

Chapter 1

John F. Enders and Measles Virus Vaccine—a Reminiscence

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Abstract Following their initial isolation in cell culture of the virus in 1954, a succession of investigators under the mentorship of John F. Enders conducted the research, development, and initial clinical studies responsible for the licensure in 1963 of a successful live attenuated measles virus vaccine. Propagation of the virus successively in human kidney cells, human amnion cells, embryonated hens' eggs, and finally chick embryo cell cultures had selected virus that when inoculated into susceptible monkeys proved immunogenic without viremia or overt disease, in contrast to the early kidney cell-passaged material, which in similar monkeys produced viremia with illness mimicking human measles. Careful clinical studies in children by the Enders group and then by collaborating investigators in many sites established its safety, immunogenicity, and efficacy. This Edmonston strain measles virus became the progenitor of vaccines prepared, studied, and utilized throughout the United States and many other countries. With appreciation of measles morbidity and mortality, most marked among infants and children in the resource-limited lands, the vaccine was incorporated into the World Health Organization's (WHO) Expanded Programme of Immunization (EPI) in 1974 along with BCG, OPV, and DTP. Successful efforts to further reduce measles' burden were launched in 2001 and are continuing as the Measles Initiative (Partnership) under the leadership of the American Red Cross, International Red Cross, and Red Crescent societies, Centers for Disease Control (CDC), United Nations Children's Fund (UNICEF), WHO, and the United Nations Foundation.

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When in 1954 John F. Enders and his two younger colleagues, Frederick Robbins and Thomas Weller, received the Nobel Prize in Physiology or Medicine for the cultivation in cell cultures of polio viruses, he had already returned to his initial interest in isolating and propagating the virus responsible for measles. Enders was a unique investigator whose career had followed a less than conventional path. The scion of a wealthy Connecticut Yankee family, he had attended an elite boys preparatory school, St. Paul's in Concord, New Hampshire, and then Yale University. Additionally, he had spent an interim year as a US Navy World War I flight instructor. Upon university graduation, he was provided a position in the family's banking enterprise, responsible for selling real estate. Recognizing his lack of interest and commitment to such a pursuit, he enrolled in the Graduate School at Harvard University studying ancient Celtic philology. A fortunate turn of events provided him a roommate in their rented Brookline apartment, Hugh Ward, a budding Australian microbiologist. Ward was apprenticed to Hans Zinsser, the eminent microbiologist, in whose laboratory he studied. Enders visited the laboratory where he became fascinated by the projects of his roommate and the views he gained through Ward's microscope. Abandoning Celtic philology, he joined Zinsser's group as a graduate student in microbiology. Here began the career for which he is widely remembered and appreciated. His early work focused on the role of complement and antibody in the response to pneumococcal infections. After, and perhaps because of, the death of his first wife from influenza virus infection, he directed his investigative efforts to viruses. Early work involved feline panleukopenia, a fatal disease of cats, mumps virus, and then polio.

Enders was a very special individual, not fitting the mold of the aggressive goal-oriented researcher, but more a contemplative, broadly interested investigator who pursued medical science for enrichment of the field and personal gratification, but not for audience acclaim. He was an ideal mentor for many young aspirants, most of whom later succeeded in developing subsequent careers as distinguished scientists. Because he believed that daily and leisurely contact with one's disciples was critical to their advancement and the productivity of his laboratory, he never accepted more than four or five fellows at any time, a marked contrast to many of his contemporaries. In addition to those from the United States, he enjoyed opening his laboratory to bright young fellows from abroad (Japan, Iran, the Netherlands, Sweden, England, Yugoslavia, Belgium, Germany, South Africa, Turkey). On the daily rounds of the laboratory benches, his question "What's new?" provided an effective stimulus to each fellow to have developed something that would then catch his interest, initiating then a 30- or 60-minute conversation in which the significance of the findings and the ways in which one might pursue further studies were discussed. One worked *with* John Enders not *for* him (Table 1).

