The demonstration of an increase in enkephalin-like activity upon electrical stimulation appears to be the first demonstration of in vivo release of an endorphin, and it supports the view that stimulation-produced analgesia in humans may be, in part, due to activation of an endogenous pain modulation system with opioid components since it is partially blocked by naloxone (*7*, *11*) and is accompanied by apparent release of opioids into the CSF. Further, it is consistent with animal findings (*12*) showing changes in concentrations of brain opioids upon electrical stimulation. Finally, the findings that baseline levels in these patients are lower than normal supports the finding (*13*) that concentrations of endogenous opioids in the CSF in patients with persistent pain may be depressed.

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10. H. Akil, S. Sullivan, S. J. Watson, J. D. Barchas, *Life Sci.*, in press. While this material cochromatographs with enkephalins in many systems and interacts with opiate receptors in the binding assay and bioassay, its quantities are too low to permit a positive identification as enkephalin. We have, therefore, chosen the term "enkephalin-like" throughout. We suggest that this opioid is similar to the fraction II opioid material identified in CSF by A. Whalstrom, L. Johansson, L. Terenius [in *Opiates and Endogenous Opioid Peptides*, H. W. Kosterlitz, Ed. (North-Holland, Amsterdam, 1976), pp. 49-56].
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## Central Nervous System Dysfunction Due to Lead Exposure

Abstract. *Central nervous system dysfunction was investigated in workers at a secondary lead smelter by means of performance tests. Correlations between test scores and zinc protoporphyrin levels, a biological indicator of lead toxicity, are statistically significant. This correlation should prove to be useful in current efforts to evaluate effects of lead exposure.*

Long-term lead poisoning has been shown to cause neurologic injury (*1*, *2*), to reduce nerve conduction velocity in adults (*3*), and to lead to mental retardation (*4–7*) and hyperactivity (*8*) in children. The question of what, if any, level of lead exposure is acceptable without risking central nervous system (CNS) dysfunction has remained unanswered, in part because of a paucity of quantitative data relating CNS effects to the magnitude of lead absorption. The study reported here provides such data.

With recent advances in understanding the biochemical effects of lead on various enzyme systems, particularly those involved in heme synthesis, the zinc protoporphyrin (ZPP) level in blood has emerged as a useful biological indicator of long-term lead effects (*9*, *10*). We report here correlations between scores for several performance tests for the evaluation of brain dysfunction and the concentrations of ZPP and lead in the blood of lead-exposed workers. Correlations between performance test scores and ZPP levels were found that were highly significant within the test population.

The data were obtained in the course of a clinical field survey of 90 workers at a secondary lead smelter in California (*11*). Each worker's occupational history was recorded, and he was given a thorough medical examination, as well as hematological, biochemical, and other clinical laboratory tests (*12*). The neurobehavioral tests used in this study included the block design (BD) (*13*), digit symbol (DS) (*13*), and embedded figures (EF) tests (*14*) and the Santa Ana dexterity test for the dominant hand (DH) and for both hands (BH) (*15*). In the BD test the subject is presented with a set of cubes whose faces are half red and half white, the halves being separated by a diagonal. The subject is asked to arrange the cubes within a time limit in a way which duplicates a pattern shown to him. In the DS test the subject is given a list in which symbols are associated with the digits from 1 to 9 and is asked to enter the symbols into the blank spaces next to a list of random digits. In the EF test the subject is shown four sets of ten superimposed outline drawings of common objects and is required to identify as many as possible. The Santa Ana dexterity test uses a metal plate with an array of square holes. Each hole holds a square peg, and the subject is required to lift, rotate through 180°, and replace as many pegs as possible within 30 seconds. This test is performed first with the dominant hand and then with both hands.

