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# MICHAEL DOUDOROFF

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*A Biographical Memoir by*

H.A. BARKER



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BY H. A. BARKER

MICHAEL DOUDOROFF WAS A general microbiologist who made major contributions to knowledge of carbohydrate metabolism in bacteria. His early studies of sucrose utilization by *Pseudomonas saccharophila*, a bacterium he isolated and made famous, established the importance of glucosyl transfer reactions in metabolism and provided the first substantial evidence that an enzyme may function as a glucosyl carrier. His investigations of glucose oxidation by extracts of *P. saccharophila* resulted in the discovery of a major pathway of glucose degradation in bacteria, the Entner-Doudoroff pathway. Other sugars were shown to be metabolized by similar, but divergent, pathways. His studies of assimilatory processes in aerobic and photosynthetic bacteria demonstrated that poly-β-hydroxybutyric acid is a major storage product formed from substrates metabolized via acetate or butyrate and is utilized by means of both intracellular and extracellular enzymes. In the latter part of his career Doudoroff and his associates extensively clarified taxonomic and phylogenetic relationships in the genus *Pseudomonas* and certain other aerobic bacteria.

Doudoroff was born in Petrograd (St. Petersburg), Russia, the son of a naval officer. In 1917 his father became a member of the short-lived Kerensky government and sub-

sequently was appointed naval attaché to the Russian embassy in Japan. The family left Russia shortly before the October revolution and lived in Tokyo for six years before moving to San Francisco in 1923. They moved to Palo Alto in 1930.

In Tokyo young Michael started his formal education in the third grade of an English-language school after having previously been tutored privately in English, French, and probably other subjects. In San Francisco he attended Lowell High School, the best college preparatory school in the city. Like many other children, Michael first developed an interest in biology by observation of curious or beautiful insects. He began collecting beetles and later butterflies in Japan and greatly enlarged his collection in California. One of the butterflies he collected turned out to be a new species and was given the specific name *doudoroffii.* On entering Stanford University in 1929, he planned to major in biology and specialize in entomology. However, as his exposure to science broadened, he was attracted to bacteriology and protozoology. As an undergraduate he carried out two short studies on aspects of bacterial variation under the guidance of Professor W. H. Manwaring. His master's thesis, done under the supervision of Dr. A. C. Giese, demonstrated that the survival of *Paramecium* at elevated temperatures is strongly influenced by its nutritional status. For his Ph.D. thesis research (1934–39), Doudoroff moved to the laboratory of Professor C. B. van Niel at the Hopkins Marine Station, where he investigated a topic of his own choosing, the adaptation of *E. coli* to elevated salt concentrations. He demonstrated that this involves both an acclimatization, independent of reproduction, and a selection of cells with an increased salt tolerance. While at the Marine Station he twice served as van Niel's assistant in the soon-to-become-famous course in general microbiol-

ogy and was introduced to the extraordinary physiological and biochemical diversity of the microbial world. This led him to undertake some studies of luminous bacteria and $H_2$-oxidizing bacteria. His main contribution to knowledge of bacterial luminescence resulted from the discovery that certain poorly luminescent strains are unable to synthesize riboflavin. Addition of a small amount of this vitamin to deficient media caused an increase in growth without affecting luminescence, whereas a larger addition increased luminescence without any further stimulation of growth or respiration. These observations provided the first evidence that riboflavin is directly involved in bacterial luminescence.

The $H_2$-oxidizing bacteria that Doudoroff isolated included a new species, *Pseudomonas saccharophila*, which can also oxidize a number of mono-, di-, and polysaccharides. Since most bacteria only oxidize di- and polysaccharides after first hydrolyzing them to monosaccharides, Doudoroff was surprised to find that cells of *P. saccharophila*, grown upon sucrose, oxidize this sugar much more rapidly than its constituent monosaccharides, glucose and fructose. His efforts to elucidate this anomaly—subsequently shown to be caused by the absence of permeases for the monosaccharides—led him in time to undertake a series of brilliant investigations on the enzymatic mechanisms of the degradation of sucrose and other sugars by bacteria.

In 1940 Doudoroff joined the faculty of the Bacteriology Department, University of California, as an instructor. His first major research contribution at Berkeley, made in collaboration with N. O. Kaplan and W. Z. Hassid, was the discovery that extracts of *P. saccharophila* catalyze a reaction between sucrose and inorganic phosphate to form glucose 1-phosphate and fructose. Since the reaction proved to be readily reversible, it was used to synthesize sucrose, a sugar not previously synthesized by either chemical or enzymatic

methods. The enzyme catalyzing this reaction was subsequently partially purified and shown not to degrade or synthesize any common disaccharide other than sucrose. However, Doudoroff and his associates found that in the reverse (synthetic) reaction fructose can be replaced by certain analogs, D-ketoxylose and L-sorbose, resulting in the formation of novel analogs of sucrose.

