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CORNELIUS BERNARDUS VAN NIEL

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A Biographical Memoir by

H. A. BARKER AND ROBERT E. HUNGATE

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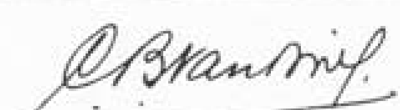

CORNELIS BERNARDUS VAN NIEL

November 4, 1897–March 10, 1985

BY H. A. BARKER AND ROBERT E. HUNGATE

CORNELIS BERNARDUS VAN NIEL —Kees to his friends and students—is best known for his discovery of multiple types of bacterial photosynthesis, his deduction that all types of photosynthesis involve the same photochemical mechanism, and his extraordinary ability to transmit his enthusiasm for the study of microorganisms to his students. His interest in purple and green bacteria developed in his first year as a graduate student. After thoughtful analysis of the confusing literature dealing with these bacteria, he carried out a few simple experiments on their growth requirements. Interpreting the results in accordance with the theories of his professor, A. J. Kluyver, on the role of hydrogen transfer in metabolism, he developed a revolutionary concept of the chemistry of photosynthesis that was to influence research on the topic for many years.

As a teacher he was unsurpassed. Although he taught in a small, somewhat remote institution with modest facilities, the force of his personality, his eloquence and scholarship made the Hopkins Marine Station a mecca for students of general microbiology throughout the western world.

EDUCATION AND EARLY LIFE

Van Niel was born in Haarlem, The Netherlands, into a family steeped in a highly conservative Calvinist tradition.

His father and several uncles were businessmen and did not have a professional education. His father died when he was seven years old, and thereafter his mother largely depended on his uncles for advice in educating her young son. Since family tradition decreed that a son should succeed to his father's business, Kees was sent to a secondary school with a curriculum designed to prepare students for a commercial career.

At the end of his third year in high school when he was fifteen years old, an event occurred that changed the course of his education. The family was spending their summer vacation as guests of a friend on a large estate in northern Holland devoted to various agricultural activities. A part was set aside for testing the effectiveness of various soil treatments on crop production, and van Niel has described how his host introduced him to the methods of agricultural research and how impressed he was to learn that "one could raise a question and obtain a more or less definitive answer to it as a result of an experiment . . . particularly because I had grown up in a milieu where any kind of question was invariably answered by the stereotyped reply: 'Because somebody (usually a member of the family) said so'" (1967,1, p. 2).

Van Niel's interest and enthusiasm for these activities led his family to reevaluate his education, and he was finally allowed to transfer to a college preparatory high school. Under the influence of one of his teachers in the new school he developed a strong interest in chemistry. He liked analytical chemistry so much that he set up a small laboratory at home and analyzed samples of fertilizer in his spare time. His academic record in high school was sufficient to obtain admission to the Chemistry Division of the Technical University in Delft on graduation without taking the usual entrance examination.

He entered the University in autumn 1916 but, after only three months, was inducted into the Dutch army, in which he served until the end of December 1918. Life in the army was both a traumatic and a highly educational experience. Removed from the protective environment of his family for the first time, he was exposed to the rough and impersonal life of military training. He later wrote that up to this time he had been "utterly unaware of the many problems to which man is exposed and with which he must learn to cope." Fortunately, he received practical and intellectual support from a former high school classmate inducted at the same time, Jacques de Kadt.

After a few days in a primitive military camp on the outskirts of Amersfoort, Jacques proposed that they rent a room in the city where they could spend their free time in greater comfort. They were soon joined by a friend of Jacques, and the three comrades spent their leisure hours discussing many subjects. Jacques was an intellectual with a cosmopolitan background. He introduced van Niel to new worlds of literature, art, and philosophy. Under his influence, van Niel read many of the works of Zola, Anatole France, Ibsen, Strindberg, Shaw, and Nietzsche. Their ideas frequently conflicted with van Niel's Calvinist background and led to what he later described as the rebellious phase of his life.

On returning to the University after army service, van Niel was undecided whether he should continue the study of chemistry or take up the study of literature. But, discussing the alternatives with an aunt whose judgment he trusted "at least in part because of her unconventional attitudes and behavior," he was finally persuaded to continue on in chemistry. Still, his mental turmoil was such that he could not immediately switch back into the normal academic routine. He spent the first six months reading French, English, Scandinavian, and Russian 19th century literature and was not prepared to take the first year chemistry examination in June 1919.

In the autumn, however, he finally settled down to serious study and by intensive effort was able in June 1920 to pass both the first- and second-year chemistry examinations. During the following year, van Niel took several courses in biology in addition to the prescribed chemistry program, including G. van Iterson's courses in genetics and plant anatomy and chemistry and M. W. Beijerinck's courses in general and applied microbiology.

By November 1921, van Niel had completed all the requirements for the chemical engineering degree except a year of work in a specialized area of his own choosing. Already strongly attracted to microbiology from his exposure to Beijerinck's courses, he decided to specialize in it after hearing the inaugural lecture of A. J. Kluyver, who succeeded Beijerinck that year.

Kluyver suggested that van Niel investigate the longevity of yeast in a medium containing sugar but little or no nitrogen. This problem provided some experience with microbiological and analytical methods and met the requirements for the degree, though the results were unimpressive.

