BIOGRAPHICAL MEMOIRS

Hilary Koprowski

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A Biographical Memoir by Charles E. Rupprecht and Stanley A. Plotkin

Figure 1 Hilary Koprowski. *Photo courtesy of Maria Colelli, The Wistar Institute.*

Hilary Koprowski (also known affectionally as HK by those who knew and worked with him), was a notable author, musician, poet, physician, and scientist—a twentieth-century iconoclast whose global contributions are still having impacts a decade after his death. He and his family emigrated from Europe before the worst ravages of the war, initiating a global journey of discovery lasting over some nine decades. Over the course of his career, numerous chapters and books would appear, including more than 820 papers in the peer-reviewed literature.

Born in Warsaw, Poland, his parentage was half-Jewish. Early in his life there, he became interested both in science and in music. Although he never explored the latter professionally, HK did train at the Academia di Santa Cecilia in Rome and remained a pianist of professional quality for the rest of his life. He would later joke that he became a physician because he was afraid he might starve as a musician. He escaped from Poland to Italy with his wife, Irena (also a physician) early during World War II. After a few detours from Italy to France and England, the family reunited in Brazil. While stationed at the Rockefeller Foundation's International Health Division in Rio de Janeiro during the 1940s, HK and his distinguished supervisor, Edwin Lennette, conducted research on several pathogens, including yellow fever, Venezuelan equine encephalomyelitis, and Herpes simplex viruses, among others. Eventually, HK was granted a visa for working in the United States, where he would move to a position at American Cyanamid, Lederle Laboratories, in Pearl River, New York, in 1945. Although HK may have been interested at that time by a major zoonotic virus, at least in theory, an unintended stop en route to New York via the Caribbean helped to cement a fascination with rabies by serendipity. Notably, Joseph Lennox Donation Pawan was a government microbiologist in Trinidad and Tobago. A fatal outbreak in cattle, and later in humans, was recorded on the island from 1925 to 1937. Initially believed to be botulism, Pawan successfully ascribed the deaths to rabies and identified vampire bats as the vector. His epidemiological studies were seminal in demonstrating the critical role of bats as reservoirs in rabies virus (RABV) perpetuation. During HK's brief layover

in Trinidad, he went to visit Pawan at his laboratory. For several hours, the Trinidadian scientist graciously regaled him with tales of his work on rabies and the localized epidemic that killed nearly forty people. This chance encounter helped focus HK on rabies, which, together with polio and other infectious diseases, would become a lifelong passion in his new environment.

Rabies is one of the oldest pathogens historically, an acute, progressive encephalitis caused by a lyssavirus, with the highest case fatality of any infectious disease, which excited HK's imagination. During the early twentieth century, rabies prevention in animals and humans was still guided by Louis Pasteur's important work from the 1880s. Rabies vaccines were rather crude, neural tissue products. Multiple passages through animal brains would attenuate RABV to a degree, with partial inactivation by drying and later chemical treatment. Given their rather low potency, rabies vaccines needed to be administered quickly after infection from an animal bite and over multiple weeks, requiring between fourteen and twenty-one doses inoculated intracutaneously across the abdomen. One major challenge that HK faced towards improvement of a rabies vaccine during the administration of human postexposure prophylaxis (PEP) was how to develop safer, more effective biologics for a disease of nature that not many scientists cared about because the primary burden was borne by lower- and middle-income countries.

