In interpreting these findings, it should be recognized that the data are derived from a standardized reporting system, and no new information was collected. Thus, the data do not depend on patient recall or other retrospective processes. Therefore, completeness and quality of the cancer case reports are unlikely to have been affected by events like the declaration of a health emergency at Love Canal in mid-1978. There are, however, limitations in the data. They are derived from a central registry that receives 65,000 new case reports annually from a population of 16.8 million people. The degree of completeness and the accuracy of reporting from a single census tract cannot be known precisely, but there is no reason to believe that reporting was unusual in the areas we studied. The possible effects of special risk factors such as socioeconomic status, smoking, or air pollution cannot be assessed in a study of this design. The effect on people who have migrated out of the area has not been studied, and it is important to emphasize that questions of long latency periods cannot yet be addressed.

Uncertain latency periods and the small size of the study population limit the findings of epidemiological studies of this type. The important uncertainties seem to be biological and etiological rather than statistical. However, there are few, if any, alternatives to the epidemiological approach to assessing potential human risk from toxic chemical waste dumps. Investigation of other dump sites may have even greater quantitative limitations than that of the Love Canal.

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References and Notes

1. This report is one part of the Love Canal research begun by the New York State Department of Health in the past 2 years. The full research context includes environmental studies of air, water, and soil contamination, hydrogeologic studies, biologic studies in humans and animals, epidemiologic studies of acute effects and reproductive outcomes, and long-term follow-up of residents for assessment of chronic effects.
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9. Because the diagnosis of primary liver cancer is difficult and cancers of many other anatomic sites metastasize to this organ, we included all malignant neoplasms reported as liver that were not specified as secondary. This ensured that we would not underestimate primary liver cancers. The initial report for only one of the four cases stated that the cancer was primary in the liver. Upon review of the case histories, it was determined that for both cases in females the cancers probably originated in the pancreas. For one of these cases, pancreatic cancer was confirmed by autopsy. Both of the cases in males were autopsied; one was malignant hepatoma, and the other adenocarcinoma of the pancreas.
10. New York State incidence data for 1974 to 1976 ranks Niagara County 5th for males and 36th for females for all cancers and 5th for males and 18th for females for lung cancer. New York State mortality data ranks Niagara County 17th for males and 22nd for females for all cancers and 10th for males and 12th for females for lung cancer. These ranks apply to the 57 counties outside of New York City.
11. We thank V. M. Gerard, V. Krimss, and T. Signorelli for clerical and statistical assistance and W. E. Schmidt for computer programming.

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Myelination of Central Nervous System Axons in Tissue Culture by Transplanted Oligodendrocytes

Abstract. *Unmyelinated mouse cerebellar cultures in which oligodendrocyte differentiation had been suppressed by exposure to cytosine arabinoside developed axonal myelin after superimposition of kainic acid–treated cerebellar explants devoid of myelin-receptive axons. The latter explants contained differentiated oligodendrocytes. The operation of a diffusible myelin-stimulating factor was ruled out by the failure of myelination in cytosine arabinoside-exposed explants not in direct contact with oligodendrocyte-containing transplants.*

Substantial myelination is achieved by 80 percent of newborn Swiss-Webster mouse cerebellar explants after 12 days of cultivation on collagen-coated coverslips in Maximow chambers (*1*, *2*). However, if similar explants are exposed to an inhibitor of DNA synthesis, cytosine arabinoside, for the first 5 days in vitro and subsequently cultured in normal nutrient medium, myelination is rare. [Only 2 of a series of 263 cerebellar cultures initially treated with cytosine arabinoside had myelin in trace amounts after 15 days in vitro (*3*).] Although Purkinje cell axons and axon collaterals, which normally myelinate, are superabundant in such cultures (*3*), ultrastructural examination reveals a reduction in the number of oligodendrocytes and a failure of the surviving oligodendroglia to differentiate fully (*4*). Thus myelination fails because of a lack of competent myelin-forming cells.

