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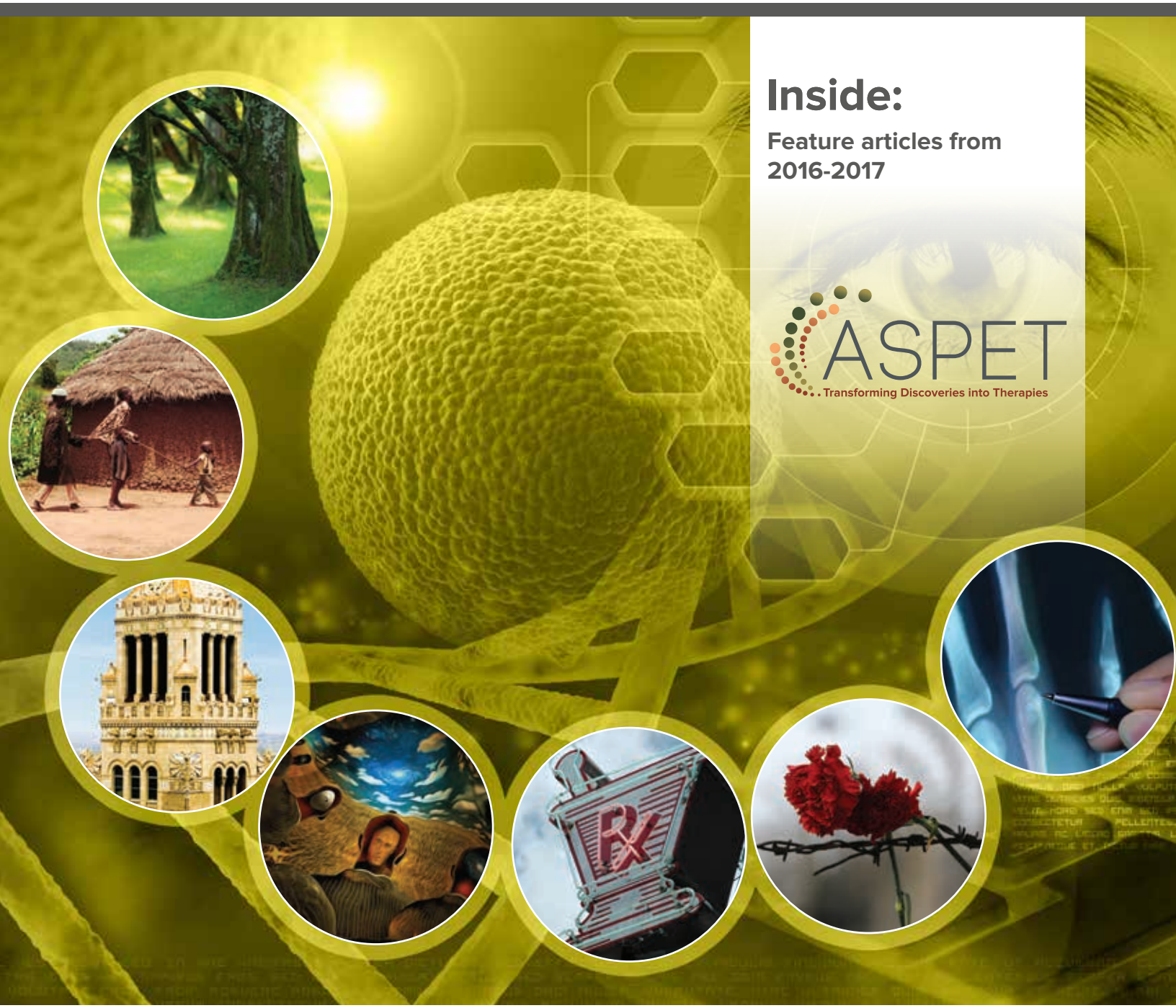
THE Pharmacologist



Inside:

Feature articles from 2016-2017

ASPET
Transforming Discoveries into Therapies

2018 Special Edition

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Adventures in Pharmacology

ASPET is pleased to present the second in a series of special editions of our quarterly news magazine, *The Pharmacologist.* This special compilation issue highlights feature articles written by ASPET member and science writer Dr. Rebecca J. 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 take us around the world. We travel from the Pacific Northwest, where researchers harvest bark from yew trees *(Taxol: Barking Up the Right Tree,* June 2016), to Sub-Saharan Africa, where much needed drugs are being developed to treat patients suffering from river blindness *(Ivermectin and River Blindness: The Chip Shot Heard Around the World,* March 2016). Rebecca's stories often provide a historical perspective, with articles touching on treating mental illness *(Chlorpromazine and the Dawn of Antipsychotics,* September 2016), as well as typhus patients during World War II (*Typhus: War and Deception in 1940's Poland,* March 2017). Her stories also cover current events that impact our daily lives, such as the story dealing with pharmacy compounding *(The Perils and Promise of Pharmacy Compounding,* June 2017). No matter what topic, Rebecca's articles 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.

Ivermectin and River Blindness:

The Chip Shot Heard Around the World

Rebecca J. Anderson, PhD

On Christmas Eve 1984, the Food and Drug Administration approved an antiparasitic drug to treat reindeer *(1)*. It was not the drug's first approval, and regulators would subsequently authorize many other indications. But the FDA's yuletide decision, though purely coincidental, somehow seems poetically fitting. The drug was ivermectin, and it was truly a gift to the world.

Satoshi Ōmura's journey would eventually end in Stockholm, but in 1971, his sights were set only on his sabbatical in the United States. He had received degrees in pharmaceutical science and chemistry, and since 1965, he had been a researcher at the Kitasato Institute.

The Tokyo-based Institute had a proud tradition of applied bioresearch accomplishments. Founded in 1914 by Shibasaburo Kitasato, who isolated the tetanus bacilli and discovered tetanus antibodies, the Institute housed many outstanding researchers. Kiyoshi Shiga discovered the dysentery bacillus, *Shigella dysenteriase*.

Dr. Satoshi Ōmura collecting a soil sample at the site where the original Streptomyces avermectinius *sample was collected over 30 years earlier.*

Sahachiro Hata, along with Paul Ehrlich, discovered Salvarsan for syphilis. Shinkichi Umeno invented the rabies vaccination system. And, Taichi Kitajima pioneered immunotherapy for cholera *(2)*.

Like all of his colleagues, Ōmura followed Dr. Kitasato's philosophy that "research should be applied as quickly as possible for the protection of people from contagious diseases" *(2)*. In his first six years at the Institute, Ōmura devised several innovative methods for isolating and culturing microorganisms from soil and other environmental samples. He also enhanced the sensitivity of bioactivity screens, which accurately detected minute concentrations of medically relevant substances produced by those microorganisms.

During his 18-month sabbatical at Wesleyan University in Connecticut, Ōmura worked alongside Max Tishler, a professor of chemistry. Tishler had retired from Merck, Sharp, and Dohme after a distinguished 32-year career, rising to senior vice president of research and development.

While the Kitasato Institute could isolate and characterize potentially useful natural products, it did not have the resources to develop and market them. Ōmura saw the value of working with an industrial partner *(3)*. With Tishler's assistance, he approached Merck and proposed a collaboration, which was formally established in 1973 *(3, 4)*.

Rebooting Antiparasitic Research

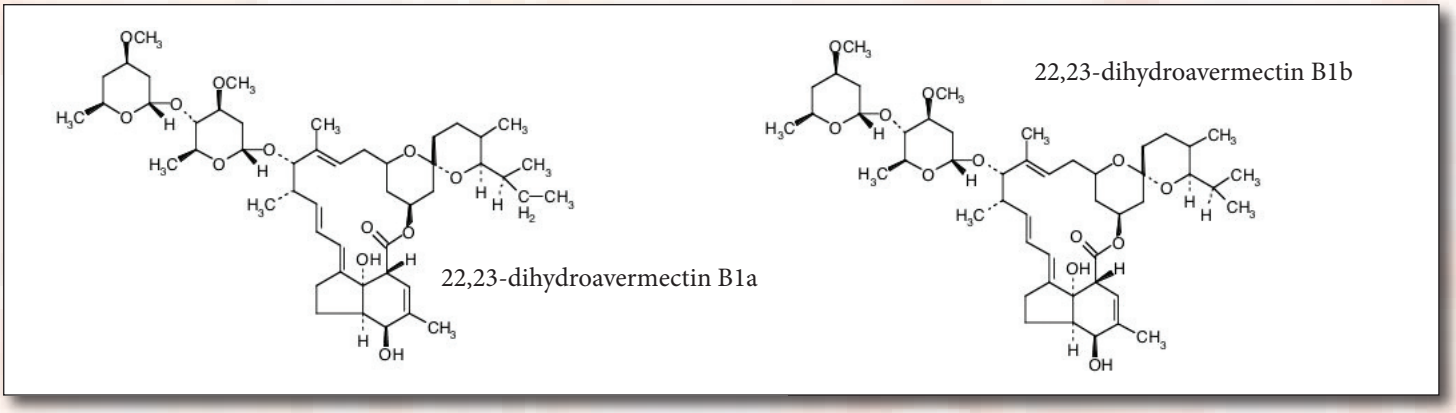
The timing could not have been better for Merck. Since 1955, scientists in Merck's animal health research division had been working to develop a profitable veterinary antiparasitic drug. At the time, veterinarians had no drugs that selectively eradicated intestinal worms *(5)*.

The best source for such compounds (as with many antibiotics and other antimicrobial drugs) was thought to be environmental microbes—particularly those found in soil. Pharmaceutical researchers encouraged their coworkers to bring back a bag of dirt whenever they traveled for business or pleasure. Microbes were extracted from the soil samples and grown via fermentation. The natural products generated by those microbes during fermentation were then evaluated for medical utility.

The Merck scientists set up several in vitro assays to detect antiparasitic activity. Unfortunately, most of the compounds registering activity in those assays were toxic—killing both the parasite and its host *(6)*. Reluctantly, they turned to an in vivo assay using mice. Although animal-based drug screening was expensive and time-consuming, the scientists could more easily distinguish between active and nonselectively toxic compounds.

Mice infected with a nematode (roundworm) were fed a lab chow that had been mixed with the experimental fermentation product. Later, fecal pellets and the animal's intestines were examined for the presence of parasitic eggs and worms, respectively *(6)*. About 1% of the experimental substances they tested showed activity. Cultures of those microbes were regrown and the fermentation products retested, but unfortunately, most of them were still extremely toxic and could not be pursued further *(6)*.

By the early 1970s, Merck researchers had tested many thousands of compounds, were facing diminished returns from their testing, and were finding it harder to justify the laborious mouse assay. When Ōmura approached Merck, they were keen to explore this new source of compounds *(3)*.



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Ivermectin (22,23-dihydroavermectin B_{1a} + 22,23-dihydroavermectin B_{1b}) is a broad-spectrum antiparasitic drug in the avermectin family.

Under the collaboration, Ōmura and his team at the Kitasato Institute were responsible for isolating microorganisms, identifying active compounds, and carrying out in vitro evaluations. Scientists at Merck's research laboratories in Rahway, NJ, handled the in vivo work and were responsible for developing any promising compounds *(2, 6)*.

In the second year of the collaboration, the Kitasato Institute sent a batch of 54 samples to Merck for testing. One of them was a fermentation broth from soil sample OS-3153, which had been collected at the oceanfront Kawana Golf Course in Japan's Shizuoka Province *(3, 5)*.

On May 9, 1975, the researchers fed a sample of the OS-3153-derived broth to a single mouse *(7)*. The broth completely eliminated larval eggs and intestinal worms in the mouse—a level of antiparasitic activity the Merck scientists had never seen before *(6)*. Over the next few weeks, they repeated and expanded their studies of the OS-3153 sample in more mice and under a variety of conditions, confirming the substance's amazing potency and selectivity.

The microorganism in the OS-3153 sample entered Merck's culture collection as MA-4680, and it was submitted for detailed characterization. It was a brownish-gray bacterium with spiral side branches bearing smooth, spherical spores *(6, 8)*. MA-4680 belonged to the *Streptomyces* genus of bacteria, but it was unlike any previously described species. They named it *Streptomyces avermitilis,* meaning the species that was "capable of separating from worms" *(6)*.

The antiparasitic substance produced by *S. avermitilis* turned out to be a mixture of eight closely related macrocyclic lactones, which the Merck scientists called avermectin *(6, 8)*. The chemical structure of the avermectins was unlike any previously known class of molecules, and they proved to have wide utility.

Reprinted with permission from Elsevier, Photo: Kitasato Institute

Photomicrograph of Streptomyces avermectinius

A Broad-Spectrum Drug

Characterizing the biological activity of the avermectins fell to an interdisciplinary team headed by parasitologist William Campbell *(3)*. Born in Ireland, Campbell conducted his doctoral research at the University of Wisconsin-Madison on a Fulbright Scholarship. In 1957, he joined the Merck Institute for Therapeutic Research and became a US citizen in 1962.

Campbell always had a "particular fondness" for parasitic worms *(7)*. The subject of his doctoral thesis was the liver fluke, a flatworm that threatened sheep in his native Ireland. Among his early successes at Merck was development of thiabendazole, a fungicide and antiparasitic drug that Merck launched in 1962 as Thibenzole® *(4)*.

After confirming the activity of the avermectins in the mouse nematode model, Campbell proceeded to test their efficacy in domesticated animals. In sheep, cattle, and chickens, the avermectins were effective against both mature and immature parasitic roundworms but had no effect on flatworms, protozoa, bacteria, or fungi *(9)*.

Campbell's group found that the avermectins were also effective against insects *(6)*. Collaborating with the Boyce Thompson Institute for Plant Research in Ithaca, New York, the Merck scientists screened 75 structurally related natural products and semisynthetic avermectin derivatives in the Institute's mite and insect screening assays under greenhouse conditions. Avermectin B_1, the major fermentation product from *S. avermitilis,* emerged as the most promising candidate for agricultural use *(6)*.

Avermectin B_1 was given the Merck development code number MK-936 and was evaluated worldwide for efficacy in protecting a number of agricultural crops, including citrus, cotton, apples, pears, vegetables, potatoes, tree nuts, and grapes *(6)*. Under field conditions, avermectin B_1 provided excellent control of many pests, including the red mite, spider mite, Colorado potato beetle, diamond back moth, pear psylla, and red fire ant *(6)*.

Avermectin B_1 was even effective against mites and insects that had become tolerant to commercially available organophosphate, carbamate, chlorinated hydrocarbon, and pyrethroid pesticides *(6)*. In 1979, Campbell's group publicly announced, "The avermectins would appear to have unprecedented potency and spectrum of biological activity" *(9)*.

The two avermectin B_1 compounds generated by *Streptomyces avermitilis* (avermectin B_{1a} and avermectin B_{1b}) both had impressive activity and could be produced in high yield *(6, 8)*. However, a double bond between C_{22} and C_{23} restricted the molecules' flexibility, and test results suggested that the structural constraint might be affecting bioactivity. Chemists therefore eliminated the double bond to produce semisynthetic 22, 23-dihydroavermectin B_{1a} and B_{1b}.

To distinguish the dihydro- compounds from the naturally generated avermectins, the researchers suggested "hyvermectin" as the new generic name. Someone pointed out that "hyver" in some language meant testicles *(7)*. They settled on ivermectin instead. Chemically, the substance that underwent further development and commercialization as "ivermectin" is actually a mixture of the two semisynthetic analogs. The manufacturing process specifies that ivermectin contains at least 80% of 22, 23-dihydroavermectin B_{1a} and not more than 20% of 22, 23-dihydroavermectin B_{1b} *(6)*.

Campbell's colleague, Lyndia Blair, tested ivermectin in an assay she had developed for evaluating compounds against heartworms in dogs. Ivermectin did not affect adult heartworms but did kill premature larvae and proved to be an effective maintenance treatment to prevent canine heartworm infection *(7, 10)*.

A Veterinary Blockbuster

Ivermectin represented a major breakthrough in veterinary medicine. Like other pesticides, it attacked insects, spiders, and parasites that live outside their hosts (e.g., fleas and mites). But importantly, ivermectin was the first "endectocide;" that is, it was effective against parasites that live inside their host *(3, 5)*.

Ivermectin was effective in unprecedented low doses and could be used orally, topically, and parenterally without producing observable toxic reactions in the animal hosts. And, it had a unique mechanism of action *(5)*.

Ivermectin forces opening of glutamate-gated chloride channels, which are common in roundworms, insects, and ticks. The uncontrolled influx of chloride ions paralyzes the organisms' pharyngeal and somatic muscles. Flukes, tapeworms, and other flatworms lack these receptors, which probably accounts for their resistance to ivermectin. In vertebrates, ivermectin stimulates GABA release. Fortunately, GABA neurons are mostly in the brain and protected by the blood–brain barrier, making the drug exceptionally safe for mammals *(5)*.

In 1981, ivermectin was introduced commercially as a veterinary antiparasitic agent, and in 1985 it was launched as an agricultural pesticide. It was immediately hailed as the most effective, broad-spectrum antiparasitic drug ever developed *(1)*. Within two years, ivermectin became the market leader, a position it has maintained ever since *(4)*.

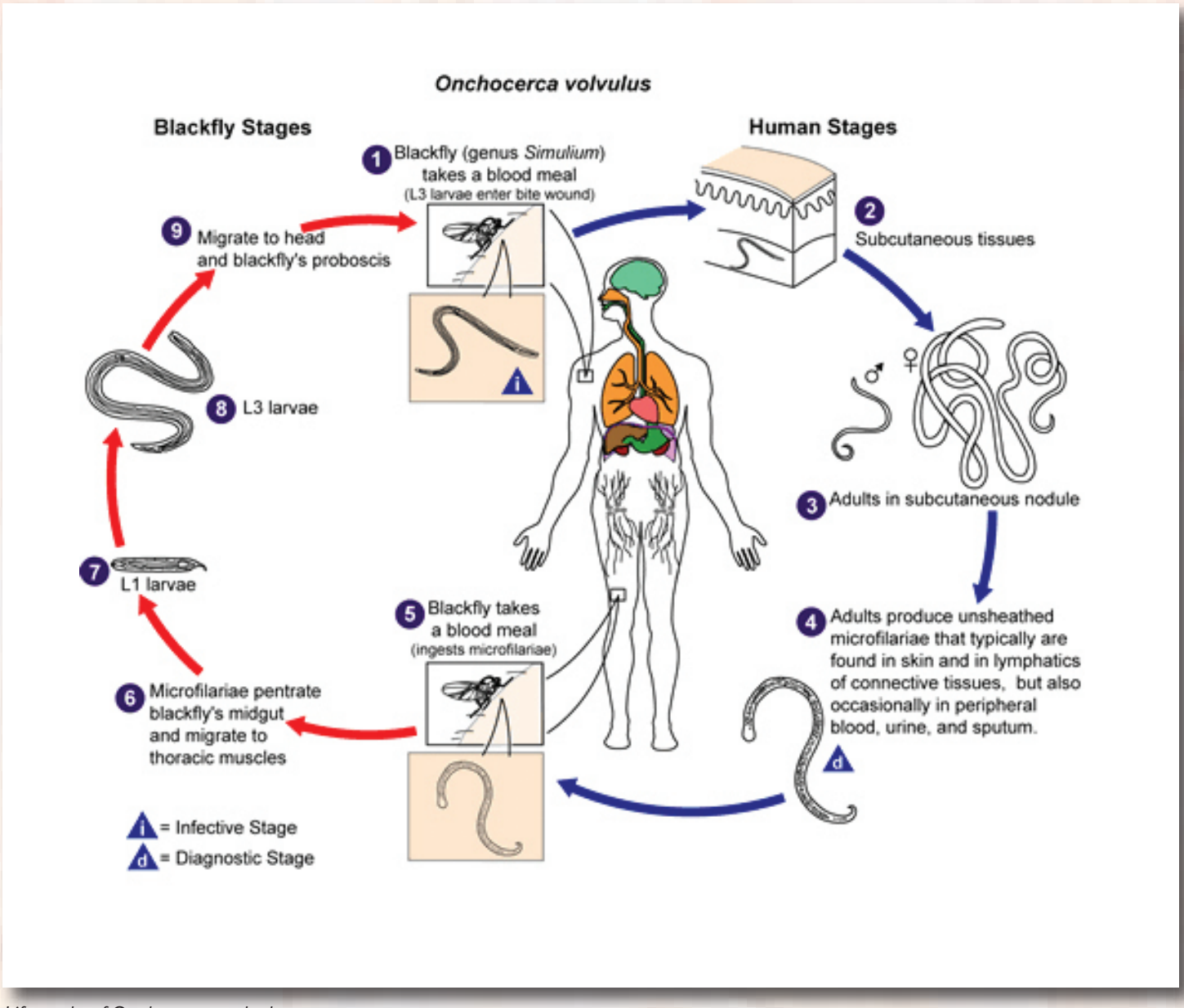
By 1987, ivermectin was Merck's second largest selling product and now has annual sales of about $1 billion. It is marketed as Ivomec® for cattle, pigs, and sheep; Equalin® for horses; and Heartgard® for dogs *(4)*. Livestock around the world are regularly treated (using dips, injections, feeds, and other formulations), and virtually every horse and pet dog in the US receives it to prevent dermatitis and heartworm infections, respectively *(2, 5)*.

The Human Connection

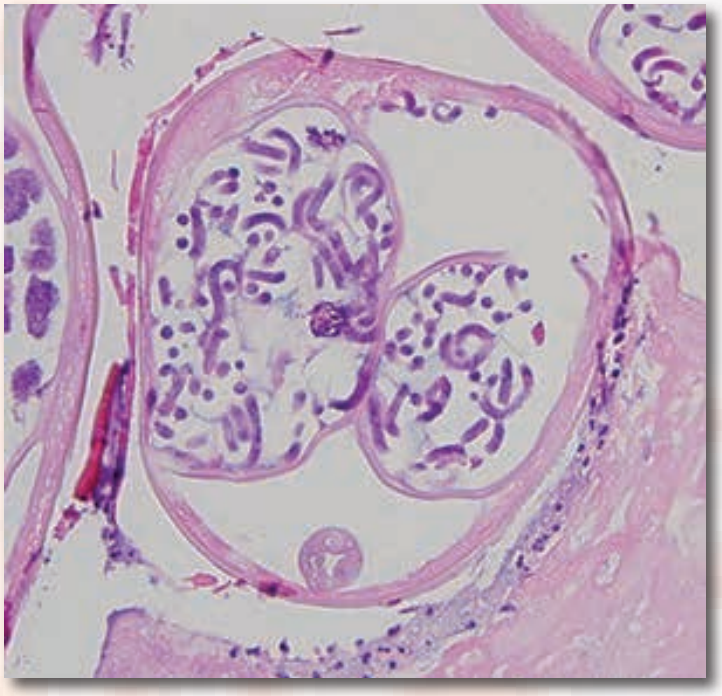
In April 1978, while conducting the screening assays to characterize ivermectin's spectrum of activity, Lyndia Blair found that the drug was effective against the larvae of *Onchocerca cervicalis,* a skin-dwelling parasite in horses *(7, 11)*. *O. cervicalis* is fairly benign to horses, and this finding was of little commercial significance. However, the horse parasite belongs to the same genus as *Onchocerca volvulus,* a human roundworm parasite. *O. volvulus* causes onchocerciasis, a disease that afflicts millions of people worldwide *(12)*.

When William Campbell saw Blair's results, he decided to explore whether ivermectin had potential as a drug for humans. It was not the first time Campbell had made this leap. While developing thiabendazole for the veterinary market, he facilitated Merck's clinical trials of the drug for trichinosis. The FDA approved thiabendazole (Mintezol®) for trichinosis in 1964, the first antiparasitic drug to spin off from veterinary to human medicine *(4)*.

In July 1978, Campbell sent ivermectin, along with the horse assay results, to researchers at James Cook University in Australia *(11)*. The World Health Organization's Special Program for Research and Training in Tropical Diseases (TDR) had contracted the Australian lab to screen drugs in cattle infected with *Onchocerca gibsoni* and *O. gutturosa*. This animal model was considered to be the best predictor of drug efficacy in humans with onchocerciasis *(12)*.



Life cycle of Onchocerca volvulus



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Onchocerca volvulus *in tissue, stained with H&E.*

The Australians reported that ivermectin was "highly effective in preventing patent infections" of the cattle parasites *(11)*.

River Blindness

Onchocerca volvulus is a roundworm parasite whose existence depends entirely on finding the right insect and human hosts at the right times in its lifecycle. When black flies bite an infected person, they ingest microscopic worm larvae along with their blood meal. The tiny larvae mature through three stages as they migrate from the black fly's gut to its head and mouth parts. The L3 larvae then enter the skin of another human victim the next time the fly feeds. The L3 larvae in the new human host then differentiate through three more stages and mature into male and female worms within about a year *(13)*. Adult male worms grow to 1.5 inches in length and females to 27 inches, forming nodules under the skin. The fertilized females produce 1,000 micro-larvae per day for up to 12 years. These microscopic larvae move easily through the skin and lymphatic vessels of connective tissues, making them accessible when black flies next feed on the human host *(13)*.

If the micro-larvae are not eaten by black flies, they cannot mature through the next larval stages. Those that remain in the infected human die after 9-18 months, and it is the dead larvae that cause the most damage to their human host *(3, 13)*. The larval residue causes skin rashes, enlarged lymph nodes, and impaired vision. In the skin, the dead larvae induce depigmentation and itching so intense that it reputedly can lead to suicide *(14)*.

Infiltration of micro-larvae in the eye leads to blindness—the second highest cause of blindness from infectious disease *(12)*. Failing eyesight develops slowly, but by the age of 50, victims' eyes become scarred and lifeless. Along the dusty roads everywhere in endemic regions, blind men are led by boys, each holding one end of a walking stick *(14)*.

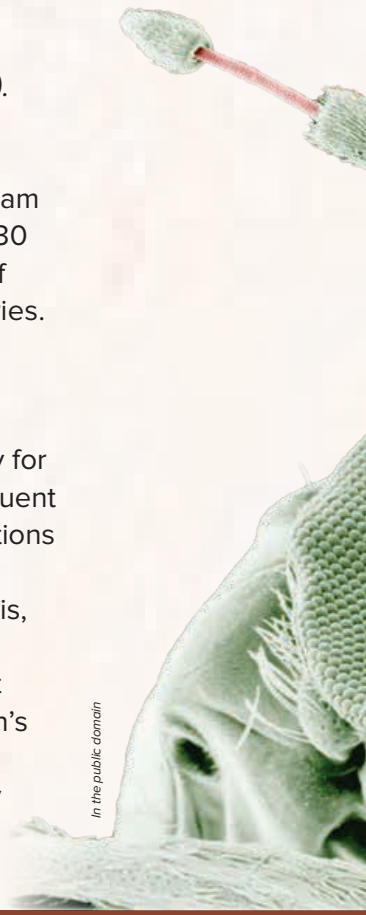
Black flies breed in the highly oxygenated, fast-flowing rivers that irrigate Africa's fertile savannas—some of the continent's most productive agricultural lands. The concentration of black flies and farmers in these regions allows *O. volvulus* to thrive and has given the disease its common name, river blindness. Of those infected worldwide, 99% live in Sub-Saharan Africa *(15)*. To a lesser extent, river blindness also afflicts people in tropical regions of six Latin American countries and in Yemen.

WHO Takes Action

In 1974, Robert McNamara, then head of the World Bank, recognized river blindness as a major disease with widespread health and socioeconomic consequences, especially in the West African savanna regions *(12)*. The World Bank joined the World Health Organization in launching the Onchocerciasis Control Program in West Africa (OCP). It targeted 30 million people who were at risk of infection in 11 West African countries.

Only two drugs were available to treat river blindness: diethylcarbamazine and suramin. Diethylcarbamazine is taken orally for 7-10 days. Because it causes frequent side effects and serious complications (lymph node pain, myalgias, conjunctivitis, hypotension, keratitis, chorioretinal damage, and optic neuritis), diethylcarbamazine must be administered under a physician's supervision *(1, 16)*.

Diethylcarbamazine effectively kills and rapidly eliminates



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micro-larvae from the eye and keeps the eye clear for a year or more. Unfortunately, the acute accumulation of dead parasitic tissue (particularly in the eye) elicits a violent and dangerous hypersensitivity response called the Mazzotti reaction *(3, 12, 16)*. This exaggerated inflammatory response often causes eye damage *(12)*. Despite its drawbacks, diethylcarbamazine was the drug of choice.

Suramin (developed 50 years earlier to treat sleeping sickness) was the only drug option for killing adult worms, but it also has several disadvantages *(12)*. It must be given intravenously once a week for six weeks and is highly toxic, often causing severe and occasionally fatal adverse reactions *(1, 3, 12)*.

By the mid-1970s, it was clear that both drugs could actually worsen eye damage, and so their use as river blindness treatments was stopped *(3)*. Instead, OCP sought to kill the parasite's vector, black flies, in West Africa. Intensive aerial insecticide spraying of the fly's breeding waters was aimed at reducing their numbers enough to break the cycle of parasite transmission *(5, 14)*.

Unfortunately, aerial spraying was not feasible or cost effective in the other regions of Sub-Saharan Africa where river blindness was also an endemic problem *(17)*. Also, some insects became resistant to the insecticides, and black flies were more mobile than expected. Consequently, people in areas that had been cleared of the parasite could be re-infected by a new wave of black flies *(4, 14)*. Aerial spraying was not a long-term solution.

A New Approach

Those afflicted with river blindness are among the world's poorest people. Their inability to pay for medication—even if there was a drug—provided little incentive for pharmaceutical companies to find treatments *(4)*.

When the World Health Organization launched TDR in 1975, its mandate was to find effective ways to combat eight neglected "diseases of poverty," including river blindness *(11, 12)*. TDR sponsored aggressive research—most significantly, a three-tiered screening program to find antiparasitic drugs. Because of the severe side effects (i.e., the Mazzotti reaction) associated with drugs like diethylcarbamazine that killed micro-larvae, TDR sought a new, nontoxic drug that killed adult roundworms *(11, 12)*.

The Australian laboratory where Campbell sent ivermectin in 1978 had been contracted by TDR to screen promising drugs in cattle, a tertiary animal model for river blindness.

Transitioning to Patients

On December 20, 1978, Campbell presented the Australian results to Merck's senior research management council as evidence that ivermectin might be effective in treating river blindness *(11)*. Roy Vagelos, Merck's senior vice president for research, was still new to the pharmaceutical industry. He had moved from his position as chairman of biological chemistry at Washington University Medical School just three years earlier and his expertise was not parasitology, but he approved Campbell's request for clinical research funds *(11, 18)*.

An adult black fly with the parasite Onchocerca volvulus coming out of the insect's antenna, magnified 100x.

Over the next year, Merck researchers met repeatedly with TDR officials and shared their ivermectin data. TDR expressed little enthusiasm and only offered some technical advice. Merck's drug selectively killed micro-larvae, and TDR's priority was to find a drug that killed adult worms *(11)*. On January 16, 1980, Merck's senior management decided to proceed independently with Phase I clinical trials *(11)*.

Campbell passed responsibility for clinical development of ivermectin to Mohammed Aziz *(4)*. A native of Bangladesh, Aziz was senior director for clinical research at Merck. Prior to joining Merck, he had worked for WHO in Sub-Saharan Africa and was an expert in tropical medicine *(18)*.

Reprinted with permission from Leedy Hoque

Dr. Mohammed Aziz

Aziz and a small group of Merck investigators went to Senegal (on the West coast of Africa) and initiated the Phase I trial at the University of Dakar on February 24, 1981 *(18, 19)*. Using a dose-escalation protocol in 32 men with mild infections (but no eye damage), Aziz found that ivermectin reduced the number of micro-larvae in a dose-dependent manner. A single oral dose of 50 µg/kg completely eliminated the larvae in skin snip specimens for a month, and all of the men tolerated the drug well *(20)*. Best of all, despite the apparent efficient killing of micro-larvae, none of the men experienced eye damage or other inflammatory responses associated with the Mazzotti reaction *(20)*.

In Paris, a second dose-ranging trial with 20 immigrants from Senegal and Mali—some with eye infections—confirmed and expanded the results. Single oral doses up to 200 µg/kg were well tolerated. Seven patients who were followed for a year maintained low skin micro-larval density and had no ophthalmological side effects *(12, 20)*.

In November 1982, Merck visited TDR and OCP in Geneva and presented results from the Phase I trials *(11, 12)*. The impressive clinical data and the growing ineffectiveness of OCP's insecticide spraying program bolstered the World Health Organization's interest in ivermectin *(11, 19)*. TDR agreed to join Merck in a collaborative research program. But each party, though hopeful, proceeded with some wariness about the other organization *(11)*.

The two sides had different motives *(12)*. The WHO agencies saw ivermectin as a new community-level tool for disrupting parasite transmission and helping to reduce the prevalence of river blindness in endemic communities. They favored community-based trials under field conditions, an essential step toward mass-treatment programs *(12)*.

On the other hand, Merck approached the ivermectin clinical trials like any other regulatory-compliant development program. The company wanted a commercial product that would generate a return on its investment *(12)*.

In 1983, Aziz proceeded with fairly standard Phase II and III clinical trials in Ghana, Guatemala, Côte d'Ivoire, Liberia, Mali, Senegal, and Togo *(11, 12, 19)*. TDR provided Merck with access to its existing network of onchocerciasis researchers and institutions *(11)*. Among these collaborations, Merck adopted a scoring system developed by Kwable Awadzi at the Onchocerciasis Chemotherapy Research Center in Ghana, a core facility supported by TDR. The method quantified commonly observed clinical responses to antiparasitic drugs *(11, 12)*.

In the trials, a single dose of 150 to 200 µg/kg effectively reduced the density of micro-larvae to near zero within a month, and the larvae remained at low levels for up to 12 months *(16, 19, 21)*. Adverse reactions were mild and transient—without triggering a Mazzotti reaction. Even in patients with severe larval infiltration in the eye, vision generally improved after the larvae cleared *(21)*.

Because most of the countries where river blindness was endemic were former French colonies or had expatriates living in France, Merck submitted its ivermectiin application to the French regulatory authorities *(5, 11, 18)*. The submitted data came exclusively from Merck's clinical trials, which had enrolled 1,200 onchocerciasis patients *(12)*. Ivermectin under the brand name Mectizan® was approved for human use on October 21, 1987, facilitating registration in other French-speaking countries *(11)*.

A New Paradigm

In parallel with the clinical trials, Merck's marketing department struggled to find ways to recover development costs and make a profit on the approved product. Based on the anticipated advantages of ivermectin, production costs, the pricing of similar antiparasitic drugs, and other factors, they arrived at a price of $3 per tablet *(4, 11)*. Unfortunately, as Roy

Reprinted with permission from Merck

Bottle of Mectizan

Vagelos explained to a reporter, "We realized that the patients who need it don't have the money to support purchase of a drug at any price" *(14)*.

By June 1986, Merck's executives had considered and rejected all of their conventional marketing options. They began exploring novel alternatives. Vagelos (who had become CEO of Merck) and his executive team searched extensively for partners willing to cover the company's expenses, so they could make ivermectin available at no cost to patients *(11)*.

They met with WHO, the US Agency for International Development, the US Department of State, European and African governments, and private organizations—all without success *(4, 11)*. Senators Ted Kennedy (D-MA), Bill Bradley (D-NJ), Frank Lautenberg (D-NJ), and Richard Lugar (R-IN) sponsored congressional funding for worldwide distribution of ivermectin, but Congress rejected their proposal *(4)*. Everyone thought the humanitarian effort was worthwhile, but no one was willing to cover the drug's costs.

Within Merck, employees began discussing the idea of donating ivermectin, citing a philosophy first expressed in 1950 by George W. Merck, the founder's son. He had told an audience at the Medical College of Virginia that "medicine is for the people...The profits will follow, and if we have remembered that, they have never failed to appear" *(4)*.

Merck had already established a reputation as a socially conscious pharmaceutical company. After World War II, Merck donated a large supply of streptomycin to the Japanese, who were suffering from high rates of tuberculosis. In 1958, the company established the Merck Medical Outreach Program, donating antibiotics, antiparasitics, and vaccines to ongoing humanitarian programs in developing countries and disaster situations *(4)*.

The same day that ivermectin was approved for river blindness in Paris, Merck announced the Mectizan Donation Program at a press conference in Washington, DC *(4, 11, 18)*. Having obtained consent from the Kitasato Institute, which agreed to forego its royalties, Vagelos said that Merck would provide ivermectin free of charge for the treatment of river blindness for "as long as it is needed" *(3, 4)*.

The impressive income generated by the various ivermectin veterinary products made Merck's decision somewhat easier. Those products were bringing in more than $300 million annually, and sales were growing at 15% per year—more than enough to cover the cost of the donation program. Offsetting the loss of income, the donation program fostered corporate good will, enhanced employee morale, and permitted an attractive tax write-off *(4)*.

For the donation program, bulk quantities of avermectin were produced at a fermentation plant in Pennsylvania and shipped to the UK or Puerto Rico for chemical conversion into ivermectin. The bulk ivermectin was then formulated into tablets in the Netherlands, and the tablets were packaged in France for distribution *(11)*. Merck paid all of the costs for production, shipping, customs, and other handling charges *(3, 11, 19)*.

Medicine is for the people...The profits will follow, and if we have remembered that, they have never failed to appear

Despite its willingness to donate ivermectin, Merck still had to resolve several other corporate concerns. The company did not want to be responsible for deciding who received the drug or how it was distributed. However, to limit its liability, the company wanted a system for monitoring side effects. Merck was also concerned about illegal rerouting of the drug to the black market or to the veterinary drug market *(11)*.

In February 1988, Merck and WHO established the Mectizan Expert Committee, comprised of seven international experts in tropical medicine and public health. This independent body was headquartered at

the Carter Center in Atlanta, Georgia, and processed applications from organizations and governments wanting to distribute ivermectin *(4, 11)*.

Community-Based Programs

TDR and OCP still advocated community-based trials under field conditions—a necessary step toward mass-treatment programs. As Merck's Phase III clinical trials were concluding, TDR and OCP pushed forward with field trials, with substantial support from Merck *(11, 12)*.

Between 1987 and 1989, thirteen Phase IV community-based trials were conducted, involving 120,000 doses of ivermentin *(11, 12)*. TDR funded five trials in Liberia, Cameroon, Malawi, Guatemala, and Nigeria. OCP funded eight other trials in Ghana, Mali, Togo, Benin, Ivory Coast, Guinea, Burkina Faso, and Senegal.

These community trials established that mass treatment with ivermectin could significantly reduce parasitic transmission *(17)*. Black flies continued to land and feed on people. But because of ivermectin treatment, there were few or no micro-larvae in the skin, thus interrupting the parasite's lifecycle and preventing its spread to new victims *(21)*.

Mass distribution of ivermectin began in 1988 *(4)*. Initially, mobile teams of health workers from OCP and government health ministries visited communities to administer the ivermectin tablets *(2, 4, 13)*. But this procedure was costly and inefficient *(17)*. The mobile teams were often frustrated because villagers did not appear when they arrived, and they needed to remain in each village for 2-3 days to check for possible side effects *(2)*.

By 1989, the accumulated experience and data made it clear that ivermectin was safe and easy to administer. WHO announced that individual medical supervision was not necessary *(2, 21)*. TDR further refined and developed the Community-Directed Treatment with Ivermectin (CDTI) program, which was formalized in 1997 *(2, 17)*.

CDTI empowered communities to organize, direct, and manage their own treatment. Selected residents in each community receive 2-3 days of training to treat themselves and their neighbors, monitor side effects, complete records, and keep track of drug distribution. Dosing is determined using a measuring stick. Children who stand shorter than the stick get one pill. Adults and adolescents get two pills *(5, 18)*.

