11, 1645 (1972); W. D. Comper and T. C. Laurent, *Physiol. Rev.* **58**, 255 (1978). 2261 (1978).
20. B. N. Preston, T. C. Laurent, W. D. Comper, G. J. Checkley, *Nature (London)* **287**, 499 (1980); G. S. Harper, W. D. Comper, B. N. Preston, *J. Biol. Chem.* **259**, 10582 (1984).
21. G. Nicolis and I. Prigogine, *Self-Organization in Non-Equilibrium Systems* (Wiley, New York, 1977).
22. Macroscopic disturbances do not occur at the boundary of the interposed collagen matrices in the absence of translocatable cells or particles (Fig. 5). Nevertheless, local effects at the boundary, such as the diffusion of fibronectin into the primary gel, could trigger macroscopic organized behavior by a "reactive" component, such as cells or beads capable of binding fibronectin. The mean square distance traveled by a diffusible substance, such as the fibronectin dimer, obeying Fick's law, is $\Delta X^2 = 2DT$. A reasonable estimate for the diffusion coefficient (D) of fibronectin is 2×10^{-7} cm^2/sec (*35*). This was also confirmed by M. Rocco and L. Zardi. *J. Cell Biol.* **97**, 324 (Abstr.) (1983). Therefore, during the time (T) of 10 seconds, after which translocation begins, fibronectin molecules could have diffused an average of 2×10^{-3} cm (one cell diameter) into the primary gel. The time course of translocation initiation (Fig. 4a) is therefore consistent with a reaction-diffusion process involving fibronectin (*35*).
23. D. R. Critchley, M. A. England, J. Wakeley, R. O. Hynes, *Nature (London)* **280**, 498 (1979); J. Wakely and M. A. England, *Proc. Roy. Soc. London Ser. B* **206**, 329 (1979).
24. J. A. Weston, *Adv. Morphol.* **8**, 41 (1970); J. A. Weston, in *Cell Behavior*, R. Bellairs, A. Curtis, G. Dunn, Eds. (Cambridge Univ. Press, Cambridge, 1982), p. 429; N. Le Douarin, *Curr. Top. Dev. Biol.* **16**, 32 (1980); D. M. Noden, in *Current Research Trends in Prenatal Craniofacial Development*, R. M. Pratt and R. L. Christiansen, Eds. (Elsevier/North-Holland, New York, 1980), p. 3.
25. J. W. Saunders, Jr., *J. Exp. Zool.* **108**, 363 (1948); in *Vertebrate Limb and Somite Morphogenesis*, D. A. Ede, J. R. Hinchliffe, M. Balls, Eds. (Cambridge Univ. Press, Cambridge, 1977), p. 1.
26. H. B. Fell and R. G. Canti, *Proc. Roy. Soc. London Ser. B* **116**, 316 (1934); P. V. Thorogood and J. R. Hinchliffe, *J. Embryol. Exp. Morphol.* **33**, 581 (1975).
27. E. D. Hay and J.-P. Revel, *Fine Structure of the Developing Avian Cornea (Monographs in Developmental Biology*, A. Wolsky and P. S. Chen, Eds. (Karger, Basel, 1969).
28. M. Kürkinen, K. Alitalo, A. Vaheri, S. Stenman, L. Saxén, *Dev. Biol.* **69**, 589 (1979).
29. R. Ross, *Biol. Rev.* **43**, 51 (1968); *Sci. Am.* **220**, 40 (June 1969).
30. P. Straüli and L. Weiss, *Eur. J. Cancer* **13**, 1 (1977); B. P. Toole, C. Biswas, J. Gross, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 6299 (1979).
31. P. C. Wilkinson, *Chemotaxis and Inflammation* (Churchill Livingstone, London, 1975); J. I. Gallin and P. G. Quie, Eds., *Leukocyte Chemotaxis* (Raven, New York, 1978); D. L. Boros in *Lymphokines 3*, E. Pick, Ed. (Academic Press, New York, 1981), p. 257.
32. B. W. Mayer, Jr., E. D. Hay, R. O. Hynes, *Dev. Biol.* **82**, 267 (1981).
33. D. Newgreen and J.-P. Thiery, *Cell Tissue Res.* **211**, 269 (1980); D. F. Newgreen, I. L. Gibbins, J. Sauter, B. Wallenfels, R. Wütz, *ibid.* **221**, 521 (1982).
34. P. Weiss, *Exp. Cell Res.* **8** (Suppl.), 260 (1961); R. O. Hynes, in *Cell Biology of Extracellular Matrix*, E. D. Hay, Ed. (Plenum, New York, 1981), p. 295.
35. S. A. Newman and H. L. Frisch, *Science* **205**, 662 (1979).
36. J. J. Tomasek, J. E. Mazurkiewicz, S. A. Newman, *Dev. Biol.* **90**, 118 (1982); S. A. Newman, H. L. Frisch, M. A. Perle, J. J. Tomasek, in *Morphogenesis and Pattern Formation*, T. G. Connelly, L. L. Brinkley, B. M. Carlson, Eds. (Raven, New York, 1981), p. 163.
37. G. C. Wood and M. K. Keech, *Biochem. J.* **57**, 588 (1960).
38. W. Dessau, H. von der Mark, K. von der Mark, S. Fischer, *J. Embryol. Exp. Morphol.* **57**, 51 (1980).
39. We thank K. M. Yamada and S. K. Akiyama (NCI, NIH) for gifts of fibronectins, T. Linsenmayer for the type II collagen, A. Springer for advice with photography and for discussions, and H. Godfrey, R. Melamede, and W. Ross for suggestions. Supported in part by a grant from the New York Medical College Intramural Research Program, and NIH by grant HD18148.

