hand, it is not always possible for an incoherent detector to distinguish the diffracted photons from those that are unscattered [J. N. Cuzzi and J. B. Pollack, *ibid.* **33**, 233 (1978)]. If the particles are large compared to the instrumental aperture, the UVS and PPS instruments do not have the angular resolution to separate the direct and diffracted waves. In this case, diffracted photons appear unscattered and the extinction measured by the stellar occultation is half the extinction measured in the radio. The rough numerical equality of the microwave and optical extinctions implies that about half of the cross-sectional area effective in reducing the starlight is in centimeter-sized and smaller particles. The vertical scales in Fig. 2 have been adjusted to reflect this difference.

13. For a discussion of the dependence of extinction efficiency on wavelength, see J. E. Hansen and L. D. Travis, *Space Sci. Rev.* **16**, 527 (1974).
14. E. A. Marouf *et al.*, *Icarus*, **54**, 189 (1983).
15. J. L. Elliot *et al.*, *Astron. J.* **86**, 444 (1981).
16. Uncertainties in the absolute scales of the imaging data made this procedure necessary for five points.
17. Theoretical precession calculated for mean radius of 87,500 km and J_2, J_4, J_6 from G. W. Null *et al.* [*Astron. J.* **86**, 456 (1981)].
18. Measurements of the ringlet shape and precession could not be obtained independently. Figure 3 was obtained by rotating all Voyager 1 measurements to the epoch of Voyager 1 closest approach, using the theoretical precession rate associated with measured values of J_2, J_4, J_6. These corrections are ~ 10°. The best-fit sinusoid to the Voyager 1 data on the outer edge was then determined. The Voyager 2 data were then corrected for differential rotation between Voyager 2 measurements, again ~ 10°, and all Voyager 2 data rotated together to give the best fit with the previously determined Voyager 1 curve. This rotation corresponds to 14.66° per day if the ambiguity in the total number of rotations is resolved by use of the approximately 11.7 rotations obtained from the theoretical value. Voyager 1 images provide sufficient information to determine the total number of rotations as between 10 and 13. Best-fit sine waves to the corrected data were then determined for both the inner and outer edges of the ringlet, constrained to have the same phase.
19. P. Goldreich and S. Tremaine, *Astron. J.* **84**, 1638 (1979).
20. J. B. Holberg, *ibid.* **87**, 1416 (1982).
21. J. N. Cuzzi, J. J. Lissauer, F. H. Shu, *Nature (London)* **292**, 703 (1981).
22. This work was supported by the National Aeronautics and Space Administration and by the Voyager Project. We thank S. Dermott, J. Elliott, F. Franklin, and P. Nicholson for helpful comments.

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5 April 1983; revised 6 July 1983

Neuron-Glia Adhesion Is Inhibited by Antibodies to Neural Determinants

Abstract. *Suspensions of embryonic chick neuronal cells adhered to monolayers of glial cells, but few neurons bound to control monolayers of fibroblastic cells from meninges or skin. Neuronal cell-glial cell adhesion was inhibited by prior incubation of the neurons with Fab' fragments of antibodies to neuronal membranes. In contrast, antibodies to the neural cell adhesion molecule (N-CAM) did not inhibit the binding. These results suggest that a specific adhesive mechanism between neurons and glial cells exists and that it is mediated by CAM's that differ from those so far identified.*

Interactions between neuronal and glial cells are believed to be of fundamental importance both in the development of the nervous system and in the mechanical and biochemical support of adult neural tissue (*1*). Neurites migrate over great distances by mechanisms that are believed to be dependent on glial cells for guidance and support (*2*), but the mechanisms responsible for these phenomena remain unknown. For example, Müller fibers in the retina and Bergmann glial fibers in the cerebellum have a long fibrous morphology spanning several cell layers and are closely associated with what appear to be migrating neurons (*1*). The search for molecules that might mediate such neuronal-glial interactions requires a specific assay, the development of which is reported below.

