

"The Limited In Vitro Lifetime of Human Diploid Cell Strains" (1964), by Leonard Hayflick

Leonard Hayflick in the US during the early 1960s showed that normal populations of embryonic cells divide a finite number of times. He published his results as "The Limited In Vitro Lifetime of Human Diploid Cell Strains" in 1964. Hayflick performed the experiment with WI-38 fetal lung cells, named after the Wistar Institute, in Philadelphia, Pennsylvania, where Hayflick worked. Frank MacFarlane Burnet later called the limit in capacity for cellular division the Hayflick Limit in 1974. In the experiment, Hayflick refuted Alexis Carrel's hypothesis that cells could be transplanted and multiplied indefinitely from any single parent cell line.

Hayflick's primary role in the early 1960s at the Wistar Institute was to provide cell cultures to the scientists there who were performing research in various areas of microbiology. While providing cell cultures to other researchers, Hayflick researched viruses and their hypothesized connections to cancer in human cells.

Hayflick's 1964 experiment arose in response to phenomena observed in a previous experiment, "The serial cultivation of human diploid cell strains," conducted with Paul Moorhead in 1961. Hayflick and Moorhead tried to develop better ways to cultivate cell populations for future experiments. In the process of culturing twenty-five strains of fetal lung cells for his experiment with Moorhead, Hayflick noticed that some of the cultures had completely stopped dividing, although they were still metabolizing. Hayflick's observations contradicted Carrel's 1912 theory that cells would continue to replicate indefinitely. At the time of the experiment's publication in 1961, Hayflick hypothesized that cells had intrinsic factors that determined aging. However, he and Moorhead did not rule out the possibility that viruses interfered with the cells and caused the cells to stop dividing.

In his 1964 experiment, Hayflick further examined the mechanisms that could cause the fetal cells to stop dividing. He created subcultures from fetal lung cell lines and adult lung tissue and named the cultures after the Wistar Institute and by number order. Hayflick cultured fetal lung tissues which he had received from male and female fetuses, and which he named the male derived cells WI-26 and the female derived cells WI-38 and WI-44. He extracted the fetal lung tissue from fetuses aborted at about three months gestation, he generated the adult strains from lung tissue of dead adults, and froze all of the tissues in a container with liquid nitrogen to preserve them. Each subculture split in a two-to-one ratio, which means that each cell in the samples divided once, producing two more cells.

Hayflick's results indicated that in a two-to-one split ratio, the average number of times a fetal cell could divide was between forty to sixty times. In adult cells, the average number of times a cell would multiply was twenty. That data indicated that in adult cells, numerous cell duplications had already occurred, as their number of duplications in the lab setting were less in comparison to the number of cell divisions in the fetal cell strains. In the experiment, Hayflick called the phase when the cell ceases to divide phase III, with phase I and II being periods in which a cell rapidly proliferates.

Hayflick investigated whether fifty was the average number of divisions for every fetal cell within the same population, or if it was just a randomly occurring number. To test his hypothesis, Hayflick randomly selected and cloned three samples of the fetal cell line, WI-38, and he recorded when each population stopped dividing. The resulting range for each clone was from fifty to fifty-four divisions. That data led Hayflick to hold that something in cells limited the amount of times a cell

can double. From the results, Hayflick concluded that cellular aging is based on the amount of times a cell doubles.

Hayflick paired adult cells and fetal cells together in culture to test whether or not something outside of the cells, rather than inside the cells, caused the cells to stop duplicating. The results did not vary from previous data. The younger cells continued to divide about fifty times. Hayflick also found that a change in a cell's duplication potential was not caused by freezing the cell samples in liquid nitrogen because the unfrozen samples obtained similar results. Researchers who used WI-38 strains in their experiments also replicated the results of Hayflick's experiment and found that the cells stopped dividing at about fifty replications.

Hayflick's results refuted Carrel's theory that cellular replication is infinite. Before Hayflick's discovery, scientists had attributed the failure in infinite replication of human cells to incorrect laboratory practices or to interference of a virus or other microorganism. Hayflick hypothesized that damage to structures in cells could cause cells to age. In particular, he claimed that this damage is likely associated with chromosomes. He called such damage as hits, or errors in DNA replication that accumulate over time, which cause a more rapid onset of phase III in a cell and result in cellular aging. The hits on the DNA were associated with telomeres by Alexey Olovnikov working at the Gamaleya Research Institute of Epidemiology and Microbiology in Moscow, Russia, during the 1970s.

Hayflick's experiment, published in "The Limited In Vitro Lifetime of Human Diploid Cell Strains," contributed to the theory that cells undergo an aging process that is influenced by telomeres. The Hayflick Limit is applicable to adult cells as well as cells found in human embryos. Into the early decades of the twenty-first century, the WI-38 fetal cell cultures established in Hayflick's experiment were still used by scientists in biological research.

Sources

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