Commentary by James J. Tuite, III

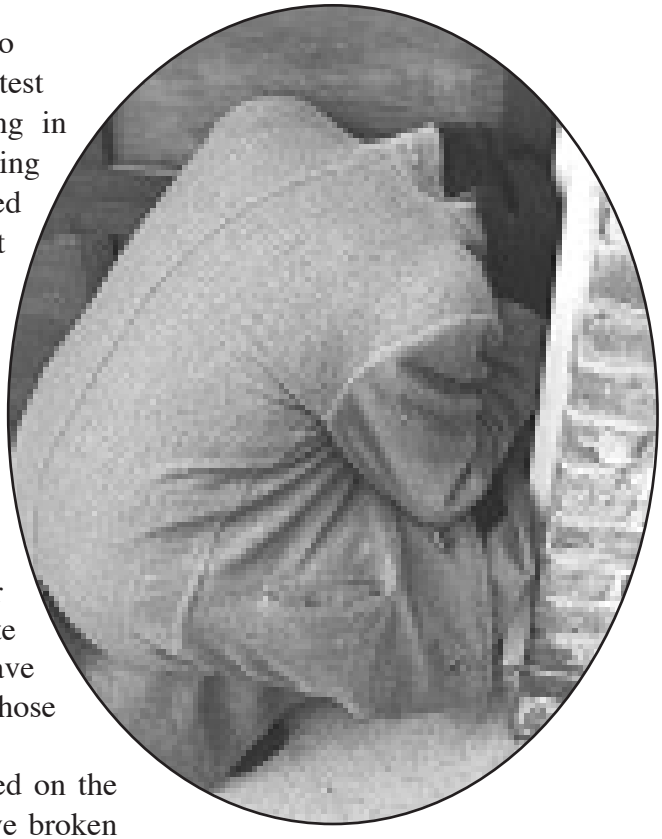
Will The Poliovirus Eradication Program Rid the World of Childhood Paralysis?

With So Little Poliovirus Detected Around the World, What Is Causing Today's Outbreaks of Acute Flaccid Paralysis?

By Neenyah Ostrom
April 20, 2001

Every child of the early '50s surely remembers the polio panics that swept the nation, invariably during the hottest days of summer, closing public pools and resulting in doctor visits at the first sign of a stiff neck or leg prone to falling asleep. My memory of the terror induced by the whispered word "polio" resides in a spot in the pit of my stomach just distal to the one recalling the Cold War era duck-and-cover drills we practiced in grammar school.

In retrospect, it seems darkly hilarious that we ever believed plywood desks and plump little arms would protect school children from a nuclear attack. Our not-quite-rational fear of the poliovirus, however, endures despite World Health Organization eradication programs [www.polioeradication.org] in the corners of the developing world where poliovirus is thought to lurk. One reason for this lingering concern is the continuing prevalence of acute flaccid paralysis, polio's most crippling symptom that can leave its victims unable to control entire muscle groups, even those that allow us to breathe.



A man demonstrates one of the US government's protocols for surviving a nuclear attack: "duck-and-cover" in the doorway of a building, c. 1955.

Worldwide polio-related public health alarms sounded on the first day of 2001 when a new epidemic was reported to have broken out on the island of Hispaniola, on which Haiti and the Dominican Republic are located. David Brown reported in the *Washington Post* that a "mutant" poliovirus, derived from strains present in the oral polio vaccine, appeared to have run amok on this Caribbean island during the latter half of 2000.[3]

When the US Centers for Disease Control and Prevention (CDC) examined these cases, another mystery was revealed: Only about one-third of the paralysis cases were associated with poliovirus. The CDC identified 19 individuals in the Dominican Republic who developed acute flaccid paralysis (AFP, the hallmark symptom of poliovirus infection as well as a syndrome unto itself) between July 12 and November 18, 2000. However, poliovirus was detected in *only six of those individuals*.

The cause of the other cases of paralysis remains unknown.[25]

The mystery deepens when we examine World Health Organization (WHO) statistics on AFP and poliovirus infection in the Dominican Republic for the last several years. [http://www-nt.who.int/vaccines/polio/case.asp] Although the number of cases of AFP in the Dominican Republic from 1996 to 1999 range from 4 to 24, *not a single case of poliovirus was detected.*

If we further examine other WHO statistics on poliovirus-associated AFP and those in which the virus is not detected, a striking fact becomes clear: *Most acute flaccid paralysis diagnosed around the world today is NOT associated with poliovirus.*

This fact raises new, disturbing questions, including whether there ever was an epidemic of poliovirus infection in the United States and Canada. There was a greatly increased prevalence of AFP, to be sure, during which many children (and some adults) tragically were paralyzed or died. Since many of those cases showed all the hallmarks of a typical poliovirus infection—fever, stiff neck and back, severe headache, muscle pain, sore throat and, in severe cases, paralysis—and occurred in clusters, they were assumed to be caused by the easily-transmitted poliovirus.

But were they? If not, what *is* the cause of so much misery today in areas of the world least-equipped to be able to deal with it? Is it correct to assume that poliovirus causes most cases of paralysis?

The Search for the Transmissible Agent of Poliomyelitis

Poliomyelitis became an important public health concern when it first spread along the eastern seaboard of the United States, as well as in industrialized areas of Europe, in the early 1900s. Its inexplicable outbreaks were frightening to the public and medical personnel alike, as Simon Flexner and Paul A. Lewis (both of the Rockefeller Institute for Medical Research in New York) demonstrated when they wrote in the *Journal of the American Medical Association* in 1909, "The cause and mode of dissemination of the disease [poliomyelitis] are unknown; and hence there exists no intelligent means of prevention. While the severity and fatality of the disease fluctuate widely, its effects are always so disastrous as to make it of the highest medical and social importance."[14]

Just a year earlier, Austrian researchers Karl Landsteiner and Erwin Popper had made a historic breakthrough in the study of poliomyelitis. Landsteiner had a nine-year-old poliomyelitis patient

What Is Poliomyelitis?

