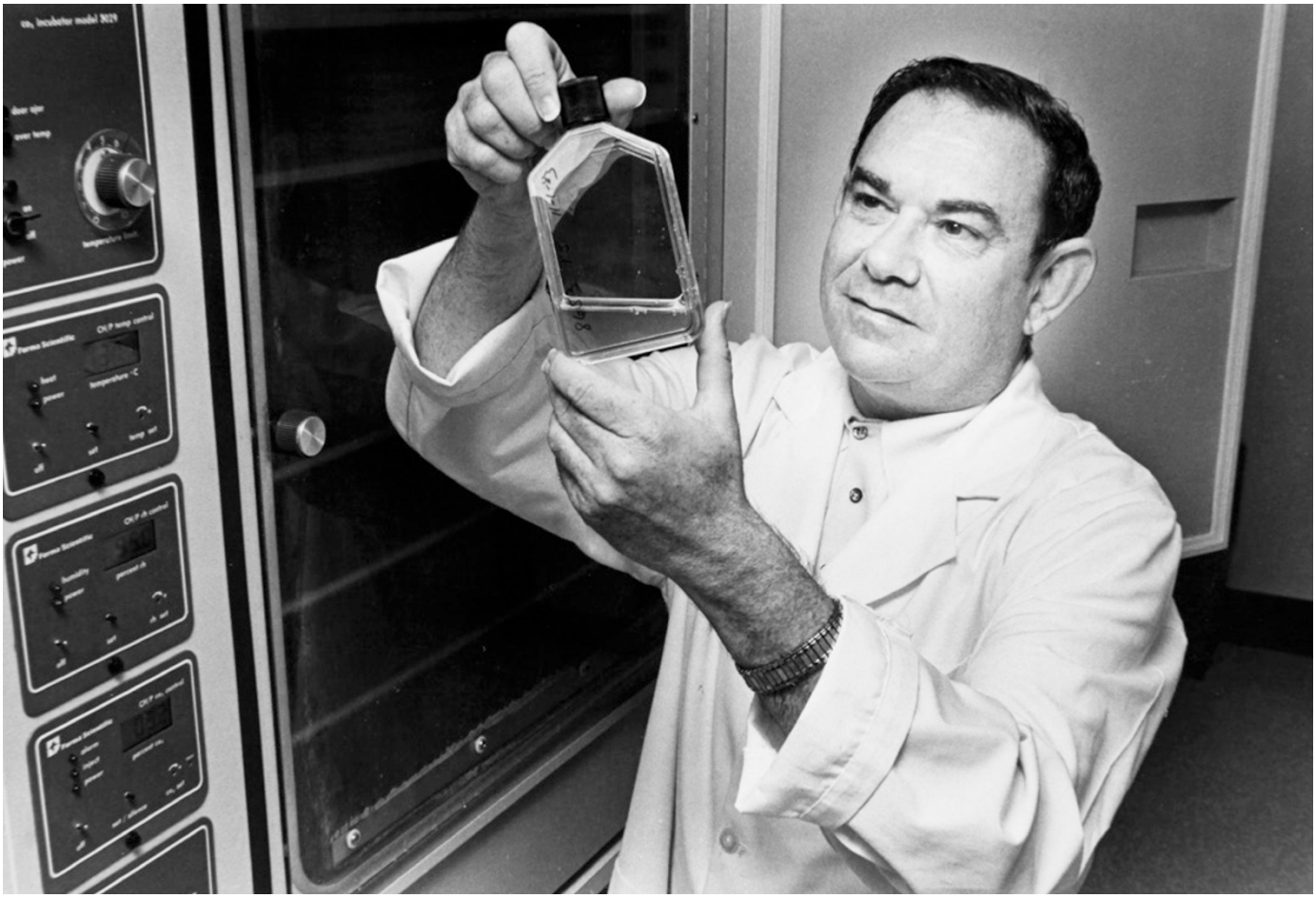

COURTESY OF LEONARD HAYFLICK

Leonard Hayflick, pictured in 1982, inspects WI-38 cells that he derived from aborted fetal lungs. The cells have been used to produce vaccines in use worldwide.

