cells (J. Acosta-Urquidi, J. T. Neary, D. L. Alkon, in preparation).
10. D. L. Alkon, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **33**, 1083 (1974); *Biol. Bull. (Woods Hole, Mass.)* **159**, 505 (1980); T. P. Jerussi and D. L. Alkon, *J. Neurophysiol.* **46**, 659 (1981); Y. Goh and D. L. Alkon, *Soc. Neurosci. Abstr.* **8**, 824 (1982). Recently, similar type B cell membrane changes produced in living animals by pairing positive current injection with light steps were shown to cause, on subsequent days, the pairing-specific behavioral changes of conditioning [J. Farley and D. L. Alkon, *Biol. Bull. (Woods Hole, Mass.)* **163**, 383 (1982)].
11. Some of these results have been communicated in preliminary form [J. Acosta-Urquidi *et al.*, *Soc. Neurosci. Abstr.* 7, 944 (1981)].
12. D. L. Alkon, *Science* **205**, 810 (1979).
13. The catalytic subunit of cyclic AMP–dependent protein kinase was obtained from Sigma (P-2645). The lyophilized powder, containing approximately 1 percent protein, 80 percent sucrose, and 19 percent potassium phosphate buffer, was dissolved in carrier solution, 0.95*M* potassium acetate, 0.05*M* tris, *p*H 9.8 (1390 enzyme units per milliliter). The *p*H of the dissolved protein solution is 8.6. Microelectrodes were first filled by capillarity with the protein kinase carrier solution and then backfilled with fresh protein kinase solution (1.38 unit/μl) 10 minutes before use. Since protein kinase is negatively charged at *p*H 8.6 (isoelectric point, *p*I, 7.6) [I. Uno, T. Ueda, P. Greengard, *J. Biol. Chem.* **252**, 5164 (1977)], negative currents (0.3 to 1.0 nA, 30 to 60 seconds) were used to eject it. Detectable enzyme concentrations ejected through microelectrodes into assay tubes by these currents were measured with the method of J. J. Witt and R. Roskoski, Jr. [*Anal. Biochem.* **66**, 253 (1975)].
14. Input resistance measured through a balanced bridge circuit agrees well with measurements obtained with two intracellular electrodes, one for current passage and one for voltage monitoring (*12*).
15. Control solution consisted of 0.26*M* sucrose, 0.097*M* K_2HPO_4, 0.95*M* potassium acetate, 0.05*M* tris; *p*H was adjusted to 8.6 with acetic acid.
16. After 10 minutes of dark adaptation, 30-second light steps (10^4 erg cm^{-2} sec^{-1}) were presented at 3-minute intervals. The LLD to the third step was compared to those to the fourth and fifth steps. The protein kinase was injected in darkness between the third and fourth light steps.
17. These two dark K^+ currents could be separated pharmacologically: I_A was preferentially blocked by 10 m*M* 4-aminopyridine and I_B by tetraethylammonium ion (*8*). I_B was also specifically blocked by perfusion in 10 m*M* Ba^{2+} (*18*). No residual I_{Na} or $I_{Ca^{2+}}$ were detected in the dark after treatments that would eliminate K^+ currents, enhance inward currents, or both. The I_B currents were estimated from current values measured with a command step (to 0 mV) occurring 30 μsec after a 5.0-second preliminary command step to − 10 mV. This method for I_B measurement was arrived at after numerous experiments (J. J. Shoukimas and D. L. Alkon, unpublished observations) that established I_B characteristics in the absence of I_A (after blocking the 4-aminopyridine).
