functions separate from those of retinol or retinal (*15*). Whether retinoic acid or a tissue metabolite derived from it is the biologically active compound is unclear (*15*). Retinoic acid, to our knowledge, has never been reported as present in brain. Nevertheless, a distinct possibility is that the gene defect in Batten disease involves an enzyme or enzymes that catabolize retinoic acid. It is of interest that the lamellated character of CLB's requires the presence of cholesterol and phospholipids and that vitamin A derivatives can readily form lamellated micelles with phospholipids (*16*). Finally, since lipofuscin or the so-called age pigments in neurons have fluorescent properties similar to those of CLB's, it seems important to reinvestigate their chemical composition in the light of this research (*17*).

LEONHARD S. WOLFE
N.M.K. NG YING KIN
R. ROY BAKER
STIRLING CARPENTER
FREDERICK ANDERMANN
Department of Neurology and Neurosurgery, McGill University, Montreal Neurological Institute, Montreal, Quebec H3A 2B4 Canada

References and Notes

1. L. Crowe and J. Stern, in *Greenfield's Neuropathology*, W. Blackwood and J. A. N. Corsellis, Eds. (Arnold, London, 1976), pp. 531–533; C. L. Dolman and E. Chang, *Arch. Pathol.* **94**, 425 (1972).
2. W. Zeman and S. Donahue, *Acta Neuropathol.* **3**, 144 (1963); P. E. Duffy, M. Kornfeld, K. Suzuki, *J. Neuropathol. Exp. Neurol.* **27**, 357 (1968); S. Carpenter, G. Karpati, F. Andermann, *Neurology* **22**, 177 (1972).
3. W. Zeman, in *Handbook of Clinical Neurology*, P. J. Vinken and G. W. Bruyn, Eds. (North-Holland, Amsterdam, 1970), pp. 588–679.
4. ———, *J. Neuropathol. Exp. Neurol.* **33**, 1 (1974).
5. C. J. Dillard and A. L. Tappel, *Lipids* **6**, 715 (1971); V. G. Malshet and A. L. Tappel, *ibid.* **8**, 194 (1973).
6. D. Armstrong, S. Dimmitt, D. H. Boehme, S. C. Leonberg, W. Vogel, *Science* **186**, 155 (1974); D. Armstrong, S. Dimmitt, D. E. VanWormer, *Arch. Neurol.* **30**, 144 (1974).
7. H. Pilz, J. S. O'Brien, R. Heipertz, *Clin. Biochem.* **9**, 85 (1976); S. M. Sumi and D. F. Farrell, *Neurology* **26**, 364 (1976); L. S. Wolfe, S. Carpenter, F. Andermann, unpublished results.
8. J. Eichberg, V. P. Whittaker, R. M. C. Dawson, *Biochem. J.* **92**, 91 (1964).
9. The conditions for pronase digestions were: 1 mg pronase (Calbiochem) added to 3 mg of P_1D pellet in 50 m*M* tris buffer at *p*H 7.5, 75 m*M* NaCl, 25 m*M* $CaCl_2$, and 0.005 percent sodium azide, followed by incubation at 20° to 21°C for 21 hours with addition of a further 1 mg of pronase after 5 hours.
10. J. Folch-Pi, M. Lees, G. H. Sloane-Stanley, *J. Biol. Chem.* **226**, 497 (1957) (extraction and separation of gangliosides); J. D. Turner and G. Rouser, *Anal. Biochem.* **38**, 423 (1970) (phospholipid separations by two-dimensional thin-layer chromatography); G. R. Bartlett, *J. Biol. Chem.* **234**, 466 (1959) (phosphate determinations); N. Crawford, *Clin. Chim. Acta* **3**, 357 (1958) (cholesterol determination).
11. We used AE1 MS-9 and LKB-9000 mass spectrometers at 70 ev with a 270°C ion source.
12. W. H. Elliott and G. R. Waller, in *Biochemical Applicatins of Mass Spectrometry*, G. R. Waller, Ed. (Wiley, New York, 1972), pp. 499–535.
13. The HCl was removed in vacuo, the residue treated with a small amount of 3*N* NH_4OH to enhance the fluorescence, and the solution applied to a small Sephadex-G10 column. All fractions were eluted before the amino acids (close to void volume) were collected.
14. An LKB-9000 gas chromatograph–mass spectrometer interfaced to a Varian MAT 100-SS computer was used. The column (6 percent OV-101 on Gas-Chrom Q) temperature was programmed at 50° to 260°C. The major product was eluted at 230°C.
15. M. Zile and H. F. DeLuca, *Biochem. J.* **97**, 180 (1965); H. F. DeLuca and A. B. Roberts, *Am. J. Clin. Nutr.* **22**, 945 (1969); H. F. DeLuca, *ibid.* **28**, 339 (1975).
16. K. D. Dreher, J. H. Schulman, O. R. Anderson, O. A. Roels, *J. Ultrastruct. Res.* **19**, 586 (1967); O. R. Anderson, O. A. Roels, K. D. Dreher, J. H. Schulman, *ibid.*, p. 600.
17. R. D. Taubold, A. N. Siakotos, E. G. Perkins, *Lipids* **10**, 383 (1975).
18. Supported by grants MT-1345 and MA-3189 from the Medical Research Council of Canada. L.S.W. is a Medical Research Associate of the Medical Research Council of Canada. We thank O. A. Mamer of the Mass Spectrometry Unit, Royal Victoria Hospital, for assistance in the mass spectrometric studies, G. Hamer for recording the NMR spectra, and S. Rothman and T. Seemeyer for clinical assistance.