The word "poliomyelitis" comes from two Greek words: "polio," which means gray, and "myelitis," inflammation of the spinal cord. Poliomyelitis can cripple and kill vulnerable individuals, especially children, within days. It often affects the very young, which is why it is also called "infantile paralysis." Some individuals develop only flu-like symptoms without paralysis; "aseptic meningitis" (swelling of the membranes surrounding the brain) can result. This "minor illness" of poliomyelitis (as it is called) is characterized by slight fever, malaise, headache, sore throat, and vomiting; patients usually recover completely in 24-72 hours. Non-paralytic poliomyelitis cannot be differentiated clinically from aseptic meningitis caused by other transmissible agents. Surprisingly, fewer than one in 100 cases (and possibly as few as one in 1,000 cases) of infection with poliovirus produces any obvious disease, even during outbreaks.[23,24]

The "major illness" of poliomyelitis usually develops suddenly, with fever, stiff neck and back, severe headache, and muscle pain. Major illness can progress to loss of tendon reflexes and asymmetrical weakness or paralysis. Poliomyelitis is generally diagnosed clinically by the concurrent presence of high fever and acute, asymmetrical flaccid paralysis, which develops in 2-4 days following the fever and muscle aches. Approximately 50% of people stricken with paralytic poliomyelitis remain disabled throughout their lives.[24]

The paralysis produced by poliomyelitis results from inflammation and destruction of motor neurons in the gray matter of the spinal cord and

who died on November 18, 1908, after just four days of illness. With his colleague Popper, Landsteiner created a suspension from the child's spinal cord and injected it into two monkeys, as well as a number of rabbits, guinea pigs, and mice. While the other animals were unaffected by the injections of spinal cord material, the two monkeys developed lesions in their spinal cords and brains that appeared indistinguishable from those found in humans suffering from poliomyelitis. One of the monkeys developed acute flaccid paralysis in both legs. Although Landsteiner and Popper attempted to transmit paralysis to other monkeys using the sick monkeys' nervous system tissues—which is called "passaging" of the transmissible agent—they were unsuccessful.[10,20]

The following year, Flexner and Lewis succeeded where Landsteiner and Popper had not: Flexner and Lewis reported in the *Journal of the American Medical Association* that they had successfully passaged poliomyelitis through several monkeys (i.e., from monkey to monkey). They began, like Landsteiner and Popper, by injecting diseased human spinal cord tissue into the brains of monkeys. After a monkey fell ill, a suspension of its diseased spinal cord tissue was injected into other monkeys. Flexner and Lewis's 1909 work was considered a breakthrough because the second monkey (and the third, and fourth, through at least six by the time of publication) developed poliomyelitis. Flexner and Lewis had successfully passaged the disease's transmissible component from animal to animal.[14]

But what was the passaged agent? "We failed utterly to discover bacteria, either in film preparations or in cultures, that could account for the disease," Flexner and Lewis reported. Therefore, they concluded, "…the infecting agent of epidemic poliomyelitis belongs to the class of the minute and filterable viruses that have not thus far been demonstrated with certainty under the microscope."[14]

Did Flexner and Lewis succeed in isolating poliovirus in 1909? Hindsight being 20/20, it is possible to see that early experiments attempting to create purified poliovirus preparations might well have contained other agents.

The debate over the nature of the causative agent of poliomyelitis continued. One research team speculated in 1919 that a type of bacteria, cautiously named "poliococcus," was either the culprit or a co-factor.[7] In early experiments, all kinds of biological materials—spinal cord, brain, fecal matter, even flies—were ground up and injected into monkeys to induce paralysis.[4,7,15,21,22,33] These early "virus preparations" were *known* to contain bacteria. The amount of bacteria was determined by seeding a tissue culture plate with some of the spinal cord (or fecal matter) emulsion to measure how long it took for bacterial colonies to appear. As F.B. Gordon and colleagues pointed out in a paper published in the *Journal of Infectious Diseases*,

brain. The type or degree of paralysis induced depends upon the location and extent of motor neuron destruction, and can range from minor to severe limb paralysis, to paralysis of the muscles that allow us to breathe. The iron lung was used in the 1940s and '50s to assist children who could not breathe on their own. As frightening as iron lungs look in the old photos, many children recovered completely. However, paralytic poliomyelitis is fatal in 2-10% of cases.[24]

With the exception of patients who go into respiratory failure, poliomyelitis treatment is symptomatic: non-narcotic pain killers, application of hot packs, and physical therapy.

What Is Poliovirus?

Despite the damage it causes to nerve tissue, the poliovirus has been placed in the enterovirus family of viruses that live in the gastrointestinal system. It is formed of a single strand of RNA enclosed in a protein coat that protects it from environmental attack (inactivation). Poliovirus is quite small by viral standards (22-32 nanometers). Humans are thought to be poliovirus's only host, which is why the WHO launched an eradication program. According to the CDC, the only confirmed cases of poliovirus-associated paralysis in the US since 1979 have been associated with the oral, live-virus vaccine.[24,31] In fact, the CDC now concludes that "Both laboratory surveillance for enteroviruses and surveillance for polio cases suggest that endemic circulation of indigenous wild polioviruses ceased in the United States in the 1960s."[24] Other investigators question the CDC's conclusion that wild poliovirus circulation truly "ceased" in the United States four decades ago.

"If there was no [bacterial] growth after approximately 22 hours incubation at 37 C., the specimen was considered suitable for inoculation into monkeys. This was not an actual sterility test, since growth would usually occur on longer incubation; it was rather an indication of the amount of bacterial contamination in the specimen."[15]

Early poliovirus researchers, then, knew that the "virus" they were injecting into monkeys also contained an undetermined amount of bacteria. They had no way of determining what else might be present.

