

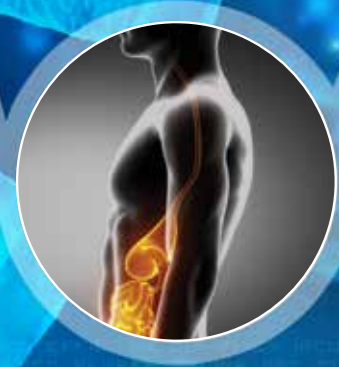


# THE Pharmacologist

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## Inside:

Feature articles from  
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# Adventures in Pharmacology

ASPET is pleased to present this special collection of feature articles from our quarterly membership magazine, *The Pharmacologist*. This compilation highlights the wonderful articles written by ASPET member and science writer Dr. Rebecca Anderson. In each issue of *The Pharmacologist*, Rebecca focuses on science stories that take us on an adventure in pharmacology.

The eight feature articles included in this collection span the globe describing travels via dogsled in treacherous conditions to deliver life-saving serum to the struggle to salvage China's iconic 2200 year old Chinese terracotta warriors ("The Great Dogsled Relay - The Race Against Diphtheria," December 2014 and "How Paul Janssen's Drugs Saved the Chinese Terracotta Warriors," March 2015). Her stories often provide a historical perspective, for example, the articles touching on the early days of drugs such as L-DOPA and methotrexate ("The Sleepy Sickness, Oliver Sacks and the Early Days of L-DOPA," September 2015 and "Methotrexate, Sydney Farber, and the Jimmy Fund: The Birth of Modern Cancer Chemotherapy," December 2015). On other occasions, they cover current events that impact our daily lives, such as the story dealing with drug tampering ("Stamping Out Drug Tampering, June 2014"). No matter which, they are always compelling and informative, providing us with stories of real-life pharmacology heroes and heroines who have made a difference both in the profession and in people's lives.

## Dr. Rebecca J. Anderson



Rebecca J. Anderson holds a bachelor's degree in chemistry from Coe College and a PhD in pharmacology from Georgetown University. She conducted postdoctoral research under an MRC fellowship at the University of Toronto. Early in her career, she conducted basic research in pharmacology

and toxicology and held faculty positions at the George Washington University Medical Center and the University of Michigan School of Public Health. In parallel with her academic appointments, she served as a reviewer on several study sections of the National Institutes of Health and as a member of a U.S. Food and Drug Administration Advisory Committee.

Subsequently, she held positions of increasing responsibility for preclinical drug research at Parke Davis & Company and Boehringer Ingelheim Pharmaceuticals and for clinical drug development at Miravant Pharmaceuticals, Kendle, Covance, and Amgen. Among her research accomplishments, she served on the teams that developed gabapentin (Neurontin®) and nevirapine (Viramune®). She belongs to Phi Kappa Phi and Sigma Xi honor societies, as well as several professional societies including ASPET.

Dr. Anderson currently works as a freelance medical writer and is the author of two books, *Career Opportunities in Clinical Drug Research* and *Nevirapine and the Quest to End Pediatric AIDS*. Her writing has been recognized by the American Medical Writers Association, the Lambda Literary Review, and the Next Generation Indie Book Awards.

# Oat Sprouts and the Topliss Tree: Rationalizing SAR

*Rebecca J. Anderson*

A pharmacology colleague of mine was fond of saying, when it comes to structure-activity relationships, “little things mean a lot.” Long before A. J. Clark formalized his receptor theory of drug action, scientists recognized that a compound’s pharmacologic activity was related to its chemical structure. But for many years, medicinal chemists faced a monumental challenge. How should they tweak the molecular structure of a marginally active compound to maximize its efficacy? More than half of all patented drugs contain a substituted benzene ring, and the medicinal chemist has an overwhelming number of synthetic choices. A large variety of possible substituents can be placed at each position around the ring, as well as various heteroatom substitutions in the ring, theoretically creating millions of structurally related analogs. Synthesizing and testing all of these analogs is obviously not practical.

Because there was no scientific rationale for choosing substituents that would optimize activity, medicinal chemists made their choices based on three things: their personal experience with other active compounds, intuition, and the availability of starting materials. It was a slow, trial-and-error process—and not necessarily fruitful. Then in the 1960s, Corwin Hansch, a chemistry professor at Pomona College in California, proposed a rational, systematic, and quantitative method for selecting substituents (1, 2).

Hansch was a most unlikely person to come up with this strategy. He received his PhD in chemistry from New York University in 1944 and immediately joined the Manhattan Project, first at the University of Chicago and then as an analytical chemist with DuPont de Nemours, a contractor at the Hanford, Washington site (3). After the war, Hansch accepted a position at Pomona, viewing the small liberal arts college only as a stepping stone to a large, research-oriented university, but “I found out it was a very fine place to stay” (4). His initial

research focused on the study of high temperature dehydrogenations. Then, by lucky coincidence, Hansch met Robert Muir, a botany professor at Pomona.

Muir had been given an office in the chemistry building, because the college's small biology building lacked room for him. He was studying plant growth

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***Hansch and Muir “fooled around” for fourteen years, trying to make sense out of how the phenoxyacetic acid analogs affected the growth of oat seedlings***

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regulators and quantitatively testing them on sections of oat sprouts. Of particular interest was 2,4-D (2,4-dichlorophenoxyacetic acid), a commercial weed killer that paradoxically causes cell elongation at low concentrations. Hansch offered to synthesize a series of simple phenoxyacetic acid analogs for Muir and his students to test. Because Hansch carried a heavy teaching load, his primary laboratory assistance in those early years came from undergraduate students. He trained them how to do research, and in the short interval before graduating, they synthesized the compounds under his direction. The college hierarchy at Pomona put no pressure on the faculty to conduct research, and Hansch and Muir “fooled around” for fourteen years, trying to make sense out of how the phenoxyacetic acid analogs affected the growth of oat seedlings (3).

**The Hansch Analysis**

They initially explored the possibility that the biologic activity of this chemical series was related to electron density at various positions around the molecule's phenyl ring. With funds from a small NIH grant, Hansch invited Toshio Fujita, who had been working on plant growth regulators at Kyoto University, to join his laboratory as a postdoctoral fellow. The landmark work of Meyer and



Corwin Hansch (Courtesy of Pomona College)

Overton, who showed that potency correlated with a compound's partition coefficient between olive oil and water, influenced Hansch and Fujita to explore the role of lipophilicity on plant growth regulation. Hansch selected octanol as an alternative to olive oil for his partition coefficient measurements because it was simpler, cheaper, and could be easily obtained in pure form. (Although many efforts have been made to find a better solvent for partition analysis, octanol remains the gold standard.)

In a quantitative manner, Hansch and Fujita showed a correlation between a compound's potency and its partition coefficient. In order to compare compounds in a chemical series, Hansch defined a new constant,  $\pi$ , which characterizes the hydrophobicity of each chemical substituent on an aromatic ring relative to an unsubstituted hydrogen. But the correlation was not perfect. Hansch and Fujita found that the combination of a molecule's hydrophobic and electronic properties provided a better correlation between chemical structure and biologic activity than either factor alone. Hansch later realized that he also needed to consider the shape/size of the molecule. Combining the data on the relative influence of those three parameters, he sought a mathematical expression that would define a simple linear relationship between structure and function.

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***To supplement the laboratory data generated by his student collaborators, Hansch combed the scientific literature. Each Saturday, he hopped into his Corvette and raced down the freeway from Claremont to the UCLA library.***

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Hansch's initial herbicide/plant growth studies led naturally to broader investigations of the correlation between chemical structure and activity in other biological systems. To supplement the laboratory data generated by his student collaborators, he combed the scientific literature. Each Saturday, he hopped into his Corvette and raced down the freeway from Claremont to the UCLA library. He wryly explained, “We simply bring [the biological data] back from the library, crank it through the computer, and interpret it” (4). That statement nicely sums up Hansch's ingenious contribution to pharmacology: the interlocking of mechanistic organic chemistry, quantitative biology,



and the use of computers—three previously unrelated lines of investigation. Quite an accomplishment for someone who had flunked high school algebra (3)!

Hansch's extensive analysis of data collected from many biological systems and many classes of structurally related compounds showed the importance of hydrophobicity ( $\pi$ ), and, to a lesser extent, the contribution of electronic and steric influences by the substituents placed on a phenyl ring. The Hammett constant,  $\sigma$ , expresses a substituent's electron-withdrawing or electron-donating effect compared to an unsubstituted hydrogen, and the Taft constant,  $E_s$ , characterizes steric hindrance. The empirical  $\pi$ ,  $\sigma$ , and  $E_s$  constants for some of the more common substituents on an aromatic ring are shown in Table 1.

**Table 1. Substituent Constant Values for Aromatic Ring Substitutions**

Substituent (R)	$\pi$ (hydrophobic)	$\sigma$ (electronic)	$E_s$ (steric)
H	0	0	0
4-Cl	0.71	0.23	-0.9
3,4-Cl <sub>2</sub>	1.25	0.52	
4-CH <sub>3</sub>	0.56	-0.17	-1.2
4-OCH <sub>3</sub>	-0.02	-0.27	-0.5
3-CF <sub>3</sub> ,4-Cl	1.59	0.66	
3-CF <sub>3</sub> ,4-NO <sub>2</sub>	0.60	1.21	
4-NH <sub>2</sub>	-1.23	-0.66	-0.6
4-Br	0.86	0.23	-1.1
3-CF <sub>3</sub>	0.88	0.43	
4-C <sub>2</sub> H <sub>5</sub>	1.02	-0.15	-1.3
3-Cl	0.71	0.37	
3-CH <sub>3</sub>	0.56	-0.07	
4-N=NC <sub>6</sub> H <sub>5</sub>	1.69	0.39	
4-SO <sub>2</sub> CF <sub>3</sub>	0.55	0.93	

To establish the structure-activity correlation for a new chemical series, Hansch synthesized an initial group of 6-12 compounds whose aromatic ring substituents gave a good discrimination between  $\pi$ ,  $\sigma$ , and  $E_s$ . He then used the corresponding biological activity data from these compounds to perform a least squares regression analysis, according to the equation:

$$\log(1/C) = k_1\pi + k_2\sigma + k_3E_s + k_4$$

where  $1/C$  is the biologic activity and  $k_1$  through  $k_4$  are constants. The calculated regression line defined the relative contribution of hydrophobic, electronic, and steric influences on biologic activity. From this

regression analysis, he could then select substituents with the appropriate hydrophobic, electronic, and steric characteristics to optimize activity. The observed biologic activity of those newly synthesized analogs also could be used to refine and strengthen the regression correlation, perhaps pointing to even more potent compounds.

This approach, which is now called the Hansch Analysis, launched the new field of Quantitative Structure-Activity Relationships (QSAR) (2, 5). The publication of Hansch's seminal papers in 1962 to 1964 generated immediate interest among chemists (1). Through Fujita's contributions and advocacy, industrial chemists around the world rapidly adopted and successfully applied the Hansch Analysis to develop new agrochemicals. Medicinal chemists also took note because QSAR provided a rational method for systematically optimizing drug potency. Among them was John Topliss.

### The Topliss Tree

John Topliss was born in England in 1930 and became passionate about chemistry in grammar school. He wanted to be a chemist. In his second year at the University of Nottingham, he secured a summer job at ICI Pharmaceuticals, which introduced him to the pharmaceutical industry and influenced his decision to pursue graduate work in organic chemistry. "The prospect of synthesizing potential new drugs was very appealing to me." After receiving his PhD from Nottingham and post-doctoral positions in Sweden and at Columbia University, he joined Schering (now part of Merck) in 1957.

At Schering and many other companies, the prevailing approach to optimize drug potency was almost entirely empirical. Topliss explored more rational approaches and initially focused, qualitatively, on the possible electronic and steric effects that influence potency. When he read Hansch's papers, he became aware of the critical importance

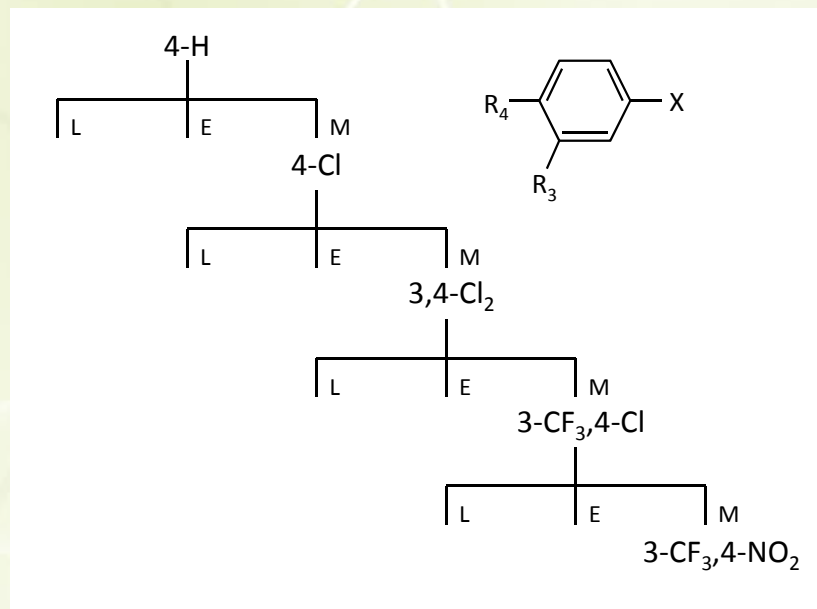


John Topliss (Courtesy of University of Michigan College of Pharmacy)

of lipophilicity, as well as the possibility of quantifying the lipophilic, electronic, and steric influences on activity. Topliss looked for opportunities to apply the Hansch Analysis at Schering, and in 1969, he gave one of the earliest presentations from industry on this approach at a meeting of the Society for Drug Research in London.

Although many medicinal chemists in the pharmaceutical industry, like Topliss, accepted that lipophilic, electronic, and steric effects were the main drivers of drug potency, few applied the Hansch Analysis in their compound synthesis campaigns. Stepwise, multiple regression analysis required mathematical, statistical, and computer methods that did not sit well with most synthetic chemists. Also, Hansch's approach required analysis of data from a sizable number of compounds before useful insights about optimal substituents could be gleaned—necessitating long, uninformative lead times that they could not afford. Their work was time critical. For each synthesized compound, even the first few in a chemical series, they needed to choose substituents that were most likely to increase potency.

Recognizing the barriers that prevented application of the Hansch Analysis, Topliss began thinking about alternative schemes that would employ Hansch's principles and rapidly optimize potency but would circumvent the need for regression analysis. He soon settled on a decision tree scheme, because the



**Figure 1.**

An example of Topliss's decision cascade, based on the lipophilicity and electronic characteristics of various substituents on a benzene ring and the corresponding biologic activity of the chemical analogs. M = more potent; E = equipotent; L = less potent;  $R_3$  = meta-substituent;  $R_4$  = para-substituent.

sequential branch points would define the most efficient way to maximize drug potency with a minimum number of synthesized compounds.

Topliss's procedure started with the unsubstituted phenyl compound and its corresponding biologic activity (measured by an appropriate bioassay). He then systematically placed substituents on the benzene ring and measured the biologic activity of the new analog. In many systems, activity increases with increasing  $\pi$  values (i.e., lipophilicity), and the 4-Cl analog is a good choice for the first aromatic substitution. In addition, 4-Cl analogs are generally easy to synthesize. The potency of the resulting 4-Cl compound, as measured by the bioassay, can be greater than (M), equal to (E), or less than (L) the activity of the unsubstituted parent compound. If the potency of the 4-Cl analog is greater, as shown in Figure 1, this could be attributed to a positive  $\pi$  effect, a positive  $\sigma$  effect, or the combined positive  $\pi$  and  $\sigma$  values. (As shown in Table 1, for 4-Cl,  $\pi = 0.71$  and  $\sigma = 0.23$ ). The 3,4-dichloro compound would be a good choice for synthesis



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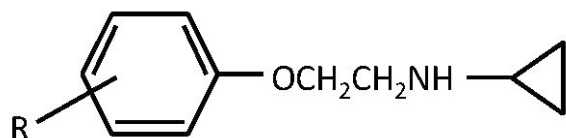


next, because this substituent has larger values for both  $\pi$  and  $\sigma$  than the 4-Cl substituent. (For 3,4-Cl<sub>2</sub>,  $\pi = 1.25$  and  $\sigma = 0.52$ ). If the potency of the 3,4-dichloro compound is, in fact, greater than the 4-Cl compound, then the next choice for synthesis would be the 3-CF<sub>3</sub>,4-Cl analog, because the  $\pi$  and  $\sigma$  values are still larger. (For 3-CF<sub>3</sub>,4-Cl,  $\pi = 1.59$  and  $\sigma = 0.66$ ). If the potency of the 3-CF<sub>3</sub>,4-Cl compound again increases compared to the earlier analogs, the next step might be to synthesize the 3-CF<sub>3</sub>,4-NO<sub>2</sub> analog. This substituent has a larger electronic value ( $\sigma = 1.21$ ) but a more modest hydrophobicity ( $\pi = 0.60$ ) and would determine the relative importance of the substituent's electronic properties. If this compound exhibits a further increase in potency, one can conclude that a + $\sigma$  is an important factor determining potency in this biologic system.

Obviously, other outcomes are possible. If the newly synthesized compound is equipotent (E) or less potent (L) than the preceding compound, it indicates an unfavorable shift due to the change in hydrophobic, electronic, or steric contributions of the new substituent. By choosing substituents that systematically increase or decrease  $\pi$ ,  $\sigma$ , and  $E_s$  at each subsequent branch point in this decision tree, the chemist can often optimize the biologic activity after synthesizing only a handful of compounds.

Topliss illustrated his scheme by presenting several specific examples taken from previously collected data. The monoamine oxidase inhibitory activity of a series of N-(phenoxyethyl) cyclopropylamines is shown in Table 2. Starting with the unsubstituted phenyl

**Table 2. Inhibition of Monoamine Oxidase by N-(phenoxyethyl)cyclopropylamines**



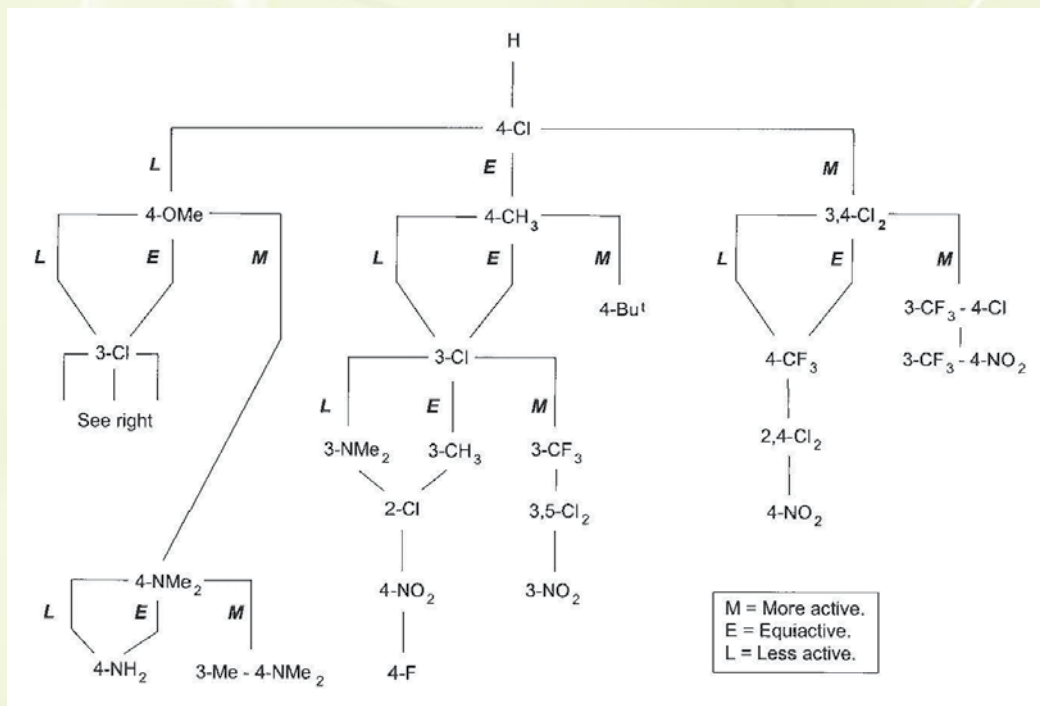
Order of synthesis (per Topliss Tree)	R	Biologic Activity
1	H	5.93
2	4-Br	6.64
3	3,4-Cl <sub>2</sub>	6.30
4	4-CF <sub>3</sub>	6.99
5	4-N=N-C <sub>6</sub> H <sub>5</sub>	7.56
6	4-SO <sub>2</sub> CF <sub>3</sub>	7.56

compound, which had a measured activity value of 5.93, the first analog in the series was the 4-bromo compound (the 4-Cl compound had not been made). The 4-bromo analog exhibited a substantial increase in activity (6.64). According to Topliss's scheme, the 3,4-Cl<sub>2</sub> compound was a logical analog to synthesize next, but unfortunately it had an activity value of 6.30 (lower than the 4-bromo compound). This result may be attributed to an adverse steric effect from meta-substitution (i.e., the 3-Cl substituent). This result would have prompted the synthesis of the 4-CF<sub>3</sub> analog, which had an activity of 6.99, representing an improvement in activity over the 4-bromo analog. With these results in mind, the indicated direction for further chemical synthesis was for other substituents in the 4-position that have highly positive  $\pi$  and  $\sigma$  values. One such compound is the 4-phenyldiazo analog, which was found to be the most active compound in the series (7.56). Another substituent with high, positive  $\pi$  and  $\sigma$  values is the 4-trifluoromethylsulfonyl substituent, which produced an analog that also had an activity of 7.56.

Topliss unveiled his decision-tree concept in August 1971 at a joint meeting of ASPET and the Medicinal Chemistry Division of the American Chemical Society held at the University of Vermont. (See Figure 2.) Encouraged by his colleagues' response, Topliss published the details of his scheme the following year (6). The article generated considerable interest among medicinal chemists. Corwin Hansch also reacted favorably to this application of his principles. Topliss was subsequently invited to speak at numerous scientific meetings and at other pharmaceutical companies to discuss his decision-tree rationale. It became a popular tool that medicinal chemists labeled the Topliss Tree.

Maybridge, a supplier of chemical intermediates, further facilitated application of the Topliss Tree methodology by marketing "Topliss Sets" for optimization of aromatic compounds. These prepackaged kits were a set of aromatic intermediates that consisted of the unsubstituted phenyl compound and the corresponding, most commonly used substituent analogs (chloro-, dichloro-, methyl-, and methoxy-). Before long, the Topliss Tree was incorporated into the curricula of medicinal chemistry courses and is now included in medicinal chemistry textbooks alongside the Hansch Analysis.

The Topliss Tree did not address all of the challenges that medicinal chemists face, but it made



**Figure 2. The Topliss Tree.**

their choices for chemical synthesis of aromatic compounds more efficient, especially in the early stages of SAR optimization. Once the most favorable type of substituent was identified by working through the Topliss Tree, the chemist could then examine similar substituents in detail, including the more unusual and synthetically difficult ones. In addition, because the Topliss Tree relied on the empirically established lipophilic, electronic, and steric constants, the strategy was compatible with other methodologies and provided a shortcut to the quantitatively rigorous Hansch Analysis.

Both Hansch and Topliss continued to make contributions to chemistry after their initial successes. At Pomona, Hansch and his students developed a computer program for calculating the partition coefficient, log P, using only the compound's chemical structure and created a database of thousands of QSAR parameters, which they have made available to other researchers. Through a collaboration with colleagues at the University of California San Francisco, Hansch further validated his methodology by demonstrating that his mathematical expressions predicted binding sites that matched the computerized 3-D surface of proteins derived from X-ray crystallography data. The use of computer graphics in QSAR is now an indispensable tool to medicinal chemists for drug design. Hansch continued his research activities and his association

with Pomona after his official retirement in 1988, a kind and generous mentor whose enthusiasm for research excellence was infectious.

Likewise, Topliss refined the Topliss Tree methodology and outlined another procedure for rapidly optimizing SAR (7). Still avoiding Hansch's statistics-intensive approach, Topliss developed a manual method of drug design in which groups of analog substituents were selected, synthesized, and tested for activity as a batch rather than sequentially synthesizing and testing

each one. The rank order of potency of these analogs, in turn, provided a rational basis for selecting new substituents that were likely to be more potent. Topliss hoped his "batchwise" method would be a faster and more advantageous strategy, but medicinal chemists more often employed the original, stepwise Topliss Tree.

In 1979, Topliss joined the Parke-Davis division of Warner Lambert (now part of Pfizer). As he advanced in his career, eventually becoming Vice President of Chemistry, he devoted less time to synthetic chemistry but oversaw development of several innovative new drugs, including the highly successful lipid-lowering statin, atorvastatin (Lipitor®). He also encouraged the chemists at Parke-Davis to apply combinatorial chemistry principles (which had only been used to generate peptides and nucleotide-based oligomers) to synthesize small molecules. They built an apparatus and developed methods for multiple, simultaneous synthesis of organic compounds in a chemical series (8). In 1992, Topliss became a full-time professor of medicinal chemistry after a longstanding affiliation with the University of Michigan and continued to make innovative contributions to drug design methodology. Along with Fumitaka Yoshida, he devised a QSAR model for predicting the human oral bioavailability of compounds, again finding that lipophilicity was a primary determinant of activity (9).

But it was the Topliss Tree that resonated most prominently with medicinal chemists. Hundreds of researchers have cited the Topliss Tree methodology in their publications, and application of the Topliss Tree during the early stages of SAR optimization has facilitated development of several commercially successful drugs. The Topliss Tree has also served medicinal chemists by redirecting their efforts away from inactive chemical analogs and allowing them to rapidly abandon unproductive compound synthesis campaigns.

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**Forty years ago, when he was sitting alone at his desk quietly sketching out his ideas, Topliss “did not in my wildest dreams expect this to be the outcome.”**

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The Topliss Tree methodology is only one of many tools used in designing a viable drug. But despite the advent of more sophisticated methods such as molecular modeling based on X-ray crystallography data and the willingness of today’s synthetic chemists to readily embrace computational chemistry, the Topliss Tree remains a valuable and frequently used tool in the medicinal chemist’s toolbox. Forty years ago, when he was sitting alone at his desk quietly sketching out his ideas, Topliss “did not in my wildest dreams expect this to be the outcome.”

For their outstanding contributions to medicinal chemistry, Corwin Hansch and John Topliss have both received many honors. Hansch received the American Chemical Society’s Smismann Award in 1976, and Topliss received the ACS Division of Medicinal Chemistry Award in 1998. Both were inducted into the ACS Division of Medicinal Chemistry Hall of Fame in 2007.

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# Stamping Out Drug Tampering

*Rebecca J. Anderson*

When Dennis and Jeanna Kellerman woke on Wednesday, September 29, 1982, they found their twelve-year-old daughter, Mary, unconscious on the bathroom floor (1-3). Paramedics rushed Mary from her home in Elk Grove Village, Illinois, to the hospital, where she was later pronounced dead. Initially, the doctors suspected she had died from a stroke (2).

That same morning, Adam Janus, a 27-year-old postal worker in Arlington Heights, Illinois, was feeling unwell at work and went home early (2-4). After eating a light lunch, he headed to the bedroom but collapsed before he got there. His wife called paramedics who desperately tried to save him. Adam's breathing was labored, his blood pressure was dangerously low, and his pupils were fixed and dilated. Nothing they did seemed to work, so they rushed Janus to the emergency room at nearby Northwest Community Hospital. He was pronounced dead at 3 pm, apparently from a massive heart attack (2-5).

Later that afternoon, paramedics were called back to Janus's home, where grieving family members had gathered to plan Adam's funeral. This time, they attended to Adam's 25-year-old brother, Stanley, who had suddenly collapsed. He was lying on the living room floor, his pupils fixed and dilated. One of the bewildered paramedics looked up at Charles Kramer, the fire lieutenant on duty, and said, "This is what happened to the first guy. It's the same thing as this morning. We're losing him" (3).

At that moment, they heard a groan in the living room, and Stanley's 19-year-old wife, Theresa, collapsed right in front of them. The Janus couple was rushed to the hospital, where Stanley died that evening. Theresa, who was brain-dead on arrival, was put on life support (3).

Helen Jensen, a village nurse in Arlington Heights, was eating dinner that evening when she received a call from Northwest Community Hospital (6). Hospital officials asked her to investigate the Janus' suspicious illnesses. Initially, she checked for a deadly bacteria or virus in the Janus house and also considered toxic gas, a poison introduced from some unknown source, and other causes. Listening closely to family members as they recounted the day's bizarre events, Jensen identified a common denominator.

Adam had stopped at a grocery store on his way home to buy a bottle of Extra-Strength Tylenol capsules and took two after eating his lunch. Later that day, Stanley took two capsules from the same bottle for back pain, and Theresa also took two for a medical condition.



Jensen located the opened Tylenol bottle in the Janus bathroom, took it to Northwest Community Hospital, and insisted that they test it (6). In parallel, a doctor at the hospital reported the suspicious Janus brothers' deaths to the Rocky Mountain Poison Center in Denver. The victims' symptoms pointed to cyanide poisoning (4).

Also on Wednesday evening, Phillip Capitelli, an off-duty Arlington Heights firefighter, was at home listening to the frenetic activity on his police scanner. He called Lieutenant Kramer at the fire station to ask for further details. When Kramer mentioned Tylenol, Capitelli recalled a conversation earlier in the day with his mother-in-law, who was a coworker of Jeanna Kellerman and had called him to ask if he knew anything about the mysterious Janus deaths (3, 4). He followed up with his friend, Richard Keyworth, a firefighter in Elk Grove Village, who told him that Mary Kellerman had taken an Extra-Strength Tylenol capsule for a runny nose and sore throat before collapsing.

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***One of the bewildered paramedics looked up at Charles Kramer, the fire lieutenant on duty, and said, "This is what happened to the first guy. It's the same thing as this morning. We're losing him"***

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By dawn on Thursday morning, the Cook County Medical Examiner's office had found cyanide in all four victims' blood, and the county's chief toxicologist found lethal concentrations of potassium cyanide in the remaining capsules of the Tylenol bottles retrieved from both the Kellerman and Janus homes (2, 4). Both bottles bore the same manufacturer's lot number MC2880 (1). But tracking down the source of the contamination would be challenging. Like all over-the-counter drugs at the time, Tylenol was displayed on retailers' shelves in unsealed bottles. The only protection was a wad of cotton inside the bottle. The two ends of the hard gelatin capsules could be easily slid open and reclosed.

In addition, potassium cyanide was readily available. It was used in industrial processes such as electroplating, film processing, fertilizer production, steel plating, and extracting gold and silver from low-grade ores. Medical and scientific laboratories also

regularly used potassium cyanide as a reagent, and drug peddlers used it to make street drug supplies of phencyclidine. Almost anyone, anywhere, could have spiked the Tylenol capsules with cyanide.

### **Putting Patient Safety First**

At 9:30 am on Thursday morning, the first calls from Chicago authorities and the media reached McNeil Consumer Products Company, notifying executives that their best-selling product was linked to several deaths in Chicago (7-9). James Burke, CEO of Johnson & Johnson, McNeil's parent company, immediately dispatched an investigative team to the McNeil plant in Ft. Washington, Pennsylvania, where the MC2880 lot had been produced (9). Their highest priority was public safety, and they recalled all 93,400 bottles of the lot. By noon, they had also issued a half-million mailgrams to physicians, hospitals, and wholesalers, alerting them to the danger. McNeil also suspended all advertising for Tylenol (4, 7, 9).