Insight into the mode of action of sucrose phosphorylase was obtained by studying the incorporation of radioactive inorganic phosphate into glucose 1-phosphate. Initially, Doudoroff and associates thought the enzyme incorporated inorganic phosphate into glucose 1-phosphate only in the presence of fructose or sucrose, which permitted the reversal of the overall reaction. However, they found that only glucose 1-phosphate, orthophosphate, and enzyme are needed to effect a rapid exchange of phosphate between the two substrates. This led to the concept that sucrose phosphorylase functions as a transglucosidase, an enzyme that transfers the glucosyl residue from a suitable donor such as sucrose or glucose 1-phosphate to an appropriate glucosyl acceptor such as fructose or orthophosphate. Supporting evidence was provided by showing that the enzyme catalyzes transfer of the glucosyl moiety of sucrose to sorbose to form glucose 1-sorboside and fructose in the absence of orthophosphate. This and other similar experiments provided some of the first evidence for the formation of a substrate-enzyme complex as an intermediate in an enzymatic reaction.

Attempts by Weimberg and Doudoroff to demonstrate directly the formation of a glycosyl-enzyme complex in the sucrose phosphorylase reaction were unsuccessful because of insufficient purification of the enzyme by the methods then available and because of an intrinsic hydrolytic activity of the enzyme. After better purification techniques were

developed, R. H. Abeles and his associates, in 1967, purified the enzyme to homogeneity and demonstrated that it does indeed bind transferable glucose, as had been postulated many years earlier.

The previously mentioned synthesis of the novel nonreducing sucrose analog, glucose 1-sorboside, by sucrose phosphorylase was followed by the synthesis of three other analogs, glucosido-D-ketoxyloside, glucosido-L-ketoarabinoside, and glucosido-rhamnoside (Doudoroff and Hassid, 1948,4). These compounds all contain 1-5 linkages between the monosaccharide units. Unexpectedly, the same enzyme was also found to catalyze the synthesis of glucosido-L-arabinose, a reducing sugar containing a 1-3 linkage. The role of sucrose phosphorylase in this synthesis appears to have been firmly established, but the mechanistic basis for the formation of this structurally distinct product could not be established.

Doudoroff, Hassid, and Barker (1947,1–4) found that arsenate can substitute for phosphate in the sucrose phosphorylase reaction as it does in the oxidation of 3-phosphoglyceraldehyde. The presumed product, glucose 1-arsenate, is unstable and is hydrolyzed to glucose and arsenate. The net result is an "arsenolytic" conversion of sucrose to glucose and fructose. In the presence of arsenate, glucose 1-phosphate undergoes a similar enzymic cleavage. Later Doudoroff, Katz, and Hassid (1948,1) showed that potato phosphorylase catalyzes an arsenolytic conversion of amylose and amylopectin to glucose.

A second type of phosphorolytic enzyme, maltose phosphorylase, was found in *Neisseria meningitidis* by Doudoroff and Fitting (1952,2). Extracts of this organism had been shown previously to catalyze a reaction between the disaccharide maltose and orthophosphate to form glucose and a phosphate ester with properties similar to those of glu-

cose 1-phosphate when the isolated ester was incubated with glucose and the *Neisseria* enzyme maltose was formed. However, the phosphate ester derived from maltose did not serve as a glucosyl donor in the sucrose phosphorylase reaction, and synthetic α-D-glucose 1-phosphate could not serve as a cosubstrate for the *Neisseria* enzyme. These observations led to the conclusion and subsequent demonstration that the phosphate ester product of maltose phosphorylase has the β rather than the α configuration. The mechanism of action of maltose phosphorylase was shown to differ from that of sucrose phosphorylase. The former, unlike the latter, is unable to catalyze a direct exchange between β-D-glucose 1-phosphate and orthophosphate or arsenate and is unable to cause an exchange between maltose and glucose in the absence of the phosphate ester. On the basis of these results, Doudoroff and Fitting proposed that the mechanism of the maltose phosphorylase reaction involves a maltose-enzyme-phosphate complex as a probable intermediate.

The discovery of sucrose and maltose phosphorylases led Doudoroff to investigate the mechanisms of synthesis or degradation of other polymeric carbohydrates by *P. saccharophila* and other bacteria. Raffinose—a trisaccharide of galactose, glucose, and fructose, and an analog of sucrose—was found not to undergo a phosphorolytic cleavage but to be hydrolyzed by the enzyme melibiase to galactose and sucrose (Doudoroff, 1945,2). Trehalose, a nonreducing glucose disaccharide, is also cleaved hydrolytically by the enzyme trehalase. Maltose was found to be neither hydrolyzed nor phosphorolyzed in *E. coli* but is converted by the enzyme amylomaltose, a transglucosidase, to glucose and a glucose polymer (Doudoroff et al., 1949,2). The latter then undergoes phosphorolysis to form glucose 1-phosphate, which is further metabolized via glucose 6-

phosphate. Doudoroff and O'Neal (1945,3) investigated the reversibility of the long-known bacterial conversion of sucrose to levulan, a fructose polymer, and glucose by an enzyme from *Bacillus subtilis*. By using invertase to detect small amounts of sucrose form in the enzymic reaction between levulan and glucose, they obtained evidence for the reversibility of levulan synthesis. Although the enzymatic mechanisms of oligosaccharide degradation elucidated by Doudoroff and his associates were of great interest, they did not account for the preferential ability to metabolize, for example, sucrose more rapidly than its constituent monosaccharides. Doudoroff was acutely aware of this lack of understanding and in two reviews (1945,2; 1951,2) proposed and critically evaluated various possible explanations. He reached the conclusion, later found by others to be correct, that the utilization of sugars is controlled by permeability mechanisms involving sugar-specific carrier proteins. His thoughtful analysis of this problem undoubtedly stimulated the development of this important area of research.

