Serial Cultivation of Human Diploid Cells in the Lab (1958–1961) by Leonard Hayflick and Paul S. Moorhead [1]

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From 1958 to 1961, Leonard Hayflick [2] and Paul Moorhead [3] in the US developed a way in the laboratory to cultivate strains of human cells with complete sets of chromosomes. Previously, scientists could not sustain cell cultures with cells that had two complete sets of chromosomes like normal human cells (diploid). As a result, scientists struggled to study human cell biology because there was not a reliable source of cells that represented diploid human cells. In their experiments, Hayflick and Moorhead created lasting strains of human cells that retained both complete sets of chromosomes. They then froze samples from the cultures so that the cells remained viable [4] for future research. They also noted that cells could divide only a certain number of times before they degraded and died, a phenomenon later called the Hayflick limit. Hayflick and Moorhead's experiment enabled research on developmental biology and vaccines that relied on human cell strains.

Hayflick specialized in culturing cells in controlled environments. In 1958, he joined the Wistar Institute in Philadelphia, Pennsylvania, a research institution that studied cell biology and viruses, at the invitation of the institute's new director, Hilary Koprowski. Koprowski tasked Hayflick with creating cell cultures for use in the experiments of other researchers at the institute. Hayflick later recalled, however, that he used his research assignment as an opportunity to study the methods and limitations of cell cultures. To assist in culturing cells, Hayflick recruited his Wistar colleague, Paul Moorhead [3], who studied the structure and function of cells and chromosomes.

The lack of longevity of normal human cell cultures in the laboratory limited research that scientists could do. With cell cultures, scientists grow populations of cells in glassware in controlled conditions for use in research. Researchers typically grow cell cultures in glassware or petri dishes that contain growth medium, a solution containing dissolved salts, sugars, and other cell nutrients. Throughout the first half of the twentieth century, researchers hypothesized that all normal, healthy cell cultures had an innate ability to divide indefinitely based on earlier findings published by biologist Alexis Carrel [5] in 1912 in the US. In practice, though, researchers did not observe endless division in normal cells. Instead, the cells in culture eventually degraded and died. Scientists posited that cells degraded because they had depleted the culture growth medium of all of its nutrients or because laboratory technicians had failed to maintain ideal environmental conditions for cellular growth. The only human cells that did divide continually in laboratory settings, called immortal cell [6] lines, were cells derived from cancerous cells. Those cell lines represented only some aspects of human cell biology.

In the mid twentieth century, medical researchers could not explain what caused cancer, and many scientists, including Hayflick and Moorhead, hypothesized that certain viruses might cause cancerous growths. Although many microbiologists used cells derived from cancerous cell lines in medical research, the cells were generally not used in the development of vaccines because scientists worried that the vaccines would be contaminated with the cancer-causing viruses possibly contained within the cells themselves. Furthermore, cells from cancerous lines, due to their constant division, were heteroploid, meaning that they had either greater or fewer numbers of chromosomes than normal human cells, which have two complete sets of chromosomes. Heteroploid cells often resulted from the continuous cell division over many generations of cells in culture.

Hayflick and Moorehead produced human cells that could be cultivated over many generations, like cells from cancerous cell lines, but also preserved the cells' diploid number of chromosomes and reduced the risk from theoretical cancer-causing viruses. They described their experiment in "The Serial Cultivation of Human Diploid Cell Strains," published in 1961.

Hayflick and Moorhead sought to develop strains of human cells that could be cultivated for long periods of time in the laboratory yet still retained their diploid number of chromosomes. They hypothesized that if they were transplanted small samples of diploid human cells from already growing cultures into a new growth environment, they would greatly increase the number of diploid cells in culture. Hayflick and Moorhead proposed that freezing small quantities of diploid cells would pause cellular growth and division without killing the cells. Frozen cells could then be stored until researchers required them, at which point they could thaw the frozen cells, restoring them to normal cellular activity. Hayflick and Moorhead predicted that after thawing, those cultivated cells would not become heteroploid like cells from other lines and would retain their diploid set of chromosomes.

