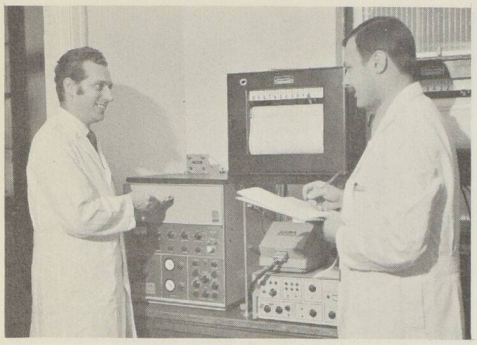
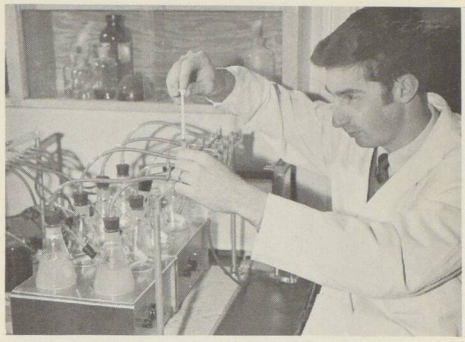


Dr. T. A. LaRue and Dr. W. G. W. Kurz test nitrogenase activity on a gas chromatograph.

Les Dr T. A. LaRue et W. G. W. Kurz évaluent l'intensité de la nitrogenèse par chromatographie en phase gazeuse.



Inhibitors of nitrogenase are tested by Clifford Mallard in flasks containing the bacteria azotobacter vinelandii. Gas chromatographic measurement of ethylene and acetylene indicate activity of nitrogenase.

Clifford Mallard étudie l'action des inhibiteurs sur les azotobacter vinelandii.

organisms," says Dr. Kurz. "Batch cultures consist of cells at random stages of development growing at different rates in a limited amount of medium. As cells multiply in such a 'closed system', they progressively alter their environment, resulting in changes in cell metabolism and composition. 'Open system' cultures, in which a continuous feed of nutrient flows into a constant volume of culture, allow cells at random stages of development to grow at the same rate, giving an average steady-state condition. However, this still does not permit investigations over the growth cycle of the cell.

"We have modified an 'open system' culture so as to cause cells to grow simultaneously through the same stages of development and at the same growth rate," Dr. Kurz says. "The result is a synchronous culture which can accurately represent the growth cycle of a single cell."

As a result of this technical accomplishment, scientists can now look at nitrogenase synthesis and activity during all stages of the growth cycle. Combined with a new assay for nitrogenase activity, this accomplishment puts NRC in a position to contribute important new advances in research on nitrogen fixation.

"Although recent techniques have given highly sensitive nitrogenase assays, they have had the disadvantage of disrupting cell metabolism," Dr. LaRue says. "This prevented continuous assay of nitrogenase activity."

Highly sensitive nitrogenase assays, rather than measuring the amount of nitrogen fixed, depend upon the ability of the enzyme system to reduce acetylene to ethylene. Originally, the assay procedure consisted of removing the normal nitrogen-containing atmosphere around growing cells, replacing it with acetylene, and measuring the amount of ethylene produced. Using gas chromatography to measure ethylene production, the assay is highly sensitive. However, an acetylene atmosphere starves the cells of nitrogen for normal growth and metabolism, and is also toxic. Such an assay is limited. It is strictly a batch assay, a 'once only proposition'.

Dr. LaRue and Dr. Kurz overcame this limitation by devising an assay which leaves growing cells undisturbed in their normal atmosphere. Trace amounts of acetylene, less than 0.001 per cent concentration, are passed through the fermenter or culture flasks. At such minute levels acetylene is not toxic to nitrogen-fixing organisms. Nitrogenase continues to fix nitrogen, but it also reduces a proportionate amount of acetylene to ethylene. Ethylene in the gaseous effluent from the culture vessels is then measured by gas chromatography. This assay does not disturb growing cells, is rapid, efficient, and — of great importance — continuous. Nitrogenase activity can be assayed every 30 seconds, if desired.

Development of these two significant advances — synchronous cultures of nitrogen-fixing bacteria and a continuous nitrogenase assay — already has enabled Dr. Kurz and Dr. LaRue to uncover new information regarding the biochemistry of nitrogen fixation.

Continuous assay of nitrogenase activity has detected transient inhibitors, compounds which momentarily suppress nitrogen fixation. Previous 'once only' batch assays could not indicate that the biochemical machinery of the cell overcomes the influence of such inhibitors to resume nitrogen fixation. These findings are forcing revisions of earlier conclusions by other workers, leading to a more accurate classification of compounds affecting nitrogen fixation.

Development of fermenters for synchronous cultures has led to improved designs which can be used to grow blue-green algae in open systems. This important nitrogen-fixing organism has been difficult to grow in a steady-state system, but a new 'dome fermenter' developed at PRL is now being used successfully for this purpose.

Using new algae cultures, Dr. LaRue and Dr. Kurz hope to compare enzymes responsible for nitrogen fixation from algae and bacteria. If these proteins from different sources prove similar, it would indicate similar biochemical mechanisms for nitrogen fixation in free-living bacteria and blue-green algae.

"If we can determine what controls nitrogenase synthesis and activity in bacteria and algae, it may lead to increased nitrogen fixation and protein production," Dr. LaRue says. "If the biochemistry of nitrogen fixation by symbiotic legumes proves similar to that in bacteria and algae, knowledge gained now may beneficially affect the agricultural scene of the entire world.

"Certain legumes, soybean for example, double their protein content every 10 days during nitrogen fiixation. If the biochemical 'on-off' switch could be found, and the period of nitrogen fixation extended for even a few days, protein production could be greatly increased."

"Industry also would appreciate knowing how nitrogen-fixing organisms convert nitrogen to ammonia," Dr. Kurz says. "If they could copy the biochemical catalysts which quickly accomplish the job at 30 to 35 degrees Centigrade and 0.2-1 atmosphere it would be a big improvement over industry's method using 450 degrees Centigrade and 250-1000 atmospheres pressure. ■