyanobacterium the two processes are separated temporally into different parts of the cell cycle.

The idea that heterocysts provide anaerobic conditions for nitrogen fixation in filamentous cyanobacteria was first put forward (Fay, P. *et al. Nature* **220,** 810; 1968) at a time when all the strains for which there was unequivocal proof of nitrogen fixation were known to possess heterocysts. But within a year the first report of nitrogen fixation by non-heterocystous cyanobacteria appeared (Wyatt, J.T. & Silvey, J.K.G. *Science* **165,** 908; 1969), clearly establishing nitrogenase activity under aerobic conditions, in a species of *Gloeothece* — then called *Gloeocapsa* — a unicellular form distinguished by its concentric layers of sheaths (see figure). If this unicellular organism could fix nitrogen under aerobic conditions, as R. Rippka *et al.* (*Arch. Mikrobiol.* **87,** 93; 1971) confirmed, why should other strains of cyanobacteria require heterocysts?

The first insight into how *Gloeothece* managed to separate nitrogen fixation from photosynthesis was provided by P.M. Millineaux, J.R. Gallon and A.E. Chaplin (*FEMS Microbiol. Lett.* **10,** 245; 1981) who found that in cultures of this organism grown under a cycle of alternating 12 hours light and 12 hours dark, 95 per cent of the nitrogenase activity occurs in the dark. There is thus a temporal separation of these two incompatible processes; during the dark period respiratory activity reduces the oxygen tension and helps protect the nitrogenase, whereas in the light period oxygen is produced by photosynthesis and the nitrogenase is inactivated. New nitrogenase must be synthesized at the start of the next dark period.



Phase-contrast light micrograph of *Gloeothece* sp. showing concentric sheaths laid down at successive cell divisions. (Bar; 10 μm.)

This explanation would hold for cells growing in the natural (or artificial) light–dark cycle but how would it account for nitrogenase activity under continuous illumination? The answer to this has now been provided by Mitsui and his collaborators.

These authors, working with another unicellular nitrogen-fixing cyanobacterium, a species of *Synechococcus*, performed experiments very similar to those of Millineaux *et al.* but managed to obtain a culture of synchronously dividing cells. As with *Gloeothece* there was little or no nitrogenase activity in the 12-hour light period and activity reached its peak near the middle of the dark period. This peak followed soon after the sharply synchronized cell division that occurred after 4 hours of darkness.

When, after three such light–dark cycles, the culture was maintained in continuous light a synchronized cell division occurred 24 hours after the previous division and was also followed by a rise in nitrogenase activity. These results clearly establish that the nitrogenase activity is associated primarily with a particular phase in the cell cycle rather than with light intensity alone. Mitsui and collaborators have also shown that within this cell cycle there is also a cycle of photosynthetic capacity, reaching a maximum during the light phase and a minimum of nearly zero in the dark phase at a time coinciding with the peak in nitrogenase activity.

Another aspect of the process still remains to be explained: how does the cell protect its nitrogenase from oxygen diffusing into the cell from the outside? Such a problem is common to other non-photosynthetic bacteria that fix nitrogen under aerobic conditions (see Bothe, H. in *The Biology of the Cyanobacteria* eds Carr, N.G. & Whitton, B.A. p.87; Blackwell, Oxford, 1982). In the case of *Gloeothece* it may be significant that sister cells, which retain a degree of synchronous development, are kept in proximity by the concentric arrangement of sheaths (see figure). They may in this way provide for themselves a synchronized micro-environment that maintains a lower oxygen tension during the nitrogen-fixing phase of the cell cycle.

We now, however, at last have a crucial part of the explanation of how such unicellular cyanobacteria are able to fix nitrogen in continuous light: each cell independently continues through its cell cycle, switching first to nitrogenase activity in the absence of oxygenic photosynthesis, and then to photosynthesis in the absence of nitrogen fixation. The temporal separation of the processes thus continues with endogenous timing by the cell division cycle. □

A.E. Walsby is Professor of Botany at the University of Bristol, Bristol B58 1UG, UK.