2 July 1984; accepted 14 February 1985

High-Affinity Uptake of Serotonin into Immunocytochemically Identified Astrocytes

Abstract. *Primary cultures of astrocytes from neonatal rat brain were incubated with tritiated serotonin. After fixation they were stained by immunofluorescence for the astrocyte-specific marker glial fibrillary acidic protein and processed for autoradiography. Silver grain density was increased over cells positive for glial fibrillary acidic protein and was reduced to background levels when sodium was omitted from the medium or the specific inhibitors of serotonin uptake fluoxetine and chlorimipramine were present. The results indicate that mammalian astrocytes can take up serotonin by a sodium-dependent, high-affinity system previously thought to be the exclusive property of serotonergic nerve endings.*

The action of most neurotransmitters released in the central nervous system is thought to be terminated mainly through reuptake by nerve endings (*1*). However, many synapses are surrounded by astrocytic processes (*2*), which could also serve as sites of uptake. Indeed, astrocytes have active systems for taking up amino acid transmitters. Many of the studies showing this were done with cultured glial cells (*3*), but uptake of exogenous labeled amino acids by astrocytes

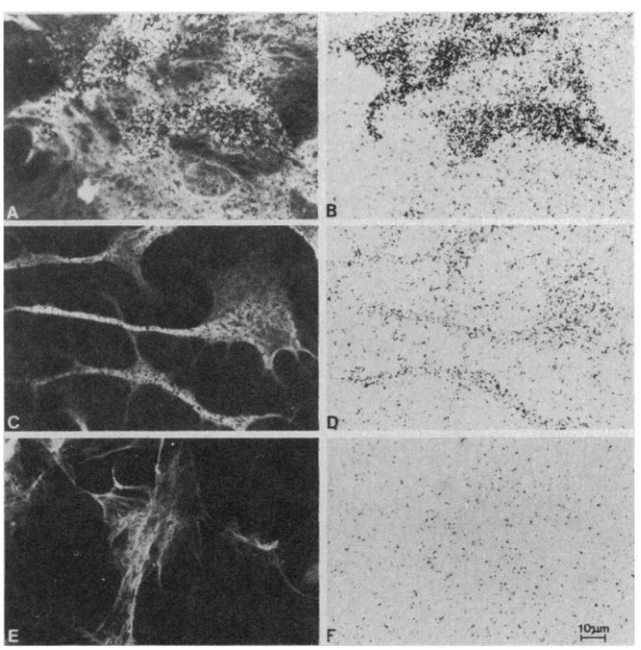

Fig. 1. Glial fibrillary acidic protein immunofluorescence (A, C, and E) and bright-field autoradiography of [^{3}H]serotonin uptake (B, D, and F) in the presence of Na^+ (A to D) and in medium in which Na^+ was replaced by choline (E and F). The cells in (A) and (B) lack processes and those in (C) and (D) are process-bearing. Primary astrocyte cultures (5 weeks in culture) were prepared from the cerebral cortices of newborn rat pups after removal of the meninges (*19*). The cells were grown on pairs of glass cover slips in 60-mm plastic dishes (Corning) in Eagle's basal medium with 10 percent fetal calf serum, supplemental vitamins and amino acids, and penicillin and streptomycin (*19*) and were used within 6 weeks. For combined autoradiography and immunocytochemistry the growth medium was removed and the cells were washed three times in buffered medium containing the following (in millimoles per liter): Na^+ (145), K^+ (4.5), Mg^{2+} (0.4), Ca^{2+} (1.3), Cl^- (127), SO_4^{2-} (0.4); HCO_3^- (25), and glucose (10) (*p*H 7.4). The cells were first incubated for 20 minutes in the same medium with $10^{-4}M$ pargyline and $10^{-5}M$ ascorbate with additions or changes as indicated in a 5 percent CO_2 and 95 percent air atmosphere at 37°C. The cells were then incubated in 2 ml of the medium containing 0.3 μM [^{3}H]serotonin (specific activity, 9 to 23 Ci/mmol; Amersham) for a further 30 minutes. To stop uptake the cells were rapidly washed seven times with 2 ml of ice-cold phosphate-buffered saline (PBS). The cells were then fixed in 4 percent paraformaldehyde and 0.25 percent glutaraldehyde in 0.1M cacodylate buffer for 15 minutes at 0°C. The cover slips in the dish were then rinsed five times with 2 ml of ice-cold PBS to remove the fixative, put into acetone at −10°C for 3 minutes to render their plasma membranes permeable to antibodies, and rinsed twice in PBS. They were then incubated for 30 minutes with a 1:200 dilution of monoclonal mouse antibody (immunoglobulin G) to human GFAP (Amersham), rinsed three times in PBS, incubated for 30 minutes with a 1:20 dilution of a rhodamine-labeled rabbit antibody to mouse immunoglobulin G, and rinsed three times in PBS. The cover slips were mounted with Fluoromount on microscope slides (cells facing up) and air-dried at 4°C overnight. The slides were dipped in Kodak NTB2 at 41°C, air-dried for 1 hour, and left at 4°C for 11 days. Then the slides were developed in Dektol (1:2 dilution) for 2 minutes, placed in 1 percent acetic acid for 30 seconds, fixed in Kodak fixer for 4 minutes, and finally rinsed in three water baths for 5 minutes each. The cells were viewed with a Nikon Labophot microscope with a ×50 oil-immersion lens, a xenon 75-W light source, and appropriate filters for rhodamine epifluorescence. Controls in which antibody to GFAP was absorbed with purified bovine GFAP showed no detectable fluorescence.

in situ has been shown by autoradiography (*4*) or by glial cell fractions from adult brain tissue (*5*). Uptake of monoamine transmitters by mammalian astrocytes in situ has not been detected (*6*), except for one mention of uptake of serotonin into astrocytic processes in the cat medulla (*7*). Uptake of serotonin into glia was also reported in the predominantly glial filum terminale of the frog (*8*). Uptake of both catecholamines and serotonin has been observed, however, in a number of glial cell cultures by measuring uptake of radiolabeled transmitters (*9*), but such studies represent uptake into transformed cell lines or average uptake into primary cultures, which may contain various proportions of other cell types. In the study reported here we demonstrated, by combined use of autoradiography and immunocytochemistry, uptake of [^{3}H]serotonin by cells in primary cultures from neonatal rat brain which also showed immunofluorescence for the astrocyte-specific marker glial fibrillary acidic protein (GFAP) (*10*). In addition, [^{3}H]serotonin labeling was sensitive to the specific serotonin uptake inhibitor fluoxetine and the tricyclic antidepressant chlorimipramine and to omission of Na^+ from the medium, clearly identifying the uptake mechanism as a high-affinity type (*1*).