The control of river blindness is now almost exclusively based on annual or semiannual treatment with ivermectin through CDTI. The program is self-sustaining and has become a way of life in Africa *(18, 19)*. Now, many local volunteers who were recruited

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"Sightless Among Miracles" by R. T. Wallen, American, 1995. The 8-foot bronze sculpture commemorates the global effort to eliminate onchocerciasis, commonly known as river blindness. The study depicts a young boy leading his father, blinded by the disease. The sculpture was commissioned by Merck & Co. and placed in their world headquarters in 1995.

for the CDTI program also distribute vitamin A (also to prevent blindness) and coordinate home-based malaria and HIV/AIDS care for their communities *(2, 18)*.

Public-Private Partnerships

Public-private collaborations existed before the Mectizan Donation Program, but they were initiated by public sector institutions that sought corporate sponsorship. In contrast, Merck's program emerged from the company's decision to develop a drug without a market, donate it, and assemble public organizations to aid in its distribution *(4)*.

The Mectizan Donation Program (comprised of Merck, WHO, 32 national governments, and 12 international nongovernment organizations) remains the largest ongoing medical donation program in history

Public-private partnerships modeled on the ivermectin program now produce and distribute many other items for which there may be no likelihood of commercial return *(2)*. But the Mectizan Donation Program (comprised of Merck, WHO, 32 national governments, and 12 international nongovernment organizations) remains the largest ongoing medical donation program in history *(11, 19)*.

In recognition of Merck's corporate philosophy and stewardship, *Fortune* magazine named Merck as America's most admired company for seven straight years, from 1986 to 1992 *(4)*.

Ivermectin Continues to Impress

Further investigations demonstrated that ivermectin's therapeutic utility extended beyond river blindness. In the 1990s, Merck and TDR reported the drug's efficacy against lymphatic filariasis, also known as elephantiasis. Infected mosquitoes transmit the parasite larvae responsible for elephantiasis to humans through bites. Larvae develop into adult worms in the individual's lymphatic vessels, causing painful, disfiguring swelling of the legs *(12)*.

Lymphatic filiariasis ranks third behind malaria and tuberculosis among the most prevalent tropical diseases—worse and more widespread than river blindness *(3)*. When combined with diethylcarbamazine or albendazole, ivermectin in a once-annual treatment decreases micro-larval density by 99% *(12)*.

In 1998, ivermectin was registered for lymphatic filariasis in France. Merck expanded its donation program to include lymphatic filariasis, and GlaxoSmithKline, the maker of albendazole, agreed to donate its drug. In 2000, WHO launched the Global Program to Eliminate Lymphatic Filariasis and adopted the two-drug combination for its community-based mass treatment program *(3, 12)*.

According to data from the World Health Organization, over 2 billion ivermectin treatments have been administered for river blindness and elephantiasis since the donation programs began in 1987 and 2000, respectively *(7)*. Each year, 110 million people receive ivermectin pills to combat river blindness and 218 million people take a dose for elephantiasis *(7)*.

Ivermectin also effectively kills other worm infestations such as ascariasis, whipworm, pinworm, and leishmaniosis, as well as schistosomiasis, chlamydia, and cutaneous parasites *(3, 5, 7, 12, 18)*. Orally administered ivermectin has become a drug of choice for the treatment of mites and head lice, superseding topical creams, which are inconvenient and impractical for full-body application *(5)*.

Tropical diseases are not a concern in the United States, but in 1996, the FDA finally approved ivermectin for human use to treat strongyloidiasis *(18, 19)*. Strongyloidiasis, a hookworm disease, is prevalent in temperate regions including the southern US, as well as in tropical and subtropical areas.

According to data from the World Health Organization, over 2 billion ivermectin treatments have been administered for river blindness and elephantiasis since the donation programs began in 1987 and 2000, respectively

New Insights

In 2002, Ōmura's research group at the Kitasato Institute published morphological, physiological, biochemical, and phylogenetic evidence arguing for reclassification of *Streptomyces avermitilis* and renamed it *Streptomyces avermectinius (3)*. The following year, they reported that the *S. avermectinius*

genome contains 9 million base pairs, the largest bacterial genome sequenced so far *(3, 5)*.

S. avermectinius turned out to be a marvel of bacterial engineering. Seventeen genes encode the necessary enzymes to produce avermectin in an elaborate 53-step synthesis *(3, 5)*. Despite decades of searching worldwide, no other naturally occurring organism has been discovered with the ability to manufacture avermectin, and the only place where *S. avermectinius* has been found is at the golf course in Japan.

Some parasites of ruminants, including cattle, have become resistant, but despite 35 years of constant worldwide use, ivermectin remains an important veterinary drug. And after more than 25 years of constant monotherapy in humans, no convincing evidence of *Onchocerca volvulus* resistance to ivermectin has emerged *(12)*. But, it looms as a possibility.

Investigators have seen a few cases of poor responsiveness, and residual micro-larvae have sometimes been observed after ivermectin treatment *(5)*. Also, because ivermectin kills only immature larvae, it must be administered repeatedly as long as the adult worms are producing new larvae. For these reasons, the search continues for alternative drugs, especially those that target adult worms.

Dr. William C. Campbell beside his wife, Mrs. Mary Campbell

Skin Snip Assay

The skin snip assay is a common diagnostic test for onchocerciasis. It involves removing some skin from an inflamed area and placing the snipped skin in saline to encourage micro-larvae to leave the skin. Microscopic examination determines the larval load.

In the meantime, the parasite responsible for river blindness is disappearing, thanks to the community-based programs. Recent studies have shown that in some areas, where ivermectin treatment has been ongoing for 15-17 years, the parasite has been eliminated. In these areas, researchers stopped treatment with ivermectin for up to 5 years and saw no re-emergence of micro-larvae in skin snip samples. The investigators thus established proof of principle that eradication of river blindness with ivermectin is possible and feasible *(17)*. This has prompted WHO to change its strategy from control of onchocerciasis to onchocerciasis elimination *(15, 17)*.

Ivermectin is a splendid gift from the earth

In 2015, Satoshi Ōmura and William Campbell received the Nobel Prize in Physiology or Medicine for their discovery of the avermectins and development of ivermectin. In his Nobel lecture, Ōmura, noting the soil origins of avermectin, said , “Ivermectin is a splendid gift from the earth” *(7)*.

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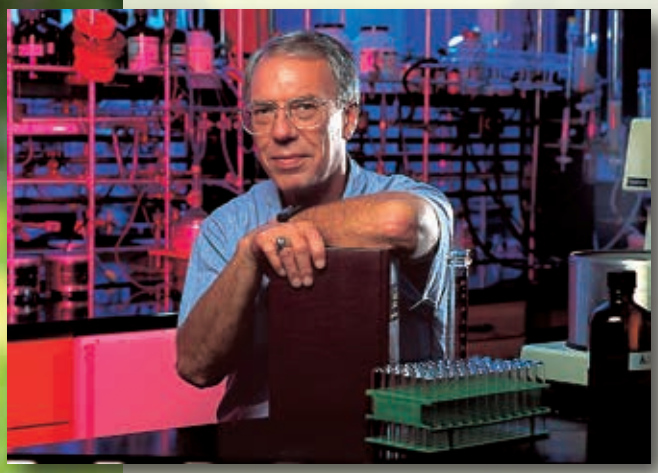
Taxol: Barking Up the Right Tree

Rebecca J. Anderson



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Dr. Robert Holton

One morning, Bob Holton discovered that his Tallahassee laboratory had been vandalized. An Iowa football coach had broken in, wanting a drug for his dying mother *(1, 2)*. Reports of innovative synthetic chemistry rarely interest the general public, but Holton's recently published paper had attracted considerable media attention. Investigators claimed that the drug suppressed ovarian and breast cancer better than anything else. And, it was exceedingly hard to get.

Holton was attending a North Carolina high school in 1960 when the National Cancer Institute (NCI) first started testing plant extracts for their potential as anticancer drugs. The NCI had created the Cancer Chemotherapy National Service Center five years earlier as a simple service for assessing small synthetic compounds. The screening program rapidly expanded, testing 30,000 small molecules annually, and then added natural products *(3, 4)*.

In June 1960, the US Department of Agriculture (USDA) began collecting a wide variety of plant specimens for the program *(4)*. Other NCI contractors then prepared plant extracts, conducted initial screening, and isolated pure compounds from crude extracts that exhibited activity. Over the next twenty years, NCI would evaluate 114,045 extracts from more than 15,000 plant species *(2, 4)*.

Barclay's Bark

Among those assigned to do the plant collections was Arthur Barclay, a 30-year-old botanist who had joined the USDA's research branch after graduating from Harvard *(3)*. Barclay's first assignment for the NCI program was collecting sunflower and daisy specimens in South Africa. In 1961, he was sent to the southwestern US and Mexico *(4)*.

On June 19, 1962, Barclay arrived in northern California and continued collecting specimens throughout northwest Nevada, Oregon, and Washington. On August 21, he ventured to a spot at the foot of Mt. St. Helens in the Gifford Pinchot National Forest *(2, 3)*. At an elevation of 1,500 feet, Barclay and his three graduate student assistants found a 25-foot Pacific yew tree, *Taxus brevifolia (3)*. They put twigs, needles, and fruit from the tree in a burlap bag labeled PR-4959 and put bark samples in another bag marked PR-4960—in all, about 15 pounds of material *(2, 4)*.

They then hiked seven miles to their base camp in Packwood, Washington, and spread the material on the floor of an abandoned house that served as their impromptu staging area. After a few days, the dried yew specimens (now less than a third of their wet weight) were packed, labeled, and shipped to the USDA's research center in Beltsville, Maryland *(2)*.

The USDA sent the yew specimens to the Wisconsin Alumni Research Foundation, one of the labs contracted by the NCI to prepare crude plant extracts *(3)*. To test for anticancer activity, the NCI arranged to have the crude extracts screened in bioassays at several other contract labs (e.g., Arthur D. Little, Hazleton Labs, and Microbiological Associates). The assays included one in vitro assay (KB cell culture) and a few in vivo assays of leukemia in mice *(4)*.

On May 22, 1964, Microbiological Associates in Bethesda, MD, reported that the PR-4960 bark extract was active in the KB assay *(3, 4)*. The lab confirmed the KB activity in June and July, but the in vivo results were inconsistent *(5)*.

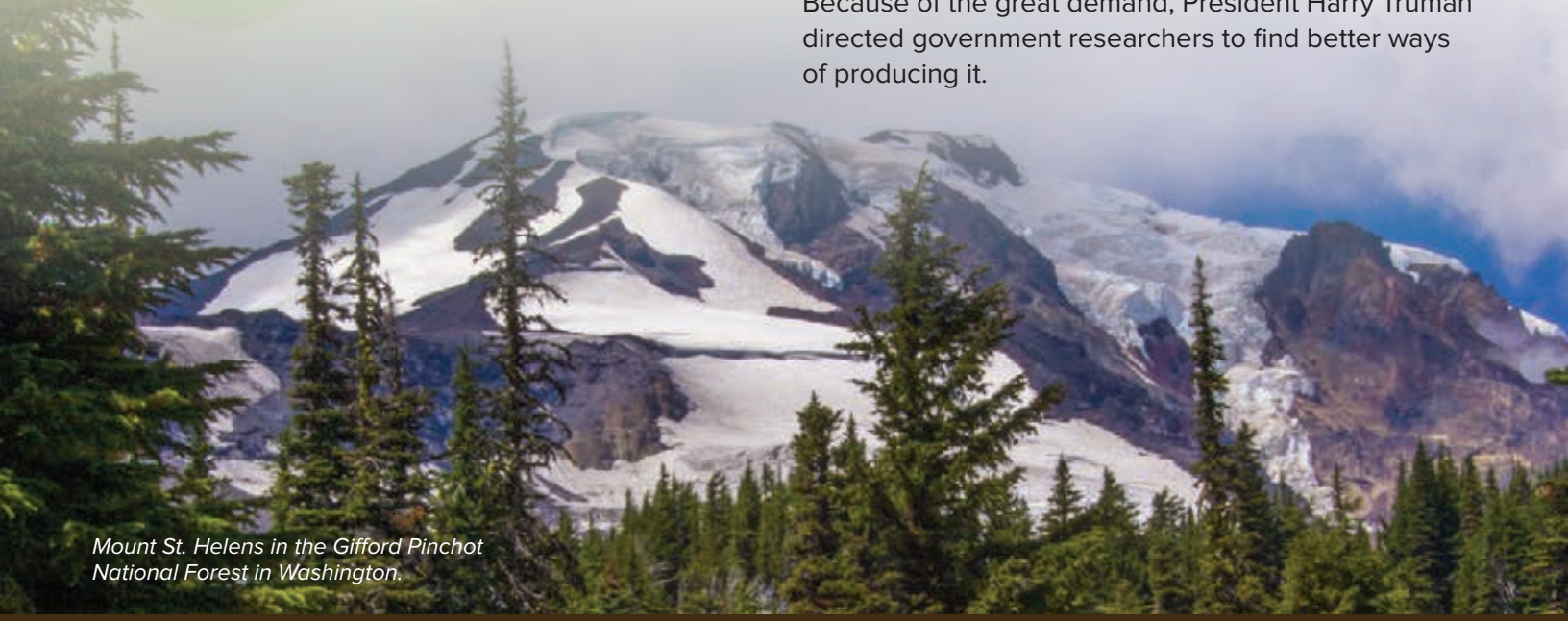
The bark extract was active in L1210 leukemia mice in one experiment but not in another. In other leukemia and lymphoma models, the extract was inactive. Nevertheless, the repeated cytotoxic effect in the KB assay met the NCI's criteria *(5, 6)*. The next step was determining the compound(s) in the extract responsible for activity. The NCI had contracted several academic chemistry laboratories to fractionate active extracts and isolate pure compounds. But for that, the chemists needed a larger sample of bark.

The USDA dispatched Barclay to the spot where he had collected PR-4960. On September 8, 1964, he bagged 30 pounds of bark from the Pacific yew tree, recording the sample as PR-8059 *(3, 4)*. It was shipped to Monroe Wall at the Research Triangle Institute in North Carolina.

The Fifth Wall

Monroe Wall began his career at the USDA in 1940. As a chemist, he was responsible for analyzing plant extracts at the agency's research branch in Philadelphia *(4)*. Ten years later, his priority shifted to cortisone, a newly discovered "wonder drug" for treating rheumatoid arthritis.

Cortisone was a steroid laboriously extracted from animal adrenal glands, and supplies were limited *(3)*. Because of the great demand, President Harry Truman directed government researchers to find better ways of producing it.



Mount St. Helens in the Gifford Pinchot National Forest in Washington.

The USDA's Philadelphia branch mounted an intensive effort to find plant steroids that could serve as a starting material for making cortisone synthetically. For nine years, Wall's team sent promising samples to the National Institutes of Health for evaluation *(3)*. Unfortunately, most of the plant extracts lacked steroid precursors.

Wall sent about 1,000 of his plant extracts to the NCI's fledgling cancer screening program *(3, 4)*. A sample from *Camptotheca acuminata,* a large shade tree that is native to China, showed impressive activity *(2, 3)*. However, because the USDA research branch's mandate was steroid chemistry for cortisone production, extracts with anticancer properties were not pursued *(3)*.

Frustrated by the USDA's lack of interest in *C. acuminata,* Wall moved to the Research Triangle Institute in July 1960. Founded just two years earlier, this new research venture linked the university towns of Raleigh, Durham, and Chapel Hill, and for Wall, the move to North Carolina was risky. He had built a stellar 20-year reputation at the USDA and left behind a state-of-the-art lab *(2, 3)*.

Starting with "nothing but four walls," the fifth Wall created a robust chemistry program and a thriving Natural Products Laboratory *(3)*. He also re-established his relationship with the NCI *(2)*.

By this time, the NCI's simple screening service had evolved into a "massive machine," capable of doing everything for drug development from animal breeding to clinical trials. The NCI also maintained banks of frozen tumors and the world's largest database of experimental drugs *(4)*.

Wall joined chemists at a handful of academic laboratories that the NCI had contracted to isolate and purify active compounds from crude plant extracts *(2, 4)*. Along with his colleague, Mansukh Wani, Wall resumed his work on *C. acuminata*. They succeeded in isolating camptothecin from a sample of wood and bark, and the NCI subsequently initiated clinical trials *(3)*.

From Trash to Treasure

In September 1964, Bob Holton was an undergraduate at the University of North Carolina—just 14 miles from Wall's laboratory—when Barclay's PR-8509 sample of Pacific yew bark arrived. It was one of seven plant samples in that shipment—all "confirmed actives" in the screening assays and all slated for fractionation and isolation *(2, 4)*.

Wall and Wani were preoccupied with camptothecin, especially making supplies so that the NCI could start clinical trials *(3)*. No one knew much about the Pacific yew, and the variable responses in the mouse assays provided little incentive for Wall to change his priorities *(4)*.

About six months after the yew bark arrived, Wall finally started the tedious process of fractionating the crude extract. In a slow, iterative process, each extract fraction was submitted for in vitro (KB) and in vivo (L1210) assessment at Hazleton Laboratories, one of the NCI's contractors *(3, 4)*.

By December 1965, Wall had exhausted his supply of yew bark and asked the NCI to arrange another collection. In May 1966, Wall received 45 pounds of bark, 135 pounds of twigs and needles, and 55 pounds of wood from Pacific yews *(4)*.

Wall continued refining the extracts. One fraction was 1,000-fold more potent than the crude extract and gave excellent results in the mouse assays *(4)*. On May 20, 1966, an excited Wall told the NCI, "This is the broadest spectrum of activity we have ever noted in our samples" *(2, 4)*. In what proved to be a prophetic observation, Wall noted that the purified yew fraction was active in an assay that led to the discovery of the first vinca alkaloids. None of Wall's previous

Dr. Monroe Wall and Dr. Mansukh Wani

Pacific yew tree, Taxus brevifolia

plant specimens had shown activity in that particular assay *(2, 4)*.

In September 1966, Wall and Wani succeeded in crystallizing the active compound, which they designated K172 *(3, 4)*. Unfortunately, yew bark contained very little K172. From 12 kg of dried bark, they could extract only 0.5 gram of pure K172, a yield of just 0.004% *(4)*.

In August 1966 and again in March 1967, Wall requested more material. "We need a lot more…if we are going to get enough product even for preliminary chemistry and the necessary preliminary antitumor studies" *(4)*. The USDA arranged for another collection, and 2,500 pounds of yew bark were shipped to Wall's lab in North Carolina *(4)*.

K172 was a complicated molecule. But even before Wall and Wani elucidated its chemical structure, they knew it contained some hydroxyl groups, making it an alcohol *(3)*. With this in mind and also drawing on the yew's botanical name, Wall named the compound "taxol" in June 1967. He thought taxol "had a nice ring to it," and the name stuck *(3, 4)*.

Bob Holton had now moved to Florida State University. His doctoral research centered on the "secondary metabolites" produced by flowering plants in the daffodil family *(2)*. Secondary metabolites do not support a plant's vital functions or growth. Rather, they play a defensive role, deterring insects and other predators. Not surprisingly, many of these complex molecules are poisonous, but they have also been explored for medicinal utility. Those with cytotoxic properties were ideal candidates for cancer treatment. Holton, who was training as a synthetic organic chemist, focused on isolating some of these enormously complicated molecules and then finding a way to make them in the lab *(2)*.

Searching for Taxol

Now that taxol had been identified as the anticancer substance in yew extracts, the NCI wanted to know the best source of it. They tested extracts from *Taxus baccata* (English/European yew), *T. cuspidata* (Japanese yew), *T. floridana* (Florida yew), *T. canadensis* (Canadian yew), *T. globosa* (Mexican yew), and *T. chinensis* (Chinese yew). Most specimens contained taxol, but the yield was somewhat lower than from Pacific yews *(4)*.

They also tested samples of Pacific yews collected throughout the Pacific Northwest, from California to Alaska. The KB results varied and could not be easily correlated with the location where the trees grew.

In a further study, many extracts of Pacific yew bark, roots, wood, and needles showed at least some activity. Needle extracts were sometimes more active than bark but other times much less. Overall, the NCI concluded that bark from the Pacific yew consistently yielded the most taxol.

Climbing Everest

Wall's main interest was elucidating the chemical structure of taxol. After years of painstaking analysis, he succeeded. The molecule consisted of a small side chain attached to a large three-ring component called a taxane *(7)*.

Wall and Wani published their paper in May 1971, and taxol's chemical structure captured the imagination of many academic researchers. At Stanford University, Robert Holton was 27 years old and just starting his postdoctoral training. When he read the paper, all he could say was, "Wow" *(2)*. Taxol was the ultimate chemical synthesis challenge—but much too complicated for postdoctoral research. For Holton, taxol would have to wait *(2)*.

Organic chemistry professors were also intrigued. Laboratory synthesis of taxol would require ingenuity and new approaches to chemical reactions. For them, taxol was "a molecular Mount Everest" *(4)*.

The National Institutes of Health provided some grant money for taxol research. A few professors explored structure-activity relationships, but most were driven by the chemical synthesis challenge *(1)*. None of them viewed taxol as a viable commercial product *(4)*.

Monroe Wall, on the other hand, thought the low yield and complex structure should not pose barriers to taxol development. In the early 1970s, he traveled to Bethesda a dozen times to advocate for taxol *(2)*.

This is the kind of chemical that only a tree would make

But the NCI decision-makers saw only modest activity in the mouse assays and had other compounds that looked more promising.

As a further handicap, Wall could not devote the time to extract all of the taxol needed for development. In February 1974, he sent his remaining supply of taxol (815 mg) to the Natural Products Branch of the NCI *(4)*.

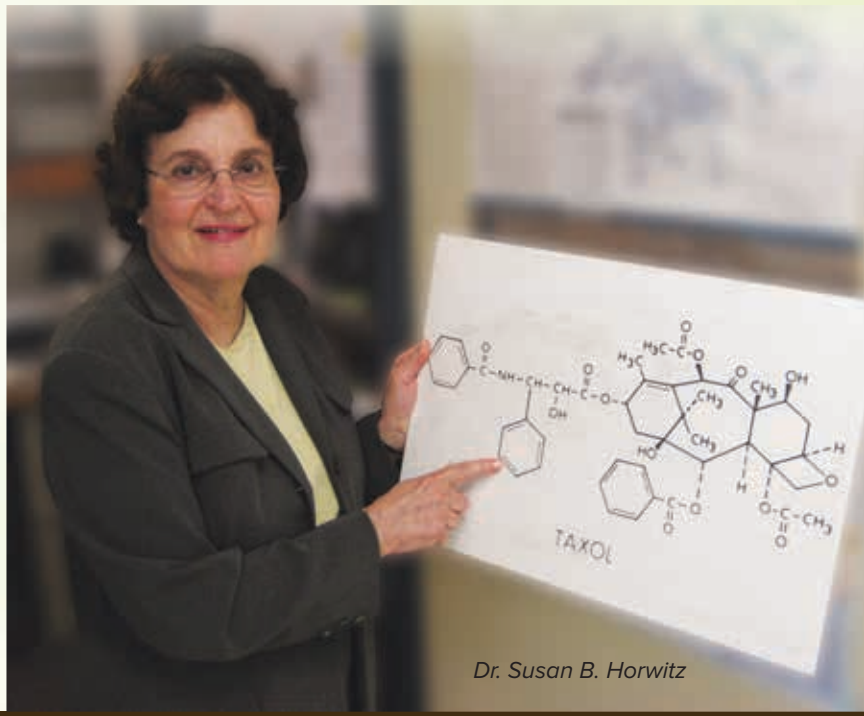
The timing was fortuitous. The NCI had begun shifting from its heavy reliance on leukemia animal models. Slow-growing solid tumors caused the majority of cancer deaths in the US, and to represent them, the NCI added the Lewis lung and B16 (melanoma) tumor models to their screening program *(4)*. Wall's taxol sample was tested in these tumor models in April 1974, September 1974, and June 1975. The results were mixed and kept taxol low on the priority list *(4, 5)*.

In October 1976, Matthew Suffness joined the Plant and Animal Products Section of the NCI's Natural Products Branch. A pharmaceutical chemist, Suffness was well acquainted with plant chemistry and extract screening. He reviewed the accumulated taxol results—some of it dating back 12 years *(2, 4)*. On the strength of the consistent activity in the KB assay and the more recent B16 tumor assay results, Suffness realized that taxol met the NCI's criteria for further development—but just barely *(5)*.

On April 18, 1977, the NCI finally decided to move forward with taxol *(4, 5)*. The next hurdle was formulating it for clinical trials, and for that, they needed more taxol. The NCI contracted Polysciences, a small industrial chemical supplier in Warrington, Pennsylvania, to purify taxol using Wall's methods.

At the end of April 1977, Polysciences received "two drums of tar" *(4)*. The 26 kg lot of concentrated extract came from bark collected in the Pacific Northwest in 1967 and 1968, and it had been sitting in storage. Polysciences successfully isolated 110 grams of pure taxol and delivered it to NCI in March 1978 *(4)*. Most of it was used for formulation development.

Clinicians needed an intravenous liquid for their patients. Unfortunately, taxol was virtually insoluble *(5, 8)*. After a year of failed attempts, the NCI team found that taxol dissolved in a solution of 50% Cremophor EL and 50% ethanol *(5, 9)*. Cremophor EL (a 35:1 mixture of ethylene oxide and castor oil) had been used to formulate other drugs, such as cyclosporine *(2)*. But when given as a large bolus, Cremophor EL could cause vasodilation, shortness of breath, and hypotension *(9)*.



Dr. Susan B. Horwitz

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Horwitz Shakes Things Up

In parallel with the formulation studies, the NCI researchers began studying taxol's mechanism of action. They also invited a few external scientists, including Susan Horwitz, to join the effort *(10)*. Horwitz was a molecular pharmacologist at Albert Einstein College of Medicine and had been studying the cytotoxic mechanism of action of other natural products, bleomycin in particular.

Although unfamiliar with taxol, she was immediately fascinated. "This is the kind of chemical that only a tree would make" *(10)*. She decided to devote a month to the project, and if nothing interesting happened, she would move on. Horwitz received a 10 mg sample in June 1977. And it was interesting. In cultured HeLa cells, taxol potently inhibited cell division *(10)*. She requested another sample in October *(4)*.

Cell division—and other cell functions—require a dynamic equilibrium between microtubules and monomeric tubulins. Any compound that disrupts this equilibrium is likely to be cytotoxic *(1, 5, 9)*. Several anticancer compounds, including colchicine and the vinca alkaloids, inhibited cell division by binding to tubulin and preventing formation of microtubules. By the 1960s, colchicine had become an indispensable tool to cell biologists, who used it to study cell division.

To Horwitz's surprise, taxol disrupted cell division differently. Instead of inhibiting microtubule formation like the other antimitotic compounds, taxol shifted the equilibrium in favor of microtubule formation and inhibited their disassembly *(10)*. The accumulated mishmash of microtubules prevented cancer cells from coordinating cell division. The cells soon collapsed and died *(2, 3, 9)*.

Horwitz published her findings in 1979 *(10)*. Cell biologists worldwide immediately recognized the value of taxol for studying cell dynamics. Taxol's novel mechanism of action complemented depolymerizing agents like colchicine, and the NCI was flooded with requests for samples of it *(4)*.

The French Connection

Horwitz's paper also sparked the interest of Pierre Potier in France. Potier had been investigating natural products and synthetic compounds that acted like colchicine *(6)*. His group assessed those compounds in a sensitive in vitro assay that measured interference with the tubulin assembly-disassembly process *(6)*.

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Dr. Pierre Potier

Potier worked at a facility near Paris, the Centre National de la Recherche Scientifique, which was located on a campus full of *Taxus baccata* (European yew). Coincidently, some of those trees had been felled to make way for a new road across the campus *(6)*. Potier took advantage of this opportunity to assess the needles, roots, bark, and wood of the European yew for taxol-like activity.

The lab prepared extracts and tested each fraction in the tubulin assay *(6)*. The most abundant active constituent in the needle extracts was 10-deacetylbaccatin III (10-DAB). Potier recognized this molecule as the large taxane ring portion of taxol. Interestingly, 10-DAB had not been active in the NCI's screening assays, but Potier's tubulin assay was more sensitive and detected mild inhibition of microtubule disassembly *(6)*.

Potier's group published their findings in 1981. Chemists immediately realized 10-DAB was an ideal starting material for synthesizing taxol *(3)*. Unlike bark, yew needles were a renewable resource, and 10-DAB was easy to obtain. A number of top-notch chemists were awarded NCI grants to find a practical way of adding the small side chain to the C13 site of 10-DAB, which would produce taxol *(2, 3)*.

In France, Potier also pursued taxol synthesis through two collaborations. The first was with Andrew Greene, who headed Centre National's research group in Grenoble. Greene's group would synthesize the taxol side chain. Potier's group in Paris would devise a synthetic route for attaching the side chain to 10-DAB *(4)*.

Potier's natural products group also signed a collaborative agreement with Rhône-Poulenc Rorer. Together, they would explore the chemistry of taxane compounds, define the structure-activity relationships, and seek new and patentable anticancer compounds *(4)*.

All of these chemists knew attaching the side chain to 10-DAB was easier said than done. The 15-membered taxane ring system of 10-DAB had many points where the side chain preferred to attach, rather than the C13 site that would create taxol *(9)*. Progress was slow.

Using Yews

Toxicology testing of taxol began in October 1980, and to keep development on track, the NCI needed more of it *(4)*. Without a viable synthetic method, the most expedient source remained natural extraction. And the best extracts, based on the NCI's assessment, came from Pacific yew bark.

Taxus brevifolia is an evergreen with reddish-purple bark and flat, inch-long needles *(3)*. Yew trees grow very slowly, reaching a height of about 30 feet in 100 years. Yew wood is hard, heavy, and slow to rot *(3)*. The English yew provided wood for longbows that were critical to the English victories at Crecy and Agincourt, and Wordsworth extolled its virtues in his poem, "Yew Trees" *(5)*. But Pacific yew wood was not of much commercial use except for fence posts *(3, 4)*.

Pacific yews grow in the shade of giant conifers, on the banks of streams, in deep gorges, and in damp ravines, but they are widely scattered. According to one Forest Service researcher, "If you walk over 100 acres of forest, you can expect to find yews on four of them" *(8)*.

Young yews are little more than shrubs—too small to harvest any bark from *(8)*. Adding to the difficulties, bark could only be collected during the spring and summer months when the sap was running *(4)*. The paper-thin bark was laboriously hand-peeled, either from the standing trees or after they were felled. Either way, stripping the bark killed the tree *(1, 3)*.

In October 1981, collectors delivered 3,366 lbs of bark to Polysciences. Over the next year, Polysciences produced about 260 g of pure taxol to support the toxicology studies *(4, 5)*.

Shifting to the Clinic

In the late 1970s, the NCI expanded its repertoire of solid tumor screening assays. Colonies of immunosuppressed mice were implanted with human tumors from the colon (CX-1), lung (LX-1), or breast (MX-1)—animal models that represented the three major types of cancer in the US. Taxol inhibited tumor growth in the colon and lung assays. Even better, taxol produced considerable regression of the breast tumors *(4)*.

Phase I clinical trials began in April 1984 at seven clinical sites involving 101 patients *(4, 5)*. Like virtually every other stage of taxol's development, things got off to a rocky start. Drug injections caused hypersensitivity reactions in about 10% of the patients. The problem was attributed to the Cremophor-ethanol vehicle *(5, 9)*. Pretreatment with antihistamines and dexamethasone and a slow, continuous 24-hour infusion minimized the hypersensitivity responses *(1, 5, 9)*.

Some of the patients with ovarian and renal cell carcinoma responded to taxol treatment, and on April 16, 1985, the NCI decided to move forward with Phase II trials *(4, 5)*. Those trials required considerably more taxol. Having ended its interagency agreement with the USDA, the NCI made arrangements directly with an Oregon fencepost dealer to collect 12,000 lbs of yew bark. Concerns of wildfires delayed collection, but 11,000 lbs of bark were delivered in the fall of 1986 *(4)*.

The Phase II trials were conducted at the same seven centers that had participated in the Phase I trials. The Johns Hopkins Oncology Center recruited ovarian cancer patients, and the Albert Einstein College of Medicine recruited renal cancer patients. Encouraged by taxol's response in the B16 (melanoma) mouse assay, the remaining five centers (which were part of the Eastern Cooperative Oncology Group – ECOG) recruited melanoma patients *(4)*.

Because taxol had shown impressive activity in the MX-1 mammary tumor screen, the NCI wanted to recruit breast cancer patients, too. But those trials were delayed. Lack of taxol supplies had become critical *(1)*.

On December 1, 1986, the NCI's Developmental Chemotherapy Section met to address the crisis. So far, three of the Phase II centers had enrolled patients. Only one of the 14 melanoma patients at the ECOG centers had responded to taxol. At Johns Hopkins, the results were more encouraging. Two of the first seven patients with refractory ovarian cancer had shown a partial response, and one had a marginal response *(4)*.

The NCI had sufficient taxol to supply the enrolled patients, but there was not enough on hand to start even one more Phase II trial. And, the next delivery from Polysciences was not expected before spring. At least seven planned Phase II trials were put on hold. ECOG stopped enrollment in February 1987, with 3 of 24 melanoma patients showing partial responses *(4)*. Johns Hopkins continued to enroll ovarian cancer patients *(1)*.

To ease the crisis, the NCI ordered 60,000 lbs of bark, which would yield about 3 kg of taxol. Patrick Connolly, a lumber contractor, collected 37,000 lbs of bark in the 1987 harvest season and delivered the remainder of the bark in August 1988 *(4)*.

Extracting and purifying that much material stretched the capacity at Polysciences to the limit.

The NCI contracted Hauser Chemical Research, a natural products facility in Boulder, Colorado, to assist *(4)*. In February 1989, Hauser completed the crude extraction from 25,000 lbs of Connolly's collection. Polysciences and Hauser then began isolating pure taxol from the extract.

The 3 kg of taxol produced from Connolly's bark harvest was sufficient to resume the suspended Phase II clinical trials. But to continue beyond that, NCI realized that they needed even more taxol.

In April 1989, NCI awarded a contract for another 60,000 lbs of bark to John Destito at Advanced Molecular Technologies in Bellevue, Washington. Destito was a capable, creative, and collaborative entrepreneur. But despite his best efforts, he experienced frustrating delays.

To speed things up, Destito decided to stockpile the yew logs through the winter and peel the bark mechanically after steaming the cut logs at 40°C. Destito's ingenious method allowed harvesting out of season, automated the debarking process, and produced the same taxol yield as hand-peeling *(4)*.

Going Commercial

The clinical results, especially from Johns Hopkins, kept getting better. William McGuire, who led the Johns Hopkins clinical trial, reported his results to the American Society of Clinical Oncology in May 1988. Noting a 30% response rate, he said, "There is no other drug that has produced this kind of response in drug refractory ovarian cancer...There were patients treated who were two to three weeks from death who are still alive today because of taxol" *(4, 11)*.

The cost of converting Pacific yew bark to taxol was draining the NCI's budget

At the NCI, one official told a colleague that public interest was intense. "People are begging for it" *(4)*. But the cost of converting Pacific yew bark to taxol was draining the NCI's budget, and many other promising drug candidates were competing for the same funds *(2)*. The NCI wanted to develop taxol but needed help.

The solution was a CRADA. Congress had recently instituted Cooperative Research and Development Agreements under the Federal Technology Transfer Act. CRADAs encouraged and facilitated the transfer of commercially promising knowledge from federal agencies to private industry.

On August 1, 1989, the *Federal Register* published the NCI's announcement for the taxol CRADA. To qualify, a drug company needed to have experience developing natural products and be willing to cover the cost of collecting bark, as well as extracting, purifying, and formulating taxol. The NCI would turn over all of its taxol data to the company, and in return, the company was expected to expedite taxol's development and regulatory approval *(1)*.

Semi-synthesis Succeeds

Working independently and largely ignored by the NCI, Pierre Potier and Andrew Greene had continued their efforts to synthesize taxol. In 1988, after nearly a decade of research, they published their "highly efficient practical approach" for attaching 10-DAB to the appropriate side chain *(12)*. They also applied for a French patent.

In the wake of the Potier-Greene paper, the NCI greatly increased funding for taxol chemistry projects. Purifying taxol from bark took more than a year, and the NCI realized that demand was accelerating much faster than yew trees grew. Soon, 30 chemistry groups were working on the synthesis of taxol *(1)*.

Robert Holton was now a chemistry professor at Florida State University. Academic tenure afforded him more freedom in selecting research projects. He chose taxol. But despite the publicity surrounding the clinical supply crisis and Potier-Greene's semi-synthetic achievement, Holton's interests were purely academic. He was driven by the intellectual challenge of synthesizing taxol from scratch *(2)*.

Holton had already succeeded in synthesizing part of the taxane ring when Matthew Suffness called him. Suffness, who had trained in the same Stanford lab as Holton, was now Chief of the Natural Products Branch at the NCI *(2)*. He assured Holton that taxol was not just a chemical curiosity. Thousands of patients needed the drug, and "somebody's gotta figure out how to make it" *(2)*. Holton put aside his total synthesis project and focused on practical solutions.

Within 18 months, Holton found a semi-synthetic route that delivered twice the yield of Potier's process *(2, 4)*. Holton patented his method in May 1991 and began contacting drug companies that might be interested in adapting it for commercial production of taxol *(1, 2)*. One of them was Bristol-Myers Squibb.

The Owl in the Tree

Concerns were intensifying that taxol extraction from Pacific yew bark was unsustainable. Since the 1940s, the logging industry had been given fairly free rein to clear-cut old-growth forests and harvest commercially valuable species such as Douglas fir, Sitka pine, and western cedar. Loggers gathered the remaining "trash trees," shrubs, and plants into "slash piles" and burned them *(4)*. The cleared area was then replanted with an even-age forest containing only commercially valued trees.

Aggressive logging had destroyed about 85% of the old-growth forests in the Pacific Northwest, including "trash trees" like the Pacific yew *(4)*. To preserve a portion of the old-growth forests, environmentalists succeeded in listing the spotted owl as a threatened species in June 1990. Unfortunately, the spotted owl was a poorly chosen surrogate for the plants and animals in this habitat. It wasn't cuddly like baby seals, and the poor spotted owl was caught in a highly politicized battle between environmentalists and workers whose livelihood depended on logging.

Everyone was now hugging the scrawny tree

Because yews had been commercially unimportant, no one had bothered to conduct a proper inventory, and estimates varied widely. Regardless, sooner or later the species would be extinct, and everyone was now hugging the scrawny tree. It was the sole source of a potent drug that could help thousands of cancer patients.

In an extraordinary move, both sides came together to support the Pacific Yew Act. Pacific yew trees were declared a public resource, and the Secretaries of Agriculture and Interior were charged with managing all yew trees on federal lands. Pacific yews could be felled only to manufacture taxol *(4)*.

Accelerating Approval

After more than a year of negotiations, the NCI and Bristol-Myers Squibb signed the taxol CRADA in January 1991—the latest step in a long-standing and special relationship *(4)*. In 1972, Bristol-Myers had been the first drug company to sign an agreement to market drugs emerging from the NCI's cancer screening program. Furthermore, Bristol-Myers Squibb was one of the few drug companies with broad experience developing cancer drugs and handling natural products *(13)*.

Under the CRADA, Bristol-Myers Squibb took over the NCI's contracts for all Pacific yew collections of bark, needles, and twigs *(1, 9)*. In addition, the Pacific Yew Act effectively reserved all Pacific yew trees on federal lands for Bristol-Myers Squibb and only for medicinal use until 1998 *(4)*.

