

AN EVIL MAN AND A THIEF, OR A PIONEER WORKING FOR A BETTER TOMORROW?

As he turns 90, **Leonard Hayflick** talks to **George F Winter** about his life.

Professor Leonard “Len” Hayflick was the first person to isolate *Mycoplasma pneumoniae*; the first person to develop cell strains of normal human fibroblasts; and in 2014 was co-recipient of the City of Philadelphia John Scott Award for his discoveries in ageing, an award “given to the most deserving men and women whose inventions have contributed in some outstanding way to the comfort, welfare and happiness of mankind”.

Yet Leonard Hayflick was described as “evil” by the Vatican.

Currently Professor of Anatomy at the University of California, San Francisco, Leonard celebrated his 90th birthday on

20 May this year. Here, he reflects on his life and times; from his early scientific development, to working in the “golden age of virology”, to overthrowing dogma and courting controversy.

Early days

“My sister and I were raised in a terraced house in Philadelphia, Pennsylvania, during the Great Depression, so times weren’t easy. Aged nine, my scientific interest was aroused when an uncle bought me a chemistry set.” With Leonard and a friend exploring the chemistry of explosives, they acquired sodium. “There was a concreted area behind our house with holes for clothes-drying racks,” he recalls. “One rainy day

A CONTROVERSIAL LIFE

Starring
microbiologist

**LEONARD
HAYFLICK**

we threw small cubes of sodium into the holes. A minute later there was an explosion, and an orange flame shot out.”

After high school, in January 1946, Leonard was accepted to study at the University of Pennsylvania, and after 18 months of army service he majored in microbiology.

University and the Wistar Institute

Leonard’s career was shaped when his adviser at university, Warren Steinbring, took a course in cell culture at a hospital in New York State. “Under Steinbring I grew chick embryo tissue into which I introduced what at that time were called pleuropneumonia-like organisms (PPOs). Today they’re called mycoplasmas. As a master’s degree student, I attended the Wistar Institute – on the University of Pennsylvania campus – the oldest biological research institute in the US. The Director was Hilary Koprowski – who I’d describe as a benevolent dictator – and it’s a little-known fact that Koprowski was the first to produce and test a live poliovirus vaccine, contrary to the popular belief that it was Albert Sabin.”

Gaining his doctorate in 1956, Leonard accepted a post at the University of Texas: “We travelled to Galveston, my wife pregnant with the first of our five children. I worked in the microbiology department with the renowned cell culturist Charles Pomerat, who pioneered time-lapse photography, and I also met a cytogeneticist called Paul Moorhead, who played an important role in my career.”

Golden age of virology

In 1958, Leonard returned to the Wistar Institute, running its cell culture laboratory. “I entered the field when cell culture was enjoying a renaissance, with the subsequent decade often called the golden age of virology.” Leonard’s daughter played an important role too. “Because my laboratory was close to the University of Pennsylvania Hospital,



Pictured: Leonard Hayflick holds a cell culture tube by a liquid nitrogen refrigerator in his lab at Stanford University, California, in November 1971.

where my wife gave birth to our third child, Susan, I arranged with the obstetrician to obtain the placenta and amnion, which I cultured. After several weeks I noticed that these normal amnion cells had converted to an immortal cell population. I named the cell line WISH (Wistar Institute Susan Hayflick), which became popular.”

Human diploid cell lines

Now investigating the role of viruses in human cancers, Leonard sought normal human cells that could be cultured, and began working with aborted fetal tissue from the University of Pennsylvania Hospital, establishing a series of fetal diploid cell strains WI-1 to WI-25. The scientific dogma was that cultured cells, if properly treated, would replicate forever. But Leonard – now working again with Paul Moorhead – found that after about 50 population doublings, the cells

replicated more slowly before stopping, having reached what is now known as the Hayflick Limit. “I discovered that, upon freezing, and after reconstitution, the normal human fetal cells retained their ‘memory’ of what population doubling level they had attained upon freezing and, when thawed months or years later, replication continued until the maximum total of about 50 population doublings was reached. This suggested that normal human cells have a mechanism for counting DNA replications, an idea later confirmed by the 2009 Nobel laureates, who discovered the molecular basis [telomere attrition] for my findings.”

Overturing dogma

Would Leonard and Paul Moorhead confront established dogma? “Paul and I were young men, starting our careers. George Gey, the man who established the HeLa cell line, warned, ‘Lenny, you’re

going to get yourself in a lot of trouble if you publish this.’ Furthermore, the publicity-seeking surgeon Alexis Carrel, working at the Rockefeller Institute in New York, claimed he had cultured cells from a chick heart for almost forty years, contradicting my observations. But we thought that Carrel had erred.”

Leonard played a masterstroke. “I contacted three top cell culture experts – including Harry Eagle, whose name is given to a widely used media formulation – and they agreed to grow my cells. After six months, they phoned: ‘Len, the cultures you sent us have stopped dividing.’ Well, I figured that if I’m wrong and go down in flames, I’ll have important company.”

Leonard and Paul submitted their paper to the *Journal of Experimental Medicine*, who rejected it, with future Nobel laureate Peyton Rous insisting that cells cultured properly *in vitro* would replicate indefinitely. “That was precisely the

dogma we thought we’d torpedoed,” recalls Leonard.

However, their paper was published, without alteration, in *Experimental Cell Research*, prompting a surge of interest in the biology of cellular ageing and becoming one of the most cited papers in biomedical science.

