tremely unlikely that the synergistic activity of the AEF-supplemented cultures is due to a carry-over of residual AEF bound to the bone marrow cells (*12*). It is noteworthy that Cohen and Fairchild reported that bone marrow cells usually lost their synergistic activity after being cultured for 2 days, the period during which residual bone marrow lymphocytes usually die in such cultures. Thus, pre-T cells were present in AEF-supplemented, long-term cultures of murine bone marrow and absent in cultures not supplemented with AEF.

An adherent cell layer, which provides an essential microenvironment for the maintenance of stem cell activity (*2*), was established much faster in the presence of AEF. This could indicate that AEF mediates its effects, in part at least, by inducing rapid development of the adherent layer. Such a layer could then provide the microenvironment necessary for the self-renewal and differentiation of residual stem cells. However, we have evidence to suggest that AEF has additional effects in these cultures. Thus, even when cultures are established under optimal conditions with two consecutive inocula of bone marrow cells (*2*), the addition of AEF together with the second inoculum (at a time when an optimal adherent layer has already been established from the first inoculum) has a cumulative effect, manifested by markedly higher cell numbers and increased proliferative and CFUC-assayed stem cell activities, compared to conventional Dexter cultures not supplemented with AEF (*12*).

Our studies are related to other recent studies in which the potential for lymphoid stem cell differentiation in vitro was investigated. It was shown that cells derived from long-term Dexter cultures can reconstitute the lymphoid system of lethally irradiated recipients (*5*) and that such cultures contain cells possessing the enzyme terminal deoxynucleotidyltransferase (*18*), considered to be a marker of primitive lymphocytes or prothymocytes (*19*). While our studies were in progress, it was reported that Thy-1-bearing cells could be found in the bone marrow cultures themselves or after exposure to TCGF (*7*).

As mentioned above, AEF-containing supernatants have some unique biological effects on T lymphocytes (*10*), which are associated with the presence on AEF molecules of Ia antigenic determinants encoded by the I region of the murine major histocompatibility complex. The Ia molecules are involved in cell-cell recognition and stimulation phenomena in the immune system (*20*). Progenitors of myeloid, erythroid, and megakaryocytic cells in human bone marrow bear the human equivalent of the murine Ia antigens (*21, 22*); it is possible, therefore, that interactions between Ia-containing molecules and/or cells are also involved in the biological effects of AEF on the bone marrow cultures.

The results presented here, together with information concerning the effects of AEF on mature lymphocytes (*8–10*), strongly suggest that AEF may prove useful for inducing lymphoid (and other) cells to differentiate from progenitors in the bone marrow. This could provide a system for analyzing the events that occur during the differentiation of immunocompetent lymphocytes from early stem cell stages and perhaps a more efficient means of culturing bone marrow for transplantation.

AMNON ALTMAN
THOMAS D. GILMARTIN
DAVID H. KATZ

Department of Cellular and Developmental Immunology, Scripps Clinic and Research Foundation, La Jolla, California 92037