As a side project, van Niel checked a published report that a nonmotile Sarcina could develop flagella and motility by repeated transfer in a special medium. His first publication (van Niel 1923) showed that the previous author had confused Brownian movement with true motility and that his so-called flagella were artifacts of the staining method.

DELFT: WORKING WITH KLUYVER

After receiving his Chem. E. degree van Niel accepted a position as assistant to Kluyver. His duties consisted of caring for a large, pure culture collection of bacteria, yeasts and fungi; assisting undergraduates; and preparing demonstrations for Kluyver's two lecture courses. One of the courses dealt with the microbiology of water and sewage in which iron and sulfur bacteria play a role. Since Kluyver was unfamiliar with these organisms, he assigned van Niel the task of learning to culture them so that he could provide material for class demonstrations. To fulfill this assignment, van Niel read the publications of Winogradsky, Engelmann, Molisch, and Bavendamm on the colorless and purple sulfur bacteria and concluded that fundamental disagreements concerning the metabolism of these organisms needed clarification. Finding the purple bacteria "aesthetically pleasing," he continued studying them after the lecture demonstrations were completed.

During the next two years, while continuing as Kluyver's assistant, and later as conservator of the Institute, van Niel demonstrated that purple sulfur bacteria could grow in glass-stoppered bottles completely filled with a mineral medium containing sulfide and bicarbonate that were exposed to daylight. (No growth occurred in the dark.) He also isolated pure cultures of a *Chromatium* species and *Thiosarcina rosea* and showed that the yield of cells was proportional to the amount of sulfide provided and much greater than that of colorless aerobic sulfur bacteria in a similar medium.

These observations and the earlier demonstration that O_2 is not produced by purple bacteria were interpreted (in accordance with Kluyver's theory that most metabolic reactions are transfers of hydrogen between donor and acceptor molecules) to mean that purple sulfur bacteria carry out a novel type of photosynthesis in which carbon dioxide is reduced by hydrogen derived from hydrogen sulfide with the aid of energy from light. Mentioned briefly in Kluyver and Donker's treatise, "The Unity in Biochemistry,"[1] without supporting evidence, this interpretation was probably based on van Niel's

[1] A. J. Kluyver and H. J. L. Donker, "Die Einheit in der Biochemie," *Chemie der Zelle und Gewebe*, 13(1926):134–90.

work. Kluyver was not a coauthor of any of van Niel's early papers on photosynthetic bacteria.

During this period Kluyver and van Niel published two papers: one dealing with a new type of yeast, *Sporobolomyces* (thought on the basis of its mode of spore formation to be a primitive basidiomycete), and another providing an explanation for the unusual morphology of a spore-forming bacterium that grew in liquid media as a tightly twisted, multistranded rope.

While van Niel expected to continue his study of purple bacteria for his Ph.D. dissertation, he also developed, as a side project, an effective method for isolating propionic acid bacteria from Swiss cheese. When Kluyver pointed out that a study of this group would provide a faster path to the doctorate than a completion of his investigations of the slow-growing purple bacteria, van Niel reluctantly agreed. He spent the next two years, therefore, studying the biochemistry and taxonomy of the propionic acid bacteria. These biochemical studies were the first to provide a quantitative picture of the products derived from the fermentations of lactate, glycerol, glucose, and starch. His taxonomic studies provided a sound basis for recognition of the species of *Propionibacterium*. Van Niel's dissertation, written in English, was published in 1928.

An unexpected byproduct of the study of the propionic acid bacteria was the identification of diacetyl as the compound responsible for the characteristic aroma of high quality butter. Van Niel noticed that cultures of one of his propionic acid bacteria grown on a glucose medium smelled like butter, then correlated this odor with the distinctive ability of the organism to produce acetylmethylcarbinol, an odorless compound that is readily oxidized to diacetyl, the actual source of the aroma.

Van Niel spent almost seven years in the Delft laboratory, a stimulating period during which Kluyver was developing his ideas about the importance of hydrogen-transfer reactions in metabolism and the similarity of basic biochemical reactions in different organisms (the "unity in biochemistry" theory). Van Niel considered these ideas to be among the most fundamental and fruitful of that era. Revering Kluyver (whom he always referred to as "the Master"), as one of the great scientists of the age, he was yet able at a later time to point out some of Kluyver's errors in the analysis of specific phenomena and his occasional excessive reliance on generalizations lacking adequate experimental support (1959,1).

PACIFIC GROVE: HOPKINS MARINE STATION

In late 1927, L. G. M. Baas-Becking of Stanford University came to Delft looking for a microbiologist to fill a position at the new Jacques Loeb Laboratory at the Hopkins Marine Station on the Monterey Peninsula. Greatly impressed by van Niel's research accomplishments and his capacity for lucid communication, he offered him an appointment as associate professor. Put off by the reputed materialism of American society, van Niel was yet attracted by Becking's enthusiasm for the new laboratory and—encouraged by Kluyver—decided to strike out on his own.

He arrived in California at the end of December 1928 and was immediately impressed by the charm of Carmel, the beautiful site of the Jacques Loeb Laboratory, and the freedom from outside pressures that the Marine Station provided. In later years he could never be persuaded to leave, even to succeed Kluyver at the Delft laboratory.

