Environmental Hydrocarbons Produce Degeneration in Cat Hypothalamus and Optic Tract

Abstract. *2,5-Hexanedione, the principal neurotoxic metabolite of the industrial solvents* n-*hexane and methyl* n-*butyl ketone causes axonal degeneration in the mammillary body and visual nuclei of cats. Prolonged, low-level exposure to hydrocarbons in the environment may cause premature deterioration in areas of the human brain vital for perception and behavior.*

Aliphatic hydrocarbons such as *n*-hexane are widely deployed in the environment and are known to produce axonal degeneration in the distal parts of peripheral nerves (distal axonopathy) in man and experimental animals (*1–3*). An experimental study with *n*-hexane demonstrated that distal axonal degeneration also occurs in the spinal cord and brainstem of the central nervous system (CNS) concomitantly with the changes in the peripheral nervous system (PNS) (*4*). The weakness and sensory loss associated with the peripheral nerve changes (neuropathy) mask the signs of the CNS degeneration which probably accompany most human toxic neuropathies. After removal from the toxic environment, the PNS axons usually regenerate whereas many of the CNS changes are permanent. This permits the individual to recover muscle strength and sensation but may leave spasticity associated with irreparable damage to spinal tracts (*5*).

In our study, which was stimulated by reports of diminished vision (*6*) and memory (*7*) in human *n*-hexane intoxication, we used 2,5-hexanedione to produce distal axonal degeneration in cats (*8*). 2,5-Hexanedione, the putative neurotoxic metabolite of both *n*-hexane and methyl *n*-butyl ketone (*9*), was used because it is readily soluble in water and lends itself to prolonged, low-level administration that resembles the situation of human exposure.

Intoxicated animals developed insidiously an unsteady gait and distal weakness in the lower extremities after 60 to 75 days. Further intoxication resulted in a progressive symmetrical weakness with foot drop in all extremities. By the time of perfusion, animals were quadriparetic and unable to walk. Visual loss, abnormal pupillary reflexes, nystagmus, titubation, or hoarseness were not noted.

The evolution of hydrocarbon-induced feline giant axonal degeneration was similar to that described in previous studies from this laboratory (*2*). The characteristic morphological changes were abnormally large fibers with swollen axons displaying abnormal accumulations of 10-nm neurofilaments, and myelin sheaths inappropriately thin for their axon diameter. Axonal swellings were present in the rostral gracile, dorsal spinocerebellar, and caudal corticospinal tracts in the early stages of intoxication and evolved concomitantly with changes in the PNS. Advanced changes consisting of distal fiber disintegration were present in the tracts at the time of perfusion. In contrast, early axonal swellings were present throughout the mammillary bodies (Fig. 1a), the lateral geniculate body and distal optic tract, and the superior colliculus. Necrosis, macrophage accumulation, and hemorrhage did not accompany the degenerative process. The optic nerves, vestibular nuclei, dorsal medial thalamic nuclei, and inferior colliculi were comparable to those of control animals. The transsynaptic neuronal breakdown seen at an advanced change in the gracile nucleus was not detected in these loci (*10*). Despite the enlargement of terminal axons in the mammillary and visual nuclei, synaptic complexes remained intact.

We now demonstrate that a neurotoxic hydrocarbon causes widespread axonal degeneration in the mammillary body, lateral geniculate nucleus, and superior colliculus of intoxicated cats. Since this change occurred concomitantly with axonal swellings in the distal optic tract with preservation of the more proximal optic nerve, these phenomena are best interpreted as further examples of the distal (dying back) axonopathy seen elsewhere in the PNS and CNS of these animals (*3*). The differential vulnerability of nerve tracts is proved to be related directly to the length and diameter of the composite axons (*2*). The earlier and greater changes seen in spinal cord pathways relative to those seen in the hypothalamic and visual nuclei correlate well with the relative shortness and small fiber size of the retina-geniculate and hippocampus-mammillary pathways presumably involved in this process.