References and Notes

1. M. J. Cline and D. W. Golde, *Nature (London)* **277**, 177 (1979).
2. T. M. Dexter, T. D. Allen, L. G. Lajtha, *J. Cell. Physiol.* **91**, 335 (1977).
3. M. A. S. Moore and A. P. Sheridan, *Blood Cells* **5**, 297 (1979).
4. T. D. Allen and T. M. Dexter, *Differentiation* **6**, 191 (1976); N. Williams, H. Jackson, E. Rabellino, *J. Cell. Physiol.* **93**, 435 (1977); T. D. Allen, in *Stem Cells and Tissue Homeostasis*, B. I. Lord, C. S. Potten, R. Cole, Eds. (Cambridge Univ. Press, Cambridge, 1978), p. 217; J. F. Eliason, N. G. Testa, T. M. Dexter, *Nature (London)* **281**, 382 (1979).
5. J. W. Schrader and S. Schrader, *J. Exp. Med.* **148**, 823 (1978); E. V. Jones-Villeneuve and R. A. Phillips, *Exp. Hematol.* **8**, 65 (1980).
6. D. A. Morgan, F. W. Ruscetti, R. Gallo, *Science* **193**, 1007 (1976); F. W. Ruscetti, D. A. Morgan, R. C. Gallo, *J. Immunol.* **119**, 131 (1977).
7. E. V. Jones-Villeneuve, J. J. Rusthoven, R. G. Miller, R. A. Phillips, *J. Immunol.* **124**, 597 (1980); J. W. Schrader, I. Clark-Lewis, P. F. Bartlett, *J. Supramol. Struct. Suppl. 4* (1980) p. 130; G. G. Tertian, Y. P. Yung, M. A. S. Moore, *ibid.*, p. 136.
8. D. Armerding and D. H. Katz, *J. Exp. Med.* **140**, 19 (1974).
9. Z. Eshhar, D. Armerding, T. Waks, D. H. Katz, *J. Immunol.* **119**, 1457 (1977); D. Armerding, D. H. Sachs, D. H. Katz, *J. Exp. Med.* **140**, 1717 (1974); D. Armerding, R. T. Kubo, H. M. Grey, D. H. Katz, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 4577 (1975); D. Armerding, Z. Eshhar, D. H. Katz, *J. Immunol.* **119**, 1468 (1977).
10. A. Altman, T. E. Bechtold, J. M. Cardenas, D. H. Katz, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 3477 (1979); A. Altman, J. M. Cardenas, T. E. Bechtold, D. H. Katz, *J. Immunol.* **124**, 105 (1980); A. Altman, J. M. Cardenas, T. E. Bechtold, D. H. Katz, *ibid.*, p. 114; A. Altman, J. M. Cardenas, T. E. Bechtold, D. H. Katz, in *Proceedings of the Second International Lymphokine Workshop*, A. L. De Weck and M. Landy, Eds. (Academic Press, New York, 1980), p. 519; A. Altman and D. H. Katz, *Immunol. Rev.* **51**, 3 (1980).
11. K. A. Smith, *Immunol. Rev.* **51**, 337 (1980).
12. A. Altman, T. D. Gilmartin, D. H. Katz, *J. Supramol. Struct.*, in press.
13. J. E. Till and E. A. McCulloch, *Radiat. Res.* **14**, 213 (1961).
14. T. R. Bradley and D. Metcalf, *Aust. J. Exp. Biol. Med. Sci.* **44**, 287 (1966); D. H. Pluznik and L. Sachs, *J. Cell. Comp. Physiol.* **66**, 319 (1965).
15. J. W. Mier and R. C. Gallo, *Proc. Natl. Acad. Sci. U.S.A.*, **77**, 6134 (1980).
16. D. Metcalf, *Immunology* **21**, 427 (1971); D. Metcalf and M. A. S. Moore, *Hematopoietic Cells* (North-Holland, Amsterdam, 1971).
17. J. J. Cohen and S. S. Fairchild, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 6587 (1979).
18. J. W. Schrader, I. Goldschnider, F. J. Bollum, S. Schrader, *J. Immunol.* **122**, 2337 (1979).
19. A. Silverstone, H. Cantor, G. Goldstein, D. Baltimore, *J. Exp. Med.* **144**, 543 (1976).
20. D. H. Katz, *Lymphocyte Differentiation, Recognition and Regulation* (Academic Press, New York, 1977), pp. 530–597.
21. R. J. Winchester, P. A. Meyers, H. E. Broxmeyer, C. Y. Want, M. A. S. Moore, H. G. Kunkel, *J. Exp. Med.* **148**, 613 (1978).
22. M. A. S. Moore, personal communication.
23. This is publication 177 from the Department of Cellular and Developmental Immunology and publication 2145 from the Immunology Departments, Scripps Clinic and Research Foundation. Supported by NIH grant CA-25803 and National Foundation-March of Dimes grant 1-540. A.A. is a recipient of American Cancer Society faculty research award JFRA-17. We thank L. Gunnill, A. Hugus, and K. Dunn for preparing the manuscript.