Unfortunately, the situation in Chicago was growing more dreadful by the hour.

In parallel with Burke's initial actions on Thursday morning, Mary Reiner, a 27-year-old telephone company employee who was recovering from the birth of her son, was found dead in her home in Winfield, Illinois (1, 2, 4). Investigators found four cyanide-laced Extra-Strength Tylenol capsules in a bottle labeled Regular-Strength Tylenol. Unable to locate the original Extra-Strength Tylenol bottle, they could not determine the lot number of the tainted capsules (4).

On Thursday afternoon, Mary McFarland, a 31-year-old homemaker in Elmhurst, Illinois, was rushed to Good Samaritan Hospital in nearby Downers Grove but was dead on arrival from cyanide poisoning (1). Investigators found a bottle of contaminated Extra-Strength Tylenol capsules in her purse and another bottle at her home marked with lot 1910MD. An empty bottle in her trash was marked lot MC2738 (4).

Authorities in Chicago grimly feared



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more bodies might be found—shut-ins and single people being the most vulnerable—and they mounted an extraordinary effort to inform and protect the public. Chicago Mayor Jane Byrne ordered removal of all Tylenol products from Chicago stores (10). Police cruised neighborhoods, shouting a warning over loudspeakers not to take Extra-Strength Tylenol capsules (2, 4). Boy Scout troops went door to door gathering bags full of the bottles, and church groups launched telephone drives to reach elderly citizens and others who might not have heard the repeated broadcasts on radio and television. School officials sent notices home with children, and transit workers on buses and trains spread the word. Far into the night, police made the rounds to taverns, and all paramedic units received anti-cyanide kits (4, 10).

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***Illinois Gov. James Thompson declared, “We have a madman out there”***

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Although the information reaching Burke and his team in New Brunswick, New Jersey, was fragmentary, they quickly became convinced that the cyanide had not been introduced, either accidentally or intentionally, during the production or distribution of their product (7, 9). Lot MC2880 had been shipped from the Pennsylvania plant to 31 Eastern and Midwestern states. Lot 1910MD had been produced at McNeil’s plant in Round Rock, Texas, and distributed in Chicago and the West (4). Yet, the cyanide-laced capsules had been found only in the suburbs of

Chicago. The most likely explanation was that someone in the Chicago area had taken bottles of Extra-Strength Tylenol from retailers, filled the capsules with potassium cyanide, and then replaced the bottles on retailers’ shelves. Illinois Gov. James Thompson declared, “We have a madman out there” (4).

On Friday, October 1, and in view of the escalating tragedy, McNeil expanded its recall to include all 171,000 bottles of the 1910MD lot that had been produced in Texas (4). Johnson & Johnson also offered a \$100,000 reward for information leading to the arrest and conviction of whoever was responsible (10). Unfortunately, also on Friday, doctors at Northwest Community Hospital abandoned their efforts to save Theresa Janus, who became the sixth fatality (4). Late on Friday night, police found the body of the seventh and final victim. United Airlines flight attendant Paula Prince, 35, died in her near North Side apartment with a bottle of Extra-Strength Tylenol capsules nearby (4, 10). Prince’s bottle came from yet another lot, 1801MA, and she was the only victim found in Chicago, not the suburbs (4).

Even in those first hectic days, personnel at Johnson & Johnson’s headquarters fully cooperated with the media because reporters seemed to have the most up-to-date and accurate information about the poisoning incidents (8, 9). Burke and his staff also worked closely with local, state, and federal authorities on the criminal investigation. On Monday, October 4, Burke and his executives flew to Washington, DC, to meet with FBI Director William Webster and FDA Commissioner Arthur Hayes (7). Burke proposed a nationwide recall of all Extra-Strength Tylenol



*Safety seal on a Tylenol bottle*



## Armor-Clad Packaging

Acetaminophen, the active ingredient in Tylenol, had been marketed by several drug firms since the 1960s as an over-the-counter analgesic and antipyretic. In 1975, McNeil launched an aggressive and highly successful advertising campaign, and by 1982, Tylenol had captured more of the analgesic market than the next three brands of acetaminophen combined (9).

While market analysts praised Johnson & Johnson for the company's quick and decisive actions—putting patient safety before corporate profits—they predicted that the Tylenol brand (the most recognizable over-the-counter product for pain-relief) was dead. Most consumers believed McNeil was not at fault, but they still associated the poisonings with Tylenol and were understandably hesitant to use it. One Madison Avenue advertising guru said, “I don't think they can ever sell another product under that name” (11).

Undeterred by the market analysts, Burke and his staff never considered retiring or replacing the Tylenol brand (7, 9). While dozens of McNeil employees manned banks of telephones and responded to thousands of inquiries from consumers and the media, all seeking information about Tylenol and the poisonings, Burke simultaneously set up several task forces to stage Tylenol's comeback. By Monday, October 11, he had finalized his re-launch strategy (9).

An easy solution would have been to abandon the capsule formulation in favor of tablets, which are much harder to contaminate, but consumers preferred Tylenol capsules. They were easier to swallow than tablets, and some consumers thought that they provided more potent pain relief—an unfounded, placebo-driven perception, presumably because capsules looked similar to prescription drugs (13). However, to reintroduce Tylenol capsules, Johnson & Johnson needed to win back consumer confidence, which in turn relied on an absolute assurance that the product was safe. Therefore, Burke's most critical task force was the one charged with developing new tamper-resistant packaging, and he assigned himself to head it (7). Various inventors had already developed more than a dozen protective-packaging methods, most of which were aimed at preventing children from opening drug bottles. Burke was determined to be the first in the industry to devise packaging that deterred willful tampering and to do more than anyone else in the industry—actions that were intended to reassure the nervous public (7).



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capsules. Although Webster and Hayes were initially concerned that such drastic action would inflame an already panicked nation, their opposition vanished when they received word of copycat strychnine-laced Tylenol capsules in California.

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***One Madison Avenue advertising guru said, “I don't think they can ever sell another product under that name”***

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On Tuesday, Johnson & Johnson announced the recall of 31 million bottles of Extra-Strength Tylenol capsules, and the FDA urged consumers nationwide not to take Extra-Strength Tylenol capsules of any lot number “until the series of deaths in the Chicago area can be clarified” (4, 7). Some retailers and government officials mirrored Mayor Byrne's directive and removed all Tylenol products from store shelves. On Thursday, October 7, and in an attempt to retrieve the millions of bottles of Tylenol capsules that had already been purchased and were sitting in consumers' medicine cabinets, Johnson & Johnson offered to exchange all those capsules for Tylenol tablets (9, 11). The company examined 1.5 million bottles of Tylenol and found three unopened bottles with tainted capsules—all retrieved from the Chicago area (12). Truckloads of the recalled capsules were eventually dumped into an incinerator in north New Jersey and burned (7).





**J&J CEO  
James Burke**

Burke's task force quickly settled on a triple seal system: an outer box with glued flaps, a plastic shrink sleeve over the cap and neck of the bottle, and a strong foil seal over the mouth of the bottle (8, 14). They decided that the first product to be

produced and shipped with the new packaging would be the now infamous Extra-Strength Tylenol capsules. McNeil's executives and engineers went into overdrive, working three shifts, seven days a week to retool production (7). They had to reconfigure carton machinery to glue as well as fold the boxes, and they madly scrambled through Johnson & Johnson's affiliated drug companies for existing machines and material (7). For the plastic shrink sleeves, they searched in vain as far as Asia and Europe for enough equipment and finally resorted to mounting the shrink sleeves by hand. They designed new graphics and wanted to call the anti-tampering device the Tylenol "safety seal," but they first had to track down the man who owned the trademark to that phrase and negotiate a license to use it (7). On October 23, 1982, less than a month after Mary Kellerman's death in Chicago, Johnson & Johnson publicly announced its intention to reintroduce Tylenol capsules in "tamper-resistant containers" (14).

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***Burke's task force quickly settled on a triple seal system: an outer box with glued flaps, a plastic shrink sleeve over the cap and neck of the bottle, and a strong foil seal over the mouth of the bottle.***

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In parallel with re-engineering Tylenol's packaging, company officials were spending a lot of time in Washington, DC. They called on more than 160 Congressional offices, urging lawmakers to make

product tampering a federal crime (8). They also cooperated with FDA officials who were drafting the first regulations for tamper-resistant packaging. On November 4, 1982, Burke and several of his top executives met with Health and Human Services Secretary Richard Schweiker and FDA Commissioner Hayes (7). During the meeting, Burke pulled out a prototype of the newly packaged Tylenol capsules, and both officials had difficulty opening the bottle. Schweiker said, "Everybody else is going to have a package. You've got an armored tank" (7).

The next day, the FDA published the new regulations for tamper-resistant packaging of over-the-counter products in the Federal Register (47 FR 50442). With few exceptions, all OTC human drug products were required to have "an indicator or barrier to entry" and a statement on the label alerting the consumer to the specific tamper-resistant feature(s). The new Tylenol packaging, with three barriers, went far beyond those requirements. One week after the FDA regulations were published and less than six weeks after the first deaths were reported in Chicago, Tylenol capsules in the triple-seal packages began reappearing on store shelves (14). Sales quickly rebounded, and within three years, Tylenol was once again the dominant brand of acetaminophen (12).

On October 14, 1983, a year after the Chicago deaths, President Reagan signed the Federal Anti-Tampering Act, making it a felony to tamper and inflict harm with any consumer product (drug, device, food, or cosmetic).

### **Deja Vu All Over Again**

On Friday, February 7, 1986, Diane Elsroth, 23, was visiting her boyfriend in his parents' home in Yonkers, New York. She complained of feeling ill, and her boyfriend opened a new bottle of Extra-Strength Tylenol and gave her two capsules (15, 16). Twelve hours later, he went to check on her and discovered Elsroth dead. On Monday, Elsroth's autopsy revealed evidence of cyanide poisoning, and the retrieved bottle of Tylenol contained three more capsules filled with potassium cyanide.

In a repeat of the Chicago crisis, Johnson & Johnson fully cooperated with FDA and local authorities, advised people in Westchester County, New York, not to take any Tylenol capsules, and suspended all television advertising (16). The company also retrieved the entire stock of Tylenol

capsules from all retail outlets within a three-mile radius of the store where the poisoned capsules had been purchased. On February 13, after testing 67,000 capsules, McNeil and FDA analysts discovered a second bottle with five cyanide-laced capsules (15). Realizing that Elstroth's death might not be an isolated poisoning, Johnson & Johnson issued a nationwide press release urging consumers not to take Tylenol capsules until further notice and requested retailers and wholesalers to remove all Tylenol capsule products from their shelves.

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***Stella had filled the Excedrin capsules with cyanide, repackaged them, and placed three of the bottles in area stores, making Bruce's death look like the work of a serial poisoner.***

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Despite Johnson & Johnson's "armor-clad" safety barriers, the compromised Tylenol packaging showed no visible signs of tampering. Burke and his staff rapidly concluded that they could no longer guarantee the safety of Tylenol in capsules. He announced that the company would stop manufacturing all over-the-counter capsule products, despite their popularity (13). Instead, Tylenol would be manufactured as caplets. Caplets are oval-shaped like a capsule but are solid like tablets and therefore extremely difficult to violate without leaving clear evidence of tampering. With a slick coating and smaller size, the new caplets were also easy to swallow (13). Other companies chose to continue manufacturing their acetaminophen products in capsules.

### **Other Corporate Headaches**

A few months later, on June 11, 1986, Susan Snow, a 40-year-old bank manager in Auburn, Washington, woke with a headache. She took two Extra-Strength Excedrin capsules and went to take a shower. Forty minutes later, her 15-year-old daughter found Snow unconscious on the bathroom floor (2). With only a faint pulse, Snow was transported to Harborview Medical Center, where she later died without regaining consciousness. An astute medical examiner detected the distinct scent of bitter almonds during Snow's autopsy and determined that she had died from cyanide poisoning, which

was traced back to the innocuous looking bottle of Excedrin capsules. Taking a page from Johnson & Johnson's playbook, executives at Bristol-Myers Squibb, the makers of Excedrin, immediately recalled the product nationally, hoping to avert more deaths.

Following Bristol-Myers Squibb's highly publicized and massive recall, Stella Nickell called police to report her suspicions about her husband's death two weeks earlier (17). Bruce Nickell had died shortly after taking four Extra-Strength Excedrin capsules. Although his death was initially thought to be due to complications from emphysema, laboratory tests of his blood subsequently detected a lethal concentration of cyanide, and investigators recovered two bottles containing tainted Excedrin capsules from the Nickells' home in Auburn, Washington. In the months that followed, a total of five bottles of Extra-Strength Excedrin containing cyanide-laced capsules were retrieved from the Tacoma, Washington, area. It seemed odd that two of them had ended up in the Nickells' home. During detailed examination of the tainted material, an FBI analyst found minute specks of a green crystal substance mixed with the cyanide (2). He identified the green substance as an algaecide, Algae Destroyer, a product used in fishponds and home aquariums.

Law enforcement officials discovered that Stella Nickell owned a fish tank and had bought Algae Destroyer from a pet store prior to the deaths of Susan Snow and Bruce Nickell. She had also taken out three insurance policies on her husband in the year prior to his death (17). When she failed a lie detector test and investigators found substantial fingerprint evidence, police charged her with her husband's murder (2).

**The names acetaminophen and Tylenol are both derived from the compound's chemical name, N-acetyl-para-amino-phenol, or APAP:**

**N-acetyl-para-amino-phenol → acetaminophen**

**N-acetyl-para-amino-phenol → Tylenol**



FBI agents and state police subsequently compiled evidence that indicated Stella had filled the Excedrin capsules with cyanide, repackaged them, and placed three of the bottles in area stores, making Bruce's death look like the work of a serial poisoner. On May 9, 1988, a jury found Stella guilty of murder under the new Federal Anti-Tampering Law, and she was sentenced to 90 years in prison (2).

### Tightening Control

As the deaths in New York and Washington demonstrated, over-the-counter capsule products continued to be vulnerable to tampering because the two ends of the gelatin capsules could be easily opened and reclosed. Consequently, FDA amended and strengthened its requirements in 1989 (54 FR 5227), requiring all two-piece, hard gelatin capsules to have a minimum of two tamper-resistant packaging features. The regulations encouraged, but did not require, manufacturers to seal the capsules as one of the two tamper-resistant features. Examples of acceptable capsule-sealing technologies included sonic welding, banding, and sealing techniques that employed solvents or low temperature heating.

The new tamper-resistant packaging did not deter Joseph Meling. In February 1991, he opened capsules of Sudafed 12-hour decongestant that had been protected in blister packs and spiked them with cyanide, intent on killing his wife. To divert suspicion, he drove along a 30-mile stretch of Interstate 5 between Tacoma and Olympia, Washington, and deposited five of the tainted Sudafed packages on the shelves of retailers located near the interstate exits (2). After taking Sudafed, Mrs. Meling was rushed to the hospital in a coma. She survived—ironically because Joseph asked the emergency room doctors if they had considered the possibility that she had been poisoned (18). They immediately pumped her stomach. Unfortunately, two other victims

who purchased and consumed the cyanide-laced capsules died within the next two weeks.

Reaction to the cyanide-laced Sudafed capsules paralleled the earlier tampering incidents (18). Burroughs Wellcome, the manufacturer of Sudafed, and the FDA ordered a nationwide recall of the product and assayed thousands of capsules for cyanide. Local, state, and federal officials cooperated in conducting the criminal investigation and quickly narrowed the suspects to Meling. In 1993, he was convicted and sentenced to life in prison under the Federal Anti-Tampering Law (2).

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***Concerns about tampering, which once discouraged the use of those analgesics, have now been replaced by concerns about consumers unwittingly taking too much acetaminophen.***

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The tainted Sudafed packaging showed obvious signs of tampering (19). The lot numbers on the blister pack and outside carton did not match. The foil on the blister pack had been pulled back and then pushed back into place, and the cyanide-containing capsules were oversized and missing the purple band that otherwise sealed all Sudafed capsules. Citing the Sudafed incident, the FDA again strengthened the packaging requirements for over-the-counter drug products “to improve consumer protection by addressing specific vulnerabilities in the OTC drug market.” The new requirements, which the FDA issued in 1998 (63 FR 59463), mandated that all two-piece, hard gelatin capsules of over-the-counter drugs must be sealed plus have at least one additional tamper-evident feature in the packaging. The product's label was required to highlight all tamper-evident features of the product,



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including those on the box, the bottle or closure, and the sealed capsules.

The agency also changed the regulation terminology from “tamper-resistant” to “tamper-evident” packaging. Despite clear signs of tampering, the Sudafed victims had perhaps been complacent, falsely assured that they were buying a tamper-resistant product. Regulators, therefore, underscored the importance of sensitizing consumers so that they would be more alert and look for tampering; terminology that implied a particular package was difficult to breach or tamper-proof was no longer acceptable.

## A Swing of the Pendulum

For more than two decades, no malicious tampering of over-the-counter drugs has been reported in the United States. The elaborate packaging features, though sometimes annoying and occasionally frustrating, have proven to be effective and fostered widespread use of acetaminophen. Tylenol continues to be the most recognizable and ubiquitous brand, but acetaminophen is now an analgesic ingredient in over 600 products, including Sudafed sinus tablets and Excedrin. Concerns about tampering, which once discouraged the use of those analgesics, have now been replaced by concerns about consumers unwittingly taking too much acetaminophen.

According to the FDA, acetaminophen toxicity accounts for 56,000 emergency room visits and an estimated 200 deaths from acute liver failure each year. Severe liver injury with acetaminophen occurs most frequently when patients take more than the prescribed dose of acetaminophen-containing drugs in a 24-hour period, take more than one acetaminophen-containing product at the same time, or drink alcohol while taking acetaminophen products.

In 2011, the FDA issued new guidelines on acetaminophen-containing prescription products (20). Some of the most popular prescription analgesics pair acetaminophen with an opiate: Tylenol with codeine, Percocet (with oxycodone), and Vicodin (with hydrocodone). The agency asked manufacturers to limit the amount of acetaminophen in prescription products to no more than 325 mg per tablet or capsule by January 2014.

*Investigators testing recalled bottle of Tylenol for potassium cyanide*





About half of the product manufacturers complied with the request. For those that have not, the FDA announced earlier this year that it would begin the process of withdrawing approval of the noncompliant prescription products. At the same time, the agency announced its intentions to start regulatory actions aimed at limiting the amount of acetaminophen in over-the-counter products as well.

Safety experts are most concerned about products such as Extra-Strength Tylenol, which has become so popular that some pharmacies no longer stock the regular strength product. Instructions for taking Extra-Strength Tylenol (a dose of 1000 mg, compared to the 650 mg regular strength dose) push the body burden of acetaminophen to the FDA's recommended limit of 4000 mg per day.

In October 2013, Johnson & Johnson became the first manufacturer of over-the-counter products to address the regulatory concerns about acetaminophen-induced liver toxicity. Tylenol bottles now sport a cap with bold red lettering: "CONTAINS ACETAMINOPHEN" and "ALWAYS READ THE LABEL." The eye-catching warning is specifically intended to alert consumers and hopefully discourage overdosing.

The packaging devices, anti-tampering laws, and FDA regulations that were launched in the wake of the Chicago murders have greatly improved the safety of all over-the-counter drugs and undoubtedly prevented many deaths. But the trail leading to the original Tylenol-tampering killer has gone cold, and Johnson & Johnson's \$100,000 reward remains unclaimed.

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# The Crohn Legacy: From Halitosis to Colitis and Beyond

Rebecca J. Anderson

On a hot summer day in 1956, Burrill Crohn was planting sweet corn in the garden of his country home in New Milford, Connecticut, when he was called to the phone (1). The editor of the *Washington Post* informed him that President Eisenhower had been rushed to Walter Reed Army Medical Center and was about to undergo emergency surgery for an intestinal obstruction. The 72-year-old Crohn was not part of Eisenhower's medical team and knew nothing about the case, except what the newspaper editor told him. Soon, though, journalists representing other prominent newspapers, news agencies, radio, and television networks, and scientific organizations also called Crohn. He was one of the most prominent gastroenterologists in the country, and they all wanted to know what he thought about the President's condition and chances for recovery.

Credit: The Crohn Family



Dr. Burrill Crohn

## Always Inquisitive

Burrill Crohn did not set out to be a gastroenterologist. During his medical school training at Columbia University, he was fascinated by biochemistry and carried out a research project that earned him a PhD in addition to his MD in 1907. He subsequently set up a small private medical practice in Manhattan, but in the mornings, he volunteered as a biochemistry laboratory assistant at Mount Sinai Hospital. Between routine laboratory tests, he had plenty of time for research, and the hospital offered a wide variety of intriguing clinical cases.

From 1913 to 1921 (before the discovery of insulin), Crohn spent much of his time studying the function and diseases of the pancreas. This work was largely driven by Crohn's desire to use a new toy that had been presented to him by a visiting physician. The device was a new type of rubber catheter for collecting upper intestinal bile. Crohn first used it to establish a "normal" baseline by collecting specimens



from himself. “Night after night at bedtime, I would swallow that 36-inch long rubber catheter, drink a glass of milk to stimulate pancreatic secretion, and go to sleep. In the morning I would aspirate the pancreatic secretions and the bile from my duodenum...every afternoon the secretions were tested and the normal pancreatic enzymes evaluated in the laboratory” (1). He then compared those results with secretions collected from a series of patients with pancreatitis. His published monograph (*Studies in Pancreatic Disease*, 1915) earned him recognition as an expert on the pancreas.

Crohn also used the catheter to study liver diseases and took advantage of a new test for quantifying sensitivity to physical pain (called the Libman Test) while examining patients who suffered from peptic ulcers. He found a correlation between pain sensitivity and patients’ awareness of their peptic ulcers. Patients who presented with gross hemorrhage were insensitive to pain. On the other hand, patients with a low pain threshold were more likely to seek medical treatment, and their peptic ulcers were managed more effectively. On the strength of his innovative findings regarding ulcer management, Crohn was inducted into the American Gastroenterological Association in 1917, and in 1922, he was appointed to head the new gastroenterology department at Mount Sinai Hospital.

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***“It has been my misfortune (or perhaps my fortune) to spend most of my professional life as a student of constipation and diarrhea. Sometimes I wished I had chosen ear, nose, and throat as a specialty rather than the tail end of the human anatomy”***

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A caring physician, Crohn treated celebrities and ordinary patients with the same attentive bedside manners and exceptional expertise. To his siblings, Burrill was the big brother, a tower of strength who listened sympathetically to their problems, provided helpful advice, and generously offered assistance (2). Despite his demanding professional duties, he devoted Sunday afternoons to his children. Together, they explored all of the historical and cultural sites that New York City offered, and Crohn turned each field trip into an adventure. Climbing rocks in Central Park,

they fought the British at the Battle of Quebec. When they made their way through hilly terrain, it became the Donner Pass.

The youngsters roamed freely in the hospital’s laboratories, watching the laboratory technicians and playing with the experimental animals. His daughter recalls, “Often he would be called out to see a sick patient. But somehow he made time for me” (1, 2). Sometimes, Crohn took her along on house calls.

### **Gut Instincts**

When Crohn entered medical practice at the turn of the twentieth century, the small intestine was not a topic of much interest. His medical school professors advised the class to skip the textbook chapter on small bowel because “there are no recognizable diseases of the small intestine except, perhaps, tuberculosis” (1). During Crohn’s internship at Mount Sinai Hospital, his mentor always required that autopsies include a dissection of the small bowel because he insisted on being thorough, but “nothing of note was ever found” (1).

Despite the lack of known intestinal ailments, Crohn was always curious and developed a keen gut instinct. A popular catch-all abdominal diagnosis at the time was “chronic appendicitis.” It covered all sorts of vague, unexplained, and neurotic abdominal pains and discomfort, including an inflamed gut. Over and over, surgeons removed a healthy appendix (often missing by inches a mass in the adjacent small bowel). One day on hospital rounds during Crohn’s residency, his chief of service presented a case of “chronic appendicitis.” Crohn paid close attention as his chief described the patient’s symptoms to the residents, but something about this case aroused his doubts. After hospital rounds, Crohn went back to re-examine the patient’s belly and noted a faint line that looked like a surgical scar. When asked, the patient confirmed that years earlier his appendix had been removed. Any question in Crohn’s mind about “the fanciful diagnosis of chronic appendicitis was dispelled then and there. The disease never existed” (1).

That was the first of many cases Crohn investigated because he thought the standard diagnosis seemed illogical. Rather than accepting medical dogma, he followed the trail of evidence, and his inquisitive mind and methodical research served him well. Most of these enigmatic cases involved the digestive system. In addition to pancreatitis and hemorrhagic peptic ulcers, he studied bulimia, pica, dyspepsia, trauma-

induced intestinal ulcers, and gallstones. He published his findings, which made significant contributions to explain the etiology of those disorders and honed his expertise in gastroenterology. He once lamented, “It has been my misfortune (or perhaps my fortune) to spend most of my professional life as a student of constipation and diarrhea. Sometimes I wished I had chosen ear, nose, and throat as a specialty rather than the tail end of the human anatomy” (1)!

In 1930, Crohn examined a 17-year-old boy who exhibited a fever, diarrhea, and a tender, palpable mass in his abdomen. Tuberculosis was still a common disease, and harkening back to his medical school training, Crohn initially diagnosed intestinal tuberculosis, the only known explanation for an irritable bowel. Fortunately, new diagnostic tests had become available, and Crohn systematically conducted skin, eye, and sputum assays. All were negative for tubercle bacilli. The chest X-ray was also negative. Having exhausted all of his noninvasive options, Crohn wanted to conduct exploratory surgery to examine the boy’s intestines directly.

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***Crohn accumulated 1000 cases over the course of his medical practice, and regional ileitis emerged as a common type of inflammatory bowel disease, second only to ulcerative colitis. It affected people across all cultures and economic groups.***

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At that time, there was no treatment for intestinal tuberculosis, and A. A. Berg, his surgical colleague and friend, initially refused to operate. Earlier, at the Trudeau Sanitarium, Berg had been persuaded against his better judgment to operate and resect the bowel of five patients with intestinal tuberculosis. Two patients were made worse, 2 died, and he did not know the outcome of the last patient—and never wanted to know.

Crohn persisted. He showed Berg his patient’s test results, which had convincingly ruled out tuberculosis. Reluctantly, Berg agreed to operate. He found an inflammatory mass and removed the terminal 12–16 inches of the boy’s ileum. In the laboratory, Crohn subjected the excised specimen to every available assay, scrutinizing stained sections for hours, but

found no trace of tubercle bacilli. He concluded that the boy’s ailment represented a new and previously undocumented medical condition, which he initially assumed was probably extremely rare. However, within 2 years, Crohn and his colleagues accumulated 14 such cases, all with the same clinical characteristics.

These cases had baffled the hospital staff, who speculated that the cause might be intestinal tuberculosis or actinomycosis (a disease characterized by granulomatous lesions), but they could not establish a definitive diagnosis. Some of those patients had been lying in the wards with their condition growing progressively worse for lack of an effective treatment—in the process developing fistulas (openings) in the gut wall.

After the test results ruled out tuberculosis in these patients, Berg operated on them. In one patient, Berg removed eleven fistulas in the abdominal wall and cured the patient in a single operation. From the symptoms, surgical observations, pathologic analysis, and successful post-operative recoveries, Crohn and his pathology colleagues concluded that they were dealing with a previously undefined disease. They called it “regional ileitis” and Crohn proceeded to share their findings with the medical community.

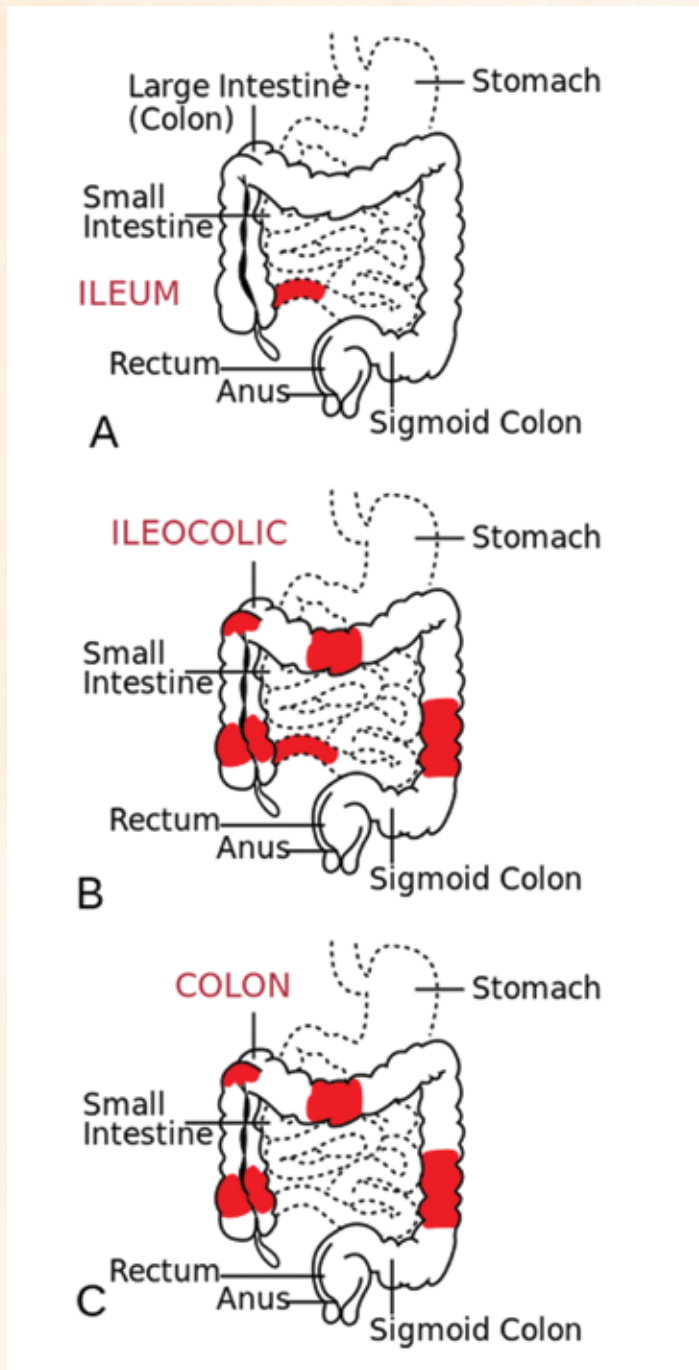
In May 1932, he traveled to New Orleans and read his paper, “Regional Ileitis: A New Clinical Entity,” at the annual meeting of the American Medical Association. Crohn explained, “Regional ileitis is an inflammatory or granulomatous disease of the small bowel, characterized by fever, diarrhea, abdominal pain, and fistula formation. It is essentially a disease of youth, slowly progressive and disabling” (1). In October, Crohn, Leon Ginzburg, and Gordon Oppenheimer published their findings in *JAMA* (3). This seminal paper, which listed the authors in alphabetical order, would be widely referenced.