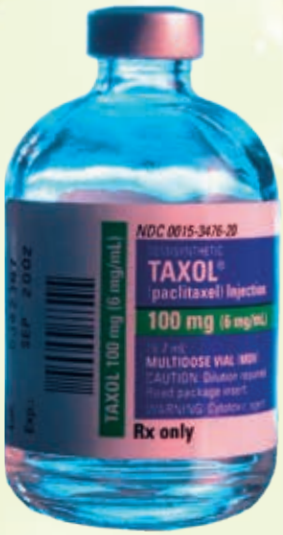
To satisfy the environmentalists, Bristol-Myers Squibb paid for research on yew ecology, the first official inventory of Pacific yew trees, and an Environmental Impact Statement assessing the effect of short-term, large-scale bark harvesting *(13)*. Bristol-Myers Squibb also contracted Weyerhaeuser, the largest supplier of timber in the US, to cultivate Taxus trees and conducted research to optimize their growth and other properties *(1, 13-15)*.

When John Destito's NCI contract expired, Bristol-Myers Squibb contracted Hauser Chemical Research in Colorado as its supplier of both yew bark and pure taxol *(2, 13)*. Hauser collected 80,000 lbs of bark in 1990, 1.6 million lbs in 1991, and another 1.6 million lbs in 1992 *(1, 13, 16)*. Hauser's process improvements doubled the yield of taxol, producing about 230 kg from 1990-1992 *(4)*.

Bristol-Myers Squibb formulated taxol (30 mg/vial) free of charge for the NCI's clinical programs *(1, 13)*. In 1991, the company supplied about 3,750 vials per month, enough to treat 500 patients *(1, 14)*.

In 1992, the company increased supplies from 5,000 to 50,000 vials per month *(13, 14)*. This permitted the NCI to establish an ovarian cancer treatment referral program, as well as a referral protocol to treat breast cancer patients *(13)*.

A mere 18 months after signing the CRADA, Bristol-Myers Squibb submitted the accumulated taxol data to the Food and Drug Administration. Taxol worked in patients who had become resistant to platinum-based therapy, which was the current "best drug" for ovarian cancer, and it was effective in patients who had been heavily pretreated with radiation and chemotherapy—factors that usually reduced responses to subsequent therapy *(9, 11)*.



On December 29, 1992, FDA approved taxol to treat refractory ovarian cancer, making it the first and only approved drug to emerge from NCI's plant screening program *(3, 17)*.

Taxol by Any Other Name

To further protect its investment, Bristol-Myers Squibb secured a trademark for its new product. In a rather controversial move, the US Patent and Trademark Office granted the company's request to register Taxol on May 26, 1992 *(4)*.

At this point, taxol had been in widespread use as a generic name for more than 20 years. Also, going unnoticed was a laxative product that Continental Laboratories had trademarked and sold as taxol in the early 20th century *(5)*.

Regardless, Bristol-Myers Squibb now had exclusive rights to call its anticancer drug Taxol. Within a couple of years, Taxol had been registered in more than 50 countries *(2)*. In 1993, the USAN authorized "paclitaxel" as the new generic name of the molecule that had formerly been called taxol.

Yew Turn

While optimizing extraction procedures, Bristol-Myers Squibb was working equally hard to reduce, if not eliminate, its dependence on Pacific yew bark *(14)*. The long-term solution to the supply problem was making taxol semi-synthetically, and the company was receptive to Robert Holton's offer *(13)*.

On April 1, 1990, Bristol-Myers Squibb signed an exclusive licensing agreement with Florida State University to use Holton's taxol patents, including an improved method that gave an 80% overall yield in just four steps *(2)*. In exchange, Florida State would receive royalties on the revenues derived from Holton's patents.

Bristol-Myers Squibb made speedy progress in scaling up Taxol production at its plant in Ireland, using Holton's improved method and 10-DAB obtained from Indena, a natural products company in Milan, Italy *(2, 8, 13, 16)*. Indena extracted large quantities of 10-DAB from renewable biomass (needles and twigs) of European *(Taxus baccata)* and Himalayan *(Taxus wallichiana)* yews *(13, 15)*.

Bristol-Myers Squibb needed regulatory approval to change Taxol manufacturing from bark extraction to the new semi-synthetic process *(13)*. The FDA approved the change on October 14, 1994, making further bark collections unnecessary *(17)*.

The Bristol-Myers Squibb contract with Hauser was not renewed, and with that, the Pacific yew crisis ended. Environmentalists rejoiced and federal conservation officials were relieved *(15)*. The little yew tree had gone from trash to treasure to trivial *(2)*.

A Quantum Leap

Abundant Taxol supplies hastened the pace of clinical trials for other cancers. The first reports of efficacy in refractory advanced breast cancer came from the MD Anderson Cancer Center in October 1990 *(1)*. The response rate of 56% was even better than in ovarian cancer *(18)*. A trial at Memorial Sloan-Kettering in 1992 confirmed the results *(19)*. Taxol was effective even in patients who had become resistant to anthracycline-based therapy, the current "best drug" for breast cancer *(9)*. The FDA approved Taxol for refractory breast cancer in April 1994 and for non-small cell lung cancer in June 1998 *(17)*.

When Taxol made its debut in January 1993, it was hailed as the most important anti-cancer drug in 15 years, but it was not perfect *(2, 8)*. As with other chemotherapy agents, bone marrow suppression and white blood cell depletion were common. Taxol also caused neuropathy, typically in the hands and feet, and cardiac disturbances *(9)*.

Nevertheless, Taxol was the best thing clinicians could offer at the time *(2)*. In 1995, it was the best-selling cancer drug in the world with more than $500 million in sales. In 2000, sales reached nearly $1.6 billion *(2)*.

The little yew tree had gone from trash to treasure to trivial

Totally Synthesized

Chemists were still lured to the challenge of synthesizing Taxol from scratch . As Robert Holton explained to a reporter, "The ring systems are unexplored ground. The stereochemistry, the variety of substituents, the conformational peculiarities, the strange reactivity...it's an incredible challenge" *(1)*. More than 100 academic groups worldwide were working on it *(1, 2)*.

Then, in a virtual photo finish, two groups succeeded *(2, 4)*. On February 17, 1994, Kyriacos Nicolaou and his team at the Scripps Research Institute reported their success *(20)*. Within a week, Holton's group at Florida State University published their work *(21)*.

Total synthesis of Taxol was a major intellectual achievement, but it was of little practical importance. Nicolaou's method involved 28 steps *(9)*. Holton's synthesis required 40 steps, and the yield was an abysmal 2% *(2, 4)*.

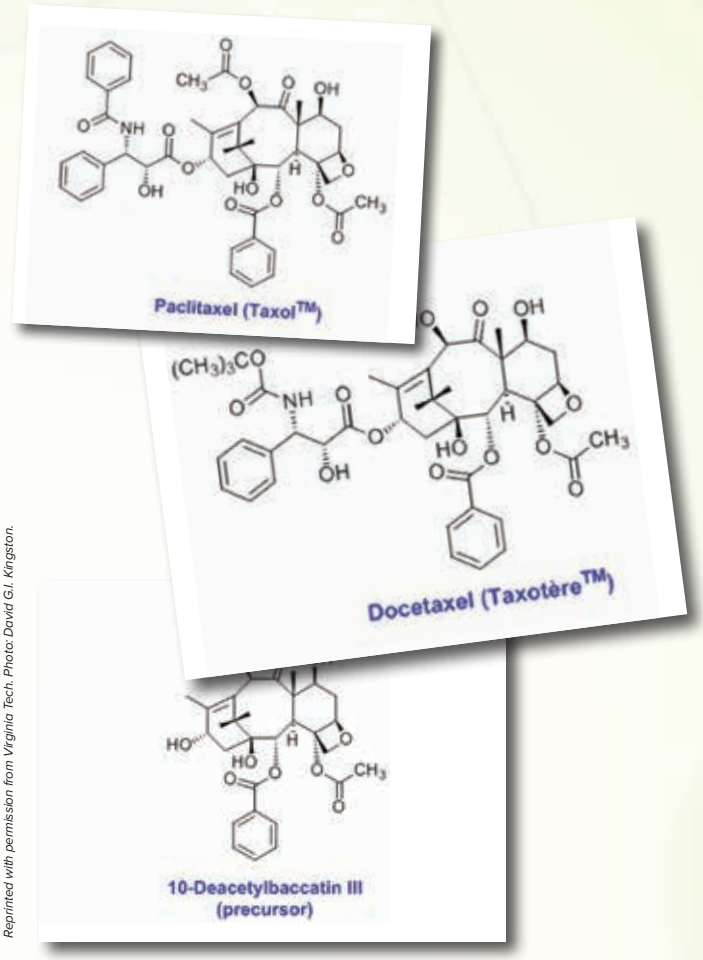
Enter Taxotere

While Taxol made headlines in the US, Potier, Greene, and their colleagues in France continued to improve their own semi-synthetic method. In collaboration with Rhône-Poulenc Rorer, they also studied the structure-activity relationships of about 40 intermediates and analogs *(1)*.

Among those compounds was RP56976, which was slightly more active than Taxol in the tubulin assay. RP56976 also exhibited significant antitumor activity *(6)*. Most impressively, it was 25% more soluble and had better bioavailability than Taxol *(1, 6, 9)*. RP56976 was named Taxotere (generic name, docetaxel).

In 1990, Rhône-Poulenc Rorer began Phase I clinical trials in Europe and the US under a research and development agreement with the NCI *(1, 9)*. Referring to their own semi-synthetic method, Potier boasted, "Our group has solved the problem of industrial production…We are today producing very large amounts of Taxotere" *(1)*.

The FDA approved Taxotere for advanced breast cancer treatment in 1995 *(4)*. It was subsequently approved alone or in combination with other agents for non-small cell lung cancer, prostate cancer, and head and neck cancer *(1, 14, 17)*.



Chemical structures of paclitaxel (Taxol™), docetaxel (Taxotere™) and 10-deacetylbaccatin III (precursor).

University Royalty

Traditionally, American universities aggressively insulated their research from all commercial influences. But the tech-transfer deal between Florida State University and Bristol-Myers Squib caught the attention of many university administrators *(2)*. In 1996, Florida State received more than $28 million in Taxol royalties, and by the end of the decade, the royalties topped $200 million. It was one of the largest patent pay-outs for a single university in history *(2)*.

Florida State used the royalties to underwrite a dozen endowed professorships. Also, under its policy, the university awarded faculty inventors 40% of the royalties arising from their patents, making Holton a very wealthy man *(2)*.

The royalties fundamentally changed Holton's perspective. His achievements were widely publicized, and hundreds of cancer patients and their loved ones contacted him. He shifted his academic chemistry pursuits and invested his royalties in applied research. He wanted to find a better Taxol analog. "If you have the opportunity to do something that could save someone's life, you just have to do it" *(2)*.

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Chlorpromazine and the Dawn of Antipsychotics

Rebecca J. Anderson, PhD

Dr. Henri Laborit

A cab driver suffering from hay fever took an antihistamine and thought nothing more about it—until he was stopped by a policeman and fined for running a red light. In hindsight, the most revealing thing about this little incident was that he told the officer he had seen the traffic light but just didn't care enough to stop.

Physicians were well aware that, in addition to alleviating allergy symptoms, antihistamines had "sedative side effects." The drowsiness, they cautioned, might impair work performance and perhaps pose a safety hazard. But this was not sedation in the usual sense. The cab driver was fully conscious. His memory was intact. But his ability to process thoughts on an emotional level had been dulled.

The anti-allergy effect of antihistamines was well understood, but the sedative side effects were not. It would take an astute surgeon, who was trying to improve surgical outcomes, to turn this special type of "sedation" into a major medical breakthrough.

Preventing Shock

Henri Laborit was born in Hanoi (then French Indochina) and received his medical training at Ecole de Santé Navale in Bordeaux, France. He became a Navy surgeon, serving in Tunisia during World War II, and returned to France after the liberation *(1-3)*.

In Tunisia and later at the French Navy's medical center in Toulon, Laborit was concerned about anesthetic and surgical shock *(1-3)*. Patients who feared or were anxious about surgery required deeper levels of general anesthesia, which in turn increased the chances of cardiovascular collapse (i.e., surgical shock), postoperative complications, and mortality.

From 1947-1950, Laborit experimented with various drug combinations that permitted surgery under lighter general anesthesia. When he prescribed promethazine to some of his patients for its antihistaminic action, he immediately recognized that it also seemed to be beneficial against surgical shock *(1, 3)*.

Promethazine calmed and relaxed patients prior to surgery. Like the cab driver, Laborit's patients were conscious and responsive, but were disinterested in the things going on around them. They also appeared to suffer less after major operations *(1-3)*. Laborit recognized that the "sedative side effect" of promethazine was quite different from other central depressant drugs and called it "ataraxy"—a tranquilizing effect *(1)*.

Potentiation and Artificial Hibernation

Laborit published his findings in the Parisian journal *La Presse Médicale,* which had the highest circulation of all French medical journals *(1, 2)*. Among those who were impressed was Pierre Huguenard, an anesthetist in Paris, who was writing his doctoral thesis in medicine. He was reading all the literature on surgery and anesthesia and wrote Laborit for more information *(3)*.

In early 1951, Laborit was transferred to Val-de-Grâce, France's famed military hospital in Paris, and given a laboratory for his research. Each week, he chaired a group discussion of civilian and military researchers. Still only in his mid-30s, Laborit expressed himself with clarity and logic, smiled easily, and was persuasive. In short, he was a charmer *(3)*.

Huguenard soon joined Laborit's weekly meetings, and the two developed a close collaboration *(3)*. Together, they worked out Laborit's technique of "potentiated" anesthesia—using synergistic drugs to reduce the amount of general anesthetic during surgery *(2-4)*.

When the operation involved lowering body temperature (e.g., icepacks for leg amputation), patients were even more resistant to surgical shock *(3)*. Laborit and Huguenard therefore proposed a method of "artificial hibernation." They first administered their cocktail of hypnotics, analgesics, curare, and an antihistamine and then cooled the patient with icepacks or air conditioning during surgery *(1, 3, 5)*. Promethazine was satisfactory, but Laborit wanted a "super-stabilizer" with a stronger, more selective effect *(1, 3)*.

Rhône-Poulenc Research

Chemically, promethazine is a phenothiazine. Since 1944, Rhône-Poulenc chemist Paul Charpentier had prepared a series of phenothiazine analogs, which his colleague, Simone Courvoisier, assessed pharmacologically. They wanted compounds with maximal antihistamine properties and minimal "sedative side effects." Weak antihistaminic compounds were quickly shelved without evaluating them for sedation *(2)*.

On October 3, 1950, the assistant scientific director at Rhône-Poulenc proposed a sharp departure from this strategy. Phenothiazines' side effect, he said, could be useful for new therapeutic indications. In a 7-page research proposal, he outlined the rationale for developing a phenothiazine that had effects predominantly on the brain *(2)*.

Citing the work of Laborit, who had kept the Parisian medical community well informed via his *La Presse Médicale* reports, the proposal suggested that such drugs might be useful as pre-anesthetics, as well as analgesics, antispasmodics, and antiparkinson drugs. The proposal ended with the prophetic statement, "we think that such substances would have an application in psychiatry" *(2)*.

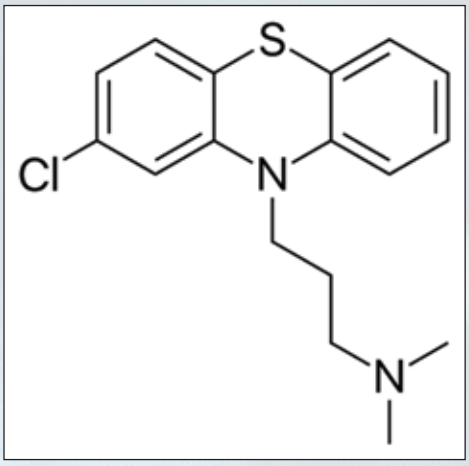
Available evidence suggested that the antihistaminic and central actions of the phenothiazines were inversely related. Pyrilamine, for example, was a strong antihistamine with weak central effects. Dimenhydrinate (Dramamine®) and diphenhydramine (Benadryl®) were weak antihistamines but had a strong central action, and in fact, were marketed as an antiemetic and sleep aid, respectively *(2)*.

Complying with the new directive, Courvoisier began pulling the failed phenothiazines off the shelf

and evaluated them specifically for their central activity. For this, she used a rope climbing test adapted by Charles Winter, a Merck pharmacologist *(6)*.

Trained rats could easily climb the rope. After treatment with a muscle relaxant, they attempted to climb the rope but appeared weak and became easily fatigued. On the other hand, promethazine made the rats confused and "unable to decide whether to climb or not" but without loss of muscle strength *(6)*. Courvoisier used promethazine as the reference standard in her search for more selective compounds *(2)*.

One compound, promazine, looked promising. It had originally been made by Charpentier in 1947 but had been promptly rejected because of its poor antihistamine activity *(1)*. To further enhance promazine's central actions, Charpentier synthesized a new compound, 4560 RP, by adding a chlorine substituent. On December 11, 1950, he sent 4560 RP to Courvoisier for pharmacologic testing *(1, 2, 7)*.



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Chemical structure of chlorpromazine

Rats treated with 4560 RP made no attempt to climb the rope, although their muscle strength and motor activity seemed unimpaired. With further testing, Courvoisier found that 4560 RP was also an antispasmodic, hypnotic, analgesic, and weak analeptic. It potentiated general anesthesia but had no antihistaminic activity *(2)*.

Rhône-Poulenc moved forward with Phase I clinical trials in April 1951. Over the next 5 months, samples of 4560 RP were sent to 35 investigators, mostly in Paris, for clinical pharmacology assessment *(2)*. Among them was Dr. J. Schneider at Broussais Hospital. On April 13, 1951, Schneider reported to Rhône-Poulenc that the drug potentiated the effects of barbiturates in a woman with acute mania *(2)*.

Laborit's Influence

During 1950-1951, Laborit made frequent visits to Rhône-Poulenc's research laboratories in Paris and discussed his success with potentiators of anesthesia *(2)*. Despite these visits, though, he knew nothing about 4560 RP, until he asked for a more selective analog of promethazine. Rhône-Poulenc had just the drug. It was 4560 RP, which Charpentier had named chlorpromazine.

On June 26, 1951, Laborit became the twelfth investigator to receive chlorpromazine *(2)*. Huguenard found that chlorpromazine eliminated patients' anxiety before surgery, and he could drastically reduce the amount of morphine and general anesthetic during surgery *(3, 7)*. It also counteracted post-operative nausea.

On October 13, 1951, Laborit and Huguenard published their progress with the artificial hibernation technique, which had been hampered by the weak and inconsistent efficacy of their drug cocktails *(2, 7)*. They enthusiastically announced, "Now we have a drug... extremely effective for its low toxicity and it greatly facilitates therapeutic techniques...It is 4560 RP" *(1)*.

From September 9, 1951 to March 10, 1952, Rhône-Poulenc shipped samples of chlorpromazine to 118 clinical investigators in Europe for the phase II trials. Rhône-Poulenc did not design or plan specific clinical trials. Rather, the company asked the investigators to explore a range of therapeutic uses, assess the patients' tolerance to the drug, and send in their findings *(2)*.

In Paris, investigators reported chlorpromazine's wide range of pharmacologic properties and prescribed it for a variety of ailments *(1)*. On December 1, 1951, Rhône-Poulenc noted use of chlorpromazine in surgery (potentiation of general anesthesia) and medicine/obstetrics (hypnotic, sedative, antispasmodic, antipyretic, and anticonvulsive). Based on the clinical observations of Schneider, Laborit, and others, as well as Courvoisier's preclinical assay results, the company added "interrupt maniacal crises" to its list of possible uses *(2)*.

Mental Illness Before Chlorpromazine

Up to the mid-twentieth century, mentally ill patients were shunted to the fringes of society. Psychotic patients experienced frightening delusions and hallucinations. Manic patients could be assaultive and destructive. In psychiatric wards, they lay strapped in their beds: arms bound in straitjackets, feet tied to the bedposts, and heads restrained with a halter *(3)*. Some were put in seclusion. They screamed, shouted, hurled abuse and insults, sang, and cried. The constant noise often disturbed people living nearby *(3)*.

Nurses grew accustomed to the habitual noise, cleaned up feces, and spoon-fed recalcitrant patients who often, laughingly, spit mouthfuls of well-chewed food back into their faces *(3)*.

Psychotic patients often stayed in asylums and psychiatric hospitals for months or years, and the institutionalized population was growing by 7% per year *(3, 8)*. By the 1950s, things had reached a crisis. Psychiatric wards were bursting at the seams *(2)*.

Many therapeutic options were tried with very limited success. In the late 1930s, surgeons performed frontal lobotomies. Some patients were calmer after surgery but others remained unchanged. Of course, lobotomy caused irreversible brain damage, whether the results were good, bad, or indifferent. And one surgeon said candidly, "The good results in some cases did not make up for the bad ones" *(3)*.

In the 1940s, malaria fever therapy proved effective against some psychoses. Patients were infected with malaria to produce a high malarial fever. After 10-13 bouts of fever, about 30% of the patients showed improvement *(3)*. Their malaria infection was then treated with quinine or other antimalarials.

Drugs had always been a large part of psychiatrists' therapy. But since nothing was known about the pathophysiology of mental disorders, all medicinal treatments were trial-and-error. These included injection of cocaine, manganese, castor oil, oil of turpentine, and even animal blood *(5)*.

Sedatives such as chloral hydrate and the barbiturates came in and out of vogue depending on the prevailing knowledge of pharmacology *(9)*. Barbiturates calmed belligerent and unruly patients, but it was merely a substitute for mechanical restraints and palliative rather than therapeutic *(9)*. In addition, patients became more violent and aggressive afterward, having found new strength during their forced rest *(3)*.

Sleep therapy proved more successful and was especially popular in Europe. Hypnotic and sedative drugs such as the barbiturates, opium alkaloids, and scopolamine were used to induce and maintain sleep for 8-12 days in a darkened room *(1)*.

Although effective in some patients, especially those in manic states, sleep therapy involved major risks and required hospitalization with continuous

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The scene at Bedlam depicted by William Hogarth in A Rake's Progress

nursing care and medical supervision *(1, 5)*. The drugs had a narrow safety margin and were addictive. Some patients developed pneumonia, an often fatal complication at a time when antibiotics were not available *(5)*.

Hypoglycemic shock therapy was introduced in 1927. Insulin was injected to induce a deep coma for 1-2 hours and then reversed by giving the patient a glucose syrup. Repeated, reversible insulin-induced comas gave more reliable therapeutic results, frequently leading to full remissions *(5)*. But, again, close monitoring was required, and according to one investigator, "Justifying these insulin comas took courage" *(3)*.

One Hungarian psychiatrist theorized that epilepsy and schizophrenia were opposite states of brain function and that inducing seizures might improve schizophrenia. At first, he induced convulsions chemically with intramuscular injections of camphor *(3)*. But the seizures were unpredictable. The patient might be walking in hazardous places such as stairways when the seizure struck *(5)*.

In 1929, intravenous metrazol was introduced as the chemical convulsant and produced faster, more favorable results. But metrazol caused patients to experience a brief period of extreme anxiety, and they remembered it. So, cooperation for follow up treatments was a major problem *(3, 5)*.

In 1938, electroshock treatment was introduced. It provided excellent and immediate control of symptoms but could cause confusion, restlessness, amnesia, and aggression *(3, 5)*. Injuries to bones and muscles were also a problem. Concurrent use of muscle relaxants avoided that problem but caused other complications (paralysis, hypoxia, etc.). As they gained experience, investigators found that the real value of electroshock was for depression, rather than psychotic states such as schizophrenia *(3, 5)*.

...since nothing was known about the pathophysiology of mental disorders, all medicinal treatments were trial-and-error.

All of these interventions sought to improve the patient's psychological state through major, often life-threatening, alterations in physiological functions: sleep, coma, or convulsions *(5)*. No treatment targeted specific neural pathways or processes because no one knew what alterations in the brain were responsible for mental illness. In fact, many psychiatrists denied that the etiology was biochemical, hormonal, or pathophysiological. Freudian psychoanalysis dominated American psychiatric practice *(2)*.

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Popular Mode of Curing Insanity! Lizzie Bonnere punishing Miss Hodson, on suspicion of taking her key

Laborit Urges Psychiatrists

Based on his observations in surgical patients, Laborit thought that chlorpromazine would work in psychiatry, in which, as in surgery, patients are stressed. "For months after I began using chlorpromazine, I urged the psychiatrists I saw daily at Val-de-Grâce, often during lunch at the cafeteria, to try it with their patients" *(2)*.

However, French psychiatrists were not easily persuaded. They had already tried innumerable sedative drugs without much success *(1, 2)*. In an effort to sway professional opinion, Laborit invited a prominent

Parisian psychiatrist to attend a demonstration of chlorpromazine's effects. C. Quarti, a psychiatrist and friend, volunteered as Laborit's experimental subject *(1)*.

On November 9, 1951, Quarti was injected with chlorpromazine and later documented her experiences, which were similar to the reactions of Laborit's surgical patients *(2)*. Unfortunately, the demonstration backfired. Quarti fainted, due to brief but severe orthostatic hypotension—an inconvenient problem for psychiatric patients. The prominent psychiatrist was not impressed, and in fact, this fainting episode worked to further dissuade psychiatrists *(1)*.

Undeterred, Laborit continued to press his case. On February 13, 1952, he and Huguenard published a 2-page article entirely devoted to chlorpromazine. The article described chlorpromazine as a "stabilizer" with unique calming effects. In conclusion, they said, "These facts let us foresee certain indications for this drug in psychiatry..." *(1, 2, 4)*.

Colonel Joseph Hamon, director of the Neuropsychiatric Service at Val-de-Grâce Hospital, finally consented to try chlorpromazine, but "without much conviction" *(1, 2)*. He was assisted by Colonel Jean Paraire and Lieutenant Colonel Jean Velluz.

Their subject was Jacques Lh, a 24-year-old severely agitated psychotic patient *(7)*. Jacques experienced his first manic attack in 1949. From September 9 to October 10, he received 15 electroshock treatments and 4 pentothal treatments at Val-de-Grâce. His mania subsided *(2)*.

On February 6, 1951, he was admitted again after suffering a similar manic attack. During his 2-month hospitalization, he received 9 electroshock treatments, followed by 15 insulin-induced comas *(1, 2)*.

On January 17, 1952, Jacques was again admitted to Val-de-Grâce, again exhibiting severe agitation. At 10:00 am on January 19, Hamon's staff administered an intramuscular injection of meperidine (an opiate) and a 50 mg intravenous dose of chlorpromazine. Jacques immediately became calm, remained responsive when awake, and at times slept *(2, 7)*.

After about seven hours, Jacques's agitation and violent behavior returned with the same intensity, until another chlorpromazine injection halted it. The behavioral pattern was the same before and after each injection. After 12 days, Jacques's periods of calmness became progressively longer, and the intervals of excitement became shorter and less violent *(2, 7)*.

Unfortunately, the chlorpromazine infusion caused irritation at the injection site, and on several occasions, Hamon's team substituted barbiturates and electroshock for chlorpromazine. Even so, after 20 days and a total of 855 mg of chlorpromazine, Jacques was discharged, "ready to resume normal life" *(1, 7)*.

Colonel Paraire reported the Val-de-Grâce team's clinical findings on February 25, 1952, at a meeting of the Société Médico-Psychologique in Paris. In March 1952, their paper appeared in the Society's official journal—the first published account of chlorpromazine in psychiatry *(7)*. Although Jacques received multiple treatments (chlorpromazine, an opiate, a barbiturate, and electroshock), this marked a turning point in psychiatry *(4)*.

Rhone-Poulenc's Psychiatric Trials

Also in March 1952, Rhône-Poulenc reconsidered chlorpromazine's potential therapeutic indications. In addition to potentiating the effects of anesthetic, analgesic, and hypnotic agents, the company included psychiatric uses (manic states, schizophrenia, detoxification cure, and sleep cure) as well as neurosis, anxiety, and epilepsy *(2)*. But clinical trials were still passive. Investigators had to take the initiative to request samples.

Some learned about chlorpromazine through Rhône-Poulenc's formal communications. Others were familiar with Laborit's work. Pierre Deniker heard about chlorpromazine from his brother-in-law, who was a surgeon *(1-3)*.

A native Parisian, Pierre Deniker received his MD from the Faculty of Medicine in Paris and was serving on its faculty, as well as on the staff at Hôpital Ste-Anne, a renowned psychiatric hospital in Paris. At St. Anne's, he was in charge of the men's department in Jean Delay's clinic *(3)*.

Dr. Jean Delay

Dr. Pierre Deniker

Jean Delay was an inspiring renaissance man. At 40, he was the youngest-ever chairman of the mental disease clinic at St. Anne's. His wide-ranging interests included philosophy, literature, and psychology, but he was first and foremost a physician. Deniker wanted to pursue experimental drug therapies, and the broad-minded Delay encouraged his efforts *(3)*.

Deniker requested chlorpromazine from Rhône-Poulenc and began treating patients at St. Anne's on March 24, 1952. In short order, he found that a daily dose of 75 mg was sufficient to control behavior and, unlike the exploratory uses at Val-de-Grâce, without the need for other drugs, treatment procedures, or icepacks *(7)*.

When Deniker informed his boss, Delay was immediately interested in chlorpromazine but insisted on a larger number of cases before they reported their findings to the scientific community. Delay recommended that all patients arriving at St. Anne's in a state of agitation, excitation, and mental confusion be assigned to Deniker's department *(3)*.

On May 25, 1952, Delay and Deniker presented their initial findings at a meeting of the Société Médico-Psychologique *(7)*. By July 1952, they had published 6 reports describing 40 psychiatric patients in whom chlorpromazine had induced a "syndrome of psychomotor indifference" *(4)*.

These were the first published reports showing that a single drug could effectively treat major psychoses *(4, 7)*. Delay and Deniker's observations were promptly confirmed and reported by other investigators in France, Italy, and Austria *(5, 7)*.

In addition to these psychiatric effects and confirming the pharmacologic properties Courvoisier had documented preclinically (analgesic, ganglionic blockade, antiemetic, antipyretic, antishock, anticonvulsant, antispasmodic), clinical investigators also reported chlorpromazine-induced orthostatic hypotension, heart palpitation, and hypothermia *(2)*. Chlorpromazine even cured hiccups *(9)*.

In November 1952, just two years after Paul Charpentier had first synthesized it, Rhône-Poulenc launched chlorpromazine as a prescription medication in France *(7)*. Because of the drug's wide range of pharmacologic properties, the company branded it Largactil®, a drug "large in action" *(4, 7)*.

1953 – A Pivotal Year

In 1953, psychiatry was completely transformed by three events. The first was the persistence of chlorpromazine's champions, Delay and Deniker in Paris and Heinz Lehmann in Montreal.

Deniker traveled to many European university centers, describing his therapeutic successes, and European psychiatrists came to St. Anne's to meet with him *(3)*. Delay and Deniker also organized the first psychiatric conference entirely devoted to chlorpromazine, held in Basel, Switzerland, in November 1953 *(1)*. By the end of 1953, chlorpromazine dominated treatment in mental institutions across Europe *(1)*.

Meanwhile, Heinz Lehmann was introducing chlorpromazine to American psychiatrists. Lehmann fled from Germany to Canada in 1937 *(7, 10)*. Working at the Verdun Protestant Hospital in Montreal and fluent in French as well as German and English, Lehmann read the publications of Delay and Deniker. In 1953, he received a "generous amount" of chlorpromazine from Rhône-Poulenc and treated 71 psychiatric patients *(11)*.

Dr. Heinz Lehmann

Lehmann confirmed Delay and Deniker's observations, saying, "The drug is of unique value in the symptomatic control of almost any kind of severe excitement" *(11)*. His paper, published in English in February 1954, introduced chlorpromazine to North American psychiatrists *(7)*.

Lehmann continued to lead efforts, along with a few others, in raising the prominence of pharmacological therapy in American psychiatry. He also helped devise a comprehensive battery of preclinical and clinical assessments for evaluating new psychotropic agents *(10)*.

SKF Partnership

The second major event of 1953 was Rhône-Poulenc's success in penetrating the US market. In the 1950s, European drug companies faced big hurdles to marketing their drugs because US regulations were more restrictive than in Europe and American physicians were skeptical of European scientific and clinical data *(2)*.

Rhône-Poulenc's first attempts to interest an American partner failed. One drug company said chlorpromazine had "no large market potential" *(2)*.

The reception at Smith, Kline & French (SKF) Laboratories in Philadelphia was more favorable. Unlike most American firms at the time, SKF was actively fostering close collaborations *(2)*. SKF had a good portfolio of drugs to offer European companies, and Francis Boyer, SKF's president, had already made several trips to Europe to establish relationships. When Rhône-Poulenc's inquiry arrived in April 1952, Boyer, who was fluent in French, quickly built a rapport with his Rhône-Poulenc counterpart *(2)*.

Coincidently, SKF scientists were exploring the interesting properties of SKF 525. SKF 525 potentiated barbiturate anesthesia and also potentiated centrally acting stimulants such as amphetamine *(2)*. Chlorpromazine, an anesthetic potentiator, fit nicely into their research plans.

Other SKF scientists were looking for a drug that was more specific for treating nausea and vomiting than Dramamine. They were interested in chlorpromazine's antiemetic properties *(2)*.

In May 1952, the two companies exchanged 200 mg samples of SKF 525 and chlorpromazine *(2)*. The swap turned out to be a better deal regarding chlorpromazine than SKF 525. The effects of SKF 525 seen in the laboratory could not be duplicated in humans. Rather than acting as a potentiator like chlorpromazine, SKF 525 inhibits CYP450. Clinical trials were shelved, but later, SKF 525 became a valuable research tool for studying microsomal metabolism.

SKF quickly confirmed Courvoisier's preclinical results and initiated clinical trials on October 28, 1952 *(2)*. Unlike Rhône-Poulenc, which allowed investigators free rein, SKF played a direct role and planned specific clinical trials. Among the clinical investigators who conducted these early trials was Louis Goodman at the University of Utah *(2)*.

By December 1952, the American clinical results showed that chlorpromazine effectively controls nausea and vomiting, markedly sedates acute manic patients, lowers refractory fevers due to head trauma or uncontrolled infections, and relieves the itching associated with Hodgkin's disease *(2)*.

With a view toward rapid market entry, SKF gave priority to the clinical trials for an antiemetic indication because it was the easiest to demonstrate *(2)*. In a study of 70 patients at Peter Bent Brigham Hospital in Boston, chlorpromazine had "a powerful selective effect against nausea and vomiting…without producing any degree of sedation" in patients suffering due to cancer chemotherapy, pregnancy, and many other emetic conditions *(12)*. This clinical report, which appeared four months before Lehmann's paper, was the first American publication of chlorpromazine results.

In contrast, the clinical data from psychiatrists trickled in, but by February 1953, SKF was impressed. Chlorpromazine induced a "strikingly unusual type of sedation…giving complete relaxation without actively inducing sleep" *(2)*. As the year progressed and the evidence grew stronger, SKF added psychiatric indications as a top priority in its development plan. More psychiatry investigators were added, including N. William Winkelman at Sidney Hillman Medical Center in Philadelphia and Winfred Overholser at St. Elizabeths Hospital in Washington, DC *(2, 9, 13)*.

Then, SKF's plans for registering and marketing chlorpromazine were nearly derailed *(2)*. In SKF's manufacturing plant, almost everyone who handled the drug experienced contact dermatitis. Workers developed skin rashes, and their eyes became irritated and bloodshot. It was a drug-induced photosensitivity reaction that was also seen in some patients. Rhône-Poulenc advised manufacturing precautions (i.e., protective clothing, goggles, and special ventilation devices), which prevented further problems at SKF *(2)*.

Another side effect problem, jaundice, was much more challenging. SKF spent considerable time and effort and added clinical trials to elucidate the safety risk *(2)*. Unfortunately, no pattern emerged to establish that jaundice was drug-related. Some investigators suspected it was coincidental and due to concurrent hepatitis infections. In any case, SKF agreed to include a warning about jaundice in its prescribing instructions, which remains on the current label.

To persuade American physicians—especially psychiatrists—to use chlorpromazine, SKF sponsored a promotional tour for Laborit and Deniker *(2)*. These two pioneers of chlorpromazine met for the first time in November 1953 when they boarded the airplane that brought them to the US *(3)*.

Laborit's demonstrations of his artificial hibernation technique on dogs were less than impressive. Most of the dogs died. This only added to the skepticism of American anesthesiologists about artificial hibernation, and SKF subsequently dropped the indication *(2)*.

Deniker's hectic tour encompassed all of the major mental institutions in North America, and he was warmly received. His articulate presentations describing chlorpromazine-treated schizophrenic patients inspired and convinced many influential psychiatrists *(2)*.

By the end of 1953, SKF had supplied over 600 physicians with chlorpromazine, by far the largest group of investigators ever to test an investigational drug from SKF. But the dataset included only 104 psychiatric patients, whereas more than 1,000 patients had clearly established chlorpromazine's efficacy as an antiemetic drug *(2)*.

On March 4, 1954, SKF submitted chlorpromazine to the Food and Drug Administration (FDA) for approval. The application contained 22 major clinical studies, of which 9 were antiemetic trials. The psychiatric data came from 6 investigators, including Lehmann in Montreal and Winkelman in Philadelphia *(11, 13)*.

On March 26, 1954, the FDA approved Thorazine® (SKF's brand of chlorpromazine) for nausea and vomiting and in neuropsychiatry *(2)*.

Reserpine

The third pivotal event of 1953 was triggered by an article in the Sunday edition of *The New York Times*. On March 15, 1953, Nathan Kline, an American psychiatrist, was reading *The New York Times* when a report from India caught his eye. R. A. Hakim had been awarded a gold medal at a medical conference in Bombay for his presentation of a paper on the cure of schizophrenia *(14)*. There was no drug in Western medicine that cured schizophrenia, so Kline was intrigued.

Hakim's potion consisted of a half-dozen herbs, but the main ingredient was *Rauwolfia serpentina,* a shrub with red blossoms that grows wild in many parts of India *(1, 3, 14, 15)*. For hundreds of years, *Rauwolfia* had been a common household remedy for insect and snake bites, insomnia, intestinal diseases, and to facilitate childbirth *(1, 14, 16)*. It had also been used for fevers, to induce sleep in children, and as a cure for insanity *(15, 16)*.

In the 1930s and 1940s, several Indian investigators reported that *Rauwolfia serpentina* was an effective treatment for hypertension *(1, 3)*. Tablets made from the dried *Rauwolfia* root were in "such unprecedented popularity" that nearly every patient with high blood pressure in India had used it *(16)*.

In 1931, Indian researchers isolated five alkaloids from the *Rauwolfia* root *(1, 3)*. In 1952, chemists at the Swiss drug company Ciba successfully synthesized reserpine (Serpasil®), the alkaloid that accounts for about half of the pharmacologic activity of the *Rauwolfia* root, for use in hypertension *(2, 3, 5)*.

The Indian reports of hypertension and psychoses efficacy were published in English and available in the US. But American psychiatrists remained unaware of reserpine until Kline's chance reading in *The New York Times (1)*.

After testing tablets of the whole *Rauwolfia* root and reserpine on himself, Kline treated over 700 psychiatric patients at Rockland State Hospital

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An advertisement from the early 1960s for Thorazine®, Smith, Kline & French (SKF) Laboratories' brand of chlorpromazine

Rauwolfia serpentina *plant*

in New York *(14)*. His measures of efficacy were observational but quantitative: fewer physical assaults, a decreased need to restrain the patients, and fewer patients put in seclusion. The wards were also less "noisy" *(14)*. Kline's report to the New York Academy of Sciences announced the first Western psychiatric use of reserpine *(2)*.