22 August 1980

Dye Coupling and Possible Electrotonic Coupling in the Guinea Pig Neocortical Slice

Abstract. *Iontophoretic injection of the fluorescent dye Lucifer Yellow CH into single neurons of guinea pig neocortical slices resulted in the staining of more than one cell. Dye-coupled neuronal aggregates were found only in the superficial cortical layers and were often organized in vertical columns. Antidromic stimuli evoked all-or-none, subthreshold depolarizations in some superficial cells. These potentials were not eliminated by manganese and did not collide with spikes originating in the soma, suggesting that they arose from electrotonic interaction between superficial cortical neurons.*

Although interactions between neocortical neurons are believed to be mediated mainly by chemical synapses, there has been speculation that electrotonic coupling might also be present (*1*). Electrotonic transmission has been demonstrated in several mammalian subcortical structures (*2*), but there is little direct evidence for its occurrence in the cortex. However, gap junctions, the morphological substrates of electrotonic coupling, have been demonstrated in the motor cortex of primates (*3*). The fluorescent dye Lucifer Yellow CH crosses gap junc-

0036-8075/81/0102-0067$00.50/0

tions in a number of preparations (*4, 5*), and this phenomenon, called dye coupling (*5*), implies the existence of a direct low-resistance pathway between cells. We now report that aggregates of neurons in neocortical slices maintained in vitro are dye coupled, and we present electrophysiological evidence that they are electrotonically coupled as well.

Experiments were performed with slices from the guinea pig sensory-motor cortex, cut in the parasagittal or the coronal plane. Slices were 350 μm thick, 3 to 6 mm wide, and extended from the pial surface to the subcortical white matter. Techniques for removing the brain, slicing the tissue, and maintaining the slices in oxygenated Ringer solution warmed to 37°C were essentially the same as those used for hippocampal slices (*6*). For intracellular recordings, glass micropipettes were filled either with 4*M* potassium acetate (30 to 50 megohms) or with a 5 percent solution of the lithium salt of Lucifer Yellow CH in water (125 to 225 megohms) (*7*).

Eighteen neuronal impalements, in as many different slices, were sufficiently stable to allow the injection of the dye (*8*). Eleven of the injections were in the superficial cortical layers (< 400 μm below the pial surface), and eight of these resulted in distinct staining of more than one neuron (Fig. 1, A to D). Seven of the injections were in the deeper layers of the cortex (> 1200 μm below the pial surface), and all of these resulted in the staining of only a single neuron (Fig. 1E). In most instances the dye filled enough of the dendritic arborization to permit morphological characterization of the stained cells and determination of their relationship to one another.

Figure 1, A to D, shows examples of groups of stained neurons in the superficial cortical layers. In three slices, a single dye injection stained groups of two or three lamina III pyramidal cells, whose cell bodies were situated at the same depth below the pial surface, 20 to 50 μm apart, and whose dendritic arborizations overlapped considerably (Fig. 1D). In five other slices, dye-coupled groups consisted of three to six lamina II and lamina III neurons that were organized in columnar clusters perpendicular to the pial surface and parallel to the radial blood vessels (Fig. 1, A to C). In most instances, these neurons were identifiable as pyramidal cells, and their axons could often be traced downward as they passed through the deeper layers toward the subcortical white matter. Their dendritic processes overlapped extensively and could be traced as far as 100 μm to either side of a central row of clearly separated cell bodies (*9*).

