Hayflick's Tragedy: The Rise and Fall of a Human Cell Line
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Hayflick's Tragedy: The Rise and Fall of a Human Cell Line

Stanford, California. A personal disaster which also raises problems of international diplomacy and public health has suddenly crashed about the head of the distinguished biologist Leonard Hayflick. Though Hayflick may yet be able to step clear of the debris, he has already, by his own decision to resign under threat of dismissal, lost his job as professor of microbiology at the Stanford University Medical Center. He is also the subject of a National Institutes of Health investigation which, unless he can refute its charges, as he says he can, could have severe repercussions on his reputation as a scientist.

The central issue in what remains a highly confused situation is Hayflick's stewardship of an important human cell line. The cells are increasingly being used for the manufacture of vaccines, as well as for research purposes. On the assumption that stocks would last for the next several decades, millions of dollars have been invested by vaccine authorities throughout the world in testing the cells' safety and suitability. Yet it now appears that there are sufficient stocks only for the next several years. Moreover, many of the surviving ampules which NIH authorities decided to remove from Hayflick's laboratories last August are proving to be contaminated with bacteria, a fact which may make them unsuitable for vaccine use and render the supply situation even more acute.

Serious issues about Hayflick's handling of the cells are raised in a report completed by NIH management accountant James W. Schriver on 30 January and released to *Science* last week under the Freedom of Information Act. The report charges that Hayflick had formed a company, Cell Associates, to sell the cells, even though they were government property, and had even entered into a large contract with the pharmaceutical house of Merck & Co., Inc. From the description of the contract in Schriver's report, it seems that if Merck had exercised all its options, Hayflick's company would have received about $1 million. Hayflick says that the sum would have been far less.

The Merck contract has not been implemented, but the sales to date of WI-38 cells amount to some $67,000. Hayflick kept the proceeds of these sales, the report says, but charged the costs of the operation, except for postage and freight, to NIH research agreements.

The report also reveals that many ampules of the cells cannot be accounted for by Hayflick's records. Nearly two-thirds of the ampules, according to the records, were contaminated with bacteria, but the contamination was not reported to NIH or the government vaccine authority. The NIH investigators, who traveled to England and Yugoslavia to verify Hayflick's sales records, also claim that there is a question of whether the cells shipped by Hayflick were always of the same age, or population doubling level, as he represented them to be.

The charges sound serious, and indeed are so if wholly true. They raise questions not only about Hayflick's stewardship of the cells, but equally of NIH's oversight of what is now claimed to be its property and a valuable national and international resource. Until the full story emerges, it is necessary to give weight to Hayflick's belief that he can refute the virtual totality of the Schriver report. In a prepared statement he denies any wrongdoing, expresses confidence that he will be totally vindicated, and says, "I urge my scientific colleagues around the world who have long relied on my integrity and that of my work to regard with great caution the statements in the [Schriver] report."

Hayflick has prepared a detailed rebuttal which he says will, and his lawyers say may, be filed with NIH by 1 April. Because of pending litigation (he is suing the NIH for denial of due process in releasing the report without his rebuttal; NIH says he failed to deliver it on time), Hayflick declined to show the document to *Science*. But during a reasoned and extensive discussion in the presence of two lawyers he provided explanations for the questions the report addresses.

The cell line in question was laid down by Hayflick in July 1962, when he was working at the Wistar Institute in Philadelphia. The cells were derived from the lung tissue of a female fetus aborted in a Swedish hospital. Because of a method Hayflick developed, they became the first normal human cells to be established in culture. They have since become the best studied cells of their kind and are known to researchers throughout the world as WI-38 cells.

Besides developing the methods for culturing normal human cells, Hayflick has two other significant advances to his credit, discovery of the organism that causes primary atypical pneumonia, and a finding of significance for the biology of aging, that embryonic human cells go through only about 50 divisions before dying out.

The importance of WI-38 cells for vaccine production is simply that they have been so intensively studied over the years that their normalcy and freedom from viruses can be relied upon. It would take years to develop a similar guarantee for a new human cell line, and only one other line, cells developed in Britain and known as MRC-5, has a comparable pedigree. When vaccines are made, the cells are allowed to multiply a particular number of times and then infected with virus. The viruses are later harvested from the cells to prepare the vaccine.