Then disaster. “My recollection,” says Leonard, “is that while the paper was on press our electrical freezer failed, and we lost everything. So I started another cell population called WI-26, since the first 25 were named WI one through 25. The WI-26 cell line came from a male fetus and it was widely circulated, nationally and internationally.” In 1962, Leonard, in collaboration with the UK’s Common Cold Research Unit, described a new rhinovirus isolated in WI-26 cells.

Ageing

“The suggestion I made,” recalls Leonard, “that the finite lifetime of cultured normal cells relates to ageing, was dismissed by many as foolish, but it’s been confirmed thousands of times since, worldwide. The relationship that this work had to ageing was then picked up by many people worldwide. However, those working in the field of the biology of ageing in the 1960s were outside the scientific mainstream.”

In 2007, Leonard referred to a prevailing belief that resolving age-associated diseases will advance our understanding of the fundamental ageing process. “It will not. The distinction between disease and ageing is also critical for establishing science policy because although policy makers understand that the funding of research on age-associated diseases is an unquestioned good, they must also understand that the resolution of age-associated diseases will not provide insights into understanding the fundamental biology of age changes.”

Interestingly, with Leonard now aged 90, he revealed that his mother lived to the age of 106 years.

LEONARD HAYFLICK IN QUOTES

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Had I patented the cells, I might own the world's supply of WI-38, which would make me sufficiently wealthy to buy London, I think

Mycoplasma pneumoniae

With news of the sensitivity of Leonard's WI diploid cell strains to human viruses spreading, a researcher named Robert Chanock, at the National Institutes of Health (NIH), Bethesda, Maryland, visited the Wistar Institute and told him that Monroe Eaton was working at Harvard with his eponymous "Eaton agent", believing that it caused primary atypical pneumonia. "I said to Bob," Leonard remembers, "have you and Eaton considered PPLOs? And Bob said, 'What are they?' Well, he educated me about Eaton agent, I educated him about PPLOs and we decided that Bob would send me embryonated egg yolk in which this organism grew, and I would test it for mycoplasmas."

While Leonard was undertaking this work, his boss Hilary Koprowski appeared in the laboratory one day: "He was worried that I would spread mycoplasmas, and said 'Len, I didn't hire you to work on mycoplasmas. I hired you to work on cell cultures, and I'd appreciate it much if you stopped your *Mycoplasma* work.' I disobeyed him. My isolation and identification of the new *Mycoplasma* - *Mycoplasma pneumoniae* - was published in *Proceedings of the National Academy of Sciences* and because it was an important discovery, it made the front page of *The New York Times*."

WI-38 cell line

In 1962, following a legal abortion at a Swedish hospital, the lungs from a female fetus were flown from Stockholm to Leonard's laboratory, where he established the WI-38 cell line of the first "normal" human cells to provide licensed human virus vaccines against

poliomyelitis, measles, mumps, rubella, varicella, shingles, adenovirus, rabies and hepatitis A.

His discovery happened to be made at the same time that primary monkey kidney cells used to manufacture poliomyelitis vaccines were found to be contaminated with simian viruses.

Last year, a report estimated that the number of cases treated or averted with WI-38-related vaccines was 4.5 billion globally. But in a letter from the Vatican dated 9 June 2005, Cardinal Elio Sgreccia questioned the ethics of parents using such vaccines on their children: "Would it not be a matter of true (and illicit) cooperation in evil, even though this evil was carried out 40 years ago?" In this context, it is perhaps noteworthy that the "evil" rubella vaccine has prevented many abortions worldwide.

Stakeholder controversy

But that was not the only controversial aspect of Leonard's WI-38s. "The cells," he says, "couldn't be patented because the patent laws in the US and most other countries prevented patenting of living materials. So I delivered ampoules of WI-38 worldwide freely to most virus vaccine manufacturers. For instance, I set up with Dr Frank Perkins in London a repository for WI-38s that I brought in a liquid nitrogen container on board an aircraft, which is impossible today. The aluminium vessel looked like a 500-pound bomb."

Meanwhile, Leonard was unaware that Koprowski had arranged with Wellcome Laboratories that in return for providing WI-38 cells, royalty payments would be made to support members of the Wistar

Institute. "Technically I wasn't a full member of the Institute," notes Leonard, "so to learn that my efforts would yield returns only to full members of the Wistar and not to me was bothersome. I decided to leave, accepting a professorship at Stanford University, California."

But what about Leonard's WI-38 ampoules? "I decided to take all the ampoules to Stanford until this matter was resolved, so that all stakeholders could have a say. The stakeholders were Paul Moorhead; the Wistar; the WI-38 embryo's estate; myself; and although the government may have had a stake, that remained undetermined. It couldn't be a decision made to favour a single stakeholder, so I put all my several hundred ampoules into a liquid nitrogen container and strapped it to the back seat of my Pontiac sedan. Two of my children sat next to it, and the third in the front seat, and off we went to California."

Now charging the same amount as the American Type Culture Collection to ship WI-38s to scientists worldwide, Leonard's untouched "Cell Culture Fund" amounted to a total of \$66,000 by the mid-1970s, when an accountant from the NIH, with no biomedical background, examined his documentation and accused Leonard of having stolen the WI-38s which the accountant said belonged to the federal government.

Following seven years of litigation, with Leonard's career on hold, an out-of-court settlement was reached. "The Cell Culture Fund, now worth \$90,000, was awarded to me and it all went deservedly to my lawyers, and I became the sole scientist in the US legally entitled to ownership of the cells. Had I patented the cells, I might own the world's supply of WI-38, which would make me sufficiently wealthy to buy London, I think." **BMS**

