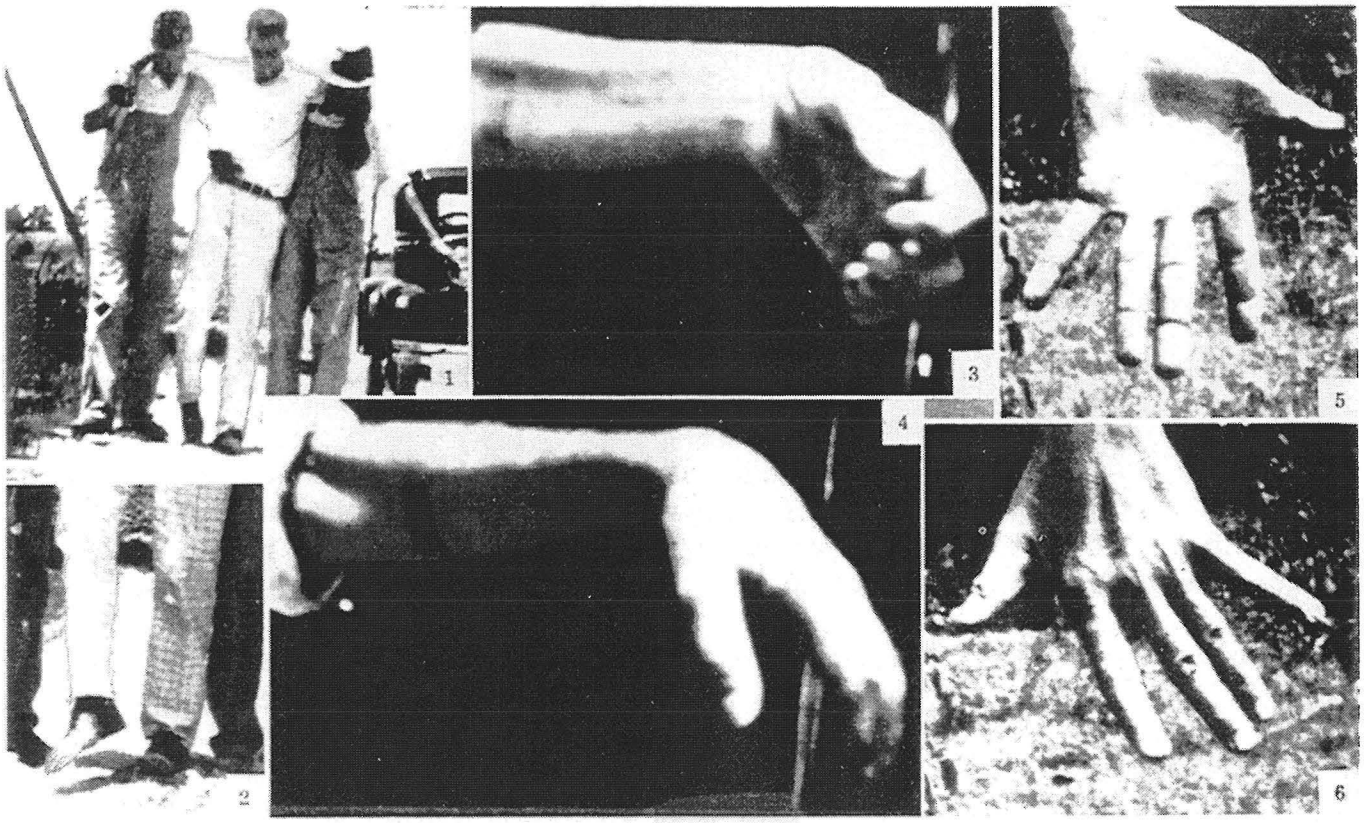
OPIDN

Organophosphate-Induced Delayed Neurotoxicity



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Internal Medicine Grand Rounds
University of Texas Southwestern Medical Center
Dallas, Texas
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Interests: Epidemiologic research on infectious diseases, particularly nosocomial infections; evaluation of the outcomes of hospital care, particularly the development of multivariate risk indexes for risk-adjusting case mix; and investigation of unusual illnesses such as the Gulf War syndrome.

Cover Photograph

Photographs of residual neurologic impairments from "Jake Paralysis" enlarged from a cinematographic film (from Kidd J.G., Langworthy O.R. Jake paralysis: paralysis following the ingestion of Jamaica Ginger Extract adulterated with tri-ortho-cresyl phosphate. *Bull. Johns Hopkins Hosp.* 1933;52:39-65).

Figs. 1 and 2. The foot-drop and the paralysis of the dorsiflexors of the ankle in a moderately severe case. The patient is quite unable to stand unless supported.

Figs. 3 and 4. The extensor musculature of the fore-arm is more severely paralyzed by the poison than the flexor. There is a wrist-drop. Atrophy of the flexor musculature is evident in both of the photographs.

Figs. 5 and 6. There is also atrophy of the intrinsic musculature of the hands seen in the thenar and hypothenar eminences and in the interossei.

Acknowledgements:

This grand rounds describes a condition that was brought to my attention by our area's medical toxicologist, Dr. Tom Kurt, who has guided my study of the subject. Dr. Al Roberts provided the case report.

Every physician is familiar with the clinical presentation, pathogenesis and treatment of the immediate cholinergic crisis caused by exposure to an organophosphate, cholinesterase-inhibiting pesticides, such as malathion. The muscarinic and nicotinic effects are covered thoroughly in the pharmacology course in medical school and occupy a half page or more in the major internal medicine textbooks (1,2). Failure to recognize and treat this immediate syndrome quickly would be considered poor medical practice.

Some organophosphate chemicals produce a second syndrome that may be as common as the immediate cholinergic crisis but sometimes more severe and long-lasting in its effects. This is the syndrome of *organophosphate-induced delayed neurotoxicity (OPIDN)*. Whereas the immediate, cholinergic syndrome usually begins within minutes or hours of exposure to the chemical, the OPIDN syndrome typically begins several days or weeks later, following an asymptomatic interval and may leave the victim permanently impaired. The two syndromes may occur sequentially in the same exposed individual, or either one may occur alone. Despite having affected tens of thousands of people in the U.S. over the past century, few physicians are aware of the delayed, chronic syndrome. In current internal medicine textbooks, it occupies no more than a footnote in the discussion of toxic peripheral neuropathies, and its relationship with the immediate cholinergic syndrome is not mentioned (1,2).

Every year, hundreds of cases of immediate pesticide poisoning are reported in Texas (3), but the incidence of delayed, chronic neurotoxicity, which often goes unrecognized and is rarely reported, is unknown. With the increasing numbers of organophosphate pesticides and other cholinesterase-inhibiting chemicals in use and the lack of scientific understanding of their interactions, the incidence of OPIDN may be increasing, possibly explaining the apparent rise in the frequency of the neurasthenic syndromes such as Agent Orange-related illnesses, chronic fatigue syndrome, multiple chemical sensitivity, and the neurodegenerative diseases (4). Suspecting OPIDN, taking a focused exposure history, and ordering the right diagnostic tests may help explain many cases of these perplexing conditions.

The general structure of organic phosphorus chemicals with cholinesterase-inhibiting properties is shown in the first figure. The various compounds are classified into four categories I-IV on the basis of their respective -X constituents.

The following is a case report of a typical patient with OPIDN presenting to an internist on our faculty.

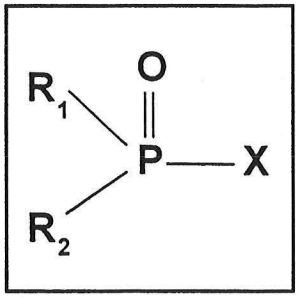

Figure 1. *General formula for cholinesterase-inhibiting organic phosphorus chemicals*

Case Report

A 66 year old North Dallas woman presented with a two-week history of weakness, aching and burning of the lower extremities. She first noticed the problem when she was unable to perform her usual isometric knee exercises. Shortly, she developed severe fatigue ("lost my usual get up'n go") and became unable to complete her regular aerobics class. This was soon followed by severe aching in both legs up to her thighs, accompanied by extreme restlessness of her legs and a burning sensation. The symptoms bothered her less when she was active. Her past health had been excellent, and she exercised vigorously. Except for a back injury eleven years earlier, she described herself as "never sick." She took vitamin supplements but no excess pyridoxine or excess doses of other vitamins. Her physical examination was completely normal. There was no objective muscle soreness, no altered sensation, and no abnormal reflexes.

On questioning, she recalled that a few days before the onset of her symptoms, the inside of her house and her yard had been heavily sprayed with pesticides, including drione (Sevin) and safrotin (Noc-out), both organophosphates. She recalled that she did not vacate the house as directed after the spraying and further that she almost invariably went barefoot when at home and in her yard. After consultation with a medical toxicologist, a red cell cholinesterase test was ordered. The result was normal, but it had been several weeks since the original exposure. The patient's fatigue and neurologic symptoms gradually abated and had subsided completely at six months. She remains in excellent health eight years later (5).

Initial Recognition of OPIDN from Poisoning with TOCP

The syndrome of OPIDN was first described in people poisoned with tri-ortho-cresyl-phosphate (TOCP). Cresyl-phosphate (Lindol or Lyndol) is a common industrial solvent and plasticizer used in making plastic, polyvinyl chloride (PVC), celluloid, cellophane, paints, and varnishes and for tanning leather (6,7). A very inexpensive industrial chemical, it is oily in consistency, soluble in alcohol, and highly heat resistant, making it ideal also for use as a heavy machinery lubricant and as a cooking oil. Unlike the organophosphates, to be discussed below, TOCP has little anti-cholinergic effect.

The First Epidemic, 1899

The first cases of delayed neurotoxicity occurred in France in 1899, when 6 tuberculous patients developed "polyneuritis" after treatment with *phospho-creosote* (8), later shown to contain TOCP. Cases of permanent neurologic impairment from this tuberculosis remedy continued to occur into the 1930s.

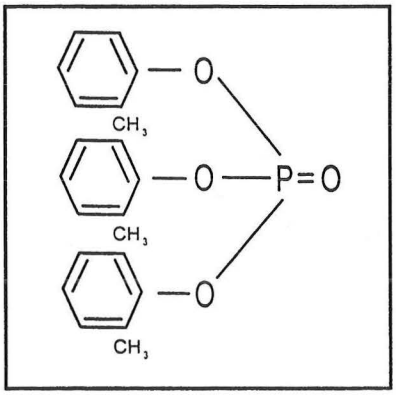

Figure 2. *Chemical structure of tri-ortho-cresyl phosphate (TOCP)*

Jake Paralysis, 1930

In late February 1930, newspapers throughout the southern and midwestern U.S. from Florida and Virginia to Texas and Missouri reported the sudden epidemic occurrence of a paralytic illness (9-11). By April, approximately 5,000 cases had been identified almost all in adults 20 to 60 years of age, and ultimately as many as 50,000 people are estimated to have been paralyzed (12,13). Given a population of the 17 affected states of 50 million in 1930 (14), the attack rate may have approached 1 per 500 adult residents.

Typically an adult man or women would suddenly collapse on the street, unable to arise due to leg weakness (6,9-11,15). In the hospital some would recall a transient episode of nausea, vomiting, abdominal cramping and diarrhea, followed by an asymptomatic interval of 1-4 weeks, and finally soreness and cramping of the calf muscles for a day or two before being stricken by profound lower extremity weakness. Almost all victims reported having consumed one or more 2-oz bottles of Fluid-extract of Jamaica Ginger U.S.P. ("Ginger Jake" or simply "Jake") 1-4 weeks earlier, about the time of the transient gastrointestinal illness; however, many had consumed Jake habitually for years. A surprising number of different name brands of Jake (e.g., Queen City, Land, Archer, Peer, Loyal, K.D., B&L, Fuller, Tommac, Peco, Superior, and Superb) were apparently involved (16,17).

