HISTORY OF CHEMISTRY IN THE NATIONAL INSTITUTE OF DIABETES AND DIGESTIVE AND KIDNEY DISEASES (NIDDK)

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Supplemental material

Abstract

The origins of the Laboratory of Bioorganic Chemistry, NIDDK, NIH can be traced to events that occurred in the early 20th century. From its beginning to the present, as the laboratory evolved through several organizational changes, many important historical contributions to organic chemistry and biochemistry were made. For example, its early precursor, the Division of Chemistry of the Hygienic Laboratory, was assigned the responsibility of safeguarding public health by analyzing environmental and other chemical risks. This review will trace important developments from the early 20th century to the present. The topics covered in this review include a historical synopsis, early work on receptors, carbohydrates, heterocycles and nucleotides, with specific emphasis on frog skin alkaloids, the NIH shift (a transfer of an aromatic hydrogen atom to a neighboring ring position during ring hydroxylation, important in the biochemical processing of aromatic substrates), the methionine-specific cleavage of proteins using cyanogen bromide (used commercially and in peptide research) as well as other fundamental contributions. Ongoing research in medicinal chemistry, natural products, biochemistry, vaccines and pharmacology, some leading to clinical applications, will be discussed.

Introduction

The National Institutes of Health (NIH), with headquarters in Bethesda, Maryland, USA, is the largest biomedical research institution in the world, with approximately 7000 researchers supporting basic and "bench-to-bedside" translational research (that is, practical application of basic science to enhance human health). The NIH, including its Intramural Research Program (IRP), is almost wholly supported by the US Federal Government, in recognition of the benefits of basic biomedical research to public health. Dozens of pharmaceuticals have been developed and introduced to the market with the direct participation of NIH scientists (1). Additionally, development of countless pharmaceuticals has been facilitated by novel concepts discovered at NIH and published in the research literature. Thus, the NIH IRP has provided many basic research discoveries that have been and continue to be instrumental in the discovery and development of new medical treatments and diagnostics. The NIH IRP has also played an important role in the mentoring of American and foreign scientists through its training programs.

Although only a handful of the laboratories inside NIH are focused primarily on medicinal chemistry, these research groups, including what is now the Laboratory of

Bioorganic Chemistry (LBC) of the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), have a major historical impact. This report describes the contributions of many chemists to the research of this Institute, including the unique roles of key researchers in the development of organic chemistry in NIDDK. Within this laboratory, there have been major efforts, both in the past and present, on G protein-coupled receptors (GPCRs) and ion channels, natural products, carbohydrates, anti-infective drugs, nucleosides and nucleotides, and heterocycles. Specifically, such topics as toxins that act at ion channels, receptors for drugs of abuse, biogenic amines, carbohydrates, and purine receptors are discussed here. We provide a synopsis of each of these diverse areas of research associated historically and currently within this laboratory.

Early Historical (Chronological) Perspective

This section discusses the origins of the organic chemistry laboratories of NIDDK. These laboratories include what are now designated as the Laboratory of Bioorganic Chemistry (LBC) and the Laboratory of Medicinal Chemistry (LMC), which have origins within a common progenitor, the Laboratory of Chemistry (LC) (Figure 1). In order to understand the origins of the LBC, it is necessary to trace the reorganizations within NIDDK (nee NIAMD, National Institute of Arthritis and Metabolic Diseases) that resulted in the current makeup of these organic chemistry laboratories. These laboratories were direct descendants of the Division of Chemistry of the Hygienic Laboratory of the US Public Health and Marine-Hospital Service (shortened to Public Health Service in 1912) in Washington, DC (2-7).

The precursor to NIH was the Hygienic Laboratory, which in 1891 moved from New York City (where it was concerned with immigrant health) to Washington, DC, where it adopted a broader role in evaluating scientific factors affecting public health. The importance of chemistry in the context of US government biomedical research became apparent in the early decades of the 20th century, especially with increased understanding of the role of chemistry in living processes. This was accentuated in Nobel prizes during that period (6, 8). Thus, the early precursor of LBC, the Division of Chemistry of the Hygienic Laboratory, was founded in 1905 and assigned the responsibility of safeguarding public health, which came to include analyzing environmental and other chemical risks and developing chemical diagnostic tests. The laboratory evolved through several organizational changes and its eventual move to the current location in Bethesda, MD, where it made many historically important contributions to organic chemistry and biochemistry.

The Division of Chemistry was established though an Act of Congress in 1902, although it began work in 1905 (6, 7). The reorganization legislation added three