About 1950 Doudoroff and his associates began a series of investigations of the oxidative degradation of various sugars by *P. saccharophila* that revealed several new pathways of carbohydrate metabolism. Doudoroff and Entner (1952,1) studied the enzymatic oxidation of glucose and identified glucose 6-phosphate, 6-phosphogluconate, D-glyceraldehyde 3-phosphate, 3-phosphoglycerate, and pyruvate as intermediate products. The novel feature of this widely used pathway is the conversion of 6-phosphogluconate to pyruvate and glyceraldehyde 3-phosphate. 2-Keto-3-deoxy-6-phosphogluconate was postulated to be an intermediate in this reaction and was subsequently shown to fulfill this role by Doudoroff and MacGee (1954,1), who isolated and characterized the compound. The enzyme catalyzing cleavage

of the keto acid, ketodeoxyphosphogluconate aldolase, was later purified and crystallized by Doudoroff and Shuster (1967).

Several other sugars were shown to be metabolized by *P. saccharophila* by similar but partially divergent pathways. L- and D-arabinose and L-galactose are all metabolized via the corresponding nonphosphorylated aldonic gamma-lactones and aldonic acids. Then the pathways diverge. L-Arabinose is oxidized via an unidentified, unstable intermediate to α-ketoglutarate in such a way that the carboxyl carbon adjacent to the carboxyl group is derived from the carboxyl carbon of arabonic acid (Doudoroff and Weimberg, 1955). Reactions of the tricarboxylic acid cycle were shown not to be involved in this novel reaction. The α-ketoglutarate is further oxidized to pyruvate. D-Arabonic acid is dehydrated to 2-keto-3-deoxy-arabonic acid, which is cleaved and oxidized to pyruvate, derived from carbon atoms 1 to 3, and glycolate, derived from carbon atoms 4 and 5 (Doudoroff et al., 1956,1,2; Doudoroff and Palleroni, 1956,3,4). D-Galacturonic acid is dehydrated to form 2-keto-3-deoxygalactonic acid, which, after phosphorylation, is cleaved by a specific aldolase to pyruvate and glyceraldehyde 3-phosphate (Doudoroff and DeLey, 1957,2; Doudoroff et al., 1957,3,4; Doudoroff and Wilkinson, 1964; Doudoroff and Shuster, 1967). Fructose, which is well utilized by certain mutants of *P. saccharophila*, is converted to fructose 6-phosphate and then metabolized in the same way as glucose (Doudoroff et al., 1956,1,2). Doudoroff and Szymona (1960) found that *Rhodopseudomonas spheroides* contains enzymes of both the Embden-Meyerhoff and Entner-Doudoroff pathways of glucose degradation but apparently uses the latter pathway preferentially. Several enzymes catalyzing the above reactions and a novel mannose isomerase (Doudoroff and Palleroni, 1956,3,4) were purified and their properties determined.

Following the recognition of oxidative assimilation, the conversion of a large fraction of an oxidizable substrate into cellular components by washed suspensions of microorganisms, Doudoroff (1940,2) investigated this phenomenon in *P. saccharophila.* During this study, the ability of the organism to metabolize sucrose more rapidly than glucose and fructose was first observed. Subsequently, Doudoroff and his associates (Doudoroff and Whelton, 1945,1; Bernstein 1944) extended their studies of oxidative assimilation in *P. saccharophila* to compare the magnitude of assimilation in growing cultures with that in cell suspensions. These studies and those in other laboratories during the 1940s established the large magnitude of assimilation in several aerobic bacteria oxidizing a wide range of substrates but did little to clarify the underlying metabolic reactions. However, Doudoroff and Wiame (1951,4) made a solid contribution to knowledge of oxidative assimilation by studying the oxidation of $^{14}C$-labeled substrates. They found that both carbons of acetate, carbons 2 and 3 of lactate, and the two methylene carbons of succinate are largely assimilated, whereas the carboxyl carbons of lactate and succinate are mainly converted to carbon dioxide. This indicated that the acetyl moieties derived from various substrates are probably a major source of assimilated carbon. Doudoroff and Stanier (1959,2) were stimulated to develop a more general explanation of oxidative assimilation by the observation of their colleague, Germaine Cohen-Bazire, that purple bacteria accumulate massive amounts of poly-β-hydroxybutyric acid during growth on certain organic acids. Poly-β-hydroxybutyric acid (PHB) was originally discovered by Lemoigne in 1927 as a major component of the cells of *Bacillus megaterium.* Doudoroff and Stanier examined the products of oxidative assimilation from glucose, acetate, and butyrate in *P. saccharophila* and of photosynthetic as-

similation from acetate and butyrate in *Rhodospirillum rubrum* and found with all those substrates that a major fraction (60 to 90 percent) of the assimilated carbon initially accumulated within the cells as PHB. When the external substrate was removed, the stored polymer was degraded intracellularly. These observations indicated that PHB can serve as an important reserve of carbon and energy as does starch or triglycerides in other organisms.