On initial examination, the victims were found to have a mild loss of sensation in the feet and hands and a severe, bilaterally symmetrical flaccid paralysis below the knees affecting the anterior tibial muscles more than the calf muscle, resulting in foot drop. Deep tendon reflexes were absent in the ankles but were hyperactive in the knees, sometimes with patellar clonus; a few patients developed fecal or urinary incontinence; vasomotor signs, including cold, sweaty and cyanotic feet, were virtually universal; and Babinsky's sign was present in some. The sensory loss would typically resolve quickly, but in severe cases weakness would develop in the intrinsic muscles of the hands and forearm one week after the appearance of the leg weakness.

Over the succeeding 1-2 years, in most victims severe muscle wasting would occur in the muscles of the anterior tibial compartment and the hands, and the weakness would gradually improve in the reverse order in which it had developed. Many victims were left with disabling foot drop and a characteristic spastic, high stepping gait (the "Jake walk").

The extent of the impairment, though not the 1-4 week incubation period, appeared to be related to the amount of the adulterated Jake consumed. For example, a woman who consumed one or more bottles per day for several months died with severe quadriplegia, bowel and bladder incontinence, and bulbar paralysis (18).

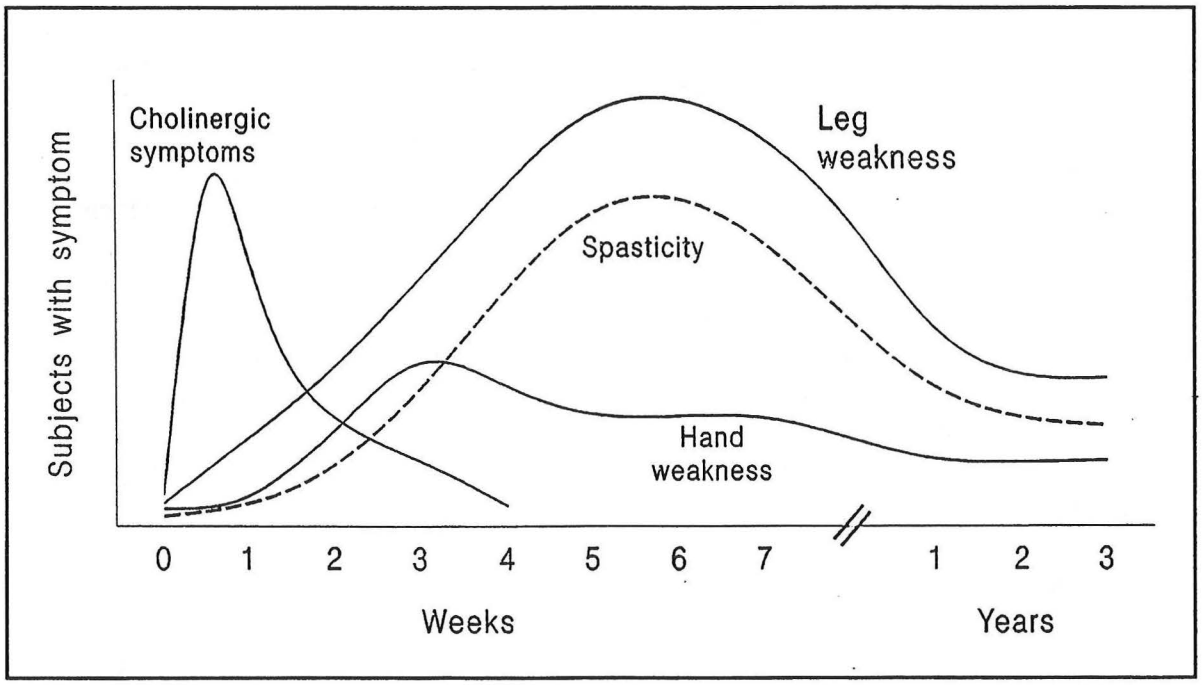

Figure 3. *Usual time course of symptoms of OPIDN from TOCP poisoning.*

Within the first weeks of the epidemic, concerns of polio and Landry's paralysis were dismissed as attention focused on the association with Jake (6,9-11,15,16). Fluid-extract of Jamaica Ginger U.S.P. was a standard over-the-counter medication widely prescribed by physicians, druggists and grocers for headache, stomach upset, and menstrual cramps since its introduction into the U.S. Pharmacopoeia in 1863 (6). A thin, dark-brownish liquid with an aromatic odor and a pungent taste (6), it consisted of oleoresin of ginger root in 70%-80% alcohol. The concentration of ginger was too strong to drink undiluted, and it was typically

taken as 1 or 2 drops in a soft drink such as ginger ale. During the era of Prohibition, the U.S.P. formulation was approved by the Prohibition Bureau because it was considered nonpotable, too concentrated to drink undiluted (19).

With the ban on the sale of alcohol in many southern states even before the federal prohibition law in 1919 (19), commercial suppliers distributed illicit formulations throughout the south and lower midwest. In these, the ginger extract was cut with oily, tasteless substances such as glycerin, molasses, caster oil, mineral oil, sages, herb extracts, and others to make the final solution potable, yet still of the regulated weight to pass government inspection (6,19). Throughout the south and midwest, people typically purchased a 2-oz bottle of Jake, poured it in coffee or a carbonated drink from the soda fountain, and consumed it on the premises of the drug store (6,19). A bottle of Jake had more punch than a shot of straight whiskey from the bar (19). While a medical student, the famous entrepreneur Dr. Armond Hammer made his initial fortune manufacturing and distributing tincture of ginger from his Bronx pharmaceutical plant while paying a friend to take notes to get him through Columbia medical school (20). Immediately upon seeing the dramatic jump in demand for Jake in 1919, he bought up the world's supply of ginger extract so that all wholesale suppliers had to buy from him.

The epidemic ended abruptly by the middle of 1930 as a result of the intense publicity of the link with Ginger Jake. Maurice Smith and colleagues of the National Institute of Health substantiated the link in a classic set of experiments in laboratory animals (16,17,21,22). In his initial experiments, administration of implicated bottles of Jake to laboratory monkeys and dogs failed to produce illness (16). At the suggestion of a midwestern veterinarian who had inadvertently paralyzed some ailing cattle by treating them with Ginger Jake, Smith finally reproduced the neurologic syndrome in calves, rabbits and eventually chickens (16,21). This not only established the etiologic link with Jake but also detected the remarkable species specificity of organophosphate poisons and established the hen as the most suitable animal model for studying OPIDN (23).

Smith's chemical analyses identified *cresyl-phosphate* as the adulterant at a 2% concentration in epidemiologically implicated bottles of Jake (16,17,21). In further animal studies he demonstrated that the *ortho* isomer, but not the *para* or *meta* isomers, had the potent neurotoxic property. Tri-*ortho*-cresyl-phosphate (TOCP), the cause of "Jake paralysis," would become the world's most deadly neurotoxin. In the 1930s, Lindol consisted mainly of the pathogenic *ortho* isomer, but in recent years manufacturers have substituted the para and meta isomers (6).

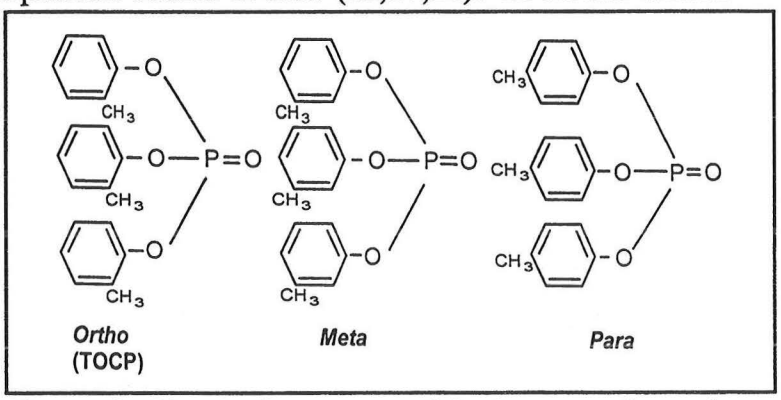

Figure 4. *Chemical structure of the isomers of cresyl-phosphate. Only the ortho isomer is neurotoxic.*

As a postscript, the criminal investigation by the Bureau of Prohibition traced the TOCP-adulterated Jake to shipments from Brooklyn and Boston and indicted 6 corporations in New York City for conspiracy to

violate federal laws (13,19). The less expensive adulterant TOCP was allegedly substituted for castor oil to undercut competition in the lucrative Jake market (23). The dramatic epidemic so influenced popular Southern culture that at least 12 popular songs, mostly in the blues idiom with titles such as "Jake Leg Wobble," "Jake Bottle Blues," and "Jake Walk Papa," were recorded and widely distributed (19). "The Jake Walk Blues," recorded in Memphis by the Allen Brothers, sold over 20,000 copies, a best seller of the day, and contributed to their rise in popularity (19). Lyrics of the various recordings suggested the certain fate of the Jake drinker,

> My daddy was a gambler, and a drunkard too,
> If he was living today, he'd have the Jake Walk too.
> When I die, you can have my hand,
> Gonna take a bottle of Jake to the promised land.

condemned the illicit practice,

> Boys, Jamaica ginger sure will do its part,
> Boys, Jamaica ginger will kill your honest heart.

or showed a lack of compassion for the self-induced ills of intemperance,

> A preacher drank some ginger,
> He said he did it for flu,
> That was his excuse
> For having the Jake leg too.

Even today, in the south a hypocritical, falsely self-righteous pastor is known as a "Jake Leg Preacher" (19).

The 1919 federal Volstead Act, establishing prohibition nationwide, was repealed in 1933, less than three years after the epidemic of Jake Paralysis.

The European Epidemic, 1931-1932

In 1931, the year following the U.S. epidemic, a large epidemic of typical TOCP-induced paralysis occurred entirely in women throughout Europe (24). Investigation linked the illness with having taken the abortifacient *apiol*. The long-used agent, derived from parsley, was shown to have been adulterated with TOCP, possibly in an attempt to increase its potency.

"The Strange Durban Epidemic of 1937"

In late 1937, an epidemic of suspected infectious paralysis of the legs affected 68 people in the port city of Durban, South Africa (25). Investigation of the first 40 cases revealed only an association with working in European-style kitchens. Public health authorities in Durban then received a report from counterparts in France of a merchantship, the *Jean L.D.*, which had been found adrift in the Bay of Biscay off the English channel a month after

visiting the port of Durban, its entire crew incapacitated by paralysis of the legs. Examination of the manifest of stores purchased in Durban for its fateful voyage was initially unrevealing. Discussion among a record turnout of the Coastal Branch of the South African Medical Society yielded no insights, "the chief highlight [being] finding of press reporters hidden outside a window, taking down all that was said."

The break in the case occurred at the home of the assistant public health officer who was studying the supply manifest from the *Jean L.D.* His wife, familiar with all the findings, glanced at the ship's manifest and remarked, "If, as you say, all the cases are associated with European kitchens, then what about this . . . Bestal superfine cooking oil . . . they mention here?" A quick telephone survey of the kitchens where some of the victims had been employed yielded the same brand of cooking oil. An attempt to reproduce the paralysis in local wild monkeys was unsuccessful, but when the monkeys inadvertently escaped, the experiment was re-initiated in cockerels. After 7 days of feeding with the cooking oil, the crow of the birds changed while the control birds crowed as usual. Two days later the affected cockerels developed paralysis of the legs. TOCP was soon identified as a contaminant in the cooking oil. Public health authorities in France likewise identified TOCP in the barrels of the same cooking oil aboard the *Jean L.D.*

Sporadic Outbreaks During World War II

Between 1940 and 1946, outbreaks of poisoning from TOCP-containing cooking oil were reported to have involved several factory workers in Germany (26), 80 men in the Swiss army (27), and sporadic cases in Liverpool (28). Three British factory workers were poisoned by inhalation of TOCP in 1942 (29).