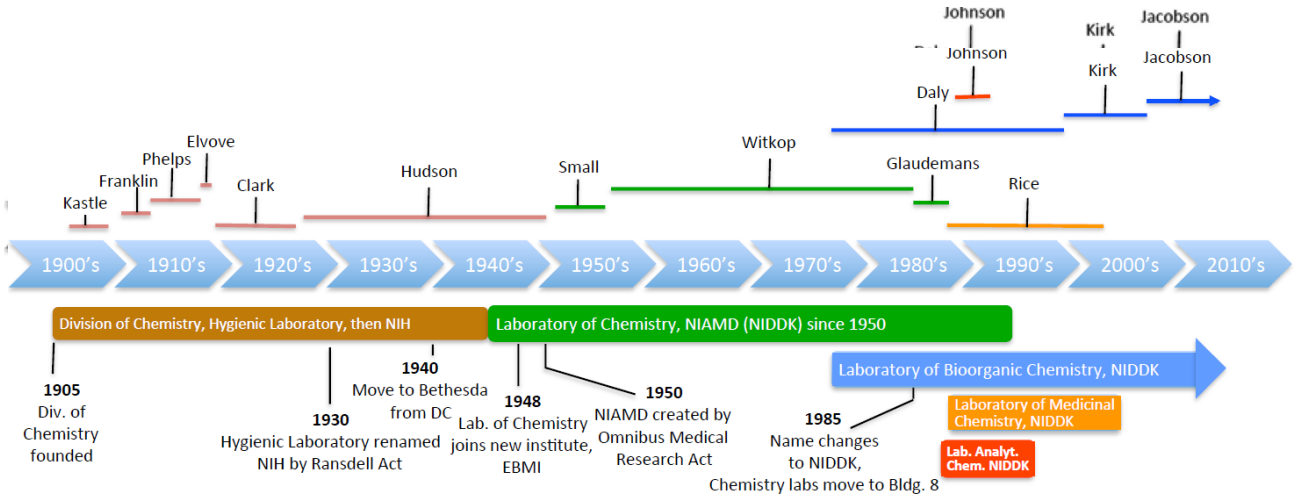


Figure 1. *Timeline of leadership (upper) and organization (lower, shade matched to leadership) of the organic chemistry laboratories of NIDDK and its precursor institutes. At the time of its founding, the Division of Chemistry of the Hygienic Laboratory was located near the present location of the Kennedy Center at 25th and E Streets, in northwest DC (2). The name of the Institute that later housed the former Division of Chemistry was changed from NIAMD (since its creation in 1950) to NIAMDD (1972), to NIADDK (1981) and then to NIDDK (1986).*

new Divisions to the Hygienic Laboratory. The existing staff, already authorized to work on infectious diseases, was designated the Division of Pathology and Bacteriology, and new Divisions of Chemistry, Pharmacology and Zoology were added to fill gaps in expertise needed for a comprehensive biomedical service organization. The Chiefs of the Divisions were to be prominent scientists who were given the title "Professor." Laboratory staff worked 48 hours per week including Saturday, and research reports were published in a dedicated periodical *Bulletin of the Hygienic Laboratory* and in *Public Health Reports*, in addition to general scientific journals (7, 9).

Figure 2. *Early Chiefs of the Laboratory and their years of service (photos E. Elvove).*

Questions that concerned chemists in the early days of the Division of Chemistry include: What makes fireflies glow? Do ingested medicines contaminate mother's milk? Why do people who drank alcoholic ginger extract during Prohibition suffer from limb weakening? How can we detect the presence of blood at a crime scene? Is fluoride in the drinking water good or bad for teeth? Thus, the Division of Chemistry initially had a responsibility to safeguard public health by studying environmental and other chemical risks, including analyses of adulterated food, water, milk, drugs and other substances. At first, the Division also provided essential chemical expertise, rare at the time, to other biomedical researchers (3) such as physician Joseph Goldberger of the Hygienic Laboratory in his classical studies of pellagra. The Division also collaborated with the Department of Agriculture, which was responsible for implementing the 1906 Pure Food and Drug Law.

The first Chief of the Division of Chemistry in 1905 was Joseph Hoeing Kastle (1864-1916), a prominent and enthusiastic educator from the University of Kentucky, (Figure 2), who held the position until 1909 (10). He encouraged the staff to cooperate with other Divisions to solve problems of public health, including routine analyses needed by the US Public Health and Marine-Hospital Service (6). Kastle invented a test for the presence of blood on forensic samples based on an enzymatic oxidation leading to a pink color reaction of reduced phenolphthalein indicator in a basic solution (Kastle-Meyer reagent) (11). His methods for measuring hydrochloric acid in the stomach (Kastle's reagent) (12) and hemoglobin in blood became standards in the field and he devised methods for determining saccharin and components of spices (13). He coauthored a study of typhoid fever in Washington, DC, with bacteriologist M. J. Rosenau and epidemiologist L. L. Lumsden of the Division of Pathology and Bacteriology (14). During this period, one of the more exotic projects was the extraction of the glowing principle of fireflies, but the work fell short of the characterization of the enzyme luciferase (15).

Kastle departed the Hygienic Laboratory after four years, displeased with the small staff, low salaries and inadequate resources for the work needed, and returned to academia (6). After a two-year interim without a Division Chief, Kastle was succeeded by Edward Curtis Franklin (1862-1937), who served from 1911-1913 (16) before returning to Stanford University. While Franklin continued Kastle's investigation of "ammonia systems," the Division continued to do analytical work, and, according to an internal report written by Elias Elvove (3) (1883-1962, Figure 3 (upper)), prepared several grams of pure tryptophan as a standard. Franklin also studied the radioactivity of the thermal springs in Hot Springs, AR, to which were ascribed curative powers. Other work consisted of abstracting scientific papers, expert testimony in court, examining the food value of a powdered infant formula called "Mammala" at the request of the War Department,

and giving highly sought public lectures (approximately monthly) sponsored by universities, other government labs, and scientific societies. His support staff consisted of one scientific assistant (Elvove), a general helper, and a glassware/laboratory cleaner.

Earle Bernard Phelps (1876-1953), already an expert on water sanitation, was chosen to serve as Chief in 1913 and served until 1918 (3, 6). He was a graduate of MIT, but with no degree more advanced than a B.S. in chemistry. In response to rising concern about industrial pollution in the United States, he studied water pollution, chlorination and waste biochemistry. Under Phelps, analytical work in the Division continued with published articles such as "The Chemical Measures of Stream Pollution and Specifications for Sewage Effluents" and "Chemical Studies of the Pollution of the Ohio River," which described research that studied re-aeration and the oxygen demand of organic pollution (17, 18).

When Phelps left the position, Elvove briefly served as Acting Chief of Division of Chemistry, Hygienic Laboratory, from 1919-1920 (3, 4). Elvove, an immigrant success story who arrived in the US from Kiev (then in the Russian empire, now in Ukraine) at age 14, worked as an expert technician in the Laboratory since the time of Kastle. Later in his career, Elvove helped to solve the mystery of Jamaican ginger (Jake) poisoning, a serious public health problem during Prohibition, in collaboration with NIH pharmacologist Maurice Smith (3, 19). Owing to its high alcohol content (70%), drinking this elixir, sold in pharmacies for various medicinal purposes, was a convenient way to imbibe. In 1930 and 1931, adulterated preparations of Jake were identified as the source of widespread episodes of weakening and paralysis of extremities. In careful analytical work in 1931, Elvove and Smith identified tri-*ortho*-cresyl phosphate as the toxic diluting agent in impure Jake, primarily produced by one disreputable Boston firm (3, 20). Elvove later developed the most advanced method for measuring fluoride in drinking water to ±0.1 ppm, which allowed dental surgeon H. Trendley Dean of NIH to establish fluoridation as a safe means of preventing dental caries (21).