While still in Brazil, Koprowski's work on yellow fever had introduced him to Max Theiler's approach to viral attenuation for vaccine development. In brief, Theiler found that propagating yellow fever virus in an unnatural host—such as in chicken embryos—resulted in a virus adapted to that host, reducing the capacity to cause disease. Later, HK would acknowledge that Theiler provided him conceptually with an encouraging model for attenuating both poliovirus and RABV. Such applications led to one of his first publications on rabies, inaugurated not at Rockefeller, but later while in the Pearl River locale.[1] Over the next decade, a related series of research papers demonstrated the attenuation, propagation, immunogenicity, efficacy, and safety of using such an avian system for RABV production. The Flury low-egg passage (LEP) and high-egg passage (HEP) RABV strains would result in vaccines useful for both human and veterinary medicine applications. The HEP modified-live RABV strain was used successfully for many years to protect domestic animals, including livestock (a major victim of vampire bat depredations throughout Latin America) and dogs (the major global reservoir). Vaccination with attenuated RABV was a much more effective method for the mass immunization of dog populations than any of the other biologics that were available by the mid-twentieth century. Immunity conferred by vaccination with modified-live RABV, as exemplified by the Flury strain, lasted at least several years in vaccinated dogs. In addition to demonstration of the safety and effectiveness of the HEP vaccine (which was even used in humans for a time), LEP became the seed rabies virus strain still used in the production of inactivated purified chick embryo cell vaccine for human prophylaxis.

In addition to focusing on RABV vaccines during the 1940s, HK also reinvigorated the importance of passive immunization in PEP and the experimental use of Syrian hamsters as an animal model.[2] Hamsters are a useful species that could be manipulated easily in the laboratory. Many of the variables of concern, such as dose, route, and timing, could be replicated in this rodent, which was a surrogate for humans. Passive immunization was important as a bridge to active immunity against RABV. After vaccination in humans, the induction of virus-neutralizing antibodies required at least two weeks to appear. Clearly, vaccines alone were therefore insufficient to prevent rabies cases with short incubation periods, such as animal bites to the face and head. When infiltrated during PEP, however, hyperimmune anti-RABV serum was able to neutralize virions deposited in the wounds immediately. These promising results produced in laboratory animals and in non-exposed humans required a real-life test. The combined concept of both passive and active immunization was demonstrated in the field during a 1954 World Health Organization (WHO) clinical trial in Iran, when eleven of twelve villagers given serum and vaccine survived after being bitten severely by a rabid wolf, whereas three of five who received vaccine only succumbed. Koprowski's analysis of the data from the vaccinated individuals provided critical laboratory support to the outcome of the trial.

In 1957, Koprowski (now a valued member of the WHO Expert Panel on Rabies) left Pearl River to become director of the Wistar Institute of Anatomy and Biology in Philadelphia, Pennsylvania. Over the next three decades, HK attracted an ensemble cast of global collaborators to focus attention on viral investigations, including Esteban Celis, H Fred Clark, Bernhard Dietzschold, Peter Doherty, Gundi Ertl, Mario Fernandes, Klaus Hummeler, Martin Kaplan, Rod Macfarlan, H. Dieter Schlumberger, Frantisek Sokol, William Wunner, Tadeusz Wiktor, and many others. The RABV work with Tad Wiktor (who was also Polish) proved especially fruitful in the years at Wistar. For example, rather than relying upon animal neural tissue for viral passage, the adaptation of RABV to WI-38 cells provided the groundwork for a major improvement in vaccine safety and efficacy.[3] After extensive preclinical and clinical trials, a human diploid cell vaccine (HDCV) was licensed in the United States during 1980. The vaccine became the global gold standard biologic for human RABV preexposure and PEP, without the risk of the severe neurologic adverse events associated with the prior generation of

biologics produced in animal brains. Another opportunity in Iran during 1976 also showed promise for a combined approach in PEP. Forty-five severely exposed persons were given RABV anti-serum and HDCV, and all were afforded complete protection.[4] This melding of basic academic viral research and applied clinical trials was a successful pathway to products licensed by industry. That strategy was repeated by the Wistar team year after year, as shown by an unprecedented number of research grants on rabies and RABV antigens supported by the U.S. National Institutes of Health.