The Mayo Clinic immediately reviewed their files and found previously overlooked cases of ileitis. Within a year, regional ileitis was being discussed in Germany at an international meeting of surgeons. The introduction of endoscopes and higher resolution radiographic images facilitated the diagnosis. Radiologist John Kantor described the “string sign,” a narrowing of the intestinal lumen that appears as a thin string on barium radiographs, and which is now generally recognized as a characteristic sign of regional ileitis. Crohn accumulated 1000 cases over the course of his medical practice, and regional ileitis emerged as a common type of inflammatory bowel



disease, second only to ulcerative colitis. It affected people across all cultures and economic groups.

By the time President Eisenhower was rushed to Walter Reed Army Medical Center, the disorder had become well recognized by the medical profession, but Crohn was the only one who still called it regional ileitis. Everyone else called it Crohn's disease. No wonder every reporter in the country wanted Crohn to comment on the President's prognosis.



The three most common sites of intestinal involvement in Crohn's disease are ileal, ileocolic, and colonic.

Based on the President's symptoms, Crohn surmised that the obstruction was a late manifestation of longstanding ileitis (1). Eisenhower had suffered from gastrointestinal distress for decades. While serving in the Panama Canal Zone as a newly commissioned officer in 1922, he experienced episodes of abdominal pain and weight loss (4). Convinced that the problem was appendicitis, he persuaded doctors in Denver to perform an appendectomy in 1923. Subsequently, he underwent a series of thorough diagnostic evaluations. While investigating his severe intestinal symptoms in 1949, doctors saw some "irregularity of caliber of the small bowel" in an X-ray (4). However, it was not until a month before his surgery in 1956 that an X-ray revealed a picture of his terminal ileum typical of Crohn's disease. His periodically inflamed ileum had healed, and scar tissue in the intestinal wall led to the obstruction (1, 4).

Eisenhower's medical team confirmed Crohn's disease as the diagnosis through direct observation during surgery and later microscopic examination of the diseased terminal ileum. They successfully bypassed the diseased segment by anastomosing the intestine above the obstruction with the transverse colon. The president made a rapid and full recovery.

### Pharmacologic Intervention

In the days before regulatory oversight of pharmaceuticals, peddlers hawked patent medicines, reinforcing the public's widely held but misguided belief that a daily bowel movement was the key to a happy life. Elixirs and pills were promoted as a remedy for spring fever, tiredness, poor blood, and depression, but most of these concoctions were a mixture of vegetable compounds (including castor oil) that were predominantly laxatives. The main ingredient in Carter's Little Liver Pills was the cathartic, bisacodyl.

Constipation was not a serious medical concern. Diarrhea was. A primary symptom of ileitis and colitis, diarrhea caused dehydration, electrolyte imbalances, and abdominal pain. Crohn saw so many cases in his long career that he once exclaimed, "When I die, I hope to be sent to a Heaven where even the angels are constipated" (1).

In the decades before the introduction of anti-inflammatory drugs, surgery was a common treatment for Crohn's disease. Crohn's first ileitis patient (the 17-year-old boy) was well for 25 years

after Berg's operation, and then he developed only a mild recurrence. In many other cases of Crohn's disease, surgical intervention gave permanent relief from symptoms. But it was a radical treatment, and there was a limit to how much small bowel could be removed if symptoms recurred.

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***The results from all of these experiments supported his view that breath odor was not produced locally in the mouth or pharynx, but rather from metabolism and subsequent respiratory excretion.***

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Current pharmacological treatment of Crohn's disease is aimed at symptomatic relief. Treatments of choice to induce remission and manage acute recurrences of inflammation are systemic glucocorticoids such as budesonide or prednisone. Remission can be maintained with immunomodulatory drugs (such as mercaptopurine and azathioprine) and broad-spectrum antibiotics (such as metronidazole and ciprofloxacin), which manage inflammation and infection, respectively. For moderate to severe Crohn's disease, as well as in patients who become refractory to the first-line drugs, TNF inhibitors such as infliximab (Remicade®) or adalimumab (Humira®) have proven effective alone and in combination with azathioprine/mercaptopurine. Because the cause of Crohn's disease is unknown and there is no cure, it remains a fertile area for pharmacologic research. Crohn's disease now affects about 500,000 people in the United States, and two of the top three best-selling drugs in the world in 2013 were anti-inflammatory drugs (adalimumab and infliximab) used to treat Crohn's disease.

### **In Search of Bad Breath**

The elucidation of Crohn's disease established Crohn as a premier gastroenterologist, and he was in great demand as a speaker. He was flattered when a dental association invited him to speak at a meeting in New York in 1941, but he struggled to find a topic in gastroenterology that would be of interest to dentists. Finally, he settled on the perfect subject: bad breath.

Until the late 1930s, halitosis was attributed primarily to decaying teeth, necrotic abscesses of the pharynx, infected tonsils, obstructed nasal passages, nasal deviations, and periodontal disease. "Even before serious consideration, these explanations did not make sense to me" (1). Improvements in oral hygiene, which minimized the role of teeth, gums, and the pharynx in persistent halitosis, reinforced his skepticism. He also noted a publication by Marion Blankenhorn who had studied a patient with a complete stenosis of the esophagus following laryngeal cancer surgery. Blankenhorn inserted garlic directly into the patient's stomach via a gastrostomy tube, and the patient developed a distinctive garlicky breath (5).

To prepare for his dental conference lecture, Crohn decided to conduct his own research and obtained a small grant from a toothpaste manufacturer to follow up on Blankenhorn's observations. With no previous experience, Crohn first had to master how to classify and quantify odors. He adapted a sniff test devised by scientists at the Massachusetts Institute of Technology for assessing industrial smells and classified breath according to type (sweet, acrid, pungent, and repulsive/nauseating) and intensity (from very faint to overpowering).

In his first experiments, Crohn asked test subjects to chew onions or garlic-loaded salami without swallowing. The odor remained on their breath for only a short time. Next, he intubated a willing subject and placed a solution of garlic or onions directly in the stomach. The subject passed the sniff test during and shortly after intubation, but a few hours later, his breath was overpowering. Like Blankenhorn, Crohn concluded that mouth exposure is not responsible for bad breath. Rather, food must pass through the intestinal tract, be absorbed into the bloodstream, and undergo metabolism in the liver. Those smelly metabolites eventually reach the lungs and are expired.

To further test this hypothesis, Crohn studied two subjects who were patients in his wards. One patient was recovering from a colostomy to treat ulcerative colitis. Crohn inserted a capsule of garlic into the patient's stoma and within hours his garlic breath was obvious to the nurses on the ward. The other patient had undergone gall bladder surgery, and a drainage tube had been inserted into his bile tract. When Crohn gave the patient garlic either orally or rectally, he could detect the distinct odor of garlic in the bile



drainage the next day. Later, the odor also appeared on the patient's breath.

Crohn repeated these experiments with whiskey—deciding it was time to jump in and serve as his own test subject. After rinsing his mouth or gargling with Scotch, Crohn noted the odor of whiskey faded within ten minutes. In his next test, he downed six shots of Scotch at bedtime. His friends did not need to be sniff test experts to whiff his whiskey breath the next morning.

The results from all of these experiments supported his view that breath odor was not produced locally in the mouth or pharynx, but rather from metabolism and subsequent respiratory excretion. He suspected that fats or fatty substances were the odiferous substances, but he was not equipped to isolate them. (Investigators subsequently identified the garlic metabolites as organosulfur compounds.) Nevertheless, when his findings were published, Crohn became an overnight expert on halitosis (6). Patients flooded to his office wanting relief from real or suspected bad breath.

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***Steve willingly returned to the clinic again and again to donate additional samples of his blood. Same result. He became known as “the man who can’t catch AIDS”***

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Because he knew the alimentary canal from top to bottom, Crohn was appointed to a panel of the American Medical Association to standardize the names of diseases of the gastrointestinal tract. In 1961, he chaired the committee of the American Gastroenterological Association that authorized and coded names of diseases of the abdominal digestive organs. Those panels established nomenclature that has now become standard at American institutions, but during the discussions, Crohn repeatedly stated his reluctance to incorporating his name into the lexicon. At an international conference in Prague, he rose to voice his objection to a resolution officially designating ileitis as Crohn's disease. He was ruled out of order, and the resolution was adopted unanimously.

### **The Other Crohn, the Other Disease**

Sixty years after Burrill Crohn published his observations of the disease that bears his name,

another member of the Crohn family made medical history. Stephen Crohn, the grandson of Burrill's brother, was increasingly puzzled why he remained healthy, while many of those around him died. In 1982, his business partner and lover, Jerry Green, died from a syndrome that had just been formally described by physicians and would later be coined AIDS. Over the next decade, Steve saw dozens of his friends become infected with HIV, develop the same symptoms, and die. In a life measured by funerals and memorials, he naturally worried that he had also become infected and frequently sought HIV testing. The results always came back negative and he remained healthy, but he constantly worried that the tests might be wrong.

Credit: The Crohn Family



*Stephen Crohn*

Intelligent and well spoken, Steve was a social activist. He marched with Martin Luther King from Selma to Montgomery and protested the Vietnam War. He found solace in Buddhism (7). His pharmacology expertise—like many counterculture baby boomers—was limited to recreational drugs.

The focal point of his life had always been the fine arts. He trained at New York's Cooper Union, the

Art Students League of New York, and City College of New York and became a talented painter and sculptor. He supported his passion for art through jobs in copyediting, magazine production, and interior design. His longest affiliation was as a proofreader for Fodor's travel guides (7, 8). In the early 1980s, he operated a restaurant with Green in Los Angeles, but for most of his adult life, he lived in Hell's Kitchen, a rough-and-tumble working-class neighborhood on Manhattan's West Side. Naturally gregarious and fun loving, Steve maintained close relationships with his family. He remembered their birthdays, cheered them when they were sick, and joined them at the beach on summer holidays. He was the favorite uncle to his sisters' children (2).

As more and more of his friends were struck down, though, Steve became increasingly conscious of every ache and pain. Could this be an early sign of AIDS? He walked faster than everyone else—so much to do, so little time (2). An advocate of self-help, he boldly faced his fears, first through support and grief groups, and then, after earning a master's degree in social work from New York University in 1992, as a counselor to caregivers and AIDS patients. Still, against all odds, he remained healthy. He wanted to know why.

### The Magic Missing Molecule

The Crohn family gathered every five years on Burrill's birthday (2). Burrill's parents had instilled the importance of education and civic duty into their 12 children. A sizeable number of their descendants pursued careers in law or, like Burrill, in medicine. Steve consulted his medically oriented relatives, and they confirmed that he embodied an interesting case. Encouraged by them, Steve persisted—for years—telling anyone who would listen that he must have some sort of natural immunity to HIV. "Why won't anybody study me?" he complained in frustration to his sister (2).

Meanwhile, Bill Paxton, a British virologist, had arrived in New York to conduct postdoctoral research at the Aaron Diamond AIDS Research Center. Looking around the center for a research project, Paxton noticed that no one was studying people who were highly exposed to HIV but had not become infected. In 1994, he began contacting AIDS activists and asking for referrals of subjects who fit that profile. Within a week, a doctor associated with the AIDS community called and told him, "I have the perfect person" (9).

Steve was the first subject to walk through Paxton's door, and they clicked instantly. Like Burrill, Steve had no qualms about being an experimental subject. He desperately wanted to help others and relished the opportunity to become part of his family's medical legacy. Paxton was impressed that although Steve had no formal research training, "he just had this empathy for science. He understood it" (9).

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***The most satisfying thing to Paxton was that he could tell Steve, "You were right. You have this molecule missing. That is advancing science"***

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Paxton exposed samples of Steve's blood to HIV—thousands of times higher than the titer normally needed for infection—but Steve's lymphocytes remained virus-free. Suspecting a laboratory error, Paxton refined and repeated his tests. Steve willingly returned to the clinic again and again to donate additional samples of his blood. Same result. He became known as "the man who can't catch AIDS" (10).

Scientists knew that T-helper lymphocytes (CD4 cells) carried a surface chemokine receptor, CCR5, that mediates HIV binding and entry into the cell. Paxton discovered that the gene that codes for



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## Comparison of Crohn's Disease Versus Ulcerative Colitis

	Crohn's Disease	Ulcerative Colitis
terminal ileum involvement	common	seldom
colon involvement	usually	always
rectum involvement	seldom	common
fever	common	in severe disease
abdominal pain	always	occasional
abdominal tenderness	always	occasional
rectal bleeding	rare	always
abdominal mass	common	never
obstruction/stricture	common	rare
fistulas	common	rare
inflammation	patchy, discrete ulcerations separated by segments of normal mucosa	continuous and diffuse
granulomas	common	never

the CCR5 receptor protein on Steve's CD4 cells was missing 32 base pairs (now called  $\Delta 32$  CCR5). The deletion did not affect his health, but the deformed receptor prevented the virus from binding (11).

Paxton soon found 12 additional people with the same defect, but Steve, with his outgoing personality, intellect, and keen sense of humor, was the most articulate and highly sought spokesperson. After the extreme sadness that he had experienced in losing so many loved ones, "he was proud of the miraculous gift of being able to provide the key to unlocking a mystery of AIDS" (2). He personified and affirmed the hope that AIDS could be conquered.

The most satisfying thing to Paxton was that he could tell Steve, "You were right. You have this molecule missing. That is advancing science" (9). The revelation about  $\Delta 32$  CCR5 triggered a flood of clinical investigations worldwide (12). Approximately 1% of the population in North America and Europe, like Steve, have the homozygous  $\Delta 32$  CCR5 mutation and are resistant to HIV infection.

In 2006, clinicians in Germany transplanted stem cells with the  $\Delta 32$  CCR5 mutation into Timothy Brown, a patient who was suffering from acute myeloid leukemia and was HIV-positive. The transplant prevented his leukemia from recurring, and the  $\Delta 32$  CCR5 mutated cells cured his HIV infection (13). The successful treatment of Brown (the "Berlin patient," the only documented case of an AIDS cure) opened new avenues for HIV/AIDS research and provided new evidence that AIDS could be cured. In 2007, the Food and Drug Administration approved maraviroc, the first CCR5 receptor antagonist, for the treatment of AIDS.

Watching these events unfold, Steve was thrilled: "Can you believe that this has happened (2)?" It was similar to the reaction of his great-uncle Burrill, who also watched in amazement as the intestinal disorder he first characterized became formally recognized and incorporated into the standard compendium of medical diseases. Each, in his own way, was extraordinary, a larger-than-life force of nature who contributed to breakthrough discoveries that have

significantly advanced medicine and improved the lives of millions of patients. But each was also a very ordinary man who enjoyed the simple pleasures in

life, performed simple acts of kindness as a matter of course, and above all else was devoted to his family, the Crohn family.

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# The Great Dogsled Relay:

## The Race against Diphtheria

Rebecca J. Anderson



Carrie M. McLain Memorial Museum, Nome, Alaska

Dr. Curtis Welch with two of his nurses.

Nome, Alaska. January 20, 1925. While Curtis Welch was making his rounds at Maynard Columbus Hospital, he realized that he had a serious problem on his hands (1). Nine days earlier, he had admitted 6-year-old Billy Barnett, who had a sore throat, swollen glands, and a fever. Welch treated Billy for tonsillitis, a common ailment in children, but then a thick grayish film began to form in Billy's throat and nasal cavity – a troubling complication.

The preceding October, Dr. Welch had treated a 2-year-old Eskimo boy for tonsillitis, but by the next morning the boy had died (2). When 7-year-old Margaret Eide came down with a severe sore throat and slight fever on Christmas Eve, Welch again suspected tonsillitis, but like the little Eskimo boy, Margaret's condition deteriorated and she died 4 days later. As the winter progressed, Dr. Welch, who had been practicing medicine in Alaska for 18 years, noted an unusually high incidence of tonsillitis and inflamed throats among children in the community. The unexpected deaths and, especially, Billy's symptoms, pointed to something much more serious than tonsillitis: diphtheria.



## Arctic Circle

Diphtheria is caused by an aerobic gram-positive bacillus, *Corynebacterium diphtheriae*. In the early stages, the patient's sore throat and low-grade fever can be easily confused with tonsillitis or a bad cold. The unambiguous sign of diphtheria is formation of a thick grayish-white membranous film over the tonsils, pharynx, or larynx, accompanied by inflammation and swelling of the neck called a "bull-neck" (3, 4). Diphtheria toxin (produced by the diseased membrane) circulates systemically, causing damage to the heart, kidneys, and nervous system. As the membrane spreads and the throat swells, the airway becomes obstructed, leading to death by suffocation (3, 5).

*C. diphtheriae* is highly contagious, spread by respiratory droplets through the air and direct contact with contaminated surfaces (3). With a 2–5 day incubation period, many people unwittingly can infect others before they show any symptoms (5). In the United States in the 1920s, more than 100,000 cases were reported each year, and it was a leading cause of death in young children, in whom the death rate was about 20 percent (5).

The native Alaskan population had less natural immunity and was particularly vulnerable to infectious diseases. Only 6 years earlier, the great influenza pandemic of 1919 had killed half of Nome's native residents (1). A diphtheria outbreak was cause for grave concern.

Diphtheria antitoxin, which had been introduced in the United States in 1891, was the standard treatment.



A vintage 1895 vial of the diphtheria antitoxin made from horse's blood.

Horses were inoculated with the *C. diphtheriae* toxin, stimulating production of specific antibodies, or antitoxin. The horses' blood was then harvested and processed to produce the antibody-containing serum (4, 6). Antitoxin will not neutralize toxin that is already fixed to tissues, but it will neutralize circulating (unbound) toxin and prevent progression of the disease (5). Patients, therefore, needed to be treated as soon as possible after a suspected diphtheria infection.

## Initial Steps

Dr. Welch had received a limited supply of diphtheria antitoxin in 1919, but it was now 5 years beyond its expiration date. He felt unjustified in using it on Billy because he "had no idea what effect it might have" (2). Instead, he employed old-fashioned remedies that doctors had used to treat diphtheria before development of the antitoxin: stimulants to strengthen Billy's heart and swabbing his throat with an astringent, ferric chloride, to break up the lesions. It worked for a while. The grayish membranes sloughed off, color returned to Billy's cheeks, and he slept more comfortably. By the afternoon of January 20, though, Billy's condition worsened. Each time he tried to draw air into his lungs, he coughed up blood. At 4:00 PM, Billy was turning blue from lack of oxygen and his breathing was labored. There was nothing else Welch could do; at this point even fresh serum could not save him. Billy died at 6:00 PM (1, 2).

The next morning, Welch made a house call to see 7-year-old Bessie Stanley in the nearby Eskimo village. She was heaving for air, and a massive membrane inside her mouth bled profusely at the touch. Whatever doubts Welch might have had about the previous cases, Bessie's symptoms conclusively pointed to diphtheria (2).

Dr. Welch gave Bessie 6,000 units of the old serum because without it, she surely would die. Unfortunately, her infection was too advanced, and she died within 48 hours. Bessie's parents and two sisters had also developed membranes in their throats, and Welch treated them aggressively with the old serum. Their throats cleared. Equally impressive, Welch noted that Bessie and her family experienced no untoward effects from the injections, raising his confidence that the antiquated serum had not degraded (2).

Immediately after Billy's and Bessie's deaths, Welch notified Nome's mayor, George Maynard. Welch expected new diphtheria cases within 24 hours, but he had only 80,000 units of serum – enough for about 6 patients. He had placed an order for new supplies the previous autumn, but the shipment had not arrived before Nome's seaport closed on November 1. No other ships would arrive until the spring thaw. To fight the impending epidemic properly, he told Maynard that he needed 1 million units of fresh antitoxin as soon as possible.



Dr. Welch telegraphed every major town and official in Alaska and made an urgent request for serum. As the territory's assistant commissioner of health, he also telegraphed the US Public Health Service in Washington, DC, which regulated production of antitoxins.

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***Welch and Morgan knew that at the current infection rate, their stock of serum would not last through the week, and without a new supply, many would die.***

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In Nome, Welch recommended that the town officials create a temporary Board of Health to deal with the crisis. The principal board members were Dr. Welch, Mayor Maynard, and Mark Summers. Summers was superintendent of Hammon Consolidated Gold Fields, a conglomerate mining company that dominated Nome's economy. To control the spread of infection, the new Board of Health immediately shut down every public building in Nome, banned all public gatherings, and discouraged travel along the region's trails (1, 2).

### **Doing More with Less**

Curtis Welch was the only doctor for hundreds of miles, but he was fortunate to have a skilled nursing staff. Among them, Emily Morgan was the most efficient and outstanding. She had served in the Army Reserve Nurses Corps for three years on the Western Front. After World War I, she returned from France to Wichita, KS, and worked as the city's first public health nurse. While in Wichita, she had contracted diphtheria and spent three weeks recovering in bed. In 1923, she inquired about missionary work, and the American Red Cross sent her to Nome to care for the native community as a public health nurse. Because Nurse Morgan had firsthand knowledge of diphtheria and her body had developed immunity, Welch and the Board of Health appointed her quarantine nurse (1, 7).

While Dr. Welch supervised medical care at the hospital, Nurse Morgan made house calls looking for people with diphtheria symptoms and reported suspicious cases to the Board of Health (2, 7). When

she discovered patients showing membranes, she posted a red and black sign, quarantining everyone in the household. She treated the most severe cases in the native villages, often working alone, and always carrying her medical bag with its precious tubes of the old antitoxin.

Late on Saturday evening, January 24, the fourth day of the crisis, Welch and Morgan summarized the situation for the Board of Health: The death toll stood at 4, there were about 20 confirmed cases, and at least 50 other people were at risk. Welch and Morgan knew that at the current infection rate, their stock of serum would not last through the week, and without a new supply, many would die.

Welch had still received no word of any available serum from Fairbanks, Anchorage, Juneau, or Washington, DC. The Board of Health could not do anything more to locate fresh serum, but they could help solve another problem: how to get the serum to Nome once it was located. Mark Summers, the superintendent of Hammon Consolidated, proposed an express delivery by dogsled.

During the winter months when the frozen Bering Sea made shipping impossible, Nome depended exclusively on dogsled teams for freight and mail deliveries. The dogsled trails connected Nome to the major settlements on Alaska's southern coast and to the east. A series of trading and military posts, roadhouses, and postal stations dotted the trails to service the sled teams, telegraph offices, and mail deliveries to settlers and miners.

Summers proposed using two fast dogsled teams, one starting from the railhead at Nenana (near Fairbanks) headed west with the serum, and the other departing from Nome headed east. They would meet halfway on the trail at Nulato for the hand-off, and the second team would return to Nome with the serum. Normally, it took about 25 days for mail teams to travel the 674 miles between Nenana and Nome. Summers's plan could shave precious days off of that time.

To handle the western, roundtrip portion of this ambitious plan, Summers's obvious choice was Leonhard Seppala, a sled driver who often ferried Hammon Consolidated employees across Alaska. The scrappy Norwegian outdoorsman was undoubtedly the fastest and strongest musher in Alaska. He and his lead dog, Togo, who was as famous as Seppala, knew the trails and had shattered a number of long-

distance records for speed and endurance. Seppala at 47 and Togo at 12 were both older than the typical sled team, but Seppala was a rare natural athlete of unusual strength and endurance, and Togo was surprisingly fast, strong, and alert. After the Board of Health meeting on that Saturday evening, Summers alerted Seppala, and he began preparations for the run of his life.

### The Rescue Begins

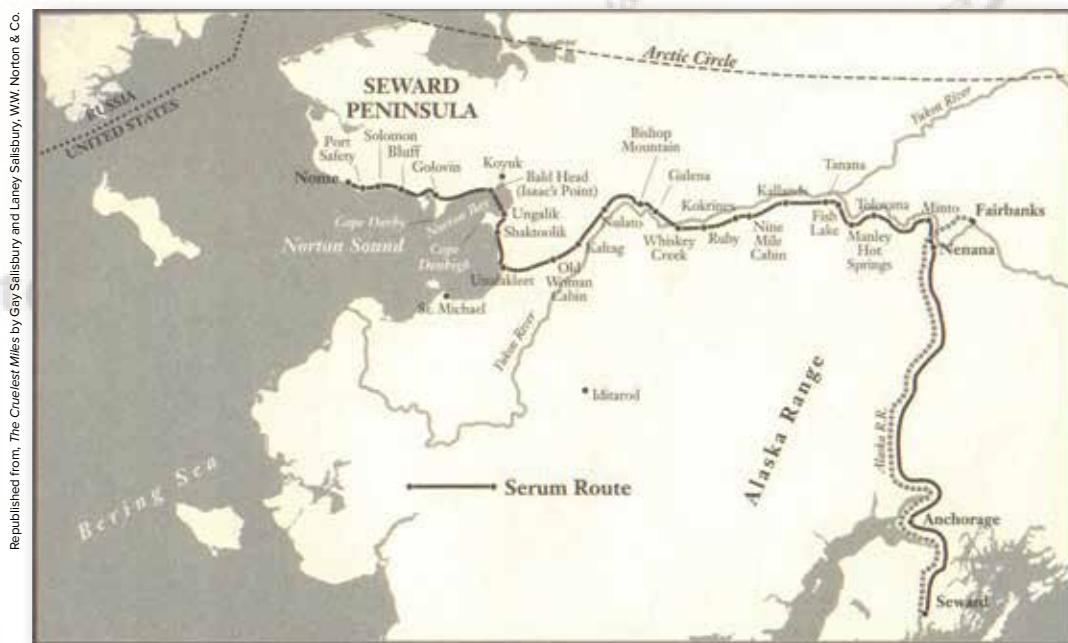
Meanwhile, as word spread about Welch's plea for serum, people responded throughout the United States, as well as at the Public Health Service territorial outposts in Alaska. On Monday, January 26, John Beeson, chief surgeon of the Anchorage Railroad Hospital, notified territorial Gov. Scott Bone that he had located 300,000 units of antitoxin. The decision about how to get the serum to Nome rested with Bone, and the first leg of the journey was obvious: Bone directed Dr. Beeson to prepare the serum at once and send it north to Nenana by train. That railroad line had been completed just two years earlier.

Beeson dutifully packed up the amber-colored glass vials of serum. As a territorial doctor, he knew firsthand the unbearable cold and jolting of dogsled trips. To protect the vials, he padded the inside of the container and then wrapped it with a heavy quilt. He placed the container in a wooden crate, covering it with thick brown cloth and pinned a note to the cloth

instructing the drivers to warm the container for 15 minutes at each stop along the trail.

Dr. Beeson carried the 20-pound package of serum to the railway station in Anchorage where the train's conductor, Frank Knight, was waiting. Beeson then notified Gov. Bone that the serum was on its way and would arrive in Nenana the following night, Tuesday, January 27. Nenana, the last stop before Fairbanks, had become a major hub connecting the rail line to dogsled trails for distribution of goods and passengers to Alaska's interior.

While the train chugged northward with the serum, Gov. Bone considered his choices for the remaining transport to Nome: traditional dogsleds versus a risky but faster airplane delivery. In Fairbanks, the terminus of the rail line, a small group of enthusiastic pilots were anxious to do their bit. But this was two years before Charles Lindbergh's historic flight across the Atlantic, and the pilots' enthusiasm far surpassed the capabilities of their aircraft. Alaska was in the midst of its worst winter in 20 years. Temperatures fell to -70 degrees, and their airplane engines had no antifreeze. Snowstorms brought shattering gusts that reached 75 mph. No pilot was hearty enough to survive in the open cockpits under those conditions, even if they could fly through uncharted mountains during the winter, with only 4 hours of daylight. And, Nome had no landing strip.



Republished from, *The Cruelest Miles* by Gay Sallsbury and Loney Sallsbury, WW, Norton & Co.

Map of the 1925 diphtheria serum dogsled relay route from Nenana to Nome.



Late on the afternoon of January 26, as the train traveled the 300 miles north to Nenana, Gov. Bone made his decision. The serum would be delivered to Nome on dogsleds. But instead of sending one team to meet Leonhard Seppala midway on the trail, as Mark Summers had proposed, Bone thought the serum would move faster if a series of fresh drivers traveled shorter distances and handed off the package in a relay. Bone contacted officials in Nenana and told them to engage relay dog teams to carry the antitoxin westward. The teams were to travel night and day with no rest, no matter how bad the conditions, until they met up with Seppala at the halfway mark. The territorial government would bear the expense. When the call went out, men all along the trail responded, reported to their posts, and stood by for their leg of the relay.

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***The teams were to travel night and day with no rest, no matter how bad the conditions, until they met up with Seppala at the halfway mark. The territorial government would bear the expense.***

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At 9:00 PM on Tuesday, January 27, Conductor Knight jumped from the moving train onto the platform at Nenana and handed the serum package to “Wild Bill” Shannon. Shannon, a mail driver with the fastest dog team in eastern Alaska, was charged with carrying the serum on the first leg of the relay. That 52-mile stretch from Nenana to Tolovana normally took 2 days, but Shannon was told to cover the route without stopping.

Dogsledders followed the “Rule of 40s,” an 80-degree window of optimal sledding temperatures. Above 40 degrees, a husky can overheat and suffer dehydration. Temperatures below -40 degrees are too hazardous for both the driver and the dogs. When Shannon released the sled’s brake and set out, it was -50 degrees.

Through the night, the temperature continued to drop. After 4–5 hours Shannon’s face grew numb and one big toe had frozen. He found it harder and harder to keep warm. To generate heat, he jogged in front of the lead dog, with the dog team following

behind. It worked for a while, but he slowly developed hypothermia. When Shannon reached the roadhouse at Minto at 3:00 AM, the temperature was -62 degrees, and parts of his face had turned black from frostbite.

Shannon spent 4 hours in Minto recuperating and resting his dogs. As Dr. Beeson had instructed, he took the serum package inside, unwrapped the layer of fur and canvas, and dangled the container from the rafters to absorb the warmth of the stove. Three of his dogs were too weak to continue and Shannon left them in Minto. At 7:00 AM on Wednesday morning, he hitched his remaining 6 dogs and headed for Tolovana, 22 miles away.

That same morning in Nome, Mark Summers telephoned Leonhard Seppala and told his Hammon Consolidated employee that it was time to begin his trek eastward. Seppala had the longest assignment: 315 miles to Nulato and 315 miles back to Nome over some of the most difficult terrain of the entire route. At midmorning, Togo led Seppala and a team of 20 dogs down Nome’s Front Street and out onto the beach trail. Nome residents were accustomed to seeing dogsled teams, but this was a rare sight. Their lives depended on Seppala successfully completing his journey.

At 11:00 AM Wednesday morning, Shannon arrived in Tolovana. His face was still creased and black from frostbite, and his dogs were exhausted. After allowing the serum to warm up in the roadhouse, Edgar Kallands began the next leg of the relay. Five hours later, he arrived in Manley Hot Springs. The temperature was -56 degrees and the roadhouse owner had to pour boiling water on the sled’s birchwood handlebar to pry loose Kallands’s gloves and frozen hands.

Over the next 2 days, the names of Dan Green, Johnny Folger, Sam Joseph, Titus Nickolai, Dave Corning, Harry Pitka, Bill McCarty, and the brothers Edgar and George Nollner crackled across the telegraph wires. Their progress generated newspaper headlines in the lower 48 states, and anxious listeners monitored radio news flashes as the sledders navigated the relay trail, which meandered westward along the Tanana and Yukon rivers.

### **A Greater Urgency**

In Nome, Dr. Welch, Nurse Morgan, and Mayor Maynard were also intently monitoring the sled teams’ progress. Six days after Billy Barnett died, his 5-year-old sister, Katherine, developed symptoms.

She received 15,000 units of the old antitoxin over several days, and the membrane in her throat slowly disappeared (2). The Stanley family's neighbor, Minnie Englestad, also became ill and received 2,000 units. Between Wednesday evening and Thursday morning, two more children in Nome came down with diphtheria. Several others were complaining of sore throats. Welch's 6-year-old antitoxin dwindled to 21,000 units, and he began to prioritize who would get the medicine (1).