18. D. L. Alkon, J. Shoukimas, E. Heldman, *Biophys. J.* **40**, 245 (1982).
19. J. Shoukimas and D. L. Alkon, *Soc. Neurosci. Abstr.* **6**, 17 (1980).
20. J. A. Connor and C. F. Stevens, *J. Physiol. (London)* **213**, 21 (1971).
21. S. H. Thompson, *ibid.* **265**, 465 (1977).
22. A number of criteria for acceptability of impalement were used: (i) the resting potentials recorded by the voltage-recording and current-passing electrodes should be approximately equal, (ii) the response to light recorded by each electrode should be the same, (iii) the leak current at 0 mV should be ≤ 10 nA, (iv) the settling time of the voltage clamp should be ≤ 20 msec, and (v) the holding current at −60 mV should be ≤ 5 nA. Corrections of I_A values for "leak" current were obtained by extrapolation from a linear portion of the current-voltage relation, which was generated with small positive and negative command pulses (±10 to 20 mV).
23. Fresh protein kinase solution was injected iontophoretically (anodal current, 0.5 to 2.0 nA; 30 to 60 seconds) through the voltage-recording electrode, while opposite and equal current was delivered through the current-recording electrode to prevent any net change in resting potential during injection. Peak current values for I_A and I_B before protein kinase injection were compared separately with the first responses after injection. Values reported in the text are the percent changes obtained from the average peak current values for five different cells (Table 1).
24. H. Kuhn, *Nature (London)* **250**, 688 (1974); A. S. Polans, J. Hermolin, M. D. Bownds, *J. Gen. Physiol.* **74**, 595 (1979); D. B. Farber, M. B. Brown, R. N. Lolley, *Biochemistry* **18**, 370 (1979); H. Shichi and R. L. Somers, *J. Biol. Chem.* **253**, 7040 (1978).
25. L. K. Kaczmarek, K. R. Jennings, F. Strumwasser, A. C. Nairn, U. Walter, F. D. Wilson, P. Greengard, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 7487 (1980); V. S. Castellucci, E. R. Kandel, J. H. Schwartz, F. D. Wilson, A. C. Nairn, P. Greengard, *ibid.*, p. 7492.
26. I. B. Levitan and W. B. Adams, *Adv. Cyclic Nucleotide Res.* **14**, 647 (1981).
27. H. Rasmussen, *Calcium and Cyclic AMP As Synarchic Messengers* (Wiley, New York, 1981).
28. J. A. Connor and D. L. Alkon, *Soc. Neurosci. Abstr.* **8**, 944 (1982).
29. J. Acosta-Urquidi, J. T. Neary, D. L. Alkon, *ibid.*, p. 825; in preparation.
30. P. Cohen, *Curr. Top. Cell. Regul.* **14**, 117 (1978); P. J. Roach, A. A. DePaoli-Roach, J. Larner, *J. Cyclic Nucleotide Res.* **4**, 235 (1978); T. P. Soderling, A. K. Srivastava, M. A. Bass, B. S. Khatra, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 2536 (1979); W. Sieghart, J. Forn, P. Greengard, *ibid.*, p. 2475; W. B. Huttner and P. Greengard, *ibid.*, p. 5402; K. Y. Walsh, D. M. Millikin, K. K. Schlender, C. M. Reimann, *J. Biol. Chem.* **254**, 6611 (1979); C. J. LePeuch, J. Haiech, J. G. Demaille, *Biochemistry* **18**, 5150 (1979).
31. We thank S. Smith and E. G. Krebs for the highly purified catalytic subunit of cyclic AMP–dependent protein kinase.