Table 1. Performance test scores, corrected for age, correlated with blood lead and zinc protoporphyrin (ZPP) levels.

| Test | Lead | | ZPP | | Slope† (%) |
|---|---|---|---|---|---|
| | *r** | *P* | *r** | *P* | |
| BD | .207 | <.03 | .294 | <.003 | 16 |
| DS | .061 | N.S.‡ | .244 | <.02 | 7 |
| EF | .216 | <.03 | .296 | <.003 | 5 |
| DH | .007 | N.S. | .005 | N.S. | N.S. |
| BH | .003 | N.S. | .007 | N.S. | N.S. |

*Multiple correlation coefficient of the fit with Eq. 2. †Percentage drop in scores for the first 100 μg of ZPP per deciliter. ‡N.S., not significant.

Blood lead levels were measured by atomic absorption spectrophotometry. The ZPP concentration in blood was measured by a hematofluorometer (Aviv Associates, Lakewood, N.J.). This instrument provides an accurate value for the concentration of ZPP from a small drop of blood obtained by finger puncture (*16–18*).

Before attempting to compare ZPP and blood lead as indicators of biologically active lead, it is useful to review briefly the origin of ZPP during erythropoiesis. Lead ions interfere with the ferrochelatase system, which catalyzes the


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insertion of iron into protoporphyrin IX to form heme, which is then incorporated into globin chains to form hemoglobin. With such interference, zinc ions take the place of iron in some of the porphyrin molecules, and zinc protoporphyrin IX takes the place of heme in a small fraction ($< 10^{-3}$) of the hemoglobin. The fact that ZPP-globin fluoresces while normal hemoglobin does not is the basis of the hematofluorometer (*18*). It has been shown (*19, 20*) that ZPP-globin, once in the erythrocyte, remains there for the lifetime of the cell (~125 days). The ZPP level in blood at a particular time thus reflects the lead level at the site of erythropoiesis averaged over the preceding 4 months. The blood lead level, on the other hand, has an equilibrium time of no more than a few days (*21*) and reflects recent lead absorption; it is therefore much more variable under conditions of variable exposure than is the ZPP level. Zinc protoporphyrin levels have indeed proved to be better correlated with symptoms of chronic lead toxicity than blood lead levels (*22*).

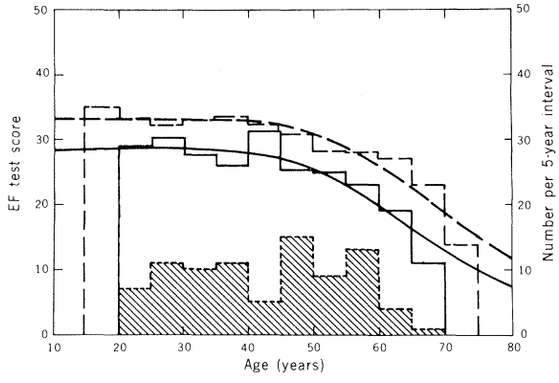


Fig. 1. The hatched histogram gives the age distribution of the lead smelter workers. The solid histogram gives the EF test scores obtained by the smelter workers averaged in 5-year intervals, the solid curve being the least-squares fit ($r = .46$; $P < .00001$) of the individual scores to Eq. 1. The dashed histogram and curve represent the corresponding data and fitted curve ($r = .60$; $P < .000001$) obtained for a control population consisting of farmers and paper mill workers.