Monitoring these events at the territorial headquarters, Gov. Bone worried about an uncontrolled epidemic that might spread diphtheria throughout Alaska. Beeson's 300,000 units of new serum had reached Ruby, but it was still 400 miles from Nome. Bone needed to get it there faster. He asked Mark Summers, Seppala's boss, to deploy sled teams along the western half of the route to relieve Seppala. Although Seppala and his team were the best in Alaska, a series of fresh teams could cover the distance faster.

Summers dispatched Ed Rohn to Port Safety, 21 miles east of Nome, and Gunnar Kaasen, another Hammon dog driver, to the mining village of Bluff, about 30 miles east of Port Safety. When Kaasen reached Bluff, he told the roadhouse keeper, Charles Olson, to hitch up his rig and drive 25 miles further east to the trading post at Golovin and wait there for the serum.

To reach further to the east – beyond Nome's telephone lines – Summers used US Signal Corps wireless operators to contact the storekeeper at Unalakleet and told him to "spare no expense" in deploying more teams along the trail (1). One was posted at Unalakleet and another 38 miles up the coast at Shaktoolik.

Summers had no way to reach Seppala, who was out somewhere on the trail. He instructed all of the new drivers to keep a sharp lookout for Seppala and his team of Siberian huskies. If they met him, they were to stop him, hand the serum over to him, and tell him to turn westward and link up with the fresh teams that were now waiting along the trail. Summers calculated that the most likely place for the handoff to Seppala would be somewhere near Shaktoolik.

### **Battling a Blizzard**

Early on Friday morning, January 30, the serum was warming near a roadhouse stove in Bishop Mountain.

Charlie Evans waited nearly an hour before starting his leg of the relay, worried that the deepening cold would freeze the precious medicine. At 4:30 AM, Evans finally set off under the green and white lights of the aurora borealis. The temperature was -62 degrees. After only 10 miles, the dogsled team encountered a thick layer of ice fog that rose to Evans's waist and swallowed the dogs and sled. Unable to see the trail, Evans put his trust in his dogs.

They navigated well on their own, but after 20 miles, his two lead dogs began tiring. Evans had not protected the dogs' groins with rabbit-fur pelts, and their legs were burned raw by the cold where the harnesses chafed their fur and skin. The two lead dogs had severe frostbite, and both were crippled. Evans put them into the basket of the sled and strapped the harness over his shoulder. For the last 10 miles, he led the remaining dogs and together they pulled the sled to Nulato.

Evans reached Nulato at 10:00 AM on January 30. He carried the lead dogs into the cabin and slumped by the stove. Both dogs were dead. The serum had now been on the trail for 3 days and had traveled 356 miles from the railhead. After a half hour in Nulato, the serum was again on the trail. Tommy Patsy, a driver named Jackscrew, and Victor Anagick took turns carrying the package from the banks of the Yukon River, over the 4,000-foot Nulato Mountains, to the trading store in Unalakleet on the Bering Sea coast. There, the serum was again warmed by the heat of another cast-iron stove. Outside, the weather was growing worse. A vicious storm was approaching.

By Saturday, January 31, another death in Nome brought the death toll to 5 since the outbreak 11 days earlier. Three more children came down with diphtheria on Saturday, pushing the caseload to 27. Dr. Welch and Nurse Morgan were also monitoring 30 suspected cases, and at least 80 people had come in contact with the diphtheria patients. But now, they were out of antitoxin.

At 5:00 AM that Saturday morning, Myles Gonangnan packed up the serum that Victor Anagick had delivered to the Unalakleet trading post. On the opposite shore of Norton Sound, Leonhard Seppala, still unaware that the relay plan had changed, made the risky decision to take a shortcut across the frozen Sound. The approaching storm blew strong northeast winds that threatened to break up the sea ice. Under these conditions, the shortcut was dangerous but



it would save him a day's travel, versus the safer but longer shoreline route. Both Gonangnan and Seppala, from opposite directions, were headed toward Shaktoolik.

For Gonangnan, the wind and massive snow drifts made progress difficult. He put on his snowshoes and tamped down the snow on the trail to give his dogs better traction, but it slowed their pace to a crawl. In 5 hours, they had managed to move only 12 miles. With the wind blowing harder by the hour, Gonangnan stopped at an abandoned hut to make a small fire and warm the serum. Fifteen minutes later, the team headed back on the trail and began the exhausting climb up to the 1,000-foot summit of Blueberry Hills. With drifts that came up to the dogs' bellies, the climb required every ounce of their energy. But Gonangnan's 8-dog team was powerful, sure-footed, and they knew the trail.

By 11:00 AM, they were battling gale-force head winds and a wind chill of -70 degrees. When they finally reached Shaktoolik at 3:00 PM, there was no sign of Seppala. It was possible he had been delayed, or that he had already passed Shaktoolik without resting. Gonangnan had not seen him on the trail, but in those whiteout conditions they could easily have missed each other.

Fortunately, Henry Ivanoff, a Russian Eskimo, had been stationed at Shaktoolik to handle the next leg of the relay. While they waited for the serum to warm, Gonangnan briefed Ivanoff on the weather conditions, which were deteriorating rapidly. Ivanoff might not have been as experienced as the other drivers, but he was determined to do his best.

Meanwhile, Leonhard Seppala and Togo had successfully crossed the iced-over Norton Sound and passed the fishing camp at Ungalik. The strong winds of the brewing storm were blowing at their backs, and they were speeding toward Shaktoolik. Nearing the Shaktoolik roadhouse, Seppala saw something through the blinding snow. As he got closer, he realized it was another dogsled team, but it wasn't moving. A reindeer had wandered onto the trail and Ivanoff's high-spirited dogs had snarled their lines as they chased playfully after it. When he saw Seppala, Ivanoff ran toward him, frantically waving his arms. Seppala had no intention of stopping. He could not afford any delays.

A determined Ivanoff continued shouting above the whistling wind, "The serum! I have it here (1)!"

When Seppala finally made out the words, he slammed on the sled brake. It took a while for him to stop his 20 dogs, turn them into the wind, and return to Ivanoff.

In the past 3 days, Seppala and Togo had traveled 170 miles. Now, Ivanoff briefed him on the change in plans and the worsening epidemic in Nome. Seppala's new orders were to carry the serum back across Norton Sound and on to the roadhouse at Golovin, where Charlie Olson was waiting. Seppala and Togo headed north and quickly covered the 23 miles to the Ungalik fishing camp.

### A Most Dire Dilemma

In Nome, Nurse Morgan was also struggling against the storm. She knew how to work in the crudest environments, but this winter was particularly tough. She continued visiting patients in the native villages until her vision was obscured by the blowing snow and the cold became unbearable.

As the extraordinary blizzard roared up the coast, Dr. Welch monitored the deteriorating weather with increasing concern. He faced a heart-wrenching choice: the need for serum versus the possibility that the shipment would be lost as the heroic drivers fought the storm. He called a meeting of the Board of Health and recommended stopping the relay; the loss of a few hours or even a few days was not as important as the safety of the serum. The Board agreed, but no one knew exactly where Dr. Beeson's package was.

Mayor Maynard phoned the roadhouse keeper in Solomon, the furthest that Nome's telephone lines reached, and told him to intercept the dogsled driver, Gunnar Kaasen, when he arrived from Bluff. Kaasen and the serum were to remain in Solomon until the storm passed.

Maynard also called Port Safety and alerted Ed Rohn, the last designated driver in the relay. Rohn unhooked his dogs, fed them, and put away his sled. Returning to his cabin, he telephoned Nome to report that the wind was blowing at 80 mph. Then, he heard the line crackle. The phone went dead. Nome had now lost its last connection with the drivers, and Mayor Maynard could only hope that his instructions had reached down the trail to Kaasen.

Late on the afternoon of Saturday, January 31, Seppala and Togo set out from Ungalik to cross Norton Sound. It was dark, and they were facing gale-

force winds with a wind chill of -85 degrees. Unfazed by the deafening wind, Togo held his head low and his body level in deep concentration. Despite the mounds of snow and slippery patches of ice covering the Sound, he carved a straight course, and they reached Isaac's Point on the opposite bank at 8:00 PM. Hours later, the storm churned the frozen Sound into an impassable icy soup. Togo and his mates had traveled 84 miles that day, half against the wind, and they needed to eat and rest before battling the wind through the next 50 miles to Golovin.

Seppala unlashed the serum package, and inside the roadhouse, he opened the fur and canvas wrappings down to the paper cartons. He was certain that the serum inside had frozen, but he placed the cartons as close to the stove as he dared.

At 2:00 AM on Sunday, February 1, the fifth day of the relay, Seppala wrapped the serum package inside his sleeping bag, covered it with sealskin, and tied it into his sled with a blanket and then covered it with more animal skins. The storm, which had been marching up the coast for 2 days, had arrived with winds of 65 mph. Seppala and Togo took the safest path, hugging the coastline and zigzagging as sea ice heaved and crashed around them – the blizzard obscuring their vision. For the last 8 miles, they turned inland, climbing 5,000 feet over a series of ridges. Having traveled for more than 4 days and with little rest, the dogs strained up the final ascent, but they did not stop. Then, they raced down the last stretch to the roadhouse in Golovin, 13 hours after leaving Isaac's Point.

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***He faced a heart-wrenching choice: the need for serum versus the possibility that the shipment would be lost as the heroic drivers fought the storm.***

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Altogether since leaving Nome, Seppala and Togo had traveled 261 miles; from picking up the serum from Ivanoff to the handoff to Charlie Olson in Golovin, they had traveled nearly double the distance covered by any of the other sled teams. They were still as fit as the day they left Nome, just a little tired.

Mayor Maynard's instructions to wait out the storm had not reached Golovin. An hour after Seppala's arrival and with a wind chill of -70 degrees, Charlie

Olson packed the serum and left for Bluff. The wind repeatedly blew Olson and his dogs off the trail, and not long after leaving Golovin, a hurricane-force gust hurtled them into a huge snowdrift. In the dark, Olson dug his way out and untangled the dogs. Despite years of rugged experience in Alaska and his heavy parkas, he could not keep warm in the blizzard.

At 7:00 PM on February 1, with the storm still raging, Olson finally reached Bluff, his hands too numb from frostbite to unleash the serum. Although he had protected his dogs with rabbit fur blankets, they were stiffened by the cold and could not have gone much further. Gunnar Kaasen helped retrieve the serum and brought the exhausted team inside, all 7 dogs limping into the cabin. Olson's fingers were white and hard as stone as he briefed Kaasen. It had taken him 4.5 hours to travel the 25 miles from Golovin to Bluff.

With his dogs crumpled on the floor and his fingers burning with pain, Olson advised Kaasen to wait for the storm to pass. They had not received Mayor Maynard's directive, but this was not weather to be traveling in, no matter how badly Nome needed the serum.

### **Balto and the Blizzard**

Gunnar Kaasen had left Norway as a young man to seek his fortune in Alaska's gold fields, and in recent years, he worked alongside Leonhard Seppala at Hammon Consolidated. A plain-speaking, practical driver, Kaasen was a good judge of sledding conditions, and he wisely waited 2 hours in Bluff. But the wind did not subside; if anything, conditions were getting worse. At 10:00 PM on February 1, the snow was coming down fast and blowing at 70 mph, stronger than Kaasen ever remembered in his 24 years living in Nome. If he delayed any longer, the trail to Port Safety would be an impassable mass of snow drifts. His 13 dogs were well fed, rested, and ready to move, including his lead dog, Balto. The Siberian husky was less experienced and slower than many lead dogs, but he was steady and strong, and Kaasen placed great confidence in him.

Eschewing the Rule of 40s, Kaasen hooked up the dogs, packed the serum, and headed out. They faced a blizzard strength that few drivers had ever dared to tackle. After only 5 miles, they plowed into a massive drift that blocked the trail. Balto tried to run through it, but the dogs bogged down. The snow



came up to the six foot two Norwegian's chest, and he could not clear a path, either. Their only choice was to retreat and detour around the obstruction. The relatively inexperienced Balto could follow trails, but now Kaasen was asking him to scout a new trail in unfamiliar territory – in the pitch dark, in a blizzard.

Balto lumbered along the back edge of the drift, trying to find the faint scent of dogs that had pattered before him that winter. He kept his nose low to the ground, his ears flattened against his head to keep out the wind, and moved slowly. After they had skirted and passed beyond the edge of the drift, Balto was still searching. Minutes felt like hours. Then, suddenly, he lifted his head and broke into a run. They were back on the trail.

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***When he reached into the sled to make sure the serum was in place, it wasn't there. It had been thrown somewhere into the darkness.***

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Well after midnight, Kaasen's right cheek began to sting with frostbite. The wind blasted thick veils of snow, obscuring his vision. He could only guess his position and turned control of the team over to Balto. Kaasen just held on.

Several times, the hurricane-force gusts hurled the sled off the trail, dragging the dogs with it. Each time Kaasen had to take off his gloves, untangle the team, and right the sled. Ten miles from Port Safety, they were slammed by a gust in excess of 70 mph, burying Kaasen in a drift. He crawled back to the sled, righted it, and fumbled with the dogs' harnesses. When he reached into the sled to make sure the serum was in place, it wasn't there. It had been thrown somewhere into the darkness. Kaasen systematically searched the snow, crawling on his knees and with his bare hands. Finally, his right hand hit something hard, his precious cargo. He leashed it back into the sled, securing it with a few extra knots of rope.

*Statue of Balto, the lead dog on the last relay team, in Central Park, New York City. The statue is dedicated to all the dogs involved in the serum run.*

By Jim Henderson (own work) [CC0], via Wikimedia Commons

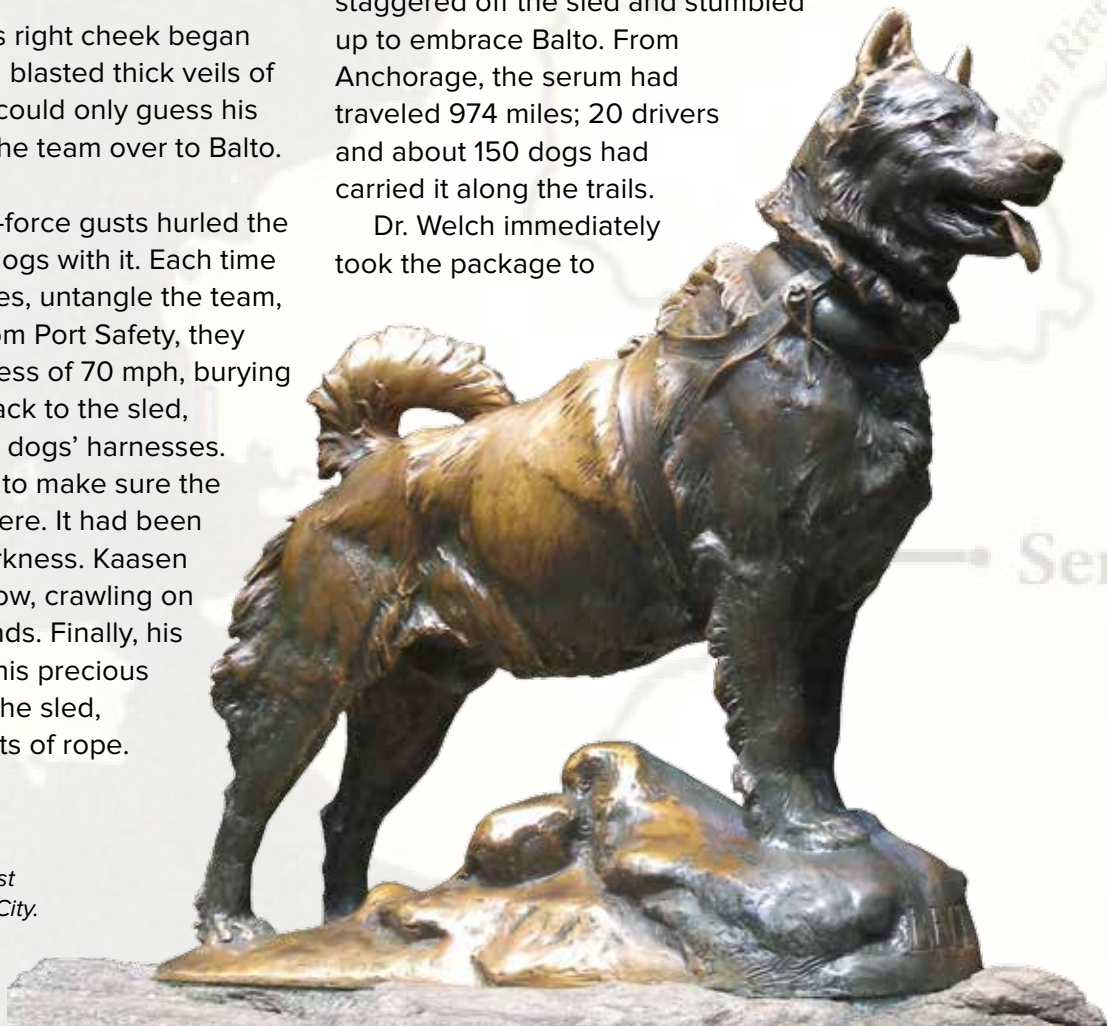
Kaasen and Balto reached Port Safety at 3:00 AM on Monday, February 2, but the roadhouse was dark. Ed Rohn had gone to sleep, assuming that Kaasen had stopped at Solomon to wait out the storm. Instead, Kaasen had missed the Solomon roadhouse, blinded by the blizzard, and proceeded straight to Port Safety. Kaasen considered waking Rohn, but harnessing and hitching his dogs would cause a further delay. The wind was easing and despite the cold, Balto and the team were still moving fast and strong. Kaasen decided to continue on to Nome.

The last 20 miles of the trail to Nome ran along the beach. The winds diminished, but the heavy snow drifts made travel slow and at times difficult. Kaasen's fingers ached from frostbite, and several of his dogs were now stiffening, but at least they could see the trail.

### **The Journey's End**

On Monday, February 2, at 5:30 AM, Balto turned onto Front Street in Nome. Kaasen stopped the team in front of the Miners & Merchants Bank. He staggered off the sled and stumbled up to embrace Balto. From Anchorage, the serum had traveled 974 miles; 20 drivers and about 150 dogs had carried it along the trails.

Dr. Welch immediately took the package to



the hospital and unwrapped the cartons. The serum was frozen solid. Fortunately, Dr. Beeson in Anchorage had allowed for expansion of the serum by using rubber and cork stoppers on each vial. Welch put them in a 46 degree room – warm by Alaska standards. By 9:00 AM, the serum had partially liquefied and not a single vial was broken. By 11:00 AM, the serum was clear and ready for use. Welch immediately treated the most severely ill patients in Nome. Nurse Morgan simultaneously headed to the Eskimo village to visit several quarantined families and injected each of them.

By early afternoon, more than 10% of the 300,000 units had been used up. By evening, several thousand more units of serum were gone. Many patients would receive a second round of injections.

Soon, news reached the world that the serum had arrived in Nome and seemed to be working. Some patients responded within hours. The repeated freezing and thawing had not affected the serum's efficacy, and on February 3 it looked as if even those who were seriously ill would recover.

A second dogsled shipment of antitoxin arrived in Nome on February 15, delivered by Ed Rohn in the middle of another blizzard. The second relay engaged many of the same drivers as the first and was also difficult, with heavy snowstorms impeding the drivers and dogs. Unrestricted use of the new serum rapidly ended the diphtheria threat, and on February 21, Dr. Welch lifted the quarantine (2).

### The Impact of the Nome Relay

A diphtheria vaccine had been introduced in the United States in the early 1920s, but it saw little use until the publicity surrounding the 1925 Nome epidemic galvanized public opinion about immunization. In the 1940s, the diphtheria vaccine was combined with tetanus toxoid and pertussis vaccine (DPT). Routine immunization of children with DPT dramatically reduced outbreaks of diphtheria. No cases have been reported in the United States since 2003 (3, 5).

In New York's Central Park, near the Children's Zoo, a statue of Balto was erected in December 1925. Generations of children have petted the head, rubbed behind the ears, and climbed on the back of the metallic Balto – its bronze surface polished down to a gold sheen by thousands of tiny hands that have never suffered from diphtheria.

### The Nome Serum Relay Participants

	Relay Leg	Distance (miles)	Driver
Jan 27	Nenana to Tolovana	52	Bill Shannon
Jan 28	Tolovana to Manley Hot Springs	31	Edgar Kallands
Jan 28	Manley Hot Springs to Fish Lake	28	Dan Green
Jan 28	Fish Lake to Tanana	26	Johnny Folger
Jan 29	Tanana to Kallands	34	Sam Joseph
Jan 29	Kallands to Nine Mile Cabin	24	Titus Nikolai
Jan 29	Nine Mile Cabin to Kokrines	30	Dave Corning
Jan 29	Kokrines to Ruby	30	Harry Pitka
Jan 29	Ruby to Whiskey Creek	28	Bill McCarty
Jan 29	Whiskey Creek to Galena	24	Edgar Nollner
Jan 30	Galena to Bishop Mountain	18	George Nollner
Jan 30	Bishop Mountain to Nulato	30	Charlie Evans
Jan 30	Nulato to Kaltag	36	Tommy Patsy
Jan 30	Kaltag to Old Woman Shelter	40	Jackscrew
Jan 30-31	Old Woman Shelter to Unalakleet	34	Victor Anagick
Jan 31	Unalakleet to Shaktoolik	40	Myles Gonangnan
Jan 31	(Shaktoolik to Seppala handoff)		Henry Ivanoff
Jan 31	Shaktoolik to Golovin	91	Leonhard Seppala
Feb 1	Golovin to Bluff	25	Charlie Olson
Feb 1	Bluff to Nome	53	Gunnar Kaasen



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# How Paul Janssen's Drugs Saved the **CHINESE TERRACOTTA WARRIORS**

*Rebecca J. Anderson*

*The Chinese Terracotta Army, dating from approximately the late third century BCE, was discovered on March 29, 1974 to the east of Xi'an in Shaanxi Province, China. Photo: Shutterstock*



Chinese museum officials gazed with dismay at their priceless army of ancient statues. For 22 centuries, the terracotta warriors had been protected and preserved in the soil of China's Yellow River valley (1). Now, less than 20 years after these old soldiers emerged from their subterranean fortress, many of them had become infected and were suffering from a mysterious rash (2). Local archeologists suspected the warriors' moldy rash was due to fungi, but they lacked specialized laboratory equipment and had only limited expertise to diagnose and treat the ailment.

The detective who stepped forward to solve this mystery and thwart an archeological catastrophe was an unlikely hero: a businessman, physician, and scientist who made and sold drugs. And most unlikely of all, he was Belgian.

Next to Hercule Poirot, Paul Janssen was probably the most famous Belgian of the 20th century, and the two compatriots had much in common. Poirot and Janssen both regularly exercised their little grey cells, saw clues that others missed, and pragmatically followed the trail of evidence. They traveled widely, often downplayed their own expertise in deference to colleagues, and chalked up a consistent record of success.

But there was one big difference. Whereas Hercule Poirot existed only in the fertile imagination of Agatha Christie, Paul Janssen was real. A little boy who grew up in war-torn Belgium, Paul had many interests, but his journey leading to the ancient Chinese warriors was anything but direct.

### **A Pharmaceutical Heritage**

Paul Janssen was raised in a family whose business was drugs. His father, Constant Janssen, had given up a successful medical practice in 1938 to devote full time to developing his own pharmaceutical business in the small Belgian town of Turnhout (2-4). Constant was the Belgian distributor of medicinal products from the Hungarian company, Richter. The product line consisted mainly of tonics, stimulants, vitamin preparations, and organic extracts.

German occupation of Belgium during World War II and the murder of Gedeon Richter (the Hungarian company's owner) by the Nazis forced Constant to increase production of his own products under the

Janssen label (3). These included repackaging and distributing generic penicillin and sulfonamides, which were increasingly in demand after the war. Paul's mother, Margriet Fleerakers, served as office manager and also supervised the production line, including quality control (3).

Paul finished high school in 1943. To avoid forced labor in the German factories, he secretly enrolled in college (at the age of 16) with the help of his uncle, Emiel Janssen (3). The 12 Jesuit teachers at the Faculté Notre-Dame de la Paix in Namur, Belgium, offered intensive courses in physics, biology, philosophy, and chemistry to a handful of students, including Paul, without the knowledge of the German occupiers (2, 3). Paul received his Bachelor of Natural Sciences degree in 1945 and began studying medicine at Catholic University in Leuven, Belgium (2-4). His medical studies and a visit to the Dutch pharmaceutical company Organon strengthened his interest in drug research and introduced him to the concept of structure-activity relationships (2).

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***The detective who stepped forward to solve this mystery and thwart an archeological catastrophe was an unlikely hero: a businessman, physician, and scientist who made and sold drugs.***

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Around the Janssen dinner table, the drug business was a frequent topic of conversation. Paul was impressed by the European pharmaceutical giants Roche and Organon and urged his reluctant father to innovate and modernize the family's Richter-Janssen product line (2). To gain a better understanding of world-class pharmaceutical research, Paul took a six-month leave during his second year of medical school and visited medicinal chemistry and pharmacology laboratories in the United States (2-4). He covered his expenses, in part, by playing competitive chess in "pick-up" matches as he traveled across the country (3).

Paul first visited Harry Gold, the well-known pharmacologist at Cornell Medical School, and then Edwin Cohn at Harvard. He also attended lectures

by Carl Pfeiffer, a well-known pharmacologist at the University of Chicago, and took a summer biochemistry course at the California Institute of Technology in Pasadena (2, 3). He rounded out the summer by visiting Searle, Upjohn, and Lederle to observe commercial pharmacology research and returned to Belgium in time to take his academic examinations, which he passed with honors (3).

Paul completed his clinical training at Ghent University and received his MD in 1951, graduating magna cum laude (3). He also stayed engaged with his family's business. One Sunday afternoon in 1951, he used his knowledge of pharmacology and pharmaceuticals to concoct his first drug product. Using a popular German analgesic as a reference, he combined acetaminophen, aspirin, and caffeine to create Perdolan. His father marketed the product, which became the most widely used analgesic in Belgium (2).

Paul fulfilled his compulsory military service at a base near Cologne, where the Belgian army formed part of the post-war allied forces (2-4). His duties as an army physician were light, and he continued his studies at the University of Cologne's Pharmacological Institute, where he synthesized his first molecules: simple chemical reactions to produce amines (2). From 1951 to 1954, he gained additional medical training in Paris, Vienna, and Heidelberg, made a number of trips to Oxford, London, and Stockholm, and visited the United States for the second time (3).

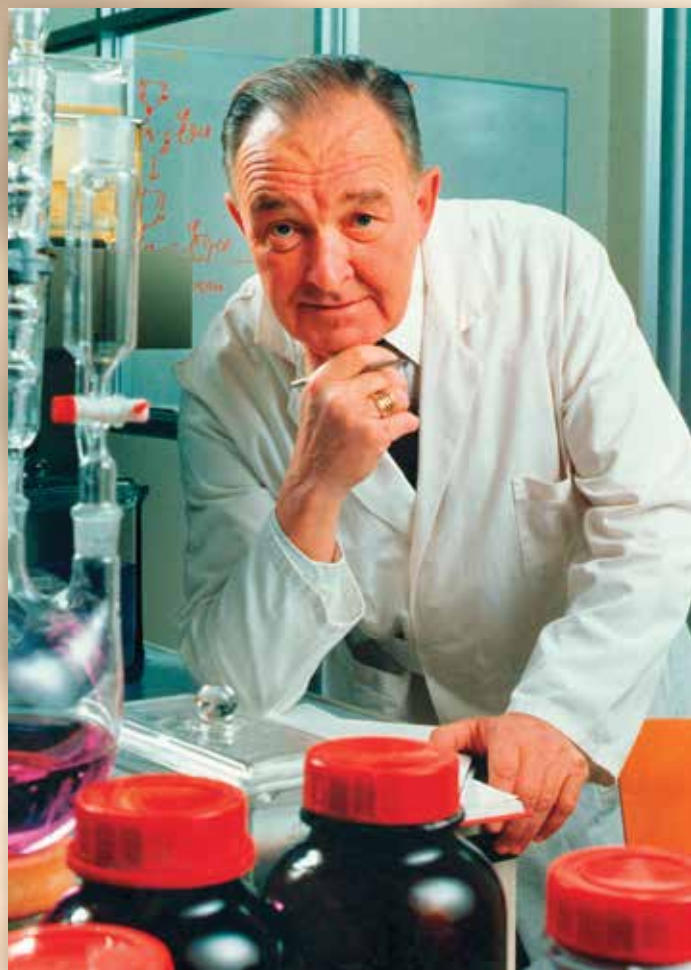
After his military service, Paul became a part-time research assistant at the Pharmacological and Therapeutic Institute in Ghent under the supervision of Nobel Laureate Prof. Corneille Heymans. In 1956, Paul was awarded his teaching certificate and a PhD in chemical pharmacology from the University of Ghent, defending a thesis on the pharmacology of propylamines (2-4).

### Joining the Family Business, With a Twist

Instead of pursuing an academic career, Paul wanted to establish an independent research facility dedicated to developing new drugs (3, 4). Constant Janssen was not interested in research himself, but he wisely did not discourage his son's ambitions. In 1953, he gave Paul 50,000 Belgian francs (\$1000) in start-up funds, and Paul set up a laboratory on the

third floor of the Richter-Janssen company's building in Turnhout. Paul was 27 years old.

As Paul later recalled, he started his research "with a small group of researchers and an equally small budget, to make new compounds that could be synthesized and purified with simple methods and equipment and which could be pharmacologically tested at minimal expense" (5). His goal from the beginning was to make his research self-sustaining: to quickly produce medically important compounds on which he could secure patents, license them to large drug companies, and use the income to recruit new associates and expand the scope of his research (2, 3). "Things had to succeed. I did what I thought had to be done: finding something that could be patented. And things had to be simple, otherwise they would get too big and take too long, and become too expensive...It was all very primitive, but...from day one, we lived off our income" (2).



*Dr. Paul Janssen in his lab.*

Photo: Copyright Janssen Pharmaceutica (2015)



For pharmacological assessment that was beyond their simple *in vitro* and *in vivo* assays, Paul and his team sent their compounds to David K. de Jongh, a physician in Amsterdam who, like Paul, had studied with Prof. Heymans at Ghent. To distinguish those compounds from the compounds generated in his own laboratory, De Jongh assigned Paul's compounds an R number (for Richter) (2, 3). The Janssen company subsequently adopted this nomenclature, Paul later explaining that the R stood for research (5).

Paul's small laboratory initially investigated treatments for painful muscle spasms. The fifth compound he synthesized was ambucetamide (R5), which relaxed uterine smooth muscle. His father's company combined R5 with Perdolan and in 1955 marketed the combination product as Neomeritine for menstrual pain (2, 3).

### Success Comes Rapidly

In 1954, the laboratory produced isopropamide (R79), a long-acting anticholinergic drug that inhibited stomach and intestinal smooth muscle spasms and blocked gastric secretion. Following his business plan, Paul licensed the drug to Smith, Kline, and French (now GlaxoSmithKline). The royalties enabled Paul to expand his laboratory and carry out more research.