That WI-38 cells are now being used to prepare an increasing number of vaccines throughout the world is doubly to Hayflick's credit. Not only did he create the cell line, but he fought a protracted battle to persuade the U.S. government vaccine authority, now known as the Bureau of Biologics, to accept the cells for vaccine use. Because of an attitude which the bureau's present director concedes was overconservative, the agency long turned a deaf ear to Hayflick's argument that WI-38 cells were superior to the virus-ridden animal cells then in universal use. Not until 1972 did the bureau follow the lead of vaccine authorities abroad in permitting a vaccine produced in WI-38 cells to be marketed in the United States. Even then, the vaccine, that for live polio virus, was manufactured in England.

Because of the government's decade long cold-shouldering of WI-38's, it is hard not to feel sympathy with Hayflick's sense of irony and outrage that the same government now claims the cells to be its own precious property. Nor is it strange to hear him say that "I felt, and I think I am justified in feeling, that these cells were like my children."

Whatever the degree of Hayflick's parenthood, there does not seem to be much dispute that the cells were developed on an NIH contract and hence were government property. This point was formalized in a memorandum drawn up in 1968, just before Hayflick left the Wistar Institute for Stanford. The memorandum stipulated that Hayflick could take 10 ampules of the cells with him (which he

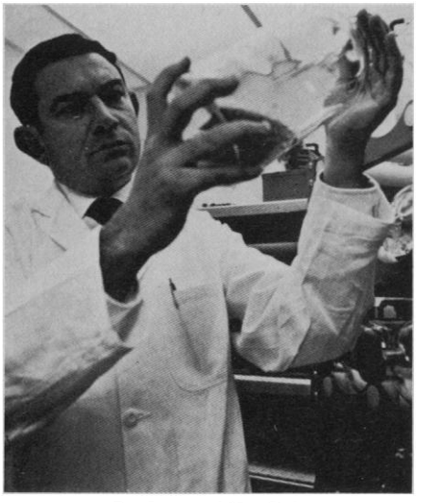
understood to mean for his personal use), and that 10 were to be left at the Wistar Institute. The remaining 400 or so were to be sent to the American Type Culture Collection (ATCC), a national cell depository in Rockville, Maryland. Hayflick took the whole lot to Stanford anyway. "As I recollect it," says a former Wistar colleague, "it boiled down to his insistence that he take the cells with him, which seemed reasonable to me at the time."

However willful it may have been of Hayflick to defy NIH's explicit instructions, NIH was not tremendously vigorous in preventing him. True, the NIH project officer and a scientist from the ATCC drove up to Wistar with a special truck to collect the cells, but Hayflick turned them away saying he wasn't ready. The project officer later went so far as to telephone him in Stanford and ask that the cells be returned. Thereafter, concedes NIH associate director Leon Jacobs, "Things sort of disintegrated. We are not completely faultless in this."

What happened to the cells between then and now is a mystery whose solution is known only to Hayflick. Even though the NIH investigation began last May, Hayflick still is not prepared to discuss the stock situation in terms of precise numbers, although all will be made clear, he says, in his rebuttal. According to the Schriver report, the original WI-38 cells were grown up to 8th division at the Wistar Institute before being put into some 800 ampules and frozen down for storage. The ampules, not quite 2 inches high, resemble tiny wine bottles in shape, and contain milky looking fluid in which the cells are suspended, at about 2 million cells to an ampule. About half of these 8th passage ampules, as they are known, were used up in the next 66 years. The collection which Hayflick took with him from the Wistar Institute amounted to some 375 of the original ampules.

When the stock was retrieved from Hayflick's laboratory last August, all that remained was about 50 of the 8th passage ampules, 46 ampules labeled passage 9, and miscellaneous ampules of higher passage. (In this context, though not always, a passage level corresponds to one population doubling of the cells. Thus from one ampule at the 8th passage level could be made two ampules at the 9th passage, or population doubling level, four at the 10th, eight at the 11th, and so forth.

The Schriver report states that there are gaps and discrepancies in Hayflick's records which make it impossible to account fully for all the ampules, and that



Dr. Leonard Hayflick

in fact a total of at least 207 remain unaccounted for. This figure includes a discrepancy between the 339 ampules which Hayflick's records show were sent to the Medical Research Council in England and the 271 which the MRC's records show were received. Hayflick says that none of these figures is necessarily correct, and that the ampules unaccounted for are mostly ones he had to destroy because of bacterial contamination.