While Flexner and Lewis may have been incorrect in assuming they had transmitted a purified form of "filterable virus" into their monkeys, they certainly transferred a disease-causing agent or agents from animal to animal. Although they could not actually visualize this agent, they described it in the greatest detail that they could. In doing so, which they undoubtedly meant to be a service to other researchers, they may have voiced their conclusions in ways that would haunt poliomyelitis research for decades.

At the beginning of the 20th century, as scientists began trying to understand and characterize viruses and viral diseases, many of them—including poliomyelitis researchers like Flexner and Lewis—overstated their findings.

Early poliomyelitis researchers were true scientific pioneers: Flexner, Lewis, Dalldorf, Landsteiner, Popper, Dulbecco, Sabin, Salk, and many others worked with unknown agents. They didn't understand the properties of the contaminated tissues they handled, and they didn't know how to protect themselves from the diseases those tissues might contain. Their bravery in undertaking these risks should never be underestimated, especially in our era when latex gloves, biosafety cabinets, and many other methods of protecting scientists from dangerous transmissible agents are readily available.

Nevertheless, these early 20th century researchers should not get a free pass for their lack of precision in describing experiments and their results.

For example, in 1948, Gilbert Dalldorf and Grace M. Sickles from the New York State Department of Health published a research report that illustrates some problems in virology that persist even today. Dalldorf and Sickles described an "unidentified, filterable agent" that they had "isolated" from the feces of paralyzed children.[6]

The problems become clear when Dalldorf and Sickles described *how* they "isolated" this agent:

> "Twenty per cent fecal suspensions, prepared by ether treatment and centrifugation, were inoculated intracerebrally into albino mice of the laboratory strain. Suckling mice, 3-7 days of age, became paralyzed, while mice 10-12 gm in weight did not. The isolations were repeated several times.…"[6]

Dalldorf and Sickles used the word "isolation" to describe their creation of a suspension of fecal matter—which was a vast overstatement, to put it mildly.

Dalldorf and Sickles then attempted to identify the agent. In 1948, antibodies, like viruses, could not be characterized as they now can. "Neutralizing serum"—the non-cellular portion of the blood, taken from a person or animal presumed to be infected with the agent—was used to differentiate between viral strains. This neutralizing serum probably contained antibodies against the agent.

According to Dalldorf and Sickles, neutralizing serum from paralyzed children inhibited paralysis in mice when they were injected simultaneously with it and the unidentified agent. This absence of evidence—that the mice did not develop paralysis—was interpreted to mean that the agent injected into the mouse had been successfully stopped by the neutralizing serum (i.e., the immune response generated by the sick child). There was no proof, as Dalldorf and Sickles asserted, that the neutralizing serum was reacting with and inhibiting *one specific agent*.

Dalldorf and Sickles believed they'd "isolated" a novel agent that could infect people, although they did not argue that it was responsible for producing the paralysis seen in their patients. "The patients we studied may possibly have been coincidentally infected with the new agent and classical poliomyelitis virus, although

isolations were not successful in [causing disease in] the rhesus monkey," they write.[6] *Again, they write of "isolation" when they are referring to taking a partially processed specimen (spinal tissue or feces) from a paralyzed person and injecting it into an animal to see if the diluted specimen produced paralysis*. True isolation did not take place.

Has Poliovirus Ever Really Been Isolated?

It is an article of faith in modern medicine that poliovirus has been isolated, characterized, is fully understood and on its way to extinction, thanks to aggressive vaccination/eradication programs. As the recent outbreak in the Dominican Republic illustrates, however, we may be further from eradicating poliomyelitis than we are generally led to believe.

Furthermore, while the agent identified as poliovirus was certainly cultured in the late 1940s, do we know for sure that it was truly isolated, i.e., grown in a pure form containing no contaminants? We now know that adventitious ("passenger") viruses like SV40 are common in the monkey tissues that early poliovirus researchers used for cell cultures. While these agents apparently cause no harm to the monkeys, their long-term effects on humans remain to be determined. [http://www.chronicillnet.org/online/bensweet.html#anchor714274]

Some 90 years after Landsteiner and Popper's report of successful transfer of poliomyelitis to monkeys, Dr. Wolfgang K. Joklik reviewed the great leaps forward during the 20th century by its defining discipline, virology.[19] The occasion was the concurrent 100th anniversaries of the American Society for Microbiology and the field of virology itself. Having served as editor-in-chief of *Virology* and an editor of *Journal of Virology* over his long career as a professor of microbiology, Joklik was uniquely placed (as he noted) to evaluate what had been learned since early experiments in virology.

Before the founding of *Virology* and *Journal of Virology* in the 1950s and '60s, respectively, Joklik noted, a number of "epoch-making discoveries in virology" appeared in journals not devoted to the field. Among the seven discoveries he singled out were two related to paralysis research. The first was "the discovery by Enders *et al*. in 1949 that poliomyelitis virus could be grown in human embryonic tissue cells cultured *in vitro*, which formed the basis of the technique of tissue culture (single cell culture)"; the second, "…the demonstration by Dulbecco, also in 1952, that an animal virus … was capable of forming plaques in monolayers of cloned cultured cells, which opened up the field of molecular animal virology."[19] While Dulbecco's 1952 study did not involve poliovirus, it led directly to his 1954 paper in which he extended the new methodology to the study of poliovirus.[8,9]

In 1949, as Joklik recounted, Harvard Medical School researcher John F. Enders, along with his colleagues Thomas H. Weller (a Fellow of the U.S. Public Health Service) and Frederick C. Robbins (a Senior Fellow in Virus Diseases of the National Research Council) showed not only that poliovirus could grow in cultured cells, but also that it could replicate in non-nervous system tissues, a stunning discovery at the time.[13] It was already suspected that poliovirus was often present in the intestines of affected individuals. However, no one had been able to propagate the virus in gut tissue, primarily because of the bacteria that naturally live there. Enders and colleagues were successful in part because they added antibiotic (penicillin and/or streptomycin) to their cell cultures to kill the bacteria—a technique that had not, of course, been available to researchers working in the pre-World War II era.