Paul noted with interest the popular opiate drug meperidine, a synthetic morphine analog that was prescribed for moderate pain and for diarrhea (3). After synthesizing and testing several hundred meperidine analogs, Paul's team noted a lack of correlation between the compounds' analgesic and constipating properties (5). In 1956, his chemists succeeded in synthesizing diphenoxylate (R1132), a potent antidiarrheal compound that had low abuse potential, and Paul sought a licensing partner.

G. D. Searle & Company was initially hesitant to license the product. Despite the recommendation of I. C. Winter, Searle's highly respected medical director, the company's business leaders were skeptical. Paul was a young, unknown doctor from a small European country (2). During the negotiations, a cousin of Jack Searle, the company's vice president and general manager, coincidentally suffered a bout of severe diarrhea. Dr. Winter administered diphenoxylate, and Jack's cousin made a speedy recovery. Searle (now part of Pfizer) soon licensed the Belgian "wonder

drug" and marketed it in the US as Lomotil®. In the 1960s, Lomotil was included in the drug supplies that accompanied the Apollo astronauts to the moon (2, 3).

By 1957, Paul had assembled a staff of 70 coworkers, and they had outgrown the lab space in his father's Turnhout factory. They moved to new laboratory quarters in Beerse, Belgium, a campus that could accommodate long-term expansion. The following year, Paul's research laboratories merged with his father's company to form Janssen Pharmaceutica, and Paul became president and director of research (3, 4). He was 32 years old.

### Velvet Glove, Steely Fist

Paul built the company's research reputation by tapping the strengths of his people (2, 3). He had both a natural authority and a deep respect for his coworkers – scientists, lab technicians, and administrative staff alike. He kept the organizational structure flat, directly stimulating, encouraging, and nurturing each person's creativity and innovative skills. Under his guidance, the younger scientists grew into well-known experts in the pharmacological treatment of a wide variety of diseases. Everyone

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***...he could sense opportunity where others might see only a failed experiment.***

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called him Dr. Paul (2).

A journalist for the industry publication *Scrip Magazine* (reporting in a 1985 article) described the Janssen organization as a collective of equals. "If a researcher wants to do something new, then all he or she needs to do is send a note to Dr. Janssen describing his or her intentions and motivations. Paul Janssen nearly always agrees. And if he doesn't, he just talks to the researcher directly to discuss things further" (2).

During his daily walkabouts, Dr. Paul engaged in lively discussions of chemistry, pharmacology, and clinical medicine. He wanted to know what the researchers were doing, the details of their results – good and bad – and their strategies for solving problems (2). Everywhere he went and of everyone he met, he asked the same question, "Anything new?"

He had an insatiable curiosity, but whether intentional or not, this simple question prompted extraordinary responses. His researchers knew they would be asked every day and stretched for fresh ideas – or at least thought hard about what they were doing. Dr. Paul's simple question constantly reminded them that research was all about finding something new (2).

Dr. Paul had an uncanny ability to amalgamate in his head all the fragments of chemistry, pharmacology, and clinical results spewing from his laboratories, and he could sense opportunity where others might see only a failed experiment. As director of research, he personally set the direction of each research project. Those projects always started with two things: a carefully reasoned concept – often inspired by unexpected laboratory observations – and a compound whose chemical structure served as a reference for targeted synthesis.

### Cyclists and Psychosis

Dr. Paul's observations and inspiration were not limited to the laboratory (6). One day while walking along a street in Belgium, his scientific curiosity was piqued by a group of competitive cyclists. Racing cyclists at that time often used high doses of amphetamine to gain a competitive advantage. However, with chronic amphetamine use, the cyclists developed taut facial expressions that progressed to grimaces. They also exhibited agitated behavior that resembled the signs and symptoms of patients with paranoid schizophrenia (2, 4).

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***Dr. Paul was skilled at recognizing core chemical structures that were biologically active and exploiting them to create a wide variety of therapeutic products.***

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The similarity between the cyclists' behaviors and clinical psychosis led Dr. Paul to speculate that an amphetamine antagonist might be useful to treat psychotic symptoms (2). His battery of simple laboratory tests included an assessment of drug-induced changes in animal behavior associated with tranquilizers (such as catatonia and sedation).

After the success of Lomotil, the Janssen chemists sought even greater separation between the neurological and constipating effects of opiates. They synthesized a series of meperidine analogs with larger and larger chemical substituents. "However," Paul admitted, "we pushed our luck too far" (5). Mice injected with these bulky molecules exhibited less of the typical opiate-like behavior (e.g., morphine-induced excitement, mydriasis, and insensitivity to pain). Instead, the mice appeared tranquilized; they became progressively calm, sedated, and slightly catatonic.

Up to this time, reserpine, chlorpromazine, and their analogs were the only compounds that produced "tranquilizing" effects in Janssen's pharmacological screening tests (5). The bulky meperidine analogs were an anomaly: compounds with tranquilizing properties but chemically unrelated to either reserpine or chlorpromazine. Dr. Paul directed his researchers to pursue this interesting series of compounds further. After synthesizing 438 analogs, the Janssen chemists produced R1625 in 1958. Better known as haloperidol, R1625 was devoid of morphine-like properties and was several times more potent than chlorpromazine as a tranquilizer. It was also faster and longer acting and had almost no antiadrenergic or other autonomic effects associated with chlorpromazine (5). Haloperidol was the most active neuroleptic yet discovered and became the prototype for a new class of psychoactive agents, the butyrophenones.

Janssen Pharmaceutica subsequently introduced 10 butyrophenone neuroleptics (including droperidol, R4749, and spiperone, R5147) for human or veterinary use (3, 5). Through further modifications of the chemical structure, the Janssen chemists also produced the long-acting neuroleptic, pimozide (R6238) (2, 4).

Despite the side-tracked research prompted by the butyrophenones, Dr. Paul continued his search for analgesics that were more potent than meperidine. Meperidine is hydrophilic and does not easily cross the blood–brain barrier. The Janssen chemists increased the lipophilicity of the molecule, and after a series of additional chemical modifications, they synthesized fentanyl (R4263) in 1960. It was 100 times more potent than morphine. Because of its rapid onset, short half-life, and minimal effect in



depressing the heart, fentanyl was widely used by anesthesiologists (2, 3).

At the other end of the meperidine spectrum, the Janssen chemists produced loperamide (R18553), which was devoid of analgesic activity because it does not cross the blood–brain barrier. Screening assays showed that loperamide was highly effective in inhibiting gut motility. Marketed as an antidiarrheal drug, loperamide (Imodium®) became one of Janssen’s most well-known products (2, 6).

Janssen Pharmaceutica continued to grow: 377 employees and affiliated companies in Germany, Holland, the Belgian Congo, Jordan, and Egypt. But the corporate headquarters in Beerse, Belgium, needed support for the company’s growing global operations. Paul explained, “A drug that doesn’t make it in America will never become an international blockbuster” (3). In 1961, Janssen joined forces with US-based Johnson & Johnson in a mutually beneficial merger. For J&J, the consumer products company best known for Band-Aids and baby shampoo now included medical research and pharmaceutical products. For Paul, the merger was a sort of insurance policy (2). Janssen Pharmaceutica expanded its global reach and acquired financial security but retained its company identity.

### Worms, Bugs, and Mold

In 1960, the Belgian Congo gained its independence, and many Belgian expatriates were forced to return to Belgium by the leaders of the new country, Zaire (now the Democratic Republic of the Congo). Many of the expatriates were scientists: pharmacologists, neurologists, veterinarians, and other specialists with extensive knowledge about parasites, fungi, and protozoa (2). Dr. Paul recruited more than two dozen of them, the first in a long line of distinguished scientists who came out of Africa and developed Janssen Pharmaceutica’s expertise in tropical medicine (2, 3).

Dr. Paul’s new parasitology team concentrated on finding broad-spectrum anthelmintics because various species of worms affect about half of the world’s population (2). Newly synthesized compounds were assessed in a simple animal model using chickens, which by nature are often infected with worms. After four years of optimizing the structure-activity of various compounds and their metabolites,

the Janssen chemists produced levamisole (R12564), which was considered a major breakthrough in parasitology (2, 6).

Similarly, the expatriate microbiologists developed a huge library of fungi and fungal spores to screen compounds for anti-mycotic activity, leading to the discovery of miconazole (R14889) in 1967 (2, 6). Miconazole was effective against a broad spectrum of fungi, molds, and some bacterial strains, including *Candida albicans*, which is responsible for vaginal yeast infections.

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### ***Ketoconazole (R41400) was the first orally active antifungal drug, a major breakthrough***

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Dr. Paul was skilled at recognizing core chemical structures that were biologically active and exploiting them to create a wide variety of therapeutic products. Lomotil, Imodium, and fentanyl were all generated from the phenylpiperidine backbone of meperidine. Similarly, levamisole and miconazole both contain an imidazole ring, which became another workhorse of Janssen chemistry. Further modifications of the imidazole series produced mebendazole (R17635) in 1968, another anthelmintic with broad-spectrum activity against roundworm, hookworm, and whipworm (2, 6).

The Janssen research initiatives to eradicate fungal, parasitic, and bacterial infections in patients evolved to include products that could also be used in veterinary medicine and for plant protection. In 1969, the Janssen chemists produced imazalil (R23979), another imidazole analog. It proved to be effective against a number of molds and fungi and was developed as an agrochemical product to prevent fungal decay in grain crops, fruits, and vegetables and to treat mildew on roses (2).

The success of these efforts led to construction of a greenhouse on Janssen’s Beerse campus in 1972 to do *in vivo* research on fruit trees, wheat, and sugar beets and to facilitate development of antifungal products to protect them. The following year – the 20th anniversary of Dr. Paul’s laboratory – Plant Protection was established as a separate division within the Janssen research organization.

Dr. Paul's staff had grown to 1,246 people, of whom 389 were researchers. They had synthesized 27,975 compounds, held 50 patents, had launched 37 commercial drugs, and were in the midst of developing 17 more drugs (2).

In 1976, the Janssen chemists synthesized another analog of miconazole with broad activity against fungi and yeasts. Ketoconazole (R41400) was the first orally active antifungal drug, a major breakthrough (2, 6). It was widely prescribed to AIDS and cancer chemotherapy patients who suffered from systemic fungal infections.

In 1979, the Plant Protection division developed propiconazole (R49362), an analog of imazalil, as an agricultural product. Propiconazole is absorbed by plants and protects them from the inside out – a more efficient antifungal delivery than topical spraying. The product is widely used to protect turf grasses, fruit and nut trees, and grain crops (2).

### The Orient Express

Paul made his first trip to China in 1976 as a member of a mission sponsored by the Belgian Chemistry Federation (2). His wife, Dora, accompanied him and was keen to explore local Chinese history and art, especially some remarkable artifacts that had been discovered just two years earlier in Xi'an. A couple of farmers had been digging a well in a persimmon orchard, but instead of water, they pulled up some clay fragments that turned out to be one of the 20th century's most spectacular archeological discoveries. When pieced together, the

fragments became the statue of a warrior from the time of China's first emperor, Qin Shi Huang.

The fledgling archeological site was not open to tourists, but through Paul's connections, he and Dora were able to arrange a private visit. The archivists were restoring the precious artifacts in a small lean-to building made from corrugated sheet metal. So far, they had assembled only two statues.

Dr. Paul subsequently made a number of trips to China, spearheading arrangements to market Janssen products in China. The Hanjiang Pharmaceutical Company in Hanzhong (a city in China's inland Shaanxi province) handled local distribution. The relationship matured, and in 1985 Janssen Pharmaceutica finally reached an agreement with the Chinese government to build a new manufacturing facility in Shaanxi province. Rather than expanding Janssen's established operations in Hanzhong, the Belgian company deferred to Chinese authorities, who preferred Xi'an, the capital of Shaanxi, as the site for the new plant. Xi'an-Janssen Pharmaceuticals opened in 1991 and was a joint venture with Shaanxi Medical Industry Company, the China Medical Industry Company, the China Pharmaceutical Foreign Technical Cooperation Company, and the Hanjiang Pharmaceutical Company (2).

The People's Republic had already consummated three other joint ventures with western pharmaceutical companies (Japan's Otsuka, the American Bristol-Myers Squibb, and a Swedish conglomerate), but the Xi'an-Janssen factory was the largest such facility (2). The eight buildings in the complex totaled 325,000 square feet of manufacturing space and produced medicines for shipment throughout China. Chinese authorities proudly showcased the Xi'an pharmaceutical plant as *the* example of successful foreign investment in the inland Chinese provinces. Much of that success was due to the warm personal relationship that Dr. Paul fostered with his Chinese friends, and the feeling was mutual. In 1993, he became the first foreigner to receive a pharmaceutical honorary doctorate in China (2, 3).



Janssen Pharmaceutica unit in Xi'an, China.



## The Chinese Puzzle

As the Xi'an-Janssen factory grew in stature, an army of ancient warriors was emerging from the Chinese soil only 15 miles away. People knew that emperors and their families had been buried in the Yellow River valley near Xi'an, a city of great historical significance. But the magnitude of what they were uncovering at the excavation site was beyond belief.

In 221 BC, Qin Shi Huang united seven independent Warring States to create China's first empire and established Xi'an as the new capital (1). Although the 39-year-old emperor ruled this empire for only ten years, the Qin dynasty was a major turning point in China's history. Qin Shi Huang abolished the feudal system and established a central government with state appointments based on merit. He standardized weights and measures as well as Small Seal Script (the form of Chinese handwriting). He also dug canals – some of which are still in use – and standardized the axle length of carts and wagons to facilitate transportation and communication (1).

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***Exposure to the 20th century atmosphere and the breath of enthralled tourists were decaying the fragile terracotta statues at an alarming rate.***

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His massive construction projects each required hundreds of thousands of laborers. One project involved linking numerous existing small defensive walls along the empire's northern border to discourage invaders, a precursor to what became the Great Wall. Another major project was construction of his own tomb. To safeguard his voyage into the afterlife, Qin Shi Huang surrounded his mausoleum with 8,000 terracotta warriors: life-sized reproductions of soldiers, some standing with chrome-plated bronze swords and spears, some kneeling with drawn bows and arrows, and still others driving chariots behind horses made out of clay (1).

What Paul Janssen saw when he stood at the edge of the excavation pits in the late 1990s was much different from his first visit. The small corrugated steel shed had been replaced by a museum of multiple



*Qin Shi Huang, the first emperor of China.*

buildings covering a site the size of 45 football fields and permitting visitors to watch the archeologists at work. In trenches below the visitors' gallery in Pit 1, long columns of soldiers stood in regimental order four abreast – each painstakingly pieced together from millions of terracotta fragments (1).

But they looked sick. The statues' colors were fading, and the mechanical properties of the terracotta had weakened. Exposure to the 20th century atmosphere and the breath of enthralled tourists were decaying the fragile terracotta statues at



an alarming rate. The temperature in the museum was typically above 70°F and the humidity ranged from 70 to 90 percent. Large areas of the gallery walls and dirt floor were covered with mold. Curators of ceramics at other museums had sometimes seen damage to their artifacts from certain fungi that produce acids, but little was known about the interaction between fungi and terracotta (2). Fortunately, Dr. Paul was now on the case.

In 1999, he returned to Beerse with a few samples of the infected terracotta (7). Fungus experts in the bioresearch laboratory of Janssen's Plant and Materials Protection division had already read about the problem in news reports and eagerly applied their extensive knowledge to these somewhat unusual patients. They isolated 19 different mycotic species, many of which were known to damage bricks and



Photo: Shutterstock

*A rank of soldiers from the excavated terracotta army.*

plasterwork, and some produced acids (8). Because these microbes also damage living organisms, they threatened not only the terracotta statues but also the museum personnel and the tens of thousands of visitors to the museum (2).

Using old flowerpots as their test subjects, the Janssen experts drew on their arsenal of antifungal products and assessed the feasibility of repelling the warriors' infections (2, 7). The flowerpots (some pretreated with antifungal agents) were contaminated with a mixture of the Chinese fungal spores and subjected to environmental conditions that mimicked those at the Xi'an museum (25°C and high humidity). After 12 weeks, the untreated control pots were covered with fungi, but the pots pretreated with Janssen's anti-mycotic agents remained fungus-free (2). Imazalil and propiconazole were particularly effective in counteracting the fungi (8).

Next, the researchers conducted field tests at the Xi'an site to determine whether the ancient terracotta could be protected like the Belgian flowerpots. To minimize damage to the delicate warriors, the scientists prepared water-based solutions of the fungicides and applied them using a simple hand-spray. They established the half-life of the antifungal effect and watched for side effects, including chemical-induced alterations in the Chinese statues' color and composition. This information led to an initial treatment plan for the warriors. Measures were also taken to control spores in the soil and air of the museum (2, 7).

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***James Black, himself a Nobel Laureate, called Paul Janssen “the most prolific drug inventor of all time... not a single researcher of medicines has done as much as he has”***

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Janssen Pharmaceutica provided its specially formulated fungicides to the museum free of charge for a two-year trial period. In addition, because of the importance of these relics, Janssen and the museum entered into a formal agreement in 2000, and Dr. Paul personally presided at the signing ceremony (2, 9). Under this “Agreement of Protection



for the Site of Terracotta Army and Relevant Relics,” Janssen Pharmaceutica not only provided its custom-formulated products but also trained several of the museum’s scientists in antimicrobial techniques and established a state-of-the-art microbiology laboratory



Photo: Shutterstock

*Decaying terracotta warriors infested with mold.*

at the museum – the Dr. Paul Janssen Laboratory for Advanced Material Protection (8).

The original 3-year cooperative agreement has been renewed twice (currently running until 2017). Wu Yongqi, the museum’s curator, describes the relationship with Janssen as “*jin shang tian hua*,” a Chinese idiom that suggests something perfect benefitting from further perfection (10). With their increased knowledge of terracotta microbiology, the Chinese technicians ultimately identified 60 different fungi growing on the statues (7, 10). Together with the Janssen specialists, they have found optimal methods for controlling fungal growth – treatment that is closely overseen by the archeologists and has kept the army fit for service (2, 10). The Chinese experts now conduct scientific research with autonomy, and the Museum of the Terracotta Warriors and Horses has become a center of excellence for research on bio-deterioration of cultural artifacts for the entire People’s Republic of China (8, 9).

### Major Products Developed under Paul Janssen’s Leadership

R-number	Name (Brand)	Date of Synthesis	Indication
R5	ambucetamide	1953	antispasmodic
R79	isopropamide (Darbid)	June 25, 1954	anticholinergic
R1132	diphenoxylate (Lomotil)	November 21, 1956	antidiarrheal
R1625	haloperidol (Haldol)	February 15, 1958	neuroleptic
R4263	fentanyl	December 8, 1960	analgesic
R4749	droperidol (Inapsine)	June 19, 1961	neuroleptic
R5147	spiperone (Spiropitan)	December 20, 1961	neuroleptic
R6238	pimozide (Orap)	January 23, 1963	neuroleptic
R12564	levamisole (Ergamisol)	February 8, 1966	anthelmintic
R14889	miconazole (Desenex, Lotrimin)	November 23, 1967	antimycotic
R17635	mebendazole (Vermox)	November 15, 1968	anthelmintic
R18553	loperamide (Imodium)	April 1, 1969	antidiarrheal
R23979	imazalil (Fungaflor)	March 28, 1969	antimycotic
R30730	sufentanil (Sufenta)	February 8, 1974	analgesic
R49362 (R35432)	propiconazole (Orbit, Tilt)	March 20, 1975	antimycotic
R41400	ketoconazole (Nizoral)	March 31, 1976	antimycotic
R64766	risperidone (Risperdal)	November 14, 1984	neuroleptic

## Peerlessly Prolific

Dr. Paul relinquished his responsibilities as Janssen Pharmaceutica's president and director of research in 1991. Under his leadership, the Janssen division of J&J had grown to more than 11,000 employees and generated 100,000 R-numbered compounds, of which 80 had been developed as pharmaceutical products for human, animal, and plant diseases (2). Those drugs included compounds for pain and anesthesiology, cardiovascular disease, allergies, all sorts of mental illnesses, and gastrointestinal disorders, as well as infestations by fungi and worms.

Dr. Paul remained an active researcher for another decade, focusing on the emerging field of

computer-assisted drug design and serving as director of Janssen's Center for Molecular Design. At the time of his death in 2003, the annual revenues of the Janssen division of J&J reached more than \$9 billion, approximately 25% of Johnson & Johnson's total sales (3).

Paul Janssen had received more than 35 scientific awards and 22 honorary doctorates (ranging from medicine to natural science, veterinary medicine, pharmacy, and philosophy) and was nominated several times for a Nobel Prize. James Black, himself a Nobel Laureate, called Paul Janssen "the most prolific drug inventor of all time...not a single researcher of medicines has done as much as he has" (2, 6). Surely, the Chinese terracotta warriors would agree.

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# Blue Bloods: How the Humble Horseshoe Crab Helps Save Human Lives

*Rebecca J. Anderson*



**Frederik Bang**

*Photo by unidentified photographer.  
Alan Mason Chesney Medical  
Archives of The Johns Hopkins  
Medical Institutions.*

It all started with one sick crab. Frederik Bang was spending the summer at the Marine Biological Laboratory in Woods Hole, Massachusetts, and contemplating his choices for research. He had his pick among a treasure trove of marine species in MBL's Supply Department, stocked daily from the local fish catch. Horseshoe crabs were plentiful, and the sick one, in particular, piqued his interest.

Bang was a pathologist and routinely used marine organisms to gain insights into biological mechanisms of clinical significance. In the US Army Medical Corps, he had directed research studies on malaria and other tropical diseases in the South Pacific. After World War II, he joined the Medical School faculty at Johns Hopkins University and spent his summers rotating between field laboratories in France (Station biologique de Roscoff); Calcutta, India; and Woods Hole. At Roscoff, he studied a marine worm that produced a thick mucus, hoping to elucidate the mechanisms responsible for cystic fibrosis. In Calcutta, he studied parasites and diarrhea. In Woods Hole, he played around with oysters and marine worms, among other things.

In 1953, Bang was appointed chairman of the Department of Pathobiology at the Johns Hopkins School of Public Health. For his summertime research, he headed to Woods Hole, where he spotted that sick horseshoe crab. When it died, Bang conducted a necropsy to determine why. He discovered that the crab's entire blood volume had clotted into a semi-solid gel (1). Thus began a cascade of landmark investigations and the discovery of a substance that has protected the lives of millions of patients.



## Characterizing the Clots

In 1885, W. H. Howell first reported that blood withdrawn from the horseshoe crab “almost immediately” forms a solid clot (2). But most of what Bang learned about the jelly-like clots came from Leo Loeb. During his summer visits to Woods Hole in the 1920s, Loeb extensively studied horseshoe crabs and characterized the clotting phenomenon.

Blood circulating in the horseshoe crab (*Limulus polyphemus*) contains only one type of cell, the amebocyte: a clear, nucleated, oblong cell that is packed with granules. When Loeb placed blood on a glass slide, he observed that the amebocytes changed their shape and released their granules, which then formed a gel (3, 4). Loeb also observed that the amebocytes collected around certain foreign bodies, but he did not pursue studies to determine whether natural pathogens could trigger granule release and gel formation. Bang suspected the trigger was marine bacteria (1).



A colony of horseshoe crabs off the Atlantic coast.

Photo by Breese Greg, U.S. Fish and Wildlife Service. In the Public Domain.

Horseshoe crabs live on the soft sandy and muddy bottoms of near-shore seawater, which is teeming with bacteria—one billion bacteria per ml (5, 6). Some sort of bacterial infection had likely made the crab sick and caused the intravascular coagulation that Bang observed. Pursuing this possibility, he followed the guidelines established by Robert Koch, who had proposed criteria for determining that bacteria cause a given disease (7).

During the summers of 1953 and 1954, Bang extracted bacteria at random from fresh seawater and injected samples into a series of horseshoe crabs.

The injected bacteria “caused an active progressive disease marked by extensive intravascular clotting and death” (1).

In subsequent experiments, he isolated bacteria from naturally diseased animals and identified *Vibrio*, a rod-shaped Gram-negative bacterium (8). Under the microscope, Bang saw that bacteria caused amebocytes to change their shape and release their granules (1). When he mixed pure cultures of *Vibrio* with amebocytes, a “much heavier gel-like material” formed (8). The gel trapped and immobilized live bacteria within minutes.

All of these findings satisfied Koch’s postulates, but some of Bang’s observations seemed to refute the idea that his horseshoe crabs were suffering from a bacterial infection. He boiled bacterial suspensions to kill the bacteria and found “to our surprise” that the sterile liquid still “caused extensive intravascular clotting in a few minutes after injection” and killed the crabs within a few hours (1, 7). Also, when he added his sterile liquid to blood removed from healthy horseshoe crabs, it induced “a stable gel” (1). Bang concluded that the pathogen responsible for these effects was a heat-stable bacterial “toxin” (1).

By the early 1960s, Bang had learned as much as he could about the pathology of bacterial infections in the horseshoe crab. To study the clotting reaction further and to characterize the putative toxin, he needed the expertise of a hematologist.

While planning his next trip to Woods Hole, Bang called C. Lockard Conley, the founder and chairman of the Division of Hematology at Johns Hopkins. Conley headed an active research group studying blood coagulation, platelets, and hemorrhagic diseases, and Bang asked if Conley could recommend someone to assist with the blood clotting experiments.

To everyone’s surprise, the rather straight-laced and old-school Conley proposed Jack Levin, a young hematologist who had only recently joined his research group. Starting research fellows in Conley’s lab just didn’t get selected to spend a summer at such a prestigious facility (9). The Marine Biological Laboratory attracted biologists from all over the world, and its productivity was “equivalent to that of many of the country’s universities combined” (10). Without exaggeration, Lewis Thomas said, “If you can think of good questions to ask about the life of the earth, it should be as good a place as any to go for answers” (10). So, when Conley asked him, Levin jumped at the opportunity. “Joining Dr. Bang,” Levin



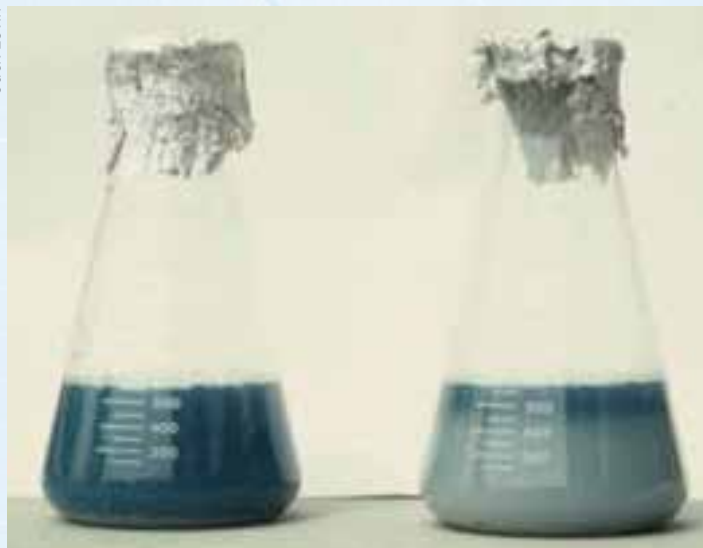
said, “was one of the smartest professional decisions I ever made” (9).

## The Big Bang

In the 1960s and 1970s, the Marine Biological Laboratory was a place “put together...by what can only be described as a bunch of people...It seems to have a mind of its own, which it makes up in its own way” (10). When Levin arrived, Bang handed him a horseshoe crab and said, “Go to it” (9). The large, intimidating creature had nine eyes, ten legs, and a long spikey tail protruding from its massive shell. But Levin soon discovered his experimental subject was docile, cooperative, and well suited to his studies.

Hemocyanin accounts for 90% of the cell-free protein floating in the plasma of horseshoe crab blood. This copper-containing protein turns blue when oxygenated, making the crab’s blood a milky shade of blue. When Levin separated the blood cells from plasma, he saw that (unlike the platelets in

Jack Levin



Flasks of blue blood collected from horseshoe crabs. The white layer in the flask on the right shows amebocytes settling from the blood plasma.

mammalian plasma) the horseshoe crab’s blue plasma did not clot. The clotting factor, whatever it was, resided in the amebocytes.

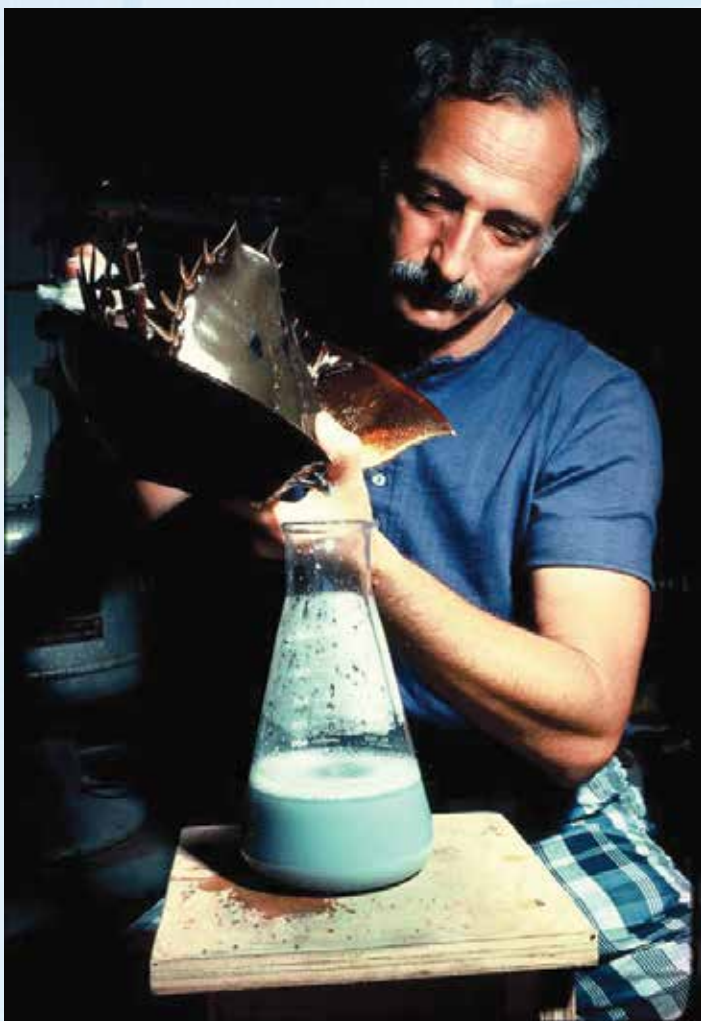
Levin also discovered, to his dismay, that the clotting process was remarkably robust. In all of his early experiments, blood extracted from the crabs clotted spontaneously. Levin tried every anti-coagulant treatment, but nothing prevented the blood from clotting (9).

Finally, as a last resort, he made use of his studies in Conley’s laboratory, where he had seen rabbit blood coagulate after exposure to bacterial endotoxins. Bacterial endotoxins survive the standard wet sterilization procedure that kills bacteria. Levin thought perhaps endotoxin contamination of his laboratory equipment was causing the persistent blood clotting.

To test his theory, he baked all of his glassware with sustained dry heat (the only way to ensure inactivation of endotoxins) and found that blood extracted and placed in his heat-treated glassware did not clot. Subsequently, he conducted all of his experiments with glassware that had been rigorously decontaminated in a drying oven at 180-190°C for over 2 h. In addition, he used only distilled water and other solutions that had been certified as endotoxin-free for human use (9).

Other than those precautions, though, Levin worked under Spartan conditions. Despite its stellar reputation, Woods Hole operated like a summer camp. One cold water spigot served the whole lab. For hot water, they boiled it over a gas flame.