During Deniker's US tour, Harvard University pharmacologists told him that reserpine-treated hypertensive patients exhibited a syndrome of "indifference" similar to his descriptions of chlorpromazine-treated patients. When Deniker returned to St. Anne's, he promptly confirmed Kline's observations, but the dose of reserpine required for psychiatric efficacy was 10-fold higher than that used for hypertension *(4)*.

In 1957, Henri Laborit, Pierre Deniker, Heinz Lehmann, and Nathan Kline shared the Albert Lasker Clinical Medical Research Award for their contributions in launching chlorpromazine and reserpine use in psychiatry *(3, 7)*.

Psychiatry Transformed

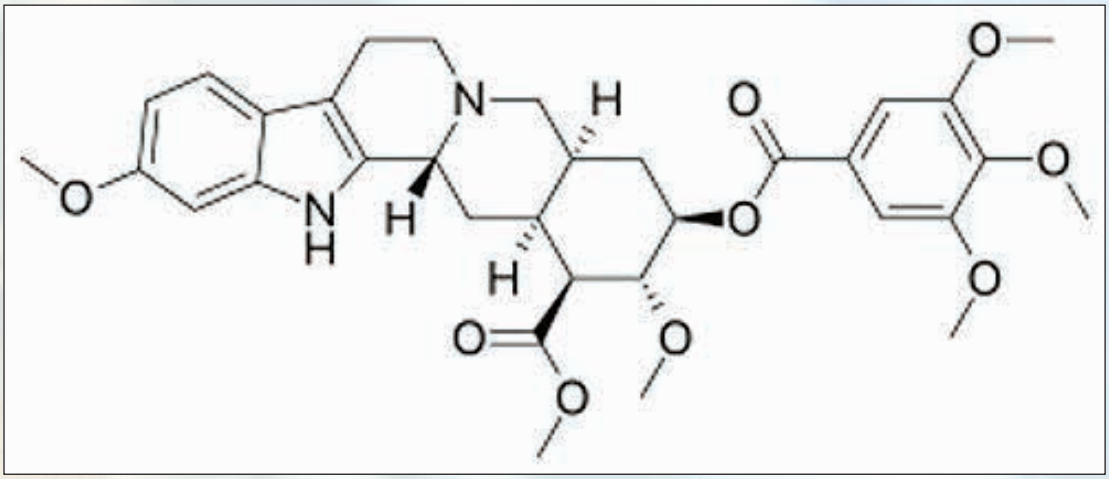
Initially, many psychiatrists favored reserpine and used it before chlorpromazine, largely because it seemed to have fewer side effects *(2, 3, 9)*. But, chlorpromazine has a faster onset of action, and use of reserpine soon declined *(2-5, 9)*.

Chlorpromazine's impact was most apparent in mental institutions *(7, 9)*. Fewer patients were subjected to shock treatments, sleep therapy, or seclusion. Straitjackets were stored away *(2, 3, 9)*. The most obvious change was the silence. In the wards, chlorpromazine's efficacy could be measured in decibels *(3)*.

Henry Brill, a New York psychiatrist, noted, "I remember walking into the dayroom and seeing this small group of patients dressed, quiet, cooperative, and in surprisingly good contact – with their psychiatric symptoms wiped away. That was perhaps the most spectacular demonstration anyone could ask for" *(2)*.

At St. Elizabeths Hospital in Washington, D.C., America's foremost mental institution, many patients who had been ill for years and were serious ward problems responded to chlorpromazine *(9)*. There was a veritable exodus of patients from mental institutions. The average length of hospital stay dropped from years to weeks, and less than 10% of schizophrenic patients remained hospitalized long term *(3, 8)*.

In 1956, the population of American institutionalized patients declined for the first time in 175 years, and the trend continued for more than 15 years *(1-3)*. Patients could expect to spend most of



Chemical structure of reserpine

their life in the community and be self-supporting *(8)*. "Asylum" virtually disappeared from the layman's vocabulary.

Chlorpromazine's phenomenal commercial success prompted drug makers to search for better analogs *(1, 3, 7)*. Phenothiazines that had been shelved and newly synthesized analogs were screened for chlorpromazine-like activity.

Within 10 years, 20 phenothiazines were in development for psychosis, as well as new drug classes, most notably the butyrophenones *(5)*. None of these compounds was superior in overall therapeutic efficacy to chlorpromazine. They differed from each other only in their potency and side effects *(5)*.

Drug Classification

From the very beginning, psychiatrists realized that chlorpromazine and reserpine were different from all of the drugs they had previously administered. "Sedative" did not properly describe them. A new pharmacologic classification was needed.

There was a veritable exodus of patients from mental institutions

In January 1955, Delay and Deniker proposed "neuroleptic" from the Greek, "that takes hold of the nerves" *(4)*. Most Europeans adopted neuroleptic, but Americans preferred "tranquilizer" *(1, 4)*.

The introduction of meprobamate, another drug with tranquilizing properties but of a different sort, forced a rethinking. "Major tranquilizer" was coined to distinguish drugs like chlorpromazine, which reduces mania and psychosis, from "minor tranquilizers" like meprobamate and the benzodiazepines, which do not *(2, 4)*.

In 1956-1957, yet another group, the antidepressants, was introduced *(2)*. Subsequently, "minor tranquilizer" was replaced with "anxiolytic" to highlight the drugs' primary effect. "Major tranquilizer" was replaced with "antipsychotic," even though the antidepressants and lithium are active in manic-depressive psychosis and might also rightly be called antipsychotics *(4)*.

Chlorpromazine also spawned a new line of research, which was directed at elucidating its unique therapeutic effects and determining its mechanism of action. This new scientific discipline, psychopharmacology, fostered a close collaboration between clinical psychiatrists, who used the drugs in patients, and laboratory researchers, who used the drugs as tools to explore the etiology of psychosis *(2, 3)*.

Extrapyramidal Syndrome

Dosing strategies for chlorpromazine varied considerably. Most French psychiatrists followed the lead of Delay and Deniker and dosed conservatively *(1)*. In the US, many psychiatrists followed the flawed philosophy that "if some is good, more is better." When moderate doses were ineffective, they escalated to as much as 3,000 mg per day *(1, 5)*. Unfortunately, some patients were simply refractory to drug treatment, at any dose.

In 1954, a psychiatrist in Switzerland, where patients were also treated aggressively, first reported seeing an "extrapyramidal syndrome" *(4, 5)*. The cluster of movement disorders included parkinsonian disturbances (tremor, rigidity, and slowed movement) along with muscle spasms and motor restlessness. (Since the 1940s, Indian physicians had also observed *Rauwolfia*-induced parkinsonism *(1)*.)

In 1959, another aspect of the extrapyramidal syndrome was first reported. Long-term antipsychotic treatment induced abnormal, involuntary mouth movements (lip smacking, puckering, and tongue movements). These rapid movements (the opposite of parkinsonism) also occurred sometimes in the limbs. This dyskinesia seemed to appear only after years of treatment, and it persisted for a long time after the drug was terminated. Because of its tardy onset and persistence after drug withdrawal, some authors began calling it "tardive" dyskinesia *(1)*.

Chlorpromazine had a broad range of pharmacologic properties—some useful, some not. Of them all, though, the decidedly unpleasant extrapyramidal syndrome, especially tardive dyskinesia, threatened to end treatment of schizophrenia *(5)*. Only after years of experience, systematic trials, and the introduction of clozapine (the first atypical antipsychotic) did researchers show that therapeutic efficacy could be separated from the extrapyramidal syndrome *(1, 5)*.

A New Age

The discovery of chlorpromazine was a major medical milestone. For the first time, a single drug

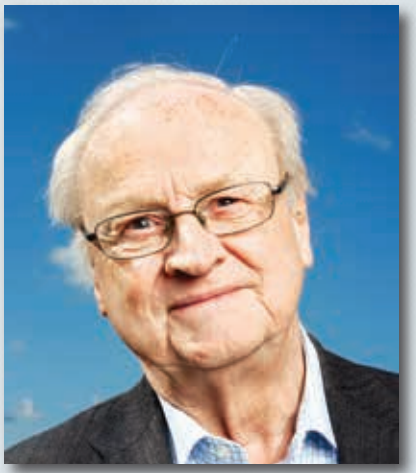
effectively controlled psychiatric disorders without relying on sleep, hyperthermia, or electric or insulin shock. Chlorpromazine achieved the long-desired objective of all psychiatrists: to quickly reduce all signs of mental illness, restore patients' mental state, and allow them to return to their families and society *(3)*.

Chlorpromazine moved psychiatry back into the mainstream of medicine *(7)*. Psychiatrists, especially in North America, began to accept that schizophrenia resulted from underlying neurochemical abnormalities, not just environmental and social influences *(5)*. Drugs became their primary treatment, but rather than eliminating psychoanalysis, chlorpromazine made patients more amenable to both individual and group therapy sessions *(7)*.

Because of chlorpromazine, psychopharmacology emerged as a research discipline. Arvid Carlsson's revelation that chlorpromazine was a dopamine antagonist provided the rationale for the "dopamine hypothesis." Chlorpromazine and other antipsychotic drugs were then used as research tools to identify neural pathways and other neurotransmitters implicated in psychiatric disorders *(7, 17)*.

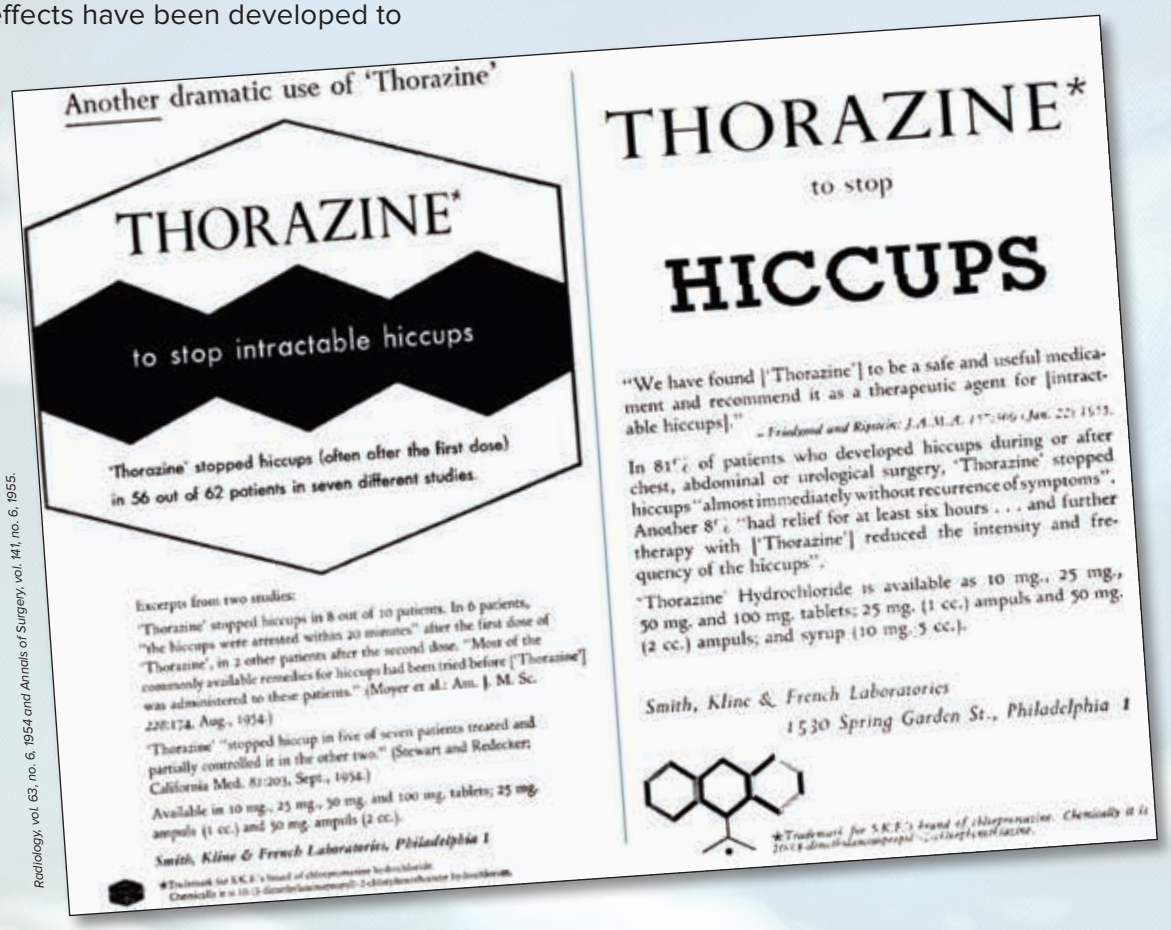
Considerable progress has been made in understanding brain mechanisms, and more selective drugs with fewer side effects have been developed to treat various psychiatric conditions. But none of them has surpassed the effectiveness of chlorpromazine. In 2007, Thomas Ban, a noted psychopharmacologist, wrote, "If an agitated and aggressive psychotic patient in the emergency room fails to respond to some of the excellent new medications that may offer distinct advantages in terms of one or another side effect, one should not hesitate in prescribing good old chlorpromazine that has remained even after 50 years one of the most reliable antipsychotic drugs" *(7)*.

And chlorpromazine is still the only drug approved by the FDA for intractable hiccups.



Reprinted from University of Gothenburg, Photo: Johan Wingborg

Nobel Prize Laureate Arvid Carlsson



Another dramatic use of 'Thorazine'

THORAZINE*

to stop intractable hiccups

'Thorazine' stopped hiccups (often after the first dose) in 56 out of 62 patients in seven different studies.

Excerpts from two studies:

'Thorazine' stopped hiccups in 8 out of 10 patients. In 6 patients, "the hiccups were arrested within 20 minutes" after the first dose of 'Thorazine', in 2 other patients after the second dose. "Most of the commonly available remedies for hiccups had been tried before ['Thorazine'] was administered to these patients." (Moyer et al.: Am. J. M. Sc. 228:174, Aug., 1954.)

'Thorazine' "stopped hiccup in five of seven patients treated and partially controlled it in the other two." (Stewart and Redecker: California Med. 81:203, Sept., 1954.)

Available in 10 mg., 25 mg., 50 mg. and 100 mg. tablets; 25 mg. ampuls (1 cc.) and 50 mg. ampuls (2 cc.).

Smith, Kline & French Laboratories, Philadelphia 1

*Trademark for S.K.F.'s brand of chlorpromazine hydrochloride. Chemically it is 10-(3-dimethylaminopropyl)-2-chlorphenothiazine hydrochloride.

THORAZINE*

to stop

HICCUPS

"We have found ['Thorazine'] to be a safe and useful medicament and recommend it as a therapeutic agent for [intractable hiccups]." — *Friedland and Ripstein: J.A.M.A. 157:305, Jan. 22, 1955.*

In 81% of patients who developed hiccups during or after chest, abdominal or urological surgery, 'Thorazine' stopped hiccups "almost immediately without recurrence of symptoms". Another 8% "had relief for at least six hours . . . and further therapy with ['Thorazine'] reduced the intensity and frequency of the hiccups".

'Thorazine' Hydrochloride is available as 10 mg., 25 mg., 50 mg. and 100 mg. tablets; 25 mg. (1 cc.) ampuls and 50 mg. (2 cc.) ampuls; and syrup (10 mg./5 cc.).

Smith, Kline & French Laboratories
1530 Spring Garden St., Philadelphia 1

*Trademark for S.K.F.'s brand of chlorpromazine. Chemically it is 2-chloro-10-(3-dimethylaminopropyl)-phenothiazine.

Radiology, vol. 63, no. 6, 1954 and Annals of Surgery, vol. 141, no. 6, 1955.

Advertisements from the mid-1950s for Thorazine®, which is used to treat stubborn hiccups

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Cortisone:
A Miracle with Flaws

Rebecca J. Anderson, PhD

Credit: John F. Kennedy Presidential Library and Museum, Boston

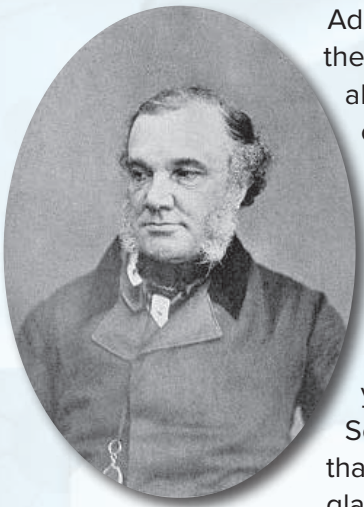
Congressman John F. Kennedy (on crutches) and his mother, Rose Fitzgerald Kennedy, at a campaign reception.

In September 1947, newly elected Congressman John F. Kennedy collapsed while visiting his sister in London and was rushed to the hospital. His symptoms weren't new, but the diagnosis was. Sir David Davis, the attending physician, told Kennedy that he had Addison's disease and estimated that he had less than a year to live *(1-3)*.

Thomas Addison (1793-1860) was one of the legendary physicians at Guy's Hospital in London, along with Thomas Hodgkin and Richard Bright. Addison was the first to describe pernicious anemia and the various skin conditions associated with diabetes, scleroderma, and high cholesterol. In 1839, he wrote one of the first modern medical textbooks, *Elements of the Practice of Medicine (4)*.

While studying pernicious anemia, Addison noticed that some of his patients exhibited atypical symptoms: no appetite, weak pulse, abdominal pain, and vomiting. Being attuned to dermatology, he also noted a striking skin discoloration. These patients progressively weakened and died. At autopsy, their adrenal glands were the "size of a hen's egg" and as "hard as stones" *(4)*. (Healthy adrenal glands are soft and the size and shape of an almond.)

The Mayo Clinic's Plummer Building in Rochester, MN.



Thomas Addison

Addison attributed the patients' adrenal abnormalities and resulting clinical condition to infection with tuberculosis, which was widespread in Europe in the 19th century *(4).* He published his observations in 1855. A year later, Charles Brown-Séquard demonstrated that animals whose adrenal glands had been removed exhibited a similar syndrome to that described by Addison. The animals, like Addison's patients, inevitably died.

The logical treatment was replacement therapy, and Archibald L. Muirhead volunteered as the first human guinea pig. Muirhead, a pharmacology professor at Creighton University School of Medicine, arrived at the Mayo Clinic in Rochester, Minnesota, in 1920. He suffered from advanced Addison's disease and was bedridden.

The only substance identified in the adrenal glands was epinephrine, which John Jacob Abel and others had isolated from the adrenal medulla and purified 20 years earlier. Muirhead received injections and rectal suppositories of epinephrine three times a day, and ate raw adrenal glands "to the point of tolerance" with each meal *(5).*

The regimen was less than optimal. Epinephrine caused weakness, tremors, and heart palpitations. The raw adrenal glands caused nausea, vomiting, and intestinal cramps. But for several months, Muirhead's condition improved *(4-6).*

Over the next decade, Leonard Rowntree and his associates at the Mayo Clinic subjected dozens of other patients to the "Muirhead regimen." More than half of them showed at least temporary improvement in their Addison's symptoms. Then, in 1929, Rowntree met with Joseph Pfiffner, who was in Rochester to attend a scientific conference. Pfiffner and Wilbur Swingle, his colleague at Princeton University, had prepared an interesting extract of bovine adrenal cortex tissue in their lab.

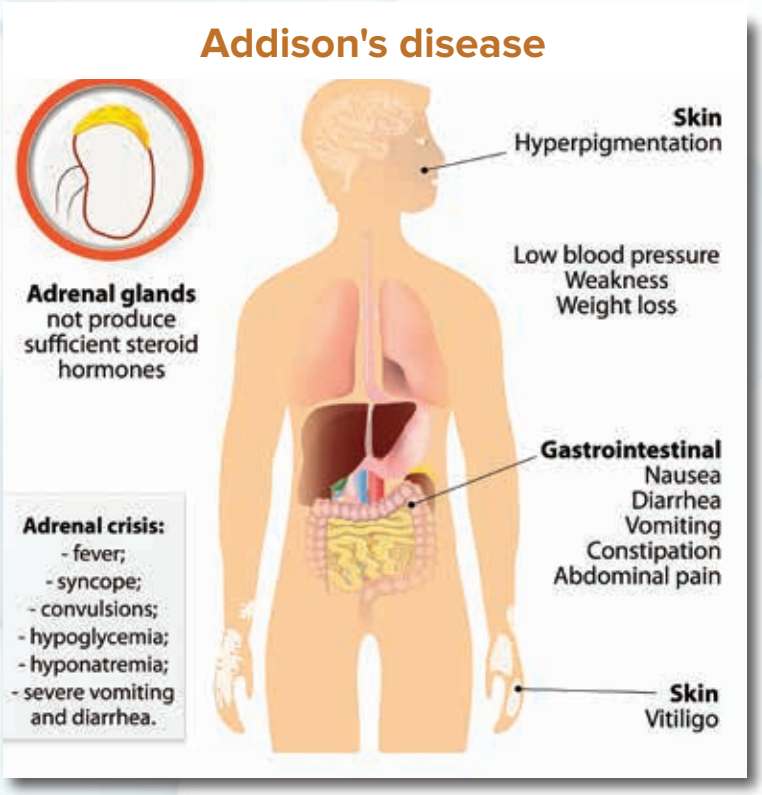
The crude Swingle-Pfiffner extract maintained the life of cats whose adrenal glands had been surgically removed. When they ran out of the extract, the cats died *(4).* Along with several other groups, Swingle and Pfiffner sought to isolate and purify the extract's life-sustaining substance, which researchers called "cortin."

In 1930, a 39-year-old farmer from Iowa arrived at the Mayo Clinic in a "state of collapse" *(4).* Rowntree had treated him previously with the Muirhead regimen, but like most patients, his addisonian symptoms had returned and progressed. With few options remaining, Rowntree requested and received a sample of the Swingle-Pfiffner extract. After two days of treatment, the farmer showed marked improvement in strength and appetite. But he also experienced severe irritation at the injection site, and when the extract ran out, his symptoms returned.

Over the next four years, the Mayo Clinic treated 48 patients with the Swingle-Pfiffner extract *(4).* Unfortunately, the Princeton researchers could produce only lab-scale samples. Rowntree asked Edward Kendall, a chemist at the Mayo Clinic, for scale-up assistance.

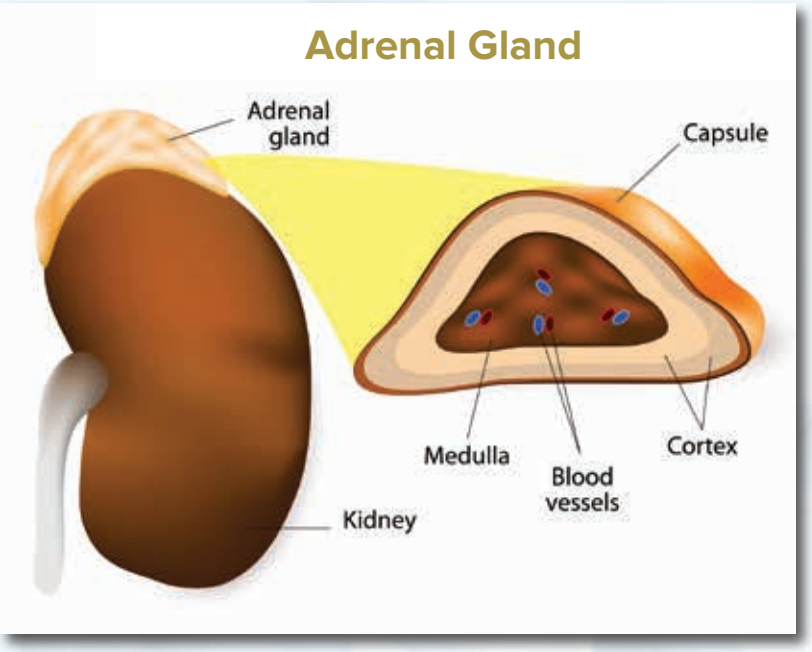
Kendall, the Compulsive Chemist

Edward Kendall began his career at Parke, Davis & Company in Detroit in September 1910. Like other leading pharmaceutical firms, Parke Davis complied with the recently enacted Pure Food and Drug Act. The chemistry department's primary responsibility was to ensure the purity of the company's products



Signs and symptoms of Addison's disease

(7). Kendall felt isolated as the only research chemist, and he stayed only a few months. But the research he started there—isolating the hormone produced by the thyroid gland—would consume all his efforts for the next 20 years *(7).*



The adrenal glands consist of two structurally different parts, the adrenal cortex and adrenal medulla.

After three years at St. Luke's Hospital in New York City, Kendall moved to Rochester to head the Mayo Clinic's newly created biochemistry laboratory. In 1916, he succeeded in isolating crystalline thyroxine from the thyroid gland. With a dogged determination that characterized all of his research efforts, Kendall spent the next 10 years trying to synthesize thyroxine *(7).* In 1926, he abruptly ended his efforts when Charles Harington at the University College London published a synthetic method.

While contemplating his next big project, Kendall received a letter from Albert Szent-Györgyi. Szent-Györgyi had isolated a compound that he called "hexuronic acid" from fruits, vegetables, and (in high concentrations) the adrenal glands of cows. Szent-Györgyi was planning a trip to the US and asked if he could join Kendall's lab temporarily as a visiting scientist *(4, 7).* The Mayo Clinic was conveniently located near the meatpacking houses in St. Paul, where Szent-Györgyi could obtain large quantities of adrenal glands. Kendall agreed.

Szent-Györgyi arrived at the Mayo Clinic in September 1929 and quickly set up shop: a wooden press, a large meat grinder, and numerous 40-gallon crocks. He tackled the messy work with a passion *(4).* When he left in May 1930, Szent-Györgyi had isolated several grams of hexuronic acid. He offered a sample to Kendall, but neither of them knew what the compound did. After returning to Hungary, Szent-Györgyi and his coworkers proved that hexuronic acid was vitamin C, a discovery for which he later received the Nobel Prize in Physiology or Medicine.

Searching for Cortin

Thanks to Szent-Györgyi's project, Kendall's lab was now well equipped to handle large-scale adrenal extractions. When Rowntree asked him for assistance in preparing the Swingle-Pfiffner extract, Kendall not only agreed but also saw a great research opportunity. Something in the adrenal cortex was responsible for maintaining an individual's weight, strength, and well-being, as well as sodium-potassium balance in the blood. If he could isolate cortin, "it should have wide [clinical] application" *(7).*

Kendall may not have been the most skilled chemist, but he had several advantages over the other groups that were searching for the elusive "cortin." First, he had access to a plentiful supply of adrenal glands. Tapping his contacts at Parke Davis, he proposed a partnership.

Parke Davis had been producing epinephrine (Adrenalin®) since 1901 and was the largest supplier to retail drugstores *(7).* The company agreed to procure adrenal glands from the Detroit stockyards and ship them free of charge to the Mayo Clinic in Rochester. In return, Kendall's lab would extract both epinephrine and cortin, ship the epinephrine to Parke Davis, and retain the cortin for its own use. Both parties benefited: Kendall got the glands for free, and Parke Davis marketed Adrenalin without labor costs.

Kendall also struck a deal with the Wilson Packing Company in Chicago to obtain an additional 300 pounds of adrenal glands per week. Kendall's production facility operated around the clock in 3 shifts for 15 years and processed a total of 150 tons of adrenal tissue *(4, 7).*

Kendall's second advantage over the other biochemistry researchers was the ability to assess his extracts physiologically. Mayo's animal research labs prepared and cared for the animals. Dogs whose adrenal glands had been surgically removed rarely survived more than 48 hours. Active adrenal extracts, such as the Swingle-Pfiffner extract, prolonged their life.

In 1933, Kendall announced at a weekly staff meeting at the Mayo Clinic that he had succeeded in isolating cortin, the adrenal cortex hormone. Although vague on details, he said the crystalline substance maintained adrenalectomized dogs in a "normal condition" *(7).*

The Turning Point

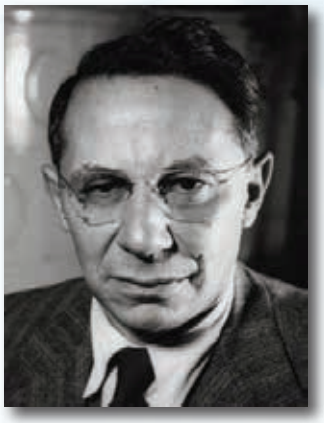
In June 1934, 17-year-old John F. Kennedy arrived in Rochester. At the Mayo Clinic and later at nearby St. Mary's Hospital, the teenager underwent a series of uncomfortable tests. His doctors originally suspected a peptic ulcer but ultimately concluded that Kennedy had colitis *(1).* Their state-of-the-art prescription was a restricted diet, reduced emotional stress, and injections of horse serum *(1).* Kennedy was discharged after a month, but he continued to suffer intestinal discomfort throughout his senior year at Choate prep school.

In September 1934, physiologist Dwight Ingle joined Kendall's lab and set up additional methods for evaluating the activity of Kendall's extracts *(7).* When Ingle electrically stimulated muscles of adrenalectomized rats, muscle twitching ceased in less than 24 hours. The Swingle-Pfiffner extract restored and maintained normal muscle twitches indefinitely *(4).*

Unfortunately, the crystalline compound that Kendall called "cortin" did not restore normal muscle activity in Ingle's rat assay. Similarly, adrenalectomized dogs treated with the compound eventually developed the symptoms of Addison's disease *(4).* Kendall's announcement about cortin had been premature.

Further research showed that the adrenal cortex produced not one but many compounds. Kendall differentiated them alphabetically in the order in which he isolated and crystallized them. Meanwhile, Joseph Pfiffner moved to Columbia University and joined forces with another highly skilled chemist, Oskar Wintersteiner. They also designated their crystalline compounds alphabetically *(8).*

Another prominent adrenal hormone explorer was Tadeus Reichstein. Born in Poland, the young Reichstein moved with his family to Kiev, Ukraine, and was educated in Zurich, Switzerland *(9, 10).* Known for his modesty, collegial style, and wide network of collaborators, Reichstein outshone his contemporaries in both brilliance and productivity *(10, 11).* In Zurich, Reichstein likewise distinguished his adrenal cortex compounds with letters of the alphabet.



Dr. Tadeus Reichstein

The publications of these investigators were confusing. Pfiffner's compound F was the same as Reichstein's compound Fa, which Kendall called compound E *(8).* To clarify the confusing nomenclature, the three labs shared samples for comparison, but they also remained competitive *(4, 7).*

By 1936, Kendall and the Pfiffner-Wintersteiner lab had each isolated five cortical compounds *(4).* The compounds were all chemically related and were conclusively shown to be steroids. Meanwhile, Reichstein had published seven papers on adrenal cortex chemistry, and everyone agreed that he was well ahead of the other groups *(4, 7).*

The following year, Reichstein identified another cortical compound, which he named substance H. Initial pharmacologic results suggested that substance H was the most active compound isolated so far. Many thought it was the long-sought "cortin."

On closer inspection, substance H proved to be the same as Kendall's compound B. Ingle's assays had already shown that compound B/substance H was active in the rat twitch test (i.e., enhancing the capacity of muscle to perform work), but it had only a slight effect on sodium-potassium balance *(7).*

At that time, most researchers thought the adrenal cortex hormone's most important influence was on sodium and potassium. Then, Cyril N. H. Long, a Yale University investigator who was primarily interested in diabetes, found that Kendall's compounds A and B had a marked effect on carbohydrate metabolism, which helped explain Ingle's findings in the rat muscle assay *(7).*

In a related experiment, Ingle found that compounds A and B, as well as an extract of the whole adrenal gland, caused the adrenal glands and thymus of normal rats to atrophy *(7).* The externally administered compounds eliminated the need to produce the hormones naturally, and the glands shrank from disuse.

DOCA

In the summer of 1938, Reichstein in Switzerland had succeeded in synthesizing a steroid using bile acid as the starting material *(7)*. He called it desoxycorticosterone. Because Reichstein had limited facilities for pharmacologic assessment, he asked Kendall for assistance. Desoxycorticosterone was 6 times more active than substance H/compound B in adrenalectomized dogs *(7)*.

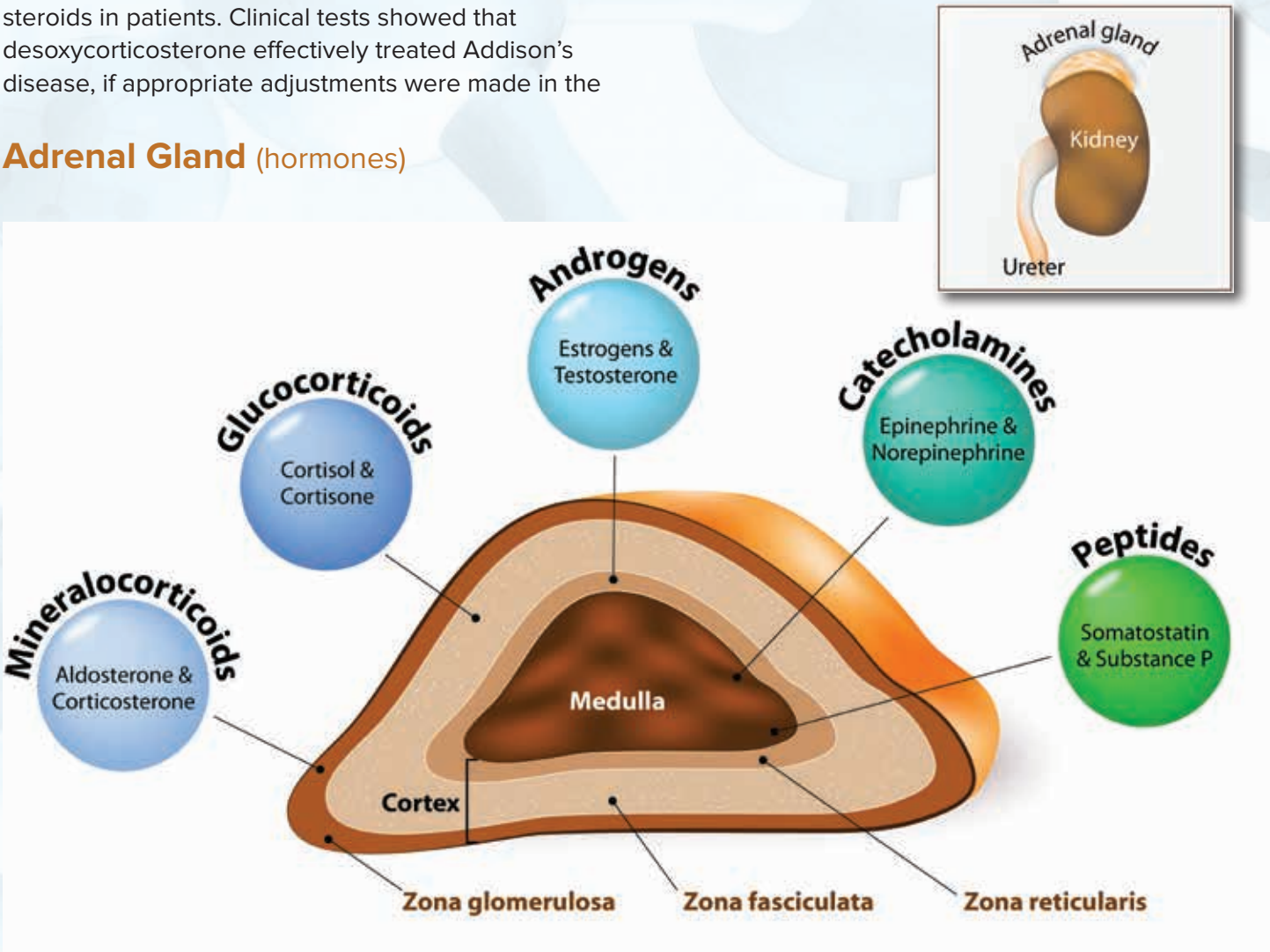
In August 1938, Reichstein reported that he had isolated desoxycorticosterone from the adrenal cortex *(7)*. This was the first time that the same steroid had been both isolated from a natural source (i.e., the adrenal cortex) and produced synthetically in the lab. In addition, desoxycorticosterone mimicked the effect of the Swingle-Pfiffner extract more closely than any other isolated steroid.

Kendall's third advantage (being affiliated with the Mayo Clinic) was the ability to test the adrenal steroids in patients. Clinical tests showed that desoxycorticosterone effectively treated Addison's disease, if appropriate adjustments were made in the patients' diet to maintain normal blood potassium and sodium levels *(7, 12)*.

Reichstein was now professor and director of the Pharmaceutical Chemistry Institute at the University of Basel *(12)*. This position involved close ties with CIBA Pharmaceuticals *(4)*. Taking advantage of Reichstein's method, CIBA and also Organon (a Dutch drug company) devised a commercially viable synthesis for desoxycorticosterone acetate (DOCA). Regrettably, the market for an Addison's disease drug was small, and DOCA was outrageously expensive *(4)*.

In February 1938 and again in February 1939, John F. Kennedy returned to the Mayo Clinic, complaining of ongoing intestinal problems *(1)*. Like the doctors in New England, where Kennedy also sought treatment, the Mayo clinicians' extensive inspection of his colon and digestive system revealed no new insights about his colitis.

Adrenal Gland (hormones)



Adrenal glands sit on top of the kidneys and are composed of an outer cortex and an inner medulla, which produce different types of hormones.

It is possible that he was offered DOCA as an experimental treatment. When Kennedy's medical records and related correspondence were unsealed in 2002, Robert Dallek and Jeffrey Kelman found that Kennedy had written his father for assistance in filling a prescription for a "very potent" drug, explaining that his doctor "seems to be keeping it pretty quiet" *(1)*.

Certainly, Kendall was actively involved in clinical investigations of DOCA for the treatment of various diseases, and Charles Code at the Mayo Clinic designed the extended-release DOCA formulation that Kennedy used. The bean-sized pellets contained a mixture of DOCA and beeswax *(4)*. Years later, Kennedy's close friend, Paul Fay, recalled watching young Kennedy make a superficial cut in his thigh with a small knife, slip the pellet underneath the skin, and cover the slit with a bandage *(1)*.

Switching Quests

On May 8, 1940, Kendall summarized the status of adrenal steroid research at the Mayo Clinic's weekly staff meeting *(7)*. After a decade of work, 28 steroids had been isolated from the adrenal cortex *(13-15)*. The accumulated animal results clearly showed that no single steroid was responsible for both carbohydrate metabolism and sodium-potassium balance. The notion that one hormone accounted for the activity observed in crude adrenal extracts, such as the Swingle-Pfiffner extract, was now widely dismissed *(7)*.

In his presentation, Kendall took a stab at defining the structure-activity relationships. Some cortical steroids, characterized by compounds A and E, predominantly affected carbohydrate metabolism (the glucocorticoids). Other cortical steroids had a marked effect on sodium and potassium (the mineralocorticoids). These included DOCA and aldosterone, which Reichstein subsequently isolated in 1953 *(7, 12)*.

DOCA was commercially available, but clinical assessment of the glucocorticoids for Addison's disease and other conditions was hampered by supply shortages. Extraction and purification of 1 g of compound A, for example, required 3,000 lbs of adrenal glands *(15)*. Kendall turned his attention to finding a way to synthesize compounds A, B, E, and F "from sources that were abundant and cheap" *(7)*.

Military Priorities

In May 1941, all of the adrenal cortex researchers, including Kendall, gathered at Yale University. They were informed that rumors were circulating within the US Army and Navy Medical Corps that the Germans had successfully developed glucocorticoid drugs. Luftwaffe pilots could fly at 40,000 feet without experiencing hypoxia *(7)*. The drug's antistress properties allegedly enabled Nazi troops to withstand the shock of severe wounds and permitted more rapid wound healing *(16)*. There were also reports that German submarines were collecting huge quantities of adrenal glands from Argentine slaughterhouses *(7, 16)*.