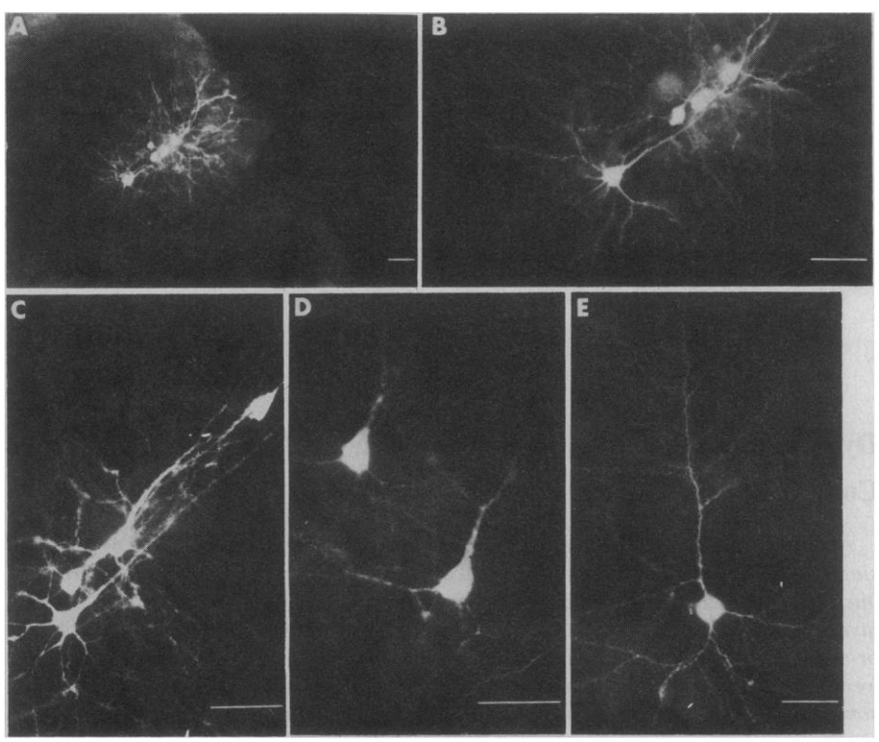

Fig. 1. Lucifer staining of neocortical neurons. (A) Intracellular iontophoresis of the dye into a single cell caused staining of a cluster of five neurons with overlapping dendrites that extend from lamina III to the pial surface. Note the vertically oriented columnar organization. (B) Higher magnification of the cluster in (A). One of the neurons is out of the plane of focus. (C) A column of four neurons was stained by iontophoretic injection of a single cell. The deepest neuron (lower left) is located 450μm beneath the pial surface, which runs perpendicular to the orientation of the apical dendrites. (D) Two superficial pyramidal neurons were stained by a single intracellular dye injection. The deeper cell (right) is 280 μm beneath the pial surface. (E) Staining of a single pyramidal neuron location 1200 μm beneath the pial surface. Scale bars, 50 μm.

In control experiments, Lucifer Yellow was ejected from a low-impedance (< 30 megohms) microelectrode into the extracellular space, with iontophoretic currents of 2 to 4 nA for more than 30 minutes; this procedure did not result in neuronal staining, confirming the results of other workers (*4, 5, 10*). Multiple staining was not due to incidental leakage of the dye into neurons penetrated and damaged by the microelectrode, since no cells were stained in slices in which many neurons were impaled and held briefly but no iontophoretic current was passed. Moreover, the neurons in Fig. 1C were stained upon first penetration of the slice by a Lucifer-filled microelectrode. It might be argued that passage of the dye from neuron to neuron was due to formation of aberrant connections after the tips of processes were severed during the slicing procedure; however, this could not explain the finding that multineuronal staining was limited to superficial neurons, even though the dendrites of deeper pyramidal cells branch extensively and extend well into the superficial layers. Some authors have suggested that multineuronal staining might be due to movement of dye across a chemical synapse after synaptic activation (*11*); this seems unlikely because dye coupling was present without electrical stimulation and was readily demonstrated in a slice bathed in a solution containing 3 m*M* Mn^{2+}, which blocks evoked chemical transmission in this preparation (Fig. 1C; see below).

Since many dye-coupled systems are also known to be electrically coupled (*4, 5, 12*), we sought evidence of electrotonic coupling in the neocortical slice. We used a modification of an indirect test for electrical interaction that entailed recording intracellularly from a single neuron while antidromically activating its coupled neighbors (*13*). An action potential generated in one neuron would be expected to propagate electrotonically to produce a small depolarization in the impaled cell.

Neurons were impaled in the superficial cortical layers, and antidromic stimuli were applied near the subcortical white matter, at least 1.5 mm from the recording site (Fig. 2A) (*14*). Chemical synaptic transmission was blocked by

bathing the slices for at least 1 hour in a solution containing 3 m*M* Mn^{2+} (*15*). The extracellular field potential recordings in Fig. 2B demonstrate the effectiveness of this treatment. In control solution, a single stimulus evoked an initial spike-like negative response with a short latency, and a subsequent slow negativity with several superimposed sharp waves (Fig. 2B, trace 1). These events were associated, in intracellular recordings, with antidromic spike invasion (*16*) and postsynaptic potential (PSP) generation, respectively (Fig. 2C, trace 1). Within 20 minutes after switching to the Mn^{2+}-containing bathing medium, the late negative wave (Fig. 2B, trace 2) and underlying PSP (Fig. 2C, trace 2) entirely disappeared.