The Moroccan Epidemic of 1959

In the summer of 1959, over 10,000 cases of lower leg paralysis occurred in several Moroccan towns west of Casablanca (30). Epidemiologic analysis revealed that the victims were generally confined to the poor sections of the city, but it spared the poorest of the poor as well as visitors attending a local festival. The cases also occurred in well circumscribed areas of the towns. After ruling out infectious etiologies, public health officials investigated the victims' concerns that the illness was due to a new type of dark colored cooking oil. Examination of cooking oils on the shelves of grocers' shops in the affected areas found bottles of the same inexpensive brand of cooking oil, some filled with the usual clear, yellow oil next to others filled with a dark oil resembling motor oil. Chemical analysis of the dark oil revealed a mixture of 33% vegetable oil and 67% lubricating oil containing TOCP.

The Vietnamese TOCP Epidemic of 1970-1971

In 1970 a physician assigned to a medical assistance team to villagers in Quang Tri Province in the northern part of South Vietnam noticed clusters of a new spastic quadriplegia involving minimal sensory loss but a characteristic spastic, stumbling gait (31). The clusters of cases often involved families. Investigation linked the illness to "black market" cooking oil laced with TOCP-containing U.S. military aviation lubricant supplied to South Vietnamese helicopter units. Even after the problem was reported, new cases continued to occur for another year of so.

Pathologic Findings from the Epidemics

The initial reports from the investigations of the Jake Paralysis epidemic (6,9-11,15,16,22) falsely concluded that the pathologic process was a purely lower-motor-neuron *polyneuritis* involving a minor, transient sensory disturbance and a more severe, often permanent motor nerve deficit. Reports of pathological examinations of the few fatal cases emphasized demyelinization of peripheral nerves including degeneration of the cell bodies of the anterior horn cells (22). All of the early descriptions of "Jake polyneuritis," however, failed to account for the clinical signs of an upper motor neuron deficit, including early *hyper*-reflexia of the patellar tendon reflexes, spasmodic contractions of the leg muscles, sphincter involvement, excessive sweating, coolness and cyanosis of the skin of the feet and hands, and Babinsky sign which were in many of the original case reports (9-11,15).

Aring in 1942 was the first to call attention to the involvement of the spinal cord (32). In a 10-year followup of over 100 patients injured in the 1930 epidemic, he recalled the clinical signs of upper motor neuron deficit in the original case reports and described the late sequelae, which included marked atrophy of muscles in the feet, legs and hands, foot drop with contracture of the achilles tendon, "clawed" hand deformity, foot deformity similar to that of Friedreich's Familial Ataxia, hypesthesia (touch, pain, temperature) below the knees, excessive diminution of vibratory sense for age, hyperactive deep tendon reflexes and patellar clonus of the knee, either absent ankle reflexes or ankle clonus, and excessively cold, moist, cyanotic skin of the feet. From autopsy material on 36 patients with the condition, he described pathologic damage to both the peripheral nerves and the spinal cord. The axons of peripheral nerves were of irregular width and decreased in number with patchy loss of myelin. In the spinal cord, there was uniformly severe degeneration of the lateral pyramidal tracts in the lower segments of the cord, degeneration and intense gliosis in the posterior columns (primarily the fasciculus gracilis) in the upper segments of the cord, and a "rim of degeneration," resembling the vacuolated type seen in subacute combined degeneration. These changes uniformly stopped short of the medulla. There was reduction in the number of anterior horn cells and of nerve cells in the brain stem and cerebrum but not in the spinal cord. The leptomeninges were often thickened. He pointed to superficial similarities to the pathological picture of amyotrophic lateral sclerosis.

In a 47-year followup of victims of jake paralysis, Morgan and Penovich (13) confirmed the late sequelae described by Aring as well as the involvement of both peripheral nerves and spinal cord.

Spinal cord lesions similar to those found in humans were later produced in rats and hens by TOCP and similar organophosphates such as triphenyl-phosphite (TPP) and *O,O*-diisopropyl-phosphorofluoridate (DFP). In the following figures, notice that different patterns resulted from poisoning with different chemicals (33).

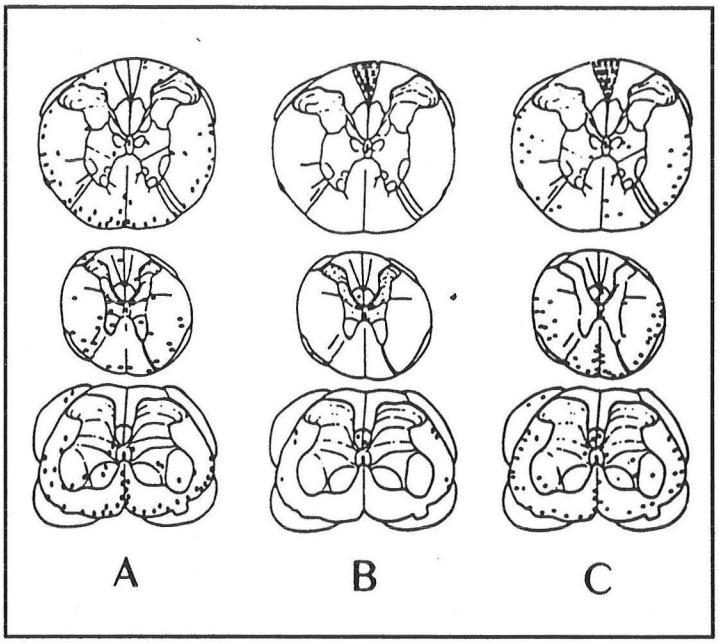

Figure 5. *Topography of spinal cord damage in rats treated with (A) TPP, (B) TOCP, or (C) both. Dots represent TPP, and X's represent TOCP.*

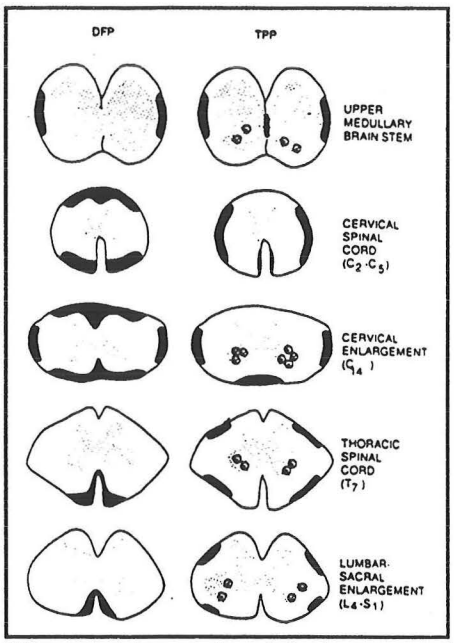

Figure 6. *Topography of brain stem and spinal cord damage in chickens treated with DFP or TPP.*

Pathophysiology of OPIDN

Organophosphates, first synthesized in 1820 and linked to adverse effects on living organisms in 1932, were first used as pesticides and later developed into chemical warfare agents during and after World War II. Two pathologic syndromes are produced by cholinesterase-inhibiting pesticides and chemical nerve agents: 1) an immediate, transient cholinergic syndrome, and 2) a delayed, chronic neurotoxicity syndrome (OPIDN). A third possible condition, the "intermediate syndrome," is of little importance and will not be discussed (34). Suspected long-term risks of cancer from pesticide exposures will also not be discussed.

Table 1. Characteristics of the two syndromes caused by exposure to cholinesterase-inhibiting chemicals.

Syndrome	Mechanism	Pathology	Symptoms	Prevention*
Immediate poisoning (transient if not fatal)	Binding and inactivation of acetylcholinesterase at the neural synapse and neuromuscular junction	Functional excess of acetylcholine produces an acute cholinergic crisis.	Muscarinic (early) Nausea, diarrhea, salivation, etc. Nicotinic (late) Muscle fasciculations cramping, paralysis asphyxiation Brain Fatigue, confusion, coma, reduced respiratory drive	Pre-exposure pyridostigmine (post-exposure atropine and an oxime)
Delayed neurotoxicity (may be chronic)	Binding to neurotoxic esterase (NTE) in brain, spinal cord and peripheral nerves, followed by "aging" of the chemical-NTE complex	Progressive axonal degeneration (Wallerian) begins 1-6 weeks after exposure.	Brain and spinal cord Cognitive symptoms, fatigue, depression, ataxia Peripheral nerves Tingling/numbness, weakness, muscle wasting	None (Pyridostigmine and other cholinesterase inhibitors might increase the risk by reducing the pool of butyrylcholinesterase.)

*The body's natural defense includes the scavenging function of butyrylcholinesterase and hydrolysis by paraoxonases in the blood.

The Immediate Poisoning Syndrome

When exposed to an adequate dose of a cholinesterase-inhibiting chemical, one may develop symptoms of poisoning almost immediately, within minutes to hours. The mechanism of the immediate poisoning syndrome is simple. The chemical binds covalently to (phosphorylates) the enzyme *acetylcholinesterase (AChE)* in the interneuronal synapse and the neuromuscular junction and inactivates it. Since the phosphorylated AChE can no longer hydrolyze ACh, there ensues an immediate functional excess of ACh, sustained depolarization of the end plate, and cholinergic symptoms (Table 1). If severe, the process leads to a cholinergic crisis.

In mild poisoning the first manifestations are muscarinic symptoms such as nausea, diarrhea, sweating, salivation, bradycardia and miosis, which can be blocked selectively by atropine. More severe poisoning causes worsening of the muscarinic symptoms and the appearance of nicotinic effects such as muscle fasciculations, cramping, and acute myopathy, progressing to paralysis and death from asphyxiation; these can be blocked selectively by hexamethonium or succinylcholine. If the cholinesterase-inhibitor crosses the blood-brain barrier, central nervous system effects progress from fatigue to disorientation, coma and loss of respiratory drive (35).

Victims who survive the episode usually recover rapidly and completely. Mild symptoms may persist for several weeks due to the gradual release of organophosphate residuals stored in fat. In severe poisoning, the sustained stimulation of the motor end plate may injure the muscle, causing a lingering myopathy.

The Delayed, Chronic Neurotoxicity Syndrome (OPIDN)

When exposed to some--*but not all*--organophosphates, an asymptomatic incubation period of 1 to 6 weeks is followed by the development of true neurologic deficits, different from the immediate, poisoning syndrome and involving virtually any component of the nervous system. The duration of the asymptomatic interval and the spectrum of the neurologic abnormalities is a function of the particular organophosphate chemical. After the chemical has been absorbed, no drugs will block the eventual development of OPIDN; although atropine and pralidoxime (2-PAM) are effective for treating the immediate poisoning syndrome, they have no effect on delayed neurotoxicity (36). Likewise, once symptoms develop, no treatment will reverse or modify them. The neurologic symptoms typically improve over time, usually over 1-2 years, but more severely affected individuals are left with permanent neurologic deficits. Presently, neuro-rehabilitation is the only approach to treatment.

The mechanism of the delayed, chronic syndrome is more complex than that of the immediate, poisoning syndrome. The symptoms and deficits are due to an injury that causes typical Wallerian degeneration of the longest and largest nerves. The damage is first evident toward the ends of the nerves and progressively extends proximally, a process known as *"dying back"* or *"distal axonopathy."* It typically affects the longest peripheral nerves, first those in the feet, legs, hands and arms; the long, efferent and afferent tracts of the spinal cord, first in the lower lumbar region and gradually ascending; and finally, the upper spinal cord and brainstem. Longitudinal section of an affected nerve typically shows normal axonal fibers next to swollen, fragmented ones at different stages of degeneration.

If the peripheral nervous system is primarily involved, the clinical picture is that seen in the early phase of TOCP poisoning: sensory dysfunction such as paresthesias and numbness and flaccid paralysis of the feet and legs (lower motor neuron dysfunction), extending after a week or so to the hands and arms. If the spinal cord is involved, one finds hyperreflexia, loss of bowel or bladder control, autonomic dysfunction and spasticity (upper motor neuron dysfunction). Involvement of the brainstem and subcortical areas adds fatigue, ataxia, and cognitive impairments.