Cell division

In 1962, Leonard Hayflick created a cell strain from an aborted fetus. More than 50 years later, WI-38 remains a crucial, but controversial, source of cells.

BY MEREDITH WADMAN

The woman was four months pregnant, but she didn't want another child. In 1962, at a hospital in Sweden, she had a legal abortion.

The fetus — female, 20 centimetres long and wrapped in a sterile green cloth — was delivered to the Karolinska Institute in northwest Stockholm. There, the lungs were dissected, packed on ice and dispatched to the airport, where they were loaded onto a trans-atlantic flight. A few days later, Leonard Hayflick, an ambitious young microbiologist at the Wistar Institute for Anatomy and Biology in Philadelphia, Pennsylvania, unpacked that box.

Working with a pair of surgical scalpels, Hayflick minced the lungs — each about the size of an adult fingertip — then placed them in a flask with a mix of enzymes that fragmented them into individual cells. These he transferred into several flat-sided glass bottles, to which he added a nutrient broth. He laid the bottles on their sides in a 37 °C incubation room. The cells began to divide.

So began WI-38, a strain of cells that has arguably helped to save more lives than any other created by researchers. Many of the experimental cell lines available at that time, such as the famous HeLa line, had been grown from cancers or were otherwise genetically abnormal. WI-38 cells became the first 'normal' human cells available in virtually unlimited quantities to scientists and to industry and, as a result, have become the most extensively described and studied normal human cells available to this day.

Vaccines made using WI-38 cells have immunized hundreds of millions of people against rubella, rabies, adenovirus, polio, measles, chickenpox and shingles. In the 1960s and 1970s, the cells helped epidemiologists to

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identify viral culprits in disease outbreaks. Their normality has made them valuable control cells for comparison with diseased ones. And at the Wistar Institute, as in labs and universities around the world, they remain a leading tool for probing the secrets of cellular ageing and cancer.

"Here's a clump of cells that has had an enormous impact on human health," says Paul Offit, chief of the division of infectious diseases at the Children's Hospital of Philadelphia. "These cells from one fetus have no doubt saved the lives of millions of people."

Few people, however, know the troubled history of the cells — one that may offer lessons for modern researchers seeking to work with human tissues. Six years after deriving his famous strain, Hayflick made off with stocks of the cells and later started to charge for shipping them, prompting an epic legal battle with the US National Institutes of Health (NIH) in Bethesda, Maryland, about who owned the cells. That struggle nearly destroyed Hayflick's career and raised questions about whether and how scientists should profit from their inventions.

What's more, the WI-38 strain has helped to generate billions of dollars for companies that produce vaccines based on the cells, yet it seems that the parents of the fetus have earned nothing. That recalls the earlier development of the HeLa cell line, named after the woman whose tumour gave rise to the cells and chronicled in Rebecca Skloot's book *The Immortal Life of Henrietta Lacks* (Crown, 2010). As with HeLa, the WI-38 case highlights questions about if, and how, tissue donors should be compensated that are still urgently debated today. Last month, for example, some scientists in the United States found themselves barred from using new stem-cell lines derived from human embryos because women had been paid for the eggs used to make them (see *Nature* http://doi.org/mv2; 2013).

The story of WI-38, unlike that of HeLa, also has its own controversial twist because it was derived from an aborted fetus. For 40 years, anti-abortion activists have protested against the use of WI-38 and vaccines developed from it. "It's still a live issue," says Alta Charo, a professor of law and bioethics at the University of Wisconsin Law School in Madison. "We still have people who refuse to take these vaccines because of their origins in fetal tissue."

SEEKING CELLS

When Hayflick opened up that icy package from Sweden in 1962, he was working at the vanguard of virus research in the United States. At the time, the Wistar Institute was led by Hilary Koprowski, a polio-vaccine pioneer who hired Hayflick to run the centre's cell-culture laboratory and supply cells to researchers. But Hayflick also began investigating whether some human cancers might be caused by viruses. To do so, he needed a resource that did not yet exist: verifiably normal human cells that could be reliably grown in the lab. Fetal cells, he thought, were an ideal candidate, because they were less likely to have been exposed to viruses than adult cells.