Doudoroff et al. (1959,1) also made a major contribution to understanding the role of organic substrates in bacterial photosynthesis. Earlier van Niel had concluded that organic substrates serve primarily, if not exclusively, as sources of reducing power for the conversion of carbon dioxide to cellular components. By using $^{14}C$-labeled acetate and butyrate, Doudoroff et al. showed that the oxidation of these substrates and the reduction of carbon dioxide are minor reactions. Most of these and other substrates are assimilated directly as poly-β-hydroxybutyric acid or as polysaccharides.

Doudoroff and his associates investigated the enzymes involved in PHB synthesis and degradation. The immediate precursor of PHB was shown to be D-β-hydroxybutyryl-coenzyme A, presumably formed by reduction of acetoacetyl-coenzyme A (Doudoroff and Merrick, 1961,1). The polymerase was found to be associated with granules of PHB and could not be obtained in soluble form. Although washed granules and associated enzyme convert β-hydroxybutyryl-coenzyme A to PHB in relatively high yields, the inability to separate enzyme and product prevented detailed analysis of the system (Doudoroff, 1966,2). *Rhodospirillum rubrum*, which stores PHB, contains a soluble intracellular enzyme system that degrades the polymer. This system also was found to be unexpectedly complex and refractory to analysis. Purified PHB is inactive as a substrate; only the PHB in washed

granules, derived from cells that synthesize the polymer, can be hydrolyzed. Even the granules proved to be very labile. They are inactivated as substrates for the soluble hydrolytic enzyme system by mild treatments such as freezing and thawing or even repeated washing by centrifugation. The soluble intracellular enzyme system that degrades PHB was shown to contain at least three separable components, a thermostable activator, a thermolabile depolymerase, and an esterase. The activator, which appears to be a protein, causes no demonstrable chemical change in granules but is essential for the activity of the depolymerase. Together these two components hydrolyze PHB mainly to D-β-hydroxybutyric acid and smaller amounts of dimer. The esterase hydrolyzes the dimer. The β-hydroxybutyric acid was shown to be oxidized to acetoacetate by a DPN-dependent dehydrogenase that was purified from both *R. rubrum* and *Pseudomonas lemoignei* and studied in some detail.

Since large amounts of PHB synthesized by microorganisms must be available in nature, Doudoroff et al. (1965,3) investigated the ability of aerobic soil bacteria to use extracellular PHB as a major energy source. They isolated over 50 strains of PHB-using bacteria, most of which belong to the genus *Pseudomonas.* The most active polymer-using strains belong to a new species, *P. lemoignei.* This organism produces extracellular enzymes that hydrolyze purified polymer to a mixture of D-β-hydroxybutyric acid, the dimeric ester of this acid, and small amounts of trimeric ester. They also form an intracellular "dimeric hydrolase" that hydrolyzes the dimeric ester. All these enzymes are produced constitutively, regardless of the carbon source on which the bacteria are grown. Both the highly specific dimer hydrolase and an NAD-specific D-β-hydroxybutyrate dehydrogenase were partially purified from extracts (Doudoroff et al., 1965,3,4). Doudoroff and Lusty (1966,3) subsequently

found that the extracellular PHB depolymerase of *P. lemoignei* can be separated into two fractions, one of which preferentially forms trimers and then hydrolyzes them to dimers and monomers in roughly equal molar quantities, whereas the other fraction preferentially forms dimers and relatively low yields of monomeric D-β-hydroxybutyric acid.

In the early 1960s, Doudoroff was persuaded by his somewhat domineering colleague Roger Stanier to undertake a collaborative study of the taxonomy of the genus *Pseudomonas.* Initially he was reluctant to participate in this undertaking, which differed greatly from his previous research and could be expected to involve the collection of a vast amount of data obtained by relatively routine methods. However, he gradually developed enthusiasm for the project, and in collaboration with Stanier, Norberto Palleroni, and several graduate students and postdoctoral fellows made extensive contributions to knowledge of the taxonomy of pseudomonads and certain other aerobic bacteria.

The first publication resulting from this investigation was a massive survey of 169 phenotypic characters of 267 strains of *Pseudomonas* (Doudoroff et al., 1966,1). The ability to utilize 146 organic compounds as sources of carbon and energy was determined. Other characteristics studied included production of extracellular hydrolases, denitrifying ability, $H_2$ chemolithotrophy, pigment production, accumulation of poly-β-hydroxybutyric acid as a cellular reserve material, biochemical pathways of aromatic ring cleavage, and the type of aerobic electron transport system. Analysis of the data so obtained permitted recognition of a relatively small number of species that can be distinguished from each other by multiple, unrelated phenotypic differences. Perhaps the most important result of these studies was the recognition that classification of aerobic bacteria by phenotype requires the determination and correlation

of a much larger number of nutritionally diverse characters than had previously been used for this purpose.