26 April 1982; revised 28 July 1982

Prorocentrin: An Extracellular Siderophore Produced by the Marine Dinoflagellate *Prorocentrum minimum*

Abstract. *Prorocentrin, a putative iron transport compound, has been extracted from the filtrates of* Prorocentrum minimum *cultures by XAD-2 resin. Production of prorocentrin can be stimulated by culturing* Prorocentrum minimum *under conditions of iron deficiency. The iron(III) complex of prorocentrin has an ultraviolet-visible absorption spectrum typical of hydroxamate siderophores.*

Marine phytoplankton, like most other living organisms, have a nutritional requirement for iron (*1*). Iron in seawater is either associated with organic chelators (*2*) or present as aggregates of the almost totally insoluble iron(III) hydroxide (solubility product constant $\approx 10^{-38}$) (*3*). Although a portion of the chelated iron(III) may be utilized by phytoplankton, the large pool of insoluble iron(III) hydroxide is presumed to be unavailable as a nutritional source.

Aquatic microorganisms—for example, bacteria (*4*) and cyanobacteria (*5–8*)—produce extracellular iron(III)-specific chelating compounds (siderophores) that enable them to solubilize and therefore acquire the iron present in the iron(III) hydroxide aggregates.

It is not clear whether eukaryotic phytoplankton acquire iron(III) in a similar manner. Spencer *et al.* (*9*) characterized a possible siderophore from a nonaxenic marine diatom, *Chaetoceros socialis*. The isolated substance had chemical properties similar to those of a hydroxamate siderophore, but definitive evidence for the origin of the compound (from the diatom and not the bacteria) was not presented. More recently McKnight and Morel (*10*) have concluded that a number of axenic eukaryotic phytoplankton are unable to produce iron(III)-specific chelators. They have suggested that the diatom *Thalassiosira weissflogii* utilizes iron(II) rather than iron(III) (*11*), because ferrous iron is more soluble in seawater and therefore it should be more nutritionally accessible. Since iron(II) is extremely labile in the marine environment, transformation of iron(III) to iron(II) at the cell membrane may be required for efficient uptake. Armstrong and Van Baalen (*7*) showed that a concentrated chloroform extract of the supernatant from an iron-depleted culture of the marine diatom *Cylindrotheca* was Csaky-positive (*12*) and that it stimulated the growth of the siderophore auxotroph *Arthrobacter flavescens* JG-9 (*13*). Circumstantial evidence for the utilization of siderophores by phytoplankton comes from numerous observations that either synthetic or natural chelators stimulate phytoplankton growth in culture and in the field, possibly by enhancing the availability of iron (*14, 15*).

To clarify whether marine eukaryotes

Table 1. Influence of culture medium on growth rate of *Prorocentrum minimum* and production of Csaky-positive compounds.

Medium and nutrient status	Growth (divisions per day)	Final yield ($\times 10^7$ cells per liter)	Csaky test* (μg)
ESNW, iron-sufficient	1.04 ± 0.08	6.27	5.9
AQUIL-Fe, iron-deficient	0.42 ± 0.01	4.01	127.2
ESNW-Fe, iron-deficient	0.75 ± 0.02	3.95	133.0

*Measured as micrograms of NH_2OH per 10^8 cells.

do produce siderophores, we conducted a survey of marine phytoplankton species in the North East Pacific Culture Collection (NEPCC) (*16*) for the production of either intracellular or extracellular metabolites that are Csaky-positive (*12*) and that satisfy the requirements of the siderophore auxotroph *A. flavescens* JG-9 (*13*). Our survey (*17*) revealed that the cell and filtrate extracts of the nontoxic, red tide dinoflagellate *Prorocentrum minimum* Schiller (NEPCC 96) showed considerable promise as a source of hydroxamate siderophores.

Microbial production of siderophores is stimulated by growth in iron-limited media (*8, 18*). We therefore grew 6-liter axenic cultures (*19*) of *P. minimum* in (i) charcoal-treated natural seawater followed by addition of all required nutrients (ESNW) (*20*); (ii) charcoal-treated natural seawater followed by the removal of most of the available iron (*15*) and then supplemented with all nutrients except $FeCl_3$ and EDTA (ESNW-Fe medium); and (iii) charcoal- and Chelex-100 resin–treated natural seawater supplemented with AQUIL nutrients (*21*), minus $FeCl_3$ and EDTA (AQUIL-Fe medium). The first medium provided an iron-sufficient control, whereas the other media provided iron-stressed conditions.

All cultures were harvested when the extracellular siderophore concentration was maximum. This occurred approximately 3 days after the culture reached senescence. Cells were harvested by continuous centrifugation and membrane filtration (0.45 μm). The cell-free filtrates were acidified to *p*H 2 and passed over XAD-2 (*2, 22*) resin to extract the Csaky-positive metabolites. Methanol elution removed the organics from the XAD-2 resin. Evaporation of the methanol under reduced pressure and partitioning of the resulting residue between chloroform and water generated an aqueous fraction that contained the Csaky (*12*) activity.

The influence of the three different types of media on growth parameters and Csaky-positive metabolite production is shown in Table 1. The reduction in growth rate and final cell yield is attributed to the iron-deficient culture medium. Cells grown under iron-stressed conditions produced more of the Csaky-positive compound. The addition of freshly prepared $FeCl_3$ to alleviate the iron deficiency in ENSW-Fe and AQUIL-Fe reestablished maximum growth rates (1.01 ± 0.07 and 0.94 ± 0.01 divisions per day, respectively) and reduced the production of Csaky-positive compound to less than 5 μg of NH_2OH per 10^8 cells.