In 1920 William Mansfield Clark (1884-1964), whose electrochemical measurements resulted in a correction of the Nernst equation, moved from the Department of Agriculture to the Hygienic Laboratory as Chief of the Division of Chemistry (9, 22). By 1925, following a major expansion in the previous 15 years, the Hygienic Laboratory employed 46 scientists and 71 support staff. Thus, Clark saw a much broader scientific scope of work than previous Chiefs. The analytical work continued, supervised by Elvove, while Clark began pioneering research on oxidation-reduction systems using electrochemical and colorimetric indicators. He was known to emphasize accuracy in physicochemical measurements in order to draw mechanistic conclusions. Clark wrote a definitive book on acid-base chemistry, *The Determination of Hydrogen Ions* (1920) (23). An important part of the Division's work during this period was the study of the toxic effects of tetraethyl lead. Tetraethyl lead was used as an additive to gasoline starting in 1923 to reduce pre-ignition engine "knocking" and valve wear. Central to the research on its toxicology was the design of a temperature-controlled oven (previously unavailable) to burn human feces, an oven constructed mainly by Clark himself (3). Subsequent research revealed its toxicity, and its use as a gasoline additive was eventually phased out (4). Clark was a skilled glassblower and machinist and aided other researchers in the lab to build complicated equipment. According to Elvove, he could "make almost any desired piece of apparatus" and did so with "utmost friendliness and kindness" (3). In 1927, Clark was appointed Professor at Johns Hopkins University where he spent the remainder of his distinguished career.

The slow process of transforming the Hygienic Laboratory into the NIH began in the wake of World War I (7). Chemical weapons were introduced during the War, and US access to German-manufactured dyestuffs and other chemical products was prevented. Consequently, there was a growing public awareness of the importance of developing domestic chemical infrastructure, especially for solving health problems. During World War I, the Chemical Warfare Service of the War Department sought to establish a private institute to apply basic chemistry to medical problems. A concerted effort to create a US government institute for basic science studies, including chemistry, in a biomedical context was championed by Charles H. Herty, a former (1915-16) President of the American Chemical Society (ACS). There was a need to centralize and coordinate government health research and to broaden the existing legislation from utilitarian science to basic research, which at the time was mainly limited to academia and private institutes (7). However, US government support of basic research was highly controversial and opposed by powerful interests. Following extended unsuccessful efforts to obtain philanthropic support for their initiative in the 1920s, backers of expanded medical research enlisted the support of Senator Joseph Ransdell (Louisiana), who introduced a bill in 1926 that mandated

federal support for this research. This effort was aided by current influenza outbreaks. After overcoming political opposition and trimming its goals, this effort culminated in the passage of the Ransdell Act, signed into law on May 26, 1930, by President Hoover. This law renamed the Hygienic Laboratory as the National Institute (singular) of Health. The act stipulated that additional resources from both public and private funds (almost nonexistent by that time during the Depression era) would be provided to the new NIH. The Division of Chemistry at that time was still one of only four NIH divisions. The mission of NIH was defined as, "study, investigation and research in the fundamental problems of the diseases of man and matters pertaining thereto." The new mission included chronic diseases and indicated a shift away from the applied study of factors contributing to disease, especially infection. Fellowships for associate researchers were to be awarded, and President Hoover himself promised to help solicit private funding toward this goal (7, p 161). Even before this formal shift away from applied questions of environmental health risk, the Division of Chemistry was involved in fundamental chemical questions such as acid-base chemistry and oxidation-reduction reactions and carbohydrate chemistry, which had no obvious disease application. US Surgeon General Hugh Cummings commented that he did not know of any possible connection that basic research on carbohydrates in the Division of Chemistry would have to public health (7, p 168). Fortunately, the mission for NIH of conducting basic research to explore complex aspects of human disease, its causes and prevention, has been realized. Thus, the current successful model of investigator-initiated research projects has prevailed at NIH, especially during its post World War II expansion (24).

In 1937 the National Cancer Institute was formed by an act of Congress, and in 1944 it was designated as a division of NIH (7). The National Heart Act brought the National Heart Institute into NIH in 1948, making NIH plural (National Institutes of Health). Meanwhile, in 1935 Luke and Helen Wilson made an initial donation of 45 acres of their estate in Bethesda, MD, to the federal government to be used for the expansion of medical research. The new Bethesda NIH campus (now occupying 70 acres) was occupied between 1938 and 1941, and the Division moved there from Washington, DC (2). The Division of Chemistry was initially located in Building 4 (in 1940, along with Divisions of Pharmacology and Zoology), where it remained for 45 years.

In response to emerging health concerns along with advances in technology, reorganization and expansion of NIH followed. In 1948, the original divisions of the old National Institute of Health were divided into two newly created institutes: the National Microbiological Institute and the Experimental Biology and Medicine Institute (EBMI). EBMI was the administrative home of the former Division of Chemistry (since World War II known as LC). On August 15, 1950, President Harry S. Truman signed the Omnibus Medical Research Act into law (Public Law 81-692) establishing NIAMD within the Public Health Service. The new institute, NIAMD, incorporated the laboratories of the EBMI, including the Laboratory of Chemistry. Thus, 1950 marked the birth of NIAMD, an event celebrated in 2010 with a symposium on NIDDK's 60th anniversary at the Fall National Meeting of the ACS (Division of Medicinal Chemistry). As Congress responded to shifting emphases on health problems, several name changes ensued that corresponded to the addition of new centers and/or the creation of new institutes. Thus, the Institute name was changed from NIAMD to NIAMDD (addition of digestive diseases, 1972), to NIADDK (addition of diabetes and kidney diseases, 1981) and then to NIDDK (removal of arthritis through formation of a new and separate institute of arthritis and neuromuscular diseases, NIAMS, 1986).

