significantly correlated with ZPP levels.

2) For the BD, DS, and EF tests, scores are correlated with ZPP at P values at least a factor of 10 lower than those for blood lead levels. This can be understood in terms of the ZPP level representing a 4-month averaging of the lead burden for exposed individuals, as discussed above. It is consistent with the finding that several other lead-related symptoms in a lead-exposed population are better correlated with ZPP levels than with blood lead levels (*22*).

3) An observable correlation of the scores with ZPP persisted to fairly low ZPP concentrations (for instance, for EF $P < .1$ for a subgroup having [ZPP] < 170 μg/dl). The slopes of the fitted experimental curves suggest that the initial decreases in performance test scores were approximately 16, 7, and 5 percent per 100 μg of ZPP per deciliter for the BD, DS, and EF tests, respectively; however, our sample size does not permit establishing statistical significance in this range.

4) Although the correlation between scores and ZPP levels is statistically significant, the fitted curves have low accountability; that is, the scatter of scores due to individual variability greatly exceeds the effect that ZPP levels have on scores for the population studied here. It is then impossible to draw conclusions about an individual's ZPP level or lead intoxication from his test scores alone.

This study is based on a group of workers whose blood lead and ZPP levels indicate that a portion of this population meet the clinical definition of lead intoxication. Erythrocyte porphyrin levels for the general (not occupationally exposed) population have been reported (*9*) to be in the range 20 to 60 μg of ZPP per deciliter. If the correlations in Fig. 2 are significant at such low ZPP levels, some degree of CNS dysfunction may occur not only in some lead-exposed workers but also in children living in lead-contaminated environments or in other groups with environmental exposures to lead (in water, food, or air).

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11. Ninety smelter workers were given the neurobehavioral tests. Their average age was 42.8 years (range, 18 to 65), and their average length of occupational lead exposure was 10.3 years (range, 0.1 to 30). Ten percent were Spanish-speaking and were given performance tests in that language. Their mean educational level corresponded to an almost completed high school education (2.5 on a scale from 0 to 8). All subjects except one were male. Other details of the cohort are given in a longer report (*12*).
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14. The EF test has been developed at the Environmental Sciences Laboratory on the basis of more than 500 subjects classified by age, sex, race, educational level, geographic area, and native language (English and Spanish).
15. The Santa Ana dexterity test has been used to investigate the effects of neurotoxic substances [H. Hänninen, L. Eskelin, K. Husman, M. Nurminen, *Scand. J. Work Environ. Health* **4**, 240 (1976); A. M. Seppäläinen, H. Hänninen, S. Hernberg, paper presented at the Second International Workshop on Permissible Levels for Occupational Exposure to Lead, University of Amsterdam, 21 to 23 September 1976.
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23. Control populations were 99 pulp and paper industry workers in Covington, Virginia, with a mean age of 43.5 ± 7.3 years (mean ± standard deviation) and a mean educational level of 2.77, and 93 farmers living in the Marshfield, Wisconsin, area, with a mean age of 41.8 ± 15.9 years and a mean educational level of 2.98. The age dependences for these two control groups were indistinguishable.
24. The correlation between ZPP and blood lead levels is discussed elsewhere (*22*).
25. Here P is the probability that a particular result was obtained as the result of a statistical fluctuation.
26. Calculations for an educationally homogeneous subset of subjects having some high school education, but no education beyond high school, lowered the significance only by an amount due to the reduction in the sample size, showing that educational level is not an important factor in these correlations.
27. The portion of this work carried out at Mount Sinai School of Medicine was supported in part by Center grant ES 00928 of the National Institute of Environmental Health Sciences. We thank Dr. Morris B. Bender, who reviewed and advised us on the battery of neurobehavioral tests and Nancy Gilbert who assisted in testing the subjects.

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Enkephalin-Containing Neurons Visualized in Spinal Cord Cell Cultures

Abstract. *Neuronal cells, axons, and terminals containing immunoreactive enkephalin have been visualized in cultures of dissociated fetal spinal cord. These cultures may provide a valuable system in which to explore the effects of chronic drug treatment on the physiology of enkephalin-containing cells and their interactions with other cells.*

The opioid peptide enkephalins, isolated from the central nervous system (*1*), appear to interact physiologically with opiate receptors (*2, 3*). Both cytochemically localized enkephalin peptides and opiate receptors are highly concentrated in the dorsal gray matter of the spinal cord (*4–6*) where the interaction of enkephalin with apparent opiate receptors on the terminals of primary afferents (*7*) may modulate pain perception (*3, 8*).