Although abortions were technically illegal in Pennsylvania at the time, they were still performed when doctors said they were medically necessary. Hayflick says he was able to obtain fetuses straight from the operating room of the University of Pennsylvania Hospital across the street from Wistar. Unless the tissue was put to some use, he reasoned, "it was definitely going to end up in an incinerator". The University of Pennsylvania says that it is unable to find records to confirm the source of fetal tissues used by Hayflick.

Hayflick developed 25 different fetal-cell strains, numbered WI-1 to WI-25. But several months into the project, he began to notice something strange. Scientific orthodoxy held that cells in culture, properly treated, would replicate forever. But his oldest cell strains were beginning to replicate more slowly. Eventually, they stopped dividing altogether.

In 1961, Hayflick and his colleague Paul Moorhead published a paper[1] that would become one of the most cited publications in biology. Entitled 'The serial cultivation of human diploid cell strains', it showed that normal fetal cells stop replicating after about 50 population doublings. The paper launched a new field: the study of cellular ageing. And the wall that the cells hit — which was later found to arrive much earlier for adult cells, which have already divided many times[2] — became known as 'the Hayflick limit'.

Crucially, Hayflick and Moorhead also showed that the fetal cells remained viable after months in the freezer and that, once thawed, they would 'remember' how many replications they had been through and would pick up where they left off. "It's apparent," the authors wrote, "that by freezing cells at each subcultivation, or every few subcultivations, one could have cells available at any given time and in almost limitless numbers." What's more, the pair's cells turned out to be easy to infect with a broad range of human viruses, suggesting that they would be perfect vehicles in which to grow viruses for vaccines.

> *"These cells from one fetus have no doubt saved the lives of millions of people."*

Hayflick decided to derive a fetal cell strain that he hoped would become both a ubiquitous laboratory resource and a substrate for industrial-scale vaccine manufacturing. He had support: in February 1962, the National Cancer Institute awarded Wistar, with Hayflick as co-principal investigator, a contract "to produce, characterize, store and study human diploid cell strains and to distribute such cell strains to all qualified investigators".

SUCCESSFUL STRAIN

By this time, Hayflick had turned to a different source for his fetal tissues: Sven Gard, chairman of the department of virology at the Karolinska Institute in Sweden, where abortion was legal. In June 1962, Hayflick received the set of lungs that would give rise to WI-38. He cultured the cells for weeks, splitting them when they covered the bottom of a bottle, so that two bottles became four, four became eight and so on. By the time the original cell population had doubled nine times, there were hundreds of bottles.

On 31 July, in a marathon session for which he recruited a small army of technicians, Hayflick dispensed the cells into more than 800 tiny glass ampoules, sealing each one with a quick pass through the flame of a Bunsen burner. Later, he transferred the precious ampoules to a liquid-nitrogen freezer in the Wistar's basement.

A year later, Hayflick received information from Sweden assuring him that the mother of the fetus and her family were free of cancer and hereditary diseases, something vaccine manufacturers would want to know. Although there is some indication that the mother consented to use of the tissue, *Nature* does not know for sure that she did. Swedish law at the time did not require such consent and, says Niels Lynöe, professor of medical ethics at the Karolinska Institute, "research ethical awareness in Sweden as well as in the US was rather low", before the Helsinki declaration, a statement of human research ethics adopted by the World Medical Association in 1964. In Sweden, "research material like tissues from aborted fetuses were available and used for research without consent or the knowledge of patients for a long time", both before and after consent rules were tightened later in the 1960s, says Solveig Jülich, a historian of medicine at Stockholm University.