Jack Levin



Jack Levin collecting blood from a horseshoe crab at the Marine Biological Laboratory.



Open windows provided the only air conditioning and ventilation, and Levin conducted all of his experiments bare-handed. Other workers routinely carted fresh marine specimens in and out for their experiments. There was no fume hood, and Levin's use of N-ethyl-maleimide (a potent eye irritant he needed to prepare his amoebocytes and prevent them from clumping) annoyed his lab mates and discouraged visitors.

Despite the makeshift environment, Levin succeeded in controlling all of his subsequent experiments, thanks to the baked glassware and

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***Horseshoe crabs are a resilient species. They can trace their ancestry back more than 400 million years to the Paleozoic era—before humans, before the dinosaurs, and even before flowering plants.***

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careful laboratory technique. "My most valuable laboratory instrument was that drying oven" (9).

Levin and Bang reported their initial findings in 1964. They demonstrated that the amoebocyte is necessary for clotting horseshoe crab blood. The clotting factors are located in the granules of the amoebocytes and not in the blood plasma. And the gel-clot reaction occurs when those clotting factors are exposed to bacterial endotoxins (11, 12).

Endotoxins are lipopolysaccharides, which are found in the outermost cell wall layer of Gram-negative bacteria. Little bits of lipopolysaccharides break off as the bacteria move in their environment. When the bacteria are killed or crushed, they release larger amounts. Levin's results were consistent with Bang's earlier observation that horseshoe crab blood clotted when exposed to dead Gram-negative bacteria. Bang had also observed that Gram-positive bacteria do not induce a clotting reaction (1). (The cell walls of Gram-positive bacteria do not contain lipopolysaccharides.)

### **Surviving in the Bacterial Soup**

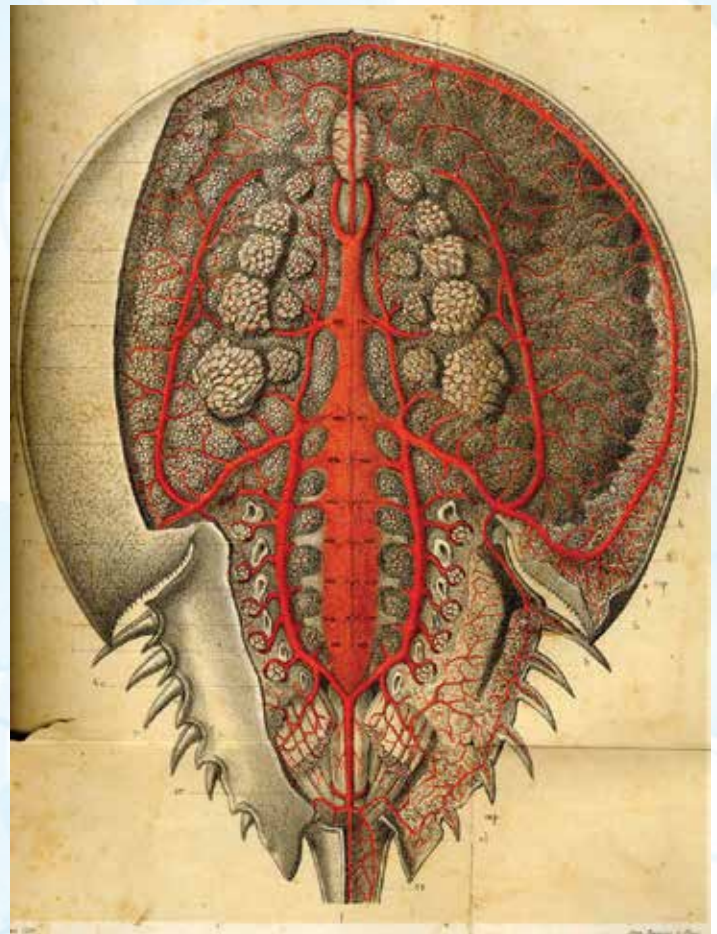
Horseshoe crabs are a resilient species. They can trace their ancestry back more than 400 million years to the Paleozoic era—before humans, before the dinosaurs, and even before flowering plants. Along the Atlantic and Gulf coasts, they contribute

significantly to the marine ecosystem. Horseshoe crabs are a predator of mollusks and marine worms, and their eggs provide a food source for shorebirds, fish, and crustaceans (13, 14). They also churn and aerate ocean sediments, which facilitates oxygenation of the estuaries and marine food production (15).

Somehow, horseshoe crabs have adjusted to life in a bacterial soup in which 95% of the single-cell organisms are Gram-negative bacteria (6). Crabs lack an immune system and cannot produce antibodies, but they have developed simple, efficient mechanisms for fending off environmental threats (5). The amoebocytes in their blood serve many of the same functions as white blood cells in mammals. Amoebocytes engulf foreign or dead cells, transport and store digested materials, and repair wound sites, among other things (16).

The horseshoe crab's rudimentary circulatory system is highly functional. Rather than an extensive network of arteries, veins, and capillaries, it is characterized by large sinuses that allow direct

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*A view of the horseshoe crab's anatomy*



contact of blood with the crab's internal tissues. However, these sinuses also give bacteria easy and extensive access to internal organs if the crab is wounded or its helmet-shaped shell is cracked. Fortunately, over the eons, the horseshoe crab has developed an exquisitely sensitive mechanism for detecting endotoxin and combatting invasion by even minute amounts of bacteria.

In response to a bacterial threat, amebocytes release their granules and liberate clotting proteins. A gel forms to trap and prevent further entry of bacteria at the trauma point, as well as block the leakage of blood. Other antimicrobial substances (such as tachyplesins and big defensins) are also released by the amebocytes and liquidate the trapped microbes (17).

### Reducing to Practice

Levin and Bang developed methods for extracting and isolating the clotting factors from the amebocyte granules (12, 18). A needle is inserted in the crab's cardiac chamber from the dorsal side, and the blue blood is collected. After centrifuging, the blue supernatant fluid is discarded and the packed amebocytes are washed with saline. The cells are then osmotically lysed by adding distilled water, releasing the substances responsible for gel formation. The cellular debris is removed by centrifugation and the supernatant lysate is stored.

The resulting protein mixture was named *Limulus* amebocyte lysate, or LAL, a very descriptive moniker



Photomicrograph of amebocytes from a horseshoe crab. Each ovoid cell is packed with granules.

comprised of the generic name of the horseshoe crab (*Limulus*), the blood cell that contains the clotting substances (amebocyte), and the process Levin and Bang used to harvest them (lysis).

The horseshoe crab is not the only species whose blood will clot in the presence of Gram-negative bacteria or their endotoxins. Investigators observed the same clotting mechanism in lobsters, oysters, and even some insects, but blood extraction from those animals was challenging (7). Levin found horseshoe crabs ideal: they are large, have a large blood volume, and have only one type of blood cell, from which the clotting substances can be easily extracted (9).

Levin was the first to use LAL in an assay for detecting bacterial endotoxins (19). A small amount of LAL was mixed with a sample solution in a test tube (18). If endotoxin was present in the sample, the solution gelled and stuck to the bottom of the tube when inverted. Although the rate of gel formation can be used to determine endotoxin concentration, more recent quantitative methods using photometric and turbidimetric endpoints have also been developed and certified by the US Pharmacopeia (20).

### LAL Lift-Off

Across Eel Pond from the Marine Biological Laboratory where Levin and Bang were conducting their experiments, Stanley Watson, a microbiologist at the Woods Hole Oceanographic Institution, was studying the role of bacteria in the marine nitrogen cycle. He had isolated membrane fractions from some Gram-negative marine bacteria and was looking for a way to assess the purity of his samples (21).



LAL test. Test tubes on the left are control samples of LAL; on the right, endotoxin has been added. The upper panel shows gel formation. The lower panel shows flocculent that forms early in the clotting reaction or in samples containing low concentrations of endotoxin.



A colleague told him about LAL, which Levin and Bang had recently isolated at MBL. Commercial sources of LAL were not available, so Watson obtained horseshoe crabs from the MBL Supply Department and set up production in his garage (9, 21). Unfortunately, his first batches were not sensitive enough for his purposes (21).

Watson decided to “spend a few weeks” trying to improve the sensitivity and reproducibility of his LAL batches (21). The weeks turned into months and a major research effort. Ultimately, he succeeded. Watson’s LAL could reliably detect as little as  $10^{-12}$  g of endotoxin (22). He not only produced LAL for his own research but also shared excess samples with other scientists who were studying bacterial endotoxins (5). When demand outpaced supply, he patented his procedure, set up a small company (Associates of Cape Cod, Inc.), and produced LAL as a lyophilized product (22).

### Protecting Patients

All Gram-negative bacteria, including *Pseudomonas*, *Salmonella*, and *Escherichia*, release endotoxin fragments from their cell walls. The immune systems of healthy people routinely handle these microorganisms when ingested, but in the bloodstream, as little as  $10^{-6}$  g of endotoxin can cause endotoxemia: a high fever, organ failure, and possibly septic shock (22, 23). Endotoxin contamination is the most common cause of fever induced by intravenous drugs and fluids, blood products, and disposable pharmaceutical devices (24, 25).

Since the 1940s, pharmaceutical manufacturers had relied on the rabbit Pyrogen Test for detecting endotoxins in injectable drugs because, like humans, rabbits exhibit a pyrogenic response to endotoxin exposure. In the Pyrogen Test, rabbits are injected with a small amount of solution from a sterile drug batch. If the animals develop a fever, the batch is considered pyrogenic and is rejected. The Pyrogen Test and a test for sterility became the two most important tools in parenteral drug manufacturing (5).

Unfortunately, the Pyrogen Test has inherent disadvantages. It is time-consuming, expensive, and nonspecific. Also, the method produces results that are only qualitative, and the induced fever varies between animals due to differences in animal handling and interlaboratory factors. Some critics raised concerns about excessive use of animals.

Regulatory officials still accept the Pyrogen Test as a method for detecting bacterial endotoxin, but they were willing to consider alternatives. Legionnaires Disease provided a compelling argument in favor of LAL use. The endotoxin of the Legionnaires’ bacillus has a different spectrum of toxicity than other, more common, Gram-negative bacteria. It induces only a weak pyrogenic response in rabbits, but it is readily detected by LAL—1000-fold greater sensitivity (24).

Regulatory officials saw the LAL test as a simple, reproducible, inexpensive, and highly sensitive alternative to the Pyrogen Test. Also, LAL could be used to assay for endotoxin in products (such as radiopharmaceuticals, cancer chemotherapy agents, vaccines, and intrathecal drugs) that are not amenable to testing in rabbits (24, 25).

The Food and Drug Administration’s Office of Biologics established a reference standard for use in determining the sensitivity of each batch of LAL, and quantitation of endotoxin was defined in “Endotoxin Units” (7, 18, 20). In 1973, the FDA published the first guidelines for the manufacture of LAL (26). In 1977, Associates of Cape Cod received the first commercial license from the FDA to manufacture LAL for use in pharmaceutical assays (5, 27).

Also in 1977, the FDA issued the first in a series of guidance documents regarding validation and use of LAL to detect endotoxins in medical products, and regulatory officials began accepting data from the LAL test as an alternative to the Pyrogen Test (5, 24, 27). In parallel, the US Pharmacopeia issued a series of monographs that established specific limits for bacterial endotoxin contamination in various parenteral products (e.g., intravenous drugs, intrathecal drugs, sterile water for injection, and radiopharmaceuticals) (20, 24).

Some manufacturers were reluctant to employ LAL because it was “too sensitive,” but most of them readily adopted LAL as their preferred quality control method for parenteral drugs (24). Unlike the Pyrogen Test, which (for practical reasons) was only used to assess the end product, LAL tests could be applied across the entire manufacturing process of both the drug substance and formulated product (7). This series of quality control tests was especially beneficial for biological drugs, which are expensive to produce. Rejecting an entire lot of finished biological product was much more costly than detecting and addressing contamination at earlier stages of production.

Virtually all intravenous drugs, as well as in-process materials (i.e., containers and closures, sterile water,



bulk drug materials, and excipients), must now pass these multiple LAL checkpoints before marketing (7). In addition, the needles and tubing used to deliver those drugs, as well as implantable devices (e.g., pacemakers) and artificial kidneys used for renal dialysis, are also checked for endotoxins using the LAL test (5).

LAL has some limitations. It cannot distinguish between live and dead bacteria, nor differentiate between species of bacteria-generated endotoxins. Fungi, as well as endotoxins, will elicit the clotting reaction. Still, the LAL test has been widely used not only for quality control of injectable drugs but also in many other situations. It is a handy method for rapid diagnosis of urinary tract infections and spinal meningitis. Other analysts have used LAL to assess food spoilage (fish, milk, and ground beef), as well as air and water quality (5).

In recognition of Frederik Bang's insightful research and its healthcare impact, the International Endotoxin and Innate Immunity Society and the Stanley Watson Foundation established the Frederik Bang Award in 1985. The biennial award recognizes scientists for lifetime achievements in endotoxin research.

### LAL on an Industrial Scale

From one sick crab, a new industry emerged based on Bang and Levin's discoveries. Specialist facilities in the United States, Japan, and China (including Associates of Cape Cod, Charles River, Lonza, Wako Chemicals, and Hyglos) now produce LAL commercially. They collect 600,000 horseshoe crabs each year and harvest the blood, which is worth \$60,000 per gallon (5, 28).

Along the eastern coast of the United States, horseshoe crabs are caught in the spring when they swim into very shallow water to spawn. Although industrial-scale production of LAL has been streamlined, the method remains essentially the same as that first described by Levin and Bang. Technicians extract no more than 30% of

each crab's blood, and the animals are then released back into the sea.

Studies have shown that the horseshoe crab's blood volume rebounds in about a week. Hemocyanin takes more than 6 weeks to recover, and the blood cell count returns to normal in about 2-3 months (5, 13). Theoretically, horseshoe crabs could be bled several times a year, but the New England LAL manufacturers collect them only once a year. This restricted bleeding schedule allows the animals to recover, and they may be recaptured and bled again in subsequent years.

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***From one sick crab, a new industry emerged based on Bang and Levin's discoveries.***

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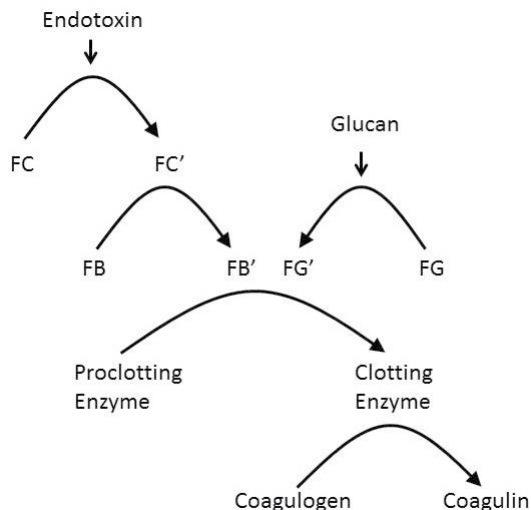
When released, the horseshoe crabs return to their natural spawning areas, but the impact of biomedical bleeding on spawning productivity is unknown. Recent studies have shown that horseshoe crabs are more lethargic, slower, and less likely to follow the tides for several weeks after being bled (13). The bleeding and catch-and-release procedures result in an estimated 8-15% mortality in males and 10-29% mortality in females (13).

Commercial harvesting of horseshoe crabs by fisheries (for bait) and by the biomedical industry (for LAL) is closely monitored and regulated in the United States (15). Of particular concern are decreases in the proportion of spawning females (from 30% to 10%) and in the number of eggs deposited in spawning beaches (13, 14). Research conducted by the US Geological Survey along the Atlantic and Gulf coasts suggested that multiple factors are likely responsible for these declines, including overharvesting of the crabs for fishing bait (a preferred bait for eels and predatory mollusks) and perhaps climate change (29).

Off the shores of Cape Cod where biomedical harvesting is concentrated, bait fishing has not been allowed since



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*The clotting cascade in LAL. Bacterial endotoxins activate Factor C, which in turn activates Factor B. Similarly, fungal glucans activate Factor G. When activated by either Factor B' or Factor G', the clotting enzyme is activated, resulting in cleavage of coagulogen to produce coagulin.*

2000 (5). Yet, despite the catch-and-release practices of LAL manufacturers, the loss of horseshoe crabs in this area, especially females, has been a growing concern (15). The Massachusetts Division of Marine Fisheries examined the factors contributing to horseshoe crab mortality resulting from biomedical bleeding. Based on the results of these studies, LAL manufacturers implemented gentler handling procedures in 2009, aimed at restoring the horseshoe crab population (15).

### Synthetic Alternatives

The declining horseshoe crab population poses a serious threat to both the marine ecosystem and pharmaceutical manufacturers who rely on the LAL test for quality control. Consequently, researchers have been exploring endotoxin detection alternatives that are not dependent on extraction of LAL from horseshoe crab blood.

Levin and his colleagues proved that the reaction between endotoxin and LAL was enzymatic and described essentially all of the endpoints that are currently in use (30, 31). They also isolated, partially purified, and described coagulogen, the gel-producing protein in LAL (31). Subsequent researchers identified five LAL proteins that are involved in clot formation. The first four proteins in the clotting cascade (Factors C, B, G, and proclotting enzyme) are serine proteinase pro-enzymes. The final substance is coagulogen, a

soluble protein that is cleaved to produce coagulin, an insoluble gel (17).

Factor C is highly sensitive for detecting the lipopolysaccharides found in the cell walls of Gram-negative bacteria, whereas Factor G is highly sensitive to the (1,3)- $\beta$ -glucan present in the cell walls of fungi. Invading pathogens trigger activation of these factors, resulting in the sequential activation of Factor B and the proclotting enzyme. In the final step of this cascade, the activated clotting enzyme converts coagulogen to coagulin (17).

Several research groups have devised assays using recombinant Factor C. The rFC reagent has been designed to activate a fluorogenic substrate in the presence of endotoxin and produce a fluorescent product. Commercial kits utilizing recombinant Factor C are available from Lonza (PyroGene™) and Hyglos (EndoZyme® rFC). Unlike LAL, which is activated by both the lipopolysaccharides of Gram-negative bacteria and the glucans from fungi, the rFC fluorescence assays are selective for bacterial endotoxins.

While the rFC assays provide researchers with a valuable tool for endotoxin detection in laboratory research, pharmaceutical manufacturers still rely almost exclusively on the LAL test. The FDA permits the use of alternative endotoxin assays if the methods have been validated according to US Pharmacopeia compendial procedures (20, 27). But compendial validation is a long and challenging process, compared to the already-accepted LAL standard (5, 7).

Consequently, the lowly horseshoe crab, with its helmet-shaped shell, prehistoric ancestry, and blue blood, remains the sole sentry protecting millions of patients from otherwise deadly endotoxin-contaminated drugs.

### Koch's Postulates

- The bacteria must be present in every case of the disease.
- The bacteria must be isolated from the host with the disease and grown in pure culture.
- A pure culture of the bacteria causes the specific disease when it is inoculated into a healthy susceptible host.
- The bacteria must be recoverable from the experimentally infected host.



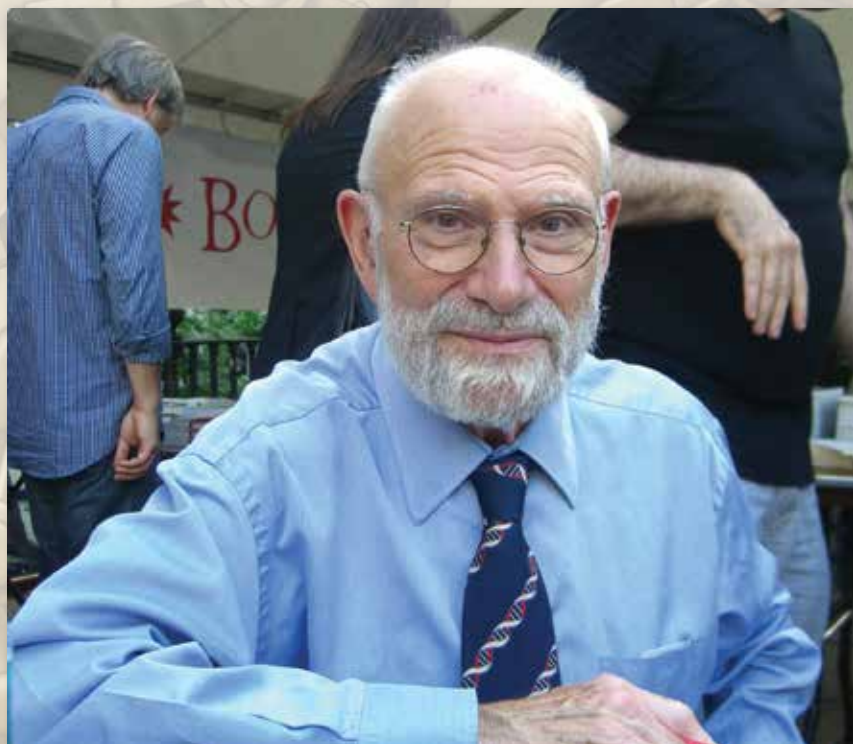
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# Sleepy Sickness, Oliver Sacks, and the Early Days of L-DOPA

Rebecca J. Anderson, PhD



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Neurologist and writer Oliver Sacks (July 9, 1933 - August 30, 2015) at the 2009 Brooklyn Book Festival

As this issue of *The Pharmacologist* was going to press, Oliver Sacks, neurologist, author, and the subject of this article, passed away. Dr. Sacks died on August 30th, 2015 at his home in Manhattan. He was 82 years old.

Even before HIPAA, clinicians always concealed the identity of patients described in their published reports and monographs to ensure patient privacy. But Oliver Sacks told tales so fascinating – and so fantastic – that a reviewer once wrote, “This is an amazing book, the more so since Sacks is talking about non-existent patients in a nonexistent hospital and a non-existent disease” (1). Yet, the patients and hospital did exist. And so did the disease.

Like his fellow neurologists, Sacks cared for patients with Parkinson’s disease, and he had seen impressive results after administering L-DOPA. However, he published cases of a different sort. The “nonexistent” patients suffered from encephalitis lethargica, an increasingly rare disorder that caused



complex nervous system deficits, including severe Parkinsonian symptoms. Sacks claimed his patients responded intensely to L-DOPA but in unusual and unpredictable ways, including adverse reactions neurologists had never seen in patients with ordinary Parkinson's disease.

### Acute Encephalitis Lethargica

In the winter of 1916–1917, a new illness suddenly appeared in Europe and subsequently spread to become a worldwide epidemic. The signs and symptoms were so varied that no two cases were exactly alike and were so strange that physicians initially ascribed various names to it: epidemic delirium, epidemic disseminated sclerosis, acute dementia, epidemic stupor, epidemic lethargic encephalitis, bulbar paralysis, hysteron-epilepsy, or just “an obscure disease with cerebral symptoms” (2).

Despite the variations from one patient to another, a “classic triad” of symptoms soon emerged: fever, somnolence, and eye signs. The abnormal eye signs took many forms, including double-vision, squint, ptosis, and pupil irregularities. The most characteristic sign was oculogyric crisis. Abruptly and without warning, the eyes would be forced downward, upward, or to either side, in a sudden involuntary attack. The patient's gaze would be fixed in that direction for several minutes before switching to another direction. These oculogyric crises would recur weekly, monthly, or at irregular intervals.

Somnolence, from which the disease eventually took its name, differed from normal sleep. Although patients appeared to be asleep, they reported that they were aware of everything that was going on around them but could not rouse themselves. They would “fall asleep” while sitting or standing, and even while walking or during meals. Their breathing pattern simulated normal sleep, including snoring. But if they were aroused by calling or shaking, they immediately “woke up,” fully oriented and conscious (3).

The lethargy typically lasted from days to a few weeks. In some cases, it lasted for months, progressing to a deeper and sometimes comatose state from which recovery was unlikely (3).

In Vienna, Constantin von Economo meticulously documented these puzzling cases and autopsied the brains of the lethargic patients who died. The curious constellation of signs, symptoms, and postmortem pathology did not fit any previously established neurological disease. To distinguish it from other forms



*In the Public Domain*

*Constantin von Economo (August 21, 1876 – October 21, 1931)*

of encephalitis (such as meningitis, polio, or rabies), some physicians called it epidemic encephalitis. Von Economo named it encephalitis lethargica, which became the official clinical designation, but the general public and lay press called it “the sleepy sickness” (not to be confused with sleeping sickness, the African parasite-born, endemic disease, trypanosomiasis).

Sleepy sickness was something of a misnomer. Although most patients were lethargic, some paradoxically exhibited intense catatonic excitement and uncontrollable impulses (4). These patients were unable to sleep and showed ceaseless movement. In the worst cases, their frenzied state of mind and body led to total exhaustion, which proved fatal within 10 to 14 days (2).

In children especially, the main characteristic of encephalitis lethargica was a profound emotional instability. They showed impulsive, tic-like, hyperactive states, including abrupt changes in personality and sudden tantrums, rages, and destructive outbursts (2).

Respiratory tics (hiccups, yawning, dry cough, sneezing, etc.) and other respiratory anomalies (hyperventilation, sighing, breath-holding, noisy expiration, etc.) were also common in the acute phase of sleepy sickness (2, 5, 6).

The number, type, and sequence of symptoms varied widely. In fact, sleepy sickness patients

exhibited a “maze of contradictory phenomena almost impossible to read anything like a rational order of events” (4). Besides the symptoms already mentioned, sleepy sickness patients sometimes exhibited paralysis, chorea, convulsions, head and limb pain, giddiness, delirium, and mania (4). Altogether, von Economo enumerated more than 500 distinct forms or varieties of these (2).

The brain pathology was consistent with the clinical profile. Pathologists saw vascular congestion leading to thrombosis, infarction, and hemorrhage in all parts of the brain. The grey matter was primarily affected, largely in the pons, basal ganglia, midbrain, and most of all, the cranial nerve nuclei, especially cranial nerve III (3, 6).

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***Between 1917 and 1930, an estimated five million patients suffered from sleepy sickness***

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Despite the limited technology of the 1920s, clinicians were convinced, based on their observations of thousands of patients, that sleepy sickness was caused by a virus (4, 6, 7). But, it was comparatively nonvirulent. Even in enclosed environments such as hospitals, asylums, military barracks, and within households, person-to-person transmission rarely occurred (3, 6). The era’s most talented researchers tried but could not isolate and identify the virus. Only recently have investigators obtained evidence suggesting that it belonged to the genus Enterovirus (9).

People from 10 to 35 years of age were most at risk (3, 6). Between 1917 and 1930, an estimated five million patients suffered from sleepy sickness, and 20-40% of them died, either from a coma that could not be reversed or from a hyperactive insomnia that could not be calmed by sedation (2, 3, 5, 6). However, the sleepy sickness epidemic was largely overshadowed by the chaos surrounding World War I and the concurrent influenza pandemic of 1919. Except for the physicians who were managing afflicted patients and the communities that were hardest hit, sleepy sickness received little notice.

The sleepy sickness epidemic ended in the late 1920s, and only a trickle of new cases have been reported since 1930 (9). Of those who survived the acute stage of sleepy sickness, some exhibited “residual troubles” that seemed to disappear over

time. Other patients continued to show clinical abnormalities, and some got worse (5).

Interestingly, some patients were not definitively diagnosed with encephalitis lethargica until months, years, or even decades later. In these cases, the initial “sleepy” symptoms were mild and dismissed as a minor complaint. About 30% of the patients who were diagnosed retrospectively had medical histories that contained no record of an initial illness resembling the symptoms of sleepy sickness (3).

Despite the baffling mixture, variability, or perhaps total absence of initial symptoms, the sequelae of sleepy sickness were unambiguous and permitted a definitive diagnosis (4, 6). Those characteristic sequelae also emerged, eventually, in the patients who had seemingly made a complete recovery from their initial sleepy sickness; after months or years of productive life, they became gravely disabled. As one physician commented in 1927, “The distressing result is that we never know when the patient is cured. (5).

### **Post-encephalitic Parkinsonism**

The clinical sequelae of sleepy sickness were called “post-encephalitic symptoms.” Post-encephalitic patients, as they came to be called, exhibited a progressive syndrome consisting of both neurological and psychiatric abnormalities. No other disorder has been shown to produce these symptoms in the same manner (3).

At the onset of this post-encephalitic stage, many patients exhibited movements that were unexpected, playful, and abnormally fast. This actually gave them a distinct advantage in sports requiring speed and agility (2). However, motor function slowly deteriorated to a Parkinsonian-like syndrome, which was the most common disability and was often called post-encephalitic Parkinsonism (5). Features typically included loss of automatic or synergistic movements, slight rigidity of all muscles, loss of equilibrium, and a running or shuffling gait (6). The face was especially affected, with a mask-like expression, slightly open mouth, staring eyes, infrequent blinking, and quivering eyelids (5).

However, the post-encephalitic syndrome was distinctly different from ordinary Parkinson’s disease. Unlike the “pill-rolling” movement in ordinary Parkinsonian patients, the tremor of post-encephalitic patients was coarse (6). Their “explosive” bursts of involuntary movement were aggravated by emotional distress and largely disappeared during



sleep (2, 5). Other common features included greasy skin, excessive sweating, drooling, attacks of hyperventilation, and – occasionally – excessive weight gain (4).

Despite the onset of movement abnormalities, patients often continued to work, though with difficulty, for a considerable time. Gradually, movement and speech became slower and less animated. Physical exertion and mental exercise aggravated the sensation of weakness, and ultimately the patients gave up the struggle, preferring to lie in bed or sit in a chair doing nothing. Rest did little to restore the patient, and insomnia was often a serious complication. The persistent fatigue was both physical and mental, and the worries of patients with family responsibilities contributed to feelings of depression, fearfulness, and irritability (5).

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***Many post-encephalitic patients spent years in state institutions and psychiatric hospitals, misdiagnosed as schizophrenic***

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Almost as common as the movement disorders, and frequently co-existing with them, were a wide range of psychotic-like abnormalities. The psychotic attacks would last a few minutes or hours and then disappear as suddenly as they came (2). Like the motor weakness, the psychotic outbursts were greatly influenced by suggestion, emotional problems, and other external events. Many post-encephalitic patients spent years in state institutions and psychiatric hospitals, misdiagnosed as schizophrenic (2). But they were not schizophrenic. Nearly half of the post-encephalitic patients suffered extraordinary crises consisting of simultaneous neurologic and psychiatric abnormalities: Parkinson-like movement, catatonia, tics, obsessions, and hallucinations, among many other things (2).

Some survivors of sleepy sickness – despite their movement abnormalities and other problems – led active and independent lives. But most of them developed catatonia, melancholia, immobility, frigidity, and apathy, a unique kind of “sleep” that would become characteristic of their clinical condition for many decades (2). They looked like living statues – totally motionless for hours, days, weeks, or years on end. Their symptoms drove them to isolation

and a timeless state, but they retained their higher brain functions of intelligence, memory, imagination, judgment, and humor (2).

A sudden event (such as fire alarms, dinner gongs, or the unexpected arrival of friends) might catch their attention and arouse them, momentarily, from their motionless, statuesque state. Shifts from immobility to motor activity were very characteristic of post-encephalitic patients, but these flashes of mental alertness were rare (2). Mostly, their thoughts and feelings were fixed at the point in time when their long “sleep” had closed in on them. They could not react or relate. Their minds remained perfectly clear and unclouded, but it was as though their brains had been put on hold (1, 2).