Reorganization of Chemistry Labs and Formation of LBC and LMC

Before a description of current and recent individual research programs and highlights, it is useful to briefly discuss the administrative adjustments to the laboratories that were primarily responsible for organic chemistry in the institute. LBC as it now exists was formed by a process of "mitosis" and "meiosis." In 1978, John W. Daly (1933-2008, Figure 3 (lower)), originally in LC under Bernhard Witkop (1917-2010) as Chief (25), was appointed Chief of the newly created offshoot, LBC. At its beginning LBC consisted of Daly's Section on Pharmacodynamics, a Section on Oxidation Mechanisms headed by Don Jerina (1940-2011), and Phil Skolnick's Section on Neuroscience. In 1985, LC was divided into two labs, consisting of LMC and the sections remaining in LC with Witkop as Chief. In 1985, the LC moved to a refurbished Building 8, where LBC is currently located.

Figure 3. *Organic chemists of the Laboratory in the lab and at leisure. Upper: Elias Elvove, seated at laboratory bench, Division of Chemistry, ca. 1938. (photo NIH Office of History, http://ihm.nlm.nih.gov/images/B06647). Lower: From left: Tom Spande, Neil Glaudemans, John W. Daly and Cyrus R. Creveling of NIDDK at the 65th Birthday Symposium in Honor of John Daly, held in Bethesda in 1998 (photo NIH Medical Arts and Photography Branch).*

After Witkop retired in 1985, the Scientific Director of the NIDDK Intramural Research Program orchestrated a complex series of reorganizations of the chemistry groups. Cornelis P. J. (Neil) Glaudemans, a carbohydrate chemist, was appointed Chief of LC and served from 1985 until 1988. The Laboratory of Analytical Chemistry (LAC) was created during that period with David F. Johnson (1932-2007), who pioneered techniques in steroid chromatography, as Chief. In 1988, the Sections remaining in LC were merged into LBC, with Daly as Chief (26), and LC ceased to exist formally. A separate Laboratory of Neuroscience (LN) had been excised from LBC in 1987 with Skolnick as Chief. At that time, Kenner Rice's group moved from LC to LN, and Rice became Chief of the Section on Drug Design and Synthesis. In 1989 Rice formed his own Laboratory (LMC, existing until 2006) and served as Chief. LMC consisted of Rice's Section on Drug Design and Synthesis and two other sections that had belonged to LC: Carbohydrates (Glaudemans) and Biomedical Chemistry (Paul Torrence). Rice's Section continued the work on drugs of abuse begun in LC by Lyndon F. Small (1897-1957) (27). The staff of LAC was merged into LBC in 1996. When Daly retired in 1998, Kenneth L. Kirk was appointed Chief, LBC, and upon his retirement in 2008, Kenneth A. Jacobson was appointed and is the current Chief of LBC.

Bridging to the Present—Topical Summary

The discussion of the research highlights that follows will be placed to some extent in the context of the organizational changes but, as a whole, should be considered a brief historical record of chemistry in LBC/LC/LMC, i.e. from the standpoint of the underlying science, not administrative moves.

Carbohydrate Research

Carbohydrate research formed one of the connecting links from the Division of Chemistry, Hygienic Laboratory, to the LC, NIAMD. Claude S. Hudson (1881-1952), who served as Chief of the Division of Chemistry from 1928 to January 31, 1951. In January 1929, the Division of Chemistry consisted of a scientific staff of 13 (including Hudson and two Fellows) (28), which increased to 15 by July 1936. Hudson served first in the Hygienic Lab and then at NIH, and was "present at the creation" of NIAMD (4, 5). He also embodied the central role of organic chemistry in the early days of NIAMD, although his Ph.D. was in physics. Prior to joining the Hygienic Lab in 1928 Hudson held positions in academia and in various Governmental agencies, including the National Bureau of Standards, War Department and the Department of Agriculture. During his tenure as Chief of the Division of Chemistry, Hudson did fundamental work on the chemistry of carbohydrates, which as food components fit the theme of the Hygienic Lab. However, Hudson went far beyond that context and mounted a large effort devoted to fundamental studies of sugars. He used carbohydrates to examine van't Hoff's hypothesis on the additive nature of optical rotatory power and explored the mechanism of mutarotation of sugars (4,5). Hudson's Lactone Rule correlates the sign of the optical rotation of aldonic acid lactones with the configuration of that carbon atom whose hydroxyl group forms the lactone. His success led to the premier research award of the Carbohydrate Division of the ACS being named after him. Hudson considered his years in the Division of Chemistry his most productive

and happy ones (4). There are many colorful anecdotes about Hudson (known as "Huddy"), for example, when his assistant failed to save a few seed crystals of a desired product for later use in crystallization, Hudson wandered for days and was heard muttering "he threw away his bait." Another story is that Hudson figuratively existed as either of two "enantiomers" (mirror images): a party-loving "levorotatory" persona that lived life to the fullest and a serious minded "dextrorotatory" one that demanded meticulousness in the lab.

Hudson's paper on xylitol derivatives in early 1944 reflected the name change from the Division of Chemistry to the Chemistry Laboratory (29). In October 1948, the Chemistry Laboratory was renamed the Laboratory of Chemistry and Chemotherapy under Hudson's leadership with Small as the Assistant Chief (30). When Hudson retired as Chief of the Laboratory and Section on Carbohydrates in early 1951, Small became Chief of the renamed Laboratory of Chemistry, and Hewitt Grenville Fletcher Jr. (1917-1973) was appointed Chief of the Section (31). Fletcher and his group began a strong synthetic program in carbohydrate synthesis, especially focusing on the synthesis of riboses, deoxyriboses, acetamido sugars, and especially the potent anti-viral nucleoside "ara-A" (32), now a widely used anti-HIV drug. Fletcher continued his research work until his death. Outside the lab, he and his successor, Glaudemans, were also known for their sailing activities.

In 1973 Glaudemans succeeded Fletcher as Chief of the Carbohydrate Section in LC and initiated a comprehensive study of the interactions of antibodies with bacterial polysaccharide antigens (33). This work was facilitated by the advent of monoclonal antibodies. Employing the use of synthetic unnatural sugar-containing derivatives (such as a series of fluoro-deoxy galactoses), Glaudemans and Paul Kovac and others in the group were able to construct models of binding sites for oligosaccharides, dextran and a number of bacterial polysaccharides with a series of monoclonal antibodies.