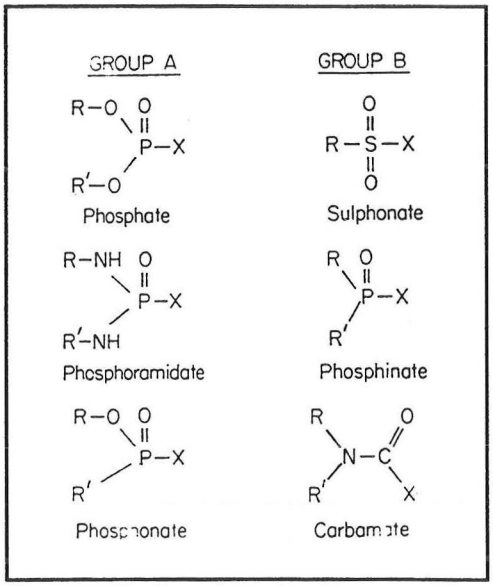

Figure 7. *Groups of AChE and NTE inhibitors. Group A chemicals cause neurotoxicity; Group B chemicals protect against it.*

Several important early clues to the complex mechanism of the syndrome came from study of the different effects of various organophosphate chemicals. Early investigations found that not all of the organophosphates that inhibit AChE produced delayed neurotoxicity. The next figure compares the chemical structures of the two groups of chemicals: organophosphates in Group A cause delayed neurotoxicity, while those in Group B do not (37,38). Organophosphates are the most important in Group A, and the carbamates, in Group B.

Moreover, if experimental animals were treated with a chemical from Group A alone, they would develop delayed neurotoxicity. However, if they were first treated with a carbamate from Group B and later by an organophosphate from Group A, they would not develop delayed neurotoxicity; the carbamate would protect them. If the Group B carbamate were administered after the Group A organophosphate, it would not protect them or limit the damage.

In the early 1970s, M.K. Johnson of the British Medical Research Council Toxicology Unit worked out the mechanism (37-39). He discovered a second enzyme throughout neural tissue, which he called *neurotoxic esterase (NTE)*. He found that NTE has enzymatic (hydrolytic) properties similar to those of AChE (e.g., hydrolyzes ACh) but, unlike AChE, it is not essential for any known neural function.

For a Group A organophosphate to produce delayed neurotoxicity, it must bind covalently to NTE and inhibit its enzymatic properties. However, both Group A and Group B chemicals readily do this. Moreover, binding of the chemicals to NTE occurs rapidly, leaving the 1-6 week delay before the occurrence of nerve damage unexplained.

All of the properties were explained by a further step in the reaction called "aging". When an enzyme, such as AChE or NTE, is exposed to an organophosphate, the organophosphate gives up its -X moiety (called the "leaving group") and binds covalently to the enzyme. This reaction inhibits the hydrolytic properties of the enzyme leaving a chemical-enzyme complex, called the "inhibited enzyme." This complex will no longer hydrolyze ACh, and it does not produce delayed neurotoxicity.

With Group B chemicals, no further reaction occurs. Chemicals in Group A have the ability to undergo a further reaction. Since this is a time-dependent reaction, requiring from 1-6 weeks to occur, it is called "aging." It leads to the loss of one of the -R groups from the chemical component of the complex. This leaves the chemical-enzyme complex with a negative charge, called the "'aged' inhibited enzyme." This complex is what injures the axon to cause delayed neurotoxicity.

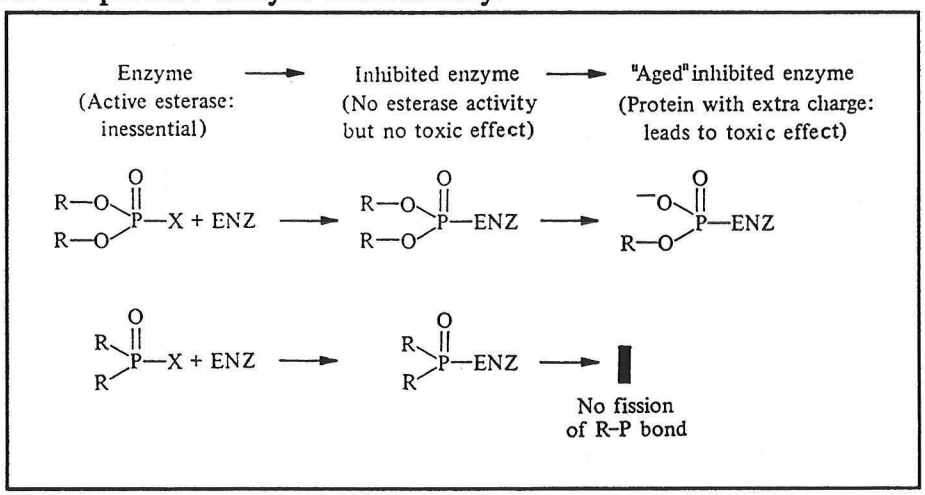

Figure 8. *Comparison of the actions of a Group A compound (top reaction: "promotion") and a Group B compound (bottom reaction: "protection") in reactions with an esterase (ENZ) such as AChE or NTE.*

This model explains the 1-6 week delay between chemical exposure and the appearance of neurologic deficits. It also explains how the Group B chemicals, if administered first, protect the host from developing delayed neurotoxicity when it is later exposed to a Group A chemical (40). Specifically, pretreatment with a Group B chemical binds to the active site of

the enzyme so that it is not available for binding when the Group A chemical comes along. Thus, the ability to undergo "aging" is the characteristic that distinguishes the chemicals of Groups A and B. In practice, whether a chemical can undergo "aging" is measured by testing whether it produces delayed neurotoxicity in laboratory hens.

Following the formulation and acceptance of the classical concept of "aging," further studies found that the sequence of dosing with Group A and Group B chemicals is more complex than originally thought (41-44). In fact, when a non-"aging" chemical (Group B) is given *before* exposure to an "aging" chemical, it protects against OPIDN ("protection"); however, if the non-"ager" is given *after* the "ager," it may actually *enhance* the potential for OPIDN ("promotion").

The Environmental Protection Agency (EPA) requires that all organophosphate chemicals be tested in a hen model, and only those that do not result in "aging" and delayed neurotoxicity can be licensed as pesticides for general use.

Ultrastructural Changes in OPIDN

The pathologic changes involved in the "dying back" or "distal axonopathy" of delayed neurotoxicity have been thoroughly investigated in experiments with hens and rats (45-50). The earliest changes in nerves occur simultaneous with the occurrence of the first neurologic signs. These include nodular swellings of the axoplasm just proximal to the nodes of

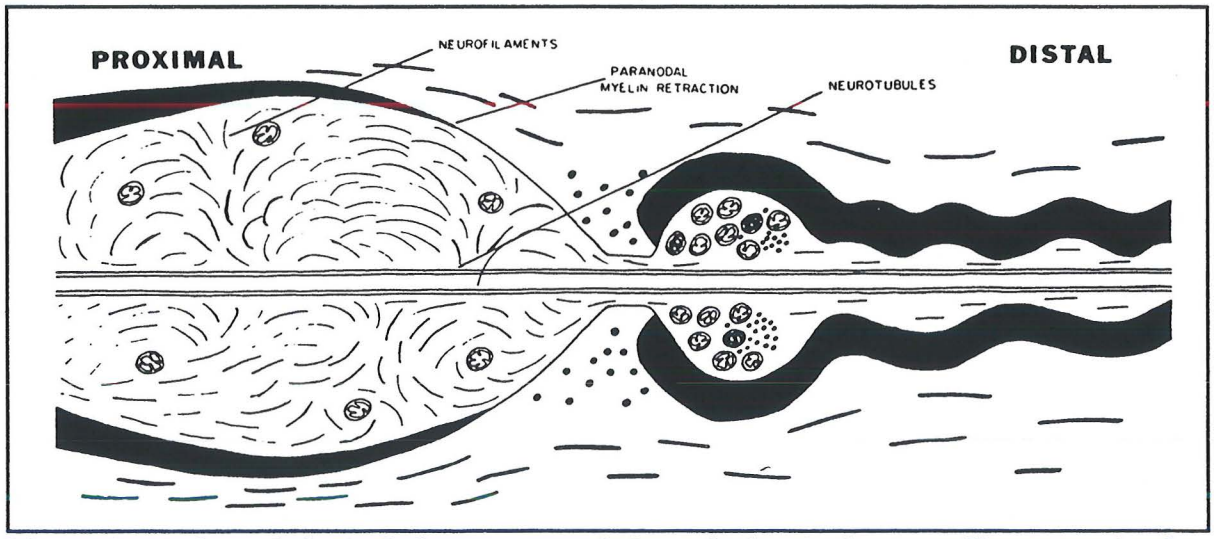

Figure 9. *Early pathological changes toward the end of a single nerve fiber. Proximal to a node of Ranvier, the axon swells with accumulation of organelles. Distally the axon is disentigrating.*

Ranvier near the end of the nerve (see the figure). The swelling comprises the formation of expanding vesicles, a proliferation of neurofilaments and neurotubules, and paranodal myelin retraction. As these changes progress over the following days, the normal space between the axonal and Schwann cell membranes collapses in spots, but the myelin sheath shows no signs of damage. Following these initial changes, the distal portion of the axon becomes shrunken, has fewer neurofilaments, and the myelin sheath