Intracellular recordings in Mn^{2+}-treated slices were obtained from 15 superficial neurons that had membrane potentials of 65 to 76 mV and input resistances of 28 to 42 megohms. In four cells, low-intensity stimuli evoked small (< 5 mV) all-or-none subthreshold depolarizations (SD's) at fixed latencies of 1.5 to 3 msec (Fig. 2, D and E). These Mn^{2+}-resistant potentials had rise times of 1 msec or less and decayed gradually over 15 to 20 msec (see below). Their latencies and peak amplitudes were insensitive to intracellularly applied depolarizing or hyperpolarizing currents (Fig. 2F).

In view of the proximity of recording and stimulating sites, we considered the possibility that SD's were due to direct excitation of the impaled neuron by the stimulus current. This seems unlikely because SD's were not evoked when the stimulating electrodes were moved laterally and toward the pial surface of the slice to a location slightly closer to the recording site, but not in the direct path of axons descending from superficial cortical layers.

A collision test was performed to distinguish SD's from electrotonic manifestations of partial antidromic invasion in the impaled neuron. In the cell illustrated in Fig. 2, E, G, and H, the antidromic stimulus evoked two consecutive depolarizations that were readily distinguished in electrically differentiated traces: a small, brief potential with a 2-msec latency (Fig. 2E, arrows), and a subsequent SD. The antidromic stimulus was preceded at various intervals by an intracellularly evoked action potential. When the interval exceeded 3 msec, both depolarizing potentials were evoked (Fig. 2G). At shorter intervals (Fig. 2H), the early, brief potential was occluded, indicating its probable axonal origin. The SD did not collide with the preceding spike, although the amplitude and wave form of the SD were affected by it.

Taken together, our evidence suggests that the SD arose as a result of direct electrical interaction between the impaled cell and another neuronal element. For example, there might be electrotonic coupling with presynaptic afferent terminals, as has been proposed to explain unusual behavior of Ia excitatory postsynaptic potentials in cat spinal motoneurons (*17*). In view of the patterns of dye coupling described above, a more plausible explanation is electrotonic coupling with an antidromically activated neighboring neuron.

The all-or-none character of the SD suggests that it reflects antidromic activation either of a small group of neurons with similar antidromic thresholds or of one coupled neuron. This correlates well with the observed patterns of multineuronal dye filling, which show discrete neuronal aggregates, each consisting of only a small number of cells. It seems unlikely that the stimulus led to antidromic invasion of every neuron in a coupled aggregate since, in the presence of 3 m*M* Mn^{2+}, spike thresholds were quite high. Furthermore, some descending axons may have been disrupted by the slicing procedure. These explanations would also account for the discrepancy between the low incidence of SD's and the high incidence of dye coupling in the superficial layers.

One puzzling feature of the SD is the gradual time course of its decay (Fig. 2, D and E). The rapid rising phase is consistent with electrotonic propagation of a distant spike. However, the slow repolarization, which often followed a plateau of maintained depolarization (Fig. 2E), might be due to an active membrane response. Since dye-coupled groups of neurons had distinctly separated somata, coupling probably occurred at points of dendrodendritic contact. The slow repolarization of the SD might thus reflect a broadened action po-