PHOTOSYNTHESIS STUDIES

At the Hopkins Marine Station van Niel continued his studies of purple and green bacteria with emphasis on the quantitative relations among substrates consumed and products formed. Progress was accelerated by the finding that the bacteria grew more rapidly under continuous artificial illumination. He demonstrated that the green bacteria oxidized hydrogen sulfide only as far as sulfur, whereas the purple sulfur bacteria further oxidized the sulfur to sulfate. Both coupled these oxidations with an essentially stoichiometric conversion of carbon dioxide to cellular materials in light-dependent reactions. The nonsulfur bacteria (Athiorhodaceace, which Molisch had grown aerobically on various organic compounds) were shown to develop anaerobically, but only in the presence of carbon dioxide and light. These and other observations led van Niel to conclude that photosynthesis is essentially a light-dependent reaction in which hydrogen from a suitable oxidizable compound reduces carbon dioxide to cellular materials having the approximate composition of carbohydrate. This was expressed by the generalized equation:

$$2H_2A + CO_2 \xrightarrow{\text{light}} 2A + (CH_2O) + H_2O.$$

According to this formulation, H_2O is the hydrogen donor in green plant photosynthesis and is oxidized to O_2, whereas H_2S or another oxidizable sulfur compound is the hydrogen donor for purple and green sulfur bacteria, and the oxidation product is sulfur or sulfate, depending on the organism. The nonsulfur purple bacteria that require suitable organic compounds in addition to carbon dioxide for anaerobic growth in light were presumed to use these compounds as hydrogen donors and to oxidize them—either partially or completely. Later, the purple sulfur bacteria were also shown to use some organic compounds in place of H_2S in their photometabolism.

These observations and interpretations, the results of some six years of investigation, were first presented at a small meeting of the Western Society of Naturalists in Pacific Grove at the end of 1929. Two years later van Niel published a detailed account of the culture, morphology and physiology of purple and green sulphur bacteria (1931,1), bringing his interpretation of their metabolism and its implications for green-plant photosynthesis to the attention of a wider audience.

All of the purple sulfur bacteria he isolated were relatively small organisms, belonging to what he called Chromatium, Thiocystis, and Pseudomonas types. In material collected in nature (and in some enrichment cultures) he observed a number of larger forms but, despite numerous attempts, was unsuccessful in isolating them. The cultivation of these organisms was not accomplished until many years later, when N. Pfennig and H. G. Schlegel, both onetime associates of van Niel, discovered that nutritional and environmental requirements are more complex than had been previously recognized.[2]

Van Niel published a large monograph covering many years of work on the culture, general physiology, morphology and classification of the nonsulfur purple and brown bacteria in 1944 (1944,2). He classified over 150 strains isolated from natural sources into six species in two genera—*Rhodospeudomonas* and *Rhodospirillum*. He described the morphology of the organisms, their pigments, nutritional requirements, and metabolism in the presence and absence of light. As in all his publications, van Niel also reviewed the historical background and current literature of the subject critically and thoroughly.

Following the recognition of several types of photosynthesis using different hydrogen donors, van Niel began to

[2] H. G. Schlegel and N. Pfennig, "Die Anreicherungskultur einiger Schwefelpurpurbakterien." *Archiv für Mikrobiologie*, 38(1961):1–39, and N. Pfennig and K. D. Lippert, "Über das Vitamin B_{12} Bedurfnisphototropher Schwefelbakterien." *Archiv für Mikrobiologie*, 55(1966):245–56.

consider how radiant energy participates in these reactions. There appeared to be two possibilities, both considered by earlier investigators: radiant energy could be used to activate either carbon dioxide or the hydrogen donor.

Initially, van Niel and Muller (1931,2) were inclined to believe that light is used primarily to activate carbon dioxide, a relatively stable compound and the common reactant in all photosynthetic systems. But they did not exclude the second possibility, that light also activated the hydrogen donor. In this connection they noted a correlation between the presence of nonchlorophyll yellow and red pigments and the nature of the hydrogen donor used by different organisms. These pigments, lacking in the green sulfur bacteria that utilize the easily oxidizable hydrogen sulfide, occur exclusively in organisms utilizing water or sulfur, then thought to require a greater activation. This led van Niel to undertake a series of studies of the pigments of the purple and green bacteria.

Van Niel and Arnold (1938,1) developed a convenient spectrophotometric method for determining the amount of bacteriochlorophyll in photosynthetic purple and brown bacteria under conditions avoiding interference by the red carotinoid pigments. They also reported that van Niel and E. Wiedemann, working in A. Stoll's laboratory, had examined the green pigments of six different strains of purple and brown bacteria and concluded that they were identical with the chlorophyll of the purple sulfur bacterium, *Thiocystis*, previously studied by H. Fischer.

Van Niel and Smith (1935,2) began a study of the chemistry of the major red pigment of the nonsulfur purple bacterium, *Rhodospirillum rubrum*. By solvent extraction and repeated crystallization, they isolated about 100 milligrams of an apparently homogeneous carotinoid they called "spirilloxanthin." Its empirical composition was found to be

$C_{48}H_{66}O_3$, and it contained fifteen double bonds, no more than one hydroxyl group, and no free carboxyl group—making it the most highly unsaturated carotinoid then known.