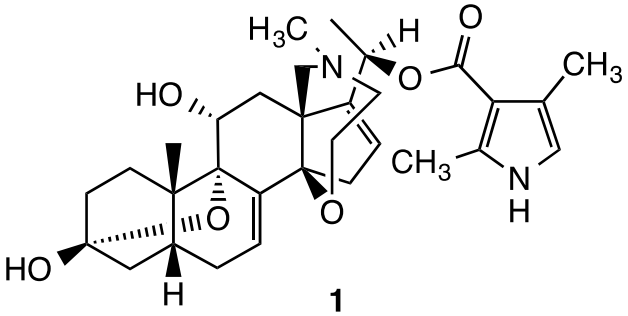
Glaudemans retired in 1998, and Kovac became Chief of the Section, which at the time was under LMC. He and his group have expanded this work to the construction of conjugate vaccines from synthetic fragments that mimic the structures of bacterial carbohydrate antigens such as those of cholera and anthrax (34, 35). The long-term goal is to develop conjugate vaccines from synthetic oligosaccharides that can provide lasting immunity from infectious bacterial diseases (34). Exploratory work in this area led to the discovery (35) that antigenic lipopolysaccharides can be directly conjugated to carrier proteins using squaric acid chemistry, which simplifies industrial vaccine production.

Natural Products

Witkop was trained at the University of Munich with a Ph.D. thesis on toxin chemistry and in 1944 was forced to retreat to a farm refuge in Southern Germany, eventually arriving in the US in 1947 to continue research work at Harvard (36). He began work at NIH in 1950, was appointed Chief of LC, NIAMD, in 1957 and served until 1988 (25). His influence and contributions to organic chemistry in NIDDK were profound, not just in the science, but also in his influence on a younger generation of scientists, both American and from foreign lands, in particular from Japan. His research group published roughly 400 papers on diverse aspects of synthetic chemistry, photochemistry, reaction mechanisms, and natural products.

In 1962, Witkop sent a visiting fellow, Fritz Märki, to Colombia to check out stories of the Colombian poison dart frog *Phyllobates aurotaenia*, an excellent example of curiosity-driven research. This expedition, which included biologist Marta Latham, confirmed the potent biological activity of extracts. The group undertook in the field preliminary characterization that identified the active principles as a steroidal alkaloid, but lacked sufficient quantity for further structural characterization. Witkop enlisted Daly to go with Latham on a subsequent expedition. This and later trips netted 2400 frogs that yielded a total of 30 mg of material from which several toxic alkaloids were isolated. Subsequent work by Daly, and an X-ray structure by Isabella Karle of a crystalline derivative prepared by Takashi Tokuyama (Figure 4), led to the characterization of the main neurotoxin, which was given the name batrachotoxin (BTX, **1**, from the Greek word "batrachos," meaning frog) by Witkop. BTX is used widely as a research probe (37). This alkaloid as the most potent nonpeptidal toxin proved to be one of the most poisonous organic substances known, with an LD_{50} in mice of 2 μg/kg. In 1971, Edson Albuquerque of University of Maryland confirmed and extended Märki's observations to conclude that sodium channels are the site of action of BTX (38). Thus, this toxic principle of the poison dart frog was shown to cause an irreversible increase in the permeability of electrically excitable membranes to sodium ions.

Figure 4. *Bernhard Witkop, John Daly and Takashi Tokuyama (left to right) examining the structure of batrachotoxin, ca. 1969. (photo NIH Record).*



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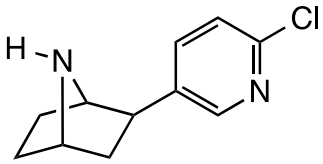
The results of these expeditions had a major impact on the course of research in LC. Thus, the discovery of BTX launched a 40-year research program at NIH on frog alkaloids that led to extremely important uses of BTX and other toxins in a host of research applications. Daly, frequently accompanied by his long-time associate Charles Myers of the American Museum of Natural History, made numerous trips to rain forests and other locales where exotic species of amphibians could be found and he collected an enormous inventory of skin extracts (26). Assisted by skilled coworkers, most recently by Tom Spande, Martin Garraffo, and Noel Whittaker, these were analyzed by increasingly sensitive mass spectrometry, chromatographic and NMR techniques, and led to the isolation and characterization of approximately 1000 alkaloids with potent and useful activities. Included are epibatadine (**2**), pumiliotoxins and many bicyclic "izidines" (39). The studies of the pharmacological profiles of these compounds, as well as refinements of analytical techniques and development of valuable microsynthetic methods, produced a wealth of scientific dividends.



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A fascinating aspect of this story involved the biosynthesis of these alkaloids. All attempts to entice frogs held in captivity to produce alkaloids proved futile. Measures tested for increasing toxin production included scaring frogs with natural predator snakes. The recognition of sequestration of alkaloids from dietary arthropods came only recently, a discovery by the Daly group that expanded the concept of his program of bioprospecting. Daly's energetic efforts continued unabated until his death in 2008. Valuable samples of extracts remain to be examined, a task left to Daly's close academic colleagues Richard Fitch, Ralph A. Saporito, and Tappy H. Jones, as well as others carrying on similar work. Aspects of this work were recently reviewed in a memorial tribute to Daly published in a special issue of *Heterocycles* (40).

The talent available to sustain a strong natural products research program remains in LBC. Carole Bewley, Chief, Section on Natural Products, joined the laboratory in 2000 and is using natural products chemistry to identify new treatments of viral, bacterial, and neoplastic diseases. Trained in the area of marine natural products, Bewley has targeted medical problems of profound significance, including tuberculosis and HIV infection, taking advantage of the vast universe of naturally occurring compounds that possess potent biological activities. Such compounds, when identified, are used as leads that, with chemical modification, can be converted to organic structures with improved pharmacological properties. For example, starting from marine invertebrates, Bewley and her group have identified several classes of novel marine natural products, also known as secondary metabolites, which potently inhibit HIV-1 infection and tumor cell growth *in vitro*. Results from these multi-faceted studies of biologically active natural products, many of which are collected from marine origins, include the discovery and full characterization of novel carbohydrate binding proteins and enzyme inhibitors from understudied sources and chemical libraries. Novel inhibitors such as the anabaenapeptins, potent protease inhibitors, and the chrysophaentins, which kill multiple strains of drug-resistant bacteria, have resulted from these efforts (41, 42).