Immunofluorescence photomicrographs of cultures incubated with [^{3}H]-serotonin under control conditions and fixed and stained for GFAP are shown in Fig. 1, A and C. Pargyline was present to inhibit the monoamine oxidase present in primary astrocyte cultures (*11*). The same cultures were also processed for autoradiography, and bright-field photomicrographs of the same fields as in Fig. 1, A and C, are shown in Fig. 1, B and D, respectively. Figure 1, E and F, shows immunofluorescence and bright-field views, respectively, after uptake of [^{3}H]serotonin from medium in which Na^+ had been omitted. Figure 1, A to D, shows localization of grains over GFAP-positive cells. Uptake was seen in both GFAP-positive "flat" cells (Fig. 1, A and B) and process-bearing cells (Fig. 1, C and D). Uptake into GFAP-positive cells was not uniform, however, and in Fig. 1, A and B, intensely labeled cells that partially mask the GFAP immunofluorescence can be distinguished from neighboring GFAP-positive cells that show lower silver grain densities. These differences may reflect specialization of serotonin uptake in different astrocytes in vivo. Such variation in uptake was also recently reported in autoradiographic studies of DL-[^{14}C]α-aminoadipic acid uptake into astrocytes in culture (*12*), and we have found that astrocyte cultures prepared from different brain regions show different capacities for Na^+-dependent uptake of [^{3}H]serotonin (*13*). Alternatively, since we had to use less than optimum fixation conditions to preserve antigenicity (*12*) [4 percent paraformaldehyde and 0.25 percent glutaraldehyde rather than 4 percent glutaraldehyde], we observed less than optimum uptake and variation in grain density may have been due to differential loss of label during fixation. The cells in the cultures were 90 to 95 percent GFAP-positive, and about 50 percent of these cells showed grain density that was above the background level. Reduction in grain density over the cells to background levels because of omission of Na^+ from the medium strongly suggests that the uptake mechanism is of the high-affinity type (*1*). This conclusion is also supported by the low concentration of [^{3}H]serotonin used ($3 \times 10^{-7} M$) and a recent kinetic study of [^{3}H]serotonin uptake in astrocyte cultures showing Na^+ and Cl^- dependence, a Michaelis constant for [^{3}H]serotonin of around 0.4 μM, and the expected rank order of inhibition by a variety of clinically effective antidepressants (*14*).

The effects of fluoxetine and chlorimipramine, specific inhibitors of [^{3}H]serotonin uptake (*1*), on the autoradiographic localization of [^{3}H]serotonin are shown in Fig. 2. Figure 2, A and B, shows control GFAP immunofluorescence and grain density in this experiment. The effects of fluoxetine and chlorimipramine are illustrated in Fig. 2, C to F, and the effect of omission of Na^+ is shown in Fig. 2, G and H. Uptake under control conditions and in the absence of Na^+ were included because in the experiment represented in Fig. 2 we used 4 percent paraformaldehyde without glutaraldehyde to fix the tissues, while in the experiment represented in Fig. 1 we used paraformaldehyde plus 0.25 percent glutaraldehyde. It is clear that the presence of the inhibitors and the omission of Na^+ reduced grain density over the cells to the background level, as seen in the dark areas where no cells were present.

While these findings indicate that [^{3}H]serotonin is taken up by mammalian astrocytes in culture, several autoradiographic studies of [^{3}H]serotonin uptake in the brain have not detected a glial component (*6*). Surgical lesions of the midbrain raphe nuclei or chemical le-