While Enders and colleagues' 1949 paper is widely acknowledged to be a turning point in poliomyelitis research—many, including World Health Organization poliovirus eradication researchers, credit this piece of science with paving the way for the development of both the Salk and Sabin polio vaccines—poliovirus was not actually isolated by these investigators, either. They successfully grew "filterable agents," which they assumed to be poliovirus, in human embryonic tissues. Like Landsteiner and Popper 40 years earlier—and like just

The Dulbecco "Isolation" Experiment

In 1954, Dulbecco and his colleague Margaret Vogt published a classic research paper that is credited with having set the standard for purifying poliovirus for decades.[9] In it, they introduced a technical innovation to the process of "purifying" viruses from tissue culture. This new technique was called "plaque purification"; a single plaque (a circular area of cells that stained differently from the surrounding culture) was considered to represent a pure virus population. Plaque purification utilized trypsinization, which involves treating the cells—in this case, monkey kidney cells—with the enzyme trypsin, breaking up any clumps of cells that might have formed and resulting in a single-cell suspension.

In the early days of poliovirus research, tissue culture was usually conducted using monkey kidneys (or, sometimes, monkey testes). Dulbecco and Vogt explained where the "virus" they grew came from:

> "The virus was supplied as a 20 per cent suspension of spinal cord of rhesus monkey in distilled water. Type 1 virus obtained from passage through the monolayer kidney cultures was used. Type 2, Yale-SK strain, and Type 3, Leon strain, were kindly supplied by Dr. J.L. Melnick in form of tissue culture supernatants."[9]

That passage clearly demonstrates that Dulbecco and Vogt *did not isolate pure poliovirus* in any of the experiments described in this 1954 report. While they write of seeding their cultures with "virus," they actually used unpurified suspensions, not pure viral isolates.

Once the monkey kidneys were ground up into "single cells, cell clusters, and cell debris," they were seeded with the monkey spinal cord emulsion. The appearance of the plaques was evidence that the virus was growing, according to the model Dulbecco had developed in 1952.[8]

The control for these experiments was to treat the cultures with monkey antiserum (derived from monkeys infected with Type 1, 2, or 3 poliovirus); if Type 1 antiserum inhibited plaque formation but Type 2 or 3 (or normal monkey) antiserum didn't, then Type 1 poliovirus was assumed to be *exclusively present in the culture*. In other words, it was assumed that no other organism or disease-associated agent was growing in the culture.

Once again, what Dulbecco and Vogt describe as “isolation” of the poliovirus is not isolation in the way we would understand it in modern microbiology. To perform their “plaque purifications,” they simply pipetted some liquid (“plaque stock”) from one culture plate and replated it onto other culture plates. When the second-generation cell cultures showed evidence of viral growth (i.e., plaques), monkeys were inoculated with the plaque stock. The inoculated monkeys developed paralysis and, subsequently, most died. Since the plaque purified viral stock both grew new plaques in second-generation cell culture and caused monkeys to develop flaccid paralysis, Dulbecco and Vogt concluded they had “isolated” poliovirus.

Like poliovirus researchers before them, it is clear that Dulbecco and Vogt were propagating disease-associated substances in their tissue cultures, and that they later transferred these substances to monkeys in whom acute flaccid paralysis developed. These were impressive accomplishments.

Dulbecco and Vogt’s claims, however, went further than they had evidence to support. They asserted not only that they had isolated poliovirus, but that, “Since each plaque stock originated from a single virus particle (as proved in the Discussion), these stocks constitute the purest lines of virus presently available.”

© PhotoDisc/Siede Preis

How could they possibly know that a “single virus particle,” something they had never seen or measured, was causing the growth of exactly one plaque in their cultures? The evidence Dulbecco and Vogt supplied to “prove” that a single virus particle produced each plaque is contained in a mathematical equation: They extrapolated the cell culture’s assumed “virus concentration” from the number of times the original fluid (for example, monkey spinal cord suspension) was diluted. The fewer times the fluid was diluted, the more plaques grew in laboratory cultures; the more times it was diluted, the fewer plaques grew. Dulbecco and Vogt’s mathematical model assumed this linear relationship between dilution of virus stocks and number of plaques formed and, when they reached the greatest possible dilution that still caused a single plaque to grow, they assumed that *only one “virus particle” was present therein*. And how did they prove that assumption, as promised? They provided their mathematical model. This is a perfectly tautological proof. Its most apparent flaw is that the mathematical model did not—could not—distinguish between a “single virus

particle" and a biological complex that may have contained a single virus.

This is made clear in Dulbecco and Vogt's description of the plaque-forming "single virus particle" they claim to have isolated:

> "Having arrived at this point, it is now possible to define properly the characteristics of the virus particle detected by a plaque. Owing to its all-or-none effect, it has the character of a particle. It corresponds to a unit of the virus which is not further subdivisible at high dilution. From the property by which it is recognized, we call it a plaque-forming particle. We do not know its morphological or genetic properties. It might be a single elementary body, or a clump of them, provided that the clump persists indefinitely at high dilution...."[9]

It is puzzling, in retrospect, that Dulbecco and Vogt raised the possibility that they were detecting a "clump" of material, but thereafter ignored it. What if another type of virus was also included in these particles? Or, what if host genetic material attached itself to the particle to form a "clump"?