Dissociated cells obtained from fetal spinal cord can be maintained in tissue culture for periods of up to several months (*9*), providing a model system for assessment of acute and chronic physiological and pharmacological manipulations. Intracellular recordings from cultured spinal cord neurons coupled with extracellular iontophoresis of leucine-enkephalin have revealed a variety of peptide effects, many of which appear to fall outside the classical definition of neurotransmitter action (*10*). We now report evidence from immunohistofluorescence studies for the presence of leucine- and methioine-enkephalin immunoreactivity in the cell bodies and processes of cultured cells from mouse spinal cord. These cultures permit the visualization of enkephalin-containing cells and processes in a planar array so that cells, axons, and terminals can be traced in detail that is not feasible in intact animals.

Spinal cords and dorsal root ganglia were obtained from fetal mice at 13 to 15 days of gestation and mechanically dissociated; the cells were cultured on collagen-coated glass cover slips for several months as described (*9*). Cultures were

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rinsed with phosphate-buffered sucrose and fixed in freshly prepared, buffered, depolymerized 4 percent paraformaldehyde (*11*). Enkephalin was stained by the indirect immunofluorescence method of Coons (*12*) as described (*13*), using 1:30 dilutions of primary rabbit antiserums directed against methionine- and leucine-enkephalins that had been characterized in radioimmunoassay (*14*). After washing, cultures were reacted with fluorescein-conjugated goat antiserum against rabbit immunoglobulin G (1:40, Cappel Laboratories), washed again, mounted, and viewed in a Zeiss Universal microscope as described (*13*). Control serum was prepared by adsorbing undiluted antiserum with a 1 m*M* concentration of the appropriate enkephalin.

A variety of morphologically discrete cell types have been described in these cultures of dissociated spinal cord cells (*9*). Resting upon one or more layers of nonneuronal flat cells are phase-bright cells that are classified as neuronal because of their morphologic and physiologic characteristics (*9*). While enkephalin immunofluorescence was observed in about 1 to 3 percent of the morphologically identified dissociated neurons in spinal cord cultures, occasional clusters of neurons were observed in which a relatively large number of cells were reactive (Fig. 1A). This fluorescence was virtually eliminated by preadsorption of the primary antiserum with enkephalin or substituting preimmune serum for the primary antiserum, and hence appeared to be specific for enkephalin. Fluorescence was seen after staining with antiserum against methionine-enkephalin and antiserum against leucine-enkephalin. Although each serum displays only about 0.05 percent cross-reactivity with the other pentapeptide in radioimmunoassay (*14*), the high concentrations of antiserums used in histochemical experiments preclude definitive conclusions as to whether individual cells contain one or both of the enkephalins.