On the other hand, if cerebellar cultures are initially exposed to kainic acid, a glutamic acid analog, all cortical neurons are destroyed except granule cells, which are selectively resistant to the neurotoxic effects of kainate, succumbing only after prolonged exposure (*5*). Such cultures also have no axonal myelination, because the only surviving axons are the parallel fibers (granule cell axons), which do not normally myelinate in mouse cerebellar cultures (*2*, *6*). However, occasional granule cells in kainate-treated cultures are observed in the living state surrounded by perisomatic myelin, which is also occasionally found in normal cerebellar explants and in cerebellum in vivo (*2*, *7*). Electron microscopic examination confirms the presence of granule cell perisomatic myelin and demonstrates normal appearing, fully differentiated oligodendrocytes in explants exposed to kainic acid (*5*).

A question can therefore be raised as to whether oligodendrocytes from explants exposed to kainic acid, in which there are no axons receptive to myelination, will, if transferred to explants treated with cytosine arabinoside, myelinate available axons that have never been myelinated because of a lack of competent oligodendroglia. The purpose of the present study was to address this question.

Parasagittally oriented cerebellar explants were prepared from newborn Swiss-Webster mice (*2*). Some explants were cultivated for the first 5 days in vitro with 5 or 10 μg of cytosine arabinoside per milliliter of nutrient medium, and subsequently with normal medium (*3*). Other explants were cultivated for the first 5 days in vitro with $10^{-4}M$ kainic acid incorporated into the nutrient medium, followed by feeding with normal medium (*5*). Still other explants served as normal medium controls. After 9 days in vitro, explants exposed to kainic acid were excised from their cover slips under a dissecting microscope, placed in a 1:1 ratio directly over explants exposed to cytosine arabinoside, and cultivated further in normal nutrient medium. Such

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transplanted cultures were observed by light microscopy through day 19 for the appearance of myelinated fibers.

A total of 43/53 (or 81 percent) of transplanted cultures developed myelinated axons, a figure comparable to that for control cerebellar explants. Myelin was observed in some cultures by 3 days after transplantation and was generally present in all myelinating cultures by 5 days after transplantation (14 days in vitro). The appearance of myelin in the living state was confirmed in stained preparations and by ultrastructural examination (Fig. 1).

It is technically difficult, even with autoradiographic or other labeling procedures, to demonstrate that myelination in the host cytosine arabinoside–treated explants is accomplished by oligodendrocytes from the transplanted kainate-exposed cultures. It is possible, however, to control for the operation of a diffusible factor emanating from the transplanted explants that might stimulate oligodendrocyte division or differentiation and myelination in the host cultures. Such controls were prepared in the following manner: (i) transplanted explants exposed to kainic acid were placed 2 mm or more apart from seven cytosine arabinoside–treated cultures, rather than directly over the latter, at 9 days in vitro; (ii) a series of six paired cerebellar explants (total of 12 explants) separated by 2 mm or more on the same cover slip were exposed to cytosine arabinoside for the first 5 days in vitro, and then only one of a pair received an overlying kainate-treated transplant at 9 days in vitro; and (iii) fragments of the outer half of newborn mouse cerebellar cortex, rich in granule cells but relatively poor in oligodendrocytes (*8*), were placed over ten cytosine arabinoside–treated explants at 7 days in vitro and cultivated in normal nutrient medium through day 19. Of these groups of controls, only the cytosine arabinoside–exposed explants with overlying kainate-treated transplants developed myelinated fibers, suggesting that diffusible myelin-stimulating factor was not operative and that oligodendrocytes from the transplants myelinated the axons of the host cultures.