Unable to work or care for themselves and difficult to look after, these patients were frequently abandoned by their families and friends. They were put away in chronic care hospitals, nursing homes, lunatic asylums, or special colonies. There they stayed, almost totally forgotten for decades, and there they died by the hundreds of thousands (2).

Autopsies showed brain abnormalities similar to patients with ordinary Parkinson’s disease (6). Catastrophic damage to the substantia nigra was the hallmark of the post-encephalitic brain: an almost total loss of neurons and pigment and replacement by a pale glial scar (6, 8). There was also calcification and hyaline degeneration of the blood vessels, most notably in the corpus striatum (6). Neurofibrillary tangles were noted in the substantia nigra, coeruleus, hippocampus, parahippocampus, and amygdala (6). In some patients, the loss of grey matter extended to the spinal cord, particularly the anterior horns; the cortex was usually spared. And, in general, the white matter was spared (8).

After 1935, the medical literature on sleepy sickness came to an abrupt end. Few attending physicians took an academic interest in post-encephalitic patients, and over time there were fewer of them to study (2). New cases rarely emerged, and the disease was all but forgotten. Consequently, the more profound forms of encephalitis lethargica and its long-term outcomes were not documented in medical journals (1, 2).

### **Renewed Interest**

During the 1920s and 1930s, hospitals around the world had been built or converted to accommodate and care for the influx of post-encephalitic patients.

One of them was Beth Abraham Hospital, which opened in 1920 in the Bronx, New York (1).

When Oliver Sacks arrived in the fall of 1966, a year out of his neurology residency, Beth Abraham's population of sleepy sickness survivors had dwindled to about 80 patients. Fortunately for Sacks, it was perhaps the largest such group remaining in the United States (1, 2). The post-encephalitic patients were scattered in various wards among the hospital's 500 residents, who suffered from a variety of degenerative neurologic diseases, including motor neuron disease (ALS), syringomyelia, Charcot-Marie-Tooth disease, Parkinson's disease, strokes, brain tumors, and senile dementia (1).

Sacks was well suited to the task before him: an unconventional physician who had always taken the road less traveled. When he was only 12, his schoolmaster had accurately predicted, "Sacks will go far, if he does not go too far" (1).

During his medical school training at Oxford University, he read the entire 12 volumes of his personal copy of the Oxford English Dictionary, along with first edition books by Johnson, Gibbon, Pope, and Darwin in the Queen's College library. The newly minted physician moved to the United States in 1961, feeling that "there were too many Dr. Sackses in London" (1). (His parents, two brothers, an uncle, and three first cousins were all British physicians.)

Sacks completed his internship and neurology residency in California while also breaking the California state squat-weightlifting record (600 pounds). On weekends, he shed his white coat for black leather and rode his motorcycle to the Grand Canyon, 1,000 miles round-trip. On Monday mornings, he reported "bright and fresh" for neurology rounds (1).

In September 1965, Sacks moved to New York to begin his fellowship in neurochemistry and neuropathology at Albert Einstein College of Medicine. He aspired to be a "real" bench scientist, but he lacked the temperament and manual skills for laboratory work. His recreational drug use, begun during his residency in California, had ramped up to huge doses of amphetamines every day and, unable to sleep, huge doses of the hypnotic, chloral hydrate, every night (1). Laboratory mistakes were, perhaps, inevitable. His bosses pulled him aside and in the kindest manner possible advised, "Why don't you go and see patients – you'll do less harm" (1).

In October 1966, Sacks reported to Beth

Abraham Hospital, and indeed he "felt better," not only because he enjoyed seeing patients but also because he had ended his self-administered drug experiments (1). Sacks was intrigued by the post-encephalitic patients. They defied the prevailing view that neurologic and psychiatric disorders were distinct. He was also surprised that most of the post-encephalitic patients, although now in their 50s or 60s, looked half their actual age. Their consciousness had been suspended at the point when the disease locked and imprisoned parts of their brains, and somehow, their suspended animation had also suspended the aging process. "It was my first encounter with disease of a depth I had never seen, read of, or heard of, before" (2).

He sifted through the patients' original charts from the 1920s and 1930s to confirm that they were survivors of the sleepy sickness epidemic. Chronically institutionalized, they were profoundly isolated, and many had no contact with anyone but the nursing staff (2). Sacks arranged to have the post-encephalitic patients moved into a single ward, hoping that this environment might foster a community atmosphere. Over the next few years, through intensive observation, Sacks got to know each of them not only as patients but as people (1).



*In the Public Domain*

*Casimir Funk (February 23, 1884 – November 19, 1967)*

## L-DOPA Changes Everything

Casimir Funk, a Polish chemist working in London, first synthesized L-3,4-dihydroxyphenylalanine in 1912 (10). The amino acid was of interest as a probable precursor of epinephrine, but the name was unwieldy. Bruno Bloch at the Basel County Hospital in Switzerland coined DOPA, an acronym derived from the chemical's German name, *Dioxyphenylalanin*.

Scientists subsequently adopted Bloch's acronym, despite its mildly scatological meaning in Polish (10).

In the 1950s, investigators found unusually rich concentrations of dopamine in the extrapyramidal system, especially the striatum, and Arvid Carlsson proposed that dopamine was involved with the



control of motor function (10, 11). In Montreal, André Barbeau showed that urinary excretion of dopamine was significantly lower in patients with Parkinson's disease (12). In parallel in Vienna, Oleh Hornykiewicz and coworkers reported that patients who died of Parkinson's disease had virtually no dopamine in the striatum and subnormal amounts in the substantia nigra and pallidum (13). The dopamine losses were more prominent in post-encephalitic patients than in patients with ordinary Parkinson's disease (12, 13).

Dopamine replacement was a reasonable therapeutic strategy. Barbeau and Hornykiewicz, working independently but frequently communicating, conducted the first clinical trials in 1961. Because dopamine does not cross the blood-brain barrier, they administered L-DOPA, the active enantiomeric precursor of dopamine.

Barbeau reported that L-DOPA rapidly reduced the rigidity of Parkinson's disease, but the effect lasted only three hours. Similarly, Hornykiewicz observed that L-DOPA reduced the akinesia of Parkinson's disease in a dose-dependent manner (13). Soon afterward, many other centers conducted similar clinical trials and confirmed the ameliorative effect of L-DOPA (10, 13).

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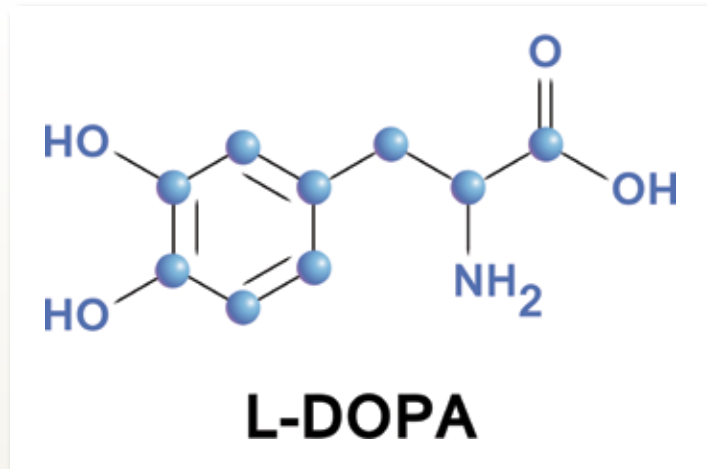
***They looked like living statues—totally motionless for hours, days, weeks, or years on end***

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Other agents had been used with limited success to treat Parkinson's disease: anticholinergics, antihistamines, apomorphine, and amantadine. Those treatments had been discovered empirically, whereas the clinical introduction of L-DOPA represented a successful example of translational research. Basic research on the central and sympathetic nervous systems had led directly to L-DOPA, the first rational therapy for Parkinson's disease (10).

Unfortunately, the pharmacologic effect of L-DOPA was short-lived, and follow-up clinical studies disputed its value as a therapeutic agent. At Brookhaven National Laboratory in New York, George Cotzias applied the ancient axiom, "if some is good, more is better." He sought "to saturate (and keep saturating) the enzyme, DOPA decarboxylase, which generates dopamine from L-DOPA in the brain" (14). Cotzias escalated the daily dose of L-DOPA until he achieved a lasting response – at doses that were up to a thousand times larger than those previously used.

Other neurologists soon adopted Cotzias's dose-escalation procedure and confirmed his findings (10).



*In the Public Domain*

*The L-dopa molecule*

To suppress the gastrointestinal and cardiovascular effects of excess peripheral dopamine, L-DOPA is often combined with Carbidopa, a peripheral decarboxylase inhibitor. The L-DOPA/Carbidopa combination remains the standard of care and most effective treatment for ordinary Parkinson's disease.

Cotzias published his findings in February 1967, and the impact was immediate (2). Patients who had been facing only misery and increasing Parkinsonian disability were suddenly given hope that they might be transformed by this new drug (1).

### **Awakening**

At Beth Abraham, Sacks read Cotzias's paper and a half-dozen other clinical reports with great interest, but he hesitated giving L-DOPA to his patients. Their post-encephalitic syndromes were far more complex than ordinary Parkinson's disease, and they had been institutionalized far longer. "I did not know what might happen" (2). Some of his patients had been violently impulsive and hyperkinetic in the early stages of their illness – before becoming enveloped in their Parkinson-like cocoon – and he worried that L-DOPA might unmask or exacerbate those psychomotor abnormalities (1, 2). He overcame his reluctance in the summer of 1968 when an especially fierce heat wave claimed the lives of some of his patients. "The need to do something became even clearer and stronger" (2).

Sacks and Beth Abraham Hospital applied to the Food and Drug Administration to use L-DOPA, which was still an experimental drug. In March 1969, he

began a 90-day double-blind, placebo-controlled trial with six post-encephalitic patients. Within a few weeks, L-DOPA produced “clear and spectacular” responses, and he could infer from the precise 50% failure rate the three patients who had received placebo (1, 2).

With such convincing results, Sacks felt he could not justify continuing the clinical trial. He abandoned the placebo treatment and offered L-DOPA to any patient who wanted to try it (1). He also abandoned the 90-day limit of L-DOPA treatment. “This would have been like stopping the very air that they breathed” (2).

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***By contrast, the post-encephalitic patients exhibited a profound and rapid awakening. Once the dose escalation of L-DOPA reached a certain threshold, the patient snapped to life***

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As Sacks expanded treatment through the summer of 1969, nearly all of his patients exhibited an astonishing, festive “awakening.” Suddenly, the ward of silent, stationary post-encephalitic patients was electric with excitement (2). After living for decades in a state of suspended memory, perception, and consciousness, the patients came back to life, fully conscious. Patients who had been mute for 40 years were talking. Patients who had spent their days motionless in their chairs now stood up and walked without effort. Their muscles functioned with full strength on their command – not the slow recovery so typical of a limb weakened by being immobilized in a plaster cast.

Most remarkably, their intellectual and emotional faculties returned, but in a sort of time-warp. They spoke of events and people from the time that their post-encephalitic symptoms had enclosed them, as if those people were still alive and those events had just happened. Even their colloquial speech and mannerisms reflected the 1920s and 1930s, but they were not disoriented or unaware of the intervening years. As one patient explained (after decades of silence), “I can give you the date of Pearl Harbor; I can give you the date of Kennedy’s assassination. I’ve registered it all – but none of it seems real. I know I’m 64, but I feel I’m 21” (2).

Sacks asked his patients to keep personal diaries. Their entries confirmed that their higher brain functions had remained intact, but for decades they had been

unable to connect with the world around them.

The FDA wanted Sacks to document these results on standard case report forms, but the responses to L-DOPA were so complex in both neurological and human terms that the standard forms “could not begin to accommodate the reality of what I was witnessing” (1). He kept detailed notes and started carrying a tape recorder, camera, and Super 8 movie camera “because I knew that what I was seeing might never be seen again” (1).

Some of the patients slept much of the day and were wide awake at night. That meant that he, too, needed to keep a 24-hour schedule. He volunteered to be the hospital’s “permanent” on-call physician for all 500 patients at Beth Abraham but spent much of his time in the post-encephalitic patient ward (1).

In addition, the hospital staff (neurologists, psychologists, social workers, physiotherapists, speech therapists, and music therapists) were in constant communication, talking to each other excitedly, phoning each other on weekends and at night, and discussing the unprecedented events unfolding before them (1, 2).

Virtually all patients with ordinary Parkinson’s disease show some sort of awakening when given L-DOPA. They improve gradually over days, reaching a plateau in about two weeks. By contrast, the post-encephalitic patients exhibited a profound and rapid awakening. Once the dose escalation of L-DOPA reached a certain threshold, the patient snapped to life. Patients with the severest disease awakened almost instantaneously (2). In addition, the post-encephalitic patients were much more sensitive to L-DOPA and were awakened with a fraction of the dose required for patients with ordinary Parkinson’s disease (2).

Patients with ordinary Parkinson’s disease are usually in excellent behavioral health, apart from their Parkinsonian symptoms, which may be mild. Their response to L-DOPA consists chiefly of a reduction or apparent abolition of the Parkinsonism. By contrast, the post-encephalitic patients suffered from a large number of disabilities. L-DOPA caused a profound reduction in both the Parkinsonian symptoms and the patients’ innumerable other problems, such as torsion-spasms, involuntary writhing, chorea, tics, catatonia, depression, apathy, and torpor – some of which were not thought to be mediated by dopamine or amenable to L-DOPA (2).

L-DOPA also reversed the post-encephalitic patients’ feelings of being cut off and withdrawn from the



world. They became intensely interested and amazed at everything around them as if they were children again or released from prison (2). In the words of one patient, “I feel so contented, like I’m at home at last after a long hard journey. Just as warm and peaceful as a cat by the fire” (2).

Unfortunately, the beneficial effects of L-DOPA, which had returned the post-encephalitic patients to near-normal mental and physical functioning, lasted only a few weeks or in some patients, only days. Then, things became much more complicated.

### Tribulation

By August 1969, almost all of the post-encephalitic patients ran into trouble. Some developed an extreme sensitivity to L-DOPA, and Sacks had to severely cut back the dose. He stopped treating some patients completely, inserting a “drug holiday” for weeks or months, and then reintroduced L-DOPA. Some of these patients did better after the drug holiday, but others reacted differently – and adversely – to the drug each time he re-administered it. He tried carefully titrating the dose to optimize efficacy, without success. “There seemed to be, with many patients, nothing between too much L-DOPA and too little” (1).

In addition, the patients’ responses grew more complicated, splitting into numerous and more bizarre abnormalities: new phenomena (chorea, tics), over-excitement (restlessness, mania), and fluctuations that rapidly developed into sudden and catastrophic oscillations (2).

Patients became increasingly excited, going from impatience and restlessness to a state of mania and frenzy. Then, they suddenly crashed into a deep depression – worse than their pre-DOPA state (2). Once these oscillating episodes emerged, their severity could sometimes be softened by increasing L-DOPA, sometimes by decreasing L-DOPA, and sometimes by neither (2). Patients would shuttle between the “up” and “down” states almost instantly, spending less and less time in an “in-between” state. Sacks called these sudden oscillations a “yo-yo effect” (1).

Sacks was dismayed by the extreme sensitivity to L-DOPA, the sudden unpredictable responses, the rapid development of oscillations, and finally, the absolute impossibility of matching dose and effect (2). “The ‘system’ now seemed to have a dynamic of its own” (2).

Despite the bizarre reactions caused by L-DOPA, stopping treatment was sometimes not an option.

Withdrawing the drug plunged some patients immediately into a stupor and life-threatening coma. The drug caused disturbing effects, but without it, they would die.

These phenomena, in 1969, had not been seen by other clinicians. Patients with ordinary Parkinson’s disease typically showed a long period of therapeutic benefit with L-DOPA and the side effects, when they came, were usually mild. The post-encephalitic patients, on the other hand, appeared much more prone to early and severe adverse reactions (2).

Sacks knew he “had been given the rarest of opportunities; I knew that I had something important to say” (1). In September 1970, he sent a letter to *JAMA*, describing his observations in 60 patients who had been maintained on L-DOPA for a year (15). Half were post-encephalitic patients and half had ordinary Parkinson’s disease. Nearly all of them had done well at first, but almost all of them, sooner or later, developed complex, sometimes bizarre, and unpredictable adverse reactions (1).

In December, several clinicians responded to Sacks in letters to *JAMA*. They had treated hundreds of Parkinsonian patients with L-DOPA and questioned his findings. One correspondent said that even if Sacks’s observations were real, he should not publish, because it would “negatively impact the atmosphere of optimism” that surrounded the introduction of L-DOPA (1).

Sacks had shaken conventional wisdom about the dose-response relationship. “The effects of L-DOPA should have been straightforward – but weren’t. They were straightforward at first and then something happened” (2). Convincing his colleagues would require more comprehensive evidence. He wrote a detailed clinical report – full of statistics, figures, tables, and graphs – and sent it to *Brain*, the oldest and most respected journal of neurology. The paper was rejected (1).

Sacks’s findings could not be easily conveyed in a medical journal report. Encephalitis lethargica was a complex disorder, and the post-encephalitic patients’ responses to L-DOPA were unpredictable and unreproducible. To describe the phenomena faithfully, Sacks turned to writing detailed, individual patient narratives. He compiled 20 of these case studies in a book, *Awakenings*, which was published in June 1973 (2).

The book was largely ignored by the medical community, but several documentary film producers

approached Sacks. He hesitated. A documentary would expose his patients' identities, which he had diligently concealed in *Awakenings*. But when asked, they said, "Go ahead; film us. Let us speak for ourselves" (1).

In October 1973, a film crew from Yorkshire Television arrived at Beth Abraham to interview the patients. In moving descriptions, they looked back on their illness and described how they were now living their lives after having been cut off from the world for so many years (1). The filmmakers supplemented the interviews with some of Sacks's Super 8 footage, showing the patients before treatment and their awakenings in 1969 after receiving L-DOPA. The documentary, also entitled *Awakenings*, was broadcast in England in 1974, the only documentary account of a forgotten epidemic and how the patients' lives were transformed, for a while, by a new drug (1).

The Yorkshire documentary was subsequently shown at a psychiatry conference (1). Neurologists who had been skeptical of Sacks's clinical observations saw, many for the first time, the dizzying array of bizarre reactions in post-encephalitic patients who had received L-DOPA treatment (2).

After L-DOPA's approval by the FDA in 1970, experience with the drug expanded to millions of patients, and a greater understanding of the complex responses to L-DOPA gradually emerged. Sacks's patients had been harbingers of the complicated pharmacological response induced by long-term L-DOPA administration. Despite the greater magnitude of the post-encephalitic patients' reactions to L-DOPA, the breadth and types of those reactions – both short term and long term – were the same in patients with ordinary Parkinson's disease (2). The adverse reactions simply took longer to develop and were usually milder in patients with ordinary Parkinson's disease (1).

The sudden oscillations, extraordinary "sensitization" to L-DOPA, and wide array of extrapyramidal effects were being reported by everyone. The yo-yo effect described by Sacks became a textbook sign of long-term L-DOPA therapy: the on/off phenomenon. Although dosing remains constant, patients rapidly fluctuate between an "off" state in which they receive no therapeutic benefit to being "on" in which they benefit from L-DOPA but may also exhibit disturbing motor abnormalities.

In 1977, Sacks partnered with P. C. Carolan to study the electrical brain activity of his post-encephalitic patients under a variety of conditions, with and without L-DOPA treatment (16). Untreated patients, during their trance-like state, generated EEGs with exceedingly slow and irregular brain activity, similar to patients who are in a stupor. After taking L-DOPA (or other anti-Parkinsonian drugs), their EEGs suddenly shifted to faster, better organized, and more rhythmical activity at the instant they become alert and animated. With continued drug use, as the patients became increasingly frenzied, the EEG showed repeated bursts of high-voltage paroxysmal activity (2).

### Accommodation

Some post-encephalitic patients never achieved a satisfactory therapeutic benefit and were forced either to cease taking L-DOPA altogether or, because of the possibility of a life-threatening coma, to accept a very miserable compromise. They were maintained on a constant or periodic schedule of L-DOPA, tolerating tics and other dyskinesias as the price for a marginally alert and functional state (2).

Fortunately, some post-encephalitic patients, as well as the majority of patients with ordinary Parkinson's disease, eventually achieved a satisfactory net benefit from L-DOPA. In these patients, L-DOPA efficacy gradually diminished, but the patients reached a sort of steady state.



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This stable response resulted from a compromise dosage that permitted a fairly satisfactory level of functioning, in between a full therapeutic response and serious adverse reactions. The patients were much better than their pre-DOPA state, but “they are no longer very well or very ill” (2).

Successful treatment of the post-encephalitic patients depended on comprehensive medical care, of which L-DOPA was only one component. They required adequate rest (12 hours or more) and needed to avoid stress. The therapeutic power of music allowed many of them an ease of movement that was not otherwise possible. Patients who were able to re-establish personal relationships enjoyed the best outcomes. Caring family members helped them restore a connection to the world around them (2).

With advancing age, the post-encephalitic patients developed a highly specific impairment in speech and motor function, but they retained their intellect, good humor, and interest in life (2). They survived “by being uncomplaining, undaunted, and finally laughing; not succumbing to nihilism or despair, but maintaining an inexplicable affirmation of life” (2).

After 1974, Sacks’s clinical interests broadened, but he continued to monitor his post-encephalitic patients at Beth Abraham and elsewhere. Perhaps no other physician had observed and cared for more post-encephalitic patients, nor observed the effects of L-DOPA for so long (2). In 1990, Columbia Pictures released a movie, also entitled *Awakenings*, starring Robin Williams as a fictional neurologist based on Sacks and Robert De Niro as one of his patients.

In 2008, Oliver Sacks was named Commander of the Order of the British Empire by Queen Elizabeth II. Although he gave up his motorcycle, he continued to swim daily and frequently trekked mountain trails. As Stephen Jay Gould wrote in a poem about his friend: “Oliver Sacks / Still lives life to the max” (1).

In addition to sleepy sickness, Sacks wrote compelling patient narratives on migraine, Tourette’s syndrome, hallucinations, autism, and other neurological conditions. But *Awakenings* remains his signature work. “I cannot think back on this time without profound emotion,” he said, “– it was the most significant and extraordinary in my life, no less than in the lives of our patients” (2).

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**Sidney Farber, Methotrexate,  
and the Jimmy Fund:**

# The Birth of Modern Chemotherapy

*Rebecca J. Anderson, PhD*



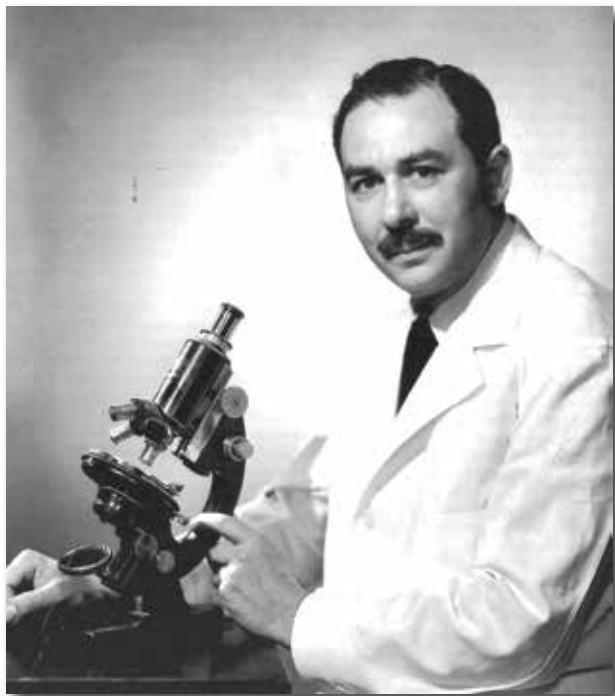
*In the Public Domain*

*President Richard Nixon signing the National Cancer Act (December 23, 1971)*

On December 23, 1971, President Richard Nixon sat at a table in the White House to sign the National Cancer Act, and with that, he declared war on cancer. Attacking cancer as a war has been controversial ever since. But at the time, intensive lobbying and publicity had generated great hope within Congress and across the nation that cancer could be cured. The enthusiasm was propelled by a string of clinical successes, all of which could be traced back to a small basement laboratory in Boston and to an indefatigable champion who was neither a lobbyist nor an oncologist.



Sidney Farber was the son of Polish immigrants who settled in Buffalo, New York. The third of 14 children, Sidney thrived in a family that put a high priority on academic excellence (1). His father, an insurance agent, often brought home textbooks, which the children studied around the dinner table. They were as fluent in German as in English and prepared detailed reports from the textbooks for their father's inspection.



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The young Dr. Sidney Farber (1947)

Sidney worked his way through the University of Buffalo studying biology and philosophy by day and playing violin in music halls at night. He graduated Phi Beta Kappa in 1923 and began medical school at the Universities of Heidelberg and Freiburg in Germany (1). The next year, he secured a seat at Harvard Medical School and graduated in 1927 (1, 2). Farber then trained in pathology at Peter Bent Brigham Hospital. By 1929 and at the age of 26, he had become the first full-time pathologist at The Children's Hospital in Boston and was also appointed instructor in pathology at Harvard Medical School (2, 3).

Even as a student, Farber conveyed a European stateliness. He stood a commanding six feet tall and was always impeccably attired (1, 4). His diction was precise and deliberate, and he addressed his colleagues formally by title (1, 2, 4).

Those who knew him well enjoyed the friendship of a warm, considerate, and generous man with a sly

sense of humor and a fondness for mystery novels (1, 2, 4). His bedside manner was compassionate, and with children, he showed a grandfatherly gentleness (4).

But he could be obstinate, argumentative, and "devastatingly objective" (1, 2). "He never looked for a fight, but he never dodged one" (1). His gentle voice was persuasive, and he excelled at separating fact from "fuzzy thinking," a mental discipline he imprinted on his trainees and junior faculty (1, 2).

In his lucid pathology lectures, Farber enlivened an otherwise dry anatomic and histologic recitation by emphasizing the dynamic biological events that occur during human disease (2). His 1937 book on autopsies, *The Postmortem Examination*, remains a classic.

Like his personal appearance, Farber's 9 x 12 foot pathology laboratory in the basement of The Children's Hospital was the epitome of precision and tidiness (2). He had an unparalleled work ethic, and during his first two decades at The Children's Hospital, Farber made a number of important contributions to pathology. He called attention to sudden infant death syndrome (SIDS) and was among the first to report that encephalitis in infants and children was caused by the Eastern equine virus (1). Along with his associates, he described cystic fibrosis as a generalized disorder, drew attention to hyaline membrane disease in newborns, and defined many of the enzymatic abnormalities associated with celiac syndrome and pancreatic insufficiency (1, 2).

### From Pathologist to Chemotherapist

In 1946, Farber became Chairman of the Division of Laboratories and Research at The Children's Hospital and in 1947 was named pathologist-in-chief. In 1948, he was appointed professor of pathology at Harvard Medical School. During this time, still working out of his small basement laboratory, Farber began focusing his attention on leukemia, a rare but invariably fatal childhood cancer. Life expectancy for children with acute leukemia usually extended only a few weeks after the onset of symptoms (1, 5).

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***Life expectancy for children with acute leukemia usually extended only a few weeks after the onset of symptoms.***

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Leukemia had been shunted to a no-man's-land between hematology and oncology, and little was

known about it because internists had no drugs to treat it and surgeons could not operate on blood (6). Some physicians tried radiation therapy, but mostly they provided supportive care to make the children's last days more comfortable.

Farber's introduction to hematology came in medical school through the inspiring lectures of George Minot, who discovered that pernicious anemia could be cured by supplemental vitamin B<sub>12</sub>. In the 1940s, Farber read several papers claiming that folic acid (vitamin B<sub>9</sub>) inhibited the growth of sarcomas and caused regression of some spontaneous breast cancers in mice (7). This prompted him to wonder whether leukemia, like pernicious anemia, was the result of a vitamin deficiency (1).

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**Minutes after the broadcast ended, nearby residents walked or drove to The Children's Hospital with cash donations.**

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Farber contacted Yellapragada Subbarow, a former Harvard colleague who had recently been appointed research director at Lederle Laboratories. Subbarow and his associates had succeeded in synthesizing folic acid and were actively evaluating a series of analogs as nutritional supplements (6, 7). Farber requested and received several of those compounds.

With the cooperation of surgical and medical associates at several Boston hospitals, he treated 90 patients who were suffering from two dozen different malignancies, including eleven children with acute leukemia (5, 7). The results were disastrous. Instead of helping the patients, folic acid accelerated tumor growth.

Meanwhile, Subbarow's team found that several of their synthesized analogs interfered with folic acid metabolism and normal cell growth, both in vitro and in chicks (1, 7). This discovery, coupled with Farber's clinical failure using folic acid, led Farber to a new hypothesis: "folic acid antagonists might be of value in the treatment of patients with acute leukemia" (8).

The two major classes of leukemia, acute and chronic, were divided into two biological subtypes, lymphoid and myeloid. However, despite Farber's expertise as a pathologist, he found it "impossible to diagnose with certainty the exact morphologic type of leukemia because of the primitive nature of the blasts"

(5). In most cases, he diagnosed the children simply with acute leukemia.

In the spring of 1947, Farber and Subbarow devised a plan for assessing the various folic acid antagonists synthesized at Lederle. They would systematically test the compounds, first in laboratory animals and then in patients, with the goal of finding the most effective and least toxic antagonist in the series (5).

In March 1947, Farber began daily intramuscular injections of the first folic acid antagonist, pteroylaspartic acid, to a four-year-old girl with acute leukemia. The girl died a week later, but Farber was encouraged because her bone marrow had greatly improved. "A change of this magnitude in such a short time has not been encountered in the marrow of leukemic children in our experience" (8). He continued his study, giving either pteroylaspartic acid or another antagonist, methylpterotic acid, to a series of 21 children with acute leukemia (8).

In November 1947, Subbarow sent Farber a new compound. It was simply folic acid modified with a 4-amino substituent, but it proved to be a powerful antagonist. They called it aminopterin. Farber, Louis Diamond, and their associates gave aminopterin to 16 children with acute leukemia. Ten of the children showed clinical, hematologic, and pathological evidence of improvement. In April 1948, Farber sent patient narratives describing the top five responders to the *New England Journal of Medicine* (8).

Although some of the children remained alive for many months, the remissions were temporary. By the time Farber's paper was published on June 3, 1948, most of them had died. Furthermore, aminopterin was toxic, causing diarrhea, ulcerations of the tongue and mouth, and other signs of folic acid deficiency. Farber emphasized that his results did not justify any suggestion of a "cure," and the scientific community reacted with skepticism and disbelief to his claim of remissions. Remissions, even temporary, were unheard of in leukemia (1).

Nevertheless, aminopterin-induced remissions were real and marked a major breakthrough. This was the first time that any cancer had responded to chemotherapy, and Farber's *NEJM* paper became a widely cited classic (7).

Unfortunately, Subbarow died unexpectedly in August 1948, essentially ending the synthesis of new folic acid analogs (7). But Farber was encouraged by the aminopterin remissions and enthusiastically continued the clinical evaluation of Subbarow's



compounds. The antagonists, amethopterin and amino-an-fol, were especially effective. By the end of the year, Farber and his colleagues had treated 60 children with acute leukemia and also received reports from other clinicians who were following his lead (5). More than 50% of the children showed clinical and hematological improvement after treatment with the newer folic acid antagonists.