A 40-liter culture of *P. minimum* grown in ESNW-Fe medium and harvested shortly after the cessation of growth provided a sufficient amount of the extracellular Csaky-positive compound to enable purification and preliminary chemical characterization. We have named this compound prorocentrin. Culture filtrates were extracted as described above. Ferri-prorocentrin was formed by adding freshly prepared iron(III) hydroxide (*23*) to the resulting aqueous fraction and heating the suspension at 80°C for 2 hours. Excess iron(III) hydroxide was removed by filtration, and the deep red filtrate was reduced in volume by rotary evaporation under reduced pressure. A white precipitate that formed in the concentrated filtrate was removed, and the mother liquor was applied to a rotary thin-layer chromatography plate [Chromatotron (Harrison Research), HF silica gel with a starch binder]. Fractionation was achieved by elution with a series of solvents (methanol and water, 1:24; methanol and water, 3:7; and butanol, water, and acetic acid, 60:25:15). Several red-orange fractions were collected in each solvent pass. After concentration by rotary evaporation, each fraction was assayed by the Csaky test. A single red-orange fraction from the butanol–water–acetic acid pass was positive.

Table 2. Comparison of ferri-prorocentrin with desferri-prorocentrin; N.D., not determined.

Item	Ferri-prorocentrin	Desferri-prorocentrin
R_f*	~ 0.60	0.18 to 0.20
I_2 vapor	+	+
$FeCl_3$ spray	None	Pink color
Perchlorate test	+	+
Csaky test	+	+
Molecular size (daltons)	N.D.	560 to 590

*In a mixture of butanol, water, and acetic acid (60:25:15).

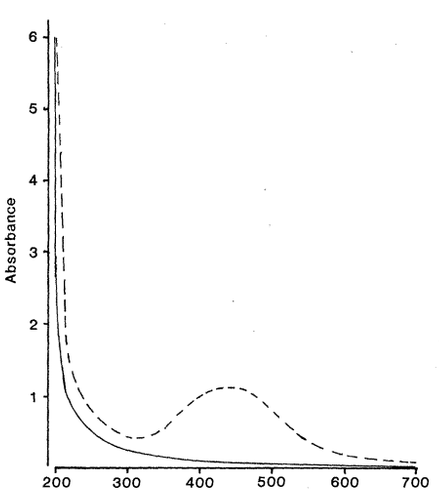

Fig. 1. The ultraviolet-visible absorption spectra of desferri-prorocentrin (solid line) and ferri-prorocentrin (broken line) in water.

Analytical thin-layer chromatography of the Csaky-positive fraction on silica gel (SIL G/UV_{254} eluted with a mixture of butanol, water, and acetic acid, 60:25:15) showed a single iodine-positive spot at a relative mobility (R_f) of approximately 0.6, corresponding to ferri-prorocentrin. The iron could be removed from the complex by treatment with 1*N* NaOH. Thin-layer chromatography of the desferri-prorocentrin showed a single spot at $R_f \approx 0.2$, which took up iodine and gave a pink spot with ferric chloride spray (2 percent $FeCl_3$ in ethanol). Prorocentrin was desalted on a Bio-Gel P-2 column to give approximately 7 mg of a white solid. Calibration of the BioGel column with several low molecular weight standards allowed us to estimate that the molecular size of prorocentrin is 560 to 590 daltons. We could not estimate a molecular size for ferri-prorocentrin because it streaked on the column.

Prorocentrin shows only strong end absorption in its ultraviolet-visible spectrum (Fig. 1). Formation of the iron(III) complex at neutral *p*H generates a new absorption band with a wavelength for maximum absorption (λ_{max}) of 440 nm (H_2O), which shifts to a λ_{max} of 450 nm upon acidification to *p*H 2. The ultraviolet-visible absorption spectrum of ferri-prorocentrin is typical of trihydroxamate siderophores such as ferrichrome (*24*).

Several observations support the claim that prorocentrin is a hydroxamate-containing siderophore produced by the dinoflagellate *P. minimum*. First, the axenic condition of our cultures and the isolation of the same compound (identical results on thin-layer chromatography) from cell extracts eliminates the possibility of a bacterial origin. Second, the positive Csaky test shown by pure prorocentrin (Table 2) and the ultraviolet-visible absorption spectrum of ferri-prorocentrin are characteristic of hydroxamate siderophores. Third, increased production of prorocentrin in an iron-limited culture medium is the required physiological manifestation of a siderophore-based iron acquisition mechanism. Finally, we have demonstrated that the desferri and ferri forms of prorocentrin can be readily interconverted.