Armed with the ampoules, Hayflick now launched WI-38 on its

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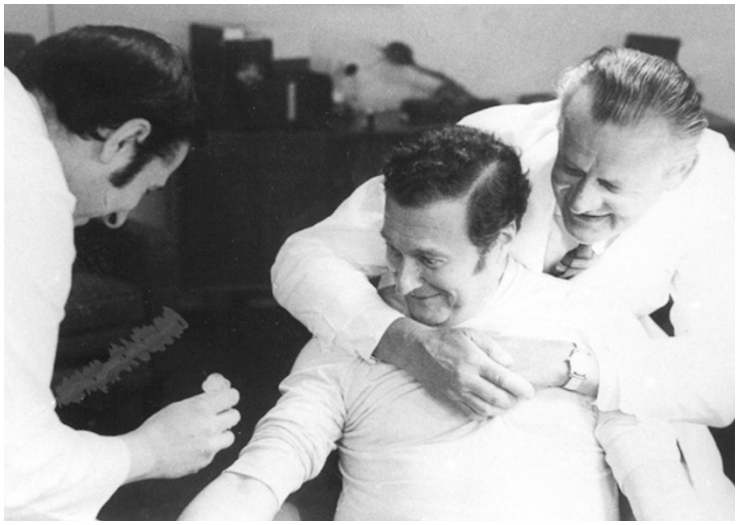
march around the globe. During his frequent flights abroad, he often toted a small liquid-nitrogen freezer bearing WI-38 ampoules. In this way, he hand-delivered the cells to colleagues in London, Moscow, Leningrad and Belgrade. He also mailed out hundreds of 'starter' cultures grown from the ampoules. Scientists were hungry for the cells in part because they were a cheap, plentiful model for studying the fundamental biology of normal human cells — and soon papers began to appear, probing everything from the cells' respiration[3] to their constituent fatty molecules[4].

WI-38 found a greater use in virology, where the ease of infecting the cells with a panoply of human viruses quickly made the strain an important virus-identification tool. In 1967, the cells became a workhorse in a World Health Organization survey of viruses causing lower respiratory tract infections in hospitalized children on four continents.

Hayflick also supplied WI-38 liberally to aspiring vaccine-makers. One was Stanley Plotkin, a Wistar scientist and a physician who had seen at first hand the effects of the huge rubella epidemic that swept the United Kingdom and the United States in the early 1960s. Rubella can be devastating to fetuses whose mothers are infected: those that are not killed *in utero* are frequently born blind, deaf, mentally disabled or with some combination of these conditions.



WISTAR INST.

Hilary Koprowski, director of the Wistar Institute, is inoculated by Stanley Plotkin with rabies vaccine developed using WI-38, in 1971.

Working at the Wistar, Plotkin grew rubella in WI-38 at 30 °C, cooler than body temperature, creating a weakened strain that still fired up the immune system enough to protect against future infections. Trials showed that his vaccine induced better immunity against rubella than competitors[5]. Plotkin's vaccine was licensed in Europe in 1970 and in the United States in 1979. A version made by the pharmaceutical company Merck, based in New Jersey, is today the only rubella vaccine available in the United States, and GlaxoSmithKline uses Plotkin's weakened virus in a rubella vaccine that it markets in Europe and Australia.

The rubella vaccine was only one of many made using WI-38. In the 1960s, a WI-38-based measles vaccine was licensed in the former Soviet Union and Koprowski developed a rabies vaccine using the cells. In the early 1970s, the pharmaceutical company Wyeth (now part of Pfizer) launched an oral adenovirus vaccine developed using WI-38 and Pfizer, based in New York, used WI-38 to make a vaccine against polio. Today, the cells are also used by Merck to make vaccines against chickenpox and the painful nerve infection shingles.

SENSE OF EXCLUSION

Despite his groundbreaking paper and the growing prominence of WI-38, Hayflick felt like a second-class citizen at the Wistar Institute. He was never promoted to a full member, and he believed that Koprowski, much as he publicly bragged about WI-38, saw him as more of a technician than a scientist. (Koprowski died last April.)

Hayflick's simmering sense of exclusion boiled over when one day, Hayflick says, he learned that Koprowski had offered a guaranteed supply of WI-38 to the British drug-maker Burroughs Wellcome (one of the companies that merged into GlaxoSmithKline), along with Hayflick's cell-culture technology for producing live polio vaccine[6], all in exchange for royalties to the institute. Hayflick says that he was shocked that Koprowski intended the institute to profit from WI-38 and believes that it had kept him in the dark.