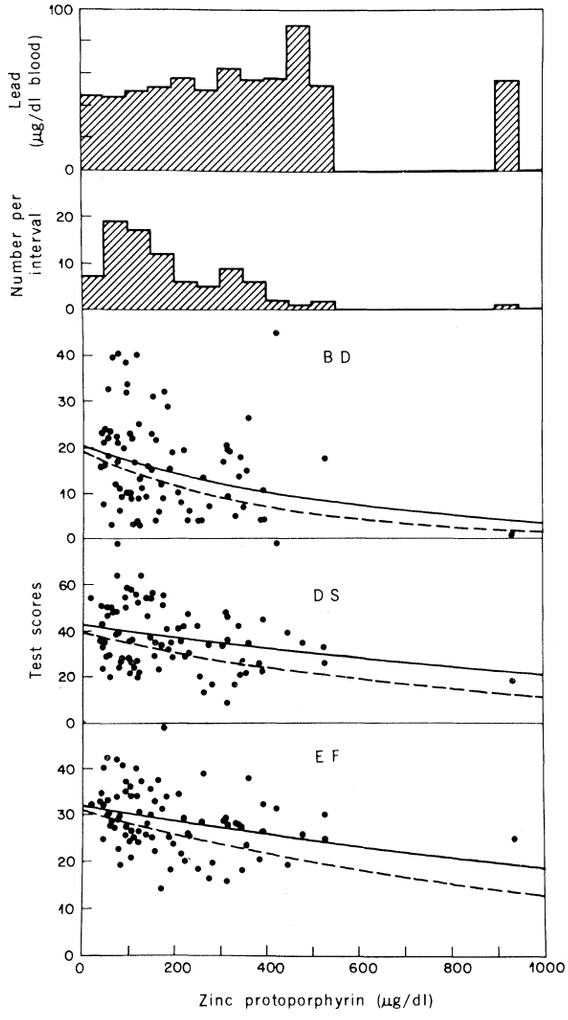


Fig. 2. The points shown give the age-corrected scores obtained by 90 lead smelter workers on three performance tests (BD, DS, and EF) as functions of their individual ZPP levels. The solid curve is an exponential (Eq. 2) fitted to these points, as discussed in the text. The statistical parameters characterizing the significance of these correlations are given in Table 1, along with initial slopes of the fitted curves. The dashed curves represent the exponentials fitted to the uncorrected scores and show that the correction is not large. The hatched histograms give the distribution of ZPP levels for the test population and the blood lead levels corresponding to 50 μg/dl ranges of ZPP.

The performance scores for the behavioral tests used have a characteristic age dependence. This is shown for one test (EF) for exposed and nonexposed populations (*23*) in Fig. 1. We obtained the age correction from the test population itself. This was possible since all ZPP levels were found to be well represented in each age group. The scores ($S$) as a function of age ($A$) were fitted by the equation

$$S = \frac{S_0}{1 + \alpha(A - 15)^5} \quad (1)$$

This form contains only two adjustable constants ($S_0$ and $\alpha$) and led to a distribution of positive and negative deviations between test scores and the curve that was nearly independent of age by the criterion of minimum autocorrelation. A least-squares fitting procedure was used to determine $S_0$ and $\alpha$ for each test, and all scores were then normalized by multiplying the actual score obtained by a subject by the correction factor ($S_0/S$).

The age-corrected scores for all tests as functions of ZPP concentration were fitted by exponential decay curves of the form

$$S = S_0 e^{-\beta[\mathrm{ZPP}]} \quad (2)$$

This equation has two adjustable parameters, as does a linear regression curve, but has the advantage of avoiding the possibility of negative scores at high ZPP concentrations. Figure 2 shows the least-squares fitted exponentials for both age-corrected and uncorrected scores. It is found that the age correction has only a small effect on the correlation coefficient and on the slope of the score-ZPP relationship. The histograms in Fig. 2 show the distribution of ZPP levels and the average blood lead levels corresponding to 50 μg/dl ranges of ZPP (*24*).

The statistical significance and the initial slope of the score-ZPP relationship for each of the tests are shown in Table 1, which also includes the corresponding results for the score-lead relationships obtained in an equivalent manner. The following results are obtained.

1) Three of the five performance tests (BD, DS, and EF) show according to Student's $t$-test a statistically significant dependence on ZPP with $P$ ranging from .003 to .02 (*25, 26*). These tests have been successfully used in the assessment of brain dysfunction. The dexterity tests scores (DH and BH) are not

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 $\mu$g/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 $\mu$g 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 $\mu$g 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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