Although the initial classification of pseudomonads was based entirely on phenotypic characters, Palleroni, Doudoroff, and associates later used the newer techniques of DNA-DNA and ribosomal RNA-DNA hybridization to investigate the genotypic and phylogenetic relations among species. On the basis of ribosomal RNA homologies they were able to divide thirty-five species or subspecies of *Pseudomonas* and one species of *Xanthomonas* into five major evolutionary lineages. Closer relationships among species within each lineage were established by DNA homologies. These comprehensive studies, which looked at bacterial classification from the point of view of phenotypic analysis, genetic relationship, and comparative biochemistry, served as a model for subsequent investigations. Doudoroff and Palleroni summarized their conclusions about the taxonomy of *Pseudomonas* in the *Annual Review of Phytopathology* (1972,3) and developed a practical scheme for identification of twenty-nine species for the eighth edition of *Bergey's Manual of Determinative Bacteriology.*

With Stanier and others Doudoroff investigated the taxonomy of other bacteria, including some denitrifying bacteria, $H_2$-utilizing bacteria, and organisms of the *Moraxella* group. The denitrifying bacteria, most of which had been previously classified as *Pseudomonas denitrificans,* were found to belong to several species and at least two genera on the basis of phenotypic characters and DNA and ribosomal RNA homologies. The $H_2$-utilizing bacteria, previously placed in the genus *Hydrogenomonas,* were shown to be a heterogeneous group; some organisms were assigned to the genus *Alcaligenes* and others to the genus *Pseudomonas.* The authors proposed that the genus *Hydrogenomonas* be discarded. Studies of the *Moraxella* group, done mainly by Paul Baumann,

supported separation of these organisms into two genera, *Moraxella* and *Acinetobacter*, on the basis of a cytochrome c-dependent oxidase reaction, and recognized one species of *Acinetobacter* and several species of *Moraxella* on the basis of correlated phenotypic properties.

Doudoroff exerted a profound influence on the teaching of bacteriology at Berkeley. When he joined the department of bacteriology as an instructor in 1940, the courses of instruction emphasized mainly the medical and paramedical aspects of the subject. Doudoroff was given responsibility for teaching the introductory lecture and laboratory courses in general bacteriology, and he proceeded to reorganize them along the lines developed by C. B. van Niel and the Delft School of Microbiology. This involved the presentation of bacteria and other microorganisms as creatures whose structures, behaviors, and metabolic activities were worthy of study independently of their roles in agriculture, industry, or disease. Doudoroff brought great enthusiasm, a broad knowledge of general microbiology, and more than a touch of drama to his teaching. He was solely responsible for instruction in general microbiology for some years until R. Y. Stanier and E. A. Adelberg joined the department. Together they later wrote the excellent and widely used textbook, *The Microbial World*, based upon the courses Doudoroff had developed and which he continued to teach until his death. Thus, his influence on the teaching of bacteriology extended far beyond the university.

Doudoroff had a warm, outgoing personality. He loved conversation, the give and take of a lively discussion. He often enlivened seminars with penetrating questions or stimulating comments. As a scientist he was an accomplished experimentalist with insight to recognize and skill to solve a variety of biochemical and biological problems.

Doudoroff's contributions to microbiology and biochemistry were recognized by several honors and awards. In 1945 he received the first Sugar Research Award of the National Academy of Sciences with H. A. Barker and W. Z. Hassid. He became a J. S. Guggenheim Foundation fellow in 1949 and collaborated with Fritz Lipmann at Massachusetts General Hospital and Jacques Monod at the Pasteur Institute. In 1960–62 he held a Miller Research Professorship at the University of California, Berkeley, and in 1963 he was awarded a National Institutes of Health Special Postdoctoral Fellowship for studies with Professor Georges N. Cohen at the Centre National de la Recherche Scientifique, Gif-sur-Yvette, France. In 1962 he was elected to membership in the National Academy of Sciences.

In the late 1930s, Doudoroff married Mary Gottlund, a painter of considerable ability. They had one son, Michael John, now a professor of Spanish at the University of Kansas. The Doudoroffs were divorced about 1944, and he subsequently married Rita Whelton, who had been one of his graduate students. She died after a few years. His third wife, Olga Fowlks, had a son and daughter by a previous marriage. They formed a happy family. The death of Olga in 1974 was a crushing blow to Doudoroff. He died of cancer the following year after a short illness at the age of sixty-three.

## SELECTED BIBLIOGRAPHY

1935

Bacterial antifibrinolysins. *Proc. Soc. Exp. Biol. Med.* 32:1467–68.

1936

Studies on thermal death in Paramecium. *J. Exp. Zool.* 72:369–85.

Association of characters among dissociates from *Staphylococcus aureus*. *Proc. Soc. Exp. Biol. Med.* 34:216–17.

Dynamics of dissociated bacterial cultures. *Proc. Soc. Exp. Biol. Med.* 35:339–41.

1938

Lactoflavin and bacterial luminescence. *Enzymologia* 5:239–43.

1940

Experiments on the adaptation of *Escherichia coli* to sodium chloride. *J. Gen. Physiol.* 23:585–611.

The oxidative assimilation of sugars and related substances by *Pseudomonas saccharophila* with a contribution to the problem of the direct respiration of di- and polysaccharides. *Enzymologia* 9:59–72.