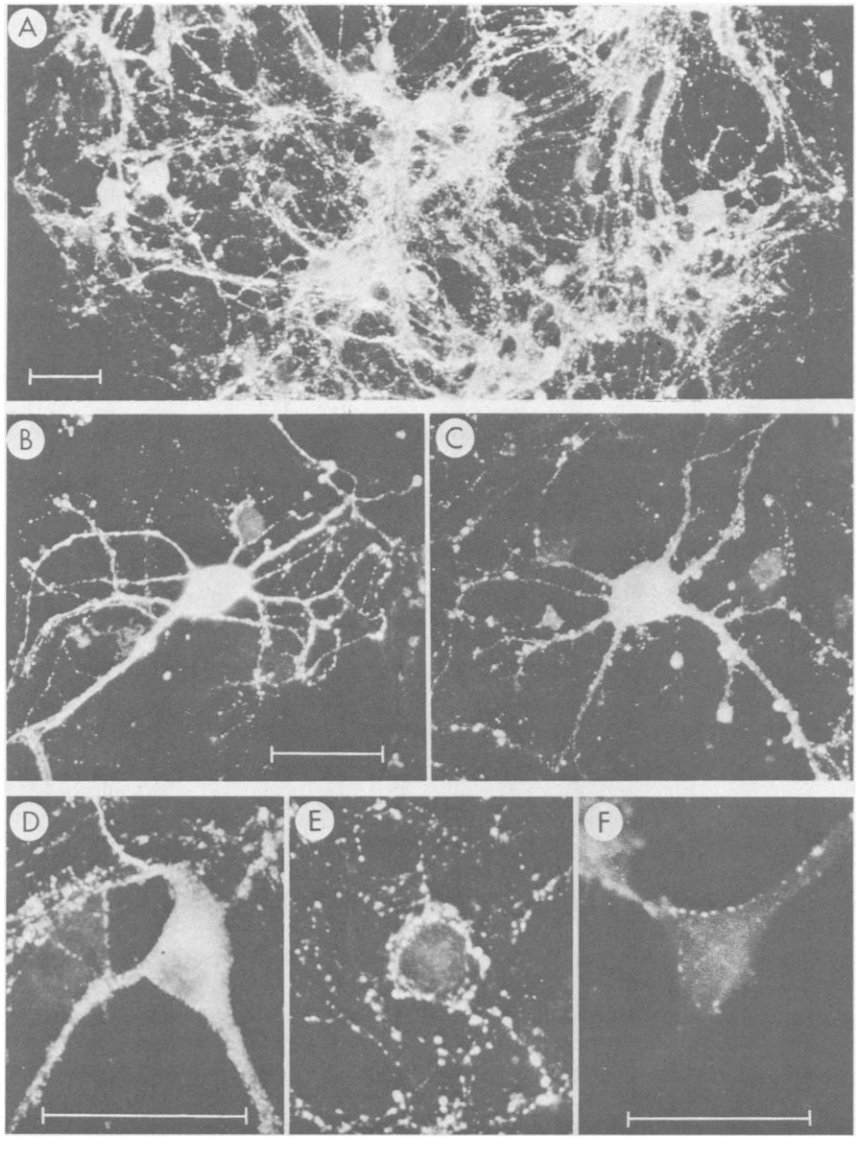

Fig. 1. Dissociated spinal cord cells staining positively for enkephalin immunoreactivity; bars, 50 μm. (A) Clusters of associated neurons containing both positive and negative cells are seen, as well as a complex of reactive neurites. (B and C) Isolated fluorescent neurons present reactivity within their perykarya as well as their extensively branching neurites. (D) Localization of fluorescence is seen in cell bodies and processes. (E and F) Neurites containing foci of positive peptide reactivity intimately invest the cell body and proximal processes of unreactive neurons.

The nonnuclear portions of perikarya and the processes of reactive cells all displayed bright fluorescence (Fig. 1, B and C), with high-intensity staining in the varicosities found along processes. Sizes and branching patterns of positive cells were widely divergent. Cells with both small and large (> 20-μm diameter) somata were fluorescent, which suggests a heterogeneity of reactive cell types.

Peptide reactivity observed in cell bodies presumably reflects binding of labeled antibody to intracellular antigens and not to surface membrane substrate, since the nuclear regions appeared relatively devoid of fluorescence. The granular nature of the fluorescence within the neuronal cytoplasm and proximal neurites (Fig. 1D), which resembles findings in vivo, suggests that the peptide reactivity is concentrated within discrete regions and not freely dispersed throughout the cytoplasm.

Culture techniques allow the localization of a neuronal cell body and all of its processes and terminals in a planar array, a phenomenon rarely found in vivo. Accordingly, neurites could often be traced for several hundred micrometers. In those instances where fluorescent processes approached other neuronal cell bodies, discrete areas of intense peptide reactivity were often apparent (Fig. 1E). These fluorescent neurites were observed to coil about the proximal processes and cell bodies of nonreactive neurons. While such an interaction cannot be captured adequately within one place of focus, an example of such "innervation" is presented in Fig. 1F. The enkephalin-containing neurite appeared to wrap about the target cell, displaying many discrete punctate foci of immunoreactivity. To determine whether these foci of enkephalin-related fluorescence represent morphologically indentifiable synaptic terminals requires techniques of higher resolution, however.