Hayflick found a new job as a professor of medical microbiology at Stanford University in California, to start in July 1968. In January that year, he met to discuss the fate of the 370-odd remaining WI-38 ampoules with Koprowski and representatives from the NIH and the American Type Culture Collection (ATCC), then in Rockville, Maryland, a non-profit organization that distributes cell cultures. The participants agreed that Hayflick could take ten ampoules of WI-38 with him to Stanford, and that ten would stay at the Wistar. The rest would remain the property of the NIH's cancer institute and were to be transferred to the ATCC, which would handle distribution from that point on.

Hayflick was troubled by the plan, which he says he felt under pressure to sign. And he felt a sense of injustice. Companies, and the Wistar, he now believed, were profiting from cells he had created and handed to them freely. "To then have [them] descend on what I had struggled so hard to give value to, and try to take it for their own benefit," he says. "I think that an average person would be capable of understanding why I was — to put it mildly — concerned." The Wistar Institute says that it acted ethically in conducting research that led to the development of WI-38 and that it received royalties from licensed vaccines grown in WI-38 cells but not from licensing the cells.

At some point after that January meeting, Hayflick made a quiet trip to the Wistar basement and packed all the WI-38 ampoules into a portable, 30-litre liquid-nitrogen tank. In June 1968, he strapped the container into the back seat of his green Buick LeSabre next to two of his children, and motored to California. "I just absconded with the cells," Hayflick says with a wry smile.

Once in Stanford, Hayflick began charging for many of the WI-38 cultures that he was sending out to hundreds of scientists who were still asking for them. His fee was US$15 — the same amount charged by the ATCC for cell shipments — and he banked the money in an account he called 'Cell Culture Fund'. By May 1975, he had accrued more than $66,000.

Hayflick was determined, he says, to keep the funds in a separate account until some independent legal authority could determine who owned the cells. The issue didn't come up until the spring of 1975, when he was interviewed at the NIH as a candidate to direct its new National Institute on Aging. The NIH decided to turn to its Division of Management Survey and Review, an office that investigated allegations of mismanagement of NIH funds. It sent three accountants to Hayflick's Stanford lab, where they spent days going over records and assessing his inventory of WI-38.

Their report became public in March 1976, when the NIH provided it under the Freedom of Information Act (FOIA) to several journalists. Accounts of its contents soon appeared in *Science* and on the front page of *The New York Times*. "Within 24 hours my career was in the sewer," Hayflick says. The report said that Hayflick had sold "the property of the United States Government" and banked the money; that the WI-38 ampoules had been poorly accounted for; and that some ampoules were contaminated with bacteria. Hayflick strongly

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disagrees with the report. He says that no legal decision gives the government title to WI-38; that he sequestered the funds received for preparing and shipping WI-38 in an account until ownership could be established; and that no evidence has ever been provided for the assertion of mismanagement. Hayflick explains that, contrary to common practice in 1962, he had not laced the cells with antibiotics at the outset because vaccine manufacturers feared allergic reactions to the drugs.

Shortly before the *Science* article[7] was published, Hayflick sued the NIH. He argued that the agency had violated the 1974 Privacy Act by making his name and the allegations against him available under the FOIA without including his rebuttal. He also sued for title to WI-38 and its proceeds. By then, Hayflick was also facing a criminal investigation: Stanford University had alerted local prosecutors that the case could be one of criminal theft of government property. (The prosecutors subsequently found no grounds for criminal investigation and dropped the case.) Meanwhile, some vaccine manufacturers, fearing that there would not be enough stock of WI-38 to meet future needs, switched much of their work to an alternative fetal cell strain, MRC-5.

Hayflick resigned from Stanford in February 1976 and was soon in an unemployment line collecting $104 a week. Not only was he jobless, he was without the cells that he described to *Science* that spring as "like my children". The NIH had taken them from his lab while he was at a conference the previous year.

CHANGING TIMES

Some months later, Hayflick landed a job across the San Francisco Bay at the Children's Hospital, Oakland, and sought to revive his research on ageing. In 1977, peer reviewers approved his application for a three-year NIH grant and, after a lengthy fight with the NIH to get both the funding and some WI-38 cells, in January 1981 he received six of the original ampoules of cells.