Rhodoviolascin, a red pigment that had almost the same absorption spectrum and melting point as spirilloxanthin, was later isolated by Karrer and Solmssen from a nonsulfur purple bacterium identified as *Rhodovibrio*.[3] This compound contained two methoxyl groups and had the empirical formula $C_{40}H_{54}(OCH_3)_2$. Polgár, van Niel, and Zechmeister (1944,1) redetermined the molecular weight and composition of spirilloxanthin using material purified by column chromatography and concluded that the formula established by Karrer and Solmssen was correct and that rhodoviolascin and spirilloxanthin are identical. They also found that spirilloxanthin is unstable and reversibly converts, under relatively mild conditions, to two compounds designated neospirilloxanthin A and B, which can be separated from spirilloxanthin chromatographically. A study of the absorption spectra of these compounds under various conditions led to the conclusion that spirilloxanthin is an all-*trans* compound, whereas neospirilloxanthin-A probably contains two *cis* double bonds, one of which is centrally located. In a broader review of the known properties of red pigments derived from various nonsulfur purple bacteria, van Niel (1944,2) concluded that, in addition to spirilloxanthin, at least two other pigments occur in these organisms, distinguishable by their melting points and absorption spectra.

When anaerobic cultures are exposed to oxygen, some strains of nonsulfur purple bacteria undergo a dramatic color change from yellow-brown to deep red. Van Niel (1947,1) investigated this phenomenon in L. Zechmeister's

[3] P. Karrer and U. Solmssen, "Die Carotinoide der Purpurbakterien I," *Helvetica Chimica Acta* 18(1935):1306–15. Parts II and III of this article appear in *Helvetica Chimica Acta* 19(1936):3 and 1019.

laboratory. Using cells of *Rhodopseudomonas spheroides* grown under semianaerobic conditions in continuous light, he isolated the two most abundant red and yellow carotinoid pigments as crystalline products. Both pigments were shown to have all-*trans* configurations and, as previously shown for spirilloxanthin, were easily converted to the *cis*-isomers. In order to follow the pigment changes associated with exposure of anaerobically-grown cells to oxygen, a spectrophotometric method was developed to determine the amounts of red and yellow pigments in a mixture obtained by extracting a cell suspension. Using this method the yellow pigment was shown to be partially and irreversibly converted to the red pigment when anaerobically-grown cells were exposed to oxygen. As previously noted by C. S. French, the conversion of the yellow carotinoid to red occurred only in the presence of actively metabolizing bacteria. The nature of the chemical transformation responsible for the color change was not determined.

Studies in several laboratories of the role of various pigments in photosynthesis and phototaxis by *Rhodospirillum rubrum* had resulted in conflicting conclusions as to whether spirilloxanthin with absorption maxima at 550, 510 and 480 nm, or another pigment with maxima at 530, 490 and 460 nm was the photoactive compound. A possible explanation for this discrepancy was provided by L. N. M. Duysen,[4] who observed that the absorption spectrum, and therefore presumably the pigment composition, of *R. rubrum* changed with the age of the culture. Young cultures showed a minor 530 nm absorbance peak, gradually replaced by the 550 nm peak of spirilloxanthin as the culture aged. This observation was confirmed by van Niel and Airth (unpublished work, 1954) with two strains of *R. rubrum*. Van Niel, Goodwin, and Sissins

[4] L. N. M. Duysens, unpublished doctoral thesis for the University of Utrecht, 1952.

(1956,1) subsequently identified the carotinoids in young cultures and showed that these indeed decreased with time, while spirilloxanthin increased from about twenty percent of the total carotinoids in a one-day-old culture to about ninety percent in a five-day culture.

These studies provided information concerning the identity and properties of the pigments of photosynthetic bacteria but did little to clarify the role of the pigments in photosynthesis.

By 1936 van Niel's interpretation of the role of the photochemical system in photosynthesis had changed radically (1936,3). He had abandoned the earlier theory that radiant energy participated directly in carbon dioxide activation when he recognized that various nonphotosynthetic bacteria, including several chemoautotrophic species, methanogenic bacteria and propionic acid bacteria, readily utilized carbon dioxide in the dark. Furthermore, the idea that each of the many inorganic and organic compounds used as substrates by the photosynthetic bacteria were directly involved in a photochemical reaction appeared unlikely, particularly since van Niel had shown that certain organic compounds used by the nonsulfur purple bacteria are oxidized both in the dark with O_2 or in the light in the absence of O_2. He later demonstrated that even the rates of organic substrate oxidation are the same in the dark and in the photosynthetic reaction, provided the light intensity is sufficiently high (1941,2; 1949,2).

Van Niel finally concluded that both plant and bacterial photosynthetic reactions have a common photochemical reaction: the photolysis of water to form a strong reducing agent and a strong oxidizing agent. He postulated that the reducing agent was used, through a series of enzymatic reactions, to convert carbon dioxide to cellular constituents; whereas the oxidizing agent was used either to generate O_2

in green plant photosynthesis or to oxidize the hydrogen donor in bacterial photosynthesis. Van Niel's unified interpretation of the photochemical event in photosynthesis is similar in principle to the current interpretation of this process, although a special type of chlorophyll (rather than water) is now considered to be the source of the light-generated oxidizing and reducing species.