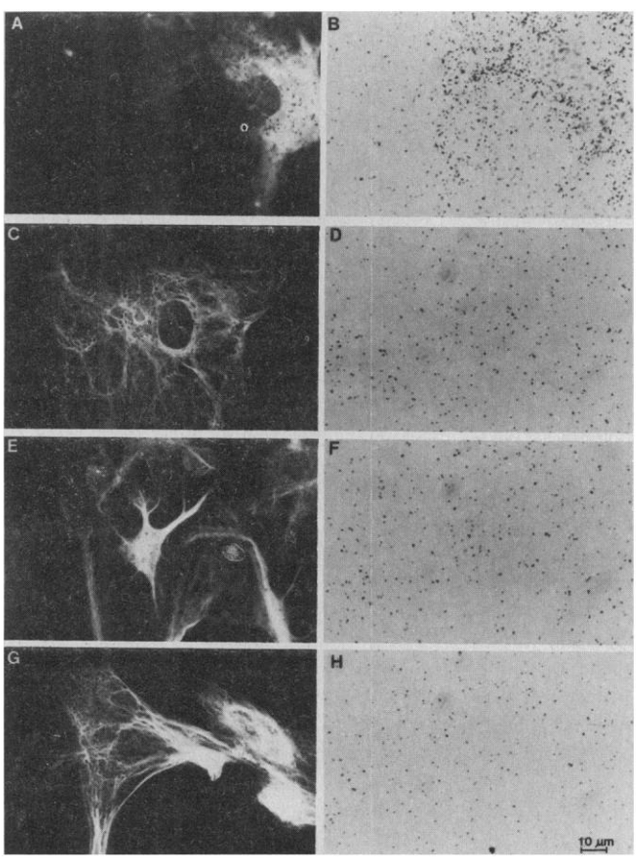

Fig. 2. Effects of fluoxetine and chlorimipramine on [^{3}H]-serotonin, as shown by autoradiography. GFAP immunofluorescence (A, C, E, and G) and [^{3}H]serotonin autoradiography for the same fields (B, D, F, and H). The micrographs show uptake of $3 \times 10^{-7} M$ [^{3}H]serotonin for 30 minutes in HCO_3^--buffered medium (see legend to Fig. 1) alone (A and B), in medium with $10^{-7} M$ fluoxetine (C and D), in medium with $10^{-7} M$ chlorimipramine (E and F), and in Na^+-free medium (G and H). The methodology was as described in the legend to Fig. 1, except cells (6 weeks of age) were fixed with 4 percent paraformaldehyde without glutaraldehyde for 30 minutes at room temperature and were developed after 26 days at 4°C. The grain density in (D), (F), and (H) over both the fluorescent cells and the fluorescent-free areas corresponds to the background level, since the black areas contained no cells (as determined by phase microscopy).

sions by injection of 5,6- or 5,7-dihydroxytryptamine [which appear to selectively destroy serotonergic neurons (*15*)] also selectively inhibited high-affinity uptake of serotonin (*16*). Although such studies certainly suggest a more intense uptake or storage in nerve endings, they cannot rule out a contribution of high-affinity glial uptake associated mainly with subsequent metabolic degradation by monoamine oxidase, since in some studies monoamine oxidase inhibitors were not added and light microscopy does not clearly distinguish between neuronal and glial processes in the neuropil. It is also uncertain whether the lesion studies were completely specific for neurons. Thus destruction of serotonergic neurons may have indirect effects on astroglia surrounding such nerve endings. Also, prolonged exposure of cultures to 5,7-dihydroxytryptamine inhibits their high-affinity uptake of [^{3}H]serotonin (*17*).

In conclusion, our results show high-affinity uptake of [^{3}H]serotonin by immunocytochemically identified astrocytes in primary culture. If our results with normal astrocytes in culture apply to astrocytes in situ, then uptake by such cells is likely to be a significant route for the termination of action of serotonin and thus a possible site of action for drugs such as the antidepressants. Uptake into astroglia presumably occurs in addition to reuptake by nerve endings. The relative contributions of neurons and astroglia to uptake could be assessed if selective inhibitors of astroglial and neuronal uptake were found, and screening for such compounds could conveniently be done in astroglial and neuronal cultures and highly purified synaptosomal preparations. It is also possible that astrocytic uptake plays a role in the etiology of psychiatric diseases involving serotonin (*18*).

