van Niel Remembered

Revered as a teacher, C. B. van Niel also contributed substantially to our understanding of the phototrophic purple and green bacteria

NORBERT PFENNIG

The purple and green sulfur bacteria first attracted C. B. van Niel's interest in the mid-1920s, when he worked with Albert Jan Kluyver in Delft, The Netherlands. In his first published work on the subject, van Niel wrote: "From olden times the purple bacteria have raised the interest of the biologist . . ." What makes them so attractive?

On the simplest level, these bacteria are extraordinary creatures to look at, with specimens ranging from purple-red to yellowish green. Moreover, they impart color to their habitats, causing shallow freshwater ponds to look purple-red and sometimes covering the sediment with that same vivid hue. Likewise, in stratified lakes and salt marshes, these bacteria can paint their immediate environment in startling colors.

Over the last century, such observations in the natural environment led microbiologists to bring specimens back to the laboratory for microscopic examination. During the early period, a wide variety of cell types was documented in careful drawings, with names given to them by biologists such as Ehrenberg, Danguard, Ray Lancaster, Cohn, and most comprehensively, Sergius Winogradsky.

While working with Kluyver in 1923, van Niel was asked to study the iron and sulfur bacteria for Kluyver's lecture course on wastewater. In preparing for this task, van Niel became familiar with the literature and the then-current controversy, which soon captured his full attention.

The seeds of that controversy were sewn several decades earlier, when Engelmann discovered the phototaxis of the purple bacteria. He noted that they accumulate in certain spectral bands when light is projected under the microscope-at bands corresponding to the major absorption maxima of these bacteria (420 to 500, 580 to 600, and 800 to 900 nm). Based on his experience in studying various types of green-, yellow-, brown-, and red-colored algae with the same methods, Engelmann concluded that the purple bacteria are photosynthetic organisms, although their absorption spectra were not the same as those of green plant chlorophyll. However, because he was unable to show oxygen evolution in purple bacteria exposed to light, his assertions that these bacteria were photosynthetic were not accepted by his contemporaries.

Meanwhile, also during the 1880s, Winogradsky advanced the concept of the chemosynthetic mode of life on the basis of his studies of the colorless sulfur bacteria, *Beggiatoa* and *Thiothrix* species. He concluded that these aerobic bacteria obtain their energy for autotrophic growth by oxidizing sulfides and sulfur to sulfates. Thus, for the first time, the assimilation of carbon dioxide, which until then had been known to occur in green plants only in the light, was thought not to require light. Instead, sulfide oxidation was considered the source of energy and thus was a substitution of chemical for radiant energy.

Winogradsky also published detailed morphological studies of the purple sulfur bacteria, observing that they store globules of elemental sulfur inside the cells, much like the colorless sulfur bacteria. He concluded that the purple bacteria also have a chemosynthetic metabolism. Although he found the purple bacteria in anoxic habitats, he assumed that they gain their needed oxygen from the various green organisms that he always detected in his preparations. Everything seemed fine to him. Winogradsky's concept of chemosynthesis and Engelmann's concept of photosynthesis for the metabolism of the purple bacteria were the conflicting ideas that attracted van Niel's interest.

While van Niel began studying the purple bacteria in Delft, Kluyver and Donker published *Unity in Biochemistry*, which outlined the possibility of a dehydrogenation and hydrogen transfer mechanism for the oxidation of hydrogen sulfide to sulfate, indicating that hydrogen acceptors other than oxygen might be used. Van Niel set out to test this concept by conducting experiments on pure cultures—a "labor of love" that he later dedicated to Winogradsky and Kluyver. He began this work in Delft, and after he had moved with his family to California in 1929, he finished it at Hopkins Marine Station.

Norbert Pfennig is a professor on the biology faculty at the University of Constance in the Federal Republic of Germany. This article is adapted from the first C. B. van Niel Memorial Lecture, 11 July 1986, in Monterey, Calif.