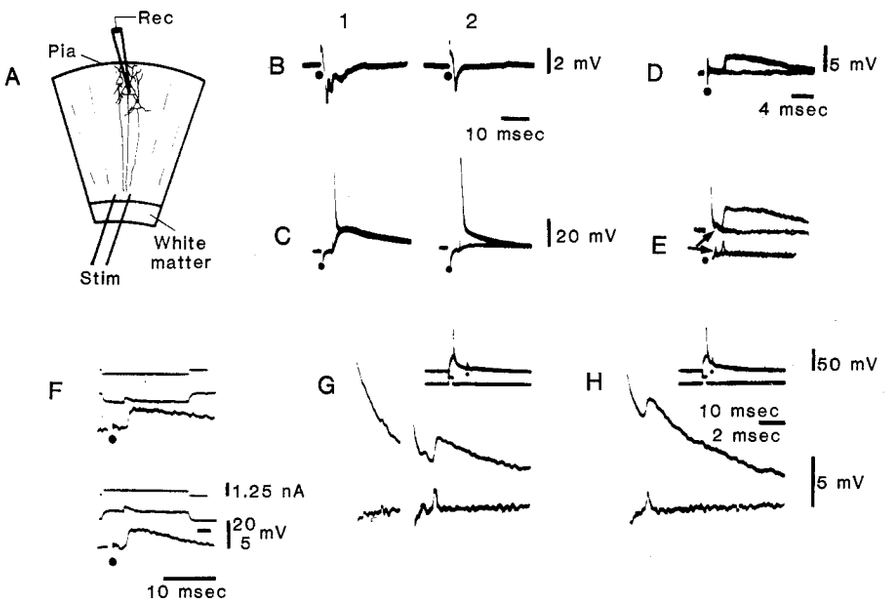

Fig. 2. (A) Diagram of experimental setup; *Stim*, stimulus site; *Rec*, recording site; *Pia*, pial surface. (B) Stimulus-evoked extracellular field potentials (trace 1) before and (trace 2) 25 minutes after transfer to bathing medium containing 3m*M* Mn^{2+}. Note disappearance of late, prolonged negativity. (C) Stimulus-evoked intracellular potentials in one neuron (trace 1) before and (trace 2) 5 minutes after transfer to solution containing 3m*M* Mn^{2+}. Each sweep shows superimposed traces at two stimulus intensities. In trace 1, the lower intensity stimulus evokes a PSP which is preceded by an antidromic spike when the intensity is increased. In trace 2, the PSP is blocked, but the antidromic spike persists. Subthreshold depolarization was not recorded in this cell. Time calibration in (B) applies to (B) and (C). (D) Superimposed traces to show SD evoked in all-or-none manner by antidromic stimuli of gradually increasing intensities. (E) Same as (D) for a different neuron. The lower trace was electrically differentiated to delineate the low-amplitude potential that precedes the SD (arrows). Calibrations in (D) apply to (D) and (E). (F) Effects of hyperpolarizing and depolarizing currents on the SD in same neuron as in (D). Upper traces, current monitor; middle traces, low gain and slow sweep speed; bottom traces, high gain and fast sweep speed. (G) and (H) Collision experiment in same neuron as in (E). Upper two traces, low gain and slow sweep speed. An intracellular depolarizing current pulse (2 msec, 0.75 nA) evokes a somatic spike that is followed at long (G) and short (H) intervals by an antidromic stimulus (dots). Third trace, expanded, high gain sweeps to show responses to antidromic stimulus. Bottom traces, electrically differentiated recording of third trace. At long interstimulus intervals (G), the antidromic stimulus evokes a brief, spikelike event followed by the SD. At shorter interstimulus intervals (H), only the SD is evoked. Calibrations in (H) apply to (G) and (H).

tential in the prejunctional dendrite or a local active response in the dendrite of the impaled neuron. Too little is known about the electrophysiological characteristics of neocortical dendrites to resolve this issue.

Our data provide both morphological and electrophysiological evidence for electrotonic coupling of superficial neurons in the neocortical slice. This form of intercellular communication may also be a feature of the neocortex in vivo (*18*), where it could be of great functional significance. It is intriguing that five of the dye-coupled aggregates were organized in vertically oriented columns, a structural arrangement of importance in current concepts of the mechanisms of neocortical information processing (*19*). Under normal conditions, the activities of electrotonically coupled neurons might not necessarily be highly synchronized, since complex interplay between electrotonic and chemical forms of transmission can result in a flexible repertoire of intercellular interactions (*13, 20*) On the other hand, in pathological conditions such as epilepsy, the presence of electrotonic connections might be an important factor in achieving the characteristic high degree of neuronal synchronization.