In collaboration with H. Larsen and C. S. Yocum, van Niel investigated the energetics of photosynthesis in green sulfur bacteria supplied with different reducing agents with the object of determining whether the energy released by oxidation of the reducing agents was used to reduce carbon dioxide (1952,3). They determined the number of light quanta used to convert one molecule of CO_2 into cell material when either H_2, thiosulfate, or tetrathionate was used as the reducing agent. Photosynthesis with H_2 was expected to require about 28,000 calories less than with the other substrates because of the large energy change associated with H_2 oxidation, but—finding that the number of light quanta required to reduce one molecule of CO_2 was approximately the same with all three substrates—they concluded that the energy obtained by the oxidation of the electron donor is not used for CO_2 assimilation.

Several other postdoctoral fellows who studied with van Niel made significant contributions to understanding the biology and physiology of photosynthetic bacteria. Providing background and inspiration for these investigations, van Niel gave encouragement and advice during the experimental work, evaluated results critically, and aided in preparing the manuscripts—but was seldom willing to become a coauthor of the final publications. Many of his own scientific contributions, consequently, are embedded in the publications of his associates, as in F. M. Muller's 1933 publications on the utilization of organic compounds by purple sulfur bacteria;

J. W. Foster's 1944 paper on the coupling of CO_2 reduction to the oxidation of isopropanol to acetone by nonsulfur purple bacteria; H. Larsen's works in 1952 and 1953 on the culture and physiology of green sulfur bacteria; and R. K. Clayton's 1955 report on the relation between photosynthesis and respiration in *Rhodospirillum rubrum*. Van Niel's influence can also be seen in Pfennig's work on the nutrition and ecology of photosynthetic bacteria.

METHANE PRODUCTION AND CARBON DIOXIDE UTILIZATION

Van Niel's studies of photosynthetic bacteria led him to consider other processes in which carbon dioxide utilization might occur. In the early 1930s he had postulated that methane formation from organic compounds by anaerobic bacteria was the result of carbon dioxide reduction. This idea was based upon the investigations of N. L. Söhngen, a student of Beijerinck who had studied the decomposition of lower fatty acids by methanogenic enrichment cultures under anaerobic conditions and found that formate and lower fatty acids with an even number of carbon atoms are converted quantitatively to carbon dioxide and methane. The identity of the products, therefore, was independent of the chain-length of the substrate. Söhngen's cultures, furthermore, could convert hydrogen and carbon dioxide to methane according to the equation:

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O.$$

Since carbon dioxide is clearly reduced to methane in this reaction, van Niel concluded that this also occurs in the fermentation of fatty acids. Carbon dioxide, in other words, was postulated to serve as hydrogen acceptor for the oxidation of fatty acids to carbon dioxide and water. This could explain why methane is the only reduced compound formed in the methane fermentation of organic compounds—a theory that received support from the 1939–1940 demonstration by H. A. Barker that a purified culture of a methanogen apparently coupled the oxidation of ethanol to acetic acid with the reduction of carbon dioxide to methane. In 1967, however, M. P. Bryant et al. found that the culture contained two kinds of bacteria—one which oxidizes ethanol to acetate and H_2, and the methanogen that converts H_2 and carbon dioxide to methane.

The formation of methane from all but a few organic compounds now appears to require a similar participation of a non-methanogenic bacterium. Van Niel's carbon dioxide reduction theory of methane formation from organic compounds, consequently, is valid only for the syntrophic association of two species.

Following the early studies of S. Ruben and M. D. Kamen at the University of California, Berkeley, on biological carbon dioxide fixation by use of the short-lived carbon isotopes ^{11}C, van Niel and some of his students collaborated in similar studies with propionic acid bacteria,[5] fungi,[6] and protozoa (1942,3). The experimenters sought to confirm and extend the unexpected discovery of H. G. Wood and C. H. Werkman that succinic acid is formed in part from carbon dioxide. The ciliate *Tetrahymena geleii* was also shown to incorporate carbon dioxide into succinate, whereas the fungi *Rhizopus nigricans* and *Aspergillus niger* incorporated carbon dioxide into the carboxyl groups of fumarate and citrate, respectively.

Van Niel's special contribution to these investigations was his attempt to understand the general requirement of non-

[5] S. F. Carson, J. W. Foster, S. Ruben, and H.A. Barker, "Radioactive carbon as an indicator of carbon dioxide utilization. V. Studies on the propionic acid bacteria." *PNAS* 27(1941):229–35.

[6] J. W. Foster, S. F. Carson, S. Ruben, and M. D. Kamen, "Radioactive carbon as an indicator of carbon dioxide utilization. VII. The assimilation of carbon dioxide by molds. *PNAS* 27(1941):590–96.

photosynthetic microorganisms for carbon dioxide and the mechanism of its fixation (1942,2). He concluded that carbon dioxide fixation generally occurs by carboxylation reactions and that carbon dioxide is probably required to counteract the decarboxylation of oxaloacetate, which "constitutes a 'leak' through which certain essential cell constituents are drained off."

In 1935 H. A. Barker, at van Niel's suggestion, undertook a study of the respiratory activity of the colorless algae *Prototheca zopfii*. His original objective was to use Otto Warburg's manometric method to identify the organic compounds that the organism could oxidize and to determine the quantities of O_2 consumed and CO_2 produced from a known quantity of each substrate. The data showed that the amounts of O_2 and CO_2 were far below those required for complete oxidation, the gas exchange accounting for only seventeen to fifty percent of that required for complete oxidation depending on the particular substrate. The rest of the substrate was apparently converted into storage or cellular materials with the approximate empirical composition of carbohydrate. This unexpectedly high conversion of respiratory substrates to cell materials became known as oxidative assimilation. In Kluyver's laboratory, G. Giesberger and C. E. Clifton subsequently obtained similar results with several bacteria.