Although electron microscopy—which would have allowed them to visualize a single viral particle—existed in 1954, Dulbecco and Vogt did not use it. Instead, they employed the time-honored technology in which viruses were assumed to be present in cultures if certain chemicals stained them, or if fluids thought to contain them produced characteristic patterns of growth, like the poliovirus-related plaques described here. Dulbecco and Vogt could not possibly determine that they were viewing single viruses in their cultures and, therefore, their assumption that they had isolated a "single virus particle" was a vast overstatement. Dulbecco and Vogt did not isolate poliovirus.

about everyone else in the field during its first 60 years or so—Enders and co-workers called this disease-transmitting suspension of tissue "virus."

Despite this overstatement, Enders, Weller, and Robbins were the first to prove that a transmissible agent associated with poliomyelitis could be propagated in cells in the laboratory, and that cell cultures could be substituted for live animals in studying such transmissible agents. In 1954, their ground-breaking work was rewarded with a Nobel Prize.

Renato Dulbecco's 1952 paper lauded by Joklik is considered to have made a significant contribution to viral research in general and, by extrapolation, to poliovirus research. Working at the California Institute of Technology (in Pasadena), Dulbecco developed a method of growing plates of cells so that "virus plaques" could be visualized. He grew Western Equine Encephalomyelitis virus plaques on a substrate of chicken embryo cells and, when he published his paper, he pointed out that it was still unknown whether all viruses could be cultured in this manner. These were truly the very earliest days of modern virological research, and Dulbecco expressed hope that investigators would some day be able to distinguish between various viruses grown in cell culture by using his methodology and examining the resulting plaques under the microscope.[8]

In 1954, Dulbecco and his colleague Margaret Vogt published a classic research paper [see sidebar, "The Dulbecco Isolation Experiment," above] that set the standard for purifying poliovirus cultures for decades.[9] Dulbecco and Vogt, like their colleagues, used monkey kidney cells to culture tissues thought to contain

poliovirus. Dulbecco and Vogt explained where the "virus" they grew came from:

> "The virus was supplied as a 20 per cent suspension of spinal cord of rhesus monkey in distilled water. Type 1 virus obtained from passage through the monolayer kidney cultures was used. Type 2, Yale-SK strain, and Type 3, Leon strain, were kindly supplied by Dr. J.L. Melnick in form of tissue culture supernatants."[9]

In other words: Dulbecco and Vogt *did not isolate pure poliovirus* in any of the experiments described in this 1954 report. While they write of seeding their cultures with "virus," they actually used unpurified suspensions, not pure viral isolates.

It is clear from this historical review of early poliovirus research papers that none of these poliomyelitis researchers truly isolated poliovirus. Additionally, they were injecting monkeys with experimental fluids that were probably contaminated with other disease-associated agents.

Further confusing the picture (but not reviewed here) is the fact that enteroviruses other than poliovirus are associated with AFP. For example, as recently as February 2001, it was shown that Coxsackie A24 is associated with nonpolio AFP.[5]

How Much Flaccid Paralysis Is NOT Caused by the Poliovirus?

There is an astonishing number of cases of paralysis around the world *not* associated with poliovirus. If you visit the World Health Organization website that tracks acute flaccid paralysis (AFP), polio and non-polio [http://www-nt.who.int/vaccines/polio/case.asp], you will see that the world is not rid of the scourge of AFP. For example, India reported 9,580 cases of AFP in 1999; 2802 of them, fewer than one-third, were associated with poliovirus. China reported 5,064 cases of AFP to WHO in 1999; only one of those cases was associated with poliovirus. Poliovirus eradication and vaccination programs have not eliminated paralysis.

WHO recently declared Egypt on the threshold of eradicating poliovirus. "We are now at the end of a polio era," a UN Children's Fund Project Officer told Reuter's news service in late February 2001. Egypt had "not a single case of the crippling virus reported so far this year" or in 2000, according to Reuters.[17]

According to the WHO AFP/polio surveillance web site, however, there were 54 cases of acute flaccid paralysis in Egypt in 2000 (the most recent year for which statistics are available). In 1999, although there were 9 AFP cases classified as due to poliovirus, 276 were classified as nonpolio. During 1998, Egypt had 295 cases of AFP, 35 of which were classified as poliovirus-related; in 1997, Egypt reported 217 cases of non-polio paralysis compared to 14 cases in which poliovirus infection was confirmed; and in 1996, the earliest year for which statistics are available, Egypt reported 309 cases of acute flaccid paralysis. One hundred of those were classified as poliovirus-related, leaving 209 cases—two-thirds of the total—probably due to a cause other than poliovirus (with the caveat that epidemiological statistics are not perfectly accurate in every country of the world). [http://www-nt.who.int/vaccines/polio/case.asp]

Afghanistan is another country in which there is an increasing prevalence of AFP compared to a decreasing incidence of poliovirus. As the U.S. Centers for Disease Control and Prevention's *Morbidity and Mortality Weekly Report* (*MMWR*) notes on March 2, 2001, "During 1999-2000, the number of AFP cases [in Afghanistan] increased from 230 to 253, and the number of wild polioviruses isolated from AFP cases decreased from 63 to 28."[28]

How does the CDC explain the increase in AFP cases in Afghanistan, in the face of a vigorous poliovirus eradication campaign? Well, it doesn't. In fact, the *MMWR* report almost makes the increase in nonpolio AFP sound like a triumph of public health: "During 1999-2000, the nonpolio AFP rate almost doubled and the number of districts reached by NIDs [National Immunization Days] increased steadily. Careful planning and

supervision of house-to-house vaccination and support from an increasing number of local partners resulted in the largest number of children ever being reached. Monitoring by nongovernment organizations, United Nations' agencies, and local authorities has increased the quality of NIDs…."[28] In other words, the more National Immunization Days there were, the more cases of paralysis appeared. Does this mean immunizations were causing paralysis? No, but neither was increased immunization preventing children from becoming paralyzed.