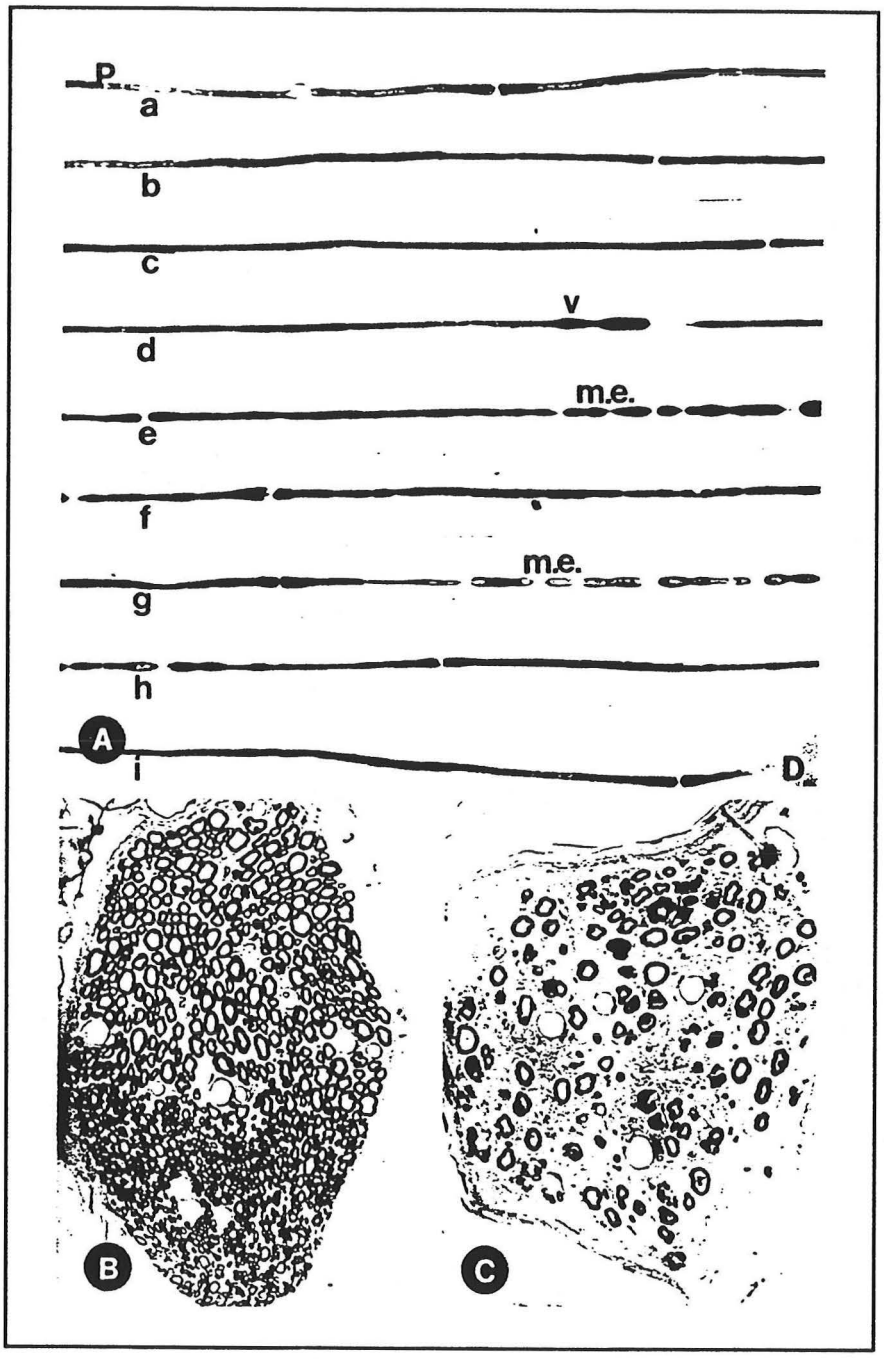

Figure 10. (A) Consecutive segments of a single, teased myelinated fiber from the recurrent laryngeal nerve of an adult cat, given a single intraperitoneal injection of diisopropyl phosphorofluoridate (DFP) (40 mg/kg) 20 d previously: *P*, proximal end; *D*, distal end. Proximal internodes (*segments a–c*) and distal internodes (*segments h and i*) appear normal. Internode in *segment d* exhibits an internodal varicosity (*v*) while segments *e* and *g* display localized internodal myelin ellipsoids (*m.e.*) (osmium tetroxide fixation, ×94). Transverse, 0.5-μm sections of proximal (B) and distal (C) ends of left recurrent laryngeal nerve from a cat, 28 d after DFP dosing. Although the distal end has marked loss and degeneration of fibers, the proximal end appears normal (modified silver stain, ×255). (Reproduced by permission from T. W. Bouldin and J. B. Cavanagh: *American Journal of Pathology*, *94*, 241, Harper & Row, New York, © 1979.)

becomes corrugated and begins to disintegrate. Finally, two or three weeks after the deficits appeared, the axoplasm becomes rarefied, often crowded to one side by huge vesicles, distal nerve endings begin disintegrating, and the myelin sheath degenerates.

Ultimately, cross-sections of affected nerves show normal numbers of axonal fibers in the proximal portions of the nerve and severely reduced numbers in the distal portions.

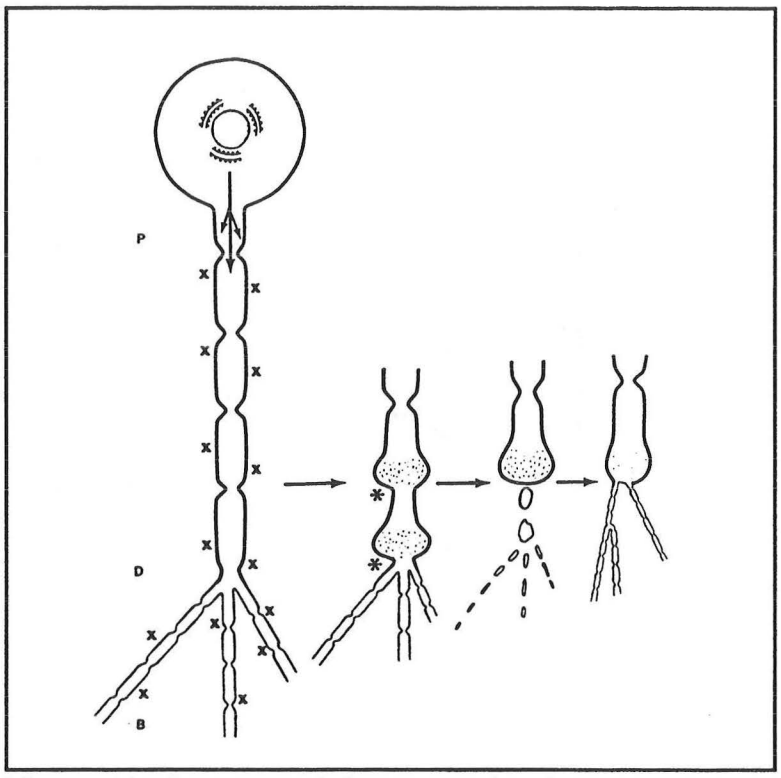

Figure 11. *Sequence of changes in distal axonopathy ("dying back"). Toxins (x) inhibit enzymes for nutrient transport. Demands met proximally (P), not distally (D). Damage occurs first at nodes of Ranvier (*).*

The Body's Defense System

The body's natural defense against poisoning by cholinesterase-inhibiting chemicals is comprised of two additional esterases, *butyrylcholinesterase (BuChE)* (51,52) and the family of *paraoxonases* (53). BuChE (also called pseudo-cholinesterase, plasma cholinesterase or serum cholinesterase) circulates in the blood and acts as a "false target," scavenging molecules of cholinesterase-inhibiting poisons to keep them from reaching the synaptic and neuromuscular junctions. The paraoxonases then hydrolyse the cholinesterase-inhibitor molecules to harmless degradation products. As the body's pool of BuChE becomes more saturated, as by a massive exposure to a cholinesterase-inhibitor or by treatment with pyridostigmine (Mestinon), more of the chemical gets by the protective systems into the synaptic and neuromuscular junction where it binds to AChE and NTE. Binding to AChE causes ACh excess and acute cholinergic symptoms, and binding to NTE may result in delayed, chronic neurotoxicity.

Pyridostigmine as a Defense against Chemical Weapon Exposure

Understanding of the pharmacology has led to strategies for protecting people from poisoning by organophosphates. It is primarily used in protecting military troops from chemical warfare agents. The most important strategy is pre-treating subjects with pyridostigmine (Mestinon) before exposure and administering atropine and an oxime after exposure. Pyridostigmine, the mainstay of treatment for myasthenia gravis in clinical practice, is a carbamate that complexes with AChE but does not undergo "aging" with AChE, BuCHE or NTE. While pyridostigmine is complexed with AChE, it protects the enzyme from attack by an organophosphate nerve agent. Several hours after pre-treatment,

the pyridostigmine spontaneously releases the AChE and restores its function.

In the Persian Gulf war, the military command, anticipating an Iraqi attack with chemical nerve agents, authorized unit commanders to order their personnel to take a low dose (25 mg 3 times per day) of pyridostigmine (Mestinon®) over the first 5 days of the air war (16 through 21 January 1991) and during the 5-day ground war (24 through 28 February 1991).(54-56) This dose was found to inactivate approximately 30% of serum BuChE and produce only mild symptoms of ACh excess, including mild nausea, abdominal cramping and diarrhea.

This agent has been shown to improve survival from exposure to certain chemical nerve agents by binding reversibly to both BuChE in blood and to AChE at the synaptic and neuromuscular junction, temporarily protecting them from being bound permanently by the chemical nerve agent. If exposure to a chemical nerve agent follows pre-treatment with pyridostigmine, a cholinesterase-reactivating drug (an oxime) can be administered to remove the pyridostigmine and some of the chemical nerve agent, restoring enough cholinesterase function to increase the probability of survival.(54-56)

By binding to and inactivating approximately 30% of the protective blood pool of BuChE, however, low dose pyridostigmine produces the same types of dose-related acute, cholinergic side effects as those that follow exposure to the chemical nerve agents, due in both instances to binding and inactivating AChE. This makes it difficult to distinguish symptoms of sublethal exposure to chemical nerve agents from the side effects of pyridostigmine. An open question is whether partially saturating the protective pool of BuChE with low-dose prophylactic pyridostigmine will allow more chemical nerve agent past the protective systems and into the brain and peripheral nerves where it can bind to NTE and cause delayed, chronic neurotoxicity. We have undertaken collaborative studies in laboratory animals to explore this issue.

Over-Susceptibility to Organophosphates

There is substantial individual variation in susceptibility to the organophosphates and carbamates. For example, patients with myasthenia gravis are highly resistant to the action of pyridostigmine and require doses of up to 600 mg per day (compared to 90 mg/day given to troops in the Gulf War).

Conversely, studies of soldiers who took pyridostigmine during the Gulf War identified a subgroup of approximately 10% who experienced particularly troublesome side effects, including more severe muscarinic effects, such as diarrhea and urinary and fecal incontinence, as well as nicotinic effects, such as muscle twitching and cramping.

The mechanism of over-susceptibility to pyridostigmine has not been well studied. It has been hypothesized that it is due to a deficiency of the protective pool of BuChE, either inborn by a genetic error or by prior exposure to other cholinesterase-inhibiting chemicals.

People with inborn errors of the protective BuChE or paraoxonases would demonstrate over-susceptibility to low dose pyridostigmine and would also be more susceptible to chronic, delayed neurotoxicity (OPIDN) from exposure to combinations of cholinesterase inhibiting chemicals. Lichtenstein et al. reported an Israeli veteran of the Gulf War who developed severe nausea, insomnia, weight loss, fatigue and deep depression while taking 30 mg of pyridostigmine three times a day during the war (57). The symptoms gradually disappeared after discontinuing the drug. He had a prior history of prolonged post-surgical apnea after

receiving succinylcholine and was found to have a genetic abnormality of BuChE.

We suggest that over-susceptibility to pyridostigmine was a marker for individuals whose protective pool of BuChE (and/or paraoxonase) was already diminished by prior exposure to other cholinesterase inhibitors such as the pesticides in flea collars, DEET in insect repellents, recurrent exposures to sublethal environmental levels of chemical nerve agents, or environmental pesticides used liberally to control insect vectors.

When those with an already diminished pool of protective BuChE enzymes took the uniform, low dose of pyridostigmine, it would more quickly saturate the reduced remnant of their protective pool of BuChE, spill over to the synaptic and neuromuscular junctions, and cause more advanced cholinergic side effects. If personnel with a diminished pool were also exposed to sublethal environmental concentrations of a chemical nerve agent, it is possible that more would get past the diminished BuChE pool and reach the NTE receptors in the brain and peripheral nerves. By this mechanism, it is possible that low dose pyridostigmine might have protected personnel from the acute cholinergic syndrome on the battlefield only to increase their susceptibility to delayed, chronic neurotoxicity (OPIDN) appearing weeks or months later.

Predicting OPIDN from the NTE Assay

Just as one orders a measurement of red blood cell AChE to detect recent exposure to a cholinesterase-inhibiting chemical and to estimate its severity, it is now possible to measure levels of NTE in circulating blood lymphocytes to predict whether delayed neurotoxicity is likely to develop (23). In hens exposed to a Group A organophosphate (one that undergoes "aging"), there must be at least 70% inhibition of NTE before delayed neurotoxicity will develop (58,59). The critical threshold level for humans is not known, but anecdotal evidence suggests that it may be lower, perhaps 60% or less (36).

OPIDN from Pesticides

In the wake of the TOCP-poisoning epidemic, great attention was turned to determining whether other organophosphate chemicals might cause similar problems. Consequently, a system arose for testing suspect chemicals for delayed neurotoxicity in adult hens, and in recent decades regulatory agencies in the U.S. and many other countries have made such testing mandatory. Despite the increased vigilance, a number of chemicals that cause OPIDN have made their way into use and have caused injury. The organophosphate pesticides have largely replaced the older chlorinated hydrocarbon pesticides (e.g., DDT), because organophosphates do not persist as long in the environment. As a result, in the future there will be increasing opportunities for injury from these chemicals.