Organic Synthesis and Organic Mechanisms

Research in organic synthesis and mechanisms from the 1960s through the 1990s touched on topics as fundamental and diverse as the NIH shift, peptide chemistry, physical organic studies of conformation in catalysis and fluorine in bioorganic chemistry. These investigations are outlined below.

The story of the NIH shift and oxidation mechanisms provides an excellent example of discovery-driven research. In the 1960s, attempts to use isotopic labeling to measure the rate of enzymatically catalyzed oxidation of 4-tritiophenylalanine were thwarted by unexpected isotope retention in the tyrosine product. A mechanism involving arene oxides was proposed and the ensuing rearrangement termed "the NIH Shift," a transfer of an aromatic hydrogen atom to a neighboring ring position during ring hydroxylation, received much support in subsequent research (43). The discovery of this process, confirmed in many subsequent experiments, was particularly important in the study of the chemistry and biochemistry of these arene oxides that are formed during the oxidative metabolism of aromatic compounds, most notably polycyclic aromatic hydrocarbons (PAH). For example, the mechanistic details of the NIH shift provided a basis for explaining why certain PAHs (present in chimney soot and bus exhaust) are highly carcinogenic and others are less so. The impressive research of Jerina and his Section on Oxidation Mechanisms in LBC, NIDDK, dramatically attested to the importance of this seminal discovery by Daly, Witkop, Gordon Guroff (1933-1999), Sidney Udenfriend (1918-2001) and others at NIH (44). In 1990, Jerina was described as a prospective Chemistry Nobel Prize winner based on his citation record for work on the role of arene oxides in carcinogenesis and drug metabolism (45).

LC occupied a critical position in biomedical research at NIH as the biological sciences revealed the increasing complexities of structure and functions of biomolecules such as amino acids and peptides. Critical to progress was an in-depth understanding of the physical organic principles involved in interactions of small molecules with macromolecular systems. In addition, having available in the Institute the knowledge and experience of practitioners of modern chemistry proved to be a valuable resource for other disciplines. Important contributions included the discovery by Witkop and Erhard Gross (1928-1981) that cyanogen bromide can selectively cleave peptide bonds at the carbonyl group of a methionine residue (46). This breakthrough in protein chemistry facilitated enormous contributions to this field, playing a part in the syntheses of hormones such as somatostatin (47). Thus, bacteria were engineered to produce somatostatin linked to galactosidase through a methionine residue. Cyanogen bromide cleaved the methionine linkage releasing somatostatin, thus completing a recombinant DNA strategy wherein a bacterium produced a polypeptide of higher organisms. A similar approach using cyanogen bromide cleavage was used in an industrial synthesis of insulin.

Witkop's extensive work in tryptophan chemistry provides another example of the application of fundamental organic chemistry to important biological systems (48), including studies of indolenine hydroperoxide intermediates involved in tryptophan oxidation to *N*-formyl kynurenines, and a novel photochemical cyclization of chloroacetyl derivatives to produce tricyclic indole derivatives. These and other studies during this period were typical of the applications of organic chemistry to understanding the fundamental chemical behavior of biological building blocks.

Another important discovery in the 1960s provided valuable tools for neuropharmacology. The false neurotransmitter 6-hydroxydopamine, discovered by Witkop and Siro Senoh (the first NIH visiting scientist from Japan) in 1959, was found to be selectively toxic to dopaminergic neurons. This chemical sympathectomy had many applications including implications for the etiology of Parkinson's disease. Daly and Cyrus R. Creveling (1930-2008) later observed similar neurotoxicity of hydroxyserotonin derivatives on serotonergic neurons (49).

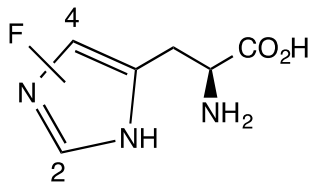
Perhaps no chemist in the laboratory was as attuned to physical organic principles as was Louis A. Cohen (1926-1996), who came to LC in 1954. He thrived on the applications of physical organic constants, especially to enzyme catalysis. In his research, he often attempted to mimic nature by the clever design of "test-tube" reactions. For example, in the 1970s and 1980s he designed compounds predicted to exist in favored conformations for lactone formation based on a "trimethyl" lock (50). This concept of "stereopopulation control" indeed produced model reactions with rate enhancements approaching that of enzyme catalysis. Much of this work was done with postdoctoral fellows Sheldon Milstien and Ronald T. Borchardt (50, 51). He also pioneered with postdoctoral fellow Leon Farber the electrochemical cleavage of proteins at tyrosine residues (52). His knowledge of

physical organic principles was a valuable resource that was used by many scientists in NIDDK and throughout NIH until his death in 1996.

Fluorinated small molecules have achieved wide use in biomedical research, particularly in the design of drugs, biochemical probes and biological tracers. In this regard, the number of medicinal agents that contain fluorine substituents is disproportionally high relative to other halogens and other functional groups. The special properties of fluorine, specifically its small size and high electronegativity, are largely responsible for its importance in biomedical research. There have been several notable contributions by NIDDK chemists to this area.

One such area is in fluorinated amino acids. (*S*)-Proline plays important and unique roles in protein structure because of its conformational rigidity. For example proline and (2*S*,4*R*)-4-hydroxyproline are critical components of collagen, and have been powerful tools in the study of the special properties of proline-containing peptides and proteins. In 1965 the first syntheses of (2*S*,4*R*)-4-fluoroproline and (2*S*,4*S*)-4-fluoroproline were reported by Witkop and co-workers in LC (53), which were used to study collagen biosynthesis. Subsequent work in several groups demonstrated the effectiveness of using fluorinated proline derivatives to study the many important roles of proline in protein structure and function.