1942

Studies on the luminous bacteria. I. Nutritional requirements of some species, with special reference to methionine. *J. Bacteriol.* 44:451–59.

Studies on the luminous bacteria. II. Some observations on the anaerobic metabolism of facultatively anaerobic species. *J. Bacteriol.* 44:461–67.

1943

With N. O. Kaplan and W. Z. Hassid. Studies on the nutrition and metabolism of *Pasteurella pestis*. *Proc. Soc. Exp. Biol. Med.* 53:73–75.

Phosphorolysis and synthesis of sucrose with bacterial preparation. *J. Biol. Chem.* 148:67–75.

Studies on the phosphorolysis of sucrose. *J. Biol. Chem.* 151:351–61.

1944

With W. Z. Hassid and H. A. Barker. Enzymatically synthesized crystalline sucrose. *J. Am. Chem. Soc.* 66:1416–19.

With W. Z. Hassid and H. A. Barker. Synthesis of two new carbohydrates with bacterial phosphorylase. *Science* 100:315–16.

1945

With R. Whelton. Assimilation of glucose and related compounds by growing cultures of *Pseudomonas saccharophila*. *J. Bacteriol.* 49:177–86.

On the utilization of raffinose by *Pseudomonas saccharophila*. *J. Biol. Chem.* 157:699–706.

With R. O'Neal. On the reversibility of levulan synthesis by *Bacillus subtilis*. *J. Biol. Chem.* 159:585–92.

With W. Z. Hassid, H. A. Barker, and W. H. Dore. Isolation and structure of an enzymatically synthesized crystalline disaccharide D-glucosido-L-sorboside. *J. Am. Chem. Soc.* 67:1394–97.

On the utilization and synthesis of sucrose and related compounds by some microorganisms. *Fed. Proc.* 4:241–47.

1946

With W. Z. Hassid, H. A. Barker, and W. H. Dore. Isolation and structure of an enzymatically synthesized crystalline disaccharide D-glucosido-D-ketoxyloside. *J. Am. Chem. Soc.* 68:1465–67.

1947

With H. A. Barker and W. Z. Hassid. Studies with bacterial sucrose phosphorylase. I. The mechanism of action of sucrose phosphorylase as a glucose-transferring enzyme (transglucosidase). *J. Biol. Chem.* 168:725–32.

With W. Z. Hassid and H. A. Barker. Studies with bacterial sucrose phosphorylase. II. Enzymatic synthesis of a new reducing and of a new non-reducing disaccharide. *J. Biol. Chem.* 168:733–46.

With H. A. Barker and W. Z. Hassid. Studies with bacterial sucrose phosphorylase. III. Arsenolytic decomposition of sucrose and of glucose-1-phosphate. *J. Biol. Chem.* 170:147–50.

With W. Z. Hassid and H. A. Barker. Enzymatically synthesized disaccharides. *Arch. Biochem.* 14:29–37.

1948

With J. Katz and W. Z. Hassid. Arsenolysis and phosphorolysis of the amylose and of the amylopectin fractions of starch. *Nature* 161:96–97.

With W. Z. Hassid, A. L. Potter, and H. A. Barker. The structure of the enzymatically synthesized reducing disaccharide D-glucosido-L-arabinose. *J. Am. Chem. Soc.* 70:306–10.

With A. L. Potter, J. C. Snowden, and W. Z. Hassid. Alpha-L-glucose-1-phosphate. *J. Am. Chem. Soc.* 70:1751–52.

With W. Z. Hassid. Enzymatically synthesized polysaccharides and disaccharides. *Fortschr. Chem. Org. Naturst.* 5:101–27.

1949

With J. N. Wiame and H. Wolochow. Phosphorolysis of sucrose by *Pseudomonas putrefaciens*. *J. Bacteriol.* 57:423–27.

With W. Z. Hassid, E. W. Putman, A. L. Potter, and J. Lederberg. Direct utilization of maltose by *Escherichia coli*. *J. Biol. Chem.* 179: 921–34.

With H. Wolochow, E. W. Putman, W. Z. Hassid, and H. A. Barker. Preparation of sucrose labeled with $^{14}C$ in the glucose or fructose component. *J. Biol. Chem.* 180:1237–42.

1950

With W. Z. Hassid. Synthesis of disaccharides with bacterial enzymes. *Adv. Enzymol.* 10:123–43.

With W. Z. Hassid and H. A. Barker. Phosphorylases: Phosphorolysis and synthesis of saccharides. In *Enzymology*, ed. Summer and Myrbach, pp. 1014–39. New York: Academic Press.

With H. P. Klein. The mutation of *Pseudomonas putrefaciens* to glucose utilization and its enzymatic basis. *J. Bacteriol.* 59:739–50.

1951

With W. Z. Hassid. Enzymatic synthesis of sucrose and other disaccharides. In *Advances in Carbohydrate Chemistry*, vol. 5, ed. C. S. Hudson and S. M. Cantos, pp. 29–48. New York: Academic Press.

The problem of the direct utilization of disaccharides by certain microorganisms. In *Phosphorus Metabolism*, vol. 1, ed. W. D. McElroy and B. Glass, pp. 42–48. Baltimore, Md.: The Johns Hopkins University Press.

