A key to an increased world protein supply

Biological nitrogen fixation

As the search continues for more efficient and abundant food resources, a parallel search goes on to determine the basic chemical processes involved in food production. While agronomists and oceanographers survey land and sea for crops to meet the world's increasing deficit in protein supply, research scientists are surveying the biochemistry of living organisms to better understand the limiting factor in protein formation — biological nitrogen fixation. This fundamental reaction is essential to life on land and in the oceans.

Although the earth's atmosphere contains an abundance of elemental nitrogen, 80 per cent by volume, plants cannot use it directly to build protein. Nitrogen must be fixed — combined in usable form — before plants can incorporate it onto a carbon skeleton. Meeting this need, a wide variety of living organisms possess specialized biochemical processes of such efficiency that an estimated 100 million tons of nitrogen are fixed annually on this planet.

Agriculturally, symbiotic legumes are the most important nitrogen-fixing group. Their root nodules, formed by bacterial infection, contain the ability to fix elemental nitrogen. Blue-green algae, which grow in oceans, deserts, lakes and Arctic regions, are the most widespread of the nitrogen-fixing organisms. Other organisms responsible for nitrogen fixation include non-legumes in close association and certain species of free-living bacteria. The biochemistry of nitrogen fixation by these various organisms is probably similar, if not identical. However, increased understanding of this biological phenomenon is urgently required, for herein may lie the key to replenishing the world's protein storehouse.

Until the first reproducible cell-free extract containing a nitrogen-fixing enzyme system was demonstrated in 1956, almost nothing was known about the biochemistry of nitrogen fixation. Since then, experiments with bacterial cell-free extracts have revealed that the nitrogen-fixing system, called nitrogenase, contains two proteins, requires energy, and a source of electrons. Similar activities, requirements and components of nitrogenase from various bacteria have been demonstrated.

Such knowledge increases understanding of the mechanism of nitrogen fixation, but serious fundamental questions remain unanswered. What turns on nitrogen fixation in living organisms? What shuts it off? What controls nitrogenase synthesis in the cell? Where does nitrogenase synthesis fit into the growth cycle of the cell? Can biological nitrogen fixation be artificially induced or controlled?

Answers to these questions have been awaiting further technical advances that would permit detailed study of what goes on in relation to nitrogenase synthesis and activity inside the cell during its growth cycle. New techniques to assay nitrogenase without disrupting biochemical machinery of living cells were required. Organisms had to be grown in synchronized cell cultures — cultures in which the cells are at the same stage of development and growing at the same rate.

Two scientists, Dr. W. G. W. Kurz and Dr. T. A. LaRue of the Prairie Regional Laboratory, of the National Research Council of Canada have grown nitrogen-fixing bacteria in synchronous cell cultures and have developed a continuous nitrogenase assay which does not disrupt living cells. These accomplishments have resulted from their preliminary attempts to clarify the biochemistry of nitrogen fixation by two free-living bacteria species, azotobacter vinelandii and clostridium pasteurianum, with a view to eventually increasing nitrogen fixation in areas of agricultural importance.

"Formerly, nitrogenase studies were limited to batch cultures of micro-

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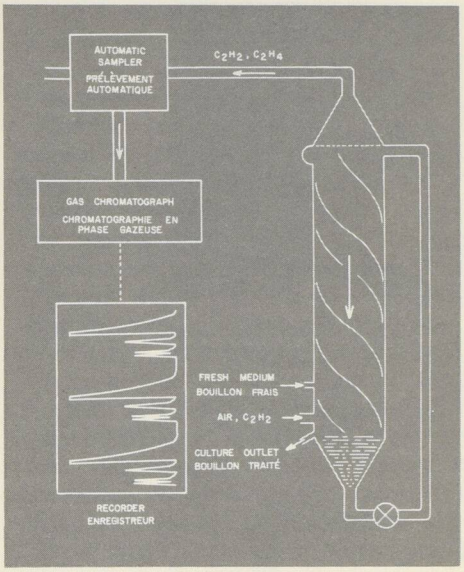

Nitrogenase is determined in microbial culture by continuous determination of acetylene reduction. Trace amounts of acetylene are incorporated into air used for aerating microbial culture. Portions of effluent air are automatically sampled and analysed by gas chromatography. Amount of ethylene formed from acetylene indicates activity of nitrogenase.

Dans des cultures microbiennes, la nitrogenèse s'évalue en mesurant continuellement la réduction des traces d'acétylène injectées dans l'air circulant dans les cuves. Les effluves sont échantillonnées automatiquement et analysées par chromatographie en phase gazeuse. La quantité d'acétylène transformée en éthylène permet d'évaluer l'intensité de la nitrogenèse.