Trail of Ironies to Parkinson's Disease

Sloppy chemical synthesis by an illicit drug producer has led to important insights into the basic cause of Parkinson's disease

The past 2 years has witnessed the unfolding of one of the most remarkable stories of recent medical history. Specifically, the sloppy laboratory practices of a man synthesizing heroin-like drugs for street sale in northern California has lead to the identification of a chemical—known as MPTP—that selectively kills cells of the substantia nigra in the brain and thereby induces clinical symptoms that are identical with Parkinson's disease in humans and in some animals.

This irony-packed trail of events has therefore led to the elaboration of the first genuine animal model of this important and baffling neurological disease of aging and to the first real insights into its basic cause. Contrary to most expectations parkinsonism might be the consequence of long exposure to an insidious environmental neurotoxin, which might be MPTP or something related to it. Perhaps the greatest irony of all, however, is that in the late 1950's, a decade after it was first synthesized in the laboratory of a major pharmaceutical company, MPTP was tested as a potential anti-parkinsonism drug.

The recent story began in June 1982 when George Carrillo, a 42-year-old drug addict, was admitted to Santa Clara Valley Medical Center in San Jose virtually unable to move or talk. Neurologists Philip Ballard and William Langston were initially baffled by their patient's condition, and Parkinson's disease was certainly not the obvious diagnosis in someone so young. The puzzle enlarged when, a week later, Carrillo's sister came to the hospital clearly suffering from a neurological condition of some sort too. Although not as badly affected as her brother, her movements were slow, she had a hand tremor, and her face was blank and expressionless.

It transpired during interviews that the two patients had recently bought and used a "synthetic heroin" from a local supplier, and it was shortly after this that their clinical symptoms set in. Ballard and Langston considered the possibility that their patients had been poisoned in some way by the drug they took, and began analysis of it. There was, however, no obvious connection.

Then, by one of those helpful twists of fate, Ballard visited a friend's house in Santa Cruz, where he met and talked with James Tetrud, a neurologist from nearby Watsonville. During conversation Tetrud mentioned he was involved in an interesting case of a young man who, for no obvious reason, had suddenly become rigid and immobile. The patient had a brother in a similar but less severe condition. The parallel with the San Jose patients was striking, particularly when it transpired that the Watsonville brothers were also drug users and had recently obtained a new synthetic heroin.

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Ballard and Langston acted quickly. They made a public announcement about the danger of the new drug and pushed harder on the chemical analysis of the material by sending it to several forensic laboratories. The break came when Halle Weingarten, a toxicologist to whom Langston had sent some of the drug, recalled having read an article in a little-known journal called *Psychiatry Research*. The article, which was published in 1979, described a 23-year-old graduate student who had developed a parkinsonian-like condition after using a meperidine-like drug, 1-methyl-4-phenyl-proprionoxy-piperidine (MPPP), which he had synthesized in his own laboratory. In making short cuts in his manufacturing process the graduate student had contaminated his chosen product, MPPP, with a by-product, 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine (MPTP), which proved literally to be a fatal error.

The student had been investigated by several doctors since 1976, including a group at the National Institute of Mental Health. Glenn Davis, then at the University of Tennessee Center for Health Sciences, had initiated the study and stuck with it throughout. He and his collaborators noted the parkinsonian-like symptoms and were able to confirm the diagnosis in 1978 when the patient died of a drug overdose: the cells of his substantia nigra were severely depleted.

The NIMH team had tested the patient's drug mixture on rats, hamsters, and guinea pigs, but couldn't get any clear-cut indication of Parkinson's disease. Nevertheless, they wrote up the case in a short paper, which was rejected first by *The New England Journal of Medicine*, because it presented just one case, then by the *Journal of the American Medical Association*, because it had too many authors for such a short note, and was finally accepted by *Psychiatry Research*, which was in its first year of publication.

When Langston called Weingarten to check on progress she told him about the Davis paper. He quickly got a copy from the library: "I knew within the first three sentences of the description that we were dealing with the same thing," he recalls. "I called Ian Irwin," who was analyzing the "synthetic heroin" at the Drug Assay Laboratory at Stanford University Hospital, "and read him the formulas from the paper."