(*S*)-Histidine also has many roles in biological structure and function. Histidine is often present in the active site of enzymes where both of the basic nitrogens of the imidazole ring have important functional roles. Hydrogen-bonding of the imidazole ring can also play important structural roles in proteins. In addition, histidine serves as the biological precursor of histamine, an important player in the immune response that also serves as a neurotransmitter. In the mid 1960s, Cohen initiated attempts to synthesize fluorinated imidazole derivatives, including histidine and histamine, recognizing that the strong electronegative effects of fluorine could evoke profound changes in biological and chemical behavior. Success in this endeavor hinged on the discovery of a new fluorination procedure and in 1969, Kirk and Cohen reported the synthesis of several ring-fluorinated imidazole derivatives using their newly developed photochemical Schiemann reaction of diazonium fluoroborates (54). This breakthrough opened the door to a myriad of research projects utilizing the unique chemical and biochemical properties of fluorinated imidazoles, in particular 2- and 4-fluoro-(*S*)-histidine (**3**). Use of these analogues to study protein structure and function continues to this day (55).



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In the 1980s, in collaboration with Daly and Creveling, Kirk and his group extended the study of fluorinated analogues to include fluorinated derivatives of biogenic amines: norepinephrine, epinephrine and related adrenergic agonists (56). Fluorine-induced selectivities towards α- or β-adrenergic receptors, depending on the site of fluorination, resulted in a broad program of synthesis and pharmacology, both to exploit these selectivities, and to attempt to determine the mechanism(s) by which fluorine exerted such a profound influence.

Subsequently the related fluorinated amino acids, viz. fluorinated 3,4-dihydroxyphenylalanine (FDOPA) and 3,4-dihdroxyphenylserine (FDOPS), were synthesized. These analogues proved to be very important in a variety of projects, especially ^{18}F-labelled analogues as agents for *in vivo* imaging of the heart and brain using positron emission tomography (PET). Particularly in the 1980s and 1990s, the Kirk research group worked closely with the PET Department of the NIH Clinical Center in developing standard metabolites of 6-FDOPA that proved critical in the implementation of [^{18}F]6-FDOPA as a biological tracer (57).

Medicinal Chemistry

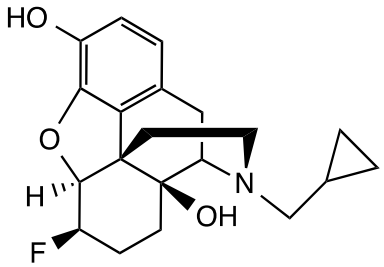
World War II brought a need to secure reliable sources of anti-malarial agents. Small was asked in 1938 by Rolla E. Dyer, Director of NIH, to refocus research efforts from his opiate program in the Division of Chemistry's Section on Chemotherapy (58) to seek synthetic antimalarials in anticipation of coming wartime quinine shortages (26). He and some of his group, including Erich Mosettig (1898-1964), moved from the University of Virginia to NIH (Division of Chemotherapy in DC) in 1939 where they began work on antimalarials. Mosettig was trained in Vienna, recruited from Ernest Spaeth's laboratory by Small and later became Chief of the Steroids Section, LC (59). Small's laboratory was one component of the Office of Scientific Research and Development (OSRD) antimalarial effort, and at the end of the war Small returned to his opiate program. In

1948 the group was attached to Hudson's laboratory in Building 4 in Bethesda (30).

Small was asked to concentrate on finding morphine and codeine replacements. Witkop's report (5) and the 1979 Smissman Award address given by Everette L. May (1914-2008) (60) describe these events in more detail. Rice's review of analgesic research at NIH (61) and a review by May and Arthur Jacobson (62) also provide considerable insight. May joined the group of Small and Mosettig in 1941, and became Chief of the Medicinal Chemistry Section in LC in 1960, where he remained until joining the faculty of Virginia Commonwealth University in 1977. Small succeeded Hudson as Chief of LC in 1951 and served until his death in 1957. Small was elected to the National Academy of Sciences in 1941, was recipient of the Hillebrand Prize of the Chemical Society of Washington in 1949, and was Editor of the *Journal of Organic Chemistry* for 13 years (1938-1951). Included in the list of important players in medicinal chemistry at this time were Arnold R. Brossi (1923-2011), who came from Hoffman LaRoche, and Rice, who began his NIDDK career in 1972 with Ulrich Weiss (1908-1992) and then as a fellow in May's section, and went on to become the Chief of the LMC.

NIDDK has been and continues to be a leading center of medicinal chemical research at NIH. Within this Institute there have been recent major efforts in the exploration of toxins that act at ion channels, receptors for drugs of abuse, biogenic amines, carbohydrates, purine receptors, and other classes of bioactive molecules. Representative examples of contributions to medicinal chemistry made by NIDDK chemists in the areas of opiates, purines, and nucleic acids are summarized briefly below.

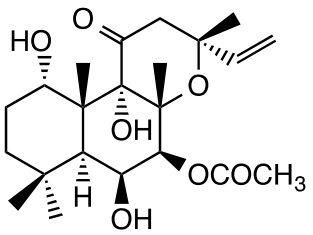
For decades LMC and its predecessors produced leading work in the medicinal chemistry of drugs of abuse (61). This laboratory inherited the role of the chemical research effort at the University of Virginia on opioids initiated in 1929 by the National Research Council of the National Academy of Sciences (62). Small's extensive work in the morphine alkaloids provided the foundation of modern structure activity relationship (SAR) in the opiate series and largely defined the chemical character of opiate reactivity. His discovery of metopon validated the program hypothesis that it was possible to separate the beneficial from the detrimental effects of morphine derivatives by chemical modification of the structure. This work provided the proof of principle for the program that still permeates contemporary analgesic research. May explored the SAR of many classes of opioid analgesics, including the 6,7-benzomorphans and the 5-phenylmorphans (60). He developed the synthetic opiate analgesics phenazocine and levo-alpha-acetylmethadol, which have been used clinically. He also introduced an antimalarial bromophenanthrene methanol, which was used as a life-saving treatment during the Vietnam War. The analgesic Talwin still in use today was developed as a direct result of May's discovery of the benzomorphans as analgesics.