Hayflick and Moorhead's experiment aimed to both create a method for growing human diploid cells in the laboratory for long-term research use, and to determine whether or not those cell strains contributed to cancerous growths. Hayflick and Moorhead first grew human cells in laboratory growth cultures, transplanted small samples of cells into new containers to grow additional cell cultures, and froze samples of cells from those cultures to preserve the cells for later research. Next, to test if the frozen cells were still viable [4] for research, Hayflick and Moorhead thawed the frozen cells and attempted to grow cell cultures from them. Finally, they implanted samples of those human diploid cell strains in living tissues to see if they led to cancerous growths.

To develop diploid human cell strains, Hayflick and Moorhead began cultivating cells from 25 different tissues retrieved from aborted fetuses. Those cells became 25 different human cell strains, named numerically WI-1 through WI-25. The WI stood for Wistar Institute, where the cell strains were developed. Hayflick and Moorhead used fetal tissues because, more than adult cells, fetal cells more readily developed into fibroblast cells, which are specialized cells that provide structural support to most body tissues. Fibroblast cells were preferable for laboratory cell cultivation because they grew rapidly and continuously in cell cultures, providing an abundance of cells for research. Hayflick and Moorhead cut each of the tissue samples into small, thin slivers and then implanted them on the inner wall of glass bottles filled with nutrient-rich growth medium. Hayflick and Moorhead then placed the tissue-coated bottles of each cell strain in a warm environment for three days, regularly replacing the growth medium with a fresh supply, to begin growing the cell culture.

Hayflick and Moorhead let the cultures grow until the cells coated the entire glass bottle. Each sample of fetal cells was then subdivided by a process called subcultivation. During subcultivation, Hayflick and Moorhead removed a small sample of cells and implanted those cells onto the wall of a new glass bottle filled with growth medium, creating a new cell culture of the same cell strain. Each new cell culture constituted a new generation of cells that could itself be further subcultivated by the same method, enabling the total number of cells to grow exponentially. Hayflick and Moorhead then divided samples of the remaining cells into small portions and froze them to pause the cells' growth and halt any further cell division.

Hayflick and Moorhead continued subcultivating cells twice a week for about ten months, at which point the cell cultures stopped growing and began to degrade. Hayflick and Moorhead hypothesized that the cells had stopped dividing because of a build-up of toxic products of cellular growth in the growth medium. Hayflick and Moorhead tried introducing fresh growth medium that was free of any possible toxins, but the cell cultures continued to degrade and die over the next several months. However, other cell cultures placed in the same growth medium did not degrade and die. The researchers concluded that something about the cells themselves, not their environment, caused them to begin deteriorating.

Hayflick and Moorhead next attempted to determine whether or not the cells that they had frozen could still be used to grow more cell cultures. After thawing small samples of cells, Hayflick and Moorhead implanted the cells onto the walls of glass bottles. They again cultivated them in growth media and discovered that, even after freezing, the cells still grew in new cultures, which could themselves be subcultivated. Regardless of previous freezing or subcultivations, researchers could use samples from diploid human cell cultures to grow more diploid human cell cultures. Hayflick and Moorhead had created a diploid human cell strain that could be grown in laboratory cultures nearly indefinitely.

Hayflick and Moorhead then investigated whether or not the cells grown in the new cultures were diploid. When writing about the experiment, they said that they were concerned that the cells may not have remained diploid because they had grown them over many generations, which can lead to heteroploidy. Using light microscopes, Hayflick and Moorhead looked at samples of cells during the metaphase of cell division, when chromosomes are distinct and easily viewed. They counted the number of chromosomes in 250 individual cells to obtain an estimate of how many cells still had a diploid number of chromosomes. Hayflick and Moorhead determined that more than 97 percent of cells were diploid even after more than twenty generations of subcultivation. Hayflick and Moorhead concluded that their process of serial cultivation and freezing of fetal cells was an effective method for preserving a diploid human cell strain.

Finally, Hayflick and Moorhead needed to show that their cell strains did not cause cancer. The problem with the immortal cell [6] lines created from cancer cells was that researchers hypothesized that the cells might contain cancer-causing viruses. In order to make sure that their new cell strains did not cause cancer, Hayflick and Moorhead tested the WI-25 cell strain in living tissue. They selected the WI-25 strain because it had undergone the most subdivisions and was the most likely cell strain to cause cancer. Therefore, if it did not, it was likely that none of the other strains would either.