The Western Hemisphere has also been impacted by an increased case load of AFP. As mentioned earlier, the island of Hispaniola (the Dominican Republic and Haiti) experienced what the CDC called an "outbreak of poliomyelitis" that began in July 2000. There were 54 cases in the Dominican Republic, 12 of which were

Polio Vaccination Recommendations

U.S. Centers for Disease Control and Prevention

Recommendations for children in the United States include a 4-dose vaccination series with inactivated poliovirus vaccine (IPV) at ages 2, 4, 6–18 months, and 4–6 years. Unvaccinated adults should receive three doses of IPV, the first two doses at intervals of 4–8 weeks and the third dose 6–12 months after the second. If three doses cannot be administered within the recommended intervals before protection is needed, alternative schedules are proposed. For incompletely vaccinated persons, additional IPV doses are recommended to complete a series. Booster doses of IPV may be considered for persons who previously have completed a primary series of polio vaccination and who may be traveling to areas where polio is endemic.

Morbidity and Mortality Weekly Report, March 2, 2001, Vol. 50, No. 8, p. 147

© PhotoDisc/C Squared Studios

"laboratory-confirmed poliomyelitis cases attributed to vaccine-derived poliovirus type 1," according to the CDC. Although the oral polio vaccine is known to cause polio in about 1 of every 750,000 infants who receive it or their mothers—unlike the inactivated Salk vaccine "shot," the Sabin oral vaccine contains live viruses—the 45 cases reported in January 2001 in the *Washington Post* are, if confirmed, clearly outside the realm of this statistic.[3]

As of the end of February 2001, the cause(s) of 33 AFP cases in the Dominican Republic and three in Haiti remained undetermined.[27] All of these cases might be due to the oral polio vaccine, in which case the mystery would be solved—leaving unanswered, however, the question of what factors contributed to such a large vaccine-associated outbreak of paralysis.

If these 36 AFP cases are not related to the polio vaccine, however, then what is causing them? What is causing other nonpolio outbreaks of AFP identified by WHO all over the world?

And in cases in which poliovirus is fingered as the culprit in an outbreak, how sensitive are the current methodologies that virologists use to isolate and identify it?

How Is Poliovirus Detected Today?

It is nearly unimaginable how sensitive and sophisticated laboratory technology has become over the last 30 years. As we examine the entire sequence of the human genome in early 2001, it's difficult to imagine that it was only in the 1970s that scientists first developed the technology that allowed the rapid sequencing of genes, including genetic sequences from transmissible agents like bacteria and viruses.

This new sequencing methodology was immediately applied to poliovirus research. During the 1970s, the CDC began routinely performing genotypic testing ("molecular sequencing" or "oligo-nucleotide fingerprinting") on stool samples collected in suspected poliovirus outbreaks to determine whether the virus was present. Using findings from the new technology to extrapolate to the prior decade, CDC documents now state that, "Both laboratory surveillance for enteroviruses and surveillance for polio cases suggest that endemic circulation of indigenous wild polioviruses ceased in the United States in the 1960s."[24]

To detect poliovirus today, according to CDC and WHO guidelines, two stool samples should be collected from each patient, 24-48 hours apart within 14 days of the onset of paralysis, and they must arrive at the laboratory in "good condition." While WHO's target is to obtain two good samples in at least 80% of all AFP cases, some areas of the world fall short of this, approaching only 50%.[28]

The CDC provides the following guidelines on how to detect poliovirus:

> "The following tests should be performed on appropriate specimens collected from persons who have suspected cases of polio: a) isolation of poliovirus in tissue culture; b) serotyping of a poliovirus isolate as serotype 1, 2, or 3; and c) intratypic differentiation using DNA/RNA probe hybridization or polymerase chain reaction to determine whether a poliovirus isolate is associated with a vaccine or wild virus.
>
> "Acute-phase and convalescent-phase serum specimens should be tested for neutralizing antibody to each of the three poliovirus serotypes. A fourfold rise in antibody titer between appropriately timed acute-phase and convalescent-phase serum specimen is diagnostic for poliovirus infection. The recently revised standard protocol for poliovirus serology should be used. Commercial laboratories usually perform complement fixation and other tests. However, assays other than neutralization are difficult to interpret because of inadequate standardization and relative insensitivity."[24]

While this procedure is a time-honored method of detecting the poliovirus and the body's response to it, it does not "isolate" the poliovirus—it simply detects poliovirus. The samples tested by the CDC and WHO should be described as "poliovirus reactive material," not as samples that contain isolated, pure poliovirus.

Once again, we have no proof that poliovirus has been isolated.

If Not Poliovirus, Then What Is Causing Today's Cases of Flaccid Paralysis?

"The history of the etiology of poliomyelitis is a history of errors."

J.F. Eggers, *Medicine*, 1954

If the majority of the U.S. population has been immunized since the 1950s, why did it take until 1979 to "eradicate" poliovirus within the United States?[24,31]

And what is causing the nonpolio cases of paralysis that continue to occur all over the world?

It is becoming clear that one culprit capable of causing not only paralysis but also other neurological conditions is organophosphate pesticides. Recent research has tied chronic organophosphate pesticide exposure to development of Parkinson's disease signs and symptoms in an animal model.[2] And researchers in Paraguay have good evidence that an outbreak of AFP among children in 1990-1991 was associated with organophosphate pesticide exposure.