Previous studies on cell adhesion have concentrated mainly on homotypic cell adhesion, that is, the binding between similar or identical cells. For example, cell aggregation studies (*3*) have resulted in the isolation of distinct cell surface glycoproteins of different specificities called neural cell adhesion molecule (N-CAM) and liver cell adhesion molecule (L-CAM); these molecules mediate, respectively, homotypic adhesion between neuronal cells and between liver cells (*4*). The study of the molecular basis of cell-cell adhesion between differing cell types (heterotypic cell adhesion) cannot, however, be accomplished unambiguously in vitro with an aggregation assay but must rely on techniques in which specifically identified cells of each type interact in a pairwise manner (*5–8*). Moreover, the methodology must ensure that such interactions be potentially distinguishable at the molecular level from homotypic interactions among cells of each type.

The binding of cells in suspension to cells in a monolayer has been used to measure adhesion between homologous and heterologous cell types (*6, 8*). In the case of N-CAM in particular, both homotypic (neuron-neuron) and heterotypic (neuron-myotube) forms of cell adhesion have been observed; in both cases, adhesion was specifically inhibited by Fab' fragments of antibodies to N-CAM (*4, 8*). The relevance of such assays as models for physiological cell-cell adhesion has been demonstrated by a number of studies on N-CAM adhesion in more complicated experimental systems including histogenesis of the retina, neuronal fasciculation, and nerve-muscle adhesion (*8, 9*). In our present study, we have used such a heterotypic cell-cell binding assay to investigate the initial adhesion between neuronal and nonneuronal cells, focusing particularly on neuron-glia binding. Neuronal cells were found to bind specifically to glial cells and antibodies to neuronal cell surface determinants inhibited neuron-glia adhesion.

Single cell suspensions of neuronal cells were prepared from embryonic chick brain and retinal tissue in media free of Ca^{2+} and Mg^{2+} ions. The majority of these cells (85 to 90 percent) were identified as neurons on the basis that they contain N-CAM (*4*), a nerve-specific protein in cells from these chick tissues (*10*). Flat nonneuronal cells were purified from cell suspensions of neural tissues by their ability to adhere preferentially to collagen-coated substrates (*11*). These flat cells were obtained in culture free of neuronal cells, had a morphology that was typical of glial cells but different from fibroblasts (Fig. 1, a and c) (*7, 11*), and were not recognized by indirect immunofluorescence with the use of either monoclonal or polyclonal antibodies to N-CAM. Moreover, we have raised a monoclonal antibody that recognizes these flat cells in culture; it did not stain neurons and fibroblasts, and it stained astroglial-like cells in tissue sections by indirect immunofluorescence (*12*). These results strongly support the suggestion of others that the flat nonneuronal cells obtained from brain by similar procedures (*11*) are astroglia. It should be noted, however, that no completely satisfactory set of criteria is currently available for the recognition of chick astroglia in culture.

The extent of binding of fluorescently labeled neuronal cells in suspension to monolayers of forebrain glial cells during a 25-minute incubation period was much greater than the rate of binding to monolayers of fibroblasts (Fig. 1). No significant difference was detected in the adhesive specificity of neurons from brain or retina to monolayers of forebrain glia (Table 1, upper part). Embryonic glial cells from several parts of the central nervous system bound neurons in significant numbers. In contrast, other types of cells in monolayers, including those from the meninges, bound few cells. Neuronal cell binding was routinely performed in the absence of Ca^{2+} and the level of binding to glial cell monolayers was not significantly higher in the presence of

Ca^{2+} (Table 1). These results confirm studies from other laboratories on binding between neuronal and astroglial cells from the brain (*7*) and suggest that a specific adhesive mechanism exists mediating binding between neuronal and glial cells. The binding of about 2 percent of the neurons added at high input levels was found to be significant and was comparable to values observed in other heterotypic adhesions (*8*).