In 1954, one pediatric fellow who spent a year in the laboratory was dispatched to a suburban school where an outbreak of measles was reported to be underway. Thomas Peebles obtained throat swabs and blood specimens from the affected youngsters and brought them back to the laboratory. In conventional Enders' fashion, never to waste material and always to utilize available opportunities, cells from human kidneys had been successfully cultured *in vitro*. These originated from a

Table 1 Enders laboratory participants in the research and development of measles virus vaccine

Thomas Peebles	Samuel Katz
Kevin McCarthy	Ann Holloway
Anna Mitus	Donald Medearis
Milan Milovanovic	Elizabeth Grogan

neurosurgical procedure then in fashion in which children with hydrocephalus had a unilateral nephrectomy with a connection then established between the cerebrospinal fluid in the subarachnoid space and the ureter of the sacrificed kidney. These kidneys came to the Enders’ lab, where they were minced, trypsinized, and put into cell culture with nutrient media. It was in these cells that measles virus was first successfully cultivated and passaged a number of times (Enders and Peebles 1954). Because the name of the young student from whom the original virus had been isolated was David Edmonston, this strain of virus has subsequently always been identified as Edmonston virus. Virus harvested from early passage of these cultures was inoculated into measles-susceptible monkeys who then developed fever, rash, viremia, and eventually measles-specific antibodies, both complement-fixing and virus-neutralizing (Peebles et al. 1957). As ventriculoureteral shunts fell out of fashion, with the development of improved technology for relief of hydrocephalus, new sources of human cells were sought. At a neighboring hospital, an obstetrical institution, 15–20 women were delivered each day of newborns and their placentas cast aside. Enders, in his customarily frugal but innovative fashion, suggested we strip the amniotic membrane from these discarded placentas and attempt to prepare cultures of human amnion cells. One of the fellows went to the obstetrical hospital to claim a placenta, brought it back to the laboratory, where it was mounted so that the amniotic membrane could be sterilely removed. The membrane was then trypsinized, and the resultant cells were dispersed, harvested, and placed in test tubes and flasks where they were successfully grown. Measles virus after 24 passages in human kidney cells replicated effectively in these human amnion cell cultures and once again produced an identifiable cytopathic effect (Milovanovic et al. 1957). With typical Enders’ imaginative approach, he then suggested that if the virus grew readily in human amnion cells, perhaps it would also replicate in a nonhuman but similar environment. Therefore after 28 human amnion cell passages, we moved to embryonated hen’s eggs and inoculated virus intra-amniotically. The eggs were obtained from a supposedly pathogen-free flock in New Hampshire. Although there was no visible resultant pathology, fluids harvested from these infected eggs displayed cytopathology when inoculated back into human amnion cells, and titers indicated the virus had not merely persisted but had multiplied (Milovanovic et al. 1957). After six passages in the fertile hen’s eggs, we prepared cell cultures from trypsinized chick embryo tissue and inoculated virus into those tubes. Although no effect was seen for the initial passages, after five, there was visible cytopathology which coincided with demonstrable replication of the virus in these cultures (Katz et al. 1958). It was 13th passaged chick cell material that was inoculated into measles-susceptible monkeys and the results compared

with the original early human kidney-cell-propagated virus. In contrast, the chick cell virus produced no rash, no detectable viremia but nonetheless complement-fixing and virus-neutralizing antibodies (Enders et al. 1960). In addition to the aforementioned studies, chick cell virus was also inoculated directly into the cistern and the cerebral hemispheres of susceptible monkeys. No behavioral changes were noted after this procedure, but the animals were sacrificed and neuropathological studies of the infected cerebral tissue were conducted by veterinary pathologists at the neighboring animal hospital. No histological changes could be identified. In contrast, monkeys similarly injected intracranially with early passaged kidney cell virus developed lesions with local mononuclear cell infiltrates, perivascular cuffing, and demyelination. In another series of experiments, monkeys that had been immunized with the chick cell virus were then challenged with the virulent human kidney cell virus and proved completely resistant to infection. After these successful studies in monkeys, the question was how next to proceed to evaluation in humans.

