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# This Week's Citation Classic®

**Chanock R M, Hayflick L & Barile M F.** Growth on artificial medium of an agent associated with atypical pneumonia and its identification as a PPLO. *Proc. Nat. Acad. Sci. USA* **48**:41-9, 1962. [National Insts. Health, Bethesda, MD; and Wistar Inst. Anal and Biol., Philadelphia. PA]

**Primary atypical pneumonia, or "walking pneumonia," was thought to be caused by a virus. This was finally disproven and, for the first time, a human disease was found to have as its cause the smallest free-living microorganism—a mycoplasma. [The *SCI*® indicates that this paper has been cited in more than 815 publications.]**

## Discovery of the First Mycoplasma to Cause a Human Disease

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**In 1961 we published a paper[1] in which we described the finite lifetime of cultured normal human cells, interpreted the phenomenon to be a manifestation of aging at the cell level, and described the use of these cells for human virus isolation and vaccine manufacture. As a result we were inundated with requests for my normal human cell strains WI-26 and WI-38.**

**One of the requestors was Robert Chanockof the National Institutes of Health, who came to visit me in Philadelphia to obtain a starter culture. During our conversation I asked him about his current research, and he described his studies on primary atypical pneumonia (PAP), which represents a significant proportion of pneumonias in young adults. The putative etiological agent was then known as the "Eaton agent," studied by Monroe Eaton,[2] who, working at Harvard University, believed that the unidentified agent would grow in embryonated eggs, cotton rats, and hamsters. However, the virology community remained unconvinced and discounted his work—a fact that caused him considerable distress.**

**Although the etiological agent of PAP was thought for many years to be a virus, I was told by Bob that the agent had not been identified as such and that the disease was amenable to treatment with broad spectrum antibiotics. These two facts, and my previous research on the mycoplasma infections of rats, triggered my curiosity. I reasoned that many of the pneumonias in dozens of lower animals were caused by mycoplasmas, and it seemed a good bet that humans should not be an exception. Mycoplasmas are the smallest free-living organisms and probably represent the minimum possible size for a cell. Bob was unfamiliar with the mycoplasmas (then called pleuropneumonia-like organisms, or PPLOs) and I asked him to send material to me containing the Eaton agent.**

**The sample arrived, fortuitously, when I was testing several different agar media in an effort to find a better mycoplasma culture formulation than what was then in use. One of these experimental media grew mycoplasmas from the Eaton agent sample.[3] Although this was encouraging, it was not proof that a new mycoplasma had been discovered because many virus preparations at that time were contaminated heavily with mycoplasmas.**

**After I succeeded in subculturing the isolate (it stubbornly resisted transplantation to fresh agar plates), Bob arranged to have my isolate examined by immunofluorescence with antisera from both acute and convalescent cases of PAP. It was the only mycoplasma isolate tested that lit up in convalescent phase antisera, unlike all of the then-known human species that we used as controls. Later, a fresh mycoplasma isolate from a patient with PAP was administered to prisoner volunteers, and the disease reproduced. Bob and I named the new organism *Mycoplasma pneumoniae.* Thus, PAP became the first human disease whose etiology was proven to be caused by a mycoplasma. It is this fact that accounts for the popularity of the paper.**

**The papers describing these mycoplasma studies and the human diploid cell strain studies are two of our four papers that appeared on the list of the 100 most-cited papers of the two million published in the basic biomedical sciences during the 1960s.[4] This anecdotal evidence supports the conventional wisdom that biologists do their best science when they are young, because we were never able to duplicate this citation feat in any subsequent decade.**

**It is also a tribute to the freedom at that time to pursue interesting leads unfettered by preconceived expectations written into grant proposals. All of the research reported in these four papers was done in my laboratory using resources "bootlegged" from grants awarded for entirely different purposes. This could not happen today without the horror of being reprimanded (or worse) for illegally misappropriating federal funds for unapproved purposes.**

1. Hayflick L & Moorhead P S. The serial cultivation of human diploid 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 1: cell biology.* Philadelphia: ISI Press. 1986. p. 144.]
2. **Liu** C, **Eaton** M D & **Heyl J** T. Studies on primary atypical pneumonia. II. Observations concerning development and immunological characteristics of antibody in patients. *J. Exp. Med.* 109:545-56, 1959. (Cited 115times.)
3. **Hayflick L.** Tissue cultures and mycoplasmas. *Texas Rep. Biol. Med.* 23(Supp. 0:285-303, 1965. (Cited 855 times.)
4. **Garfield** E. Most-cited articles of the 1960s. 3. Preclinical basic research. *Essays of an information scientist.* Philadelphia: ISI Press', 1981. Vol. 4. p. 370-8.

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