Previous studies on cell-cell adhesion have demonstrated the value of Fab′ fragments of specific antibodies prepared against cell surface molecules in inhibiting cell-cell adhesion, in defining cell-cell binding specificities, and in purifying glycoproteins involved in cell-cell adhesion (*3*, *13*). To obtain antibodies that might be specifically useful in the study of neuron-glia adhesion, we immunized rabbits with membranes purified from chick embryo brains; and the Fab′ fragments from the antiserums of the animals were then tested in our cell-cell adhesion assay. When incubated with neurons, these Fab′ fragments inhibited binding of neurons to monolayers of glial cells (Table 1, lower part). Binding of neuronal cells was not inhibited with Fab′ fragments prepared from serums of nonimmune rabbits (Table 1, lower part). Although some (but not all) antiserums to neuronal membranes were found to contain antibodies to N-CAM (anti-N-CAM) that blocked N-CAM–mediated adhesion in neuronal cell aggregation assays, their activity in our experiments could not be attributed to anti-N-CAM. Moreover, a mixture of anti-N-CAM

Table 1. Binding of neuronal cells to cells in monolayers; N.D., not done.

Heterotypic binding between		Fab′ present during binding*	Number of cells bound to monolayer		Inhibition by Fab′ (%)
Cell in monolayer	Neural cell in suspension		$-Ca^{2+}$	$+Ca^{2+}$	
Forebrain glia	Brain	None	110	121	
Forebrain glia	Retinal	None	124	129	
Hindbrain glia	Retinal	None	123	131	
Retinal glia	Retinal	None	115	N.D.	
Skin fibroblast	Retinal	None	9	27	
Meninges	Retinal	None	6	13	
Forebrain glia	Brain	Nonimmune	110	N.D.	
Forebrain glia	Brain	Anti–N-CAM	118	N.D.	0
Forebrain glia	Brain	Anti-brain	52	N.D.	53
Forebrain glia	Brain	Anti-(brain extract depleted of N-CAM)†	55	N.D.	50

*Binding was measured in duplicate samples to 1 mm^2 of the monolayer and the average of those numbers is shown. SME-medium was used for Ca^{2+}-free medium and Eagle's minimal essential medium with Earle's salts was used for Ca^{2+}-containing medium. Meninges were dissected from 10-day chick embryo brains, trypsinized, and cultured on collagen-coated tissue culture dishes. These cultures were free of neurons and had a fibroblastic morphology (see legend to Fig. 1). Monovalent Fab′ fragments were prepared from the IgG fraction of rabbit serum, and antibody to purified N-CAM was prepared as described (*4*). Polyspecific antibody (anti-brain) was obtained by biweekly injection of rabbits with membranes (10 mg of protein) from 10-day chick embryo brains, and rabbits were bled weekly after the third injection. As shown in the lower part of the table, neurons were first incubated in 1 ml of medium containing 1 mg of Fab′ for 15 minutes at 4°C. These results were compiled from eight individual experiments. †See text.