A further wrinkle to the stock situation lies in the contract with Merck. The contract, executed in October 1974, calls for Hayflick's company, Cell Associates, to supply Merck with 100 ampules of the 9th population doubling from the primary 8th passage WI-38 cells and 50 ampules of the 10th doubling, at a cost of $5000 and $2500 per ampule, respectively. Merck also had the right of first refusal on another 50 ampules each of the two doubling levels as soon as Cell Associate's inventory fell below a certain level. If Merck were to pay the same price as before, though presumably it would have been higher as supplies shrank, the total contract would appear to have been potentially worth about $1 million to Cell Associates, of which Hayflick and his wife are the sole stockholders.

The Schriver report raises the question of how Hayflick proposed to fulfil the Merck contract, which according to Schriver's calculations would have required approximately 100 8th passage ampules. This is more than the 10 which were arguably Hayflick's personal property and indeed exceeds the number of ampules found in Hayflick's laboratory. Hayflick absolutely denies either that he entered into a contract he could not fulfil, or that he has a secret supply hidden away. The explanation, he says, is simply that Schriver has failed to understand a widely held convention that cells customarily designated as 9th population doubling level may in fact have doubled several more times, depending on the size of the bottle in which they are grown. For this reason the Merck contract could have been fulfilled with far fewer ampules than Schriver believes. Hayflick estimates that between 5 and 30 8th passage ampules would have sufficed, depending on what bottle size was agreed with Merck.

It is hard to find anyone who agrees with Hayflick's calculations. One cell culture specialist says his argument would be laughed out of court, another describes the reasoning as "Jesuitical" and "terribly feeble." Maurice Hilleman, a vaccine scientist who as vice-president of Merck ordered the contract to be negotiated, said that while he didn't have the contract at hand, one would expect by geometric doubling to derive 100 9th passage ampules from 50 of 8th passage and so forth. James E. Shannon, the American Type Culture Collection scientist who now has custody of the WI-38 ampules, says that the numbers specified in the Merck contract, as described in the Schriver report, could not be fulfilled from Hayflick's stocks. Since the exact wording of the contract is not available, however, it is impossible to be certain whether or not it could have been fulfilled.

The issue of contamination raises several questions, such as when Hayflick first discovered it and what his policy was for coping with it. It is not in fact surprising that the ampules should be contaminated, considering the technology available at the time they were laid down, but the point is that the scientific community has assumed until now that they were sterile. Hayflick explains that the contamination was for long masked because he routinely treated the cells with an antibiotic in order to kill mycoplasma, but which presumably killed the bacteria as well. Hayflick says he never actually found any mycoplasma in WI-38's but used the antibiotic as a prophylactic.

He was first alerted to the problem of bacterial contamination in 1968 by Frank Perkins, a close colleague who was then head of the British vaccine authority. Perkins, now with the World Health Organization in Geneva, says Hayflick's response was that all the contaminated ampules would be destroyed. Hayflick says that he did destroy a lot of ampules. "But then it just occurred to me that if a lot of cells had been inadvertently cleaned up without the contamination ever being detected, that was solid ground for preserving those remaining contaminated ampules and using them for re-

search purposes." Thus Hayflick continued to clean up cells from the contaminated ampules and send them out to researchers. As far as vaccine manufacturers were concerned, he says that "they were never given ampules or starter cultures that knowlingly came from contaminated pools, to the best of my knowledge."

One problem is that even after 1968 Hayflick never made public the fact that some of the WI-38 ampules were contaminated. In fact on 20 April 1972 he told a Senate subcommittee that "The human diploid cell strain WI-38 tested in hundreds of laboratories throughout the world has never been found to contain an indigenous contaminating microorganism." Hayflick told *Science* that certain "key people" were informed, but the list does not include the Bureau of Biologics, for example.

What if either contaminated ampules or ampules cleaned up by antibiotics had accidentally been used by a manufacturer for vaccine production? According to Bureau of Biologics director Harry Meyer, there is unlikely to have been a public health hazard because antibiotics are regularly used in the later stages of cell multiplication. Nonetheless, the general custom is always to start with initially sterile cells. This is the practice followed by the British vaccine authority, for example, with the live polio vaccine made from WI-38 in England cells for the United States.

Future users of WI-38's may not have the same option because the stocks removed from Hayflick's laboratory appear to be heavily contaminated. According to preliminary tests performed by Shannon, of the nine 8th passage ampules opened so far, three are clean, five contain a *Micrococcus* bacterium, and one has a *Pseudomonas* species.