The researchers implanted cells from the WI-25 cell strain into the cheek pouches of five living hamsters. As an experimental control group, they also implanted five other hamsters' cheek pouches with cells derived from cancerous cell lines. At first, nodules, an early sign of developing cancer, appeared in the check pouches of both sets of hamsters. However, after three weeks, nearly all nodules in the hamsters implanted with the WI-25 cells had disappeared while the nodules in the hamsters implanted with cells from cancerous cells lines had all increased in size. Hayflick and Moorhead performed biopsies of the remaining nodules to confirm their prediction that their subcultivated cells did not cause cancer. The biopsies showed that the nodules in hamsters implanted with cells from cancerous cell lines indeed were cancerous, while the WI-25 cell nodules were

due to inflammation and bleeding at the implantation [7] site.

Hayflick and Moorhead also implanted a similar human cell strain, WI-1, into the muscle tissues of five dying cancer patients. He also implanted cells from cancerous cell lines into five other terminal cancer patients. Like in the hamsters, at first nodules grew at the implantation [7] sites of both groups. After a couple of days, the WI-1 cell nodules began to recede, while the nodules multiplied in patients implanted with cells from cancerous cell lines. At the end of a week, nearly all the WI-1 nodules had disappeared, and Hayflick and Moorhead biopsied the remaining nodules in both groups. The results of the biopsies showed that the cancerous cell nodules were heteroploid cells, while the WI-1 nodules were diploid and non-cancerous. Hayflick and Moorhead's findings, which they published in 1961, demonstrated that human diploid cells could be propagated over many generations, like cancerous cell lines, without becoming heteroploid or cancerous themselves.

The results had an immediate and lasting impact on the understanding of developmental biology and scientific research. The results of their experiment disconfirmed Carrel's 1912 hypothesis that normal cells grew indefinitely in culture, despite observations of cell cultures degrading over time. Carrel had suggested that the reason that cells, in practice, appeared to deteriorate and age over time was due to imperfect laboratory growth conditions for the cells. Carrel claimed that under ideal conditions, cells in cultures could divide indefinitely, effectively acting as an immortal cell [6] line. Hayflick and Moorhead's findings, however, indicated that aging in organisms happens on a cellular level, a process called senescence [8], and that cells could only undergo a limited number of divisions before they degraded and died. During their experiment, Hayflick and Moorhead attempted to continually culture fetal cells, replacing old growth medium with fresh, nutrient-rich medium. However, they found that after about forty or sixty generations, the cells began to die rather than reproduce, a phenomenon later called the Hayflick limit. That result demonstrated that the cells could not grow indefinitely in culture, even under ideal conditions.

Hayflick and Moorhead's method of serial cultivation of human diploid cells also helped scientists to maintain an abundant supply of diploid human cells for research. By Hayflick and Moorhead's calculations, a single human cell strain could be subcultivated enough times to produce nearly 20 metric tons of viable [4] cells. While not technically immortal, that supply would be close to inexhaustible for practical research purposes.

Moreover, the creation of viable [4] diploid human cells enabled vaccine research. Because Hayflick and Moorhead showed that their human cell strains did not cause cancer in hamsters or humans [9], those strains could be used in vaccines without threat of contaminating the vaccine with some cancer-causing agent. Subsequent similarly derived human cell strains, such as WI-38, became the basis for vaccines for childhood diseases like rubella and chickenpox.

While many researchers welcomed the development of human cell strains by Hayflick and Moorhead, some, including the Catholic Church, disapproved of the use of aborted fetal material in the development of vaccines on religious grounds. The Vatican later stated that they objected to the method of development of vaccines derived from fetal tissues, not to individual's use of those vaccines to prevent diseases.

Hayflick and Moorhead demonstrated not only that human cells could be successfully cultivated in the laboratory while still maintaining a diploid number of chromosomes, but also that they could maintain the cells almost indefinitely through serial subcultivation. Furthermore, their experiment laid the foundation for Hayflick to further research the limit of cell division in culture, later called the Hayflick limit. Hayflick and Moorhead's discoveries allowed for further experiments in developmental biology and vaccine development by providing abundant human cells for research.

Sources

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[3] https://embryo.asu.edu/search?text=Paul%20Moorhead
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