One month earlier, the Bayh–Dole Act had become law, giving institutions the right to claim title to inventions made using government funds, as long as they gave the inventors a piece of the royalties. Hayflick's invention predated the law, but the new mindset that Bayh–Dole represented made it harder for the government to justify the continued legal fight over WI-38, which by then had stretched on for nearly five years. In summer 1981, the Department of Justice wrote to Hayflick's lawyers, offering to settle the lawsuit out of court, and Hayflick assented. With both sides agreeing that the issues were in reasonable dispute, and neither side admitting liability, the settlement allowed Hayflick title to the six original WI-38 ampoules now in his possession, and to their progeny. The government would retain title to the 19 original ampoules in its hands. As for the proceeds from his sales of WI-38, which, with interest, had grown to around $90,000, Hayflick would keep it. He spent it all, he says, and more, to pay his lawyers; he has never profited financially from WI-38, he says.

Scientists, meanwhile, were continuing to benefit academically from the cells. By the mid-1980s, thanks to revolutionary new tools in molecular biology, WI-38 was helping them explore everything from gene expression in human leukaemias[8] to the effects of the just-cloned tumour necrosis factor[9], an important immune regulatory protein.

> *"We still have people who refuse to take these vaccines because of their origins in fetal tissue."*

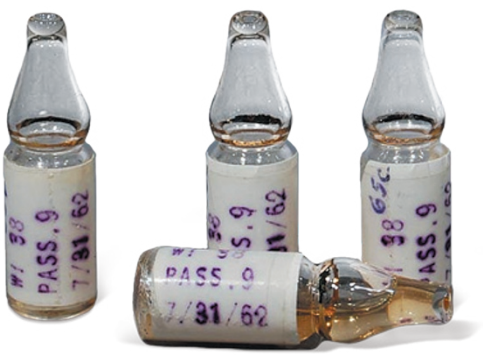
The cells have played "a very critical role in studying cellular senescence," adds Rugang Zhang, who works in this field at the Wistar Institute. That's because they so reliably stop replicating after about 50 divisions and because scientists have, over time, built up a wealth of knowledge about the reasons why. In the 1990s, for instance, WI-38 was used to discover the most widely used marker of cellular senescence[10]. More recently, Zhang's team used the cells to discover a pathway by which the complex of DNA and proteins known as chromatin controls cell proliferation[11].

But the controversies surrounding the cells have rumbled on. Back in July 1973, Hayflick received a call at home from a senior medical officer at NASA. Skylab 3 had taken off several hours earlier from the Kennedy Space Center in Florida, bound for the Space Station. The NASA physician was contending with anti-abortion demonstrators who were protesting about the presence aboard of WI-38 cells, which were going to be used to detect the effects of zero-gravity on cell growth and structure. Once Hayflick explained that the abortion from which the cells were derived had occurred legally in Sweden, the physician said that he would defuse the situation — but concerns among anti-abortionists about WI-38 have lasted to this day.

"Other vaccines are produced in a completely morally non-objectionable way. So why aren't we doing this with all vaccines?" says Debi Vinnedge, the executive director of Children of God for Life, a group based in Largo, Florida, that opposes the use of WI-38 in vaccine-making. In 2003, Vinnedge wrote to the Vatican asking for an official position on whether Catholics could ethically receive vaccines made using cells from aborted fetuses. She waited two years for an answer. The letter, when it came, concluded that where no alternative exists, it is "lawful" for parents to have their children immunized with vaccines made using WI-38 and MRC-5, to avoid serious risk to their own offspring and to the population as a whole.

Still, the Vatican wrote, faithful Catholics should "employ every lawful means in order to make life difficult for the pharmaceutical industries" that use such cells. Merck, a major producer of Plotkin's rubella vaccine, has been a perennial target of abortion opponents, who have pressed the issue at Merck's US shareholder meetings. (Merck said in a statement to *Nature* that "it would be exceedingly difficult, if at all possible, to develop and test an alternative", and emphasized the vaccine's long record of safety and effectiveness.) The irony of the protest is not lost on Plotkin. "I am fond of saying that rubella vaccine has prevented thousands more abortions than have ever been prevented by Catholic religionists," he says.