Because of its apparent relation to the synthesis of cell materials in photosynthesis and the general problem of the utilization of the products and energy of respiration for assimilatory purposes, van Niel maintained a continuing interest in this phenomenon. He and his students studied assimilation reactions of both yeast and bacteria, the most interesting result being the demonstration that yeast but not lactic acid bacteria assimilate about thirty percent of the glucose decomposed under anaerobic conditions—a process they called "fermentative assimilation."

Bacterial Taxonomy

One of van Niel's most enduring scientific interests outside of photosynthesis and photosynthetic bacteria was bacterial taxonomy. In his doctoral dissertation he had reviewed what he called "the main features of bacterial taxonomy" and proposed a possible sequence for the evolution of various morphological types of bacteria. Starting from a presumably primitive, nonmotile, spherical cell, it progressed along three postulated evolutionary lines to polarly flagellated spirilla, peritrichously flagellate sporulating rods, and permanently immotile rods forming conidia.

With small modifications, this concept of morphological evolution formed the basis of the taxonomic system proposed by Kluyver and van Niel (1936,4). Four morphological families defined by cell shape, type of flagellation, and sporulation were subdivided by morphology into eleven tribes. The organisms in the morphological tribes were further assigned to sixty-three genera on the basis of types of energy metabolism, substrate utilization and—among chemo-heterotrophic anaerobes—products of metabolism. Although recognizing that this taxonomic system was an oversimplification, the authors believed that it was more rational and "natural," i. e., phylogenetic, than previous systems.

In 1941, van Niel and R. Y. Stanier undertook an analysis of the problems of classification of the larger taxonomic units among microorganisms (1941,3). After pointing out glaring deficiencies in the definitions of major microbial groups in *Bergey's Manual*,[7] they concluded that for larger taxa, morphological characteristics should be given priority over physiological characteristics. On this basis they decided that the blue-green algae (Myxophyta) and the bacteria (Schizomy-

[7] *Bergey's Manual of Determinative Bacteriology*, ed. D. H. Bergey, Baltimore: Williams and Wilkins Co., 5th edition, 1936.

cetae) should be combined in the kingdom, *Monera*, which comprises organisms without true nuclei, plastids, and sexual reproduction. The Schizomycetae were then separated into four classes: Eubacteriae, Myxobacteriae, Spirochaetae, and a heterogeneous group of organisms not falling into the other classes. The Eubacteriae were further separated into three orders (Rhodobacteriales, Eubacteriales, Actinomycetales) on the basis either of type of metabolism (photosynthetic, nonphotosynthetic) or cell organization (unicellular, mycelial). Each of these groups was defined as precisely as possible with the information available, the authors emphasizing that the proposed system was a first draft and subject to revision as new information accumulated.

By 1946, van Niel no longer believed that a taxonomic system based on phylogenetic considerations was possible in view of the relatively few morphological properties of bacteria, the general absence of developmental processes, and the probability of the occurrence of both convergent and divergent evolution in the development of existing groups (1946,1). He pointed out that attempts to classify bacteria in a single system by the use of morphological, physiological, nutritional, and ecological properties was only partially successful. Since different properties often overlapped, a single organism could be assigned to more than one taxonomic group or could not be readily assigned to any. He concluded that attempts to accommodate all known bacteria in a single taxonomic system should be abandoned until more information on phylogenetic relationships was available. He went so far as to suggest that the use of binomial nomenclature should be discontinued until phylogenetic relationships could be firmly established and proposed, in the meantime, bacteria could be identified more readily by multiple keys based upon any of several conspicuous and readily determinable properties.

By 1955, van Niel had become skeptical of the possibility of separating bacteria and blue-green algae from other organisms on the basis that they lacked nuclei, plastids, and sexual reproduction. New developments had weakened or destroyed these negative criteria as differential characters. He noted that some bacteria contained "discrete structures that might be considered, on the basis of their behavior and chemical nature, as nuclei"; that photosynthetic pigments of some purple bacteria and blue-green algae were located in uniform spherical particles rather than being distributed evenly throughout the cells; and that, in *E. coli*, an exchange of genetic characters between cells had been clearly demonstrated (1955,1).

On the basis of new information developed since van Niel's 1955 paper, Stanier and van Niel (1962,2) again examined the criteria used to distinguish bacteria and blue-green algae from viruses and other protists. In agreement with Lwoff,[8] they noted that the structures and modes of reproduction of viruses differ from those of bacteria and that no ambiguity existed as to the taxonomic position of rickettsia, pleuro-pneumonia-like organisms, and other obligately parasitic bacteria. The bacteria and blue-green algae were separated from all other protists by the procaryotic nature of their cells. They distinguished the procaryotic from the eucaryotic cell by the absence of internal membranes separating nuclear material and—when present—respiratory and photosynthetic apparatuses from each other and from the cytoplasm. In addition, the nuclei of procaryotes divide by fission rather than by mitosis, their cell walls contain mucopeptides as a strengthening element, and the structure of the flagella, when present, is unique. The authors concluded that there was no adequate basis for separating bacteria from blue-green algae.

[8] A. Lwoff, "The concept of virus." *Journal of General Microbiology*, 17(1957):239–53.

