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This Week's Citation Classic

Cristofalo V J & Sharf B B. Cellular senescence and DNA synthesis: thymidine incorporation as a measure of population age in human dipioid cells. *Exp. Cell Res.* **76**:419-27, 1973. [Wistar Institute of Anatomy and Biology, Philadelphia, PA]

In this paper aspects of the kinetics and dynamics of cell replicative aging were established along with a method for defining cell culture replicative age. [The *SCI*® indicates that this paper has been cited in more than 410 publications.]

Cell Senescence in Culture: The Definition of Replicative Age

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I first joined the faculty of the Wistar Institute in the fall of 1963. I went there primarily because I was enamored of the opportunity to work with normal human cells in culture. The benchmark experiments of L. Havflick and P.S. Moorhead[1] and, later, Hayflick[2] had established that fibroblast-like cell cultures, derived from normal human tissues, would proliferate in culture while maintaining a stable normal human karyotype and normal growth characteristics. However, after a period of vigorous growth, the proliferation rate of the culture would decline, the cells would begin to deteriorate and eventually would cease to proliferate. Hayflick and Moorhead interpreted this declining proliferative activity as a manifestation of senescence in vitro. At the time, this view was controversial, especially among the community of biogerontologists who were invested in the notion that organisms aged, not isolated somatic cells, and that cells exhibited senescence as a result of the artificial conditions of culture. (Indeed now, 30 years later, these caveats are still raised, even though by any definition of aging, human cells in culture age.)

Although I had my own set of reservations about how human fibroblast aging might relate to human aging, I focused my research on the fundamental aspects of the regulation of proliferation.

In the spring of 1969, Hilary Koprowski, then director of the Wistar Institute, asked me to attend, in his place, a meeting on aging, in Gatlinburg, Tennessee. Upon my arrival, the meeting organizers informed me that I must fill in for Harry Eagle, who was listed in the program as a discussant for a presentation by Leonard Hayflick, but who was unable to attend due to a family emergency.

In attempting to prepare for the meeting a well-balanced, critical appraisal of how the so-called "Hayflick limit" related to the biology of senescence in organisms, I was forced to confront the fact that precious little was really known about the senescence of these human fibroblast cultures. For example, whether the declining proiiferative capacity of the culture was due to a parasynchronous increase in the generation time of all of the cells, a change in the fraction of cells which were in the proliferating pool, or both, was not known. There were some interesting clues; G.S. Merz and J.D. Ross[3] at the Wistar Institute had shown that the percentage of single cells that could grow and form multicellular clones was lower in older populations. Also, A. Macieria-Coelho et al.[4,5] had reported that both the average G1 and G2 periods of the cell cycle were longer in cultures from older populations.

Another problem which confounded the interpretation of studies on cell cultures was that there was no method for normalizing the data on cell culture aging from different laboratories. Hayflick's studies[2] on WI-38 cells showed a range of replicative life spans from 39 to 58 doublings. Thus doubling (or passage) level as a measure of the age of a culture was not adequate. What was needed was a precise measure of in vitro age which would normalize for this variation.

The study reported in the paper established that both a decline in the time to traverse the cell cycle and a decline in the fraction of cells participating in replication combined to give the reduced proliferative potential of the population which characterized senescence. The paper has been highly cited most probably because it provided a kind of normalized biomarker for aging in cell culture. However, other aspects of the data formed the basis for later studies on changing cell cycle parameters, signal transduction, and gene expression during the G1 period of the cell cycle.

1. **Hayflick** *L* & **Moorhead P** S. The serial cultivation of human dipioid cell strains. *Exp. Cell Res.* 25:585-621, 1961. (Cited 2,150 times,) [See also **Hayflick L.** Citation Classic®. (Barrett J *T.* ed.) *Contemporary classics in the life sciences. Volume I: cell biology.* Philadelphia: ISI Press, 1986. p. 144.]
2. **Hayflick L.** The limited in vitro tifetime of human dipioid cell strains. *Exp. Cell Res.* 37:614-36. 1965. (Cited 1,735 times.) [See also: **Hayflick L.** WI-38—from purloined cells to national policy. Citation Classic. *Current Contents®/Life Sciences* 33(3):14. 15 January 1990.]
3. **Merz G S & Ross J D.** Viability of human dipioid cells as a function of in vitro age. J. *Cell. Physiol.* 74:219-21. 1969. (Cited 155 times.)
4. **Macieria-Coelho** A, **Ponten** J & **Phillipson** L. The division cycle and RNA synthesis in dipioid human cells at different passage levels in vitro. *Erp. Cell Res.* 42:673-84, 1966. (Cited 245 times.)
5. ---------- . Inhibition of the division cycle in confluent cultures *of* human fibroblasls in vitro. *Exp. Cell Res.* 43:20-9. 1966.

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