With war on the horizon, the US National Research Council (NRC) set three research priorities. Combat efficiency trumped all other considerations, and the NRC's top priority was synthesis of a performance-enhancing glucocorticoid. The second priority was production of penicillin, and the third project was development of drugs for malaria *(7, 15, 17)*.

Kendall had a substance for which he did not have a disease, and Hench had a disease for which he did not have adequate treatment

On October 7, 1941, the glucocorticoid committee met in Washington, DC, to organize its work. The 14 committee members represented CIBA, Merck, Schering, E. R. Squibb & Sons, several prominent university labs, and the Mayo Clinic (i.e., Kendall) *(7)*.

Compounds A and E enhanced the ability of muscle to perform work in Ingle's muscle twitch assay. Both compounds also influenced carbohydrate metabolism and glycogen deposition. Compound E appeared to be 2-3 times more potent than compound A and became the ultimate goal. But compound A seemed simpler to synthesize. The committee decided to proceed stepwise: first, synthesize compound A and then compound E *(7)*.

Because of his extensive experience with adrenal extracts, Kendall was heavily engaged in the NRC project and soon established a close working relationship with the chemists at Merck. Early in 1942, Lewis Sarett, a young Merck chemist, spent three months in Kendall's lab and made rapid progress in preparing key chemical intermediates. When Sarett returned to Merck, the two chemists harmonized their efforts. Kendall's lab focused on preparing compound A. Sarett attempted conversion of compound A to

compound E *(7)*. They labored for years, but progress was slow.

In the fall of 1943, the glucocorticoid committee received word that Reichstein had succeeded in making compound A. Despite his expertise, though, Reichstein's yield was only 0.04%, making compound A prohibitively expensive and available in only small amounts *(7)*. Everyone assumed that this skilled chemist would also soon succeed in synthesizing compound E, but World War II had now turned in the Allies' favor. It was improbable that compound E would be available in time—and in sufficient quantities—for use by soldiers and marines. In addition, the US government now knew the German rumors were completely false *(7)*.

During the war years, penicillin production surged, and it was available in ever-increasing quantities. Quinacrine and other antimalarials had also been successfully developed. But the government's top research priority, compound E, failed to deliver, and the NRC terminated the project in June 1944 *(7)*.

The Price of Persistence

Merck had invested heavily in the compound E project and continued to collaborate with Kendall's lab after the committee disbanded. Over the next two years, the Merck chemists managed to scale up production and improve the yield of compound A.

In April 1946, Kendall and the Merck project leader decided to share their accumulated data on compound A. They organized a special session in conjunction with the FASEB meeting in Atlantic City *(7)*. The session was well attended, including Reichstein, who was visiting the US.

The audience was mostly interested in clinical results, which were only preliminary, rather than the extensive laboratory data. Mayo clinician Edwin Kepler and other investigators reported that compound A was of no value in treating patients with Addison's disease *(7)*.

It seemed unlikely that compound E, which differed from compound A by only an additional hydroxyl group, would perform any better *(7)*. But despite the audience's lack of interest, Kendall returned to his lab and proceeded with compound E.

From DOCA to Compound E

In the 1940s, John F. Kennedy continued to suffer bouts of colitis, DOCA treatment notwithstanding, and he developed two more problems: osteoporosis and Addison's disease. In 1944, Navy surgeons removed "some abnormally soft disc interspace material" to relieve persistent back pain *(1)*.

After his Addison's disease diagnosis in 1947, Kennedy regularly implanted a DOCA pellet every 3 months *(2)*. When Dallek and Kelman reviewed Kennedy's medical records, they concluded that long-term steroid treatment had caused his adrenal glands to atrophy, just as Ingle had demonstrated in laboratory rats. (Kennedy never had tuberculosis.) Pathologists who participated in Kennedy's autopsy confirmed that his adrenal glands had been reduced to "a few individual adrenal cortical cells immersed in a sea of fat" (*2, 3, 18*).

In a more recent examination of Kennedy's medical records, Lee Mandel suggested that Kennedy's Addison's disease was the result of an endocrine autoimmune disease, APS 2 *(3)*. Regardless, Kennedy's case presented a medical dilemma. Steroid treatment had caused (or exacerbated) his adrenal glands to shut down and atrophy, making him dependent on continued steroid use. But those same steroids would further weaken his bones, especially in his lower back, and cause a host of other steroid-related adverse effects.

In 1947, Lewis Sarett, with assistance from Kendall, succeeded in devising a much-improved method for making compound E *(16, 17, 19)*. The 37-step synthesis was a major chemistry achievement, but most researchers were skeptical that compound E would be of much value, except perhaps for Addison's disease—and that was a small market *(16)*.

Merck organized an investigator conference in New York City on April 29, 1948, to drum up interest *(7)*. Although the assembled physicians were interested in adrenal cortical hormones, they remained skeptical about compound E. Only Randall Sprague, a Mayo clinician, requested a sample to treat one Addison's disease patient. Afterward, Merck's project leader advised Kendall that Merck would likely end its compound E efforts unless someone found profitable clinical uses for it *(7)*.

Undaunted, Kendall returned to his lab and continued working on a simplified synthetic method. In August 1948, he bumped into Philip Hench in the lobby of Mayo's Plummer Building *(7)*. The two Mayo staff members were casually acquainted but had never worked together. Hench inquired about the compound E project, and Kendall rather elusively said he was making progress *(4)*.

Hench's Hunch

Archiv Werner Stuhler / Lindau Nobel Laureate Meeting

Prof. Dr. Philip Showalter Hench at the 10th Lindau Nobel Laureate Meeting.

Philip Hench, a tall, solidly built man, had been born with a severe cleft palate. The deformity affected his speech but did not stop him from being a loquacious talker *(4).* He spent hours perfecting his elocution and practicing his formal presentations.

Hench received one of the first postgraduate fellowships offered by the Mayo brothers and joined the staff in 1923 *(4, 15).* In 1926, he founded and became the head of the Mayo Clinic's Department of Rheumatic Disease.

In 1929, as Kendall was embarking on his adrenal cortex research, Hench became intrigued by a 65-year-old patient who experienced a prolonged remission of his rheumatoid arthritis during and after an attack of jaundice *(4, 15).* At the time, rheumatoid arthritis was thought to be progressive and irreversible.

Over the next 20 years, Hench accumulated and reported many cases of jaundice-induced remission of rheumatoid arthritis. He also noted that pregnancy, starvation, injection of typhoid vaccine, and general anesthesia (even without surgery) likewise produced remissions *(4, 15)*.

Hench suspected an endogenous substance was responsible for the remissions and began calling it "substance X" *(15).* Thinking that substance X was associated with the liver, he tried to mimic the therapeutic effect of jaundice by injecting cholesterol, liver extracts, bile acids from humans or cattle, and even blood from jaundiced patients *(4).*

In 1931, Charles Slocumb joined Hench as the Mayo Clinic's second rheumatologist. Howard Polley became the third in 1942 *(4).* Both supported Hench's clinical investigations of rheumatoid arthritis. Among the possible treatments was lactophenin, a new jaundice-producing agent. Investigators in Sweden reported that about half of their lactophenin-treated patients developed jaundice, along with relief from their arthritis symptoms *(4, 15).*

In July 1948, two patients with long-standing rheumatoid arthritis volunteered for Hench's lactophenin experiment at St. Mary's Hospital. The first patient developed jaundice, along with a dramatic remission of arthritis symptoms *(4).*

The second patient, 29-year-old Mrs. G., from Kokomo, Indiana, had participated in Hench's earlier clinical trials without success. She could not raise her arms over her head or lift a book. Sometimes, she could not even roll over in bed. Unfortunately, lactophenin did not induce jaundice, and her arthritis remained unchanged *(4).*

Hench had no other treatments to offer and wanted to discharge Mrs. G., but she refused to leave (15). She had seen the dramatic relief that jaundice produced and was willing to try anything. For weeks, Hench, Slocumb, and Polley racked their brains for ways to help their stubborn but extremely cooperative and affable patient *(4).*

Then, Hench encountered Kendall in the clinic lobby. It was a longshot. There was no data suggesting that compound E affected inflammation, pain, or any other aspect of rheumatic disease.

A Hail Mary at St. Mary's

Hench called Randall Sprague, the Mayo endocrinologist who had received 9 g of compound E for his Addison's patient *(13).* Sprague was in the middle of hospital rounds and impatiently listened to Hench's long-winded pitch. Sprague thought it was "an absurd idea" and refused to share his precious aliquot *(4, 13).* Hench then called Kendall's office.

Kendall had planned a long weekend at his cottage on Lake Zumbro. On Thursday afternoon, he swung by his office to pick up his messages, including one from Hench. Surely, the issue, whatever it was, could wait until Monday. But that evening at the cottage, Kendall said, "a sense of urgency came over me" *(7).*

The nearest phone was an old-fashioned hand-crank monstrosity at a farmhouse two miles away. For 45 minutes, Kendall stood uncomfortably speaking into the wall-mounted mouthpiece as Hench recounted Mrs. G.'s case in excruciating detail *(4).* Kendall was receptive, but he did not have enough compound E for clinical testing.

Kendall followed up by appealing to Merck, but before releasing its limited clinical supplies, the medical department wanted a formal justification. Kendall knew nothing about rheumatoid arthritis. Hench knew nothing about compound E. Basically, as Charles Slocumb later noted, "Kendall had a substance for which he did not have a disease, and Hench had a disease for which he did not have adequate treatment" *(4).*

Drs. C. H. Slocumb, H. F. Polley, E. C. Kendall, and P. S. Hench

With assistance from Kendall, Slocumb, and Polley, Hench wrote the medical rationale. He kept the letter simple and uncharacteristically brief because the rationale was convoluted and lacked scientific rigor *(7)*. Fortunately, Merck was satisfied and on September 4, 1948, shipped 5 g of compound E, worth more than $1000 ($10,000 in today's currency) *(4, 16)*.

Thus began the successful collaboration between Kendall and Hench. They complemented each other's work habits. Hench approached research conservatively and meticulously, and he analyzed results impartially. Kendall, on the other hand, was reckless, constantly improvised, flew by the seat of his pants, and habitually made premature announcements of his laboratory successes *(4)*. Hench was receptive and sympathetic to the ideas of others; Kendall resisted suggestions. But both had a compulsive work ethic, displayed boundless optimism, confidently defended their ideas, and could be stubborn *(4)*.

Because Hench was preparing for a European lecture tour, Slocumb and Polley took charge of Mrs. G.'s case. On Tuesday evening, September 21, 1948, Slocumb injected the first 50 mg dose of compound E intramuscularly. He continued with twice-daily injections, and by Friday, he found Mrs. G. exercising and raising her hands over her head. Her painful stiffness was gone *(4, 7, 15)*.

Slocumb repeatedly urged Hench to visit Mrs. G. before he left town, but Hench was intensely focused on his lectures. He knew Mrs. G.'s case well, his expectations were low, and he had no time for the half-mile trip from his office to St. Mary's Hospital. Finally, on Friday evening and now somewhat irritated, he yielded to Slocumb. When he walked into Mrs. G.'s room and saw her moving with ease, he was flabbergasted *(4, 13)*.

The following week, Hench stopped in New York on his way to Europe to meet with the Merck project director. He described Mrs. G.'s response and without overpromising, he persuaded Merck to supply compound E to treat four additional rheumatoid arthritis patients *(4, 7)*.

To conserve the precious and expensive drug, Slocumb flushed out every syringe, needle, and bottle, and Kendall recovered and recycled every bit of compound E residue *(4)*. Hench stayed in touch with his team, and when he returned from Europe in December 1948, he immediately expanded the clinical trial. Over the next 3 months, they treated 18 more patients *(7)*.

The results continued to be spectacular, but Merck insisted on treating patients at other sites before they announced their results *(4)*. In March 1949, Hench chose five highly regarded rheumatologists representing clinics spread from coast to coast. Because of the limited supplies, each investigator could treat only two patients. All of them—in every geographic region—experienced a dramatic remission of their rheumatic disease *(4)*.

The Blockbuster

Rumors began circulating of a breakthrough in arthritis treatment. Hench, Kendall, and their Merck counterparts planned a formal announcement at the Association of American Physicians (AAP) meeting in Atlantic City on May 3, 1949 *(16)*. Prior to that, Kendall and Hench presented their findings at Mayo's weekly staff meeting.

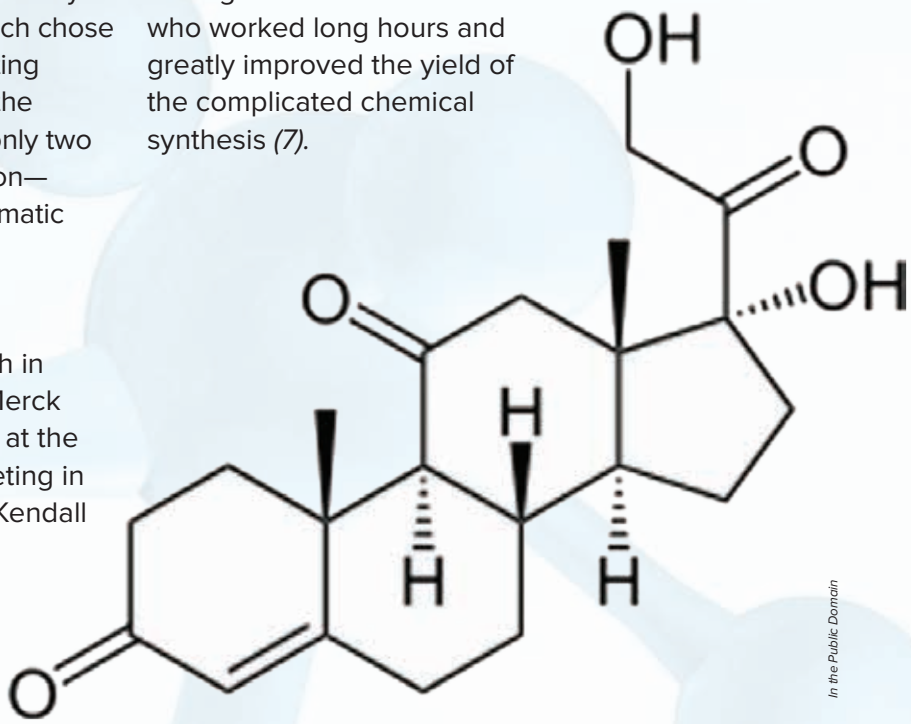
On April 20, 1949, every seat in Mayo's Plummer Auditorium was taken. Chairs clogged the aisles, and people sat on window sills and the speaker's platform. Others crammed the hallway out to the elevators. Mayo's staff meetings were closed to the press, but the country's most famous science writer, William Laurence of *The New York Times,* wrangled a seat in the front row and furiously took notes *(4, 7)*.

The Mayo team had filmed all 23 of their patients before and after treatment with compound E. Hench showed clips at the staff meeting and the AAP meeting, as well as the June meetings of the American Medical Association and the International Congress of Rheumatologists *(7, 15)*. He emphasized that compound E was not a cure for rheumatoid arthritis and should be viewed as just a new research tool. What everyone saw was a wonder drug.

Press coverage was extensive—and somewhat confusing *(7)*. Many in the media and public mistakenly equated compound E with vitamin E. One day in May 1949, Hench stopped by Kendall's office, and they brainstormed a more distinctive name—settling on "cortisone" *(4, 7)*.

Cortisone was hailed as "among the biggest advances that medicine has ever made in a single leap" *(16)*. Congress appropriated $1.1 million ($27 million in today's dollars) to the National Institutes of Health specifically for research on cortisone and its analogs *(20)*.

Merck, the only company with the know-how to make cortisone, was flooded with requests. Max Tishler directed Merck's efforts to scale up production, adding dozens of chemists who worked long hours and greatly improved the yield of the complicated chemical synthesis *(7)*.



In the Public Domain

Chemical structure of cortisone

Still, demand outstripped supply and fostered a black market for fake cortisone *(13, 15)*. Merck turned to the National Academy of Sciences, which formed a committee to evaluate requests and equitably distribute the drug until adequate supplies became available *(4, 7)*. In June 1950, Merck, followed by Schering, introduced cortisone commercially, and by October, the price dropped to $22.40 per gram *(16)*.

Cortisone production was further streamlined when large supplies of progesterone (produced from an extract from Mexican yams) became available. Using progesterone (a cheap starting material), Merck, CIBA,

Upjohn, and Pfizer manufactured large quantities of cortisone and related corticosteroids *(4)*.

In 1952, Upjohn implemented a new low-cost process using a microbiological step *(Rhizopus nigricans)* and sold cortisone for $4 per gram *(15, 16)*. The once-rare and expensive cortisone was now ubiquitous. It also stimulated research and development of a series of more potent and selective glucocorticoids.

The Crash

In 1950, Kendall, Hench, and Reichstein were awarded the Nobel Prize in Physiology or Medicine—just two years after the first arthritis patient had been treated with cortisone. By that time, though, physicians realized that cortisone was no panacea. The miracle drug had become a curse. Physicians were warned to "avoid the temptation to premature use" because corticosteroids could do more harm than good *(21)*.

All of the rheumatoid arthritis patients in Hench's original study experienced unpleasant side effects from cortisone treatment *(15)*. Within a few weeks, Mrs. G. became bloated, with a "moon face" and streak-like lesions on her body (called striae). Mentally, the previously affable patient now alternated between depression, euphoria, and psychosis *(4)*.

Janis Larson, a 16-year-old high school junior from Elkader, Iowa, was also in Hench's clinical trial *(22)*. Because cortisone was in short supply, Hench first tried other treatments, including salicylates and physical therapy. But her arthritis remained severe and uncontrolled.

On September 15, 1949, Janis received her first injection of cortisone *(22)*. Within a day, she could raise herself out of bed without assistance, and her pain was greatly reduced. Knee biopsies showed a marked decrease in inflammation. It was an exciting time at St. Mary's, and occasionally, Janis was wheeled into the hospital auditorium to be photographed *(22)*.

However, by October 16, 1949, Janis had developed a moon face, which persisted until the mid-1950s. Despite reducing the cortisone dose, she developed striae, and on November 20, treatment was discontinued. Janis immediately "crashed." She felt worse than before taking cortisone. Shortly before Christmas, she resumed cortisone treatment, along with salicylates, physical therapy, and frequent doses of morphine *(22)*. She was discharged in February 1950, vowing not to return to the Mayo Clinic.

In 1951, Janis was treated at University Hospital in Iowa City. Her doctor began estrogen treatment, which allowed her to taper her daily cortisone dose. In 1956, she discontinued cortisone altogether *(22)*. Her rheumatoid arthritis had become inactive and has remained so since. But her joints had badly deteriorated. She endured corrective surgery to replace both hips, both ankles, and both knees, as well as operations on her fingers and both elbows *(22)*.

By 1954, John F. Kennedy was suffering unbearable back pain. He was taking cortisone daily, along with the DOCA implants, and they had weakened his spine. His fifth lumbar vertebra had collapsed. Surgery to fuse the bones in his lower back would strengthen his spine, but his doctors advised against it. In an Addison's patient, surgical stress and post-operative infections could be fatal. The alternative was paralyzing pain and Kennedy insisted on surgery *(1)*.

On October 21, 1954, surgeons at Cornell's Hospital for Special Surgery conducted the three-hour operation. Before, during, and after surgery, Kennedy's endocrinologist, Ephraim Shorr, monitored his metabolism and administered cortisone, hydrocortisone, and desoxycorticosterone to compensate for his adrenal insufficiency *(23)*. Kennedy's survival was significant enough to warrant a case study in the *AMA Archives of Surgery (23)*.

By that time, though, physicians realized that cortisone was no panacea. The miracle drug had become a curse.

Afterward, corticosteroids continued to control Kennedy's addisonian symptoms, but they also progressively weakened his spine. During the White House years, he took hydrocortisone, prednisone, and fludrocortisone, along with drugs for his colitis (anti-diarrheals), recurring urinary tract infections (antibiotics), a thyroid deficiency (liothyronine), weight loss (testosterone), and chronic back pain (procaine) *(1, 3)*.

A New Norm

Glucocorticoid treatment of rheumatoid arthritis is now extremely limited, but cortisone and its structurally related analogs have proven useful in more than 70 conditions involving inflammation and hypersensitivity. These include asthma, burns, skin rashes, organ transplants, lupus erythematosus flares, and eye inflammation. Intra-articular, inhaled, and topical formulations restrict side effects and are now preferred over systemic administration *(15-17)*.

Ultimately, Hench's positioning of cortisone as a research tool proved to be correct *(15, 21).* In the 1940s, research for arthritis drugs was "moribund, if not dead" *(16).* Then, cortisone electrified scientific interest, drew many talented researchers into the field, and led directly to development of today's disease-modifying arthritis drugs. But that's another story.

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Typhus:

War and Deception in 1940's Poland

Rebecca J. Anderson, PhD

In German-occupied Poland during World War II, life was harsh and uncertain. The Nazis considered Poles an "inferior race" and set out to exploit them and systematically demolish their society. Polish Jews and other "undesirables" were rounded up, sequestered in ghettos, transported to concentration camps, or simply shot *(1)*.

The remaining young adults were exploited as a free natural resource. Many thousands of them were deported to Germany and forced to work under abysmal conditions in support of the Nazi war machine *(1, 2)*. Faced with these grim realities, one lucky Pole found himself in the right place at the right time, thanks to Stanislaw Matulewicz.

At the time of the German invasion of Poland in 1939, Matulewicz was a physician in general practice in Rozwadow, a village on the marshy banks of the San River, about 125 miles southeast of Warsaw *(3, 4)*. Among his duties, Matulewicz was required to comply with an ordinance imposed by the new German-run government to report all suspected and confirmed cases of epidemic typhus *(2)*.

Map of World War II concentration camps and death camps

Epidemic Typhus

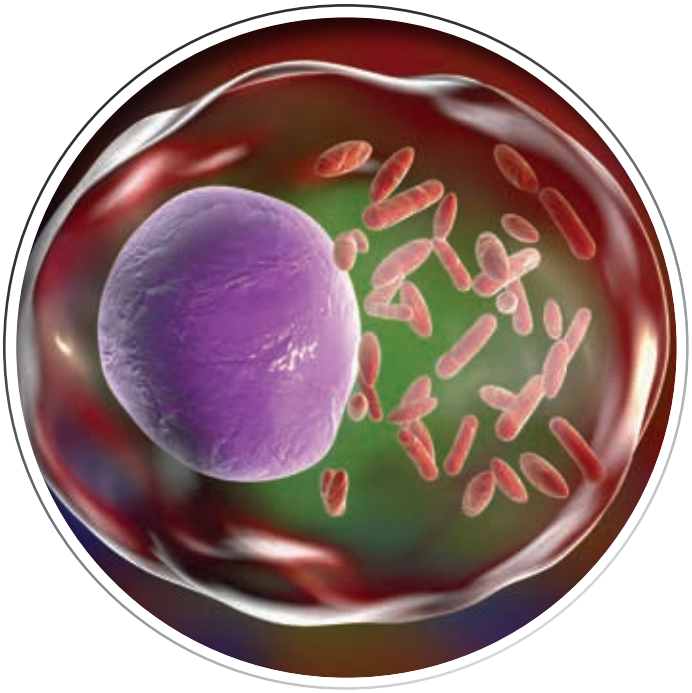
Until the mid-19th century, typhus and typhoid fever were indistinguishable *(3).* Both induce fever, headache, and a skin rash. But despite the similarity in names, the two diseases are distinctly different. Typhoid fever is an intestinal infection caused by *Salmonella* and is characterized by abdominal pain, intestinal lesions, and diarrhea. The infection spreads from person to person, most commonly through contaminated food or water. Some patients carry the bacteria without symptoms and can unwittingly infect others—Typhoid Mary being the most famous example.

On the other hand, epidemic typhus (also known as trench fever, jail fever, or louse-borne typhus), is caused by *Rickettsia prowazekii.* Rather than direct human contact, typhus is transmitted by human body lice, which can live on clothes and thrive under poor hygienic conditions. Lice ingest rickettsial bacteria when feeding on the blood of an infected person. The bacteria multiply in the louse's gut and spill into the louse feces. When infected lice defecate during their next blood meal, the new victim is infected through the bite wound or by broken skin from scratching. Even dead lice can harbor and transmit the disease *(3).*

Source: CDC/ Frank Collins, Ph.D. Photo Credit: James Gathany.

Female body louse

Male body louse



Bacteria Rickettsia *(small red rods) inside human cell*

The typhus skin rash is not easily distinguished from measles and other rashes, but typhus patients go on to develop serious symptoms including muscle pain, mental confusion, kidney damage, gangrene, multiorgan failure, coma, and cardiovascular collapse. Death is due to dehydration and shock *(4).* In the era before antibiotics and vaccines, epidemic typhus could decimate populations *(2-6).*

For thousands of years, epidemic typhus has thrived in prisons, refugee camps, military barracks, and anywhere that people are exposed to cold weather and confined in a densely-populated environment under unsanitary conditions *(3, 6).* During World War I, typhus plagued louse-infested soldiers in the trenches along the Western Front *(3, 7).*

Post-war sanitation eliminated epidemic typhus in Germany. However, two decades without exposure lowered the natural immunity of the German population to typhus, compared to people in Poland

and Russia *(2, 4, 6)*. Consequently, Germans in the occupied territories took extraordinary steps to protect themselves and prevent the disease from entering Germany *(2, 5, 6)*.

The occupation troops conscientiously screened Poles during mass deportations to identify those who might be carrying "infected" lice on their bodies *(2, 5)*. After registration at Auschwitz, for example, concentration camp prisoners were sent to quarantine for 6-8 weeks. Those suspected of having typhus were killed to prevent spreading the infection *(3)*.

As the war progressed, precautions loosened and typhus spread rampantly through the camps. At Bergen-Belsen, it is believed that Anne Frank and her sister died of typhus.

Polish physicians, including Matulewicz, were required to send blood samples of all suspected cases to the German State laboratories for analysis. If the results were positive for typhus, the lab promptly notified the German authorities as well as the physician who provided the specimen *(1, 2)*. Non-Jews who tested positive were quarantined or sent to special hospitals. Infected Jews were shot and their homes burned *(1)*.

Matulewicz knew that a positive typhus test result amounted to a death sentence for his Jewish patients *(1)*. He therefore set up his own typhus test so that he could diagnose patients. If the patient was Jewish or someone else who was hiding from the Germans, he did not send the blood sample to the German labs *(1, 7)*. The assay Matulewicz devised was the Weil-Felix test, the same assay used by the German State laboratories.

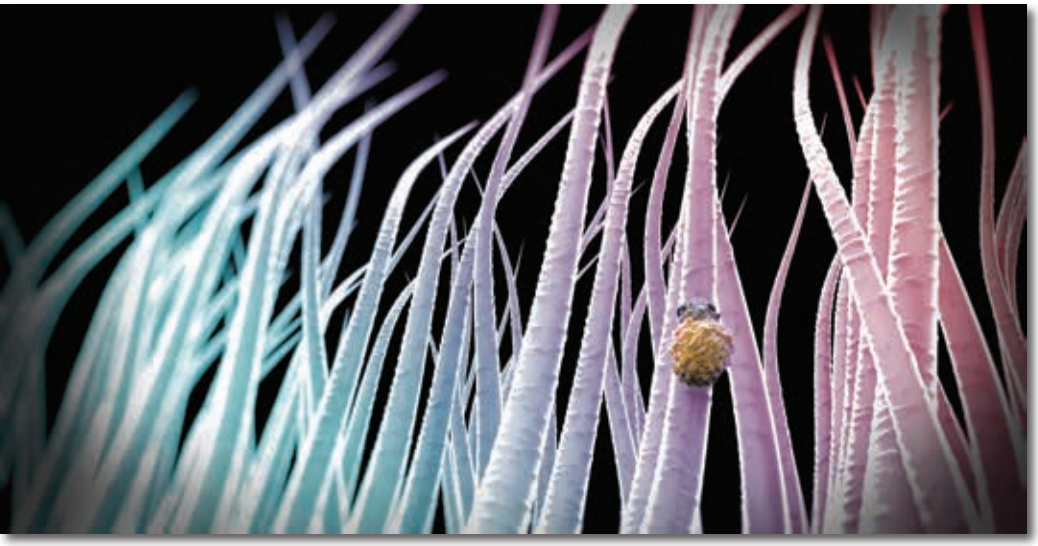
Matulewicz knew that a positive typhus test result amounted to a death sentence for his Jewish patients

Weil-Felix Test

In 1916, Edmund Weil, a Pole, and Arthur Felix, a Czech, discovered that a cell wall O-antigen of certain strains of *Proteus vulgaris* bacteria cross-reacts with antibodies of *Rickettsiae (2, 4)*. [*Proteus vulgaris* can cause urinary tract infections but is otherwise largely benign *(4, 7)*.] The OX-19 strain of *Proteus* and *Rickettsia prowazekii* (the epidemic typhus organism) both trigger human antibodies that recognize the *Proteus* OX-19 cell wall antigen *(3, 4, 7)*. Rocky Mountain spotted fever *(Rickettsia rickettsii)* also induces antibodies that cross-react with *Proteus* OX-19, but this rickettsial organism is not present in Europe *(2)*.

The reagent for the Weil-Felix test is a suspension of *Proteus* OX-19 bacteria that has been killed with formalin *(2, 6)*. This reagent is mixed with a serum sample from an ill patient. If the patient is infected with epidemic typhus, the serum will contain rickettsial antibodies that have been generated to fight the typhus infection, and those antibodies will bind to the OX-19 polysaccharides on the *Proteus* cell surface *(4)*. The antigen-antibody complex clumps (that is, agglutinates), and the serum sample turns cloudy *(7)*. This positive test result, along with the appropriate clinical symptoms, leads to a patient diagnosis of epidemic typhus.

The Weil-Felix agglutination reaction was a simple lab test for epidemic typhus and was quickly adopted by both sides during the latter stages of World War I *(2)*.



Microscopic view of lice in the hair

During World War II, the Germans again employed the Weil-Felix test to confirm typhus in symptomatic patients in the occupied territories *(2)*.

Matulewicz's only motive for using his homemade Weil-Felix assay was to intercept the blood samples of typhus-infected Jews and save their lives. But one day in 1942, a desperate young man came to see Matulewicz. He was among those who had been deported to Germany to work in the forced labor camps, but recently he had been granted permission to return to Poland to visit his family. His 14-day leave was almost up and if he did not return to Germany on time, he and his whole family would be hunted down, arrested, and sent to a concentration camp *(2, 7)*.

A New Twist on an Old Test

The laborer was looking for any excuse to escape the misery of slavery in Germany. He had even considered committing suicide, but even that would not spare his family from retaliation by the Gestapo *(2, 7)*.

Because the Germans feared epidemic typhus more than bullets and bombs, a typhus diagnosis would certainly allow the laborer to remain in Poland. He came to Matulewicz to request a physician's certificate, an official document that was used to verify the medical diagnosis of a serious disease *(2)*. Matulewicz hesitated, because falsifying the certificate was risky. If the German authorities discovered a deliberate misdiagnosis, the consequences would be dire for both him and his patient *(2)*.

Equally unacceptable was intentionally infecting the laborer. Medical ethics prevented Matulewicz from causing harm to any patient, especially propagating a disease like typhus, which was highly contagious and often fatal.

But there was a third possibility. Matulewicz knew that the Weil-Felix test relied on cross-reactivity between the *Proteus* OX-19 antigen and typhus antibodies. A person who was infected with *Proteus* OX-19 would also produce antibodies, and those antibodies would obviously react with the Weil-Felix reagent—indistinguishable from the positive result of a typhus-infected patient *(2, 4)*.

Matulewicz reasoned that an injection of the *Proteus* OX-19 reagent would cause a healthy person to develop antibodies that would most likely generate a positive Weil-Felix result. Because *Proteus* bacteria in the reagent suspension had been inactivated, the injection would not cause a urinary tract infection. Other possible side effects from the injection were unknown, but Matulewicz thought the risk was low. He proposed to test his idea, and the laborer gladly agreed to be his experimental subject *(2)*.

Matulewicz injected 1 ml of the *Proteus* OX-19 suspension intramuscularly *(1, 2, 5, 7)*. The laborer, indeed, developed antibodies to the *Proteus* OX-19 inoculation, and subsequently, Matulewicz observed a Weil-Felix agglutination reaction in a sample of the laborer's serum. Of course, it was a false positive. The laborer did not have typhus. The positive result simply reflected agglutination with *Proteus* OX-19 antibodies *(2)*.

To save the laborer, Matulewicz needed to get an official diagnosis. Now confident of the outcome, he sent the laborer's blood sample to the German State laboratory for analysis. Soon, a telegram arrived with the official result: "Weil-Felix positive" *(2, 3)*. The telegram was submitted to the local German authorities, and the laborer was officially released from his work in Germany.

In addition, all of the laborer's family members who had been in contact with him were excluded from future deportation *(2)*. The Germans feared that "infected" lice might be carried by the family during the bacteria's incubation period *(2, 5)*.

A short time later, Matulewicz confided his experiment and the successful ruse to Eugene Lazowski, a fellow physician in Rozwadow.

Lazowski's Journey

Eugene Lazowski came from a Catholic family who actively supported the Polish Underground *(8)*. His parents hid Jewish families in their home and were later named Righteous Gentiles by Yad Vashem *(9, 10)*.

When the Germans invaded Poland, Lazowski had just finished medical school at the University of Warsaw. He became a soldier in the Polish army, served as a medic, and for a while, was held in a

Eugene Lazowski

prisoner-of-war camp *(8)*. The camp was surrounded by a 10-foot wall topped with barbed wire *(9)*. One night, seizing an opportunity, he ran toward a section where he saw a break in the barbed wire, scaled the wall, and leaped over *(7, 9)*. On the other side, he spotted an unattended horse and cart. He stopped and groomed the horse as if he owned it and then calmly walked away without attracting attention *(9)*.

After his escape, Lazowski settled in Rozwadow and worked as a doctor with the Polish Red Cross *(1, 4, 7, 8)*. Although he did not shelter Jewish refugees like his parents, Lazowski supported the Polish resistance. He supplied information and provided medical care to bands of saboteurs and guerrillas who were hiding in the woods *(1, 10)*.

The rear fence of his home backed up to the Jewish ghetto in Rozwadow *(1, 9, 11)*. Although it was against German orders and punishable by death, Lazowski provided medical care to many Jews in the ghetto *(7, 9)*. To request his assistance, they would hang a white cloth on the back fence *(9, 11)*. At night, Lazowski would sneak through the fence and treat them *(7, 11)*.

The German authorities closely monitored the drugs and medical supplies that physicians used. To reconcile the discrepancy caused by the supplies that Lazowski used in the ghetto, he devised a creative accounting scheme *(1)*. His office was close to the town's railroad station, and he was often asked to treat patients who were traveling through. In his inventory reports, he exaggerated the amounts of drugs and supplies that he used to treat the travelers, knowing that the Germans could not easily verify those entries *(1, 7)*.

When Matulewicz told Lazowski about the laborer he had rescued, Lazowski immediately saw that the same procedure could be used to save others from deportation *(2, 10)*.

The Grand Deception

Deteriorating sanitary conditions in Poland had facilitated the spread of epidemic typhus. Hospitals became overcrowded, and most infected patients were cared for at home by family members *(2)*. When the number of cases was concentrated in one area, the German Public Health Authority declared it to be an "epidemic area" *(2)*. Germans tended to avoid such areas, and consequently, the quarantined population was relatively free from Gestapo atrocities *(2, 4)*.

Playing on the Germans' fears, Lazowski and Matulewicz faked a typhus epidemic and used the Germans' own laboratories to make it "official." They called it their private immunological war—a war aimed at saving lives rather than causing deaths *(1, 2, 6)*. Their only weapon was a syringe.

They knew it was a dangerous undertaking. If their ruse was discovered, they would be considered conspirators in league with the Polish Underground and punished accordingly. So, they selectively administered their *Proteus* OX-19 injections, with a carefully planned strategy in mind. First, they injected only non-Jews, because they knew the Gestapo would kill Jews who tested positive for typhus *(1)*.

Second, they selected patients who already exhibited symptoms (e.g., fever, headache, skin rash) that were consistent with epidemic typhus *(2)*. At that time, it was common for physicians to give sick patients intramuscular injections of pharmaceutical products to stimulate the patients' immune system. Some vaccines and "protein suspensions" (e.g., bovine bile extract, lipids, and bacterial proteins) were used for this purpose *(2)*.

Matulewicz and Lazowski's patients did not question the injection of the Proteus OX-19 suspension, because they assumed it was simply a routine shot to boost their immunity. The doctors never told them that the injection would induce a false typhus response *(2)*. In fact, they kept their activities secret from everyone—including their wives *(11)*.

Third, Lazowski and Matulewicz strictly controlled the number of injections and the number of patients they infected, so that the cohort reflected the well-accepted seasonal variation of epidemics. They increased their injection schedule during the winter, diminished the number of patients during the spring, and increased their numbers again in the fall *(2)*.

Fourth, Matulewicz and Lazowski knew the Germans would naturally suspect that a Polish physician might try to "game the system" by mislabeling blood samples. A sneaky physician could use the blood from one actual typhus patient and re-label it as the blood of many other suspected cases.

Bundesarchiv, Bild 101I-134-0782-35
Foto: Knobloch, Ludwig | Mai 1941 ca.

Because of the cross-reactivity of the Weil-Felix reaction, Lazowski and Matulewicz were confident that the patients whom they had infected would biologically test positive for typhus (created by an artificial method). Consequently, they always submitted blood that corresponded to patients who had been injected with *Proteus* OX-19—no sample switching required *(2)*.

Fifth, to further deflect suspicion, Lazowski and Matulewicz referred some of their patients (after injecting them with the *Proteus* OX-19 suspension) to other doctors who were not aware of the scheme. These doctors would "discover" the typhus on their own and report it separately *(1)*.

Finally, when Lazowski and Matulewicz found a patient who really did have typhus, they publicized the case as much as possible, but only if the patient was not Jewish *(1, 2)*.

Within a few months, the number of reported cases was sufficiently large to declare the area, which consisted of about a dozen villages, an "epidemic area" *(7)*. The local German authorities began posting "Achtung, Fleckfieber!" (Warning, Typhus!) signs in Rozwadow and the surrounding villages *(1)*. Deportation of workers to Germany from these quarantined villages was stopped, and German troops kept their distance.

Facing Fear with Defiance

Before the war, Jews accounted for at least 10% of the area's population. By the time Matulewicz and Lazowski began their fake epidemic in 1942, most of them had already been rounded up by the Germans *(1)*. However, many Jews were still hiding in the countryside, including a large contingent that had fled Warsaw and other urban areas *(1)*. The area-wide typhus quarantine thus protected them, as well as the villages' residents.

Villagers began to feel more relaxed, but the doctors—knowing there was no actual epidemic—lived in constant fear. As Lazowski later explained, "I didn't know if I would be arrested and tortured by the Gestapo. So I carried a cyanide pill in case I was arrested" *(1)*.

Typhus warning sign

Jan Hryniewiezki, who was 15 at the time and later became a surgeon, remembered the injections. After a while, he said, people figured out what was going on because no one died. But to ensure continuation

I carried a cyanide pill in case I was arrested

of the protective faux-quarantine, everyone kept quiet *(1)*.

When a patient asked why he recovered so quickly from such a serious disease, Lazowski said, "I just told him he was a lucky man" *(1)*.

After one year, Matulewicz moved away from the area. Lazowski stayed and continued his "private immunological war" for two more years *(2)*. During this time, the greatest danger to Lazowski was the possibility that German doctors might conduct their own physical examinations of the fake typhus patients *(2)*. Cross-reactivity in the Weil-Felix test ensured that the German labs would always report a typhus diagnosis. But the symptoms and extremely poor health of actual typhus patients could not be easily faked, and a direct physical exam of the fake typhus patients would most likely expose the ruse.