Another scientific issue raised by the Schriver report is that of whether the cells distributed by Hayflick were always at the passage level represented. Hayflick's belief that population doubling levels are by convention designated as less than in fact they are would speak to some of the differences on this score, though an instance in which the Yugoslav vaccine authority received cells labeled as 9th passage which Hayflick's sales invoices record as 19th passage is something else again. Hayflick says Schriver misunderstood the significance of the "19th," which referred to the date.

Hayflick's contention that the exact population doubling level does not greatly matter may have caused two types of problem. It could have made a difference to researchers studying the biology of aging if the cells received were older than they understood them to be. With vaccine manufacture, there are no regulations on the age of cells, but it is nevertheless conventional international practice to produce vaccines in cells of a particular doubling level well before they enter senescence. According to Bureau of Biologics director Meyer, culture of vaccines in cells of a higher population doubling level "is maybe not an important issue from the point of view of human hazards, but it would sure psych me up in terms of quality control." Hayflick says that a difference of one or two doublings either way could hardly be significant either in aging research or in vaccine production.

Though the Schriver report would appear to raise problems for Hayflick, it is entirely possible that he will, as he says, refute all its allegations. The root of the confusion, after all, lies in the incomplete state of Hayflick's records. Though his habits of documentation might leave something to be desired for the custodian of an important cell line, he would not be the first scientist to have kept haphazard records. Another pertinent difficulty seems to have been that he developed a personal antagonism for Schriver early on in the investigation, after which the two conversed through lawyers, an unhappy medium for the full exchange of ideas.

The Schriver report was not the basis for action of the Stanford University Medical Center, which had completed an independent inquiry into Hayflick's affairs before receiving Schriver's conclusions. The inquiry in part concerned the placement by Hayflick of fees for mycoplasma testing in his own bank account. Hayflick contends that these were in the nature of consultancy fees, and had been collected openly for 7 years with the knowledge and consent of his NIH contract officers, Medical Center dean Clayton Rich nevertheless told Hayflick that he planned to recommend some disciplinary action, probably dismissal, following which Hayflick resigned on 27 February. At least one faculty colleague, radiology professor Henry Kaplan, feels that Hayflick was harshly dealt with and should have been allowed simply to return the disputed fees. "In view of the complexity of the case, I would have hoped that the university officers could have coped with Dr. Hayflick in a more compassionate way," Kaplan says. Medical Center counsel John J. Schwartz counters that Hayflick had the right of a hearing before his faculty collegues, which he forwent by his resignation.

But the real tragedy for Hayflick is not what the NIH inquiry or Stanford has done to him but what he has apparently done to the future of WI-38's. Although he contends that the stocks now at the ATCC are ample for 10 years or so, if properly managed, others think differently. "I was very, very surprised to learn there were only 50 ampules left," remarks Hayflick's English colleague Perkins. Stanley Plotkin, a vaccine developer at the Wistar Institute, says, "I was really just bowled over when I heard it."

The Bureau of Biologics believes the stocks will last for only a few years at best and is already looking at the only available alternative, a human cell line known as MRC-5 developed by the English Medical Research Council. Hayflick, when pressing to get WI-38's accepted for vaccine use, often used to say that the stocks would yield up to 20 million metric tons of cells. Now, just as that battle was won and vaccine makers were turning increasingly to WI-38 cells, the stocks turn out to be severely limited. Thus the credit for the next generation of vaccines will go to MRC-5 instead of to Hayflick and WI-38.

The huge contract with Merck was of course tangible proof that WI-38 cells had at last come into their own. If fulfilled, the contract might have given Merck a near monopoly on a vital world resource. But Merck vice-president Maurice Hilleman says that the purpose was simply to assure Merck's own supplies. "It was not our intention to corner the market," he says. "We had no idea what the total available supply was. We just said that this was what we would like to have to guarantee the future for 10 years. We certainly would not want to take the lion's share." Hilleman adds tht it was also a "bad situation" to have the world's main supply held by one man in a place where all could have been destroyed in an accident.

Why did Hayflick start selling the cells and taking such apparently careless management of the stock? According to Plotkin, many people warned him about the sales, but he was not open to any kind of remonstrance. "I think that in the really classical Greek sense it was a tragedy, because it is a man who at the height of his powers brought about his own downfall," Plotkin says. Yet Hayflick's setback may well prove only temporary. The Schriver report recommends that he not be chief recipient of any more NIH contracts. But associate director Jacobs says the recommendation is unlikely to be accepted. "In the long run," he says, "things will straighten out for this guy, and if he has continued usefulness as a scientist he will get awards from us."

—NICHOLAS WADE