Systematic Approach

van Niel soon marked progress because he instituted a systematic approach-highlighted by the use of defined mineral media, morphologically and physiologically characterized pure cultures of purple and green sulfur bacteria, quantitative determinations of the substrates and products of anaerobic photometabolism, and proper evaluations of the stoichiometry of the reactions. Thus, he showed unequivocally that the anaerobic metabolism of both purple and green sulfur bacteria is light dependent. Moreover,, the amount of carbon dioxide converted into cellular material is strictly dependent on the amount of hydrogen sulfide provided. The reaction stoichiometry under anaerobic conditions in the light is as follows:

$$CO_2 + 2H_2S \rightarrow (CH_2O) + 2S + H_2O$$

$$3CO_2 + 2S + 5H_2O \rightarrow 3(CH_2O) + 2H_2SO_4$$

$$\text{net: } 2CO_2 + H_2S + 4H_2O \rightarrow 2(CH_2O) + H_2SO_4$$

The similarity between the first reaction and the photosynthetic reaction of green plants and algae (below) is striking:

$$CO_2 + 2H_2O \rightarrow (CH_2O) + H_2O + O_2$$

Noting this similarity, van Niel described photosynthesis in a more general formula, calling it a light-dependent transfer of hydrogen from an appropriate donor, H_2A, to CO_2:

$$2H_2A + CO_2 \rightarrow 2A + (CH_2O) + H_2O$$

van Niel's formulation carries certain important implications. First, it leaves open the possibility that hydrogen donors other than water and hydrogen sulfide, including thiosulfate, molecular hydrogen, and even organic substrates, can serve in CO_2 assimilation. Second, oxygen produced during green plant photosynthesis comes exclusively from water, not from carbon dioxide as was then believed. The use of isotopically labeled oxygen by Ruben, Randall, and Kamen confirmed this second conclusion in 1941. Third, the assimilation of carbon dioxide proceeds through light-independent reactions. This conclusion was confirmed 25 years later through the efforts of Bassham, Benson, Calvin, and Racker. The reactions became known as the reductive pentose phosphate cycle, and it was first established for green plants and algae and then later for purple bacteria. Arnon and his collaborators later outlined the reductive tricarboxylic acid cycle as an alternate means for carbon dioxide assimilation in the green sulfur bacteria.

Interests in Photochemistry, Species Diversity, and Purple Nonsulfur Bacteria

After 1932, van Niel devoted himself to the problem of the photochemical step in photosynthesis, developing the idea that the energy of light is used exclusively for the cleavage of water molecules into reduced and oxidized components. The reduced portion participated in the carbon dioxide assimilation, whereas the oxidized part either yielded molecular oxygen in green plants or oxidized the reduced substrates of the purple and green bacteria. Thirty years of extensive biochemical research in numerous laboratories were necessary to uncover the underlying photosynthetic electron transport systems responsible for these biochemical reactions.

The methods for isolating pure cultures of bacteria were less refined in the 1930s than they are now. Thus, several peculiar forms, which were described by Winogradsky, Lauterborn, and others, frustrated the efforts of researchers to grow them in a pure culture. van Niel nevertheless urged microbiologists to develop the necessary methods- with Helge Larsen, his studies of the green sulfur bacteria proved fruitful during the 1950s. Pure cultures of many of the purple sulfur bacteria were not obtained until later, in about the mid-1960s.

During the mid-1930s, van Niel concentrated on the culture and physiology of the purple nonsulfur bacteria, resuming work initiated by Molisch in 1907. This effort on pure cultures was capped in 1944 with a taxonomic treatment of the entire group, based on the morphological, physiological, and biochemical studies.

At the Hopkins Marine Station, van Niel maintained the largest culture collection of phototrophic bacteria, enabling many laboratories to conduct biochemical studies and to characterize the carotenoids, bacteriochlorophylls, cytochromes, and also details of the biochemical pathways for the assimilation of both carbon dioxide and organic substrates.

Much later, the availability of the electron microscope brought new insights about the photosynthetic membranes of these bacteria, indicating a fundamental difference between them and the cellular organization of photosynthetic eucaryotes. These differences were expressed succinctly as early as 1962 by Stanier and van Niel: "In eucaryotic cells, respiration and photosynthesis take place in specific membrane-bounded organelles, the mitochondria and chloroplasts, respectively.

In the procaryotic cell, there is no equivalent structural separation of major subunits of cellular function. . . . In fact, one can say that no unit of structure smaller than the cell in its entirety is recognizable as the site of either metabolic unit process."

