# Evidence for a Nitrogenase System in the Photosynthetic Bacterium *Rhodospirillum rubrum*

Martin D. Kamen and Howard Gest[1]

*Mallinckrodt Institute of Radiology and Department of Chemistry, Washington University*

IT HAS BEEN DEMONSTRATED that the non-sulfur purple bacterium, *Rhodospirillum rubrum* (strain SI) will produce molecular hydrogen as a major photosynthetic product during illumination in certain media (*1*). This phenomenon does not occur when nitrogen is present either in the elementary form or as ammonium ion (*1*). These observations suggest the existence in these organisms of a nitrogenase or nitrogen-fixing system, hitherto unsuspected.

Evidence has been obtained using molecular nitrogen labeled isotopically with $N^{15}$ which appears to provide unequivocal support for the existence of a light-stimulated nitrogenase system in *Rhodospirillum*. In one set of experiments, 3-day-old $H_2$-producing organisms were harvested by centrifugation, washed twice with *m*/20 phosphate buffer (pH 7.2) and suspended in a neutral medium containing $MgSO_4$, phosphate buffer, trace elements, biotin and D,L-malic acid. The bacterial suspension was divided into three 10-ml portions. Two portions were placed in 75-cc Warburg vessels on a vacuum line. The third portion was boiled and the dead organisms so obtained were placed in the third vessel on the vacuum line to provide a control. After evacuating and flushing the vessels and vacuum system several times with helium, the vessels were filled to 0.1 atmosphere with labeled $N_2$ (30% $N^{15}$) and 0.9 atmosphere helium. The nitrogen was generated from Eastman Kodak $NH_4NO_3$ (30% $N^{15}$ in the $NH_4$ group) with alkaline NaOBr and freed of all combined labeled nitrogen ($NH_3$ or N oxides) by passage through two liquid air traps. Control experiments revealed no detectable $NH_3$ in the elementary nitrogen ($< 2 \times 10^{-3}$ mg $NH_3$ in a total of 6 mg as $N_2$). One vessel was illuminated, one was kept in the dark. After 6 days, the organisms were separated, and total Kjeldahl nitrogens obtained. These were analyzed for $N^{15}$ content.[2] The organisms maintained in the light showed an $N^{15}$ content in total cellular nitrogen of 3.14 atom percent excess; those in the dark, 0.189 atom percent excess; and the boiled controls 0.008 atom percent excess. In another set of experiments almost identical results were obtained with the additional observation that small amounts of ammonia (0.5 mg $NH_4Cl$/ml) inhibited $N^{15}$ uptake from the labeled molecular nitrogen.

These observations appear to be the first reported in which there have been demonstrated (1) a nitrogenase system in photosynthetic bacteria, (2) a specific effect of molecular nitrogen on hydrogenase activity, and (3) a stimulating effect of light on turnover of molecular nitrogen. These observations have been confirmed in the laboratories of R. H. Burris and P. W. Wilson at the University of Wisconsin. We wish to express our appreciation for their wholehearted cooperation.

It has been ascertained that the $N^{15}$ uptake observed is the result of a net fixation of nitrogen. An illuminated suspension of *R. rubrum* maintained for 30 days in an atmosphere of molecular nitrogen and hydrogen and in a medium devoid of all combined nitrogen except for a minimal quantity of yeast extract has shown a seven-fold increase in cellular nitrogen gained at the expense of molecular nitrogen.

### Reference

1. GEST, H. and KAMEN, M. D. *Science*, **1949**, **109**, 558.

[1] Predoctoral fellow of the American Cancer Society, recommended by the Committee on Growth of the National Research Council, 1947–1949. Present address, Department of Microbiology, Western Reserve University Medical School, Cleveland, Ohio.

[2] We are indebted to Prof. D. Rittenberg and Mr. I. Sucher, Columbia University, and to Dr. Mildred Cohn, of Washington University, for the $N^{15}$ analyses.