The changes in mammillary body and optic tract are probably the pathological correlates of the alterations in memory and vision that have been infrequently reported in human *n*-hexane neuropathy. Degeneration of the human mammillary bodies may also result from thiamine deficiency (Wernicke's disease) and has been proposed as one of the lesions associated with the loss of recent memory that characterizes the Wernicke-Korsakoff syndrome (*11*). Indeed, the distribution of the changes evoked by 2,5-hexanedione is strikingly similar to some of

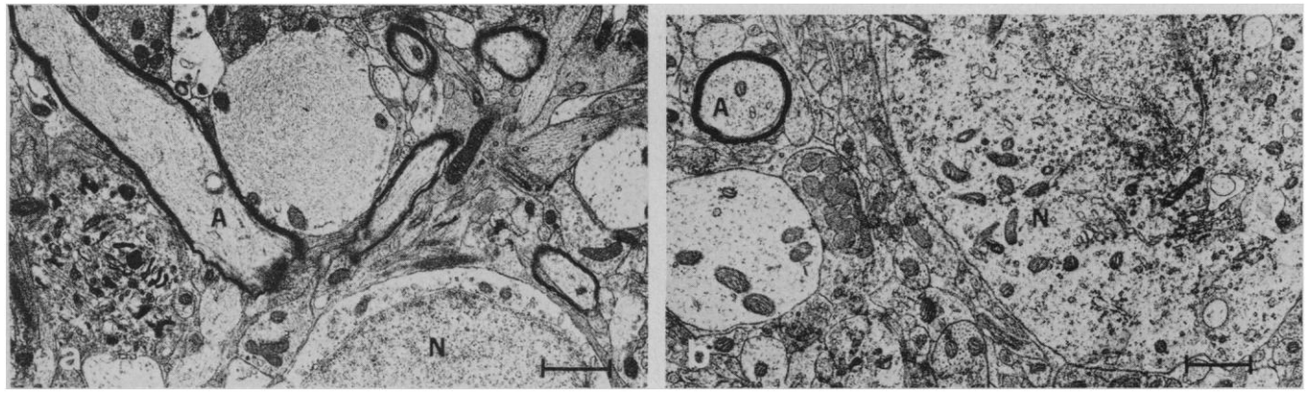

Fig. 1 (a) Section from the mammillary body of a cat intoxicated with 2,5-hexanedione for 69 days. Axons (*A*) with abnormal accumulations of neurofilaments are prominent. The neuron cell body and other neurites appear unremarkable. (b) Mammillary body from control animal with axon (*A*) with normal complement of neurofilaments and normal-appearing neuron (*N*). Scale bars, 1 μm.

0036-8075/78/0113-0199$00.50/0

those described in human and experimental Wernicke's disease where the mammillary bodies, anterior cerebellar vermis, gracile nuclei, and peripheral nerves are involved. It seems unlikely that 2,5-hexanedione damages these areas because of an induced thiamine deficiency, since many of the principal abnormalities of Wernicke's disease (macrophage response and periaqueductal and periventricular degeneration) were not present.

Pathological changes in visual nuclei and mammillary bodies probably accompany many human toxic neuropathies of the distal axonopathy type, yet there has been scant documentation of visual or mental state deterioration. Two factors may explain this discrepancy. (i) Few of the clinical reports included detailed analysis of visual function (none has reported visual evoked responses) or serial mental status evaluation. (ii) Many of the case reports involved short, high-level exposures and the clinical manifestations were mild and readily reversed (*1*).

The importance of these findings may lie in the field of public health. *n*-Hexane is widely used as an industrial solvent and is one of the many hydrocarbon components of gasoline. From this study it seems likely that prolonged, low-level industrial or environmental exposure to *n*-hexane or to the other environmentally prominent compounds that evoke distal axonopathy (acrylamide, carbon disulfide, cresyl phosphate) will provoke subtle changes in areas of the nervous system which may be vital to memory and vision. As the neuronal function and population decline in the course of the normal aging process, individuals exposed to such compounds might experience premature or accelerated deterioration in vision and mentation.

Note added in proof: Pilot studies have shown that acrylamide monomer, a chemical widely used in the plastic industry, also produces in rats distal axonal degeneration in the hypothalamus, optic tract, and anterior cerebellar vermis concurrent with a peripheral neuropathy.