The local Gestapo chief was closely watching Lazowski's movements, and the young doctor walked a fine line, trying to stay in the good graces of the Germans at the same time he was deceiving them. He positioned himself as a sort of hero, because he bravely and selflessly provided medical care in a typhus-infested region—something their doctors were reluctant to do. "They needed me"*(1)*.

In late 1943, a Pole who was collaborating with the Nazis informed the local Gestapo chief that the typhus outbreak wasn't what it appeared to be *(1, 2, 4, 7)*. The Gestapo chief, in turn, notified the German health authorities, who dispatched an investigative commission and two carloads of soldiers to the quarantined area *(1, 7)*. If the fake typhus patients were discovered, the Germans would kill them—and Lazowski, too *(1)*.

Fortunately, Lazowski was ready. He had gathered the oldest, sickest, and most unhealthy-looking people he could find and put them in filthy huts in Rozwadow. They had all been injected with *Proteus* OX-19 *(1)*.

When the visitors arrived, Lazowski warmly greeted them at the edge of town and invited them to a big party hosted by the townsfolk. Vodka flowed, kielbasa was plentiful, and music played *(1)*.

The senior German doctors stayed at the party and sent their younger colleagues to conduct the investigation. Lazowski led them to the huts where the sick patients awaited their physical exams. But he cautioned the doctors "to be careful because the Polish are dirty and full of lice, which transfer typhus" *(1)*.

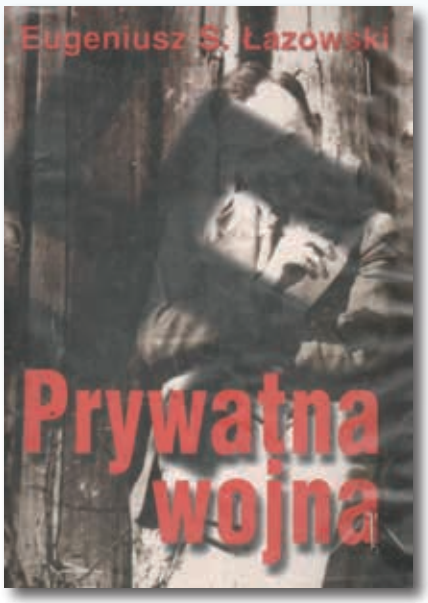
The young doctors rushed through their inspection and took blood samples from only a few patients—without checking for actual symptoms of typhus *(1, 7)*. Of course, those blood samples later tested positive for typhus. Lazowski was not bothered by the German health authorities for the rest of the war *(1)*.

The Big Reveal

Near the end of the war, as the Soviet army approached from the east, the Germans began fleeing. One of them, a young military policeman, roared up on his motorcycle and stopped at Lazowski's office. Lazowski had secretly treated him for venereal disease and as a gesture of gratitude, the policeman passed along a friendly warning that he was on the Gestapo hit list *(1, 10)*. Lazowski had always been careful to display his loyalty to the Germans and assumed that he had nothing to fear. He was surprised when the policeman puckishly quoted a specific date and place where Lazowski had been seen treating members of the Underground *(1)*.

Lazowski fled from Rozwadow with his wife and daughter and lived for a while with relatives *(11)*. When the German occupation ended, he settled in Warsaw and continued to practice medicine under communist rule (1, 11). But he kept his secret about the fake typhus "epidemic," fearing retaliation from Poles who had collaborated with the Germans *(1, 8)*.

In 1958, he moved with his family to Chicago and only then did he confide everything to his wife *(1)*. He studied to earn an American medical license and continued to practice until his retirement in 2004



Private War

(8). In 1984, he became professor of pediatrics at the University of Illinois Chicago Medical Center, where he taught and published over 100 research papers in Polish and English *(6, 8).* Meanwhile, Matulewicz had resettled in Zaire, where he became a professor of radiology. Later, he retired in Poland *(1, 2).*

The Weil-Felix test has now been largely replaced by diagnostic methods that offer much better sensitivity and specificity. Indirect immunofluorescence antibody testing is now the gold standard. But the Weil-Felix test is still used in some developing countries because of its low cost *(5).*

In 1977, Lazowski and Matulewicz finally broke their silence. They published their story for members of the American Society for Microbiology, detailing Matulewicz's discovery and how they exploited it to save their patients *(2).* In 1993, Lazowski published *Prywatna wojna (Private War),* which became a best-selling book in Poland.

During the 6 years of the German occupation, 6 million Polish citizens (one-fifth of the population and half of whom were Jewish) died as a result of mass executions, imprisonment, concentration camps, or other misfortunes of the occupation *(2).* In the last three years of that occupation, Lazowski and Matulewicz saved an estimated 8,000 Poles, including many Jews-in-hiding, and proved that sometimes the syringe is mightier than the sword *(2, 7).*

A glimpse of Rozwadów, Poland today

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The Promise and Perils of Pharmacy Compounding

Rebecca J. Anderson, PhD

On September 18, 2012, April Pettit, an internist at Vanderbilt University Medical Center, sent an email to the Tennessee Department of Health, describing a patient with a rare form of meningitis *(1).* The patient had contracted a fungal infection after receiving a contaminated epidural injection for back pain. The contaminated product was a steroid solution made by a compounding pharmacy in Massachusetts. Soon, other meningitis cases emerged, leading to what one lawyer called "the deadliest catastrophe in the history of modern medicine" *(2).*

Compounding through the Ages

Compounding is a pharmacy term that describes the process of combining ingredients to produce a medication tailored to meet the needs of an individual patient. It is a practice that dates back thousands of years. The earliest descriptions of compounded medicines are contained in the cuneiform tablets of Mesopotamia *(3).* The ingredients in these ancient prescriptions, which were written in 2600 BC, included about 1,000 plant-derived compounds *(4).*

Traditional Chinese medicine began even earlier, but the first text was the *Huang Ti Nei Ching* (The Yellow Emperor's Classic of Medicine), which was written around 300 BC. The *Nei Ching* documented medicinal preparations that had been in use since 2600 BC and encompassed diet and acupuncture as well as drugs *(3, 4).*

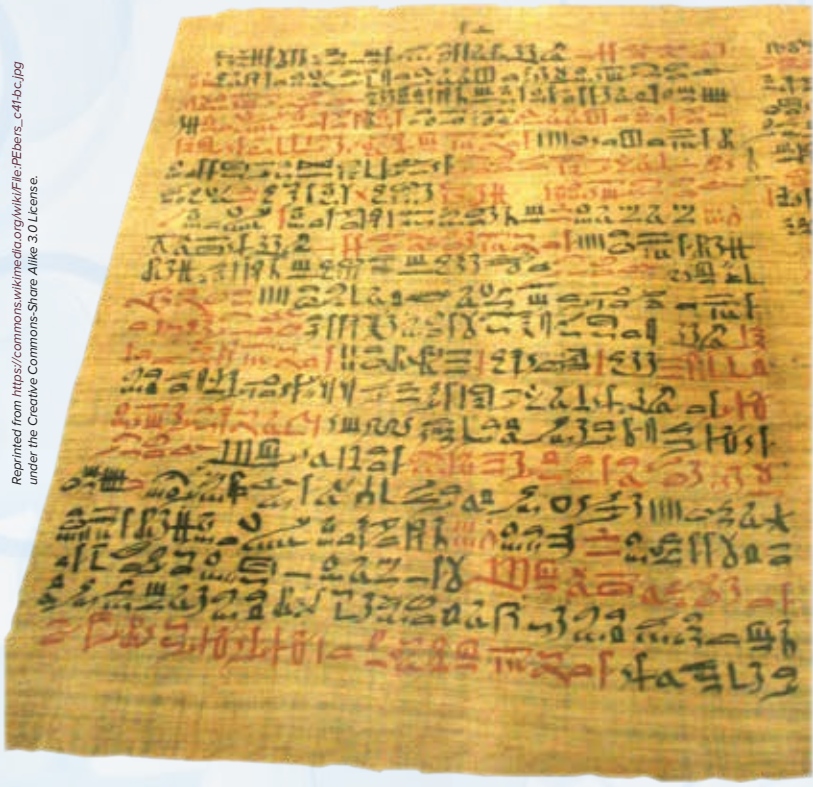
Egyptian medicine began around 2900 BC, but the first known medical text, the Kahun Papyrus, was written around 1900 BC *(3, 4).* The Ebers Papyrus and Smith Papyrus date from 1550 BC (*4, 5).* Collectively, these papyrus fragments, written in hieroglyphs, contain roughly 800 prescriptions, many prepared through compounding *(3-5).*

Among the plant ingredients that the Egyptians incorporated in their medicines were the resins of pine, fir, and cedar trees. The most important of those resins were frankincense and myrrh, which came from a small region bordering the Gulf of Aden: the eastern Horn of Africa and the South Arabian coast *(3).*

In India, the Ayurveda, a holistic system of medicine, emerged in 1000 BC *(4).* The medicinal component of the Ayurveda relied on plant-derived substances. Prominent among the plant ingredients were spices, especially cinnamon and pepper *(3).*

Knowledge of medicinal ingredients in the Western world was based mainly on the Greek and Roman cultures. Greek prescriptions date from the time of Socrates (469-399 BC) and Hippocrates (460-380 BC). Hippocrates favored myrrh, which has bacteriostatic properties, but his prescription books also include thyme, cinnamon, and other spices as ingredients *(3).*

The most significant Greek contribution to compounding came from Galen (130-201 AD), a Greek physician who practiced in Rome *(4).* He wrote a 22-volume compendium that dominated medicine for 15 centuries. Among the Galenic remedies were a variety of colored salves that were loaded with the salts of arsenic, mercury, and lead *(3).*

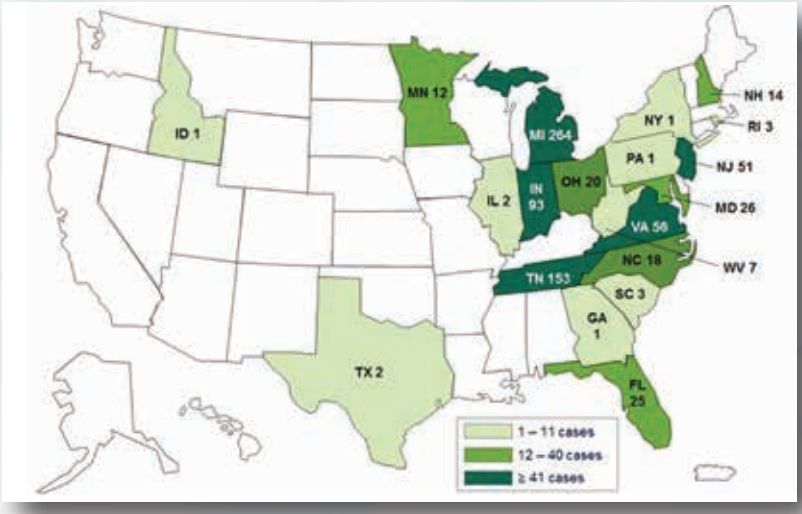


The Ebers Papyrus (c. 1550 BC) from Ancient Egypt

The Roman physician Pliny the Elder (23-70 AD) is most noted for *Historia naturalis* (Natural History). Rather than an original work, Pliny's book compiled thousands of medical facts written by 100 authors and was revered as a pillar of human knowledge for 1600 years. Some of those folk remedies proved to be very effective drugs (e.g., fern for intestinal worms and ephedra for asthmatic cough) *(3).*

The Roman Cornelius Celsus (15 BC-50 AD) also wrote an encyclopedia, *De medicina* (On Medicine). Like the medicines of the Greeks, Celsus's ingredients included myrrh and heavy metal salts (alum, copper acetate, lead oxide, and sulfides of mercury and antimony), which were mixed with resins, pitch, bitumen, and wax to produce salves and ointments *(3).*

By the 16th century, medicine in Western Europe had evolved into a hierarchy of practitioners: physicians, surgeons, apothecaries, and midwives *(6).* The recently invented printing press greatly facilitated their ability to acquire knowledge about medicinal compounding. Among Johannes Gutenberg's



Source: Centers for Disease Control and Prevention

Persons with fungal infections linked to NECC-manufactured steroid injections, by state.

most influential and widely distributed books in Europe were those that described Greco-Roman herbal medicines *(4)*.

Lady Grace Mildmay, a well-to-do 16th century English woman, collected compounding recipes from various reliable sources and tweaked them based on the outcome of the patients she treated. Like her contemporaries, she used a wide range of ingredients: plants, metal salts, minerals, animal parts (hooves, horns, and claws), ale, and wine. Her compounding methods often involved rituals, as well as procedures. For example, Mildmay's "precious balm" consisted of more than 160 ingredients, required 10 distillations in a complicated ritual of 14 steps, and took at least 5 weeks to prepare *(6)*.

The most enduring of all medicinal compounds was theriac, which dates from the time of Nero (37-68 AD). Fearful of poisoning, Nero directed his physician, Andromachus, to develop new and better antidotes *(3)*. Andromachus took a traditional and already effective antidote and increased the number of ingredients to 64, including chunks of viper flesh. He also increased the opium content by 500% *(3)*.

Vessel for storing Theriac

Theriac became wildly popular—no doubt due to opium addiction. Galen wrote a whole book about it, *Theriaké*. Those who could afford the expensive preparation took it for everything, from the Black Death to routine prophylactic use for almost anything *(3)*.

By the 13th century, theriac had been adopted in China, and versions of it were also available in India. In Europe, it survived the Renaissance, with even more elaborate ceremonies required for its preparation. Theriac was included in the official German pharmacopoeia until 1872 and in the French pharmacopoeia until 1884 *(3)*.

Compounding in the US

In the US, compounding pharmacies began emerging in the early 1800s *(5, 7)*. Several of today's well known drug companies originated as 19th century shops owned by pharmacists: George Merck (Merck & Co.), William Warner and Jordan Lambert (Warner-Lambert—now Pfizer), John K. Smith (GlaxoSmithKline), and Eli Lilly *(7)*.

The medicines these pharmacists compounded were crude mixtures from natural sources, such as opium and belladonna. To increase the potency of their remedies, they often performed an extraction using water or alcohol and concentrated the solution through evaporation (e.g., laudanum, a tincture of opium).

An estimated 80% of all prescriptions were made by compounding up to the 1920s *(5)*. By the 1940s, compounding accounted for about half of all medications *(7, 8)*. As modern pharmaceutical manufacturing became established, compounding declined and pharmacists simply dispensed formulations that had been manufactured by drug companies *(5, 8)*.

Although compounding now represents only about 1% of all US prescriptions, it remains an integral part of the pharmacy profession and is practiced in the pharmacies of hospitals, chain drug stores, and local communities *(9)*. Customized drugs are needed by patients who may be allergic to a manufactured drug's ingredients (e.g., preservatives and dyes), need a liquid to alleviate difficulties in swallowing pills, or need a nonstandard dosage strength *(9, 10)*.

Sterile Compounding

Intravenous (IV) drug administration dates back to the London cholera epidemic of 1832. William O'Shaughnessy replenished cholera patients' fluid depletion by IV infusion of normal saline *(11)*. In the 1880s, Sydney Ringer, a British physician and physiologist, improved the electrolyte formula by including the chlorides of calcium and potassium, in addition to sodium. He used the solution to perfuse isolated organs in the laboratory. The subsequent addition of sodium lactate resulted in lactated Ringer's solution, which is still widely used *(11)*.

To ensure sterility, IV solutions were originally sealed in steam-cleaned glass vacuum bottles. In 1933, Baxter Travenol introduced the first commercial product, but throughout the 1930s, only the sickest and most critical patients received fluids intravenously *(11)*. During World War II, demand for intravenous fluids grew substantially to treat injured soldiers, and in the late 1950s, sterile plastic bag containers were developed *(11)*.

In the 1960s, hospital-based pharmacies were established to provide a centralized center for compounding sterile solutions for hospitalized patients *(12)*. Typically, these hospital pharmacists took commercially available sterile solutions and added ingredients (such as electrolytes and vitamins) to meet the needs of individual patients. As the number of marketed injectable medications increased, so did sterile compounding at hospitals. By the 1980s, nearly 70% of hospital pharmacies prepared customized IV solutions for their hospitalized patients *(12)*.

In the 1990s, preparation of the sterile compounded products used in hospitals shifted from local hospital pharmacies to large pharmacies that offered outsourcing services

Demand for compounded sterile solutions grew further with the introduction of total parenteral nutrition (TPN) and cardioplegia solutions. TPN by its very nature is a complex solution, requiring multiple additives. Cardioplegia solutions, which are used to perfuse tissues when the heart is stopped during open-heart surgery, typically include just electrolytes, but premixed cardioplegia solutions were not commercially available until 2000 *(12)*.

Compounded sterile drugs for infusion pose more risks than orally administered drugs, because pharmacies must implement special safeguards to prevent injury or death from microbial contamination *(9)*. The risk of contamination increases with the complexity of the compounding process *(12)*.

Opportunity Knocks

In the 1990s, preparation of the sterile compounded products used in hospitals shifted from local hospital pharmacies to large pharmacies that offered outsourcing services *(9, 12)*. Several factors contributed to this shift. Schools of pharmacy were placing less emphasis on the compounding skills of their students. In addition, the required standards for sterile compounding facilities and procedures and training personnel had become more stringent. Periodic shortages of commercial injectable medications pushed hospitals to find alternative sources *(9, 12)*.

To meet these needs, a new industry emerged: large compounding pharmacies that specialized in outsourcing *(12)*. Like community- and hospital-based pharmacies, these large outsourcing operators were regulated by state pharmacy boards. They were required to comply with laws for recordkeeping, certifications, and licensing of the state where they are located *(1, 9, 12)*. There was little federal oversight.

As the large compounding outsourcers began producing drugs beyond what had historically been done within traditional compounding, officials at the Food and Drug Administration (FDA) became increasingly concerned (10). The outsourcers were engaging in interstate commerce—large scale manufacturing that was normally the purview of the FDA *(9, 12)*.

In 1997, Congress passed the FDA Modernization Act (FDAMA) to clarify the scope of federal oversight. Over the strong objections of FDA Commissioner David Kessler, FDAMA (Section 503A) exempted compounded drugs with a valid prescription from FDA's requirements for Good Manufacturing Practices *(1, 13)*.

The FDA could not proactively gather information about pharmacy practices and procedures. Without

Compounding Pharmacy Safety Incidents Investigated by the FDA (10)				
Year	**No. deaths**	**No. Injuries**	**Drug**	**Comments**
1997	0	2	Riboflavin	Contaminated sterile injection
2001	3	35	Steroid	Contaminated sterile injection
2002	1	5	Methylprednisolone acetate	Contaminated sterile injection
2005	3	5	Cardioplegia solution	Contaminated sterile solution
2007	3		Colchicine	Super-potent compounding (640% of labeled strength)
2010	0	> 12	Avastin	Contamination from repackaging sterile injection product
2011	9	19	Total Parenteral Nutrition	Contamination of sterile product
2012	0	43	Ophthalmic drugs	Contaminated sterile products (29 suffered vision loss)
2012	64	753	Methylprednisolone acetate	Contaminated sterile injection

knowledge of the actual conditions at compounding pharmacies, FDA regulators could not address violations before a crisis erupted (1). The agency's authority was limited to reacting once a problem became "obvious" (i.e., "for-cause" inspections) *(1, 9)*.

Ambiguities in the law gave both NECC and the FDA a legal rationale.

Kessler was concerned that poor and inconsistent manufacturing standards—especially for sterile drugs—would cause unnecessary patient injuries and deaths. Unfortunately, his concerns proved correct, and a steady stream of incidents, mostly related to contaminated sterile products, began appearing (see table). All of these incidents resulted from poor manufacturing procedures at compounding pharmacies that specialized in outsourcing *(10)*.

A Compound Problem

One pharmacy that took advantage of the new FDAMA provisions was the New England Compounding Center (NECC), which was founded in 1998 in Framingham, Massachusetts, by the Conigliaro family (headed by Carla and Douglas Conigliaro). NECC was run by Barry Cadden, his wife (Lisa Conigliaro Cadden), and her brother (Gregory Conigliaro). The Conigliaro family's broader business operations included a recycling plant next door to NECC *(1, 14)*.

Barry and Lisa Cadden, who were both pharmacists, specialized in producing sterile injection solutions. From the beginning, they ran afoul of regulatory standards. In 2002, FDA inspectors cited NECC for failing to resolve consumer complaints, address adverse drug reactions, and correct product defects *(1, 15)*.

Over the next few years, state pharmacy inspectors also visited NECC (sometimes accompanied by FDA officials) and also found safety problems at the facility. NECC negotiated a settlement with the Massachusetts pharmacy board and avoided disciplinary action *(1)*.

Because outsourcing pharmacies could legally refuse to turn over their records to the FDA, even when FDA officials received support from state inspectors, NECC resisted FDA's requests *(9, 10)*. Ambiguities in the law gave both NECC and the FDA a legal rationale.

The 5th and 9th Circuit Courts and the US Supreme Court had responded to challenges of the FDAMA legislation by issuing separate decisions, but from state to state, the courts' interpretations of the law were conflicting and contradictory *(1, 9)*. FDA's influence over large compounding pharmacies depended on where the pharmacy was located. Despite this confusion, the FDA was always able to obtain warrants and proceed with "for-cause" inspections of pharmacy records, facilities, and practices, but it was a drawn-out and tedious process *(10)*.

In addition, unlike the state pharmacy boards, the FDA lacked the authority to enforce corrective actions. In the case of NECC, publicly available correspondence suggests that NECC was less than cooperative in correcting the deficiencies cited by FDA inspectors *(16)*. Cadden cited statues to support his position, and FDA officials rebutted those arguments by citing other regulations.

No More Mickey Mouse

Despite significant overlap and an expanded gray area, one distinction between drug manufacturers and the large compounding pharmacies remained legally clear. Drug companies (with FDA oversight) manufactured drugs in batches and could distribute them to wholesalers, retailers, and other customers. On the other hand, compounding pharmacies—large and small—were required to prepare medications for individual patients, and in each case, the compounded drug needed a doctor's prescription *(1, 9)*.

This prescription requirement posed a bureaucratic challenge for compounding pharmacies when hospitals and clinics placed orders for large quantities of commonly used injectable solutions. A typical example was methylprednisolone, a steroid that is routinely injected to relieve joint and back pain. Orthopedic practitioners and pain clinics stock ample supplies of methylprednisolone and inject many patients with it every day.

Methylprednisolone is manufactured by Pfizer and several generic pharmaceutical companies *(1)*. The St. Thomas Outpatient Neurosurgery Center in Nashville, Tennessee, had been purchasing its methylprednisolone from Clint Pharmaceuticals, a generic drug company. But when Clint raised its price to $8.95 per 1 ml vial in June 2011, Debra Schamberg, the clinic's director, contacted NECC's persistent regional salesman. She said if he was still offering a price of $6.50 per vial, they had a deal *(17)*. The large shipments from Clint Pharmaceuticals did not require individual prescriptions, but NECC was a pharmacy and licensed only to sell drugs to patients who presented a prescription.

Healthcare providers wanted the convenience of having drugs like methylprednisolone in stock. The pharmacy-prescription process created an additional layer of paperwork and interfered with how they practiced pain management for their patients. To accommodate these customers, NECC began selling large shipments of drugs without prescriptions as early as 2009 *(17)*.

Realizing that NECC's shipments needed prescriptions, Barry Cadden, NECC's president and head pharmacist, suggested a compromise. He told his national sales manager that perhaps names could be attached to the orders after the drugs were injected. This linked each dose to a patient, but it clearly stretched the intent of the law. NECC assumed that the names they received corresponded to legitimate patients, but clinics that treated many patients each day found shortcuts. They sent NECC names like Calvin Klein, Jimmy Carter, Octavius, Burt Reynolds, Filet O'Fish, and Coco Puff *(17)*.

At the St. Thomas Outpatient Neurosurgery Center, they printed out the daily patient schedules and submitted those names with each NECC order *(17)*. But some employees got creative, and one patient name they submitted was Mickey Mouse. Cadden was not amused and issued a stern internal memo saying that the names "must resemble 'real' names... no obviously false name! (Mickey Mouse)" *(17)*.

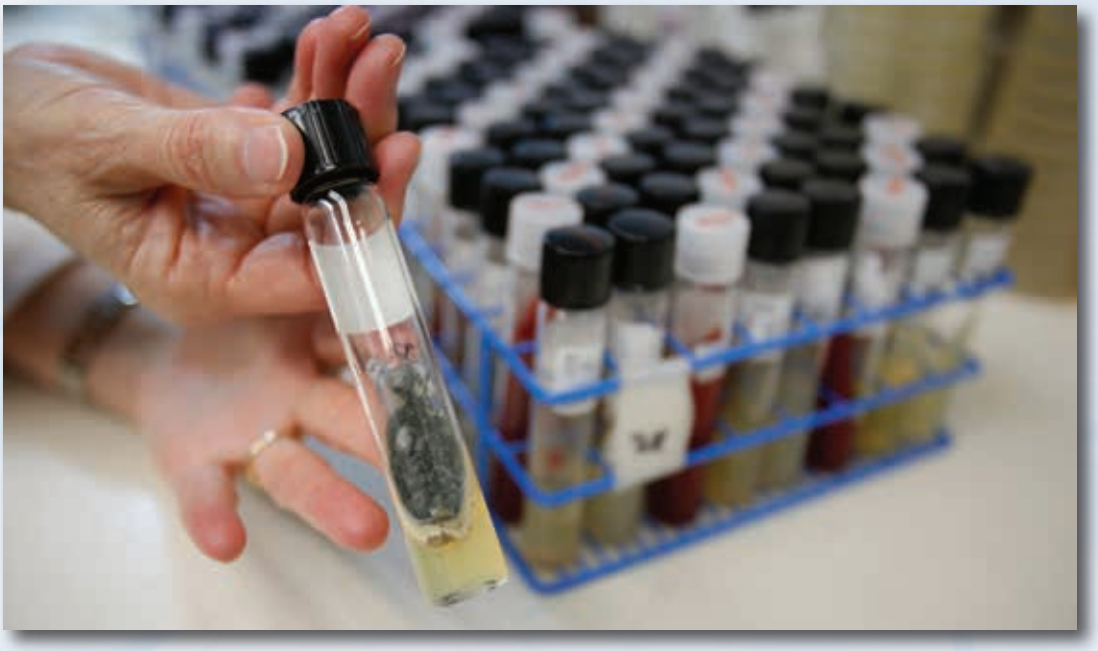
The Index Patient

On July 30, 2012, Thomas Rybinski checked in to the Outpatient Neurosurgery Center at St. Thomas Hospital *(17)*. The 56-year-old autoworker from Smyrna, Tennessee, suffered from chronic back pain caused by degenerative spinal disks. His doctor gave him a 1 ml epidural injection of methylprednisolone *(17, 18)*.

The St. Thomas Outpatient Center administered thousands of injections each year, and on its website, epidural steroid injections were listed as its "top procedure" *(19)*. Experts say that doctors overprescribe invasive back-pain therapy. Although some patients clearly get much-needed relief, most academic researchers say there is no conclusive

Minnesota Department of Health

Vials of methylprednisolone acetate manufactured by NECC



Harrison McClary / Reuters Pictures

A sample of Aspergillus fumigatus in the Vanderbilt Clinical Microbiology Lab for patient care

evidence that steroid injections are useful in easing straightforward chronic low back pain *(1, 19)*.

Despite the weak evidence, the use of steroid injections to treat back pain has skyrocketed in the last 15 years, much more rapidly than the number of patients with back pain or the aging of the population *(1, 19)*. Some patients receive more than 10 shots per year *(19)*.

NECC had been shipping its drugs to the St. Thomas Center for about a year when Rybinski arrived for his steroid treatment. His injection came from a 12.5 l batch of methylprednisolone acetate that NECC had manufactured two months earlier *(17)*. It was labeled as a sterile solution, but unlike the vials produced by Pfizer and other drug manufacturers, NECC's solutions were preservative-free *(9)*. Even more troublesome, NECC's compounding pharmacists had sidestepped batch testing that would have ensured the methylprednisolone solution was sterile *(17)*,

A month later, Rybinski went to the Vanderbilt University Medical Center in Nashville, complaining of headache, neck pain, nausea, fatigue, and decreased appetite *(18)*. Based on blood tests, a spinal tap, and a CAT scan, April Pettit and her team diagnosed meningitis *(17, 18)*. The most common cause of meningitis is a bacterial infection, and Pettit's team prescribed antibiotics for the infection and opiates and NSAIDs to control his pain. Routine cultures of Rybinski's blood and spinal fluid showed no bacteria, but the drug treatment eased his symptoms and he was discharged *(18)*.

Despite continuing his antibiotic treatment at home, Rybinski's headache and back pain worsened *(18)*. A week after his discharge, he returned to Vanderbilt and was obviously ill, uncomfortable, and agitated. His speech was incomprehensible. The analysis of a new spinal tap was still consistent with meningitis, but now an MRI revealed brain inflammation. Intravenous antibiotics improved his mental state over the next two days *(18)*.

Unfortunately, by his sixth day in the hospital, Rybinski was increasingly drowsy, he stared intermittently, and the right side of his face drooped. A brain scan showed mild hydrocephalus *(18)*. Pettit's team began to suspect that Rybinski's meningitis symptoms might stem from a far rarer fungal infection, and they began treatment with the antifungal agent, amphotericin B *(17, 18, 20)*.

The following day, Vanderbilt's microbiology lab confirmed the diagnosis. A culture of the spinal fluid that had been drawn on the day Rybinski was re-admitted to the hospital showed *Aspergillus fumigatus (18)*. The doctors began intravenous voriconazole and continued treatment with liposomal amphotericin B. An MRI revealed newly damaged blood vessels in his brain *(18)*.

Sleuthing the Cause

Unlike bacterial meningitis, fungal meningitis is not contagious *(21)*. Petitt's team investigated the possible source of Rybinski's infection. *Aspergillus* species are ubiquitous in the air, soil, and organic matter. The fungus typically enters the body through the sinuses, lungs, or a break in the skin, but it rarely

causes illness in people with a healthy immune system *(18)*. Rybinski showed no signs of infection on his skin, in his respiratory tract, or in his blood. That left direct contact with his spinal meninges.

Pettit tracked Rybinski's activities in the weeks before his symptoms appeared, and his family mentioned the steroid injection at St. Thomas *(17)*. Rybinski had likely been exposed to the fungus from the epidural injection of methylprednisolone. Presumably, the solution was contaminated and spread the fungus into the intradural space, causing his meningitis *(18)*.

Pettit emailed a copy of Rybinski's lab results describing the fungal infection to the Tennessee Department of Health *(1, 17)*. At that point, Rybinski had suffered brain damage from hemorrhages and increased intracranial pressure *(17, 18)*. He was unresponsive and began shaking his head rhythmically. The doctors put him on a respirator and inserted a catheter to drain spinal fluid and relieve the pressure on his brain. They also began anticonvulsant drug treatment, which controlled his seizures, but his brain function continued to decline *(18)*.

After obtaining more information from Vanderbilt, Tennessee state officials contacted St. Thomas Hospital, where they discovered two patients were also being treated for meningitis. Both had received steroid injections *(17)*. Prior to those patients and Rybinski, 78-year-old Eddie Lovelace had been treated at Vanderbilt for what seemed to be a mild stroke. Unfortunately, he quickly deteriorated and died on September 17, 2012—the day before Pettit contacted state health authorities. Lovelace had also received a recent steroid injection at the St. Thomas clinic, and, in retrospect, he was the first fatality of what would become the largest outbreak of healthcare-related infections ever reported in the US *(20)*.

On September 24, 2012, the Tennessee Department of Health contacted the Massachusetts Department of Public Health. Working with the Centers for Disease Control and Prevention (CDC), the Tennessee health officials had identified eight cases of meningitis, all of which had been traced to methylprednisolone acetate manufactured by NECC *(1, 17, 20, 21)*. The Tennessee officials also contacted NECC directly to request the lot numbers of the methylprednisolone acetate vials associated with the meningitis patients who received shots at St. Thomas *(17)*.

On September 26, 2012, Massachusetts investigators arrived at NECC to begin an inspection of the facilities, and NECC voluntarily recalled three lots of methylprednisolone acetate *(1, 20, 21)*. The meningitis patients had received injections from lots manufactured by NECC in May, June, and August 2012 *(1, 20)*. Federal authorities promptly contacted all clinical centers that had received those three lots, which could have exposed an estimated 13,500 patients to fungal contamination *(2, 20, 22)*.

Clinics, along with state and local health officials, then began the tedious process of contacting each of those patients by telephone, home visits, or letters *(20)*. Unfortunately, their efforts were hampered because the drug lot number was often not recorded in the patients' medical records *(20)*. Nevertheless, they managed to contact more than 99% of the patients at risk *(20)*.

On September 27, 2012, the CDC received a report from North Carolina that Elwina Shaw, a patient at High Point Regional Hospital, was suffering from meningitis, strikingly similar to the symptoms seen in the Tennessee patients. Shaw had received a steroid injection a few weeks earlier at the High Point Surgery Center, another NECC customer *(17, 20)*.

At Vanderbilt, Thomas Rybinski continued to suffer brain hemorrhages. The damage was irreparable, and his family elected to discontinue life support. He died on September 29, 2012 *(17, 18)*.

The Investigation

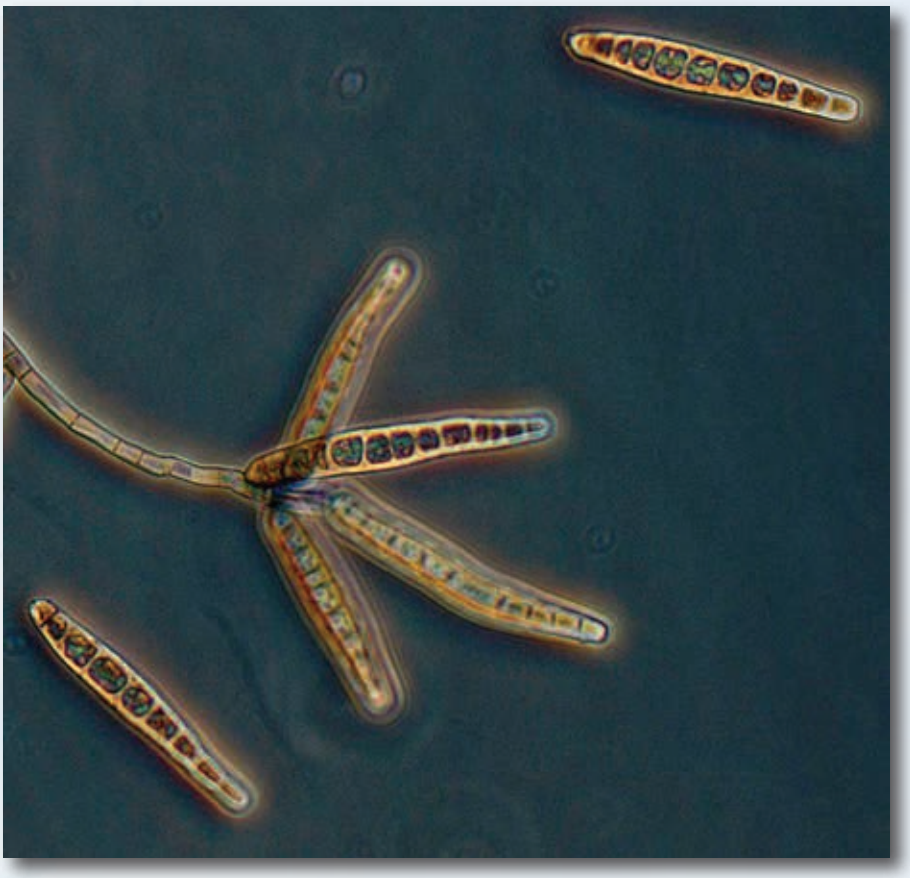
This cluster of reports now gave the FDA sufficient "cause," and on October 1, 2012, the agency sent a team to begin its own inspection of the NECC facilities *(1, 16, 23)*. In a bin of 321 vials of methylprednisolone manufactured in August 2012, the inspectors saw visible signs of contamination in 90 vials. The records of NECC's lab analysis indicated that the lot was sterile, but FDA analysts found microbial growth in all 50 of the vials they tested *(23)*. Further FDA analysis identified the microbe as *Exserohilum rostratum,* a black fungus *(16, 20)*.

It seemed that everywhere the state and federal inspectors looked—clean rooms, prep rooms, weigh stations, laminar flow hoods—they found evidence

of contamination: greenish yellow discoloration, white filamentous substances, yellow residue, cloudy brown discoloration, and dark hair-like discoloration *(23)*. NECC's own environmental monitoring of the labs, lab equipment, and workers' hands had documented unacceptable levels of microbial contamination. But the inspectors found no records indicating that NECC had investigated these "out-of-spec" results or taken corrective actions to prevent contamination of the pharmacy's sterile products *(23)*.

In addition, NECC's air intake units were within 100 feet of the Conigliaro family's recycling facility. Excavators and freight trucks at the facility kicked up dust, presenting another possible source of contamination to the pharmacy *(14, 23)*.

On October 3, 2012, while the state and federal officials were in the midst of their inspection, NECC voluntarily ceased all operations *(16)*. The following day, NECC expanded its voluntary recall to include all of its compounded products *(1, 21, 22)*. On October 9, 2012, NECC surrendered its license to the Massachusetts pharmacy board *(1)*.



Source: Centers for Disease Control and Prevention

Exserohilum rostratum

On October 5, 2012, the FDA issued the first in a series of public alerts to doctors, patients, and the general public. The CDC had received reports of 35 cases of meningitis, including 5 deaths *(1, 16)*.

Further testing by FDA and CDC labs of NECC's unopened vials revealed a potpourri of microorganisms: *Bacillus* bacteria and various species of fungus, including *Aspergillus, Exserohilum, Cladosporium,* and *Penicillium (20, 21)*. On October 11, 2012, the FDA issued another MedWatch Alert, expanding its warning to include other NECC products: preservative-free injectable betamethasone, triamcinolone, and cardioplegia solution. A few days later, the MedWatch Alert was further expanded to include NECC's sterile ophthalmic drugs *(21)*.

No Easy Treatment

Although Thomas Rybinski, the index patient, had been infected with *Aspergillus,* the major culprit in the subsequent meningitis cases was determined to be *Exserohilum rostratum,* a black mold that is widely found on plant debris, in soil, and in water *(20, 21, 22)*. It rarely causes invasive infection in people, but direct exposure to nervous system tissues can cause meningitis *(22)*. The incubation period for patients who developed meningitis was 1-14 weeks after their steroid injection (*20, 22)*.

The rapid alerts and frequent updates posted by the FDA and the CDC undoubtedly saved many lives *(20)*. Because laboratory results often could not provide a conclusive diagnosis, doctors and clinics were advised to begin aggressive treatment for patients who showed even subtle signs of fungal infection *(16, 20, 22)*.

As the outbreak evolved, diligent monitoring and rapid treatment of the fungal infections circumvented meningitis. In these later cases, manifestation of the fungal infection was often confined to the injection site *(20)*. Epidural abscesses and bone inflammation at the injection site caused back pain, which differed in quality from the patients' chronic

back pain *(22)*. Fewer patients who received steroid injections in their joints became infected, but those who did experienced increasing pain for several months after injection *(22)*.

Initially, the CDC and the FDA recommended aggressive treatment with the antifungal agents voriconazole and liposomal amphotericin B for 3-6 months, based on the *Aspergillus fumigatus* infection diagnosed in Thomas Rybinski. Unfortunately, the high doses of amphotericin B required to clear the spinal infection caused a host of adverse reactions and drug-drug interactions *(22)*.