Immunofluorescence studies of neurons derived from dorsal sensory ganglia

showed no specific immunoreactivity. This difference between dorsal root ganglia and spinal cord cultures is consistent with in vivo studies showing that enkephalin is not present in sensory afferent neurons (*15*), but that it is localized to neuronal cell bodies, processes, and presumptive terminals in the dorsal horn of the spinal cord (*5, 6*).

In frozen sections of rat brain, satisfactory visualization of enkephalin immunofluorescence within most of the perikarya that apparently contain enkephalin has required prior treatment of the animals with colchicine (*16*), although several cell body groups may be seen dimly without such manipulations (*6, 17*). In contrast, we have found many examples of immunoreactivity in perikarya of untreated cultured neurons. This demonstration of enkephalin in spinal cord cells grown in tissue culture suggests that these neurons either retain or indeed develop the ability to synthesize opioid peptides while maintained in vitro.

The present results, together with the electrophysiologic responsiveness to enkephalin (*10*) indicate the presence of functional receptors and suggest that dissociated neurons grown in tissue culture may be a suitable model system in which both the chronic application of drugs and physiological manipulations may be effectively used to investigate cellular mechanisms underlying the neuronal functions of opioid peptides.

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The Jet Stream Microbeveler: An Inexpensive Way to Bevel Ultrafine Glass Micropipettes

Abstract. *Ultrafine glass micropipettes can be easily beveled in a jet stream of grinding compound suspended in saline. The beveling is gradual and continuous, highly reliable, and can be accomplished with common laboratory apparatus. The beveled electrodes are comparable in performance to those prepared with expensive commercial bevelers.*

The beveling of glass microelectrodes by grinding has recently become standard practice in many laboratories where intracellular studies are in progress. Beveling increases the electrode pore size and yet permits the retention of small tip dimensions. Such electrodes penetrate tissue more smoothly, have a lowered impedance, and are less noisy than unbeveled electrodes of comparable tip size (*1–3*). The increase in tip pore size that results from beveling is associated with improved ejection of substances by electrophoresis or pressure (*4*), and yet the small, sharp tip facilitates cell penetration (*2*).

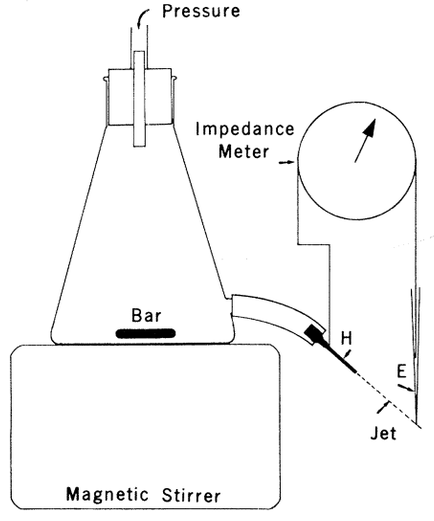

Fig. 1. Diagram of the beveler. A container of micropolish suspension is placed on a magnetic stirrer. Pressure is applied to form a jet of solution from a 20-gauge hypodermic needle (*H*). The impedance meter is connected from the needle to the electrode (*E*), which is held in the jet stream by a micromanipulator.

The commercial electrode bevelers currently available can be traced to the work of Barrett and Graubard (*5*) and the modification of their technique by Kripke and Ogden (*3*) and by Brown and Flaming (*6*). A wobble-free surface in which the grinding compound is embedded is rotated with great precision against an electrode held in a precision advancer. This technique, which requires a substantial investment in equipment and a skilled operator, is inconvenient, results in electrode breakage, and is not suitable for electrodes with long flexible shanks of the type used for most intracellular work.

We have discovered, to our surprise, that very fine beveled electrodes can be produced quickly and reliably if a jet of a grinding solution is simply squirted against the electrode tip. Electrodes thus produced are comparable in performance to those beveled by a rotating grinding surface and we have used them to penetrate and inject horseradish peroxidase (HRP) (E.C. 1.11.1.7) into cells of the frog retina.

The technique we use is illustrated in Fig. 1. As in the earlier procedures, it is essential to monitor electrode impedance during grinding. In essence, the grinding compound (*7*) is directed against the electrode tip at an angle of about 45° in a stream of saline. The suspension is squirted from a 20-gauge hypodermic needle under enough pressure to form a smooth stream. The electrode is lowered into the stream by a micromanipulator of mundane design. Contact with the

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