MICHAEL J. GUTNICK
DAVID A. PRINCE

Department of Neurology, Stanford University School of Medicine, Stanford, California 94305

References and Notes

1. K. J. Roney, A. B. Scheibel, G. L. Shaw, *Br. Res. Rev.* **1**, 225 (1979).
2. C. F. L. Hinrichsen and L. M. H. Larramendi, *Brain Res.* **7**, 296 (1968); C. Sotelo and S. L. Palay, *ibid.* **18**, 93 (1970); R. Baker and R. Llinas, *J. Physiol. (London)* **212**, 45 (1971); C. Sotelo, R. Llinas, R. Baker, *J. Neurophysiol.* **37**, 560 (1971); R. M. Wylie, *Brain Res.* **50**, 179 (1973); H. Korn, C. Sotelo, F. Crepel, *Exp. Brain Res.* **16**, 225 (1973); Y. Yarom and R. Llinas, *Neurosci. Abstr.* **5**, 109 (1979).
3. J. J. Sloper, *Brain Res.* **44**, 641 (1972); ——— and T. P. S. Powell, *Proc. R. Soc. London* **203**, 39 (1978).
4. W. W. Stewart, *Cell* **14**, 741 (1978).
5. M. V. L. Bennett, M. E. Spira, D. C. Spray, *Dev. Biol.* **65**, 114 (1978).
6. C. Yamamoto, *Exp. Brain Res.* **14**, 423 (1972); P. A. Schwartzkroin, *Brain Res.* **85**, 423 (1975). The standard perfusion solution contained NaCl, 124 m*M*; KCl, 5 m*M*; NaH_2PO_4, 1.25 m*M*; $MgSO_4$, 2 m*M*; $CaCl_2$, 2 m*M*; $NaHCO_3$, 26 m*M*; and dextrose, 10 m*M*.
7. Lucifer Yellow CH was provided by W. W. Stewart.
8. Lucifer injection was attempted in neurons that had membrane potentials greater than 50 mV. With use of a bridge circuit, dye was subjected to electrophoresis by injection of 1- to 2-nA, 750-msec negative current pulses at 1 Hz for 5 to 10 minutes. Because of the high resistance of the Lucifer electrodes, they could not be used for reliable electrophysiological measurements. After the injection period, the slice was left in the chamber for 5 minutes to 2 hours and was then removed and fixed in 4 percent buffered Formalin. The tissue was dehydrated in alcohols, cleared in xylene, whole-mounted, and examined and photographed with an epifluorescence microscope and appropriate filters. The tissue was then counterstained with cresyl violet to verify the laminar distribution of dye-filled neurons. Neurons whose resting membrane potentials had fallen below 35 mV by the end of the injection period did not stain.
9. Under the microscope, the three-dimensional character of the columnar-shaped dye-coupled aggregates was evident when the plane of focus was shifted back and forth. Also, examples of this dye-coupling pattern were obtained in slices cut in the sagittal as well as the coronal plane.
10. B. A. MacVicar and F. E. Dudek, *Brain Res.* **196**, 494 (1980).
11. U. Kuhnt, M. J. Kelly, R. Schaumberg, *Exp. Brain Res.* **35**, 371 (1979).
12. W. R. Lowenstein and T. Kanno, *J. Cell Biol.* **22**, 565 (1964); G. D. Pappas and M. V. L. Bennett, *Ann. N.Y. Acad. Sci.* **137**, 495 (1966); A. Kaneko, *J. Physiol. (London)* **213**, 95 (1971); M. V. L. Bennett, in *Intracellular Staining Techniques in Neurobiology*, S. D. Kater and C. Nicholson, Eds. (Elsevier, New York, 1973); I. Imanga, *J. Membr. Biol.* **16**, 381 (1974); W. R. Lowenstein, *Cold Spring Harbor Symp. Quant. Biol.* **40**, 49 (1976).
13. For references, see: M. V. L. Bennett, in *Handbook of Physiology*, E. R. Kandel, Ed. (Williams & Wilkins, Baltimore, 1977), vol. 1, section 1, pp. 357-416; E. Korn and D. S. Faber, in *The Neurosciences: Fourth Study Program*, F. O. Schmitt and F. G. Worden, Eds. (MIT Press, Cambridge, Mass. 1979).
14. Extracellular stimulation was applied with bipolar electrodes consisting of side-by-side electrolytically sharpened and insulated tungsten wires with an intertip distance of about 100 μm. Constant current stimuli were 25 to 250 μA and 50 to 250 μsec.
15. U. Meiri and R. Rahamimoff, *Science* **176**, 308 (1972).
16. Antidromic latencies corresponded to axonal conduction velocities as slow as 0.5 m/sec, which is well within the range reported for axons in the mammalian central nervous system [S. G. Waxman and H. A. Swadlow, *Prog. Neurobiol. (Oxford)* **8**, 297 (1977)].
17. R. Werman and P. Carlen, *Brain Res.* **112**, 395 (1976).
18. The presence of gap junctions in primate somatosensory cortex has been described (*3*). However, dye coupling after intraneuronal injection of Lucifer Yellow was not reported in a recent study on cat cerebral cortex [M. Takato and S. Goldring, *J. Comp. Neurol.* **186**, 173 (1979)]. This might reflect differences between the in vivo and the in vitro preparations, or a true species difference. Alternatively, it may reflect the tendency to select for deeper neurons in the in vivo intracellular studies.
19. For references, see V. Mountcastle, in *The Mindful Brain*, G. M. Edelman and V. Mountcastle Eds. (MIT Press, Cambridge, Mass. 1978), pp. 7-50.
20. M. S. Berry and V. W. Penroth, *Comp. Biochem. Physiol.* **57A**, 289 (1977).
21. Supported by grants NS 06477 and NS 12151 from the National Institute of Neurological and Communicative Disorders and Stroke, and by the Morris Research Fund. M.J.G. is on leave from the Faculty of Health Sciences, Ben Gurion University of the Negev, Beer Sheva, Israel. We thank U. J. McMahan and B. Wallace for critically reviewing the manuscript.