Success in isolation of the siderophore can be attributed to the use of iron-deficient media and the concentration of prorocentrin on XAD-2 resin. Although the recovery with XAD-2 is less than

ideal (~ 50 percent), it is a necessary step in isolating and quantifying extracellular metabolites from this marine dinoflagellate (*25*). Without concentration, iron(III)-specific chelators from marine eukaryotes may remain undetected, whereas these compounds from marine cyanobacteria can be measured (*7*). Whether or not marine eukaryotic phytoplankton produce less siderophore than do marine cyanobacteria is unclear. Although cyanobacteria have a higher specific requirement for iron (especially when fixing nitrogen) (*26*) than do marine eukaryotic phytoplankton, quantitative comparisons between the two groups cannot be made until further information is available on the production, excretion, and cellular uptake of siderophores.

Prorocentrin is the first siderophore to be isolated from a eukaryotic marine phytoplankter. Its discovery suggests that the iron uptake mechanism of dinoflagellates may closely parallel that of prokaryotic siderophore-producing organisms (*8, 18*). The production of a strong trace metal chelator by a red tide organism has many important ecological implications. Blue-green algae can effectively eliminate competing algal species in freshwater lakes by sequestering all the available iron as a siderophore complex (*5*), and there is some indication that exogenous siderophores can both positively and negatively affect the growth of marine phytoplankton (*6*). Strong trace metal chelators can also reduce the toxicity of cupric ions to sensitive marine phytoplankton species (*27*). It will be interesting to test whether this dinoflagellate siderophore is readily available to prokaryotic organisms.

CHARLES G. TRICK
Department of Oceanography, University of British Columbia, Vancouver, British Columbia, Canada V6T 1W5
RAYMOND J. ANDERSEN*
Departments of Chemistry and *Oceanography, University of British Columbia*
ANDREW GILLAM
Department of Oceanography, University of British Columbia
PAUL J. HARRISON
Departments of Botany and *Oceanography, University of British Columbia*

References and Notes

1. C. A. Price, *Annu. Rev. Plant Physiol.* **19**, 239 (1968); J. C. O'Kelley, in *Algal Physiology and Biochemistry*, W. D. P. Stewart, Ed. (Univ. of California Press, Berkeley, 1974), p. 610.
2. Y. Sugimura, Y. Suzuki, Y. Miyake, *Deep-Sea Res.* **25**, 309 (1978).
3. J. B. Neilands, *Struct. Bond.* **11**, 145 (1972).
4. E. R. Gonye and E. J. Carpenter, *Limnol. Oceanogr.* **19**, 840 (1974).
5. T. P. Murphy, D. R. S. Lean, C. Nalewajko, *Science* **192**, 900 (1976).
6. K. M. Bailey and F. B. Taub, *J. Phycol.* **16**, 334 (1980).
7. J. E. Armstrong and C. Van Baalen, *J. Gen. Microbiol.* **111**, 253 (1979).
8. F. B. Simpson and J. B. Neilands, *J. Phycol.* **12**, 44 (1976).
9. L. J. Spencer, R. T. Barber, R. A. Palmer, in *Food and Drugs from the Sea*, L. R. Worthen, Ed. (Marine Technology Society, Washington, D.C., 1973), p. 203.
10. D. M. McKnight and F. M. M. Morel, *Limnol. Oceanogr.* **24**, 823 (1979).
11. M. A. Anderson and F. M. Morel, *Mar. Biol. Lett.* **1**, 263 (1980).
12. A quantitative test for hydroxamate siderophores [T. Z. Csaky, *Acta Chem. Scand.* **2**, 450 (1948); A. H. Gillam, A. G. Lewis, R. J. Andersen, *Anal. Chem.* **53**, 841 (1981)].
13. B. F. Burnham and J. B. Neilands, *J. Biol. Chem.* **236**, 554 (1961).
14. R. Johnston, *J. Mar. Biol. Assoc. U.K.* **44**, 87 (1964); R. T. Barber and J. H. Ryther, *J. Exp. Mar. Biol. Ecol.* **3**, 191 (1969); R. T. Barber, in *Trace Metals and Metal-Organic Interactions in Natural Waters*, P. C. Singer, Ed. (Ann Arbor Science, Ann Arbor, Mich., 1973), p. 321; R. T. Barber, R. C. Dugdale, J. J. MacIsaac, R. L. Smith, *Invest. Pesq.* **25**, 171 (1971).
15. J. Lewin and C.-H. Chen, *Limnol. Oceanogr.* **16**, 670 (1971).
16. The North East Pacific Culture Collection is maintained at the University of British Columbia and contains more than 60 dinoflagellate isolates.
17. A. H. Gillam, A. G. Lewis, M. J. LeBlanc, R. J. Andersen, unpublished work.
18. J. B. Neilands, in *Inorganic Biochemistry*, J. B. Neilands, Ed. (Academic Press, New York, 1973), vol. 1, p. 2.
19. Continuous light was provided by daylight fluorescent bulbs at an irradiance of 160 $\mu E \cdot m^{-2} \cdot sec^{-1}$ and a temperature of 18°C. Cells were grown with constant stirring (60 or 120 rev/min).
20. P. J. Harrison, R. E. Waters, F. J. R. Taylor, *J. Phycol.* **16**, 28 (1980).
21. F. M. M. Morel, J. G. Rueter, D. M. Anderson, R. R. L. Guillard, *ibid.* **15**, 135 (1979).
22. H. Narahara, *J. Soc. Brew. Jpn.* **65**, 340 (1970).
23. C. J. Carrano and K. N. Raymond, *J. Am. Chem. Soc.* **100**, 5371 (1978).
24. J. B. Neilands, *Struct. Bond.* **1**, 59 (1966).
25. R. J. Andersen, M. J. LeBlanc, F. W. Sum, *J. Org. Chem.* **45**, 1170 (1980); C. G. Trick, P. J. Harrison, R. J. Andersen, *Can. J. Fish. Aquat. Sci.* **38**, 864 (1981).
26. W. D. P. Stewart, *Annu. Rev. Microbiol.* **34**, 497 (1980).
27. E. Steemann Nielsen and S. Wium-Andersen, *Mar. Biol.* **6**, 93 (1970); S. A. Huntsman and R. T. Barber, *J. Phycol.* **11**, 10 (1975); W. G. Sunda and R. R. L. Guillard, *J. Mar. Res.* **34**, 511 (1976).