DENITRIFICATION

Van Niel published two papers dealing with aspects of the chemistry of denitrification. Allen and van Niel (1952,1) investigated the pathway of nitrite reduction by *Pseudomonas stutzeri*. Initially they tested the possibility that the conversion of nitrite to N_2 may involve a reaction between nitrite and an amine, but no supporting evidence could be obtained. They then tested possible intermediates in nitrite reduction by the technique of simultaneous adaptation and the use of various inhibitors and found that neither N_2O nor hyponitrite could fulfill this role. Nitramide, $H_2N.\ NO_2$, however, was found to be reduced readily to N_2 at about the same rate as nitrite and the utilization of both compounds was inhibited by cyanide to the same extent. Nitramide, consequently, was considered to be a possible intermediate in denitrification.

In 1920 Warburg and Negelein reported that algae exposed to light in a nitrate solution produce O_2 in the absence of added carbon dioxide. They postulated that the algae used nitrate to oxidize cellular organic compounds to carbon dioxide, which was then used for O_2 production by photosynthesis.[9]

Van Niel, Allen, and Wright proposed the alternative interpretation that nitrate replaces carbon dioxide as the electron acceptor in photosynthesis (1953,1). They showed that when nitrate-adapted *Chlorella* is exposed to high light-intensity in a medium containing excess carbon dioxide, the rate of O_2 production increased with the addition of nitrate. This increased rate could not have been caused by an increase in carbon dioxide production, since the reaction was already saturated with this compound. The higher rate, then, could

[9] O. Warburg and E. Negelein, "Über die Reduktion der Saltpetersäure in grünen Zellen." *Biochemische Zeitschrift,* 110(1920):66–115.

only result from the utilization of nitrate as an additional electron acceptor.

VAN NIEL THE GENERALIST

As his reputation as a scientist and teacher spread, van Niel responded to many invitations to lecture and write reviews. In the early part of his career these mostly dealt with bacterial photosynthesis and its relation to plant photosynthesis. Later he often dealt with broader topics such as "The Delft School and the Rise of General Microbiology" (1949,4), "The Microbe as a Whole" (1955,4), "Natural Selection in the Microbial World" (1955,3), "Evolution as Viewed by the Microbiologist" (1956,2c), and "Microbiology and Molecular Biology" (1966,1). He always displayed an impressive command of historical background and current literature and a notably clear, analytical, and elegant style of presentation.

"On radicalism and conservatism in science" (1955,2), his presidential address to the Society of American Bacteriologists in 1954, was a clear statement of van Niel's personal philosophy—a strong preference for the heretical and unconventional over established and accepted dogma, despite his recognition of the weaknesses and strengths of both. For him the essence of science was the development of an attitude of mind that "accepts experience as the guiding principle by which it is possible to test the relative merits of opposing viewpoints by means of carefully conducted, controlled experiments," and "recognizes equally keenly that our knowledge and capacities are exceedingly limited, not merely if considered from the standpoint of the individual, but even with reference to the combined experience of the human race." He concluded that the most desirable mental characteristics of a scientist are objectivity and tolerance, and that his greatest satisfaction should derive from "having enriched the experience of his fellow men."

Van Niel's chapter on "Evolution as Viewed by the Microbiologist" (1956,2c) provided a stimulating synthesis of ideas concerning the origin of life and the relation of living to nonliving systems. By the application of both logic and intuition to the available scientific information and theory, he developed the hypothesis that life is a special property of matter that inevitably appears when chemical systems reach a state of sufficient complexity under suitable conditions. His generalized concept of evolution comprised physical, chemical, biochemical, and biological phases of which only the last corresponds with evolution in the Darwinian sense.

TEACHER AND COLLEAGUE

In addition to being an outstanding investigator, van Niel was a superlative teacher, and his greatest contribution to science may well have been his teaching of general microbiology and comparative biochemistry.

Soon after coming to the Hopkins Marine Station he began offering a ten-week laboratory course in microbiology. Initially, the content of the course was similar to that given at Delft and consisted of an introduction to methods of isolating and identifying microorganisms in commercial yeast, milk, water, and soil. But van Niel soon realized that neither Beijerinck's elective culture methods—based on the principle of natural selection—nor Kluyver's ideas about comparative biochemistry were appreciated in this country. He therefore undertook to develop a course emphasizing these approaches to microbiology and biochemistry. Van Niel's students learned how numerous morphological and physiological types of bacteria, when their nutritional and environmental requirements were known, could be enriched and isolated from natural sources. He discussed the metabolism of each group, emphasizing the most recent findings regarding intermediary metabolism, similarities and differences in degradative pathways, and the chemical and energetic relations between degradative metabolism and the synthesis of cellular components. He examined the structure of bacterial cells, aspects of bacterial genetics, variation and adaptation, bacterial and yeast taxonomy, and the philosophy of science.

The course was organized as a series of relatively simple experiments for which van Niel provided the background, rationale, and interpretation of results. He was always in the laboratory guiding the work and commenting on each student's observations and results and often used the Socratic method, stimulating students to make judgments about the meaning of their observations and sometimes intentionally leading them to some plausible but incorrect conclusion so that a later experiment, already planned, would reveal the error. After a topic or phenomenon had been introduced in a laboratory experiment, he would launch into a presentation of its historical background, usually starting with the most primitive ideas and progressing to the latest developments. He always placed great emphasis on possible alternative interpretations of the available information at each phase of scientific development and on the frequently slow and difficult process of moving from clearly erroneous to more nearly correct—but never immutable—conclusions.