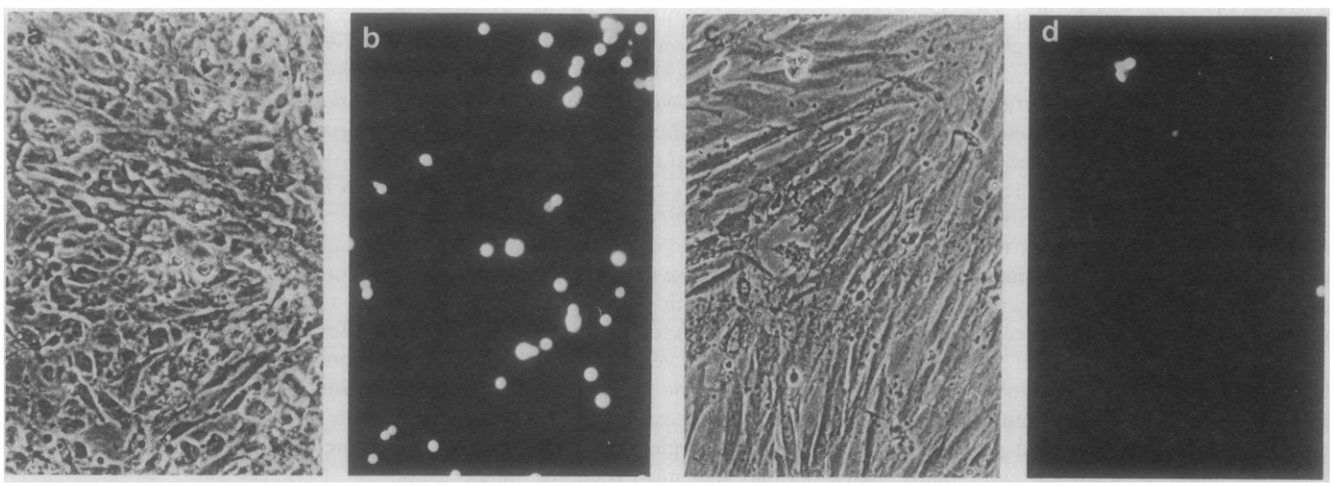

Fig. 1. Adhesion of neuronal cells to glia and fibroblasts. The suspension cell to monolayer cell binding assay (*6*, *8*) was used to measure binding between different cell types. Neurons in suspension (5×10^6) were added to a confluent monolayer of cells (35-mm dish) in 5 ml (final volume) of Eagle's minimal essential medium with Spinner salts containing deoxyribonuclease I (20 μg/ml) (SME-medium) and incubated (with rotation at 50 rev/min) for 25 minutes at 25°C. Unbound cells were removed by five successive washings in phosphate-buffered saline (PBS). (a) Phase and (b) fluorescence micrographs of forebrain neuronal cells bound to a monolayer of forebrain glial cells. (c) Phase and (d) fluorescence micrographs of forebrain neuronal cells bound to a monolayer of skin fibroblasts (×260). Suspensions of neurons were prepared from 10-day chick embryo tissues by trypsinization in the absence of Ca^{2+} and labeled with fluorescein diacetate (*6*). Flat nonneuronal cells were isolated from 10-day chick embryo neural tissues (brains were prepared free of meninges) that were dissected in Hepes-buffered calcium-free medium containing deoxyribonuclease I (100 μg/ml) (CMF), incubated at 37°C in CMF containing 0.1 percent trypsin and 1 m*M* EDTA for 45-minutes at 70 rev/min and centrifuged at 2000 rev/min in a clinical centrifuge for 3 minutes. The pellet was resuspended in 5 ml of Dulbecco's modified Eagle's medium (DMEM) containing 10 percent fetal calf serum and D-glucose (5 g/liter), and triturated 20 times with a Pasteur pipette. The cell suspension was layered on 8 ml of PBS containing 3.5 percent bovine serum albumin and centrifuged at 2000 rev/min for 3 minutes. The pellet was washed twice in DMEM, resuspended at approximately 10^6 cells per milliliter in DMEM supplemented with 25 percent conditioned medium, and transferred to tissue culture dishes that were coated with collagen (*8*). The cell suspension was incubated for 4 hours at 37°C with intermittent rotation (15 seconds every 6 minutes) at 90 rev/min. These conditions allowed neurons to aggregrate in suspension, while the glia adhered to and spread on the collagen-coated substrate. After 4 hours the medium was removed, the dishes were gently washed twice with PBS, and fresh medium was applied to the dishes. After 3 days, the medium was removed (conditioned medium) and replaced with fresh DMEM. Fibroblasts were prepared from the skin of 10-day chick embryos by trypsinization as described above for brain tissue and cultured in DMEM on collagen-coated tissue culture dishes. Cell-cell binding experiments were performed with secondary cultures of cells on 35-mm Costar dishes. Secondary cultures were prepared by trypsinization of primary monolayers (0.25 percent trypsin, containing 0.02 percent EDTA and collagenase at 1 mg/ml) for 10 minutes at 37°C. Cells were washed in fresh DMEM and grown on 35-mm collagen-coated dishes in DMEM.