3 June 1980; revised 8 September 1980

Identification of Tissue-Specific Nuclear Antigens Transferred to Nitrocellulose from Polyacrylamide Gels

Abstract. *Nonhistone protein antigens resolved by electrophoresis in sodium dodecyl sulfate were identified immunochemically after being transferred to nitrocellulose. Use of antiserum to dehistonized chromatin from Novikoff hepatoma revealed numerous protein antigens specific to the chromatin of Novikoff hepatoma in comparison to that of normal rat liver.*

Nonhistone protein antigens specific for cell types, both normal and transformed, have been repeatedly detected with the use of antiserums to dehistonized chromatins from their respective sources (*1-3*). The presence of such specific antigens is consistent with a role for some of the chromosomal proteins in cellular differentiation. Procedures for the identification, isolation, and characterization of the proteins responsible for the immunological specificity are facilitated by combining the sensitivity and specificity of immunochemistry with the resolution capability of protein electrophoresis in polyacrylamide gels. Recently Towbin *et al.* (*4*) reported methods for transfer of proteins by electrophoresis from polyacrylamide gels to nitrocellulose sheets. By combining this approach with a peroxidase antiperoxidase (PAP) antigen localization technique (*5*), we can now rapidly visualize those nonhistone proteins exhibiting antigenic activity.

The methods for preparation of chromatins (*6*) and antiserum to dehistonized chromatin (*1*) have been described. The materials include Novikoff ascites hepatoma (transplanted in 150- to 200-g male Sprague-Dawley rats) and normal rat liver (excised from healthy 150- to 200-g male Sprague-Dawley rats). The sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) method (*7*) was used to separate chromatin proteins without extraction or enzymatic digestion of the DNA. Chromatin samples in deionized water and 0.1 m*M* phenylmethyl sulfonyl fluoride, at a concentration not exceeding 1 mg/ml in DNA as determined by absorbance at 260 nm, were treated with a Sonifier cell disruptor (model W185) at 70 W eight times for 10 seconds each time, with the samples being cooled on ice for 20 seconds between sonications. The samples were mixed with 0.9 volume of a solution containing 0.139*M* tris-HCl (*p*H 6.8), 4.44 percent SDS (Bio-Rad), 22.2 percent glycerol, and Pyronine Y at 25 μg/ml and with 0.1 volume of 2-mercaptoethanol; they were then subjected to sonication without cooling for 90 seconds and heating in boiling water for 2 minutes. The samples were applied to

0036-8075/81/0102-0070$00.50/0