Myelination of peripheral axons of trembler mutant mouse by transplanted normal mouse Schwann cells has been demonstrated in animal studies, as has myelination of normal mouse peripheral axons by Schwann cells transplanted from human nerves (*9*). In other in vivo investigations, demyelinated central nervous system (CNS) axons have been remyelinated by transplanted Schwann cells (*10*), and regenerating peripheral axons were myelinated by transplanted oligodendrocytes (*11*). In recent tissue culture studies, the neurites of dissociated rat dorsal root ganglia neurons were myelinated by glial cells from rat optic nerve transplants (*12*), and myelination was increased in myelin-deficient *jp*^{msd} mutant mouse cerebellar cultures by the addition of normal mouse optic nerve glia (*13*). In our tissue culture model, normal mouse cerebellar cultures in which oligodendrocyte differentiation was suppressed by exposure to cytosine arabinoside were myelinated by transplanted oligodendrocytes from normal mouse cerebellar cultures without axons receptive to myelination as a consequence of exposure to kainic acid. The demonstration of the myelination in vitro of CNS axons derived from normal animals by transplanted oligodendrocytes also derived from normal animals further illustrates the capacity of myelin-forming cells to function under a variety of conditions when presented with the stimulus of myelin-receptive axons. The cytosine arabinoside–treated CNS explant provides an alternative model to the dissociated dorsal root ganglia neuron preparation (*12*) for evaluation of the myelinating capacity of isolated oligodendrocytes (*14*).

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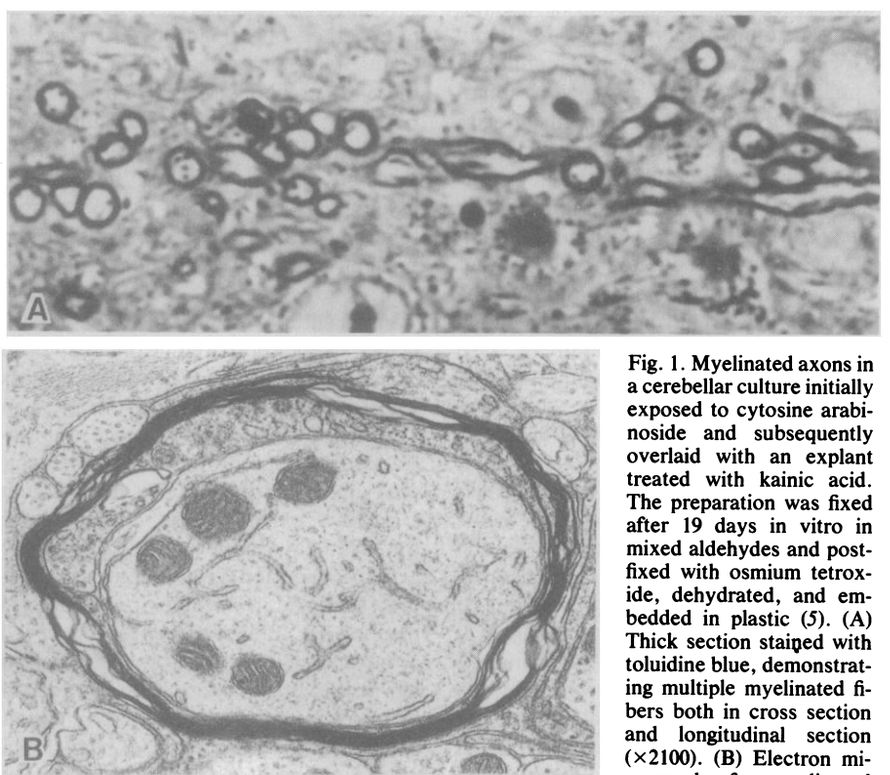

Fig. 1. Myelinated axons in a cerebellar culture initially exposed to cytosine arabinoside and subsequently overlaid with an explant treated with kainic acid. The preparation was fixed after 19 days in vitro in mixed aldehydes and post-fixed with osmium tetroxide, dehydrated, and embedded in plastic (*5*). (A) Thick section stained with toluidine blue, demonstrating multiple myelinated fibers both in cross section and longitudinal section (×2100). (B) Electron micrograph of a myelinated axon. The three small axons lying outside the myelin, above and to the left of the myelinated axon, are probable unmyelinated parallel fibers (×28,000).

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