Scientific Legacy

Many of van Niel's concepts have been substantiated by later studies. For example, together with Kluyver he showed extraordinary foresight, anticipating the scientific importance of a phylogenetic system for classifying bacteria. Although in 1936 it was entirely uncertain how the system would take shape, the correct goal already was formulated. It took another three decades before it became clear what properties of bacteria could best serve as a basis for describing their genetic relationships.

The concepts gradually emerged after the biochemistry of protein synthesis became known. In 1965, Zuckerkandl and Pauling differentiated three types of semantophoretic molecules: DNA, mRNA, and proteins. "The most rational, universal, and informative molecular phylogeny will be built on the semantophoretic molecules alone," they wrote.

One such effort to elucidate the natural relationships among the purple nonsulfur bacteria involved comparing the amino acid sequences of their cytochrome *c* proteins. Several laboratories participated between 1970 and 1980, leading to the assignment of the purple nonsulfur bacteria to three major groups of genetic relatedness.

During this period, Carl Woese and his colleagues were developing a technique for cataloging of the oligonucleotide sequences of the 16S ribosomal RNA molecules of bacteria. Significantly, in 1979, they used this system to describe the genetic relatedness of the purple nonsulfur bacteria, finding surprisingly good agreement with the relations derived from the similarities of the cytochrome c_2 amino acid sequences. Because the correspondence was based on two types of functionally unrelated semantophoretic molecules, they could rule out lateral gene transfer as accounting for the findings.

The most remarkable result of these studies of systematics is that many well-known chemotrophic gram-negative bacteria proved to be genetically more closely related to the phototrophic bacteria than are the groups of phototrophs to themselves. This finding indicated a polyphyletic origin of respiring bacteria from different lines of phototrophic ancestors. Such a possibility was first enunciated by Broda in 1971 in his "conversion hypothesis."

The current picture of genetic relatedness of the phototrophic bacteria with anoxygenic photosynthesis can be summarized as follows: all potentially photoautotrophic bacteria are eubacteria; no such phenotypes have been found among the archaebacteria. The filamentous gliding green bacteria, the green sulfur bacteria, and the purple bacteria are only distantly related to one another. The purple bacteria comprise the three subdivisions alpha, beta, and gamma. The first subdivision is dominated by purple nonsulfur bacteria, including the genera *R hodospirillum, R hodopseudomonas, R hodomicrobium,* and *R hodobacter.* The beta subdivision comprises the genus *Rhodocyclus* together with many genera of gram-negative chemotrophic bacteria. The gamma subdivision comprises the purple sulfur bacteria with the two families *Chromatiaceae* and *Ectothiorhodospiraceae,* as well as several groups of chemotrophic bacteria.

Spiritual and Philosophical Conclusions

From this picture of how these bacteria evolved, let's return to what van Niel wrote 30 years ago in a chapter entitled "Evolution as Viewed by the Microbiologist." At first van Niel discusses the origin-of-life problem. In trying to comprehend the two opposing lines of thought on this question, namely, that organisms always have existed or that they originated by spontaneous generation, Samuel Butler had suggested the idea of carrying both ideas down ad infinitum and then formulating a kind of "scientific Athanasian Creed." (The historical Athanasian Creed, enunciated by the church in the fourth century, reconciled the incomprehensibles of the identity of God in the Trinity). After detailed representations, van Niel then shows that the generalized concept of evolution, as comprising physical, chemical, biochemical, and biological phases, together with the principle of integrative levels, provides a rational basis for thinking about life and its origin. This theory also implies a solution to Samuel Butler's enigma. van Niel then continues: "Thus we are left with the final problem of formulating a 'scientific Athanasian Creed.' If we contemplate biochemical and organismic evolution to man, the most complicated organism known to us today, with powers even to destroy himself and the civilizations he has built up over a period of thousands of years, then the inevitable question arises: from man to . . . what?"

Photo courtesy of Hopkins Marine Station Library

C. B. van Niel

van Niel answers this question with a quotation from Gide's journals: "If I had to formulate a credo, I should say: God is not behind us. He is to come. He must be sought, not at the beginning, but at the end of evolution. He is terminal and not initial. He is the supreme and final point toward which all nature tends in time."

For van Niel, this came close to a scientific Athanasian Creed. □