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8. Four young adult cats were intoxicated and two were used as controls. All animals were housed in cages provided with smooth floors designed to prevent trauma to plantar nerves. The neurotoxin was administered by mouth by allowing the animals free access to drinking water containing 0.5 percent 2,5-hexanedione for periods up to 136 days. Control cats drank water as desired. All animals were weighed periodically, examined for signs of physical or neurological deterioration, and perfused through the aortic arch with 4 percent paraformaldehyde followed by 5 percent glutaraldehyde, each in 0.1*M* phosphate buffer (*p*H 7.4). After perfusion, tissue was removed from the peripheral and central nervous systems, postfixed with 2 percent Dalton's chrome osmium, embedded in epoxy resin, and processed for light and electron microscopy.
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10. Lesions of the optic tract and hippocampus are known to produce irreversible transsynaptic neuronal degeneration in the lateral geniculate body and hippocampus of man. See W. Blackwood and J. Corsellis, *Greenfield's Neuropathology* (Arnold, London, ed. 3, 1976).
11. J. Barbizet, *J. Neurol. Neurosurg. Psychiatry* **26**, 127 (1963). Other lesions have been proposed as responsible for memory loss after human thiamine deficiency. For example, M. Victor, R. Adams, and G. Collins [in *The Wernicke-Korsakoff Syndrome* (Davis, Philadelphia, 1971), pp. 164–170] have stressed the importance of the dorsal-medial thalamic degeneration. Considerable controversy still surrounds the anatomy of human amnestic syndromes. There is general agreement that the hippocampal-fornix projections are important to memory, and a recent study [W. Torch, A. Hirano, S. Solomon, *Neurology* **27**, 351 (1977)] has correlated unilateral hippocampal-fornix-mammillary-anterior thalamic transsynaptic atrophy with progressive memory loss and dementia. Hemorrhage in the human posterior hypothalamus with bilateral destruction of the mammillary bodies also has been associated with failure to record memories, even after a 7-year follow-up [R. G. Ojemann, *Neurosci. Res. Program Bull.* **4**, 1 (1966)].
12. Supported by NIH grants OH-00535, NS-08952, NS-03356, and NS 02255.

30 June 1977; revised 12 September 1977

Estrogen Formation in the Early Rabbit Embryo

Abstract. *Androgen formation (3β-hydroxysteroid dehydrogenase activity) was detectable in the rabbit blastocyst on day 5 of gestation (before implantation); estrogen formation was first detectable on day 7. The capacity to form estrogen on the day of implantation suggests that estrogen formation in the blastocyst may play a role in the implantation process.*

Indirect evidence suggests that estrogen action is involved in establishing direct contact between mother and embryo at implantation. In the rat, progesterone and estrogen in the maternal circulation are required for the initiation of blastocyst implantation, and estrogen appears to facilitate implantation even in species (such as the rabbit) that apparently lack an absolute requirement for maternal estrogen (*1*). The observation that antiestrogen inhibits implantation in intact (*2*) and ovariectomized, progesterone-treated rabbits (*3*) suggests that small amounts of estrogen are essential even in this species. Although the origin of estrogen for implantation of the blastocyst in the ovariectomized rabbit has not been defined, embryos of several species are capable of a variety of steroid hormone transformations before and after implantation (*4–6*). On the basis of histochemical studies of 3β- and 17β-hydroxysteroid dehydrogenase (E.C. 1.1.1.51) activities and the measurement of estradiol content in rabbit blastocysts, Dickmann *et*

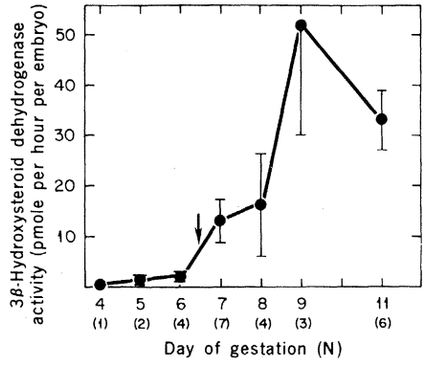

Fig. 1. Developmental pattern of 3β-hydroxysteroid dehydrogenase activity in the early rabbit embryo. Rabbit blastocysts and early embryos with membranes were recovered at various times during gestation and incubated in the presence of 5 μ*M* [7-^{3}H]dehydroepiandrosterone (30 c/mmole) for 2 hours at 37°C. At the end of the incubation, the reactions were stopped with chloroform; methanol (in a ratio of 2:1) and the reaction products, androstenedione and testosterone, were isolated and quantified (*8*). Six to eleven embryos (days 4 and 5), three embryos (day 6), or one embryo (days 7 to 11) were used for each determination. The data are presented as the mean ± the standard error of the mean for the number of determinations shown in parentheses, with the exception of day 4, which is a single determination, and day 5, which is the mean with the range of two observations. The arrow indicates the approximate time of implantation.

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