In 1976, Cesar Milstein, from the University of Cambridge, supplied myeloma cells to HK for producing monoclonal antibodies (MAbs). Once again, the laboratory collaboration with Tad Wiktor would result in another primacy—this time related to hybridomas producing antibodies against RABV and other lyssaviruses.[5] The generation of murine MAbs against RABV structural proteins allowed the differentiation of distinct species of lyssaviruses around the world. Previously, these lyssaviruses were all thought to be fairly similar and only differentiated serologically. In addition, the use of MAb panels provided the identification of antigenic variants and epidemiological insights to better understand emergence and association of these pathogens with specific host species. Such antigenic typing is still used as an aid in the identification of RABV associated with dogs and wildlife and in recognition of the potential source of viral exposure of human and domestic animal cases in Latin America. In addition to their use in diagnostics and viral characterization, MAbs would also provide a means of isolation of apathogenic variants of RABV for use as a second generation of vaccines for the oral vaccination of free-ranging dogs and wildlife. Moreover, some four decades after their genesis, neutralizing human MAbs are now available for PEP as an effective alternative to expensive human rabies immune globulin.

One of the last major RABV accomplishments by Koprowski and the Wistar Institute team occurred during the 1980s, when one of the authors (CER) joined the laboratory. After the eradication of smallpox, alternative uses of vaccinia virus were pursued for the expression of foreign genes. Once again, the unique partnership between Wiktor and Koprowski would make history. The development of a vaccinia-rabies glycoprotein (V-RG) recombinant virus created novel avenues for wildlife vaccination, without a risk of RABV acquisition.[6] To date, hundreds of millions of V-RG doses in attractive vaccine-laden baits have been distributed by aircraft within Africa, Eurasia, and North America for the prevention and control of rabies among coyotes, foxes, jackals, raccoons, and raccoon dogs.

From the 1990s into the new century, Koprowski would continue his focus on rabies while at Philadelphia's Thomas Jefferson University (TJU), as the head of the Center for Neurovirology (now the Center for Neurovirology and Gene Editing). Additional research into pathobiology, experimental therapeutics, and plant biopharming of vaccines and MAbs rounded out his introspection of the "incurable wound" until his retirement from TJU as a pioneer of what is now encompassed in the One Health concept, clearly ahead of his time.

As recounted above, Koprowski was responsible for improved vaccines that now prevent rabies in exposed humans and animals throughout the world, as well as many other related accomplishments in the lyssavirus field. Although not generally acknowledged, however, he was also responsible for the launch of efforts to develop live, attenuated oral polio vaccines, a success for which he is presently little known. Before Wistar, while he was at Lederle, Koprowski became interested in polio, which at the time was a disease raging throughout the world with no effective vaccine in sight.

Polio is caused by three filtrable viruses that multiply in the intestinal tract and spread from there to the central nervous system. Early attempts at polio vaccine development had failed and indeed had caused rather than prevented polio. Koprowski decided to attempt viral attenuation through adaptation to cotton rats. He isolated what was later characterized as a type 2 polio virus and adapted it to growth in rodents. This virus, called TN, had reduced neuropathogenicity in monkeys but grew in the intestinal tract of humans and induced serum antibodies against type 2 poliovirus. HK followed this work by studies with a type 1 virus, which he constructed by mixing two type 1 strains grown together in tissue culture. This strain was shown to not cause polio after intracerebral injection in monkeys of the original mixture as well of virus excreted by those vaccinated. Later in the 1950s, Koprowski obtained a type 3 virus from epidemiologic studies by John Fox. Thus, he had candidate viruses from each of the three poliovirus serotypes by 1957.

The situation in 1950 when Koprowski had started his studies on polio vaccine was that polio was endemic and often epidemic in the United States, and no vaccine was available to prevent the disease, despite prior attempts. Koprowski's idea was to attenuate the three strains of poliovirus, despite the evident risk that attenuated viruses could still cause paralysis. Key to Koprowski's efforts was to collaborate with George Jervis, a physician in charge of an institution for children with intellectual disabilities. Obviously, such a collaboration required considerable reflection and discussion with authorities at the institution and at Lederle laboratories.

In HK's first human trial, completed in 1950, twenty children received the TN polio type 2 strain.[7] But this work was little known until he announced it in 1951 at a meeting sponsored by the March of Dimes (National Foundation for Infantile Paralysis). Attenuation was accomplished

by serial passage in mouse brain tissue and also by passage in cell culture.[8] The resultant viruses were attenuated when injected into the brains of monkeys. Replication in the intestinal tracts of humans after oral administration was necessary for induction of polio antibodies. The type 2 virus had been attenuated by passage in cotton rats, originally discovered by Armstrong to be susceptible to polio virus.

