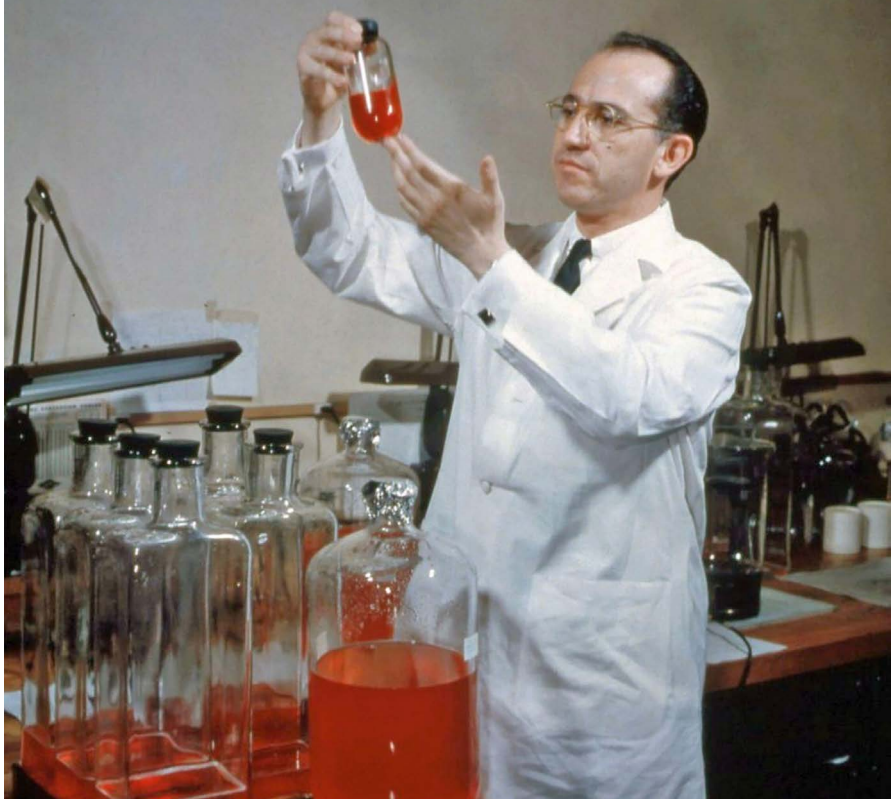
MILESTONE 9

Culturing poliovirus in cells



Jonas Salk (1914–1995), who developed one of the first polio vaccines. Credit: Pictorial Press Ltd / Alamy Stock Photo.

The disease poliomyelitis (polio) is suspected to have been around for centuries and was initially defined as an infantile spinal paralysis. By the start of the 20th century, there were severe and frequent polio outbreaks in both the USA and Europe. In 1908, Karl Landsteiner and Irwin Popper demonstrated that polio was spread by a virus, by injecting two Old World Monkeys (*Cynocephalus hamadryas* and *Macacus rhesus*) with a suspension of spinal cord from a polio patient. The suspension was bacteriologically sterile; nevertheless, following inoculation the monkeys exhibited lesions in the spinal cord similar to those seen in humans with poliomyelitis. Additionally, the rhesus monkey developed paralysis of both legs.

Having been diagnosed with polio in 1921, President Franklin D. Roosevelt founded the National Foundation for Infantile Paralysis to combat polio in 1938. This provided funding to John R. Paul and James D. Trask, who found poliovirus in the faeces of both patients and their healthy contacts. By testing sewage water in New York it was later estimated that there was a ratio of 100 subclinical infections for each paralytic case, indicating widespread infection. Widespread vaccination therefore seemed an appropriate strategy to tackle the spread of the disease.

Researchers were keen to find a method to culture poliovirus to facilitate development of a vaccine. Chicken eggs had been used to grow other viruses (MILESTONE 7); however, attempts to grow polio in chicken eggs were unsuccessful. Albert B. Sabin and Peter K. Olitsky grew a monkey-adapted strain of poliovirus (the MV strain) in fragments of human embryonic brain. However, there were concerns that such a culture system would not be suitable for growing large volumes of poliovirus for use as a vaccine. The major pathological features of patients with polio were of central nervous system origin and there was concern vaccine recipients could be at risk of central nervous system damage. Thomas H. Weller and John F. Enders had previously cultured mumps virus in non-neural tissue growing in test-tubes by suspending fragments of chick amniotic membrane in a salt solution containing components of ox serum. The amount of haemagglutinin, which is produced by the virus, could be measured in the culture fluid following inoculation of measles virus, providing a method to monitor production of virus. They found that by replacing part of the medium regularly they could maintain viability of the cells whilst growing and harvesting virus. Funded by the National Foundation for Infantile Paralysis, they joined forces with Frederick C. Robbins to attempt to grow the human Lansing strain of human poliomyelitis virus in skin, muscle and connective tissue removed from human fetal arms and legs. In the *Science* paper in which their results were published, they reported maintenance of these cultures for 67 days. Over 52 days of culture they calculated at least 1,016 more virus particles were obtained than had been initially inoculated into the culture.

In addition, intracerebral inoculation of fluid from the cultures produced paralysis in mice and two rhesus monkeys. Microscopic examination of the spinal cords of the monkeys revealed lesions similar to those seen in humans with poliomyelitis. Earlier epidemiological studies had suggested the human intestine was also capable of producing large amounts of virus, excreted in the faeces. This was confirmed by production of large quantities of virus in cultures of fetal intestinal tissue that also induced polio-like symptoms in mice. The authors had succeeded in their aim to culture poliovirus in cells other than neurons. They also noticed that the morphology of cells grown in the presence of virus differed from their control cultures and concluded that the virus could be reducing the viability of the cells.

Long-term culture in different human tissues and in different culture conditions provided a starting point for the development of differing, attenuated strains of virus. It also enabled production of large quantities of virus, paving the way for Salk to produce a successful polio vaccine for human use (MILESTONE 10). The importance of the methodological advance of Enders, Weller and Robbins was recognized by the award of the 1954 Nobel Prize in Physiology or Medicine. Also following the successful introduction of polio vaccination, the funding body that funded their research, the National Foundation for Infantile Paralysis, was able to change its name to the March of Dimes and continues to this day to fund research to improve the health of babies.

Katharine Barnes, *Nature Protocols*

ORIGINAL ARTICLE Enders, J. F., Weller, T. H. & Robbins, F. C. Cultivation of the Lansing strain of poliomyelitis virus in cultures of various human embryonic tissues. *Science* **109**, 85–87 (1949)
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