In view of this, and the discovery that *Exserohilum rostratum* was the primary culprit, the treatment regimen was modified in favor of monotherapy with voriconazole *(22)*. Although voriconazole is usually well tolerated, it caused adverse reactions at the doses needed to treat fungal meningitis. So, the sickest patients and those who had substantial side effects from voriconazole were given liposomal amphotericin B alone or in combination with lower voriconazole doses *(22)*.

Poor Prognosis

Eventually, the CDC compiled 753 cases of infection and reports of 64 deaths spread across 20 states, all traced back to NECC's contaminated methylprednisolone acetate *(21)*. Although the first reports came from Tennessee, Michigan was hit hardest, with 264 cases and 19 deaths *(2, 17, 21)*. About half of the victims developed fungal meningitis, and more than 30 suffered a stroke. The other half acquired joint or spinal infections *(13, 20)*. Interestingly, no infections or deaths were reported in Massachusetts, where NECC was located.

The CDC compiled 753 cases of infection and reports of 64 deaths spread across 20 states, all traced back to NECC's contaminated methylprednisolone acetate

For many of those who survived meningitis, recovery was long and painful due to residual effects of the initial fungal infection, adverse drug reactions, or both *(22)*. Some suffered blinding headaches and burning pain *(19, 24)*. Two years after receiving the contaminated steroid for back pain, one woman told reporters, "My head is always in a vice. Even if I get the pain under control with medication, I still feel the grip" *(24)*. In Howell, Michigan, 64-year-old John Nedroscik struggled to recover and experienced nightmares *(24)*.

In Nashville, 71-year-old Joan Peay recovered after contracting fungal meningitis in the fall of 2012 *(2)*. But a year later, she was again in the hospital with meningitis. "The whole month of October my family thought I was going to die. And I was so sick I wish I would've" *(2)*. As a result of the recurring infection, Peay suffered hearing loss and still deals with the back pain that brought her to the clinic in the first place. She looks and feels 10 years older than her age, but she says she has learned to cope with the lingering consequences of her infection and treatment *(2)*.

Cracking Down

The widespread injuries and deaths from NECC's contaminated drugs focused public attention on compounding pharmacy practices. State pharmacy boards and national pharmacy organizations strengthened their oversight of drug compounding and increased their pharmacy inspections. The National Association of Boards of Pharmacy and state pharmacy boards began coordinating efforts to ensure regulatory compliance of compounded drugs shipped across state lines *(9)*.

Public pressure also prompted Congressional hearings. In testimony before the US House of Representatives, FDA Commissioner Margaret Hamburg and FDA Center for Drug Evaluation and Research Director Janet Woodcock both cited the confusing and conflicting statutory constraints that complicated FDA's ability to regulate large compounding pharmacies such as NECC *(10)*.

"We believe there are hundreds of other firms operating as compounding pharmacies, producing what should be sterile products and shipping across State lines in advance of or without a prescription," Woodcock said *(10)*. The FDA's lack of tools for oversight and/or enforcement had resulted in a string of unnecessary patient injuries and deaths of which the NECC incident was only the most recent example.

On November 27, 2013, the Compounding Quality Act (which was part of the Drug Quality and Security

Act) was signed into law *(25)*. Five days later, the FDA held a press briefing and released several documents that provided guidance on implementing the Act.

Under the Act, pharmacies that compound sterile drugs on a large scale were defined as "outsourcing facilities." These large pharmacies may elect to register with the FDA *(25)*. Registration as an outsourcing facility requires the pharmacy to comply with FDA inspections and recordkeeping requirements. And all of the outsourcing facility's compounded products must also be registered, tested, and labeled according to FDA requirements *(25)*. The FDA maintains a publicly available list of registered outsourcing facilities along with the results of FDA inspections.

Legislators hoped that, given a choice, hospitals and other healthcare providers would prefer to purchase their compounded sterile drugs from an FDA-compliant source, thus encouraging compounding pharmacies to seek registration *(25)*. Currently, 68 compounding pharmacies have registered with the FDA, most of them have been inspected, and they are cooperating with FDA officials to correct any deficiencies in their outsourcing operations *(16)*.

Seeking Justice

Hundreds of lawsuits were filed against NECC, its executives, and their related companies, as well as the outpatient centers and hospitals that administered the tainted drug *(17)*. Many of those suits were settled on May 19, 2015.

NECC had declared bankruptcy, and its assets had been frozen by a court order since December 2012 *(17, 22)*. In the settlement, a federal bankruptcy judge approved liquidation of NECC's assets to create a $200 million compensation fund *(13)*. About 3,300 victims and creditors qualified for compensation, with the largest payments going to those most seriously impacted *(13)*.

On September 4, 2014, Glenn Chin, a supervisory pharmacist at NECC, was arrested at Boston's Logan Airport as he and his family prepared to board a flight to Hong Kong *(17)*. Chin oversaw the rooms where NECC's sterile drugs were compounded *(2)*.

On December 17, 2014, federal agents launched a series of predawn raids and arrested 13 other NECC executives, owners, and staffers including company president Barry Cadden *(14, 17)*. The 131-count indictment encompassed a wide assortment of crimes, including racketeering, fraud, conspiracy, violating federal drug laws, and financial crimes. In addition, Cadden and Chin were charged with second-degree murder *(14, 17)*.

Owners Carla and Douglas Conigliaro were accused of transferring $33 million in assets to 8 different bank accounts after the pharmacy declared bankruptcy and the court-ordered freeze on the company's assets *(14)*.

Regarding the racketeering charges, the prosecutors said that NECC's executives and staff had devised a scheme for producing methylprednisolone acetate in unsanitary conditions and sold it, knowing that it posed a risk to patients *(14)*. The indictment also said that employees and managers mislabeled batches and shipped vials that they knew had expired or had never been tested *(14)*. These federal racketeering charges represented the largest US criminal case ever brought over contaminated medicine *(13)*.

The indictment also referenced the FDA inspectors' reports, which showed that NECC had consistently failed cleanliness tests. Prosecutors said Chin instructed his technicians to "prioritize production over cleaning and inspecting" and that he told them to "fraudulently complete cleaning logs" *(14, 17)*.

In bringing murder charges, the prosecutors' aim was to portray Cadden and Chin's actions as a broad pattern of criminal fraud that went beyond routine regulatory violations *(14)*. Cadden's trial began on January 9, 2017, in Boston. He was charged with 25 counts of second-degree murder connected to deaths in 7 states, along with the racketeering crimes *(2, 14)*.

On March 22, 2017, the jury found Cadden guilty of racketeering, conspiracy, mail fraud, and introduction of misbranded drugs into interstate commerce with intent to defraud and mislead. He faces up to 20 years in prison on each of the mail fraud and racketeering counts. The jury found Cadden not guilty of second-degree murder (*2, 14, 17*).

At press time, Cadden's sentencing was slated for June 21, 2017, and Glenn Chin's trial was scheduled to begin on August 9, 2017.

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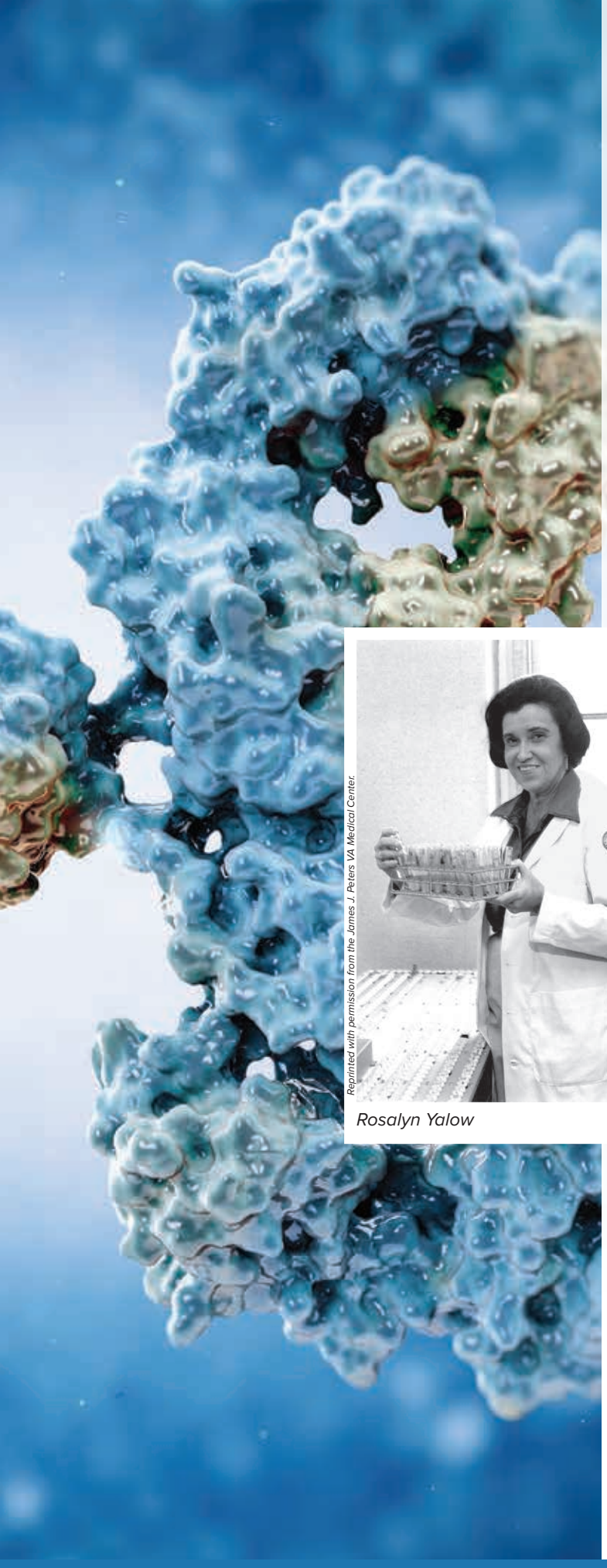

Breaking Barriers:

The Life and Work of Rosalyn Yalow

Rebecca J. Anderson, PhD

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Rosalyn Yalow

Forty years ago, Rosalyn and Aaron Yalow sat across from each other at a long banquet table in Sweden. Rosalyn had been invited from among the Nobel laureates to deliver the traditional address to university students at the beginning of this royal banquet *(1, 2)*. The young man assigned to escort the speaker had been given a seating chart, which showed two Dr. Yalows. He strode into the room wearing his crisp student uniform and confidently stood behind Aaron, assuming he was the speaker.

Rosalyn threw back her head in laughter. She rose and proceeded to the end of the long table. The red-faced student trailed along the opposite side, to the muffled amusement of the assembled notables. When he reached the end, she took his hand and whispered something that restored his self-esteem. Then, *she* escorted *him* to the podium.

This one anecdote sums up Rosalyn Yalow: researcher, mentor, spokesperson for science, and mother. Rosalyn's road to Stockholm was cluttered with obstacles, but she took no detours. She accomplished everything she set out to do, both in her career and her personal life, no matter how formidable the challenges. And she did it her way.

Driven by Ambition

Rosalyn came from a family of strong women. Her grandmother, Bertha, defied her prominent and affluent German family by marrying a tradesman. The couple immigrated to the US when their daughter, Clara, was

four years old *(3)*. After bouncing around the US, the family settled in New York City. Bertha had been well educated in Germany, but the family's nomadic travels through Europe and the US to find a place to make a living left Clara with only a sixth-grade education *(2)*.

Clara was the most defiant of Bertha's six children. She had boundless energy and inherited her mother's pluck. Both were tall, strong, and intelligent *(2)*. And like her mother, Clara married a hardworking tradesman, Simon Sussman. The Sussmans lived on the Lower East Side of Manhattan, where Rosalyn Sussman was born in 1921. Even as a child, Rosalyn showed the same matriarchal characteristics as her elders. She was outspoken, supremely confident, and fearless. Her brother called her "The Queen Bee" *(2)*.

For Rosalyn, learning shorthand was a small concession for a career in science.

Although both Simon and Clara lacked a high school education, they were voracious readers and never doubted their two children would complete college *(3)*. Rosalyn excelled in mathematics and chemistry at Hunter College, the college for women in the New York City system. In her last semester, Hunter College added physics to their curriculum and in January 1941, Rosalyn became the first student to graduate with a major in this discipline *(2, 3)*.

Nuclear physics was the hottest scientific field at the time, and Rosalyn wanted to be a part of it. With the encouragement of her professors, she applied to a number of graduate schools. But as a Jewish woman, her acceptance into those programs with financial support was unlikely *(3)*.

Jerrold Zacharias, one of her Hunter professors and, later, a physicist with the Manhattan Project, recommended her to Rudolf Schoenheimer, a leading biochemist at Columbia University's College of Physicians and Surgeons. Rosalyn excelled at typing as well as chemistry, and she accepted Schoenheimer's offer as his part-time secretary. The job's fringe benefit was the opportunity to take graduate courses at Columbia, but shorthand was a job requirement *(3)*. For Rosalyn, learning shorthand was a small concession for a career in science.

In February 1941, she was accepted in the physics graduate program at the University of Illinois, along with a teaching assistantship of $70 per month and free tuition *(2)*. She could hardly believe it—Illinois was the most prestigious school she had applied to. She immediately quit her shorthand course but continued the secretarial job until June *(3)*.

Becoming a Physicist

Rosalyn always maintained that World War II, which provided opportunities for so many women, had made her career possible. But the US had not yet entered the war, and she was the only woman in the university's College of Engineering. In fact, she was the first woman there since 1917 *(3)*.

To supplement her limited physics background, she had taken two physics courses at New York University during the summer *(3)*. Even so, she was still at a disadvantage, compared to her first-year classmates. In the fall term, she audited two undergraduate courses, in addition to her three graduate courses *(2, 3)*.

Benjamin Yalow

Rosalyn Yalow and Aaron Yalow in the 1940s.

Benjamin Yalow

Rosalyn Yalow on her wedding day.

Her teaching assignment was a freshman physics course. Like the other first-year teaching assistants, she had never taught before. But unlike them, she identified a young instructor who had an excellent reputation and refined her teaching skills by observing him in his classes *(2, 3)*.

For her thesis research in nuclear physics, Rosalyn spent long days and many nights in the laboratory. In the process, she learned to make and use apparatus for measuring radioactive substances—skills that were in high demand during the war. She earned her master's degree in 1942 and her Ph.D. in February 1945 *(2-4)*.

Because commercial instrumentation did not exist, she made or designed much of the equipment they used

In addition, Rosalyn had met and married a physics classmate, Aaron Yalow. Although he had arrived better prepared for graduate school, she finished a full semester before him—and everyone else in their class. With the war still raging, she took a position in New York City in the Federal Telecommunications Laboratory of IT&T, a European firm. She was the lab's only female engineer *(3, 4)*. In September 1945, Aaron finished his Ph.D. degree, joined her in New York, and accepted a position in medical physics at Montefiore Hospital in the Bronx *(2)*.

After the war, the IT&T research group moved to Europe, and Rosalyn returned to Hunter College as a temporary assistant professor. She taught physics to the undergraduate women and to returning veterans in a pre-engineering program that had been established under the GI Bill *(3, 5)*. But the job did not fill her time or further her research interests.

Applied Research

Researchers were increasingly being drawn to peaceful applications of radioactivity, particularly for clinical diagnosis and therapy *(4)*. This emerging field of nuclear medicine needed nuclear physicists who knew how to produce and handle radioisotopes *(2)*. At Aaron's suggestion, Rosalyn met with Edith Quimby, a leading medical physicist at Columbia's College of Physicians and Surgeons, and arranged to observe Quimby's lab workers *(2, 3)*. Everyone noticed Rosalyn, who was analytical and quickly learned clinical radioisotope tracer techniques *(2-4)*.

One day, Quimby received a call from Bernard Roswit, Chief of Radiotherapy at the Bronx Veterans Administration Hospital. Roswit was seeking advice about starting a clinical radioisotope service *(2, 4)*. Quimby took Rosalyn to see her boss, Gioacchino Failla, a pioneer in biophysics and radiobiology. After a short discussion, Failla picked up the phone and said, "Bernie, if you want to set up a radioisotope service, I have someone here you must hire" *(3)*.

Roswit had already launched the Radioisotope Unit at the Bronx VA Hospital, but little was done until Rosalyn arrived in December 1947 *(2)*. She was still teaching full-time at Hunter College, but this energetic part-time consultant soon turned an old janitor's closet into a functioning radioisotope service *(3, 5)*.

For Rosalyn's research aspirations, the timing could not have been better. Paul B. Magnuson, the new

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Old Bronx VA Hospital

Chief Medical Director of the Veterans Administration, was transforming what had been old soldiers' and sailors' homes into a progressive healthcare system of VA teaching hospitals linked to universities. He saw the synergistic value of close interactions between medical practice and clinical research *(2)*.

In addition to producing radioisotopes for the hospital, Rosalyn collaborated on research projects with Roswit and other VA physicians. Because commercial instrumentation did not exist, she made or designed much of the equipment they used *(4)*. These early collaborations produced eight publications describing various clinical applications of radioactive isotopes *(2, 3)*.

In January 1950, Rosalyn left her teaching position at Hunter College and joined the Bronx VA full-time *(3)*. The first physician under whom she worked—not seeing a career path in nuclear medicine—quit after only six months in the unit. Although Rosalyn was still navigating her way through clinical research, the open position gave her the opportunity to define his replacement. The young physicist had already gained Roswit's respect and confidence, and he approved her request to seek a physician who would complement and support her nuclear medicine research *(2)*.

The Partnership

Rosalyn went to Bernard Straus, the Bronx VA Chief of Medicine, and asked for his recommendation. They had already met. She had attended his conferences to improve her understanding of biology and medicine, and he had been impressed by her questions. She was focused, thought quantitatively, and spoke with precision *(2)*.

At this meeting, though, Straus discovered she had other skills. This confident woman knew how to work the system, had common sense, and understood that the new hire would be her boss *(2)*. Her goal, which Roswit supported, was to build an independent Radioisotope Service, separate from the Radiology Department's Radiotherapy Service. She wanted someone who would be a partner in reaching that goal.

Straus recommended Solomon Berson. After Berson completed his residency in internal medicine under Straus, they had become good friends. They shared many interests beyond medicine, including music, history, and art *(2)*. Berson was charismatic, a violinist, and played a strong game of chess. He had been Straus's very best resident, but he had a quick temper and was impatient with those who could not keep up with his sharp mind and deep insight on medical and scientific matters. Those traits undercut his effectiveness as a private practice physician, and he was considering a job offer from the VA hospital in Bedford, Massachusetts *(2, 5)*.

Berson complied with his friend's request and met with Yalow in the spring of 1950 *(3)*. According to both of them, their partnership was forged at first sight. Yalow recalled, "After half an hour I knew he was the smartest person I had ever met" *(2)*. Berson was equally impressed with Yalow, and he canceled his plans to move to Massachusetts *(2, 5)*.

Soon after Berson joined the Radioisotope Service in July 1950, Yalow gave up her other collaborations *(3)*. Neither of them had specialized postdoctoral training in research, but they learned from each other. Yalow's expertise spanned chemistry, mathematics, nuclear physics, and training as an engineer. Berson had vast clinical knowledge, deftly applying his biological insights of physiology and anatomy to clinical medicine *(4)*. They also unflinchingly disciplined each other. "We were probably each other's severest critic" *(3)*.

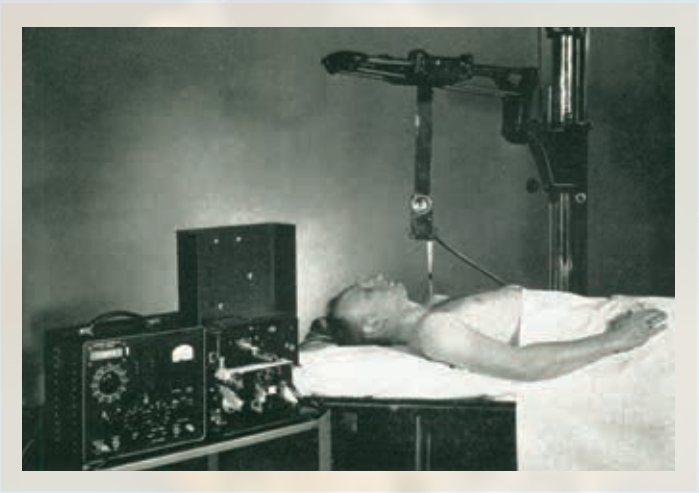
Clinical Problems to Solve

Their first investigation used radioisotopes to develop a satisfactory method for estimating circulating blood volume *(6, 7)*. Their results resolved much of the confusion about blood volume measurements made with earlier and less accurate methods *(2)*.

Berson may have been Yalow's boss, but from their first paper, which was published in July 1951, titles played no role. Authorship was determined only by their relative contributions to the work. Sometimes, Berson was first author. Other times, it was Yalow.

They next applied their technique to trace the distribution of albumin and other serum proteins tagged with ^{131}iodine. They developed mathematical constructs and experimental methods for measuring protein clearance rates, as well as the rates of protein synthesis and degradation *(3, 4)*. They also evaluated albumin versus globulin as plasma expanders *(2, 3)*.

In parallel with these studies, Yalow and Berson began studying thyroid function. Other investigators



Apparatus designed by Rosalyn Yalow to measure thyroid uptake of radioactive iodine.

had given radioactive iodine orally to diagnose hypo- and hyperthyroidism, but the procedure took several days, and the results were difficult to interpret *(2)*. Yalow and Berson injected ^{131}I intravenously to assess iodine uptake by the thyroid gland and plasma clearance. As she had done for their other studies, Yalow designed the instrument for measuring radioactivity over the thyroid gland *(2, 8)*.

Their method determined plasma clearance and thyroid uptake rates of ^{131}I in a single 35-minute sitting and was independent of various extraneous factors. This direct and reliable index of thyroid function was immediately hailed as "the most important contribution to the problem of diagnostic tracer procedures" yet published *(2)*.

The study, which incorporated data from 110 subjects, had been brilliant in concept, meticulous in design, and backed up by thorough mathematical analyses—features that became the hallmark of all their work.

Insulin

In 1954, Berson was named chief of the first independent Radioisotope Service in the VA system. Yalow and Berson had the freedom to pursue any research direction they wished, but they were still responsible for running the Bronx VA Hospital's nuclear medicine service. This included producing radioisotopes and providing a full range of lung, brain, liver, thyroid, and bone scans, as well as running a thyroid clinic using their new 35-minute technique *(2, 3)*.

They masterfully juggled their service and research activities and took pride in running their small mom-and-pop shop without ever submitting a grant proposal. Their research was funded entirely through their modest departmental budget and the VA Medical Research Program *(2, 3)*.

Yalow and Berson's experience with serum proteins and radiolabeled iodine could be applied to studies of other circulating proteins. With its great sensitivity and accuracy, a radiolabel could, potentially, measure small peptides that were present in the blood in very low concentrations, such as hormones. While they continued to investigate thyroid function, Yalow and Berson increasingly turned their attention to the small peptide hormone, insulin *(1)*.

Neither of them had any special expertise with insulin. Berson's internal medicine residency included a working knowledge of endocrinology. Rosalyn's understanding was limited to personal observations of Aaron, who had been diagnosed with type 1 diabetes at the age of 12 and took insulin daily *(2)*.

They chose insulin over other hormones because of a large unmet medical need. Next to hyper- and hypothyroidism, diabetes was the most common endocrine disorder. Yet, insulin metabolism was largely a mystery *(4)*. Their decision was also influenced by feasibility. Insulin was the hormone most readily available in highly purified form *(2, 3)*.

Type 1 diabetes is characterized by a lack of insulin production by the pancreas. On the other hand, the pancreatic beta cells in type 2 diabetes are normal, and no one knew why those patients' blood sugar was too high. In 1952, I. Arthur Mirsky proposed that, in type 2 diabetes, insulin disappeared from the bloodstream faster than normal, perhaps due to aggressive degradation by an insulin-metabolizing enzyme in the liver *(1, 3, 4)*.

Yalow and Berson could easily test Mirsky's hypothesis by measuring the plasma clearance of radiolabeled insulin. They injected ^{131}I-labeled insulin into diabetic and non-diabetic subjects and measured the radioactive counts in blood samples collected over several hours *(1, 4)*. To their surprise, the ^{131}I-labeled insulin remained in the blood of diabetic patients longer, not shorter, than in the blood of the control subjects *(9)*.

This refuted Mirsky's hypothesis, and Yalow and Berson wanted to know why. An important clue came from another puzzling observation: The rate of

disappearance of ^{131}I-insulin was the same in control subjects and in diabetic patients who had never been treated with insulin *(9)*.

Antibody Breakthrough

Their brainstorming approach to this problem was the same intertwined collaboration that characterized all of their studies. Yalow and Berson shared an office that opened into their lab. Their desks were pushed together so that they faced each other across a large, cluttered surface of books and papers—more cluttered on his side. Speculations, new approaches, and inventive methods would fly between them, and then they would go to the lab and try it. The resulting experiments were so integrated that it was impossible to dissect who came up with which idea or technical solution *(2)*.

They had some technical help, but they preferred to do the radiolabeling themselves. In a kind of ritual in the iodination room, they chatted about buffer ionic strengths or binding site saturation, as they pipetted and handed small vials back and forth *(2)*.

Yalow and Berson spent more time at the lab bench than anyone else, tediously processing and analyzing radioactivity in urine, plasma, and packed red blood cells. Day and night, they did electrophoretic separations in the cold room, centrifuged and washed hundreds of protein precipitates, cut and pasted countless electrophoresis strips, and changed thousands of tubes in the radiation counter *(2, 4)*.

Benjamin Yalow

Rosalyn Yalow working in her Bronx VA Hospital lab.

Hammering out some technical kinks took weeks. Others took months. To speed up the electrophoresis, they developed innovative methods using both paper and thin layer chromatography. In the end, they discovered that ^{131}I-insulin in the blood of insulin-treated patients was not "free" but rather was bound to a gamma-globulin *(1, 9)*.

They immediately speculated that this gamma-globulin was an antibody. At that time, both type 1 and type 2 diabetic patients were treated with insulin extracted from animal pancreatic tissue. (Bovine insulin differs from human insulin by three amino acid residues, and porcine insulin differs by one.)

Convincing the scientific community that the isolated gamma-globulin was an antibody proved to be difficult

Further experiments in animals and testing plasma from patients supported their conclusion that patients who had been repeatedly treated with bovine or porcine insulin developed insulin-specific antibodies. Antibody binding explained the increased plasma half-life of insulin in these patients *(3, 5)*.

Yalow and Berson's 20-page report is so comprehensive it could pass as a doctoral dissertation. It presented the first direct proof that such a small protein (i.e., insulin) could stimulate an immune response *(4)*.

However, convincing the scientific community that the isolated gamma-globulin was an antibody proved to be difficult. Reviewers and journal editors initially rejected their manuscript because they said insulin was simply too small to confer immunogenicity *(1, 3)*.

Conventional wisdom at that time asserted that only large proteins could be antigenic. In addition, the only way to identify an antibody was to observe the large antigen-antibody conglomerates that form and precipitate out of solution. Soluble antigen-antibody complexes of smaller proteins were invisible and more difficult to detect. Many experts thought they simply did not exist *(2)*.

A flurry of correspondence flew back and forth for several months *(1)*. Finally, the two sides reached a compromise. Yalow and Berson agreed to replace "antibody" in the title of their paper with "globulin." In the text, though, they called their binding gamma-

globulin an antibody, noting that it met the definition of "antibody" as stated in a standard textbook of bacteriology and immunity *(1, 3, 9)*.

After the paper was published in 1956, other researchers quickly confirmed Yalow and Berson's observations, and it caused a paradigm shift in immunology. Their sensitive radioisotopic technique detected soluble antigen-antibody complexes, which proved that even small peptides like insulin can be antigenic, and launched a new era in immunology research.

By demonstrating that animal-derived insulins trigger antibody production and that those antibodies attenuate insulin's effectiveness, Yalow and Berson's results led to improved diabetes treatment. It would be better for diabetic patients to take human insulin, which would not generate antibodies. Today, manufactured insulin is genetically engineered to be precisely the same as human insulin *(4, 5)*.

Yalow and Berson's main objective was to isolate, identify, and quantitate the gamma-globulin (i.e., the insulin antibody) that they found in the patients' blood. But they reported another important observation in their 1956 paper. The binding of ^{131}I-labeled insulin to a fixed concentration of antibody is a quantitative function of the amount of insulin present *(9)*. They realized that they could reverse their procedure and use the antibody to measure the amount of insulin in a patient's blood *(1)*.

RIA Is Born

Yalow and Berson spent the next three years developing a practical method for measuring insulin in circulating blood. They optimized the conditions for antibody production and found that guinea pigs were the best species. Early in the morning, before anyone else arrived, Yalow would take each guinea pig from its cage and cuddle it, thinking that happy animals would produce high quality antibodies. When the animals were injected with antigen or bled for their antibody-containing blood, she would gently hold, stroke, and calm each one *(2)*.

They systematically evaluated the species specificity of antibodies triggered by cow, pig, horse, and sheep insulins. Next, they honed their assay, first in rabbits and then with human blood samples *(1)*. The work required meticulous studies and quantitative analysis of the interaction between insulin and antibody. They calculated equilibrium constants and binding affinities *(3)*.

Finally, in 1959, they reported that they could accurately measure insulin in human blood *(10)*. Their assay, for the first time, measured a hormone in a test tube, without the need to expose the patient to radioactivity. This spectacular achievement had combined immunology (antigen-antibody binding), nuclear medicine (tracer technique), mathematics, physics, and chemistry *(4)*.

Yalow and Berson recognized RIA's broad potential, and they believed that scientific discoveries should be shared to benefit society.

And the procedure was simple *(1)*. The antibody and radiolabeled insulin concentrations are held constant. When varying amounts of unlabeled insulin are added, it displaces a corresponding amount of labeled insulin from the antibody. A standard curve is created by counting the radioactivity of bound/free insulin for each known concentration of unlabeled insulin. The insulin in a human plasma sample will also displace some of the labeled insulin from the antibody, and the amount can be quantitated by interpolation from the standard curve.

Yalow and Berson's first application of this method, which they called radioimmunoassay (RIA), was a study that measured plasma insulin in subjects under various conditions: glucose tolerance tests in nondiabetic and early diabetic subjects, patients with functioning islet cell tumors, and patients with leucine-sensitive hypoglycemia *(11)*.

This paper reported several important discoveries, but the most striking finding was that type 2 diabetic patients release more insulin and have higher plasma insulin concentrations than nondiabetic subjects. Yalow and Berson suggested that, in type 2 diabetes, patients are somehow resistant to the action of their own insulin *(11)*. This concept of insulin insensitivity is now accepted as a key feature of type 2 diabetes. It also shifted the strategy for treating type 2 diabetes from insulin treatment to diet management, exercise, and treatment with glucose sensitizing drugs *(4)*.

In a more general sense, Yalow and Berson's carefully executed studies provided the foundation for a principle that is now central to all receptor binding

assays: the sensitivity, specificity, and competitive binding of antibodies. The radiolabeled ligand (^{131}I-insulin, in their case) provided exquisite sensitivity, detecting a substance down to 1 picogram. The antibody conferred exquisite specificity. By choosing an appropriately matched antigen and antibody, RIA could measure all sorts of substances amid a myriad of other substances that were present in a blood sample in billion-fold higher concentrations. And best of all, RIA was easy and quick. Thousands of samples could be assayed as easily as one or two *(2)*.

Yalow and Berson recognized RIA's broad potential, and they believed that scientific discoveries should be shared to benefit society. Rather than pursuing a patent, they made every effort to get RIA into common use *(2, 12)*. They welcomed physicians and researchers who came from all points of the globe: from Montreal to Santiago and from Brussels to Auckland. Some stayed a few days; others stayed for a month. Under Yalow's careful guidance, they acquired hands-on experience with this new method, and many left with a precious sample of guinea pig plasma containing specific antibodies that would enable them to begin work quickly in their own labs *(2)*.

Making a Home

While Yalow and Berson were deeply immersed in their groundbreaking RIA research, the Yalows were raising two children. The VA required pregnant women to resign in their fifth month, with no expectation of returning to their jobs. Yalow and Berson ignored the requirement. Her "fifth month" lasted for four more.

Benjamin Yalow

(From left to right) Benjamin Yalow, Aaron Yalow, Rosalyn Yalow, and Elanna Yalow in 1977.

She worked until the day before she delivered *(2)*. A week later, she returned to work. Two years later, she did it again.

Aaron had become a physics professor at Cooper Union's School of Engineering, and Rosalyn held traditional views of a woman's role and responsibilities as a homemaker. Fortunately for her as well as the children, their elementary school was just a couple of blocks from home. Each morning, Rosalyn would rise absurdly early to go to the VA, which was a mile away. She would return home briefly to fix breakfast and get the children ready for school. Between experiments, she met them at home for lunch and returned again to make dinner for the family. Then, back to the lab, to work late into the night *(2, 12)*.

The Yalows employed a housekeeper who greeted the children home from school when Rosalyn couldn't, but they never had a nanny. Rosalyn did the shopping and cooking in their kosher home. Sometimes, she would take the children to the lab, so she could watch them while she worked, and they helped by feeding the animals and doing other small chores. When they grew older, she showed them her experiments and explained the scientific rationale and methods. "That's how we learned science" *(12)*.

Rosalyn discussed her research with Aaron over dinner, and when the children could keep up, they joined in the discussion. The Yalows did not take conventional vacations. Instead, the family accompanied her on her speaking tours, and they would take an extra day to sightsee. As teenagers, the children were allowed more independence than their classmates. Rosalyn's only requirement was that they should always do their best. And they did. But when she became frustrated listening to her son's hunt-and-peck typing, she would take his handwritten report and type it herself *(12)*.

For Rosalyn, there was no balance between work and family. She was an overachiever who wrapped time for her family around her work. Overall, though, her daughter says, "She was a pretty wonderful mom" *(12)*.

Professional Family

Initially, Yalow and Berson were not interested in accommodating research fellows in their lab. Yalow was happy working exclusively with Berson and concentrating on their work without distraction. Yet, as their lab morphed from the janitor's closet to a

small but efficiently run research facility, it was Yalow who convinced Berson to take them on *(2)*. The first of this revolving cadre of research fellows assisted and coauthored the "insulin globulin" paper.

To the research fellows, they were simply Sol and Ros. Sol was volatile, whereas Ros was stable and politically savvy. Sol moved seamlessly from bench to bedside—always with a firm grip on technology, science, and philosophy—but he was somewhat aloof. Ros was the research fellows' main lab mentor. She called them her "professional children" *(2, 3, 12)*.

Ros was unpretentious and could talk to anyone, regardless of their background. Even as a graduate student, she had a knack for explaining the most complicated concept in terms that anyone could understand. She immediately connected with the research fellows and was always ready with suggestions and guidance *(2)*.

Rather than lecturing, she was a good role model and offered constant encouragement. But it was tough love. She judged her own success by the discoveries she made, and she measured others by the same yardstick. Ultimately, her research fellows, as well as science-oriented young women—and even her own grandson—had to make it on their own merit. She gave no free passes *(12)*.

Ros infused the research fellows with scientific curiosity and fostered the "chain of discovery," so that this next generation could build on her accomplishments *(12)*. And they did. Many of her professional children became leaders in medicine and clinical research *(2, 3)*. She took pride and, rightly, a measure of credit for their success.

Ros was comfortable around men. She earned their respect and admiration through hard, high-quality work, and she never backed down. As one of them said, "Anyone planning to argue with Rosalyn Yalow would be well advised to be properly prepared" *(2)*.

Her relationships with women were more complex. By her own account, she was stubborn and aggressive—traits that did not endear her to many women. She refused awards for the "best woman (anything)" *(2, 12)*. She aspired to be the best—period!

Ros proactively encouraged bright young women to pursue a career in science, as she had done. But she was critical of women scientists—even fellow Nobel Laureates—who had no children. She also criticized women who had relinquished their careers to become soccer moms. She maintained a woman could and should do both.

First commercial radioimmunoassay kit

Successes and Consequences

The first applications of RIA were in endocrinology. Peptide hormones could be detected at 10^{-40} to 10^{-42} molar concentrations. In addition to insulin, Yalow and Berson studied the modulation of gastrin, which triggers gastric acid secretion, and their findings greatly facilitated diagnosis and treatment of thyroid, growth, and fertility hormone dysfunctions *(3, 4)*.

In 1965, Amersham produced the first commercial RIA kit (for insulin), and by the end of the decade, RIA had become an indispensable tool. Labs around the world were using RIAs to detect and quantitate minute amounts of enzymes, drugs, and other substances, as well as hormones *(1, 4)*. Everyone working in a biochemistry lab wore a dosimeter.

In Yalow and Berson's lab, John Walsh developed the first RIA for a virus. This assay of hepatitis-associated antigen was a breakthrough in infectious disease management *(2)*. Blood banks quickly adopted it to screen donated blood and prevent transfusion-transmitted hepatitis *(3, 4)*.

RIA made Berson and Yalow famous in the scientific community. In 1957 and 1961, they received the Lilly Award of the American Diabetes Association—the first of many honors and awards.

They were intellectual equals, and their work was seamlessly integrated. But Berson was the physician. Berson belonged to the professional medical societies, which at that time included few women and no Ph.Ds. And the charismatic Berson cultivated a broad clinical network. Yalow was less flashy—the steady, analytical partner. She was more interested in the lab than developing social contacts *(2)*.

Eloquent and genial, Berson wrote the first drafts of most of their papers and delivered virtually all of the invited lectures. But while he was willing to stand at the podium and make the acceptance speeches, he insisted that Yalow be named as a corecipient on their awards *(2)*.

Several times, Berson had refused chairmanship offers from medical schools. Finally in 1968, he agreed to become chairman of the Department of Medicine at the new Mount Sinai School of Medicine *(2)*. Although he continued to collaborate part-time at the Bronx VA, Yalow assumed the leadership of their lab, in title as well as in practice. In April 1972, Berson suffered a fatal heart attack while attending the FASEB meeting in Atlantic City.

Benjamin Yalow

Rosalyn Yalow and Solomon Berson after receiving the Lilly Award from the American Diabetes Association in 1957.

AP

Dr. Rosalyn Yalow

Emerging Solo

For Yalow, Berson's death was devastating, but any doubts about her contributions to their partnership were soon put to rest. She assumed full responsibility for writing and speaking. Over the next five years, her lab published 60 papers. She stepped out of Berson's shadow to speak at scientific conferences, and she was good at it *(2)*.

Frequently, she turned to Aaron for advice. He preferred teaching to research, but he read and critiqued every paper and every speech she wrote. His soft-spoken, scholarly demeanor belied a strength of character. He steadfastly supported his ambitious wife and was genuinely proud of her accomplishments *(2)*.

Like Berson, Yalow fully acknowledged her partner's contributions. She arranged to have the lab renamed the Solomon A. Berson Research Laboratory, ensuring that every paper she published would include his name, as long as she was there *(2, 3)*. The Berson Laboratory conducted key studies of parathyroid and gastrointestinal hormones and identified multiple molecular forms of peptide hormones (e.g., gastrin-34, gastrin-17, and gastrin-14) *(1, 2)*.

Building on the work of other investigators, Yalow and her research associate, Eugene Straus, reported that cholecystokinin (CCK) in the brain is identical to that found in the gut *(3)*. Then, using immunohistochemical techniques, they established

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that the highest concentration of CCK is in the cerebral cortex *(1)*. These findings provided the first evidence that CCK is endogenous in the brain, suggesting its role in neuroregulation and broadening the concept of neurotransmitters *(4)*. Subsequently, many gastrointestinal peptides, including somatostatin, substance P, and vasoactive intestinal peptide, were also found in the brain *(1, 3)*.