Irwin by that time had some nuclear magnetic resonance spectra from the sample but had been unable to identify what was present. A search through a list of 40,000 chemical spectra at the National Institutes of Health had not turned up anything. "As soon as Bill read me the formula of MPTP I could see how it could fit the fragmentation pattern I had," says Irwin. "That paper really broke it for us," adds Langston.

Langston called Sanford Markey, one of the NIMH authors, to ask whether the MPTP by-product had been tested on the animals. "Sandy paused, and then said, 'No, we didn't test that one,' and I then knew we had it." That telephone call put Langston on the track he needed, and rekindled an interest in the problem at NIMH. "You couldn't have got more activity had you walked up there, pulled a pin from a grenade, and rolled it through the door," says Langston.

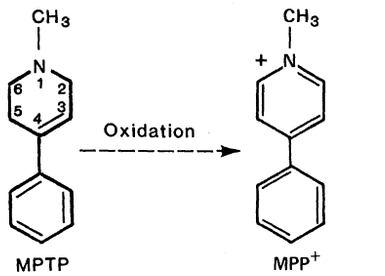
Markey, Stanley Burns, Irwin Kopin, and several other NIMH researchers in

the Laboratory of Clinical Science quickly went into action and were in constant touch with Langston and his colleagues. One of the NIMH team went so far as to fly down to Watsonville, where he told Tetrud's two patients that he had Langston's permission to see them and take them back to Bethesda. He had two extra plane tickets in his pocket dated for the following day. Langston was alerted to what was happening by a call from the patients' family doctor. "Things were a little difficult for awhile, but it was eventually sorted out amicably and two patients did go up to NIMH, which was well set up for a clinical study," says Langston. "What happened there does give you an idea of the level of interest in the subject," he adds laconically.

Meanwhile, Langston began to look into the literature about the synthesis of MPTP, where he stumbled across the tracks of the man who had been making the synthetic heroin. "I looked in the Stanford library for the 1947 paper that described the first synthesis of MPTP, and found that it had been carefully razor-bladed out. Ian had to get it from a library in San Francisco. And when I later looked for some related papers, they too had been razor-bladed out." Presumably the drug maker didn't want any competition in his quest for money to be made on the street.

Langston realized that the author of the original MPTP paper, A. Ziering, who had worked at Hoffmann–La Roche, might have had considerable exposure to the chemical, and decided to call him to find out if he was all right. Ziering was able to report that he was in good health, and it was during this conversation that he recalled vaguely having heard of some toxic properties of the chemical. Following the trail to Peter Sorter, the current chief of research for Hoffmann–La Roche, Langston learned of the company's long interest in the drug.

Because of MPTP's structural similarity to certain neurotransmitters, Roche decided to test its effect as an anti-Parkinson drug, both in animals and humans, in the late 1950's. The standard L-dopa treatment was still in the future at this time, and many pharmaceutical companies were routinely trying many potential drugs. Sorter told Langston that the only information available on the trials was a 1960 summary sheet, which recorded dosage regimes to six people, two of whom died during or shortly after the study. Although the report does not confirm it, Langston assumes that the test subjects were Parkinson patients, which would explain why the very specific neurological effects were not noticed.



Anatomy of a neurotoxin

MPTP rapidly undergoes oxidation to MPP+. The toxin is thought to be one of the intermediates in the conversion.

Not so readily explained is why progressive limb stiffness, leading to complete rigidity, in two dosed monkeys was not recognized as an animal equivalent of the human disease. Perhaps the researchers were more impressed by the more extensive results on rats, which don't apparently respond to MPTP as humans do. "These two monkeys probably represent the first good animal model for Parkinson's, and yet it wasn't recognized," says Langston. "This is so ironic, because those people must have been thinking about the disease when they were doing the tests. It wasn't as if they were working on something totally unconnected."