Fab′ fragments from different rabbits, which strongly inhibited neuron-neuron interaction (*4*), did not inhibit adhesion (Table 1, lower part), suggesting that the N-CAM present on the surface of these neurons is not involved in neuron-glia adhesion.

Although it is possible that inhibition by the Fab′ fragments from antiserums to brain membranes is due to nonspecific coating of the cell surface, this is an unlikely explanation because anti-N-CAM Fab′ fragments had no effect on the adhesion, and N-CAM represents approximately 1 percent of the neuronal cell surface protein (*4*).

To address further the possibility that some form of N-CAM might be involved in neuron-glia adhesion, rabbits were immunized with a soluble fraction from brain membranes from which N-CAM antigens had been removed by affinity chromatography (*4*). The IgG fraction that was purified from these rabbit antiserums recognized several proteins from brain membranes but did not recognize N-CAM by immunoblotting techniques (*14*). Preincubation of neurons with Fab′ fragments from these antibodies reduced the number of neurons bound to monolayers of glial cells by 50 percent (Table 1). These results support the suggestion that N-CAM is not involved in adhesion between these neuronal and glial cells. Instead, it appears that antibodies against other neuronal cell surface molecules are responsible for the inhibition of neuron-glia adhesion. The identification and isolation of these molecules should permit a more detailed analysis of their relation to N-CAM, allow us to definitively exclude N-CAM as a ligand, and also permit the analysis of specific interacting molecules on the glial cell surface.

Although it is possible that the cells that specifically bind to monolayers of glial cells might represent a minor population of nonneuronal cells, this possibility seems unlikely because greater than 85 percent of the small round cells bound to the monolayer were N-CAM–positive by indirect immunofluorescence, indicating that they are neuronal cells and not glial cells. In view of the fact that N-CAM has not been seen on the surface of glial cells in culture, the possibility that glia-glia adhesion contributes in a major fashion to the counts of bound cells is unlikely.

In summary, adhesion was measured between embryonic chick neuronal and glial cells, each of which was identified by a different specific monoclonal antibody. Polyspecific rabbit antibodies to neuronal cell determinants have been obtained that block calcium-independent adhesion between neuronal and glial cells in vitro. The inhibitory activity of these antibodies cannot be attributed to the presence of anti-N-CAM. The results indicate that a specific mechanism mediates adhesion between neuronal and glial cells and suggest that neuronal cell surface antigens different from N-CAM are responsible for heterotypic cell-cell adhesion. This hypothesis could be tested by identifying specific cell surface molecules that neutralize the ability of Fab′ fragments against brain membranes to inhibit neuron-glia adhesion.