H. K. KIMELBERG
Division of Neurosurgery and
Departments of Anatomy and
Biochemistry, Albany Medical College,
Albany, New York 12208

D. M. KATZ
Division of Neurosurgery,
Albany Medical College

References and Notes

1. S. N. Snyder, *Biol. Psychiatry* **2**, 367 (1970); L. L. Iversen, *Biochem. Pharmacol.* **23**, 1927 (1974); F. Fonnum *et al.*, in *The Cell Surface and Neuronal Function*, C. W. Cotman, G. Poste, G. L. Nicolson, Eds. (North-Holland, New York, 1970), pp. 455–504; F. Fonnum, *J. Neurochem.* **42**, 1 (1984).
2. A. Peters, S. L. Palay, H. de F. Webster, *The Fine Structure of the Nervous System: The Neurons and Supporting Cells* (Saunders, Philadelphia, 1976), pp. 231–263; S. Palay and V. Chan-Palay, *Cerebellar Cortex: Cytology and Organization* (Springer, Berlin, 1974).
3. B. K. Schrier and E. J. Thompson, *J. Biol. Chem.* **249**, 1769 (1974); V. J. Balcar, J. Borg, P. Mandel, *J. Neurochem.* **28**, 87 (1977); A. Schousboe, G. Svennby, L. Hertz, *ibid.* **29**, 999 (1977); D. L. Martin and W. Shain, *J. Biol. Chem.* **254**, 7076 (1979); D. N. Currie and G. R. Dutton, *Brain Res.* **99**, 473 (1980); J. Drejer, O. M. Larsson, A. Schousboe, *Exp. Brain Res.* **47**, 259 (1982); G. Levi *et al.*, *Dev. Brain Res.* **10**, 227 (1983).
4. T. Hökfelt and Å. Ljungdàhl, *Adv. Biochem. Psychopharmacol.* **6**, 1 (1972); J. de Barry *et al.*, *Neuroscience* **7**, 1289 (1982); G. P. Wilkin, J. Garthwaite, R. Balazs, *Brain Res.* **244**, 69 (1982).
5. F. A. Henn and A. Hamberger, *Proc. Natl. Acad. Sci. U.S.A.* **68**, 2686 (1971); F. A. Henn, M. N. Goldstein, A. Hamberger, *Nature (London)* **249**, 663 (1974); R. D. Gordon and R. Balazs, *J. Neurochem.* **40**, 1090 (1983).
6. G. K. Aghajanian and F. E. Bloom, *J. Pharmacol. Exp. Ther.* **156**, 23 (1967); N. J. Lenn, *Am. J. Anat.* **120**, 377 (1967); K. Fuxe *et al.*, *Histochemie* **16**, 186 (1968); T. Hökfelt and Å. Ljungdàhl, *Prog. Brain Res.* **34**, 87 (1971); L. Descarries and Y. Lapierre, *Brain Res.* **51**, 141 (1974).
7. M. A. Ruda and S. Gobel, *Brain Res.* **184**, 57 (1980).
8. T. Ritchie, S. Glusman, B. Haber, *Neurochem. Res.* **6**, 441 (1981).
9. R. L. Suddith, H. T. Hutchison, B. Haber, *Life Sci.* **22**, 2179 (1978); E. W. Pelton *et al.*, *ibid.* **28**, 1655 (1981); M. Tardy *et al.*, *Dev. Neurosci.* **5**, 19 (1982); E. Hansson, *Brain Res.* **289**, 189 (1983); H. K. Kimelberg and E. W. Pelton, *J. Neurochem.* **40**, 1265 (1983); P. M. Whitaker, C. K. Vint, R. Morin, *ibid.* **41**, 1319 (1983).