Knowledge that some common organophosphate pesticides cause OPIDN comes from a series of well documented human cases reports. These document a spectrum of neurologic deficits from the severe spinal cord/peripheral nerve injury typical of TOCP poisoning to a mild, peripheral sensory neuropathy with cognitive slowing. Beyond these case reports, several controlled studies addressed the question of whether workers exposed chronically to low levels of organophosphates develop cognitive disturbances without overt neurological deficits.

Case Reports of Classical OPIDN from Pesticides

In 1951 *mipafox (Isopestox)* was a promising pesticide with an oral toxicity in laboratory animals a twenty-sixth that of parathion. In a pilot test of the manufacturing process, two workers were hospitalized after developing typical symptoms of the immediate poisoning syndrome (60). On the fourth and ninth days, respectively, while the acute symptoms were subsiding, they developed typical delayed neurotoxicity similar to that from TOCP poisoning, involving both peripheral neuropathy and spinal cord damage. Both recovered partially but suffered permanent disability.

In the same year, Petry reported a case of delayed, permanent peripheral neuropathy in a French greenhouse worker poisoned by *parathion* (61). Besides the typical peripheral neuropathy and spinal cord dysfunction, this person complained of memory loss, inability to concentrate, and impotence, suggesting subcortical or brainstem involvement.

In 1958 the medical examiner Charles Petty reported two cases who developed persistent neurologic deficits, including prominent CNS symptoms after acute poisoning with approved pesticides (62). The first was a 44 year old agricultural worker exposed repeatedly to *parathion* and *EPN* (two organophosphate pesticides) as well as DDT, dieldrin and lead arsenate. Over two years after plasma cholinesterase levels had returned to normal, he continued to experience generalized weakness, malaise, fatiguability, insomnia, disturbance of equilibrium, tinnitus, a slight tremor of his hands, nerve type deafness, and hyperactive deep tendon reflexes. Whereas DDT and dieldrin are not known to cause such impairments and exposure to heavy metals was ruled out, Petty suggested that the unusually severe impairments may have resulted from a synergism among the pesticides to which the patient was exposed.

Petty's other case was a 44 year old physician who developed delayed neurotoxicity after repeatedly spraying his lawn and garden with *malathion* (62). He had sprayed regularly with DDT for 10 years without problems, but shortly after switching to malathion he developed unusual irritability, tiredness, generalized weakness, tremor, unsteady gait, and ultimately facial paresthesias, numbness of face and mouth, and weakness of legs and the right shoulder girdle. After avoiding all pesticides for a year, he continued to have fatiguability, marked weakness in the same muscle groups, anorexia, weight loss, and paresthesias of the right side of the face and oral cavity, which restricted his ability to practice medicine.

In 1959 Healy described a case of "ascending paralysis" in an 18-month old child who had been repetitively exposed to *malathion* (63). In 1977 and 1987, cases assumed to be Guillian-Barre syndrome from organophosphate poisoning were almost certainly typical delayed neurotoxicity (64,65).

Leptophos was a widely used pesticide, which had been cleared through routine toxicologic testing in hens. Although suspected as the cause of paralysis in large herds of water buffaloes in the Nile Delta and of cases of delayed neurotoxicity in chemical workers in Baytown, Texas, in the 1970s, the link was hard to prove and the agent remained in use (7). In 1974, Abou-Donia et al. demonstrated conclusively that it causes delayed neurotoxicity typical of that from TOCP (7,66). Although the severity of impairment was dose-related, the interval from a single dose to the onset of neurotoxicity was never less than 8 days, even at the highest doses (66).

In 1978 Hierons and Johnson reported a typical TOCP-like delayed neurotoxicity in a

young man who ingested the pesticide *trichlorphon (Dipterex)* (67). Despite earlier reports of similar cases in Russia and Japan, trichlorphon remained on the market because it did not produce neurotoxicity in standard safety tests in hens. The authors concluded that, in contrast to other organophosphates, trichlorphon is more neurotoxic in humans than in hens (ratio TD_{50}:LD_{50} is higher in humans). This case was also instructive in that, despite severe neuropathic weakness in the legs, measured nerve conduction velocities were normal, emphasizing that in OPIDN the surviving nerve fibers next to the damaged ones often conduct impulses normally (67).

Further cases of typical delayed neurotoxicity from *trichlorphon* continued to be reported (68,69).

In 1982, 10 cases of delayed neurotoxicity typical of TOCP poisoning were reported after exposure to the pesticide *methamidophos (Tamaron)* in Sri Lanka (70). Despite flaccid paralysis below the knees and spasticity above the knees, nerve conduction velocities were normal.

Chlorpyrifos (Dursban) is presently one of the most widely used organophosphate pesticides in both agricultural and domestic use. Having passed the standard laboratory safety tests, it is available for sale in hardware stores, is commonly applied in and around homes by commercial pest control companies, is the active ingredient in most pet tick and flea collars, and is considered safe when used as directed.

In 1986 Lotti et al. reported a typical, mild case of delayed neurotoxicity in a 42 year old man who drank a solution of chlorpyrifos (Dursban) in a suicide attempt (36). Since the elimination of chlorpyrifos from the body of humans is unusually slow, he suffered prolonged symptoms of immediate poisoning, requiring mechanical ventilation for 17 days. At 3-1/2 weeks he became asymptomatic. At 6 weeks, however, he developed weakness and paresthesias of both legs with reduced deep tendon reflexes, reduced vibratory sensation, and reduced sensory nerve velocity. At 9 weeks, the weakness was more severe. Sural nerve biopsy confirmed swollen axoplasm from accumulation of axoplasmic organelles, and teased nerve fiber preparation demonstrated some myelin ovoids arranged in linear rows, typical of distal axonopathy. The authors attributed the long incubation period of the delayed neurotoxicity to the slow elimination of chlorpyrifos; they reasoned that the persistence of high blood levels of the pesticide caused progressive inhibition of NTE over the first 20 or 30 days until NTE levels were finally reduced below the threshold required to cause neurotoxicity.

In 1993 Kaplan, Schaumburg et al. reported 8 cases of delayed neurotoxicity from exposure to exterminator-applied *Dursban* (44). All 8 patients experienced distal paresthesias in a stocking-glove distribution approximately 4 weeks after their homes or offices had been sprayed with the pesticide. Examinations showed mild sensory loss, confirmed by finding slowed sensory nerve conduction velocities. Of particular importance, 5 of the 8 patients complained of cognitive dysfunction that began at the same time as the paresthesias (see below).

Evidence of Cognitive Damage from Pesticides

In much of the early anecdotal reports, psychological, cognitive and neurological symptoms indicative of brain or brainstem damage were attributed to organophosphate exposures, but these were often difficult to separate from the persistence of the immediate pharmacologic effects of poisoning (71-75). This literature, including case reports of poisoning or suicide victims and studies of pilots involved in aerial spraying, was reviewed by Ecobichon (7). The following studies, however, provide more convincing evidence of actual brain damage from organophosphate exposure.

In 1965, Davignon et al. reported a large study of apple growers in which they compared 441 who sprayed pesticides (the applicators) and 170 who lived and worked in pesticide-contaminated areas (the environmentally exposed) with 162 who had no contact with pesticides (the controls) (76). They found higher rates of chronic miosis, diminished reflexes, tremors and balance disturbances in the applicators (16%) and the environmentally exposed (13%) than in the controls (6%). Among the applicators, those who had been exposed for more than 5 years were more likely to have the abnormalities than those exposed less than 5 years.

In 1969, Metcalf and Holmes reported the results of a study of 56 men with organophosphate exposure histories (details of exposures unspecified) and 22 controls (77). The exposed men complained more of forgetfulness, difficulty thinking, visual difficulty and persistent aches and pains. Drowsiness, fatigability and loss of interest in work were complained of by 45% of the exposed and 5% of the controls. Standard neurologic examinations revealed no differences between the groups. Electroencephalogram (EEG) showed no "hard" abnormalities but the exposed group had a higher prevalence of low- to medium-voltage slow activity in the Theta range (4 to 6 Hz activity during light drowsiness in episodes of 2 to 4 seconds). Auditory evoked responses showed more variability than did visual responses, and both tended toward lower amplitudes and longer peak latencies in the exposure group. EEG-sleep studies showed sleep abnormalities. The authors interpreted the findings as indicating organophosphate-induced damage to the midbrain and pons, and suggested that combinations of chemicals might have acted synergistically in producing the damage. In all, however, the reporting of the results was not sufficiently quantitative to substantiate the conclusions.

In 1977, Korsak and Sato described supporting findings from a study of 32 men with occupational exposure to organophosphates, 16 with extensive high risk exposure and 16 with low risk exposure (78). They found significantly greater evidence of brain dysfunction on the Halstead battery of tests for brain damage and EEG in the high-chronic exposure group than in the low exposure group.

In 1988 Savage et al. compared 100 individuals from registries of people with prior organophosphate poisonings from Colorado (1950-1976) and the Texas Rio Grande Valley (1960-1976) with 100 carefully matched controls (age, sex, education, occupation, socioeconomic level, and race) with no history of pesticide exposures (79). With the investigators blinded to group identities, the cases and controls underwent physical examinations, neurologic evaluations, audiometric testing, ophthalmic examination, blood testing, EEG and neuropsychologic testing. Matched statistical analysis showed that the cases were significantly more impaired on neurologic tests of short-term memory, the Halstead-Reitan neuropsychological indexes of organic brain damage, and MMPI measures of

psychological dysfunction, as well as by standardized reports of distress and disabilities by the subjects themselves and assessments by their closest family members.

Rosenstock et al. reported a similarly complete neurological and neuropsychological evaluation of an individual after *parathion* poisoning (80). Two years after recovering from the episode, the subject continued complaining of headaches and severe fatigue and his family confirmed his poor attention and memory. Extensive testing found severe impairment of short-term memory and moderate impairment of psychomotor and visual-spatial function. They concluded that he had diffuse organic brain syndrome with secondary depression.

On the basis of this and other observations, Rosenstock et al. designed and carried out a matched cohort study to explore epidemiologically whether past organophosphate poisoning is associated with later chronic brain dysfunction (81). They compared 36 Nicaraguan agricultural workers, each of whom had had a single episode of acute, unintentional poisoning with an organophosphate on average two years earlier, with matched controls. The exposed group performed substantially worse on 5 of 6 subtests of the World Health Organization's neuropsychological test battery and on 3 of 6 tests assessing verbal and visual attention, visual memory, visuomotor speed, sequencing and problem solving, and motor steadiness and dexterity.

In 1989, Michotte et al. published an important case report of a 60-year-old woman who ingested approximately 30 g of *bromophos* (Brofene, Brophene or Nexion), an organophosphate pesticide approved for public use (82). Despite severe depression of the plasma BuChE level, she developed no cholinergic symptoms of the immediate poisoning syndrome. Five weeks later, however, she developed severe ataxia of the legs. After improvement over succeeding weeks, she was left with chronic ataxia and dysmetria, thought by the authors to be cerebellar in origin.

Kaplan, Schaumber et al.'s report of delayed neurotoxicity from home or office spraying of *chlorpyrifos (Dursban)*, referred to above, is of particular importance for cerebral effects (44). Five of the 8 patients complained of cognitive dysfunction that began at the same time as the sensory disturbance. Four were members of a family whose home was sprayed with Dursban, one of the most widely used pesticides in American civilian life. All 4 complained of short-term memory loss, which was confirmed on clinical neurological examination (recall of objects). Both children, ages 14 and 15, experienced a decline in school performance over the succeeding 6 months. Followup examination at 6 months found all complaints and deficits to have disappeared.