Yalow had never taken a course in biology. She learned physiology, anatomy, and clinical medicine from Berson *(3)*. Yet, her depth of understanding and clinical insight were highly regarded. Harold Rifkin, a diabetes expert, sought her recommendations on new insulin formulations, and Morton Grossman, a leading gastroenterologist, consulted her about clinical syndromes involving gastrointestinal hormones *(2)*.

Benjamin Yalow

Rosalyn Yalow receiving the Nobel Prize from Sweden's King Carl XVI Gustaf.

Recognition

In 1975, Yalow was elected to the National Academy of Sciences. In 1976, she was the first woman to receive the Albert Lasker Basic Medical Science Award. And in 1977, she was already working in her office at 6:45 am when the phone rang *(4)*. She had just become the first American woman to receive the Nobel Prize in Physiology or Medicine. In her acceptance speeches, she emphasized that Berson deserved equal recognition for their accomplishments.

Yalow continued to lead her lab, accept research fellows, and make research contributions until 1991, when she became emeritus senior medical investigator at the Bronx VA. Although physical limitations increasingly restricted her laboratory activities, she regularly went to the office, read scientific literature, wrote commentaries, and continued to serve on the Bronx VA's research committee *(2, 12)*.

Yalow and Berson's legacy is profound. RIA was one of the most important clinical applications of basic research during the 20th century. It permitted new insights in endocrinology, immunology, cardiology, gastroenterology, nephrology, neuroscience, and many other disciplines *(4)*.

RIA was also the blueprint for more advanced immunoassay methods, notably ELISA (enzyme-linked immunosorbent assay), which incorporates enzymes in place of radioisotopes to detect the presence of substances in blood. These newer methods rely less heavily on radioisotopes and have all but replaced RIA in many applications. They are less dangerous and less

costly, but all of them are based on the fundamental concepts first worked out by Yalow and Berson *(4)*.

And there is one other legacy. Despite the obstacles she faced, Rosalyn Yalow never complained or made excuses. Barriers were made to be broken. She just worked harder and did better, as if to say, "Bring it on" *(12)*. The sign on her office wall read, "Whatever women do they must do twice as well as men to be thought half as good. Luckily, this is not difficult" *(2)*.

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Enbrel:
A biotechnology breakthrough

Rebecca J. Anderson, PhD

When they called her name, Elizabeth Petersen navigated her way to the microphone. The ballroom at the Holiday Inn in Gaithersburg, Maryland, was packed with researchers, industry executives, and government officials. Elizabeth, by contrast, was just an ordinary citizen, permitted a few minutes to speak during the open public hearing part of this meeting *(1)*.

Craig Smith

Enbrel vial

The impressive thing was not that Elizabeth came as an unsolicited participant, nor that she had paid her own travel expenses from Chicago. The impressive thing was that she walked effortlessly to the microphone, free of the crippling and painful arthritis that she had suffered for 36 years. She had been taking an experimental drug, and she wanted to tell the US Food and Drug Administration (FDA) officials—in person—that they should approve this drug for all rheumatoid arthritis patients.

Enbrel's success in treating patients like Elizabeth was all the sweeter because it had survived, despite skeptical experts and several serious setbacks.

A New Era

In 1980, the US Supreme Court ruled that genetically engineered microorganisms could be patented. Immediately, a generation of pioneering molecular biologists, full of bright ideas and entrepreneurial spirit, left academia and launched the biotechnology industry.

Among them were Steven Gillis and Christopher Henney, immunologists who aimed to make immune-response-based

ARCH Venture Partners

Steven Gillis

medicines *(2, 3)*. In 1981, they left the Fred Hutchinson Cancer Research Center and founded Immunex in Seattle, WA. Gillis recruited the best researchers in their fields and fostered a "collegial-critical" environment. He defined "the boundaries of the sandbox" and encouraged everyone to be creative within those boundaries *(3)*.

Energized researchers at Immunex would meet by chance in the hallway and end up discussing science for hours *(4)*. They employed a broad suite of innovative technologies, cloned a long list of genes, and expressed the corresponding recombinant proteins *(2)*. Among their early products were interleukin-based drugs and GM-CSF (granulocyte macrophage colony stimulating factor).

Stephen Duzan, an entrepreneur with no science background, joined the management team and used his business savvy to keep Immunex solvent *(2, 3)*. Through the 1980s, Duzan sold or licensed the company's proprietary technologies, which funded the ambitious research program, but profits remained elusive *(2)*.

Clinical trials of GM-CSF began in 1987, with Hoechst Roussel Pharmaceuticals assisting the young Immunex clinical team *(2, 5, 6)*. GM-CSF proved to be effective in accelerating white cell recovery following bone marrow transplantation in cancer patients and was approved by the FDA in 1991 *(2)*.

Immunex's stock skyrocketed *(2)*. Anticipating demand, the company had invested heavily in a large GM-CSF manufacturing plant *(3)*. Unfortunately, Amgen's Neupogen, a direct competitor product, was approved a month before GM-CSF for the much larger chemotherapy market. Neupogen maintained a 10-fold sales advantage over GM-CSF, and Immunex's new manufacturing facility sat underutilized *(2, 3)*.

Immunex next concentrated on developing PIXY 321, a synthetic molecule that incorporated the properties of GM-CSF and Interleukin-3. PIXY 321 was intended to stimulate platelet and white cell counts *(2)*.

Climbing the TNF Wall

When Craig Smith joined Immunex in 1988, researchers in the Receptor Biochemistry and Biophysics Department were focused on Interleukin-2 *(7)*. But Smith was intrigued with another cytokine, tumor necrosis factor (TNF).

"Tumor necrosis factor" had been coined as a term in the 1960s by researchers who found evidence of something that induced tumor regression. In 1984, Bharat Aggarwal and colleagues finally succeeded in isolating two cytotoxic substances, subsequently named TNF-α and TNF-β—the first members of what was to become a superfamily of cytokines that can cause cell death *(8, 9)*.

Craig Smith

Enbrel structure – a molecule of TNF (red) bound to Enbrel (yellow and green)

After researchers confirmed that TNF causes rapid necrosis of experimental cancers, they cloned the gene and produced recombinant TNF for clinical trials *(9, 10)*. Unfortunately, rather than attacking the patients' tumors, TNF had a paradoxical tumor-promoting effect *(7, 10)*. Consequently, biotech companies saw no commercial value in TNF, and researchers turned to other biologic drug candidates.

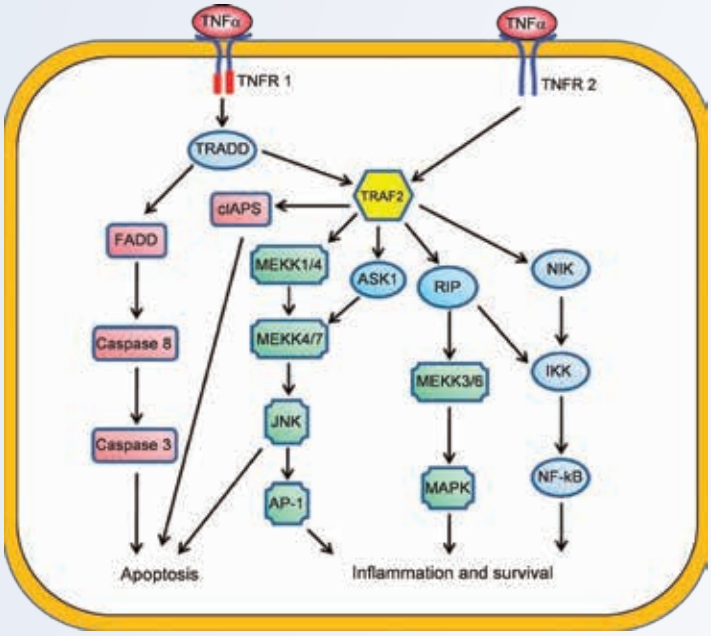
To Smith, "tumor necrosis factor" was really a misnomer. Less than 1% of primary tumor cells or tumor cell lines are killed by TNF *(7)*. Instead, TNF's primary role is to orchestrate the immune response to any challenge—whether it be a virus, bacteria, or fungus. In the oncology clinical trials, recombinant TNF had sent the patients' immune system into pathological overdrive, producing a condition similar to shock *(11)*.

Similarly, up-regulation of TNF could explain the chronic inflammation seen in autoimmune diseases *(7, 11)*. Immunex's ongoing immune-suppressor-factor program focused primarily on the interleukins *(3)*. Smith thought that suppressing TNF would also be therapeutic *(11)*.

At that time, TNF was not commercially available, and production of monoclonal antibodies was still in

its infancy *(7)*. Researchers at Centacor had created a chimeric TNF antibody called infliximab (Remicade®) by attaching the variable region of the mouse TNF antibody to the Fc region of human IgG1 *(12)*.

Taking advantage of the Gillis-authorized sandbox, Smith, in his spare evenings, began expressing and purifying recombinant forms of TNF *(7)*. Then, he used radiolabeled TNF to isolate, clone, and express the TNF receptor *(13)*.



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TNF signaling pathways

TNF binds to two receptors: TNFR-1 and TNFR-2. The extracellular (i.e., the binding site) portion of these membrane-bound receptors is functional, like the intact receptors. Extracellular TNFR-1 (p55) and TNFR-2 (p75) circulate as "soluble receptors" and can bind to TNF everywhere in the body *(13, 14)*. In a similar manner, the soluble (extracellular) portion of the IL-1 receptor specifically binds to IL-1 *(15)*.

The soluble TNF receptors expressed by Smith and his colleague, Ray Goodwin, bound to TNF with relatively low affinity *(14)*. TNF is a homo-trimer, and on the cell surface it normally binds to 2-3 receptor molecules. This multiplicity increases receptor affinity for TNF through interlocking "cooperative binding" *(7)*. Smith aimed to construct a molecule that mimicked the membrane-bound receptor configuration and would have much higher affinity for TNF than the monomeric soluble receptor.

Smith clipped human IgG1, leaving just the Fc stem and a portion of the two hinge regions. He then fused a human p75 soluble TNF receptor to each of the hinges and called the resulting molecule a "TNFR:Fc fusion protein" *(11, 16)*. The two soluble receptors in this configuration accommodated 1-2 binding domains of the TNF molecule *(7)*. As predicted, TNFR:Fc had up to a 1000-fold greater affinity for TNF than the monomeric p75 soluble receptor and was equivalent to the interlocking membrane-bound receptors' affinity for TNF *(7, 14)*.

Craig Smith

Craig Smith (left) and Raymond Goodwin

Cindy Jacobs

Cindy Jacobs

Assessing Activity

To determine the biological activity of his molecules, Smith collaborated with Cindy Jacobs in Immunex's preclinical labs. Jacobs had joined Immunex in 1985 as a part-time scientist while she was still in medical school and initially supported the GM-CSF and interleukin projects *(17)*.

Jacobs, who held a PhD in veterinary pathology/microbiology, set up animal models to test the efficacy of the soluble IL-1 receptor and TNFR:Fc. Each of the molecules was effective in animal models of antigen-induced arthritis, and they were more effective given together than either one alone *(15)*. TNFR:Fc also protected mice from otherwise lethal injections of lipopolysaccharide, an animal model of sepsis *(14)*.

Using radiolabeled TNFR:Fc, Jacobs followed its pharmacokinetics and distribution in serial blood and tissue sections collected from the animals. She found that TNFR:Fc had a 5-fold longer serum half-life than the soluble p75 receptor *(14, 15)*. Smith and Jacobs moved quickly to secure Immunex's patent rights to TNFR:Fc *(16)*.

Looking for Winners

Despite Duzan's efforts, GM-CSF's flagging sales disappointed investors. At the same time, Immunex's research expenditures were, if anything, increasing. In 1993-1994, in rapid succession, American Cyanamid acquired majority ownership of Immunex, and then American Home Products purchased American Cyanamid *(2)*.

In parallel, clinical trial results indicated that PIXY 321 was ineffective – a crushing blow to both the clinical team and the new managing partners. In October 1993, the project team shut down the PIXY 321 program *(6)*.

Immunex researchers continued interleukin development, exploring the soluble IL-1 receptor for asthma/allergy, rheumatoid arthritis, and inflammatory bowel disease *(15)*. Preliminary clinical trials showed that the molecule protected healthy volunteers from a cutaneous allergic challenge *(18)*.

With TNFR:Fc, the animal model results justified clinical trials in several therapeutic areas, but Immunex's decision makers gave sepsis top priority.

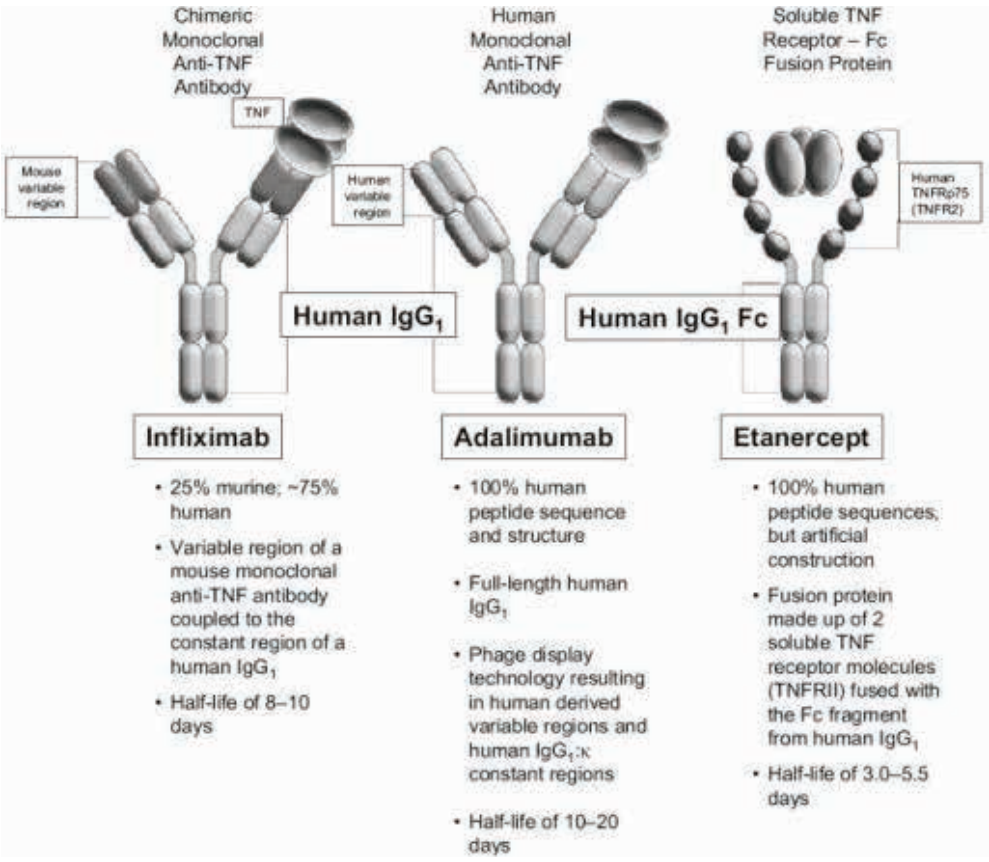

Figure 1: Etanercept structure in comparison with other TNF antagonists.

The sepsis mortality rate was about 40%, and clinicians had virtually nothing to treat it *(19, 20)*.

Immunotherapy was just emerging as a promising new approach to tackle sepsis, and many companies were developing immunology-based treatments *(7, 17)*. Some of those companies, like Immunex, had created fusion proteins by mix-and-match combinations of the p55 or p75 TNF receptor with the Fc region of IgG1 or IgG3. These fusion proteins were effective in animal models, but no one knew whether the animal results predicted efficacy in sepsis patients *(14)*.

Clinical trials for sepsis were usually completed rapidly, with a clear endpoint that required relatively few patients. If TNFR:Fc worked, it would be quickly approved *(7, 17)*. Then, Immunex could consider clinical trials for rheumatoid arthritis, inflammatory bowel disease, and other disorders *(15)*.

Sepsis

The initial clinical results were encouraging *(19)*. In healthy subjects, TNFR:Fc was safe at doses that were subsequently used in sepsis patients. In addition, when the subjects were challenged with endotoxin, TNFR:Fc bound to all TNF-α circulating in their blood *(21)*.

Immunex's clinical team, led by Janis Agosti, moved quickly to launch a large blinded, randomized, placebo-controlled trial *(19, 21)*. Because sepsis is life-threatening and progresses rapidly, the lag between enrolling and treating patients was short. Throughout the winter-summer of 1992-1993, Immunex clinical associates traveled continuously from coast to coast to monitor and support the 15 clinical sites *(19)*.

Immunex had also chartered an independent Data Monitoring Committee, which was charged with ensuring the safety of these critically ill patients and periodically reviewing the incoming trial data. The clinical investigators had enrolled about half of the targeted number of patients when the Committee became concerned *(5)*. Immunex's data management group was asked to decode the treatments of the patients who had died.

Unfortunately, most of the deaths occurred in TNFR:Fc-treated patients. And it was a dose-dependent effect *(21)*. For the clinical team, it was "very scary to think that the drug may be causing deaths" *(5)*. Immunex immediately stopped the trial. Steve Gillis, as acting CEO, had the unenviable task of notifying American Cyanamid, the FDA, and the public of the results *(3)*.

...it was "very scary to think that the drug may be causing deaths".

Through the 1990s, dozens of clinical trials of various anti-inflammatory agents failed to show a benefit in roughly 15,000 sepsis patients, despite impressive efficacy in animal models *(20)*. It now appears that TNF's predominant role in sepsis is protective, helping the patient combat systemic bacterial toxicity *(1, 20)*. One by one, each of the sponsoring drug companies, including Immunex, moved away from sepsis therapeutics.

At Immunex, it was a tumultuous time *(6)*. The company had invested heavily in the sepsis trial, and its failure was an especially hard blow—more than PIXY 321 *(6, 7)*. Further investment in TNFR:Fc did not make good business sense. "There were discussions whether to run or walk away" *(17)*. The company decided to sell its ownership of the product, and researchers moved to more promising drug candidates *(5, 22)*.

Limping Along

Despite the company's focus on sepsis, Craig Smith thought of TNFR:Fc as an innovative treatment for autoimmune diseases, and rheumatoid arthritis (RA) was always at the top of his list *(7)*. He vividly remembered his Irish-Catholic grandmother, who had raised a large family in the Midwest during the Depression, despite suffering from severe rheumatoid arthritis. She made a deep impression on her young grandson, and now his TNFR:Fc might conquer the disease that had plagued her *(7)*.

Disease-modifying antirheumatic drugs (DMARDs), such as methotrexate, sulfasalazine, and hydroxychloroquine, were available and could retard disease progression. But many patients did not adequately respond, and many stopped treatment due to toxicity *(23)*.

In parallel with the sepsis trial, Cindy Jacobs (at this time a clinical research director) oversaw small clinical trials to probe IL-1-receptor and TNFR:Fc efficacy in rheumatoid arthritis *(15)*. Seeking interested investigators, Immunex representatives attended the


Larry W. Moreland

Larry W. Moreland

Keystone Symposium in Colorado. Among those they approached was Larry Moreland, a young rheumatologist from the University of Alabama *(24)*.

Moreland headed the university's RA intervention program and had been a clinical investigator for trials of several biologic drug candidates. "None of them worked very well" *(24)*. But the results were presented or published, and Moreland, along with his chairman, William Koopman, became widely recognized as rheumatology clinical investigators *(24)*.

At the Keystone meeting, the Immunex representatives asked Moreland which molecule he wanted to test. He picked TNFR:Fc *(24)*. Richard Pope, a rheumatologist at Northwestern University, took the IL-1-receptor. The main objectives of these phase 1 trials were safety and pharmacokinetics, but Moreland and Pope also collected efficacy data on the patients' pain and swollen joints, as well as biochemical markers of arthritis (i.e., erythrocyte sedimentation rate and C-reactive protein) *(25, 26)*.

After receiving the sepsis trial results, American Cyanamid executives wanted to cease all work on TNFR:Fc. Gillis convinced them to at least continue the ongoing trials, and the FDA agreed *(3)*. Most of that work was outsourced to contractors.

Unfortunately, the results from Northwestern only added to the gloom at Immunex. The soluble IL-1 receptor provided no benefit to Pope's arthritis patients, up to doses that produced dose-limiting toxicity *(26)*.

Moreland's TNFR:Fc trial gave "a glimmer of hope" *(24)*. But Immunex needed more than a "glimmer" of RA data to attract a pharmaceutical buyer. Gillis explained, "We had the guts to go ahead" *(3)*. They used their remaining clinical supplies of TNFR:Fc to conduct a phase 2 trial *(22)*.

Consuelo Blosch, the Immunex clinician now in charge of the RA program, called Moreland and asked some very specific questions *(24)*. Over the phone, they reviewed the results of the phase 1 trial: First and foremost, the drug was safe. The only significant finding was that some patients experienced an injection-site reaction, but that was mild and manageable. Second, the patients had exhibited an overall 45% clinical improvement, and TNFR:Fc decreased the patients' C-reactive protein levels. When drug treatment stopped, these effects reversed *(25)*.

In that half-hour phone call, Moreland and Blosch designed the phase 2 clinical trial, which would include three dose levels of TNFR:Fc *(24)*. At Immunex, Blosch finalized the randomized, double-blind, placebo-controlled clinical protocol *(27)*. Within a few days, Moreland received it—the fastest he had ever initiated a new clinical trial *(24)*.


Ann Dugan

Ann Dugan

But Ann Dugan, the sole Immunex clinical associate assigned to the study, had difficulty persuading other rheumatologists to participate *(22)*. The positive data on TNFR:Fc were slim, biologic drugs from other companies had performed poorly, and rheumatologists, in general, were reluctant to use an injectable biologic drug. "Most of the published rheumatologists wouldn't even return my calls" *(22)*.

Aside from the trial's co-leads (Moreland and Scott Baumgartner at the Physician's Clinic of Spokane), most of the investigators whom Dugan successfully persuaded "just wanted to be on the cutting edge of research for their patients" *(22)*. Moreland and Blosch specifically set criteria that would attract patients: "They were the worst of the worst" *(24)*. Still, Dugan constantly traveled to the clinical sites, urging reluctant investigators to enroll patients in the trial *(22)*.

I Feel So Good

One of those patients was Elizabeth Petersen in Chicago *(1)*. She had been diagnosed with rheumatoid arthritis at the age of 29 and was told there was no cure. Her joints were so tender she avoided walking

and shaking hands. Sometimes, her attacks eased, only "because it's hurting more someplace else" *(1)*.

Her doctors first prescribed vitamin B_{12} injections to alleviate the anemia that accompanied her arthritis. Then, she tried a variety of NSAIDs (nonsteroidal anti-inflammatory drugs). Some of them provided no relief. Others made her violently ill. She took gold injections until she tasted metal, then prednisone, which made her moon-faced and depressed, and finally cortisone, which contributed to bone loss *(1)*. When her "preexisting condition" resulted in a loss of insurance coverage, she resorted to home remedies: aspirin, vitamins, exercise, and "healthy" foods.

In 1994, Elizabeth enrolled in Immunex's phase 2 trial, and by chance she landed in the group receiving the highest drug dose. The only side effect she experienced was an injection-site reaction, consisting of "a minor itch that lasted about 5 minutes" *(1)*. Soon, she found herself skipping down the alley while walking her dog. "I couldn't stop grinning…I think I was smiling in my sleep. I felt so good" *(1)*.

After 12 weeks, Elizabeth and the other patients stopped treatment and were monitored until their symptoms returned to pre-treatment levels *(28)*. Elizabeth's symptoms returned slowly. She was then offered methotrexate but feared its side effects *(1)*.

In a way, the drug worked too well.

Most patients in the high-dose group, like Elizabeth, experienced noticeable symptom relief after the first few doses. In a way, the drug worked too well. Critics later challenged the study design, claiming it really wasn't blinded, because patients improved so much they knew they were not in the placebo group *(24, 27)*. "There was no subtlety in the response" *(24)*. Some patients asked to meet Dugan to say thank you—but for confidentiality reasons, she could not see them *(6, 22)*.

Blinded or not, the endpoints chosen by Blosch were definitive and withstood all criticism *(27)*. The primary endpoint was ACR 20, a quantitative assessment of clinical function. The American College of Rheumatology (ACR) had just published this tool and encouraged its use in RA clinical trials *(29)*.The scheme had been validated using data from previous methotrexate clinical trials, but it had not yet been used to evaluate experimental drugs.

The ACR said that drug efficacy could be claimed if a patient experienced a 20% reduction in tender and swollen joint counts and a 20% improvement in 3 of 5 other "core" measures: patient and physician global assessments, pain, disability, and an acute-phase reactant biomarker such as C-reactive protein *(29)*.

By the end of 1994, Gillis, Henney, and Duzan had all left Immunex for other opportunities, but the RA trial continued. With the rest of the company assigned to other projects, Dugan almost single-handedly kept the phase 2 trial on track. "I knew the clinical details of every patient on that study" *(22)*. She ensured that the sites accurately recorded the patients' data, and then statisticians at Statprobe, Immunex's contractor in Michigan, conducted the final analysis – all according to standard procedures *(5, 22)*.

The Turning Point

Abbe Rubin, Immunex's head of statistics and data management, still remembers the day in 1995 when the Statprobe statistician called to report the TNFR:Fc results *(5)*. Three-fourths of the patients in the high-dose group achieved ACR 20 improvement *(28)*. In fact, the clinical improvement was so great that the Immunex team added another level, ACR 50, which represented a 50% improvement in the ACR-defined criteria. "We created that endpoint. No one had ever seen this level of efficacy before" *(6)*.

Remicade had been reported to improve RA symptoms, but the mouse-human chimeric molecule also induced antibodies that attenuated the drug's effect and produced an allergic response in some patients. None of the TNFR:Fc-treated patients generated detectable antibodies *(25, 28)*. This suggested that TNFR:Fc might actually be better than Remicade in RA.

Rubin gave the results to Ann Hayes, Immunex's Senior Vice President of Medical Development, and Hayes rushed the news to Immunex's senior management *(5)*. "Everything changed overnight" *(27)*. The company decided to keep TNFR:Fc rather than sell its ownership rights, and resources were shifted to aggressively continue the RA clinical trials for fast-track approval *(5, 19, 22, 27)*.

After completing her experimental treatment, Elizabeth learned that the drug—previously known by its code, TNFR:Fc—was called etanercept (Enbrel®), and she wanted to continue taking it *(1)*. Fortunately, she would not have long to wait.

Enbrel Strategy

Coming as it did on the heels of the failures with PIXY 321 and the sepsis trial, the RA results greatly boosted morale *(6)*. To manage the rapidly expanding team, Leslie Garrison was designated the Enbrel project leader. She joined Immunex in 1989 and had been involved with multiple clinical development projects, including GM-CSF *(6)*.

Unfortunately, the Enbrel team faced a major hurdle. Because Immunex had planned to hand off Enbrel to another company, the drug inventory was depleted. Clinical supplies for the phase 1 and 2 trials had been produced in Immunex's Seattle laboratories *(22)*. The ambitious phase 3 program required larger batches, and scaling up production at the company's manufacturing plant in Bothell, WA, was not trivial. It delayed the trials by many months *(22)*.

Although frustrating, Garrison and the team used this time wisely. Constantly mindful of the patients, they established cutting-edge efficacy endpoints and defined detailed categories for all adverse events *(6, 27)*. "We never lost sight of who we were working for" *(27)*. They added ACR 70 to the ACR 20 and 50 endpoints—an unprecedented level of symptom relief, but one that Enbrel achieved *(6)*.

In addition, Barbara Finck, a rheumatologist who joined Immunex in 1994, implemented a definitive set of radiographic endpoints based on the Sharp score. First proposed in the 1970s by John Sharp, the Sharp score quantified the erosions and joint space narrowing seen in X-ray films *(30)*. Previous clinical trials had followed radiographic progression of RA using the Sharp score, but Immunex took it a step further *(4)*.

Finck closely collaborated with Sharp, who was retired but coincidently lived in the Seattle area, to adapt his method for digital reading machines *(4)*. They selected experienced radiologists as their "readers," and Sharp personally trained them to read and score the digital films *(4, 31)*. Each of the readers was then given a remote X-ray station, so that they could read the films at home *(4)*.

Immunex engaged BioImaging, a vendor specializing in digital imaging, to collect, digitize, blind, randomize, distribute, and archive the X-ray films. RA radiographs had never before been managed at this level of detail.

Immunex also kept the FDA informed of these procedures, and the agency was fully engaged in the method development. The Enbrel submission was the first time that the FDA reviewers received indexed digital RA films, which greatly facilitated their data review *(4, 6)*. Subsequently, this procedure became the standard for assessing disease progression in RA drug trials *(6, 31)*.

Safety First

Critics had good reason to raise safety concerns about every biologic drug that acted on the immune system. Genentech's lenercept (a fusion protein that combined the p55 soluble TNF receptor with IgG1) was only transiently effective in RA patients because of the rapid appearance of anti-lenercept antibodies *(32)*.

In addition to producing anti-Remicade antibodies and inducing an allergic hypersensitivity response, Remicade could increase infection susceptibility and unmask latent infections like tuberculosis *(12)*.

Anticipating such questions about Enbrel's safety, the Immunex team diligently documented the exact type and severity of each injection-site reaction and allergic response (supplemented with photos), as well as infections and other side effects *(6, 33)*. They also collected comprehensive antibody data *(6, 23)*.

No Days Lost

The phase 3 trials of Enbrel alone and in combination with methotrexate completed enrollment very quickly because now physicians wanted to participate, and RA patients rushed to sign up *(5, 19, 22)*. Initially, though, drug supplies were very limited. Dugan kept track of every vial, juggling shipments to match enrollment *(6, 22)*. Excitement at Immunex ran high, where the team, pioneers in adopting electronic data capture, followed the trials' progress almost in real time. "We didn't waste any time" *(6)*.

At first, patients came to the clinic twice a week for their injections. Later, nurses at the clinical sites trained the patients to deliver their own subcutaneous injections and gave them a bag containing ice and vials of the prepared drug solution *(19)*. Patients willingly complied with the detailed written instructions for refrigerating the vials and injecting themselves because Enbrel worked.

The phase 3 trials confirmed the earlier findings. Patients experienced rapid and sustained symptom relief. An injection-site reaction was the most common side effect, but in most patients, it was mild, infrequent, and resolved quickly. Auto-antibodies were found in a few serum samples, but none of the patients

developed immunogenicity, signs of autoimmune disease, or loss of efficacy *(23)*.

To address questions about Enbrel's long-term safety, Immunex launched an open label safety trial *(34)*. When patients completed their 6-month blinded trial, they were invited to continue treatment in the open label trial, and most patients did. Patients who had participated in the phase 2 trial were also invited to enroll, and Elizabeth signed up *(1)*.

Don't Forget the Kids

As soon as drug supplies became available, the team began staging expansion of Enbrel's therapeutic indications. In parallel with the RA trials, they conducted clinical trials for psoriatic arthritis, plaque psoriasis, ankylosing spondylitis, Crohn's disease, and juvenile rheumatoid arthritis.

Rheumatoid arthritis can affect children as young as 1 year old, and it is devastating. Inflamed joints can accelerate bone growth, leading to differences in leg length during childhood development, and consequently, to significant long-term disability. The standard of care for these children had been NSAIDs and low dose methotrexate, but they needed something better *(4)*.

The FDA encouraged all drug companies to collect pediatric data in their development programs if the medical condition affected children as well as adults. But pediatric clinical trials are quite challenging for both ethical and feasibility reasons, and most companies delayed – or even avoided – this work.

As a rheumatologist who had seen juvenile RA firsthand, Barbara Finck became a strong advocate within Immunex to start the pediatric trials earlier rather than later. She succeeded in getting corporate support. It was a risky and bold management decision, given the company's already heavy investment in Enbrel, and considering that a bad outcome in a pediatric trial could derail the entire development program *(4)*.

Daniel Lovell and Edward Giannini at the Children's Hospital Medical Center in Cincinnati spearheaded the pediatric trial *(35)*. The main objective was to collect pharmacokinetic data. Because the children were too small to draw multiple blood samples, the study employed a population-PK design *(4, 35)*.

The Cincinnati group saw some of the most severe cases of juvenile RA, and in the first segment of the trial, all children received Enbrel *(4)*. Then, in the second and blinded segment, some children received placebo injections while the rest continued taking Enbrel. If children in the placebo group experienced a flare response, they resumed Enbrel treatment *(4, 35)*. This innovative study design had never been previously used for pediatric pharmacokinetics trials, but it is now standard *(4)*.

The Path to Approval

Enbrel faced stiff competition from other biologic drug candidates. In addition to Remicade, Abbott Laboratories was proceeding with adalimumab (Humira®), a fully humanized TNF-α antibody. The Immunex team presented Enbrel data at every rheumatology-related venue – large and small – and published a steady stream of clinical study reports *(6, 36)*.

But by far, the most important document the Enbrel team prepared was the Biologics License Application (BLA), requesting market approval from the FDA. The most important presentation they made was to the FDA's Arthritis Advisory Committee.

Immunex submitted the BLA to the FDA on May 7, 1998 *(33)*. As part of the BLA review and approval process, the FDA requested input and recommendations from its Arthritis Advisory Committee, an independent panel of experts. It was customary for the Advisory Committee to invite the sponsoring company to present summarized data and respond to their questions.

For the Enbrel team, this was a critical meeting, and they did their homework. Many of them had no experience with the regulatory process, though some, including Leslie Garrison, had worked toward GM-CSF's approval. They attended Advisory Committee meetings where other products were discussed, including Centacor's Remicade presentation in May 1998 *(5, 6)*.

Through the summer of 1998, they diligently prepared *(5, 6, 36)*. Ann Hayes and Garrison would make the formal slide presentation and field the Advisory Committee's questions. Many of the Advisory Committee members were rheumatologists who were familiar with Remicade and knew its problems. Their questions would reflect that experience, as well as concerns about investigational biologic drugs in general.

In a series of practice sessions, the Enbrel team and their clinical consultants brainstormed every possible contingency. Sometimes, that required additional

statistical analyses and new slides *(5)*. In addition to a concise presentation slide deck, they compiled a mind-boggling set of 1,000 backup slides *(36)*. Each one addressed a single, clearly stated and visually crisp result or data summary *(6)*.

Then, they "drilled like crazy" *(36)*. It was a close and supportive group, and they turned it into a kind of game, practicing quick retrieval of the right slide, as Hayes or Garrison answered each question *(19, 36)*. After many hours of rehearsals over weeks of fine-tuning, Hayes and Garrison knew every nuance of the Enbrel data *(19)*. They could request from memory the number of the specific slide to accompany their response to any question *(1)*.

After many hours of rehearsals over weeks of fine-tuning, Hayes and Garrison knew every nuance of the Enbrel data.

D-Day: The Meeting

On Wednesday, September 16, 1998, the Immunex contingent, along with four of their clinical consultants, arrived at the Holiday Inn in Gaithersburg for the FDA's Arthritis Advisory Committee meeting. In Seattle, the rest of Immunex anxiously watched the proceedings via a live-streamed video link *(6, 22)*. They all had a lot riding on the Committee's recommendations.

The morning session ran like clockwork. Hayes and Garrison made their presentations and responded to the Advisory Committee's questions, just as they had rehearsed. In the afternoon, the Advisory Committee discussed six questions posed by the FDA reviewers regarding Enbrel's efficacy, safety, and precautions for use *(1)*. It quickly became clear that some Advisory Committee members were not satisfied with the size of the safety database.

Side effects from Enbrel were few and infrequent, but assessment of safety was confounded by a smaller-than-traditional placebo-control group. As often happens, many placebo patients voluntarily withdrew from the clinical trials because they were not improving and wanted to explore other treatment options *(1)*. Consequently, Immunex compared Enbrel-treated patients to the long-term natural course of RA in an appropriately matched demographic group—data that had been collected by the Mayo Clinic *(6)*.

Ironically, the Advisory Committee's greatest concerns were the lack of serious side effects and the absence of serum antibodies. Had Enbrel been given to enough patients and had it been given long enough to assess the drug's safety? Jeffrey Seigel, the FDA's medical reviewer, said, "Because TNF plays a role in host defenses, blocking TNF could theoretically have an effect on the number of infections and the severity of infections" *(1)*. Immunex's ongoing long-term safety trial would eventually either refute or confirm this theory, but for now, all they could say for sure was: so far, so good *(34)*.

Elizabeth Petersen injected some humor into the proceedings. She had been taking Enbrel for 3 years, and "the only side effect that I've noticed is that I seem to be deeply in love with the entire Immunex Corporation, especially the scientists" *(1)*.

After a long discussion that afternoon, the Advisory Committee chairman concluded by saying, "Sometimes we get so bogged down in the safety issues that we forget to say how enthusiastic we are, and I think everyone on the committee is extremely enthusiastic about seeing Enbrel being developed and becoming available to our patients with rheumatoid arthritis"*(1)*.

The Advisory Committee unanimously recommended Enbrel approval for patients with moderate to severe rheumatoid arthritis who had failed DMARDs, and a majority recommended its use both alone and in combination with methotrexate *(1)*.

The Immunex team was elated. Later that day, the company's senior vice president invited everyone to her Gaithersburg hotel room. Champagne corks popped. Emotions ran high *(5, 6)*. Enbrel was finally nearing the finish line.

Approval and Beyond

On November 2, 1998, Enbrel became the first biologic drug approved by the FDA for rheumatoid arthritis in adults. Six months later, approval was expanded to include juvenile rheumatoid arthritis. FDA officials required only one contraindication in the label: "Enbrel should not be given to patients with sepsis" *(37)*.

Enbrel represented a groundbreaking achievement *(31)*. From the first RA trials onward, it had transformed patients' lives *(27)*. But Enbrel's unprecedented efficacy was a double-edged sword.

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The Immunex Board had considered building a large Enbrel manufacturing plant, but remembering the GM-CSF experience, they decided this budget-busting capital investment was unwise *(3)*. After Enbrel's approval, patients demanded the drug, and once they started treatment, they didn't stop. The Bothell, WA, manufacturing plant's capacity was insufficient. Immunex and American Home Products scrambled to set up manufacturing contracts with plants in Germany, Ireland, and Rhode Island *(3, 38)*. It took several years for those facilities to become operational and satisfy the overwhelming demand.

Champagne corks popped. Emotions ran high. Enbrel was finally nearing the finish line.

In the meantime, Immunex's top priority was to ensure that patients who were already taking Enbrel could continue uninterrupted treatment *(36)*. Those patients were issued patient ID numbers, which were required to fill their prescriptions. As production increased, additional patients received ID numbers *(36)*.

After Enbrel, Remicade and Humira were also approved for rheumatoid arthritis. However, they are co-administered with methotrexate to decrease and delay the production of anti-drug antibodies and an allergic response *(12)*. Anti-drug antibodies are less problematic with Enbrel, but methotrexate boosts its efficacy, compared to single-drug treatment *(39)*.

Enbrel, followed by Remicade and Humira, was also approved for psoriatic arthritis, plaque psoriasis, and ankylosing spondylitis. But further clinical trials revealed differences between them. Enbrel is approved for juvenile RA, whereas Remicade and Humira are not. Remicade and Humira are effective in treating Crohn's disease, whereas Enbrel is not *(40, 41)*. All three drugs now rank in the top 10 for worldwide drug sales.

TNF inhibitors revolutionized the treatment of rheumatoid arthritis. More than relieving symptoms, they sent the disease into remission *(24)*. And some of the adults and children who took their first dose as experimental subjects 20 years ago are still taking Enbrel, with no loss of efficacy *(4, 36)*.

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