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References and Notes

1. S. S. Varon and G. G. Somjen, *Neurosci. Res. Prog. Bull.* **17**, 1 (1979).
2. P. Rakic, in *Brain Mechanisms in Mental Retardation*, N. A. Buchwald and M. Brazier, Eds. (Academic Press, New York, 1975), pp. 3–40.
3. G. M. Edelman, *Science* **219**, 450 (1983).
4. R. Brackenbury, J.-P. Thiery, U. Rutishauser, G. M. Edelman, *J. Biol. Chem.* **252**, 6835 (1977); J.-P. Thiery, R. Brackenbury, U. Rutishauser, G. M. Edelman, *ibid.*, p. 6841; S. Hoffman *et al.*, *ibid.* **257**, 7720 (1982); G. M. Edelman and C.-M. Chuong, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 7036 (1982); U. Rutishauser, S. Hoffman, G. M. Edelman, *ibid.*, p. 685; W. J. Gallin, G. M. Edelman, B. A. Cunningham, *ibid.* **80**, 1038 (1983).
5. B. T. Walther, R. Ohman, S. Roseman, *Proc. Natl. Acad. Sci. U.S.A.* **70**, 1569 (1973).
6. R. Brackenbury, U. Rutishauser, G. M. Edelman, *ibid.* **78**, 387 (1981).
7. M. Sensenbrenner and P. Mandel, *Exp. Cell Res.* **87**, 159 (1974); B. Foucaud, R. Reeb, M. Sensenbrenner, G. Gombos, *ibid.* **137**, 285 (1982).
8. M. Grumet, U. Rutishauser, G. M. Edelman, *Nature (London)* **295**, 639 (1982).
9. D. R. Buskirk, J.-P. Thiery, U. Rutishauser, G. M. Edelman, *ibid.* **285**, 488 (1980); U. Rutishauser and G. M. Edelman, *J. Cell Biol.* **87**, 370 (1980); U. Rutishauser, M. Grumet, G. M. Edelman, *ibid.* **97**, 145 (1983).
10. U. Rutishauser, J.-P. Thiery, R. Brackenbury, G. M. Edelman, *J. Cell Biol.* **79**, 371 (1978).
11. K. D. McCarthy and L. M. Partlow, *Brain Res.* **114**, 391 (1976); R. Adler, M. Manthrope, S. Varon, *Dev. Biol.* **69**, 424 (1979).
12. M. Grumet, J.-P. Thiery, G. M. Edelman, unpublished observations.
13. W. Frazier and L. Glaser, *Annu. Rev. Biochem.* **48**, 491 (1979); G. Gerisch, *Curr. Top. Dev. Biol.* **14**, 243 (1980).
14. H. Towbin, T. Staehelin, J. Gordon, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 4350 (1979).
15. Supported by NIH grants HD-16550, HD-09635, AI-11378, AM-04256, and a postdoctoral fellowship to M.G. AI-06414. We thank H. Hjelt and S. Igdaloff for technical assistance.

13 July 1983

In vivo Determination of the Pyridine Nucleotide Reduction Charge by Carbon-13 Nuclear Magnetic Resonance Spectroscopy

Abstract. *An intracellular coenzyme has been observed by carbon-13 nuclear magnetic resonance spectroscopy. The pyridine nucleotides in* Escherichia coli *were specifically labeled with carbon-13 from the biosynthetic precursor, nicotinic acid. The intracellular redox status and metabolic transformations of the pyridine nucleotides were examined under a variety of conditions. A highly reduced nicotinamide adenine dinucleotide pool was observed under anaerobic conditions only in cells that were cultured aerobically on glycerol.*

Since the initial use of isotopic labeling in combination with nuclear magnetic resonance (NMR) detection to study glucose metabolism by yeast cells (*1*), applications of NMR for in vivo metabolic studies have been extended to a wide variety of microorganisms, cells, perfused organs, and, most recently, intact animals (*2*). In general, the focus of these studies has been on metabolic regulation, since in most instances the pathways involved have been established for some time. In ^{13}C labeling studies, information pertinent to metabolic regulation may be obtained by direct observation of time-dependent signal intensities, direct observation of metabolic intermediates, and analysis of label distributions and multiplet intensities in the various metabolites (*3*). In parallel with the development of ^{13}C NMR for in vivo metabolic analysis, ^{31}P NMR has been shown to provide useful data on metabolic regulation in terms of intracellular *p*H and phosphonucleotide energy charge (*4*). Simultaneous ^{13}C and ^{31}P NMR detection appears to represent a very promising avenue for detailed studies of metabolic regulation in vivo (*5*). We report here the application of ^{13}C labeling and NMR detection to the study of an intracellular coenzyme pool, the pyridine nucleotides.

The redox status of the di- and triphosphopyridine nucleotide pools is an important regulatory parameter. Concentrations of important metabolic intermediates such as pyruvate-lactate, and metabolic flux through critical branching steps have been proposed to be dependent on the redox status of these two pools (*6*). Anderson and von Meyenburg (*7*) have suggested a convenient quantitative characterization of the status of these pools as the catabolic reduction charge (CRC), [NADH]/([NADH] + [NAD^+]), and the anabolic reduction charge (ARC), [NADPH]/([NADPH] + [$NADP^+$]), where NAD^+ is nicotinamide adenine dinucleotide and $NADP^+$