The 50 Paraguayan children identified in this study—given that it was conducted in a rural, isolated area meant that quite a number of affected children might have been excluded from the study, as investigators noted—developed a type of AFP named Guillain-Barré Syndrome, or GBS. As is the case in other forms of AFP, the myelin sheath that surrounds and protects nerves is damaged in GBS. The disease's causes are unknown, but it's generally believed to be an autoimmune condition provoked by infections, toxins, or a combination of both.[16]

The children became ill during the Paraguayan summer (January to April), with weakness, upper respiratory tract infection, fever, and gastrointestinal symptoms. Three children developed difficulty breathing, and two of them required mechanical help to breathe (intubation). "Weakness progressed in an ascending pattern in 95% of the children, and simultaneously in all limbs in 5%; the average time to reach the nadir was 7 days (range, 2-12 days)," the investigators reported. Of the 35 children observed while they were in the acute stage of AFP, 18 were unable to walk, 10 walked with assistance, four walked independently, and three were too young to walk. The children exhibited full or partial paralysis of facial muscles and their bladders; they also experienced autonomic nervous system changes that created fluctuations in blood pressure, erratic heartbeat, flushing of skin, and intestinal motility. One child died.[16]

The study was conducted as part of the Pan American Health Organization's effort to eradicate poliomyelitis. David E. Hart of the U.S. National Institute of Neurological Disorders and Stroke at the National Institutes of Health was the lead investigator working alongside researchers from the Paraguayan Ministry of Health.[16] The majority of cases, they point out, were clustered in a rural, farming province named Concepcion.

"The clustering of patients in Concepcion could be related to the use of organophosphate pesticides in the cotton fields," Hart and colleagues suggest. "Farmers use great amounts of these pesticides, often in concentrated form, and empty containers serve as toys. Also, the maximum usage of organophosphates occurs during the summer (December-March)," when these children became ill.[16]

Although they note that retrospective measurement of organophosphate exposure is very difficult, Hart and co-workers cite a report that the cotton industry officially spent approximately US$ 6.7 million on organophosphate pesticides in 1991. However, more than half of the pesticides used in Paraguay are obtained "unofficially," according to this report.

"Four children were excluded from this study because of definite exposure to this product and presentation with concurrent acute cholinergic syndrome," the severe disease produced by organophosphate pesticide exposure. Hart and colleagues added, "Their clinical course, however, was similar to that of the children included" in the study.[16]

By examining the possibility that the AFP observed in these Paraguayan children might be associated with organophosphate pesticides, Hart and colleagues took that extra step that is so often omitted. Clusters of illnesses in communities can arise from any number of causes; they are not exclusively due to transmissible agents. Toxins in the environment are significant factors in many illnesses.

Since the time of Koch, bacteriologists have used the gold standard he described for the assignment of the disease process to single organisms. Bacteria and fungi can be truly isolated and grown independently on artificial media; they don't require the presence of human or other cells. One problem that researchers have faced in describing non-bacteriologic related diseases has been the assumption that a single entity can cause them, without interaction from the cells in which they are grown, the human genome, or the environment.

We live in a important time: We are about to redefine much of what we know about medical science. In early 2001, two stunning reports on the Human Genome Project, published simultaneously in

February issues of *Science* [http://www.sciencemag.org/feature/data/genomes/landmark.shl] and *Nature* [http://www.nature.com/genomics/], turned much of what we thought we knew about the human genome on its head. Instead of possessing 100,000 genes, for example, we learned that the human genome is made up of only about 30,000 genes—fewer than the number possessed by rice.[1]

Our new understanding of the human genome was produced, in part, by new technologies that we can now apply to revisiting many of the assumptions of modern medicine. One of the most important lessons learned from the challenge of decoding the human genome is that scientists need to describe laboratory experiments and results accurately. Technologically advanced tools can provide detailed and precise information, but the researchers using them must describe those results with equal precision. When a sample is laboratory reactive, it should not be assumed to be infectious. Likewise, suspensions of diseased brain tissue should not be called "virus" and dilutions of brain tissue material should not be called "isolations."

As the human genome comes more precisely into focus, our understanding of how our genes interact with one another, the environment, and other organisms will also become more precise.

Precision should also be applied to research objectives. Clearly, it is incorrect to state that poliomyelitis has been eradicated from many countries. The surprisingly large number of cases of nonpolio acute flaccid paralysis around the world warrants continued pursuit of the original objective of the March of Dimes: the elimination of infantile paralysis. On its website, the March of Dimes takes some well-deserved credit for helping to limit the amount of paralysis in the world today. “Historians have called the conquest of polio one of the great achievements of this century,” a fact sheet on the website states. “Thanks to the March of Dimes, and the millions of people who supported it, we no longer have the devastating epidemics that terrorized generations.” [http://www.modimes.org/HealthLibrary2/factsheets/Polio.htm]

Clearly, the original objective of the March of Dimes has not yet been met, or there would not be so much acute flaccid paralysis around the world today. Examining the last 50 years of poliomyelitis research shows that the objective of eliminating infantile paralysis has been replaced with the objective of eliminating poliovirus. As governments, international health organizations, and charitable foundations pour hundreds of millions of dollars into poliovirus eradication efforts, shouldn’t we also invest in basic research that will prevent *all* cases of childhood paralysis?

Acknowledgement: The author wishes to thank Dr. Howard Urnovitz, Science Director of the Chronic Illness Research Foundation, for bringing this story to her attention and approving the scientific content of this article.