The fifth patient with cognitive complaints was a 42 year old physician whose basement exercise room had been sprayed repeatedly with Dursban over 3 weeks while she, but not other family members, continued using it for exercising. Shortly after the end of the spraying, she experienced paresthesias in hands, feet and legs; sensory loss and diminished ankle reflexes in both feet; and impaired memory and slowed thinking. At 18 months, the sensory problems had disappeared but she continued to complain of cognitive slowing, problems with short-term memory, and problems finding words. Neuropsychological testing demonstrated a substantial reduction in intellectual functioning and particular impairment on tests of recent memory.

OPIDN from Chemical Warfare Nerve Agents

Organophosphates, discovered in 1854 and first used as pesticides, were exploited as chemical warfare agents by Germany--tabun (GA, 1936), sarin (GB, 1938), soman (GD, 1944)--and then by England--VX (1952) (54). Despite the fact that the Allied armies were unprepared to defend against chemical nerve agents in WWII, the Germans did not use them. Iraq's use of them against Iran in the 1980s is the only documented wartime use, although they have been used in various minor conflicts.

These agents kill by binding irreversibly to (organophosphorylating) the active site of AChE, thereby inactivating it and precipitating a cholinergic crisis from ACh excess (54). There is little information on the long-term effects of these agents on their victims, although rumors of hospitals full of neurologically impaired victims from unpublicized use in other countries abound. The best evidence for trying to predict the long-term effects on humans is from animal studies and studies of accidental human exposures in chemical weapons manufacturing plants.

Studies in Experimental Animals

In 1983, Gordon et al. reported the defining work on the potential for all known chemical nerve agents to produce delayed neurotoxicity (83). After protecting standard experimental hens from lethal effects of ACh excess with high doses of physostigmine, atropine and the oxime P2S, they administered a one-time dose of a given organophosphate nerve agent and measured the development of delayed neurotoxicity. They found that sarin produced it at 30-60 times its LD_{50}; but soman did not produce it at 38 times its LD_{50}, and tabun did not at 82 times its LD_{50}. From the degree of NTE inhibition produced, they estimated that soman and tabun would produce delayed neurotoxicity at 100-150 times their LD_{50}'s.

The next year Willems et al. repeated the experiments with higher doses and confirmed that soman produced it at between 100 and 150 times its LD_{50}, but tabun required even higher doses (84).

These studies were the basis for the view that delayed neurotoxicity was an unlikely consequence of exposure to chemical nerve agents in military situations (54). Gordon et al., however, warned that, in view of the apparent effectiveness of pharmacologic means of protecting troops from the immediate cholinergic effects of nerve agents, "consideration must be given to the possibility of the occurrence of neuropathic symptoms in the survivors of poisoning in man due to sarin, soman or tabun but not by VX" (83).

Whereas the studies of Gordon et al. and Willems et al. were based on the premise of a single massive exposure, in 1993 and 1995 Hussain et al. reported experiments in mice and hens in which low doses of nerve agents were administered daily for 10 days to model the more likely occurrence of repeated sublethal exposures (85,86). In mice they found that repeated inhalation exposures to sarin caused no cholinergic signs but produced significant inhibition of NTE in brain, spinal cord, and platelets and the appearance of hind limb weakness and slight ataxia on the fourteenth day after the start of the exposures (85). Histologic examination confirmed focal distal axonal degeneration in the spinal cords of the

exposed animals. In hens they found that 10 daily subcutaneous injections of sarin produced delayed neurotoxicity of severity intermediate between mipafox (severe) and parathion (none) (86).

It is noteworthy that delayed neurotoxicity developed in the mice and hens despite inhibition of NTE in brain and spinal cords of only 40% to 50%. This finding is in direct disagreement with those of Johnson and Lotti who found that 70%-90% inhibition of NTE was necessary before delayed neurotoxicity would develop in hens given repeated small doses of other organophosphates. This suggests that other properties of the chemical nerve agents allow them to produce neurotoxicity at lower levels of NTE inhibition.

Accidental Exposures in Humans

The reports of long-term effects of accidental human exposures to chemical nerve agents are contained in three articles. In the mid-1950s, Spiegelberg examined personnel of the German Wehrmacht who had been involved in the manufacture or handling of chemical nerve agents during World War II (87,88). Since none had been exposed to such agents since the end of the war, the findings indicated the possible sequelae 5 to 10 years after the last exposure. Although no controls were examined, he concluded that the greater majority of subjects had persistently lowered vitality with marked reduction in drive, defective autonomic regulation, intolerance to alcohol, nicotine and medicines, and the appearance of premature aging. A subset of those also had depressive disorders, syncopal attacks, slight to moderate memory loss, and organic neurologic defects (micro-symptoms and extrapyramidal signs).

In 1974, Sidell of the Edgewood Arsenal in Maryland reported the first 5 cases ever of acute, one-time poisoning from laboratory accidents with nerve agents (89). Two of the subjects, one poisoned with sarin and the other with soman, experienced psychiatric symptoms for several months after recovering from the immediate poisoning episodes. The subject poisoned by soman apparently recovered by 6 months, but the other remained psychiatrically impaired at 6 months and was then lost to follow-up. The lack of objective assessment of mental and cognitive function and of long-term follow-up precludes definitive interpretation of these cases.

The most useful study is that of Duffy et al. reporting a group-matched cohort study of workers in a chemical weapons manufacturing facility (90). In the study, 77 workers with histories of accidental exposures to sarin (E group), and 41 of these (the M subgroup) had at least 3 such exposures. Thirty-eight age, sex and socioeconomically matched workers with no past exposures to sarin (the C group) were also studied. The exposure episodes had occurred between 1 and 5 years previously. Compared with the C group, the E group had significantly increased beta activity, increased delta and theta slowing, decreased alpha activity, and increased amounts of rapid eye movement sleep. Earlier they had found a similar increase in beta activity in the temporal lobes of monkeys one year after exposure to sarin (91). These observations were made in both the humans and the monkeys at a time when their tissue AChE levels were within normal limits. This and the time since prior exposures suggested that the findings were not due to the immediate effects of the nerve agent.

OPIDN from Combinations of Cholinesterase-Inhibiting Chemicals

Until recently, all animal testing of organophosphate chemicals for the ability to produce OPIDN was performed on chemicals one at a time. When a chemical was found not to produce neurotoxicity when administered alone to hens was considered safe for humans and was approved for general use.

Based on evidence that U.S. military personnel were exposed to at least five organophosphate or carbamate chemicals in the Persian Gulf war, Kurt hypothesized that combinations of these agents might act synergistically to produce OPIDN, thus accounting for the post-war symptoms known as the Gulf War syndrome.

Abou-Donia, Kurt and colleagues tested this hypothesis in a series of laboratory studies in which groups of hens were exposed to the organophosphate and carbamate chemicals present in the Gulf War individually and in various two-way and three-way combinations (92,93). Doses equivalent to those possibly experienced by U.S. military personnel during the war were derived by preliminary dosing experiments (92). In these doses, pyridostigmine, DEET, permethrin, and chlorpyrifos (Dursban) were found not to cause OPIDN in the hens when administered alone but to cause moderately severe OPIDN in two-way combinations and severe OPIDN in three-way combinations (92,93).

The clinical end points observed in the hen experiments were the development of gait disturbances, tremor and paralysis. As shown in the graph, the frequency and severity of these findings increased with the number of chemicals the hens were receiving.

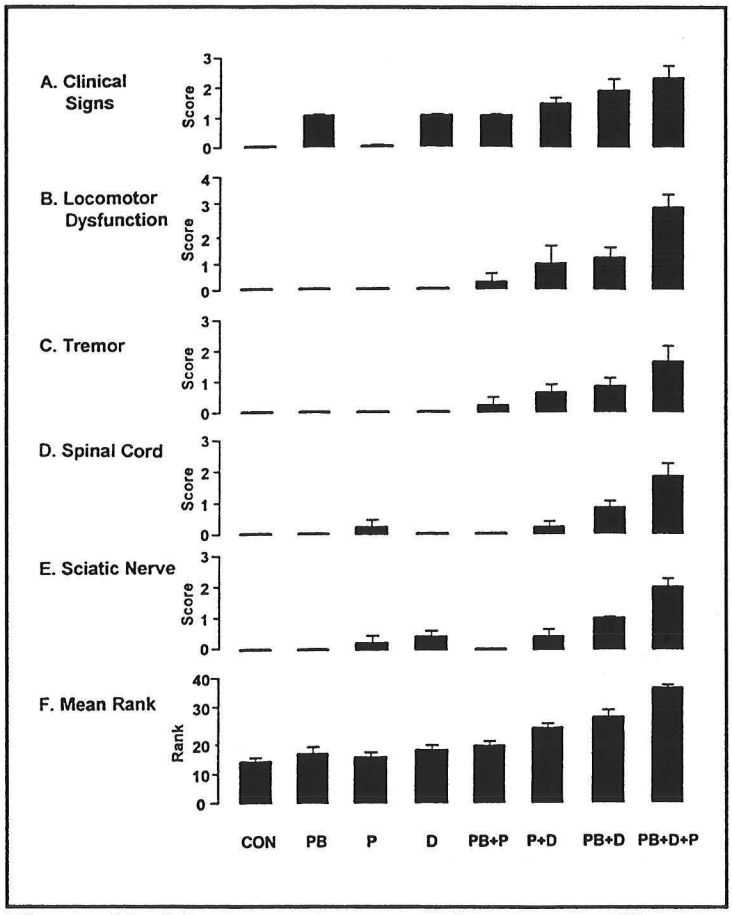

Figure 12. *Mean severity scores for groups of hens exposed to pyridostigmine bromide (PB), permethrin (P), DEET (D) and various 2-way and 3-2ay combinations of them, compared to controls (CON).*

By sacrificing the hens at the end of the study, the clinical findings were found to correspond with axonal swelling and fragmentation in spinal cord and peripheral nerves (see photograph of cross-section of postior columns of hen spinal cords).

These findings establish the biological plausibility of the hypothesis that organophosphate and carbamate chemicals, which are safe by themselves, may act synergistically to produce OPIDN. That is, chemicals previously considered safe may readily produce OPIDN when experienced in combinations. Whether this phenomenon occurs in humans remains to be determined by epidemiologic and clinical studies.

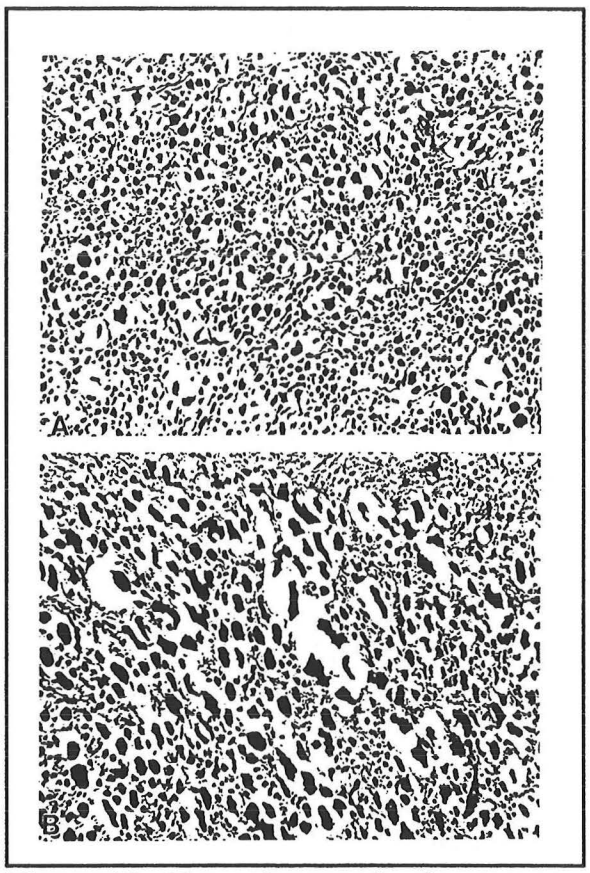

Figure 13. *Photomicrograph of cross sections of the dorsal columns of the spinal cord of controls (A) and hens receiving all 3 chemicals (B). Axons in B appear swollen, indicating OPIDN.*

Ill-Defined Syndromes Possibly Due in Part to OPIDN

The existing evidence indicates that the syndrome of OPIDN includes a spectrum of neurological abnormalities from the dramatic TOCP-induced peripheral and spinal neuropathies to a vague CNS dysfunction involving fatigue, cognitive disturbance and other central nervous system disturbances. The different clinical constellations along this spectrum are determined by the chemical involved, the magnitude and duration of exposure, certain genetically-determined dimorphisms (e.g., BuChE deficiency), and other, as yet undefined, variations of individual response. Chemicals considered safe may become highly neurotoxic by adulteration with toxic additives or by inadvertent changes in the proportion of stereo-isomers from errors in manufacturing. Exposure to supposedly harmless chemicals in combination may produce new, unusual syndromes along the OPIDN spectrum.