His lectures often lasted for several hours and were presented with such clarity and histrionic skill as to capture the complete attention and stimulate the enthusiasm of his students. As the course developed over the years along with the literature of microbiology, lectures took up a larger proportion of the available time. The course expanded from three afternoons to three days a week, with class hours often extending from eight in the morning to well into the evening, with time out only for lunch and tea and coffee breaks. The course was very strenuous for van Niel, who was never particularly robust, and in his later years he was so exhausted by its end he needed some weeks to recuperate.

During the early years, only a few students attended, but as van Niel's reputation as a teacher spread, the class had to be limited, initially to eight, and later to fourteen students—the number that could be accommodated in the small Marine Station laboratory. The students were initially undergraduate or graduate students from Stanford, but later a large proportion came from other institutions. In 1950, for example, only one of the thirteen students was from Stanford. The others were from Washington University, Wisconsin, Michigan, Missouri, California Institute of Technology, Connecticut, Illinois, Cambridge, and the University of California at Los Angeles. In addition there were eleven auditors of the discussions and lectures who did not do the experiments—mostly postdoctoral fellows or established scientists who wished to extend their background in general microbiology. The lists of students and auditors who attended van Niel's course between 1938 and 1962 reads like a *Who's Who* of biological scientists in the United States, with several, as well, from other countries. Both directly, and indirectly through his students, van Niel exerted a powerful influence on teaching and research in general microbiology for a generation.

Although his own research was concerned mainly with photosynthetic bacteria, van Niel was interested in the biology and metabolism of many other groups of microorganisms. He did not believe in directing the research of his younger associates but rather encouraged them to follow their own interests, some of which had been stimulated by his lectures and personal discussions. As a consequence, the range of phenomena investigated in his laboratory was exceedingly wide and included the culture and physiology of blue-green algae and diatoms, nutritional and taxonomic studies of plant-pathogenic bacteria, biological methane formation, pteridine and carbohydrate metabolism of protozoa, germination of mold spores, biology of caulobacteria, cultivation of free-living spirochetes, induction of fruiting bodies in myxobacteria, decomposition of cellulose, the role of microorganisms in the food cycle of aquatic environments, adaptation of bacteria to high salt concentrations, cultivation of spirilla and colorless sulfur bacteria, bacterial fermentations, thermophylic bacteria, denitrification, pyrimidine metabolism, and the thermodynamics of living systems. To all students van Niel gave freely of his time, advice and enthusiasm, drawing on his own extraordinary knowledge of the literature.

RETIREMENT

Following his retirement from the Marine Station in 1962, van Niel held a visiting professorship at the University of California at Santa Cruz from 1964 to 1968, teaching part of a freshman-level biology course in collaboration with K. V. Thimann and L. Blinks.

After 1972, van Niel gave up teaching and research entirely and disposed of his scientific library and large collection of reprints. Thereafter he lived quietly with his wife, Mimi, in Carmel and spent his leisure reading classical and modern literature and listening to classical music, which he greatly enjoyed. He was often visited by former students who continued to be impressed by the warm hospitality of his home, the charm of his personality, the breadth of his understanding, and the comprehensiveness of his memory.

HONORS AND DISTINCTIONS

DEGREES AND HONORARY DEGREES

1923 Chemical Engineering, Technical University, Delft
1928 D.Sci., Technical University, Delft
1946 D.Sci. (Honorary), Princeton University
1954 D.Sci. (Honorary), Rutgers University
1968 LL.D., University of California, Davis

FELLOWSHIPS AND PROFESSIONAL APPOINTMENTS

1925–1928 Conservator, Laboratorium voor Microbiologie, Delft
1928–1935 Associate Professor of Microbiology, Stanford University, Hopkins Marine Station
1935–1936 Rockefeller Foundation Fellow
1935–1946 Professor of Microbiology, Stanford University
1945 John Simon Guggenheim Fellow
1946–1963 Herstein Professor of Biology, Stanford University
1955–1956 John Simon Guggenheim Fellow
1963–1985 Herstein Professor, Emeritus, Stanford University
1964–1968 Visiting Professor, University of California, Santa Cruz

AWARDS AND HONORS

1942 Stephen Hales Prize, American Society of Plant Physiology
1964 Emil Christian Hansen Medalist, Carlsberg Foundation of Copenhagen
1964 National Medal of Science
1966 Charles F. Kettering Award, American Society of Plant Physiology
1967 Rumford Medal, American Society of Arts and Sciences
1967 Honorary Volume, Archiv für Mikrobiologie
1970 Antonie van Leeuwenhoek Medal, Royal Netherlands Academy of Sciences

LEARNED SOCIETIES

1945 National Academy of Sciences
1948 American Philosophical Society
1950 American Academy of Arts and Sciences
1952 Charles Reid Barnes Life Membership, American Society of Plant Physiology

1954 President, American Society for Microbiology
1954 Corresponding Member, Academy of Sciences, Göttingen, Germany
1958 American Academy of Microbiology
1963 Honorary Member, Société Française de Microbiologie
1967 Honorary Member, Society of General Microbiology
1968 Honorary Member, Royal Danish Academy of Sciences and Letters

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1927

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1929

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