10. A. Bignami, D. Dahl, D. C. Rueger, *Adv. Cell. Neurobiol.* **1**, 285 (1980); M. Schachner *et al.*, *J. Cell Biol.* **75**, 67 (1977); L. F. Eng and S. J. DeArmond, *Adv. Cell. Neurobiol.* **3**, 145 (1982); M. C. Raff, R. H. Miller, M. Noble, *Nature (London)* **303**, 390 (1983).
11. P. Levitt, J. E. Pintar, X. O. Breakefield, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 6385 (1982); P. H. Yu and L. Hertz, *J. Neurochem.* **39**, 1492 (1982); E. Hansson and A. Sellstrom, *ibid.* **40**, 220 (1983).
12. S. Huck, F. Grass, H. Hortnagl, *J. Neurosci.* **4**, 2650 (1984).
13. We recently found that, in astrocyte cultures prepared from the corpus striatum, cerebral cortex, and hippocampus of 1-day-old rat pups, the uptake of [^{3}H]serotonin over 30 minutes was 13.7, 10.0, and 5.1 pmol per milligram of protein, respectively, in the presence of Na^+ and 1.2, 1.4, and 1.3 pmol/mg in the absence of Na^+. The corpus striatum was also found to have the highest [^{3}H]serotonin uptake in a study of slices from different regions of rat brain [E. G. Shaskan and S. H. Snyder, *J. Pharmacol. Exp. Ther.* **175**, 404 (1970)].
14. H. K. Kimelberg and D. M. Katz, *Soc. Neurosci. Abstr.* **10**, 766 (1984).
15. G. R. Breese and B. R. Cooper, *Brain Res.* **98**, 517 (1975).
16. M. J. Kuhar, R. H. Roth, G. K. Aghajanian, *J. Pharmacol. Exp. Ther.* **181**, 36 (1972); A. Björklund, A. Nobin, U. Steveni, *Brain Res.* **53**, 117 (1973).
17. After exposure of cultures to $10^{-4}M$ 5,7-dihydroxytryptamine for 3 days in the absence of serum there was only a 10 percent reduction in cell protein relative to serum-free controls but a 47 percent reduction in the Na^+-dependent component of [^{3}H]serotonin uptake.
18. D. S. Charney, G. R. Heninger, D. E. Sternbert, *Arch. Gen. Psychiatry* **41**, 359 (1984); N. Brunello *et al.*, *Neuropharmacology* **21**, 1145 (1982).
19. M. V. Frangakis and H. K. Kimelberg, *Neurochem. Res.* **9**, 1685 (1984).
20. Supported by grant NS 19492 from the National Institute of Neurological and Communicative Disorders and Stroke. We thank G. Banker, R. Bourke, C. Bowman, P. Del Vecchio, D. Martin, J. Mazurkiewicz, W. Shain, and R. Waniewski for reading the manuscript and P. Del Vecchio and the Department of Ophthalmology, Albany Medical College, for generously allowing us to use their Nikon Labophot. We also thank J. Huston for a gift of purified bovine GFAP, Eli Lilly for fluoxetine, and CIBA Pharmaceuticals for chlorimipramine.