Profits from Merck's rubella vaccine represent a big slice of the billions of dollars that have been made from products that have involved the use of WI-38. Among the other companies that have made money from WI-38 are Barr Laboratories (now part of Teva Pharmaceuticals, based in Petach Tikva, Israel), which today makes the adenovirus vaccine given to all US military recruits, and Sigma Aldrich in St Louis,



LEONARD HAYFLICK

Some original glass ampoules of WI-38 cells, created in 1962.

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RAMIN RAHIMIAN/GETTY IMAGES

Leonard Hayflick today at his house in Sea Ranch, California. "We all owe a moral debt to the tissue donors," he says.

Missouri, which charges $424 in the United States for a vial of the cells.

Legal experts say it is unlikely that the parents of the fetus, or their heirs, would have any legal grounds to demand compensation for tissue collected over 50 years ago. At the time that WI-38 was derived, use of tissue without consent was routine in the United States, as it was in Sweden. Under current rules, researchers supported by US government grants are free to make use of surgically removed tissue — including aborted tissue — that has been stripped of its identifiers, without consent. However, some states have stricter rules.

But, says Charo, "if we continue to debate it entirely in legal terms, it feels like we're missing the emotional centre of the story". It could be argued, she says, "that if somebody else is making a fortune off of this, they ought to share the wealth. It's not a legal judgment. It's a judgement about morality."

The scientists and academic institutions that have worked with WI-38 and that commented for this story say that they do not see their work on the cells as unethical, in part because of the standards that existed at the time the cell strain was created. It is unfair, say some, to examine past acts by today's more stringent ethical expectations. "At the time [the fetus] was obtained there was no issue in using discarded material," says Plotkin. "Retrospective ethics is easy but presumptuous." Most companies in this story declined to comment; GlaxoSmithKline says that it is committed to upholding high ethical standards.

Regarding the situation today, Scott Kominers, a research scholar at the Becker Friedman Institute at the University of Chicago, Illinois, argues that offering donors a share in future profits from their tissues could encourage them to donate and fuel medical progress[12]. "We think that if you offer some sort of value-based compensation you'd be likely to boost tissue supply," he says. But Steven Joffe, a paediatric oncologist who directs the ethics programme at Harvard's translational medicine centre in Boston, Massachusetts, is concerned that compensating donors may paradoxically decrease their willingness to donate tissues, by taking altruism out of the equation. What's more, he says, the one-to-one relationship of WI-38, or of HeLa, to a donor, is rare. Far more often, modern medical products — such as therapeutic proteins extracted from donated blood — come from many samples combined. In these cases, he says, "trying to account for all these multiple holders of rights to income streams would just bring science to a standstill".

If nothing else, the WI-38 story highlights the benefits of discussing the issues of compensation and consent with tissue donors at the outset. In the case of WI-38, suggests Charo, returning to the donor now, even with an offer of compensation, "may also be a way of reopening an experience that may for her have been painful. You have to be careful."

Hayflick argues that there are at least four stakeholders with title to WI-38 or any human cell culture: the tissue donors, the scientists whose work gave it value, the scientists' institution and the body that funded the work. "Like me", he adds, "hundreds of other scientists had their careers advanced using WI-38 and other human cell cultures so we all owe a moral debt to the tissue donors."

Now 85 and regarded as a grand old man of ageing research, Hayflick hung onto his ampoules of WI-38 for decades, keeping them for many years in the garage of his home in California. But in 2007, weary of monthly treks to collect fresh liquid nitrogen, he donated them to the Coriell Institute in Camden, New Jersey, which, he says, he trusts to bank them safely.

In the end, he says, letting the cells go was no more traumatic than launching his own five biological offspring into the world: "It was about time that my 'children' — now adults — should leave home." ■

Meredith Wadman *is* Nature*'s biomedical reporter in Washington DC.* SEE EDITORIAL P.407.

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