Initially, we prepared lots of serum-free vaccine virus carefully scrutinized and tested for any contaminating agents and for sterility, to inoculate one another. Although this was not a test of efficacy, it was a determinant of possible toxicity and safety. With the successful completion of these preliminary studies, we then considered how best to proceed to study the vaccine in susceptible children. At a nearby state Institution for physically and intellectually challenged youngsters, outbreaks of measles occurred every 2 or 3 years, resulting in serious morbidity and a number of deaths. Following discussions with the institutional director, we were able to meet with the parents of several dozen children who had not yet suffered measles. After explaining to them the background of our potential vaccine and our plans for a clinical trial, most of them agreed to have their children participate. Using the same materials with which we had inoculated one another in the laboratory, we proceeded to inject subcutaneously a dozen susceptible children with the vaccine and several with sterile tissue culture fluid as placebo. We examined them daily, obtained nasopharyngeal cultures and venous blood samples on alternate days and followed them carefully over the next 3 weeks. Five to 8 days after inoculation, many of them developed fevers that persisted for several days and were then followed by an evanescent rash. Throughout this time, they nevertheless remained well and went about their normal activities. No virus was recovered from the throat cultures or blood, but within 2 weeks all had detectable measles virus-neutralizing and complement-fixing antibodies in their sera (Katz and Enders 1959; Katz et al. 1960a). The nursing personnel and others responsible for these children attested to the absence of any apparent disability during this time. Buoyed by these initially successful studies, we enlisted colleagues in Denver, New Haven, Cleveland, New York, and Boston to conduct similar studies among home-dwelling children under their care. The successful completion of these studies resulted in the *New England Journal of Medicine* reports in 1960 describing the background and development of the vaccine virus and the clinical observations of the vaccinated children (Katz et al. 1960b).

Throughout the years of this laboratory and clinical research (1954–1963), the Enders laboratory made available to any and all legitimate investigators who were interested in pursuing related studies varied materials for their use. These included

virus, cell cultures, and sera. The Enders philosophy was that the more people working on a problem the sooner solutions would be found. There was never any intent to patent the virus or to seek monetary return. As a result, within a short period of time, many university groups pursued measles vaccine investigations and seven different pharmaceutical firms in the United States and several abroad were producing their versions of the Edmonston measles virus vaccine (Table 2; Fig. 1). To attenuate further the clinical results of the initial vaccination (the aforementioned fever and exanthem), protocols were initiated in which the injection of vaccine was accompanied by a simultaneous tiny dose of human immunoglobulin (0.02 mg/kg body weight), which reduced these manifestations to approximately

Table 2 US Firms that produced measles virus vaccines

Pfizer	Lilly
Parke-Davis	Lederle
Philips-Roxane	Pitman Moore-Dow
Merck (Sharpe and Dohme) ^a	

^aThe sole remaining US producer



Fig. 1 First International Conference on Measles Immunization. 8 November 1961 at the National Institutes of Health, Bethesda Maryland. *Left to right:* Samuel Katz, Ann Holloway, Kevin McCarthy, Anna Mitus, Milan Milovanovic, John Enders, Gisele Ruckle, Frederick Robbins, Ikuyu Nagata

10%–15% of susceptible recipients. A number of investigators (initially Anton Schwarz in 1965 at American Home Products-Pittman Moore Dow and later Maurice Hilleman in 1968 at Merck) further attenuated the Edmonston virus by an increased number of passages in chick embryo fibroblasts at reduced temperature (32°C in contrast to the usual 35°–36°C). Additionally, several firms prepared formalin-inactivated, alum-precipitated measles vaccine from the Edmonston strain and studied its use in a three-dose schedule (Rauh and Schmidt 1965). The Enders group remained committed to live vaccine, convinced of its advantages over the inactivated preparation (Enders et al. 1962). Both the live attenuated and this inactivated vaccine were licensed in the United States on 21 March 1963. Over the ensuing several years, it was discovered that the killed vaccine did not produce enduring immunity and that when recipients were exposed to wild measles, many developed a severe atypical measles infection characterized by high fever, unusual rash beginning most prominently on the extremities, pneumonia with residual pulmonary nodules, and some central nervous system obtundation (Fulginiti et al. 1967; Annunziato et al. 1982). This inactivated vaccine was therefore withdrawn from use in 1967.

Fortunately it was not until 1969, 6 years after the licensure of measles virus vaccines, that the responsibility of wild measles virus for subacute sclerosing panencephalitis (SSPE) was discovered (Horta-Barbosa et al. 1969; Payne et al. 1969). By then, millions of American children had received live-attenuated measles virus vaccines with no resultant central nervous system complications resembling SSPE, and annual measles cases had been reduced by more than 90%. If the association of measles with SSPE had been appreciated prior to 1963, it is questionable whether licensure of a live-virus vaccine would have been so readily approved. Reassuringly, not only has SSPE become an extreme rarity in the United States and other countries with widespread childhood coverage by measles vaccine, but Bellini and colleagues at CDC have demonstrated that all the few cases identified in recent years are attributable genotypically to wild-type virus distinct from the vaccine strain (Bellini et al. 2005).