4 December 1984; accepted 26 February 1985

Retinal S Antigen Identified as the 48K Protein Regulating Light-Dependent Phosphodiesterase in Rods

Abstract. *Retinal S antigen chromatographically purified from whole retina, induces experimental autoimmune uveoretinitis in laboratory animals. The 48K protein, a soluble protein found in rod outer segments, is purified through its specific binding to photoexcited rhodopsin and is involved in the quenching of light-induced guanosine 3′,5′-monophosphate–phosphodiesterase activity. Biochemical, immunological, functional, and pathological tests showed that retinal S antigen and the 48K protein are identical.*

Certain inflammatory diseases of the retina and uvea are thought to involve autoimmune mechanisms, either as primary mechanisms or secondary to a local injury (*1*). Retinal S antigen, a soluble protein of about 50 kilodaltons (kD) found in abundance in the photoreceptor cells of the retina, has a role in the autoimmune response (*1, 2*). This protein has been isolated and purified from the retinas of different mammals (*2, 3*). Immunization of laboratory animals with a few micrograms of purified S antigen in adjuvants induces experimental autoimmune uveoretinitis, an ocular inflammatory disease (*2, 4, 5*).

We report that the S antigen is identical with the so-called 48K protein characterized in rod outer segments (ROS) by its light-dependent binding to the disk membrane (*6*). The 48K protein binds specifically to photoexcited and phosphorylated rhodopsin (R*-P) (*7*) and quenches the activity of the light-dependent guanosine 3′,5′-monophosphate (cyclic GMP)–phosphodiesterase (*8*). This suggests that the 48K protein has a regulatory role in the light-induced amplifying cascade that controls cyclic GMP hydrolysis in ROS—a major step in the phototransduction process (*9*). This protein is highly soluble in dark-adapted ROS and represents 2 to 7 percent of the total protein content of ROS (*10*).

We applied biochemical, functional, immunological, and pathological tests to compare S antigen, prepared from total retina by a standard procedure (*3*), with the 48K protein, purified from ROS by a procedure based on its binding to illuminated disk membranes (*11*).

Both proteins migrate in sodium dode-