Among the many etiologic mysteries that frustrate practicing physicians today is the spectrum of neurasthenic conditions including chronic fatigue syndrome (94), fibromyalgia

(95), Agent Orange-related illnesses (96), and, most recently, the Gulf War syndrome (97). These conditions are typified by chronic, debilitating fatigue; cognitive complaints such as forgetfulness and difficulty concentrating; depression; emotional lability; sleep disturbance; myalgias and arthralgias; intermittent diarrhea; and headaches. Physical examination and laboratory tests usually show no objective abnormalities other than a few non-specific neurologic signs. A recently demonstrated association of chronic fatigue syndrome with autonomic dysfunction and postural hypotension has rekindled interest in a neurological etiology (98).

In addition, a recent study showed that a single intraperitoneal dose of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), a toxic contaminant in the defoliant Agent Orange during the Vietnam war, in rats produced abnormalities of nerve conduction traced to severe axonal degeneration by histological examination of peripheral nerves (99,100). Although the time of appearance of the abnormalities and the spinal cord histology were not reported, the study has raised the question of OPIDN in sick Vietnam veterans exposed to Agent Orange.

The mainstay of treatment of myasthenia gravis is pyridostigmine (Mestinon). Myasthenic patients report a gradual loss of cognitive abilities and short-term memory that has been poorly characterized. Some ascribe the change to the underlying abnormality of ACh production. On the other hand, all myasthenics on Mestinon are cautioned by their physicians to avoid exposure to pesticides because the chronic depletion of BuChE from their high doses of Mestinon make them highly susceptible to immediate poisoning by other cholinesterase-inhibiting chemicals. If this is the case, why would they not be more susceptible to OPIDN with its possible cognitive deficits?

Defining and Testing for OPIDN

There is presently little evidence one way or the other to address whether these poorly understood human syndromes are due to variants of OPIDN from exposure to organophosphate chemicals. This is due partly to difficulties in defining the syndromes and partly to the lack of sensitive and specific tests to detect diffuse axonal damage in humans during life.

The problems with defining these syndromes have been addressed by case definitions agreed upon by blue ribbon committees. Well defined case definitions now exist for chronic fatigue syndrome (94) and fibromyalgia (95) but not for Agent Orange illness (96) and Gulf War syndrome (97). If these conditions ultimately prove to be single, homogeneous clinical entities, the case-definitions will have been important contributions. If, however, these conditions prove to be composed of several pathogenetically distinct component syndromes, then the case definitions may prove counter-productive. This issue is being addressed by the use of statistical techniques, such as factor analysis of symptoms, to explore for potentially important subgroups.

Testing for the dramatic OPIDN variants like that from TOCP-poisoning is obviously simple; for all but the mildest cases, a thorough neurological examination is adequate. Clinical methods to detect diffuse axonal damage at the other end of the OPIDN spectrum, however, is presently unsatisfactory and controversial. Potentially useful testing methods include neuropsychological, neurophysiologic, and audiovestibular testing.

Neuropsychologic Testing

Objective neuropsychological testing is presently used to assess abnormalities of higher cerebral cortical, subcortical and brainstem function. Standardized psychological tests, such as the Minnesota Multiphasic Personality Inventory (MMPI) (101) and the Personality Assessment Inventory (PAI) (102), are administered to detect or rule out the classical psychiatric conditions such as major depression, thought disorders and malingering or compensation neurosis.

Opinions differ on the best testing approaches for detecting organic brain dysfunction, with the issue of balancing sensitivity and specificity being at the heart of the controversy. The Halstead-Reitan battery of tests is the oldest and most widely validated method (103). When positive, the tests reliably distinguish organic brain dysfunction from psychological disturbances; however, they may not be sensitive to some important, though mild forms of brain dysfunction. Newer neuropsychological tests developed to detect subtle subcortical dementia and early Alzheimer's disease (104), appear to be more sensitive to mild, diffuse brain damage, but their specificity is of concern.

Neurophysiologic Testing

Neurophysiologic tests involve electronic measurements of physiologic nerve impulses and of responses to various stimuli (105,106). The posterior columns of the spinal cord and sensory pathways of the brainstem and cerebral cortex can be evaluated by *somatosensory evoked potentials* (105,106). This test involves stimulating the posterior tibial nerve at the ankle (or other nerves) and measuring the rate of conduction at the popliteal fossa, over the cauda equina, and at the top of the skull. Slowing in any of the measured segments can localize areas of damage.

Functioning of the visual pathway can be assessed with *visual evoked potentials* (105,106). These are measured by stimulating the retina with changing patterns of light and measuring the electrical waves from scalp electrodes. This primarily detects lesions of the optic nerves and optic radiations.

Functioning of the brainstem can be assessed by *brainstem auditory evoked potentials* (105,106). This test involves stimulating the auditory system with a series of clicking sounds and measuring the electrical spikes recorded by cutaneous electrodes. Slowing or bilateral asymmetry can localize dysfunction to several levels of the brainstem.

Higher cognitive functioning may be assessed by the *event-related potential* (also called the late latency or P300 wave) (107). This is measured by having the subject respond to auditory stimuli by counting or other mental maneuvers and making a prolonged recording out to at least 300 msec.

Functioning of the peripheral nervous system, muscles and the neuromuscular junction can be evaluated with *peripheral nerve conduction studies*, *electromyography (EMG)* and *single-fiber EMG*, respectively. In OPIDN, however, these studies may be falsely negative. In contrast to other types of peripheral neuropathy (e.g., from infection, inflammation or other toxins), in OPIDN the damage affects nerve fibers within a large nerve haphazardly leaving surviving nerve fibers functioning normally beside damaged ones. Since the

surviving nerve fibers conduct impulses normally, nerve conduction studies may be normal in patients with severe neuropathic muscle weakness (67,108).

Sural nerve biopsy is very sensitive in detecting peripheral nerve damage, but the test carries variable morbidity.

Electroencephalography (EEG) has been used to detected toxic brain damage for decades (106), but the difficulty of quantifying EEG patterns, the variability of its results, and the widespread abuse by charlatans have led many to questions its value.

Audiovestibular Testing

Electronystagmography, positional testing for nystagmus, sinusoidal harmonic acceleration, bithermic caloric testing, and dynamic platform posturography are potentially useful for detecting brainstem damage from OPIDN (109).

Neuroradiologic Testing

Magnetic resonance imaging, SPECT and PET scanning, and CT Scanning of the brain and spinal cord have generally not been useful in detecting OPIDN, but they have been abused by charlatans who attribute diverse complaints to pesticide-induced damage.

Problems with Normal Limits

Presently, a major limitation of many of the diagnostic techniques is the quality of the "normal limits" used for interpreting the results. Normative values are generally derived by performing a standardized measurement, such as a neuropsychological test or an evoked potential, in a "normal population" and choosing values corresponding to 2 or 3 standard deviations of the population as "normal limits." In evaluating an individual patient, the physician interprets the test result as normal if it is within the normal limits or abnormal if it is outside them.

This presents at least three problems for assessing patients with suspected toxic neurological damage. First, traditional normal limits are quite useful for distinguishing focal neurological lesions which cause gross abnormalities of neurophysiologic tests, far outside normal limits (e.g., brain tumors, infarcts, plaques from multiple sclerosis). Chemically induced neurotoxicity, however, tends to cause diffuse axonal damage with subtle functional disturbances that cause smaller decreases in neurological function that may not exceed the normal limits. Second, the normal populations who were tested in setting the normal limits for a particular test may not be comparable to a particular patient being tested. Distributions of results on many tests vary normally by age, sex or occupation. If the results from all members of the referent group are aggregated into one set of normal limits, the normal limits may be far too broad to detect small, but meaningful, decreases in function in persons with toxic neurological damage. Third, when testing methods change in a laboratory from a change in equipment, technicians or testing environment, the normal limits should be re-established in a new control group. In the present era of cost-containment and managed care, this is unlikely to occur. Consequently, some types of subtle dysfunction may only be demonstrated by comparing the ill individuals with carefully matched control groups, possibly under blinded testing conditions.

Conclusions

The syndrome of organophosphate poisoning should be viewed as a two-stage illness (44). The first stage is the immediate occurrence of possibly fatal cholinergic symptoms that are reversible as soon as the poison is eliminated from the body. The second stage follows the first by several weeks and involves a sensory-motor, distal, peripheral neuropathy (flaccid paralysis) often combined with upper motor neuron dysfunction (spasticity) from spinal cord damage and cognitive dysfunction from sub-cortical or brainstem damage.

Epidemics of OPIDN have occurred periodically, and individual cases undoubtedly occur sporadically from inapparent errors in the application, handling or storage of pesticides, possibly from exposure to combinations of anti-cholinesterase chemicals, and possibly from the use of chemical weapons in warfare. Most sporadic cases probably go unrecognized because the condition is not well understood by physicians or by the public. The possibility that combinations of chemicals, found safe individually, might cause OPIDN may also have added to under-recognition.

According to the classical theory, if the poisoning involves a "non-aging" organophosphate, the first stage occurs without the second stage. If it involves prolonged, low-level exposure to an "aging" organophosphate, the second stage may occur without the first stage being evident. However, the large number of reports of OPIDN caused by pesticides and nerve agents that did not cause it in laboratory hens has called the calssical theory into question. *Today, all organophosphate chemicals should be assumed to cause OPIDN under the right circumstances (e.g., high enough dose, long duration at a low dose, in combination with other compounds).*

The syndrome of OPIDN is composed of a spectrum of manifestations (variants) from severe TOCP-induced peripheral nerve and spinal cord damage to subjective cognitive, psychologic and balance complaints with no objective findings from recurrent pesticide exposures. Tests to confirm the diagnosis, when it is suspected, are insensitive for all but the most dramatic manifestations. Consequently, the incidence of new cases and the prevalence of chronic impairment from OPIDN in the population are unknown.

The idea that OPIDN is a severe polyneuropathy affecting only the peripheral nervous system is a widely held myth, mistakenly established during the 1930 epidemic of Jake Paralysis and perpetuated by numerous scientific publications since then. The manifestations of peripheral nerve involvement are easier to detect clinically than the largely subjective manifestations of brain, brainstem and spinal cord involvement. Controlled studies, however, have established the validity of central nervous system involvement with or without manifestations in the peripheral nervous system. Failure to understand that OPIDN affects axons in all parts of the central, peripheral and autonomic nervous systems in variable, and perhaps unpredictable patterns, contributes to its under-recognition.

When methods for detecting OPIDN are more fully developed, it is possible that this condition, perhaps co-linked with genetic predispositions, might explain many cases of chronic fatigue syndrome, Agent Orange-related illness, post-traumatic stress disorder, Gulf War syndrome and perhaps even some cases of the neurodegenerative disease such as Alzheimer's disease and amyotrophic lateral sclerosis. If so, the questions regarding safety testing, storage, use, and disposal of chemicals will be prodigious.

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