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Early days with Carl

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Science is all about making discoveries. That's it! It was my good fortune and Carl's good fortune to share an experiment that produced an unexpected result. In the 1960s, Carl became interested in the classification of bacteria with the ultimate goal of defining the relatedness of bacterial groups as well as events in the evolution of these organisms. He proposed to do this by studying the sequence of monomers in proteins or nucleic acids. Study of the sequence of amino acids in conserved proteins had severe limitations and could not serve Carl's purpose. However, the publication by Sanger of a technique for analysis of RNA caught Carl's attention. His previous experiments with the ribosome had convinced him that this organelle was of very ancient origin; it had only one role in the cell and so was "insulated" from the vast phenotypic variations of bacterial cells.

Analysis of the RNAs of the ribosome appeared to hold the potential for exactly what Carl was seeking: monomer sequences in an RNA polymer could lead to the study of very ancient events in evolution. Carl modified the Sanger technique, and by adding radioactive phosphorus to growing bacterial cells, each nucleotide in an RNA molecule could be labeled. Paper chromatography of oligonucleotides of different complexities yielded a unique pattern for each species; analysis of the sequence of nucleotides in each oligonucleotide defined its structure. Carl found that the 23S RNA of the ribosome was too large and complex and the 5S RNA was too small to be statistically useful. However, the 16S RNA was a molecule that could be readily analyzed, and its 1500 nucleotides provided a "statistical ensemble" (Carl's words) that was useful in studying bacterial taxonomy and ancient events in evolution. I suggested that he test his system by analysis of the genus, *Bacillus*. This analysis took many months to complete, but the results convinced me that his technique had great potential. I was probably the first believer outside of Carl's laboratory. For the first time it now seemed possible to put taxonomy on a scientific basis and to rescue it from what I considered to border on witchcraft. Carl proceeded to study various groups of bacteria and by the early 1970s had completed the study of 60 different species of bacteria.

I was not interested in bacterial classification. I was simply interested in how bacteria made a living. A group of poorly studied and poorly understood bacteria, the methanogens, that made methane as their metabolic product, attracted my attention. Study of these organisms presented real challenges, for they were extremely sensitive to oxygen and would only grow in media where the reducing potential was maintained below -330 mV. One of my students, William Balch, accepted my challenge to develop a simple technique for growing methanogens in a pressurized atmosphere of hydrogen and carbon dioxide, or nitrogen and carbon dioxide. Carl was interested in studying the methanogens, especially so when I mentioned to him that the seven or so pure cultures available at that time represented all of the different morphological types of bacteria, but each of them produced only the product methane.

So with this new technique, high levels of radioactive phosphorus could be injected into a culture of a methanogen in perfect safety. This was Carl's first assay with a methanogen. We were asking the question; where does a methanogen belong in Carl's classification? Carl couldn't believe the results of the first experiment; so it was repeated. This time Carl exclaimed: "Wolfe, these cells are not even bacteria!" Of course they are Carl; they look like bacteria in the microscope. "Well, they are not related to anything I've seen." This was the pivotal statement that opened a new era in biology. Methanogens were the first archaea, and Carl would spend the remainder of his scientific career pursuing the archaea, new species from extreme environments, RNA structure, and construction of a tree of living organisms, which reflected ancient evolutionary events.

A major disappointment of Carl's scientific life was the refusal of the biological community to accept the archael concept. Since ancient times, living things had been grouped and classified by their appearance, their morphology. That analysis of a macromolecule could be used to define groups of organisms or specifically a group of organisms distantly related to bacteria or eukaryotes was simply too much for the biologist. So, all through the 1980s and into the '90s Carl became very bitter. Some of his publications during this time reflect his frustration. Then in 1997, 23 y after the first experiment, the authors of a popular textbook in microbiology presented a full treatment of Carl's system. Other microbiology textbooks followed. Authors of biology textbooks were reluctant to take a stand in what they considered to be a controversial area. Biologists couldn't believe that animals and plants could occupy only a small portion at the end of one branch of Carl's vast evolutionary tree. However, they slowly joined in, at first only a paragraph, but by the early 2000s Carl finally began to receive the recognition that he deserved.

This issue is a celebration of Carl's life. I would like to conclude by saying that the scientific experiments with Carl were a high point in my research career. I developed a deep respect for Carl. I admired his total dedication to science. Our association was a truly rewarding experience.

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