28 September 1976

Nitrogen Fixation in Grass-*Spirillum* Systems

Smith *et al.* (*1*) indicated that nitrogen fixation by *Spirillum lipoferum* reduced the fertilizer requirement for two grasses by up to 0.6 kg of nitrogen per hectare per day. The number of bacteria applied was not exactly specified, but assuming rows 18 cm apart, about 4×10^{12} bacteria would have been applied per hectare. Each applied bacterium thus seems to be responsible for replacing (but not necessarily fixing) on the order of 1.4×10^{-10} g of nitrogen per day, a rather astounding feat for an organism which must at the onset weigh 1/10 to 1/100 of that. Since the growth rate of *S. lipoferum* and its efficiency of root infection are unknown, it is impossible to estimate the true efficiency of nitrogen fixation. Nonetheless, the association seems to be very efficient indeed. Smith *et al.* do not address this point and do not report complete nitrogen balances. Moreover, they do not begin to satisfy the spirit of Koch's postulates of microbial causality by correlating nitrogenase activity with growth enhancement in the putatively infected plants. In the light of the recent demonstration by Brown (*2*) that *Azotobacter paspali* may enhance grass growth by producing growth regulating substances, it seems to me premature to conclude that it is *S. lipoferum*'s ability to fix nitrogen which is enhancing grass growth.

ALLEN C. ROGERSON
Division of Agriculture, Fort Valley State College, Fort Valley, Georgia 31030

References

1. R. L. Smith, J. H. Bouton, S. C. Schank, K. H. Quesenberry, M. E. Tyler, J. R. Milam, M. H. Gaskins, R. C. Littell, *Science* **193**, 1003 (1976).
2. M. E. Brown, *J. Appl. Bacteriol.* **40**, 341 (1976).

16 September 1976

Carefully controlled experiments have demonstrated that the bacteria used in our experiments can invade grass root tissue (*1*) and that the colonies that result can reduce acetylene to ethylene (*2*). Further, Burris *et al.* (*3*) have demonstrated Koch's postulates working with monoaxenic cultures. These observations clearly justify testing under field conditions to determine whether inoculation may induce nitrogen fixation. We did not suggest that the cells applied to the soil, to achieve inoculation, could fix a significant amount of nitrogen. We assumed that a dynamic population increase would occur, as is well known with *Rhizobium* systems.

The report by Brown is not the first to demonstrate production of plant growth substances by bacteria. Indeed, many bacteria are known to produce such substances. However, this has not been shown with *Spirillum lipoferum*, whereas the ability to fix nitrogen has. We agree that the data at hand are limited and do not show unequivocally the reason for enhanced plant growth. We hope that other scientists will be encouraged to contribute to the solutions of problems confronting a nitrogen-deficient world.

REX L. SMITH, J. H. BOUTON
S. C. SCHANK, K. H. QUESENBERRY
Department of Agronomy, University of Florida, Gainesville 32601
M. E. TYLER, J. R. MILAM
Department of Microbiology, University of Florida
M. H. GASKINS
Agricultural Research Service, U.S. Department of Agriculture, University of Florida
R. C. LITTELL
Department of Statistics, Institute of Food and Agricultural Sciences, University of Florida

References

1. M. Garcia, D. Hubbell, M. Gaskins, paper presented at the International Symposium on the Environmental Role of Nitrogen-Fixing Blue-Green Algae and Asymbiotic Bacteria, Uppsala, Sweden, 1976; G. C. Weiser, J. Bouton, S. C. Schank, K. H. Quesenberry, R. L. Smith, unpublished data.
2. M. H. Gaskins, C. Napoli, D. H. Hubbell, *Agron. Abstr.* (1976), p. 71.
3. R. H. Burris, Y. Okon, S. Albrecht, paper presented at the International Symposium on the Environmental Role of Nitrogen-Fixing Blue-Green Algae and Asymbiotic Bacteria, Uppsala, Sweden, 1976.

4 January 1977