Early in development of the vaccine, after several presentations at national and international meetings, we began to receive a number of communications from Dr. David Morley, a British pediatrician who was developing child health programs in Nigeria, where he informed us that mortality from measles frequently approached 10%–20%. Of 555 children at his clinic 125 died of measles! He urged us to come to Nigeria and study the vaccine there. Judiciously, however, John Enders cautioned us to wait until the vaccine had proven its safety and efficacy in US youngsters before embarking on such a mission. His concern was that premature studies would be regarded as taking advantage of human guinea pigs rather than as a humane medical mission. Responding eventually to Morley's entreaties, Katz went in 1960 with Edmonston vaccine provided by Merck, which was then involved in its initial commercial production. The clinical trial was conducted in Imesi-ile, a tiny village outside Ilesha, a larger market town north of Ibadan. When informed of the project, local mothers keenly aware of measles' morbidity and mortality, eagerly brought their infants and children to participate. Many of these youngsters had malaria,

protein malnutrition, and intestinal nematode infestations. Despite these severe compromises, the initial 26 recipients responded favorably to the vaccine, had no adverse events, and developed antibodies at the expected time (Katz et al. 1962). A secondary benefit of this experience was our personal awakening to an awareness of the serious morbidity and mortality of measles among infants and children in the resource-limited nations. Our previous perspective had been a rather parochial one, of measles in the United States where nearly every child by age 7 had acquired the infection. Complications including otitis media, pneumonia, and gastroenteritis were common, requiring hospitalization in as many as 20%, but mortality was unusual, approximately one in 500 cases. Progress in the Americas had been remarkably successful, with transmission in the United States halted in 1993 (Katz and Hinman 2004) and in the entire Western hemisphere by 2002 (de Quadros et al. 2004). The few cases identified since then have been attributable to importations from countries where measles remains endemic. Although initial success in control was mainly the result of a single dose schedule, it became apparent that the 5%–10% of recipients who failed to seroconvert after this administration soon constituted a significant cluster of susceptibles in whom such a highly transmissible virus could ignite an outbreak. Therefore, beginning in the early 1990s, a two-dose schedule became the routine and has been continued worldwide in those nations where measles vaccination is practiced.

The experience in Nigeria stimulated our endeavors to place measles vaccine on the global scene, resulting eventually in its inclusion in the Expanded Program on Immunization (EPI) of the World Health Organization (WHO). However, there were still millions of deaths each year and no international effort was initiated, whereas the global focus was on polio eradication (Katz 2005). However, by the year 2000, the American Red Cross and International Red Cross and Red Crescent Societies, joined by the Centers for Disease Control (CDC), the United Nations Children's Fund (UNICEF), the United Nations Foundation, and the World Health Organization (WHO), formed the Measles Partnership (Measles Initiative) with its goal of reducing measles mortality from 873,000 annually (WHO figures for 1999) to half in the next 5 years. Remarkably, in the initial 5 years they exceeded their goal with vaccination of 297 million infants and children (ages 9 months to 5 years) and a resultant 68% overall decrease in measles mortalities (Wolfson et al. 2007). Most of this was in sub-Saharan Africa, where only 126,000 deaths were recorded in 2006 compared to the 506,000 in the first year of the Initiative (Partnership). For 2008–2010, the measles endemic countries of Southeast Asia are the targets of continuing campaigns.

Fortunately, measles virus has remained a monotypic agent with remarkably stable surface proteins that are responsible for induction of immunity. Forty-five years after introduction of the vaccine in 1963, it continues to provide solid, enduring immunity to vaccine recipients today, neutralizing measles viruses of all lineages. Even in those areas where exposure to wild measles viruses have been absent for many years, antibodies and resultant protection have persisted. An attack of natural measles conferred lifelong immunity to those who acquired it. Although it is tempting to predict that successful vaccination with attenuated measles virus will

provide equivalent immunity, it is premature to make such a prediction with the passage of less than five decades since its initial availability. In an era where many individuals are living to their eighties and nineties, the senescence of their immune systems may not maintain what has been assumed to be lifelong immunity. Only by continuing longitudinal studies will the answer to this question be provided.

In September 1985, at age 88, John Enders died peacefully at his home while reading poetry. His vision of a measles-free world has come closer to reality than he anticipated, but the challenges of elimination and eradication of so highly transmissible a virus will continue to confront us for many more years. His legacy, however, endures without challenge.

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