Literature Cited

1. Abate, Tom. February 11, 2001. Genome discovery shocks scientists. *San Francisco Chronicle*.
2. Betarbet, R., T.B. Sherer, G. MacKenzie, M. Garcia-Osuna, A.V. Panov, and J.T. Greenamyre. December 2000. Chronic systemic pesticide exposure reproduces features of Parkinson's disease. *Nature Neuroscience* 3(12):1301.
3. Brown, David. January 1, 2001. Ready to let go of polio? Mutant virus in Caribbean outbreak alarms health experts. *Washington Post*, p. A7.
4. Burnet, F.M. and J. MacNamara. 1931. Immunological differences between strains of poliomyelitic virus. *The British Journal of Experimental Pathology* 12(2):5.
5. Chaves, S.S., S. Lobo, M. Kennett, and J. Black. February 24, 2001. Coxsackie virus A24 infection presenting as acute flaccid paralysis. *The Lancet* 357:605.
6. Dalldorf, G. and G.M. Sickles. 1948. An unidentified, filtrable agent isolated from the feces of children with paralysis. *Science* 108:61.
7. Davis, W.M. 1919. The demonstration of immune opsonins for the pleomorphic streptococcus in the experimental poliomyelitis in monkeys. *Journal of Infectious Diseases* 24:176.
8. Dulbecco, R. 1952. Production of plaques in monolayer tissue cultures by single particles of an animal virus. *Proceedings of the National Academy of Sciences USA* 38:747.
9. Dulbecco, R. and M. Vogt. 1954. Plaque formation and isolation of pure lines with poliomyelitis viruses. J. Exp. Med. 99:167-182.

10. Eggers, H.J. 1999. Milestones in early poliomyelitis research (1840-1949). *Journal of Virology* 73(3):4533.
11. Enders, J.F. 1954. Some recent advances in the study of poliomyelitis. Talk given on the occasion of receiving the Passano Award, June 3, 1953. Published in *Medicin*e 33:87, May 1954.
12. Enders, J.F. 1972. Early observations on cytopathogenicity of poliovirus. *American Journal of Clinical Pathology* 57(6):846.
13. Enders, J.F., T.H. Weller, and F.C. Robbins. 1949. Cultivation of the Lansing strain of poliomyelitis virus in cultures of various human embryonic tissues. *Science* 109:85.
14. Flexner, S. and P.A. Lewis. 1909. The transmission of acute poliomyelitis to monkeys. *Journal of the American Medical Association* 53:1639.
15. Gordon, F.B., F.M. Schabel, Jr., A.E. Casey, and W.I. Fishbein. 1948. Laboratory study of the epidemiology of poliomyelitis. *Journal of Infectious Diseases* 82:294.
16. Hart, D.E. et al. 1994. Childhood Guilliain-Barré Syndrome in Paraguay, 1990 to 1991. *Annals of Neurology* 36(6):859.
17. Hassan, Abdalla. February 22, 2001. Egypt appears close to wiping out polio scourge. Reuters News Service.
18. International Human Genome Sequencing Consortium. February 15, 2001. Initial sequencing and analysis of the human genome. *Nature* 409:860.
19. Joklik, W.K. When two is better than one: Thoughts on three decades of interaction between *Virology* and the *Journal of Virology.* 1999. *Journal of Virology* 73(5):3520.
20. Landsteiner, K. and E. Popper. 1909. Uebertragung der poliomyelitis acuta auf affen. *Z. Immunitatsforsch* 2:377.
21. Melnick, J.L. and R. Ward. 1945. Susceptibility of vervet monkeys to poliomyelitis virus in flies collected at epidemics. *Journal of Infectious Diseases* 77:251.
22. Melnick, J.I. and N. Ledinko. 1952. Vaccination as a provoking factor in poliomyelitis: An experimental approach. *Journal of Infectious Diseases* 90:279.
23. *Merck Manual*, Fifteenth Edition. 1987. Merck & Co., Inc., Rahway, NJ, USA.
24. *Morbidity and Mortality Weekly Report.* May 19, 2000. Poliomyelitis prevention in the United States: Updated Recommendations of the Advisory Committee on Immunization Practices (ACIP). U.S. Department of Health & Human Services, Centers for Disease Control and Prevention, Atlanta, GA 30333.
25. *Morbidity and Mortality Weekly Report.* December 8, 2000. Outbreak of poliomyelitis—Dominican Republic and Haiti, 2000. U.S. Department of Health & Human Services, Centers for Disease Control and Prevention, Atlanta, GA 30333.
26. *Morbidity and Mortality Weekly Report.* January 26, 2001. Circulation of a Type 2 vaccine-derived poliovirus—Egypt, 1982-1993. U.S. Department of Health & Human Services, Centers for Disease Control and Prevention, Atlanta, GA 30333.
27. *Morbidity and Mortality Weekly Report.* March 2, 2001. Outbreak of poliomyelitis—Dominican Republic and Haiti, 2000-2001. U.S. Department of Health & Human Services, Centers for Disease Control and Prevention, Atlanta, GA 30333.
28. *Morbidity and Mortality Weekly Report.* March 2, 2001. Progress toward poliomyelitis eradication—Afghanistan, 1999-2000. U.S. Department of Health & Human Services, Centers for Disease Control and Prevention, Atlanta, GA 30333.
29. Morales, E.G. 1930. Acute anterior poliomyelitis at Vega Baja, Porto Rico. *Journal of Infectious Diseases* 46:32.
30. Schabel, F.M., Jr., H.T. Smith, W.I. Fishbein, and A.E. Casey. 1950. Stool virus recovery in subclinical poliomyelitis during incubation, febrile, and convalescent periods. *Journal of Infectious Diseases* 82:294.
31. Strebel, P.M., R.W. Sutter, S.L. Cochi, et al. 1992. Epidemiology of poliomyelitis in the United States one decade after the last reported case of indigenous wild virus-associated disease. *Clin. Infect. Dis.* 14:568.
32. Venter, J.C., et al. February 16, 2001. The sequence of the human genome. *Science* 291:1304.
33. Wenner, H.A., R.W. Menges, and G.S. Harshfield. 1954. Sporadic bovine encephalomyelitis: 3. Reproduction of the disease, with particular reference to the role of poliomyelitis viruses in experimental infection in calves. *Journal of Infectious Diseases* 94:284.