Milestones... in Investigative Pathology

by William K. Funkhouser, MD, PhD

Microscopes, Bacteria, and DNA: Connecting three centuries of scientific discovery from van Leeuwenhoek and Hooke to Flemming and Avery

Translated in Dobell C., Anthony van Leeuwenhoek. New York, Harcourt Brace, 1932. Reviewed in Gest H, Notes Rec. Roy. Soc Lond. 58:187-201, 2004.

Antonj Van Leeuwenhoek

Scientific knowledge is like a fabric in which threads of discovery are woven both by contemporaries and over time. Parallel discovery by contemporaries is at once both competitive and synergistic. Serial discovery over time connects scientists of like instinct who have never met, yet share both discovery and indebtedness ('standing on the shoulders of giants', etc.).

Nowadays, we refer to the 'thread' of conversation, the 'thread' of an argument, and the 'thread' of a concatenated email. This Milestone's topic threads together microscopes, bacteria, and DNA, and connects the scholarly work of 3 scientists over 3 centuries. The novel Milestone topic concerns Antonj van Leeuwenhoek and his contemporary Robert Hooke, and the thread extends through previously discussed Walther Flemming (see *ASIP Pathways*, Volume 7, October 2012) and Oswald Avery (see *ASIP Pathways*, Volume 3, February 2008).

If you can see, you can observe, measure, hypothesize, manipulate, experiment, model, understand, categorize and conclude. Since there's a lower limit of resolution with the naked eye, magnifying lenses had to be crafted before anyone could observe microscopic organisms. Available technology (convex lenses), engineers (Hooke), and practical end-users of simple microscopes (van Leeuwenhoek) synergized to discover and document protozoa, fungi, and bacteria within the 20-year period 1660-1680.

Thread #1 is literally van Leeuwenhoek's work as a fabric merchant, during which he used simple biconvex magnifying lenses to measure fabric thread densities. The historical narrative is that his interest in microscopic biology was triggered by Hooke's *Micrographia* (1665), in which simple and compound microscopes were used to visualize plant, insect, and fungal structures. (Hooke is credited with the word 'cells,' an example of a word that fundamentally changed human understanding of the physical world.) We presume that van Leeuwenhoek made his own microscopes, although he didn't publish this aspect of his work (we're all proprietary about something). His written observations in Dutch were regularly submitted throughout his adult life for publication in English by the Royal Society of London. His competitor Hooke supported publication of van Leeuwenhoek's work after doing confirmatory experiments in parallel. Van Leeuwenhoek is a good example of an entrepreneur without a formal background who makes and publishes critical early discoveries that lead to the development of a new field, in his case Microbiology. In letter 18, he documents a number of protozoans and bacteria in stagnant water. He was surprised at the number, variety, and mobility of these organisms, and had objective observers write on his behalf to confirm the findings of tens of thousands of organisms in a drop of rainwater. His little animals ('animalcules') were sufficiently well-described for subsequent biologists to name and recognize the organisms he described, so his hand-made microscopes were of good quality.

Tools for purification and experimental manipulation of bacteria allowed characterization and use. Although Simmelweiss clinically proved that something microscopic was responsible for puerperal fever in the 1840s, it required the culture techniques of Pasteur and Koch in the latter half of the 19th century to purify and speciate *Streptococcus pyogenes*. Likewise, although *S. pneumoniae* (pneumococcus) was almost certainly the old person's friend during the 17th and 18th centuries as well, it wasn't until the association of certain infectious agents with certain clinical presentations that medicine began to test for the etiologic role of bacteria using Koch's postulates. Both *S. pyogenes* and *S. pneumoniae* were isolated in culture by Pasteur in the 1880s. Culture methods were perfected for speciation, multiple types of *S. pneumoniae* were recognized, and antibodies were raised, allowing manipulation of different types.

Thread #2 is Walther Flemming's 1882 publication regarding eukaryotic mitosis, derived from the Greek work mit for thread, in which he described the accumulation of chromosomes during the M phase of the cell cycle (see previous Milestones article in *ASIP Pathways*, Volume 7, October 2012). It was subsequently recognized that chromosomes are made of both nucleic acids and proteins, with a prevailing hypothesis in the first half of the 20th century that the chromatin proteins, rather than the nucleic acids, bore the information required to transmit hereditary characters to offspring. It wasn't until 1944 that we understood which polymeric component of chromatin bears the template information of heredity.

S. pneumoniae evolved into an experimental model in the first half of the 20th century. Smooth/encaptulated/virulent and rough/non-virulent strains were found to cause disease in mice. Griffith found that infections of mice with heat-killed virulent/ smooth stains could transform the viable rough/non-virulent strain to become smooth/virulent (1928).

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Thread #3 is the Avery group's methodical biochemical dissection of the polymer responsible for this pneumococcal transformation. Avery's group was able to purify DNA by precipitation (as threads, of course) in 100% ethanol, with demonstration of pneumococcal type transformation using DNA alone as the putative transforming agent. This work culminated in 1944's "Studies on the chemical nature of the substance inducing transformation of pneumococcal types", J Exp Med79:137, 1944 (see discussion in previous Milestones article, *ASIP Pathways*, Volume 3, February 2008). Avery published one paper that year, but that paper stands as an example of thorough scientific proof of cause and effect. It is DNA, not protein, which is responsible for the type transformation of rough pneumococcus to smooth pneumococcus.

Avery's critical publication was not critically embraced by the scientific community for another 10 years. I guess ten years is better than the 200 years it took for van Leeuwenhoek's discovery of bacteria to lead to Simmelweiss, Pasteur and Koch. It led to the base pairing studies of Chargaff (1950), the dsDNA structure solution of Watson and Crick (1953), the semi-conservative DNA synthesis experiments of Meselson and Stahl (1958), and the demonstration of the 3-base genetic code by Crick, Nirenberg and others that mapped DNA sequence to amino acid sequence (1961). Thus, allelic threads of DNA ultimately encode allelic threads of protein, explaining both DNA replication and hereditary transmission of character allelism in the population. We now know that coding sequence is a minor component of total DNA in eukaryotes, that coding sequence can be alternatively transcribed by splicing, that the steady state abundance of mRNA is regulated by both transcription rates and specific microRNA abundance, and that steady state protein abundance is regulated by both translation rates and targeted proteasomal degradation.

Perhaps the best part of this thread of a story is that some of the bacteria with which we co-exist can be put to work making human proteins for treatment of human diseases, e.g. cloned human insulin for diabetes mellitus (1981) and cloned human anti- human VEGF Ig for wet macular degeneration (2004).

Our current understanding of our genome may be akin to the earliest observations of van Leeuwenhoek about his rainwater animalcules – 'surprising with respect to the number, variety, and mobility.' Those static threads of our DNA may be much more dynamic than we think. Just ask Barbara McClintock.