Following the attenuation of a type 2 polio virus, Koprowski turned his attention to the more virulent type 1 virus. He chose to mix the Sickle and Mahoney strains and to passage them in mice twenty-two times until they caused no paralysis when introduced into monkeys by intracerebral injection. Three individuals given the virus preparation orally did not develop clinical signs, but all developed antibodies against type 1 poliovirus.[9] The presentation of the first human trial with the type 2 virus was received with consternation at the meeting, including by Albert Sabin.

The type 3 virus ultimately used by Koprowski in his studies was a strain obtained by John Fox in his studies of inapparent poliovirus infection in humans. Thus, armed with attenuated strains of all three serotypes of polio, Koprowski launched studies of vaccination in his native Poland and in what was then the Belgian Congo. The Congo trials were made possible through collaboration with Ghislain Courtois, a Belgian physician who worked in the Congo.[10]

By 1957, Koprowski also had two serious competitors. The first was Albert Sabin, who had given up his skepticism and had isolated and attenuated by tissue culture passage each of the three poliovirus serotypes. The second competitor was Herald Cox back at Lederle laboratories. Both Sabin and Cox had attenuated the three polioviruses by passage in cell culture. By the early 1960s, all three investigators were performing clinical trials. The outcome was straightforward. The Lederle-Cox strains caused some cases of polio in Germany, whereas Sabin's strains appeared to be harmless. Although later rare cases of polio caused by Sabin strains were observed, the results of monkey neurovirulence tests of those strains satisfied the American licensing authorities. Unfortunately, Koprowski did not have enough data on his type 2 and type 3 strains. Thus, the general verdict was in favor of the Sabin strains, despite the apparent successful use of Koprowski's vaccine in Poland and the Belgian Congo. Although Koprowski's concept of an attenuated virus vaccine against poliovirus did succeed, it was not his strains that were eventually used worldwide. Nevertheless, millions of individuals received Koprowski's polio vaccine strains in the Belgian Congo, Poland, and Switzerland.

Koprowski's type 1 strain, called CHAT, did eventually become a part of history when a British journalist named Edward Hooper conceived the idea that HIV had been introduced into humans via the CHAT vaccine.[11] His accusation was that pools of the CHAT vaccine had been manufactured in a laboratory in Stanleyville using chimpanzee kidney cells contaminated with a virus related to HIV, which was then given to thousands of people in the Belgian Congo. Furthermore, Hooper alleged that the virus adapted itself to humans and began spreading in the Congo. Hooper wrote a book called *The River* that was based on that accusation and that attracted a great deal of attention.

As frequently happens, this attractive idea was torpedoed by the facts, but a great deal of time and effort was necessary to accomplish that, as follows:

1. The head of the Stanleyville laboratory categorically denied that the vaccine was made in his laboratory.
2. Analysis of CHAT virus pools found no contaminants.
3. Genetic analysis of HIV showed that adaptation of simian SIV strains to humans occurred no later than the 1930s.
4. Additional analysis showed that HIV probably originated in Cameroon, the result of SIV passing from chimpanzees to humans.
5. The chimpanzee cells available in the Stanleyville lab came from animals free from SIV infection.

The above points were thoroughly discussed at a meeting of the Royal Society in 2001.[12] It had taken much effort by many people to disprove Hooper's accusation, but eventually it went down the tubes with other false hypotheses. Hilary was largely unperturbed by this affair, and in any case by then his research had left polio and passed on to rabies, multiple sclerosis, MAbs, and many other topics. Notably, he was the recipient of numerous honors over his career, including the Belgian Order of the Lion, Finland's Order of the Lion, the French Order of Merit and Legion of Honour, the Order of Merit of the Republic of Poland, and the Philadelphia Award. Clearly, HK was an exceptional individual, leader, and scientist, whom it was a pleasure to know and work with throughout the years.

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