With E. R. Stadtman and F. Lipmann. The mechanism of acetoacetate synthesis. *J. Biol. Chem.* 191:377–82.

With J. M. Wiame. Oxidative assimilation by *Pseudomonas saccharophila* with $^{14}C$-labeled substrates. *J. Bacteriol.* 62:187–93.

1952

With N. Entner. Glucose and gluconic acid oxidation of *Pseudomonas saccharophila*. *J. Biol. Chem.* 196:853–62.

With C. Fitting. Phosphorolysis of maltose by enzyme preparations of *Neisseria meningitidis*. *J. Biol. Chem.* 199:153–63.

1954

With J. MacGee. A new phosphorylated intermediate in glucose oxidation. *J. Biol. Chem.* 210:617–26.

With R. Weimberg. Studies with three bacterial sucrose phosphorylases. *J. Bacteriol.* 68:381–88.

1955

With R. Weimberg. The oxidation of L-arabinose by *Pseudomonas saccharophila*. *J. Biol. Chem.* 217:607–24.

1956

With N. J. Palleroni, J. MacGee, R. Contopoulou, and M. O'Hara. Metabolism of carbohydrates by *Pseudomonas saccharophila*. I. Oxidation of fructose by intact cells and crude cell-free preparations. *J. Bacteriol.* 71:196–201.

With N. J. Palleroni, J. MacGee, R. Contopoulou, and M. O'Hara. Metabolism of carbohydrates by *Pseudomonas saccharophila*. II. Nature of kinase reaction involving fructose. *J. Bacteriol.* 71:202–7.

With N. J. Palleroni. Mannose isomerase of *Pseudomonas saccharophila*. *J. Biol. Chem.* 218:535–48.

With N. J. Palleroni. Preparation and properties of D-rhamnulose (6-deoxy-D-fructose) and glucosyl rhamnuloside. *J. Biol. Chem.* 219:957–62.

With N. J. Palleroni. Characterization and properties of 2-keto-3-deoxy-D-arabonic acid. *J. Biol. Chem.* 223:499–508.

1957

With N. J. Palleroni. Metabolism of carbohydrates by *Pseudomonas saccharophila*, III. Oxidation of D-arabinose. *J. Bacteriol.* 74:180–5.

With J. DeLey. The metabolism of D-galactose in *Pseudomonas saccharophila*. *J. Biol. Chem.* 227:745–57.

With R. Contopoulou and S. Burns. Galactose and L-arabinose dehydrogenases of *Pseudomonas saccharophila*. In *Proceedings, International Symposium on Enzyme Chemistry*, Tokyo and Kyoto, pp. 313–17.

1959

With R. Y. Stanier, R. Kunisawa, and B. Contopoulou. The role of organic substrates in bacterial photosynthesis. *Proc. Natl. Acad. Sci. U.S.A.* 45:1246–60.

With R. Y. Stanier. Role of poly-β-hydroxybutyric acid in the assimilation of organic carbon by bacteria. *Nature* 183:1440–42.

1960

With M. Szymona. Carbohydrate metabolism in *Rhodopseudomonas spheroides*. *J. Gen. Microbiol.* 22:167–83.

1961

With J. Merrick. Enzymatic synthesis of poly-β-hydroxybutyric acid in bacteria. *Nature* 189:890–92.

Disaccharide phosphorylases. In *The Enzymes*, vol. 5, ed. P. Boyer, H. Lardy, and K. Myrbaech, pp. 229–36.

1962

D-Galactose dehydrogenase of *Pseudomonas saccharophila*. In *Methods in Enzymology*, vol. 5, ed. S. P. Colowick and N. O. Kaplan, pp. 339–42. New York: Academic Press.

L-Arabinose dehydrogenase of *Pseudomonas saccharophila*. In *Methods in Enzymology*, vol. 5, ed. S. P. Colowick and N. O. Kaplan, pp. 342–44. New York: Academic Press.

With C. W. Shuster. A cold-sensitive D(-)β-hydroxybutyric and dehydrogenase from *Rhodospirillum rubrum*. *J. Biol. Chem.* 237:603–7.

1963

With G. Cohen, J. C. Patte, P. Truffa-Bachi, and C. Sawas. Repression and end product inhibition in a branched biosynthetic pathway. *Colloq. Int. C. N. R. S.* pp. 243–53.

1964

With J. M. Merrick. Depolymerization of poly-β-hydroxybutyrate by an intracellular enzyme system. *J. Bacteriol.* 88:60–71.

1965

With N. J. Palleroni. Identity of *Pseudomonas saccharophila*. *J. Bacteriol.* 89:264.

With D. J. Niederpruem. Cofactor-dependent aldose dehydrogenase of *Rhodopseudomonas spheroides*. *J. Bacteriol.* 89:697–705.

With F. P. Delafield and K. E. Cooksey. β-Hydroxybutyric dehydrogenase and dimer hydrolase of *Pseudomonas lemoignei*. *J. Biol. Chem.* 240:4023–28.