* To whom correspondence should be addressed.

15 June 1982; revised 12 October 1982

Multiple Sclerosis: Distribution of T Cell Subsets Within Active Chronic Lesions

Abstract. *The distribution of T cells and T cell subsets was examined within the human central nervous system in active lesions from seven patients with chronic multiple sclerosis. The monoclonal antibodies anti-T11, anti-T4, and anti-T8 were used to detect total (whole) T cells, helper T cells, and suppressor-cytotoxic T cells, respectively, and a monoclonal antibody against human Ia was used for macrophages and B cells. Lesion progression was associated with large numbers of $T4^+$ cells at the lesion margin and these extended great distances into the adjacent normal-appearing white matter. $T8^+$ cells were most commonly concentrated around the lesion margin and displayed a preferential perivascular distribution. Within the lesion center, only a few T cells were found. Ia^+ macrophages were most numerous within the centers of active lesions and were always present in the adjacent normal white matter. The monoclonal antibodies to T cells did not cross-react with glial cells including oligodendrocytes. These results indicate that $T4^+$ cells are actively involved in lesion extension and Ia^+ cells, in demyelination.*

Evidence from several sources has implicated cell-mediated immunity in multiple sclerosis (MS), the paradigm of the human demyelinating diseases. For example, levels of circulating T cell subsets fluctuate according to disease activity (*1*), lymphocyte studies support sensitization against nervous tissue (*2*), and the lesion pathology is similar to that occurring during T cell–dependent autoimmune demyelination (*3*). However, with the exception of a few studies on humoral factors and plasma cells in MS (*4*), little is known about immunologic events at the level of the target organ, the central nervous system (CNS). Lacking in MS (and, indeed, in any CNS condition) are studies in situ on T cells and their subsets, since these cells have never been identified within human nervous tissue. The present study has demonstrated T cell involvement in the brains of patients with MS by means of monoclonal antibodies against whole T cells, T cell subsets, and Ia-positive cells (macrophages and B cells). In CNS tissue containing active chronic MS lesions, that is, chronically demyelinated, gliotic lesions displaying abundant inflammation at the margins, we found distinct variations in T cell distribution in the CNS, suggesting that certain T cell subsets predominate at different stages of lesion development.

Samples of CNS tissue were obtained at autopsy from seven patients with documented MS for 3 to 13 years. For control purposes, CNS tissue was taken from one case each of systemic lupus erythematosus (SLE), leukemia, and carcinoma of the lung. Seventy blocks of MS tissue containing grossly visible le-