Farber found that the folic acid antagonists also induced a “temporary, definite but inconsistent” improvement in patients with related tumors such as lymphosarcoma and Hodgkin’s disease, as well as with apparently unrelated forms of incurable cancer, such as neuroblastoma and metastatic bladder cancer (5).

From these early studies, amethopterin would emerge as the best folic acid antagonist. Chemically, it was strikingly similar to aminopterin. Subbarow had simply added a methyl group to the aminopterin molecule. Today, amethopterin is more commonly called methotrexate.

### Jimmy Hits a Home Run

In May 1947, a group representing the Variety Club of New England toured The Children’s Hospital. Founded by members of the entertainment industry, the Variety Club’s philanthropic mission was to assist children, and the club was already providing funds to operate the hospital’s blood bank (3).

On their tour, the group stopped at Sidney Farber’s basement laboratory, where the enthusiastic physician described his ongoing leukemia studies. Intrigued by Farber’s progress and ambitious plans, the Variety Club established the Children’s Cancer Research Foundation and over the next few months, raised modest funds to support Farber’s small outpatient clinic (3, 4).

Bill Koster, the Variety Club’s executive director, felt that a “poster child” would enhance their fundraising efforts, and Farber chose a 12-year-old boy for this purpose. He was treating Jimmy for Burkitt’s lymphoma, a rare intestinal tumor that is fatal in about 85 percent of pediatric patients. Jimmy was the perfect poster child for Koster’s campaign: a tall, blond farmer’s son from New Sweden, Maine, and a fan of the Boston Braves (3).

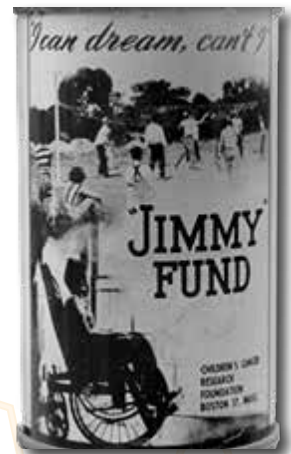
The Variety Club members arranged for Jimmy to speak with Ralph Edwards, the host of the popular radio program, *Truth or Consequences*. On May 22, 1948, through the magic of radio, Edwards in Hollywood interviewed Jimmy in his Boston hospital

bed. During the eight minute live broadcast, the entire starting lineup of the Boston Braves trooped into Jimmy’s room and presented him with the first baseman’s bat, a baseball autographed by the team, and a custom-tailored official Boston Braves uniform (3, 4).

The nationwide audience immediately responded to Edwards’s request for donations to the Children’s Cancer Research Fund. Minutes after the broadcast ended, nearby residents walked or drove to The Children’s Hospital with cash donations. Telegrams and letters brought additional contributions, some addressed simply to “Jimmy, Boston, Mass” (3). Koster hoped to raise \$20,000. The tally came to more than \$231,000 (\$2.2 million today).

The broadcast, coupled with Farber’s *NEJM* article, which appeared a few weeks later, generated great interest among pediatricians throughout New England. They wrote to Farber for help and advice, and he answered each one personally (4). By the end of 1948, patients were arriving in overwhelming numbers at Farber’s outpatient clinic—still just a single room in the Pathology Department of The Children’s Hospital. To accommodate the influx of patients, Farber moved to a nearby apartment building. The “Children’s Medical Center Tumor Therapy Clinic” occupied five apartments on the building’s first floor (3).

In 1949, Koster and the Variety Club launched a second fundraising drive. They placed red and white canisters in the lobbies of movie theaters and outside baseball stadiums. Little League players in their uniforms went door-to-door with collection cans (3, 6). The “Children’s Cancer Research Fund” was simplified to the “Jimmy Fund,” but Jimmy himself had quietly slipped into the background. Without fanfare, he continued making trips from New Sweden to Boston for treatments and checkups, hitchhiking in cars, trucks, and delivery vans with anyone who was driving up or down the coast (6). The public Jimmy was now a generic child with cancer (3).



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Jimmy Fund coin canister



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*The Jimmy Fund Building*

The donations allowed Farber to expand his research and clinical studies. In the fall of 1949, construction began on the Jimmy Fund Building, one block from Farber's original laboratory in The Children's Hospital.

Farber oversaw every detail of the new building. The wide cement steps leading up to the front door were graded so that children could easily climb them and were steam-heated to stay dry during Boston's brutal winters. The main waiting room had boxes of toys, a whirling carousel, and an electric toy train (3, 6). Hundreds of books filled the library, along with several rocking horses and bicycles (6). Hallway murals of 39 Disney characters (Snow White, Pinocchio, Bambi, Jiminy Cricket, etc.) guided patients and caregivers to the various patient rooms (3, 6).

When the five-story Jimmy Fund Building was dedicated in January 1952, it was the first facility of its kind in the country: a center dedicated entirely to the research and treatment of childhood cancers (3). The ground floor housed the Jimmy Fund Clinic. Farber wanted everyone involved in caregiving—physicians, nutritionists, social workers, and other specialists—to make decisions as a team. The concept would become known as “total patient care,” and it was remarkable that this innovation came from a pathologist whose training had not focused on living

patients (3, 4). Upstairs were state-of-the-art research labs, and their proximity to the Clinic reflected Farber's belief that scientific advances and clinical care must go hand-in-hand (3). It was another innovation that is now commonplace: translational medicine.

Among the Clinic's early research successes was a remedy for Wilms' tumor, a rare form of kidney cancer. In 1954, Farber tested a number of antibiotics on a series of mouse tumors, thinking he could repurpose some of the more cytotoxic antibiotics as cancer drugs. Actinomycin D emerged as the best candidate. In mice, it attacked not only leukemia and lymphomas but also breast cancers (1, 6). Next, Farber began a clinical trial in children with a variety of cancers and found actinomycin D was particularly effective against Wilms' tumor.

Standard treatment was surgical removal of the Wilms' tumor, followed by radiation to kill residual cancer cells surrounding the extracted kidney (1). However, the cancer sometimes metastasized, usually to the lungs, and resisted therapy. Collaborating with radiologists, Farber and his research fellow,



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*Sidney Farber, MD, with a young patient*



Donald Pinkel, found that intravenous actinomycin D, in conjunction with radiation therapy to the lungs, eradicated the lung metastases (1, 6). Overall, the 2-year survival in the 68 treated children was 80%—another milestone for Farber's group (1). Wilms' tumor was the first metastatic solid tumor to respond to chemotherapy (6).



In the Public Domain

Bottle of methotrexate

### Methotrexate Inspires Cures

Among those who noted Farber's success in treating childhood leukemia with antifolates were Min Chiu Li and Roy Hertz, researchers in the obstetrics service at the National Cancer Institute. They were studying choriocarcinoma, a cancer of the placenta that was even rarer than leukemia. In abnormal pregnancies, choriocarcinoma can grow out of the placental tissue and metastasize to the lung and brain—a lethal malignancy. In the first metastatic patient they treated with methotrexate, Li and Hertz saw miraculous results (9). The woman's chest X-ray improved, she looked healthy, and by every standard indicator, her bleeding lung tumors had vanished (6).

But Li was troubled by one puzzling observation. He could still detect a tiny amount of human choriogonadotrophin (hCG) in the patient's blood. Because hCG is secreted by choriocarcinoma cells, he reasoned that miniscule amounts of cancer were still present. So, Li continued to administer cycles of methotrexate, ignoring the mounting adverse reactions of extended treatment, until, at last, the hCG level sank to zero.

Li's patients remained free of cancer, even months after they completed his prolonged methotrexate regimen, and some never relapsed (6, 9). Li was

the first to use a valid biomarker (i.e., hCG) to guide drug treatment, and his strategy resulted in the first chemotherapeutic cure of cancer in adults (6).

At Burroughs Wellcome, Gertrude Elion and George Hitchings discovered another promising chemotherapeutic agent, 6-mercaptopurine. Like methotrexate, 6-mercaptopurine induced a rapid remission of acute lymphoblastic leukemia in children. But like methotrexate, the benefit was short-lived. The leukemia cells soon developed resistance and the patients relapsed.

Investigators at NCI decided to take a page from the recent successes in treating tuberculosis. Like leukemia cells, TB developed resistance to single drug treatment, but pulmonologists found that they could overcome TB resistance by using a combination of antibiotics. Multi-antibiotic treatment effectively stopped bacterial division, staving off resistance, and the TB infection could be eradicated.

In a similar manner, cancer investigators sought to attack leukemia at multiple points of cellular metabolism, hoping that synergistic drugs would reduce the emergence of resistance. Clinical trials established that the combination of methotrexate and 6-mercaptopurine produced longer leukemia remissions compared to single-drug treatment (6, 10). Expanding on this result, they next wanted to see if a multi-drug combo could eradicate leukemia. But finding the optimal dose and treatment duration of each compound in these multi-drug regimens would require too many patients and take too long. There were simply too many variables to manage.

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***Cancer investigators sought to attack leukemia at multiple points of cellular metabolism, hoping that synergistic drugs would reduce the emergence of resistance.***

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In Alabama, Howard Skipper developed an animal model using laboratory mice that had been implanted with L1210 cells (a lymphoid leukemia cell line developed by Lloyd Law). Using his animal model, Skipper worked out optimal drug combinations, doses, and treatment schedules to cure mouse leukemia (11).

At NCI, Emil Frei and Emil Freireich applied Skipper's empirically derived principles and began

a clinical trial in September 1962, aimed at curing childhood leukemia. They chose four cytotoxic compounds with different mechanisms: vincristine (a microtubule toxin), amethopterin (i.e., methotrexate, a folic acid antagonist), 6-mercaptopurine (an anti-purine), and prednisone (an anti-mitotic steroid). The regimen was known by its acronym, VAMP.

VAMP catastrophically suppressed bone marrow. Children were brought to the brink of coma and required intensive care, including artificial respiration. Some died from infection because VAMP destroyed their white blood cells. The remaining children, after enduring weeks of excruciating side effects, slowly improved. Their bone marrow rebounded and started producing normal red and white blood cells and platelets—all without any trace of leukemia (6, 11).

As news spread of Frei and Freireich's success, a few additional centers embraced the VAMP regimen. When doctors in Boston diagnosed 13-year-old Karen Lord with acute leukemia, they opted to try VAMP (12). Karen began the regimen in June 1964, and during the unrelenting chemotherapy, she grew progressively weaker, suffered nerve damage, and at times was delirious from pain. Her weight dropped from 108 to 50 pounds. For months, she hovered near death, and priests administered the church's Last Rights three times. But then, she began to recover. After a year in the hospital, she was discharged, cancer-free (6, 12).

Fifty years later, Karen still shudders when she leafs through her collection of photos and medical records, recalling that terrible ordeal (6, 12). But she is still healthy—one of the VAMP-treated children who established that leukemia could be cured.

Sidney Farber was not involved with Karen's treatment. Like many other physicians and hospital administrators, he was unwilling to give the extremely harsh VAMP regimen to children who were already very sick (12). Instead, Farber continued studies of single-drug treatments. At the same time, though, he was impressed with the VAMP results. While Karen was still being treated in Boston, he went to Washington to advocate larger appropriations for the National Cancer Institute. Photos of patients in remission were irrefutable proof that chemotherapy could conquer cancer (6).

Unfortunately, only about 5% of those who completed the year-long VAMP treatment were "cured" (6). Remissions for most of the children lasted only a year or two. Then, they returned to

the clinic with new symptoms: headaches, seizures, numbness, and paralysis (6, 13). Their bone marrow and blood remained free of cancer, but leukemia cells had colonized in their brains—beyond the reach of the drugs (10, 13). By 1968, most of the children in the original VAMP trial had died (6).

To kill the leukemia cells sequestered in the brain, Donald Pinkel embellished the VAMP regimen. After a year with Farber, Pinkel had become chief of pediatrics at Roswell Park Cancer Institute. In 1961, he was hired as the first medical director at St. Jude Children's Research Hospital, a new hospital in Memphis. Pinkel called his multifaceted regimen "total therapy" (10, 11, 14).

At St. Jude's, he treated leukemia with a multi-drug regimen (methotrexate, 6-mercaptopurine, vincristine, prednisone, and the alkylating agent, cyclophosphamide). To circumvent the blood-brain barrier, he injected methotrexate directly into the spinal column and irradiated the patient's brain with high doses of X-rays. The cycles of treatments lasted three years and involved alternating chemotherapy and multiple exposures to radiation (14). Keeping the children alive required intensive supportive care, including platelet transfusions to prevent bleeding and aggressive use of antibiotics to treat infections (14).

"Total therapy" was the harshest anti-leukemia regimen yet devised. But in eight consecutive trials over more than a decade, Pinkel racked up an impressive record of success. Through sequential refinements of his regimen, he achieved a disease-free survival rate of 80% (10, 14). By 1979, as far as anyone could tell, those patients had been "cured" (6, 10).

### MOPPing Up Hodgkin's Disease

Hodgkin's disease patients also benefitted from combination chemotherapy, and methotrexate again led the way. Hodgkin's lymphoma is characterized by enlarged lymph nodes and spleen, and without treatment, advanced Hodgkin's disease was uniformly fatal (11, 15). In the 1950s, investigators reduced the size of the tumors using radiation (15). In the 1960s, physicians began administering nitrogen mustard, an alkylating agent (11). About 25% of the patients benefitted from these procedures, but the remissions were brief and usually incomplete (11).

In parallel with the VAMP trials in leukemia patients, Vincent DeVita at NCI began the first multi-drug clinical trial in patients with Hodgkin's disease, using another four-drug combination:



nitrogen mustard, vincristine (brand name, Oncovin), methotrexate, and prednisone. The treatment was given the acronym, MOMP. DeVita soon modified the regimen, lengthening treatment from 2.5 months to 6 months and replacing methotrexate with a newer and more powerful compound, the alkylating agent, procarbazine. The new combination's acronym was MOPP (11).

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***Hodgkin's disease thus became the first advanced cancer of a major organ system in adults to be cured by chemotherapy***

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After three years of clinical trials with MOMP and MOPP, DeVita saw remissions in 80 percent of the patients (16). He followed the patients in the original MOPP trial for more than 40 years. About 60 percent of those who had attained complete remission never relapsed (11, 15). Hodgkin's disease thus became the first advanced cancer of a major organ system in adults to be cured by chemotherapy (11).

But like VAMP and the other early chemotherapy combinations, MOPP was harsh. Many investigators refused to use it because MOPP could cause secondary acute leukemia, heart disease, and sterility (11, 13, 15). Subsequent refinements in drug combinations reduced the toxicity and boosted the cure rate, which is currently 90 percent for patients with limited-stage Hodgkin's disease (15).

### **Pushing the Agenda**

In addition to Bill Koster, who joined the Children's Cancer Research Foundation as executive vice president and chief fundraiser, Mary Lasker became one of Sidney Farber's strongest allies (3). An entrepreneur and philanthropist, Lasker made a fortune in merchandising and began her mission against cancer when her longtime housekeeper was diagnosed with breast cancer (17). At that time, "cancer was a word you simply could not say out loud," and Mary was outraged when her housekeeper was sent to a home for incurables (17).

Her first coup was to make the American Cancer Society a more action-oriented body. The small organization's board was dominated by physicians, and none of its \$102,000 annual budget was devoted to research (17). In 1944, Mary and her husband,

Albert, who was a supremely successful Madison Avenue advertising executive, maneuvered to place a group of businessmen and other well-connected friends on the ACS board of directors, and they used their advertising and publicity acumen to dramatically increase fundraising (6, 17). Donations soared to \$14 million in 1948, a quarter of which was earmarked for research (17).

Mary first met Sidney Farber in Washington, shortly after his aminopterin results were published, and like Farber, she was fascinated by the notion that chemicals might be able to cure cancer outright (6). They subsequently maintained a regular correspondence. Farber, the willing tutor, wrote meandering "scientific treatises" on his findings, and Lasker, the intelligent, enthusiastic student, absorbed and acted on what she learned (6).

In 1951, Albert Lasker was diagnosed with colon cancer and underwent surgery. He died in May 1952 (17). About the same time, Farber quietly underwent surgery to remove an inflamed colon. He had suffered from chronic inflammatory bowel disease (likely ulcerative colitis, a precancerous disorder that predisposes to colon cancer) (3, 6). Farber never discussed the surgery, and few people knew about the colostomy bag that he expertly concealed under his lab coats and tailored three-piece suits (3, 6). Lasker and Farber emerged from these events more determined than ever. Their cancer crusade was now personal, and it assumed a more urgent, tenacious tone (6).

They shared a common, pragmatic view about how cancer research should be done. The early successes with childhood leukemia and Wilms' tumor convinced them that a systematic search could identify effective drugs, and laser-focused clinical trials of those drugs could optimize treatment regimens to cure all types of cancer. Achieving that goal required only an iron-willed national effort underwritten by Congress (4).

While Lasker lobbied behind the scenes, Farber testified at Congressional appropriations hearings (4). He was compelling, convincing, and animated—and not given to understatement. He had a flair for dramatic, carefully crafted anecdotes and shared poignant stories of cancer patients cured by drug treatment (4).

Their first political success came in 1954. Speaking for the American Cancer Society and the National Cancer Institute, Lasker and Farber pushed Congress to fund an organized search for more anticancer

drugs. The result was the Cancer Chemotherapy National Service Center, a centralized national drug screening program that was established within NCI in 1955 (1, 2, 11). It proved to be one of the government's most successful programs (11).

By 1974, the Service Center had a budget of \$68 million and annually screened 40,000 compounds in about 3 million tumor-bearing mice (11). Vincent DeVita, former director of NCI, claimed that the Service Center was responsible entirely, or in conjunction with the pharmaceutical industry, for all of the cancer drugs discovered and developed between 1955 and 1990 (11).

## Declaring War

Through Lasker and Farber's efforts in Washington, NCI's annual budget almost quadrupled from \$48 million in 1957 to \$176 million in 1967 (1, 3, 4). In the late 1960s, they expanded their messaging beyond Washington to the general public. And, they began referring to their effort as a war on cancer. In December 1969, a full page ad ran in the *New York Times* and *Washington Post*, boldly stating, "Mr. Nixon:

You can cure cancer." The ad quoted Farber, "We are so close to a cure for cancer. We lack only the will and ... the money."

Through their influence on an advisory panel commissioned by President Nixon, Lasker and Farber also pushed to have the National Cancer Institute restructured. They wanted NCI to operate independently from the rest of NIH and to concentrate exclusively on applied cancer research. As examples, they pointed to the Manhattan Project and NASA's Apollo space program, independent federal agencies that had achieved lofty technical goals through total commitment and generous appropriations.

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**Both houses of Congress overwhelmingly passed the National Cancer Act, and President Nixon signed it, with Mary Lasker looking on. In his remarks, Nixon officially declared war on cancer.**

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Not everyone agreed. Critics argued that the science behind cancer was not understood well enough to implement simple solutions. In the words of one cancer expert, Sol Spiegelman, "An all-out effort at this time would be like trying to land a man on the moon without knowing Newton's laws of gravity" (17).

Nevertheless, the publicity blitz, along with Lasker and Farber's incessant lobbying, were generating tremendous momentum on Capitol Hill. In 1971, both houses of Congress overwhelmingly passed the National Cancer Act, and President Nixon signed it, with Mary Lasker looking on. In his remarks, Nixon officially declared war on cancer.

Although NCI remained within the National Institutes of Health—a bitter disappointment to both Farber and Lasker—their influence was apparent in the mandated appropriations. The new law allocated \$1.5 billion over three years to NCI, most of which went toward a crash program of clinical trials to investigate various chemotherapy combinations.

Generous funds also supported the search for universal causes of cancer, such as cancer viruses. Farber was intrigued by the discovery of cancer-causing viruses in animals. In 1964, he pushed NCI to create the Special Virus Cancer Program. The program consumed 10% of the NCI budget, but after

**Mr. Nixon:**  
**You can**  
**cure**  
**cancer**

**CITIZENS COMMITTEE FOR THE CONQUEST OF CANCER**  
400 United Nations Plaza, New York, N.Y. 10018

**Do you agree?** If you agree, please sign and send this card to the Citizens Committee for the Conquest of Cancer, 400 United Nations Plaza, New York, N.Y. 10018. Your signature will be added to a list of citizens who support the fight against cancer.

**Name** \_\_\_\_\_  
**Address** \_\_\_\_\_  
**City** \_\_\_\_\_  
**State** \_\_\_\_\_  
**Zip** \_\_\_\_\_

**Do you agree?** If you agree, please sign and send this card to the Citizens Committee for the Conquest of Cancer, 400 United Nations Plaza, New York, N.Y. 10018. Your signature will be added to a list of citizens who support the fight against cancer.

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**State** \_\_\_\_\_  
**Zip** \_\_\_\_\_

In the Public Domain

Full page ad from the *New York Times* (December, 1969)



a decade of work, it failed to identify a single human cancer virus (6).

To the chagrin of academic scientists and public health officials, the War on Cancer pushed basic research and cancer prevention programs to the sidelines. And a skeptical *Chicago Tribune* editor noted, “A crash program can produce only one result: a crash” (6).

Interestingly, the first notable clinical trial funded under the new Cancer Act was conducted outside the United States (6). NCI researchers had shown that methotrexate, combined with cyclophosphamide and the anti-pyrimidine, 5-fluorouracil, using the acronym CMF, could produce impressive, but temporary, remissions in metastatic breast cancer (11). Paul Carbone at NCI thought it would be better to use chemotherapy to attack the microscopic cancer cells at the margins of the resected breast tumor as an adjunct to surgery rather than as a separate treatment after the cancer had metastasized. He called it “adjuvant chemotherapy.” It was the same strategy that Farber and Pinkel had used a decade earlier to cure Wilms’ tumors (13).

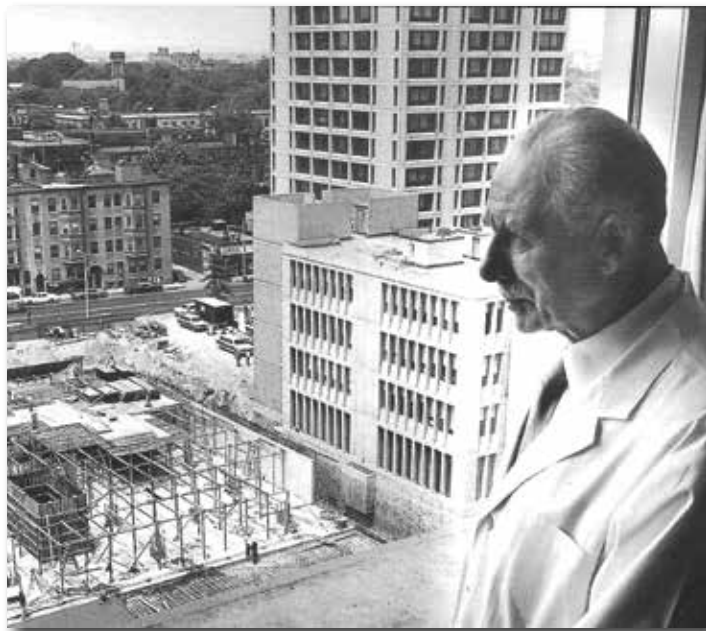
In preliminary experiments, Carbone obtained encouraging results with the CMF combo as adjuvant chemotherapy in conjunction with mastectomy. But surgeons in the United States were not interested in collaborating on a controlled clinical trial. At that time, they thought a radical mastectomy was sufficient to eradicate breast cancer.

Gianni Bonadonna, an oncologist at the Instituto Tumori in Milan, Italy, and his colleague, Umberto Veronesi, the chief breast surgeon at the Instituto, were interested. Bonadonna visited Carbone at NCI to study the CMF protocol and was subsequently awarded a contract under the new Cancer Act to conduct a clinical trial in Italy (11).

The randomized controlled trial began in 1973 and showed that women who received the CMF adjuvant regimen had longer disease-free survival than those who received no CMF treatment (13, 18). Adjuvant chemotherapy with various drugs was subsequently incorporated alongside breast, bone, and colorectal surgeries and led to a significant decline in cancer mortality (11, 13).

### Farber’s Legacy

Meanwhile, Sidney Farber continued to expand the Children’s Cancer Research Foundation in



*Reprinted with permission from the Dana-Farber Cancer Institute*

*Dr. Sidney Farber watching construction of the Charles A. Dana Building in June, 1971*

Boston. In 1958, the Jimmy Fund Building grew from five to nine stories, providing additional laboratory and office space, along with a special suite for Farber on the top floor (3). In the 1960s, Charles A. Dana and his family’s foundation made a series of substantial donations to the Jimmy Fund. In 1969, Farber’s cancer clinic and “total patient care” philosophy were opened to adult patients as well as children (3). To accommodate this expanded mission, the Cancer Research Foundation began construction of the 17-story Charles A. Dana Cancer Center in June 1971. Farber watched construction progress with pride from his office window across the street (3).

Although Farber retired from the Harvard Medical School faculty in 1970, he remained director of the Foundation. Two heart attacks in the late 1960s forced him to slow his workaholic schedule, but he still saw patients and led the Foundation’s ambitious and prolific research agenda (3). On March 30, 1973, he suffered a third and fatal heart attack while working at his desk in the Jimmy Fund Building.

The Children’s Cancer Research Foundation was renamed the Sidney Farber Cancer Center in his honor in 1974 and then the Sidney Farber Cancer Institute in 1976. In 1983, it was renamed the Dana-Farber Cancer Institute, in recognition of the contributions of both Sidney Farber and the Charles Dana family (1).



*Reprinted with permission from the Dana-Farber Cancer Institute*

*Bronze statue depicting Dr. Sidney Farber and "Jimmy" (2013)*

## Jimmy Returns

In May 1998, Einar Gustafson returned to Boston to help Dana-Farber celebrate the 50th anniversary of the original Jimmy Fund radio broadcast. Einar had been the star of that broadcast, but Sidney Farber had chosen to call him Jimmy, partly to protect Einar's privacy and partly to make him a generic poster child, representing all childhood cancer patients. In New England, "Jimmy" was a nickname synonymous with "the boy next door" (6).

Whether through Farber's treatments at the Clinic, spontaneous remission, or both, Einar survived his Burkitt's lymphoma. Farber kept in touch with his young patient, and Einar's family made modest donations to the Jimmy Fund every year. But Einar had returned to a quiet life in Maine (3).

The 62-year-old grandfather of six made no secret that he was Jimmy, but he was inherently modest and spent his time raising three daughters and running his trucking business. When his sister contacted Dana-Farber in 1998 to reconnect the Institute with Jimmy, Einar was more relieved than annoyed. He willingly re-entered the spotlight and represented Dana-Farber in a series of personal appearances as the now-grownup Jimmy (3, 4, 6).

With elucidation of the human genome, the discovery of oncogenes, and most recently the immunotherapy approach, scientists and clinicians continue to make progress, and Dana-Farber remains at the forefront of cancer research, therapeutics, and prevention. Sidney Farber's philosophy of translational medicine and total patient care has now been widely adopted, and NCI's network of Comprehensive Cancer Centers ensures that laboratory discoveries move seamlessly to the clinic as innovative treatment and supportive care for all cancer patients.

Curing cancer was always Sidney Farber's goal. Although he could not claim victory, in his lifetime and largely through his efforts, acute childhood leukemia, Wilms' tumor, Hodgkin's disease, choriocarcinoma, and metastatic breast cancer went from incurable to diseases with dramatically high survival rates. And methotrexate, the compound that emerged from his original folic acid antagonist research more than 60 years ago, remains a mainstay (now often coupled with leucovorin rescue) of combination chemotherapy regimens as well as a treatment for autoimmune diseases such as rheumatoid arthritis and psoriasis. Methotrexate also remains on the World Health Organization's Model List of Essential Medicines.





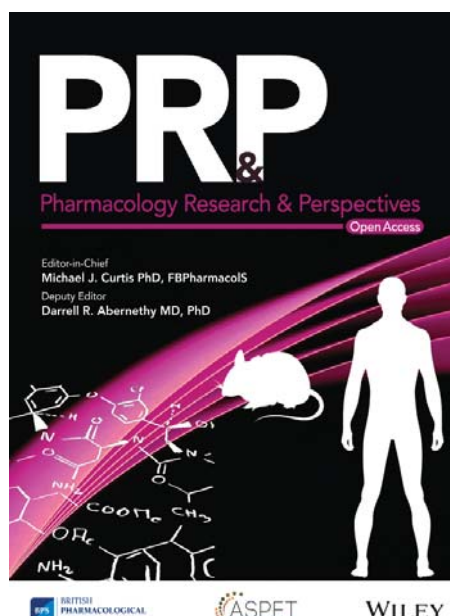
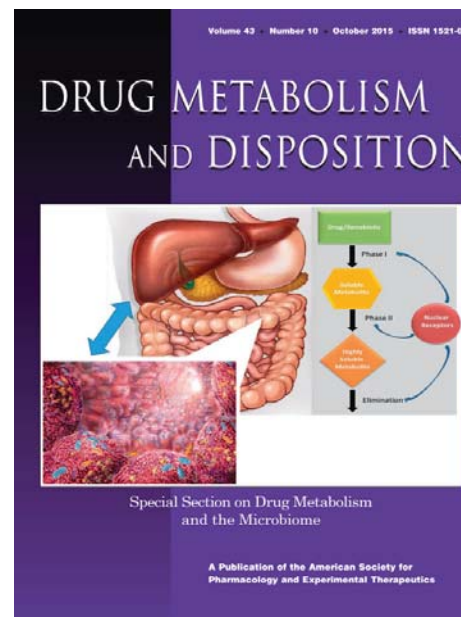
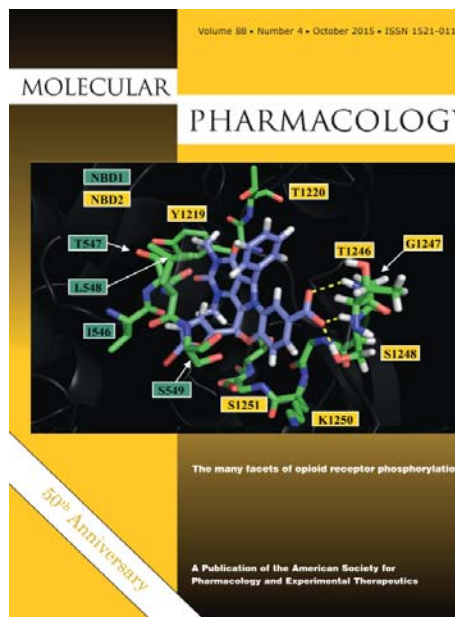
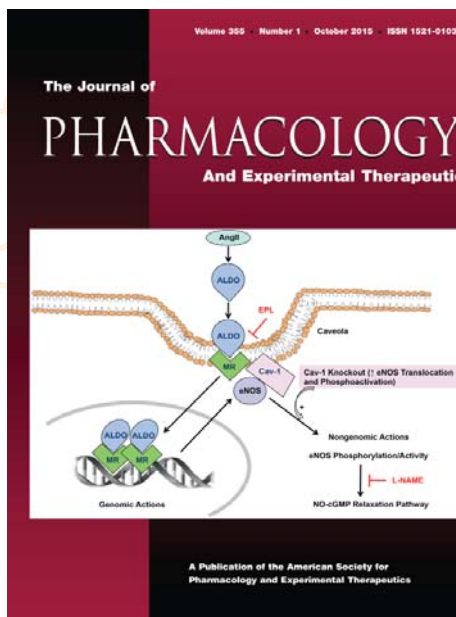
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