With the identification of MPTP as the probable cause of selective parkinsonian-like neurological damage, the race for publication was on. Langston, Ballard, Tetrud, and Irwin got to the publication line first, with a 23 February 1983 report in *Science* on the connection between the drug and the disease-like condition. Burns and his NIMH colleagues followed a few months later with a *Proceedings of the National Academy of Sciences* paper on the production of an animal model of Parkinson's disease, using MPTP in rhesus monkeys.

These papers ignited an explosion of interest, with many reports being published during this past year and many more in press. The identification of this highly specific neurotoxin has dramatically shifted the focus of Parkinson research back to the basic level. Ever since highly successful management of the disease through L-dopa dosage began in the late 1960's, research has primarily been on discovering ever-better drug therapy. The disease itself remained a total mystery.

The pathology of Parkinson's disease is classical, and involves extensive cell death in the zona compacta region of the substantia nigra, an area in the brainstem. Typically, several other brain loci are affected too, including the locus coeruleus and the dorsal motor vagus nucleus, but to lesser degrees. The cells of the substantia nigra send long processes up to higher brain centers, known collectively as the corpus striatum, where they pump out dopamine at high levels. Without a constant supply of dopamine in this region, motor function fails and people freeze up. Why? No one really knows.

The MPTP revelations have already begun to throw a little light on this puzzling disease. There has for a very long time been discussion about what aspect of the brain pathology causes the behavioral defects: the nigral degeneration alone, or this in combination with the other cell loss? Now, MPTP-induced parkinsonism shows all the symptoms of the natural disease, including oily skin, or seborrhea. The brain damage, however, is restricted completely to the substantia nigra. "This observation allows us for the first time to say definitively that the symptoms derive solely from the cell death in the substantia nigra," says Langston.

This finding has clear implications for the development of a brain graft approach to treatment of parkinsonism: the replacement of a dopamine supply, from adrenal tissue, for instance, might well be successful. Had symptoms derived also from other tissue damage, such therapy potentially would be incomplete.

Langston is of course interested in the origin of the differences between drug-induced and natural Parkinson's disease. Perhaps the cell loss in areas outside the substantia nigra occurs as a secondary degeneration in the usually very slowly developing disease? As the drug-induced condition is a much more rapid insult there is probably insufficient time for secondary degeneration to occur. "We can test this idea by long-term experiments in our animals," Langston points out.

Another possible source of the difference, however, relates to an old adage of neurotoxicity, which says that acute effects of a toxin are often different from chronic effects. Once again, the animal model can be used to test this, by giving very low doses over a very long time. A third possibility is that natural Parkinson's disease is caused not by MPTP but by some related chemical whose selectivity is less focused. "You can imagine a whole range of substances that might act roughly like MPTP."

The animal model can also be used to settle another long-running debate among researchers, namely, the effect and course of L-dopa therapy. As treat-

ment progresses it becomes more and more difficult to get the drug dosage within acceptable limits: too low, and rigidity occurs; too high, and dyskinesia, distressing writhing movements, results. Consistent therapy eventually becomes impossible. Some authorities say the deterioration simply reflects progressive degeneration of the nigra. Others contend it is due, in part at least, to a toxic effect of the L-dopa itself. Long-term regimes with MPTP-induced parkinsonian monkeys, some treated with L-dopa, some not, should give important data on what really is happening.

The question to be resolved now, of course, is whether or not MPTP has anything at all to do with the natural disease. The unfolding story of MPTP happened to coincide with the accumulation of as many as 80 twin pairs in several studies relating to the genetic component of the disease. The answer seems to be fairly clear: "for most patients genetic factors are not important," wrote Langston and Donald Calne, of the University of British Columbia, in a recent review. "We conclude that environmental factors are likely to play a part in the etiology of Parkinson's disease in most patients."

Langston notes that most major neurological diseases can be identified from descriptions of symptoms in literature going back to the Bible and beyond. Not so for Parkinson's disease. "There doesn't seem to be any recognition of it before James Parkinson's description in 1817, so perhaps it is related to the advent of industrial society." Irwin notes that MPTP can be synthesized from a mixture of very simple chemicals and under very mild conditions. Incidental synthesis at low levels during other processes is therefore conceivable. He and Langston plan to look for traces of the chemical in autopsy specimens of Parkinson's patients.