With F. P. Delafield, N. J. Palleroni, C. J. Lusty, and R. Contopoulou. Decomposition of the poly-β-hydroxybutyrate by pseudomonads. *J. Bacteriol.* 90:1455–66.

1966

With R. Y. Stanier and N. J. Palleroni. The aerobic pseudomonads: A taxonomic study. *J. Gen. Microbiol.* 43:159–271.

Metabolism of poly-β-hydroxybutyrate in bacteria. In *Current Aspects of Biochemical Energetics*, ed. N. O. Kaplan and E. P. Kennedy, pp. 385–400. New York: Academic Press.

With C. J. Lusty. Poly-β-hydroxybutyrate depolymerases of *Pseudomonas lemoignei*. *Proc. Natl. Acad. Sci. U.S.A.* 56:960–65.

1967

With C. W. Shuster. Purification of 2-keto-3-deoxy-6-phosphohexonate aldolases of *Pseudomonas saccharophila*. *Arch. Mikrobiol.* 59:279–86.

1968

With P. Baumann and R. Y. Stanier. A study of the *Moraxella* group. I. The genus *Moraxella* and the *Neisseria catarrhalis* group. *J. Bacteriol.* 95:58–73.

With P. Baumann and R. Y. Stanier. A study of the *Moraxella* group. II. Oxidative-negative species (genus *Acinetobacter*). *J. Bacteriol.* 95:1520–41.

With R. W. Ballard, R. Y. Stanier, and M. Mandel. Taxonomy of the aerobic pseudomonads: *Pseudomonas diminuta* and *P. vesiculare*. *J. Gen. Microbiol.* 53:349–61.

1969

With D. H. Davis and R. Y. Stanier. Proposal to reject the genus *Hydrogenomonas*: Taxonomic implications. *Int. J. Syst. Bacteriol.* 19:375–90.

1970

With N. J. Palleroni, R. Y. Stanier, R. E. Sola'nes, and M. Mandel.

Taxonomy of the aerobic pseudomonads: Properties of the *Pseudomonas stutzeri* group. *J. Gen. Microbiol.* 60:215–31.

With F. Gasser and R. Contopoulou. Purification and properties of the NAD-dependent lactic dehydrogenases of different species of *Lactobacillus*. *J. Gen. Microbiol.* 62:241–50.

With D. H. Davis, R. Y. Stanier, and M. Mandel. Taxonomic studies on some gram-negative polarly flagellated "hydrogen bacteria" and related species. *Arch. Mikrobiol.* 70:1–13.

With R. W. Ballard, N. J. Palleroni, R. Y. Stanier, and M. Mandel. Taxonomy of the aerobic pseudomonads: *Pseudomonas cepacia, P. marginata, P. alliicola* and *P. caryophylli*. *J. Gen. Microbiol.* 60:199–214.

1971

With N. J. Palleroni. Phenotypic characterization and deoxyribonucleic acid homologies of *Pseudomonas solanacearum*. *J. Bacteriol.* 107:690–96.

1972

With E. Ralston and N. J. Palleroni. Deoxyribonucleic acid homologies of some so-called "*Hydrogenomonas*" species. *J. Bacteriol.* 109:465–66.

With N. J. Palleroni, R. W. Ballard, and E. Ralston. Deoxyribonucleic acid homologies among some *Pseudomonas* species. *J. Bacteriol.* 110:1–11.

With N. J. Palleroni. Some properties and taxonomic subdivisions of the genus *Pseudomonas*. *Annu. Rev. Phytopathol.* 10:73–100.

1973

With D. C. Hildebrand and N. J. Palleroni. Synonymy of *Pseudomonas gladioli* Severini 1913 and *Pseudomonas marginata* McCulloch 1921, Stapp 1928. *Int. J. Syst. Bacteriol.* 23:433–37.

With N. J. Palleroni, R. Kunisawa, and R. Contopoulou. Nucleic acid homologies in the genus *Pseudomonas*. *Int. J. Syst. Bacteriol.* 23:333–39.

With E. Ralston and N. J. Palleroni. *Pseudomonas pickettii*, a new species of clinical origin related to *Pseudomonas solanacearum*. *Int. J. Syst. Bacteriol.* 23:15–19.

With A. H. Neilson. Ammonia assimilation in blue-green algae. *Arch. Mikrobiol.* 89:15–22.

1974

With R. Contopoulou, R. Kunisawa, and N. J. Palleroni. Taxonomic validity of *Pseudomonas dentrificans* (Christensen) Bergey et al. Request for an opinion. *Int. J. Syst. Bacteriol.* 24:294–300.

With N. J. Palleroni. *Pseudomonas.* In *Bergey's Manual of Determinative Bacteriology,* 8th ed., ed. R. E. Buchanan and N. E. Gibbons, pp. 217–43. Baltimore: Williams and Wilkins Co.

With J. J. Sanchez and N. J. Palleroni. Lactate dehydrogenases in cyanobacteria. *Arch. Mikrobiol.* 104:57–65.

1980

With A. B. Champion, E. L. Barrett, N. J. Palleroni, L. Soderberg, R. Kunisawa, R. Contopoulou, and A. C. Wilson. Evolution in *Pseudomonas fluorescens. J. Gen. Microbiol.* 120:485–511.