The very high selectivity of the neurotoxicity is a puzzle and a challenge. The cells of the substantia nigra are not the only ones in the brain to produce dopamine, and yet this group is the only one affected in the disease. Substantia nigra cells have high levels of the black pigment neuromelanin, but then so do other cells that are spared. What is special about the substantia nigra?

These cells produce large amounts of dopamine, which are pumped up processes, released onto striatum cells, and then taken up again. This secretion/reuptake cycle in the striatum might well intensify the dopamine association, thus making a quantitative difference between the cells of the substantia nigra and other dopaminergic brain centers. The adrenal medulla also makes dopamine, but in this case as an intermediate in the synthesis of epinephrine. The adrenal medulla is also unaffected by MPTP.

Langston and his colleagues have recently been looking at the metabolism of MPTP in the hope of pinning down its toxicity. First, they noted the removal of the double bond in the pyridine ring makes the product completely innocuous, thus indicating that oxidation in this ring might be important in the toxicity. Indeed, it turns out that MPTP is very rapidly oxidized to the 1-methyl-4-phenylpyridinium ion (MPP+). Langston and Irwin suspect that MPP+ itself is not the toxin they seek but that the culprit lies somewhere in between it and MPTP.

Blockage of the MPTP to MPP+ oxidation in a test animal, by pretreatment with a monoamine oxidase inhibitor, prevents the development of the condition. As it happens, monoamine oxidase inhibitors are used in Europe, but not in the United States, in the treatment of Parkinson's disease. "The fact that here we have come across a drug that blocks MPTP neurotoxicity and is used in treatment of Parkinson's is of more than passing interest," notes Langston.

Progress has been like dismantling a Chinese puzzle, says Langston: "Every step forward we make we seem to push the problem back a step. We've found the metabolite, and we've found out how to block the metabolite and so prevent the disease. We seem to know more, but we still have no idea why the nigral cells are specifically affected."

—ROGER LEWIN

Crystal Anisotropy Directs Solidification

Simplified mathematical models of solidification elevate crystalline anisotropy to main player in selecting dendritic growth patterns

Two groups of theoretical physicists have produced evidence for the essential role of crystalline anisotropy in determining the feathery dendritic growth patterns assumed by many materials in growing from the melt or vapor. "You need to have anisotropy to get the side branching characteristic of dendritic growth," says Eshel Ben-Jacob of the Institute for Theoretical Physics at the University of California at Santa Barbara, a member of one of the groups.

No one who has marveled at the intricacies of snowflakes, whose hexagonal patterns reflect the symmetry of ice crystals, would be surprised at the findings. Nonetheless, previous work had declined to treat anisotropy effects, partly because the problem was fierce enough without them. Because of the simplified nature of the mathematical models used by the theorists, however, some researchers wonder if they are realistic enough for the asserted role of anisotropy to be accepted without further study.

Not all solids exhibit dendritic growth. Consider a molten, elemental metal encased in a container that is maintained at a temperature just below the melting temperature. Solid will freeze out on the walls of the container and grow smoothly inward at a rate limited by how fast heat can flow from the hot liquid through the solid to the container walls.

If, however, for some reason such as the presence of a seed crystal, solid forms initially in the center of liquid, it will grow outward toward the cool walls through liquid that has cooled to below the melting temperature. Growth into an undercooled liquid of this type is unstable. Small bumps or perturbations on the surface of the solidifying material grow faster than neighboring areas, forming fingers of solid that project into the liquid. Later, perturbations on the fingers result in side branches and so on. Eventually, the feathery dendritic patterns exemplified so well by snowflakes result.

Economically important instances of dendritic growth occur in multicomponent metal and semiconductor systems even when growth is not into an undercooled liquid. Here chemical diffusion of minority components plays a role analogous to heat diffusion and leads to dendritic growth, which is undesirable. For