4

Rice developed Cyclofoxy (**4**), a narcotic antagonist labeled with ^{18}F as a PET imaging agent for opioid receptors. Cyclofoxy was the first PET ligand that was designed on paper, synthesized, and studied in preclinical pharmacology and toxicology and introduced in humans all at a single institution, namely NIH (63). He and his associates published the first images of opioid receptor occupancy in a living primate in 1984 (63). Rice also developed the first practical total synthesis of opium products in 1980 (64). This methodology offers independence from foreign sources of opium, and such independence would enable opium poppy eradication as a strategy to eliminate heroin abuse. In 2006, Rice moved his program from NIDDK (30) to the National Institute on Drug Abuse (NIDA) (61) where it is now the Chemical Biology Research Branch.

As described above, John Daly made ground-breaking discoveries in the area of natural products isolation and characterization. In addition, in work closely related to his research in natural products, he earned acclaim in areas that included the investigation of the SARs for agonists/antagonists at adenosine, adrenergic, histamine, serotonin, and acetylcholine receptors. His pioneering research included studies on the modulation and functional relationships for systems involving calcium, cyclic nucleotides, ion channels and phospholipids. Two of the most important and widely used pharmacological probes introduced by Daly and coworkers were the activator of adenylate cyclase forskolin (**5**) and nicotinic acetylcholine receptor agonist epibatidine (**2**), which has spurred much research in new treatments for pain and dementia.



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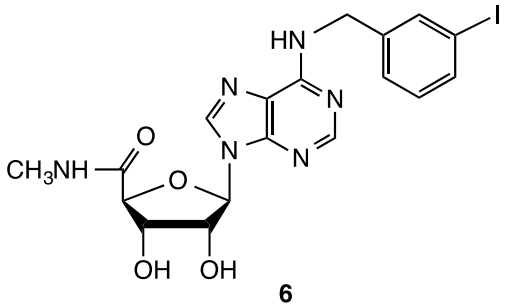
Daly's seminal studies on the mechanism of actions of caffeine and other xanthines, research that defined adenosine receptors as an important target for drug discovery, exemplified his ability to merge organic chemistry with pharmacology. His systematic work characterized the effects of adenosine analogues on cyclic AMP in the brain. These effects that were antagonized by methylxanthines established the concept of adenosine receptors in the brain. He also played a decisive role in establishing these receptors as *bona fide* biochemical entities and contributed to the discovery of receptor heterogeneity (65).

This research on adenosine receptors was facilitated greatly by Daly's development of a technique for prelabeling ATP in brain slices that allowed direct measurement of the conversion of the radiolabeled ATP into labeled cyclic AMP (66). Thus, one of the most efficient stimulators of cyclic AMP accumulation in brain slices proved to be adenosine. Extended studies revealed that cyclic AMP accumulation was antagonized by theophylline and other methylxanthines. It was enhanced by dipyridamole and papaverine, phosphodiesterase inhibitors that also prevent adenosine uptake into cells and thereby increase effective extracellular adenosine concentrations. Subsequent preparation of a series of analogues by Daly and his group helped to establish the fundamental SARs for these biologically important receptors (67).

K. Jacobson greatly extended research on adenosine receptors in particular, and on purinergic receptors in general. For example, he has explored SARs to introduce many widely-used ligand probes for the pharmacological study of GPCRs, e.g., the four adenosine receptors and eight P2Y receptors, which respond to extracellular nucleotides. These compounds have been essential in countless biological studies that are furthering delineation of the physiological role of extracellular purines as transmitters and modulators, particularly those actions mediated by GPCRs. Thirty seven compounds, including selective radioligands, designed and synthesized by Jacobson and his group are currently available from commercial sources as research tools. These ligands, most of which became available during the past decade, have had a clear influence on the course of industrial and academic medicinal chemistry of purine receptors. For example, the discovery of the first A_{2B} adenosine receptor-selective antagonist in LBC has led to new preclinical candidates for treatment of asthma and diabetes later under development by several pharmaceutical companies (68).

Early in the studies of purine receptors at NIDDK, the potential value of structural exploration of these elusive proteins in drug design was recognized. Soon after cloning of the receptors in 1990, Jacobson and his group began molecular modeling of the adenosine receptors and their putative binding sites for agonists and antagonists. The computational probing of the architecture of purine and pyrimidine receptors was supported by site-directed mutagenesis. These structural insights, gained initially by using the relatively primitive modeling techniques available at the time were refined in stages by Jacobson and colleagues and have successfully guided ligand design. The recent determination of the X-ray structure of an agonist-bound A_{2A} adenosine receptor (69), which is a target for neurodegenerative diseases and inflammation, validated much of the preceding modeling work carried out in Jacobson's lab.

Basic research labs in the NIH can partner with pharmaceutical industry through a formal collaborative research and development agreement (CRADA). The ongoing program in LBC to develop selective adenosine receptor agonists has led to clinical trials of two adenosine receptor agonists originally synthesized by Jacobson in 1993, including the A_3 receptor-selective agonist IB-MECA (**6**). From 2006 to 2010, a CRADA was established with Can-Fite Biopharma to advance understanding of the therapeutic benefits of A_3 adenosine receptor agonists, which they promoted to clinical trials. These ongoing Phase II/III trials target inflammatory diseases such as rheumatoid arthritis, psoriasis and dry-eye disease and also liver cancer (70). IB-MECA and its 2-chloro analogue have been found to be well-tolerated in humans with promise as a broad based treatment for many chronic diseases.



6

Jürgen Wess is a pharmacologist and Chief of the Molecular Signaling Section of LBC. He has made seminal contributions to GPCR structure and function, particularly with respect to the muscarinic acetylcholine receptors (71).

Application of organic chemistry in the nucleic acid field has received considerable attention in NIDDK. Torrence, who served as Chief of the Biomedical Chemistry Section in the LAC of NIDDK from 1989 to 1999, carried out research in drug discovery for a variety of viral diseases. As part of this research, he studied the use of oligonucleotides, in particular based on the 2',5'-oligoadenylate system, as potential antiviral therapeutics (72). In 1999, Torrence joined the faculty of Northern Arizona University as chair of the chemistry department where he continued research into antiviral therapeutics until his retirement.

Dan Appella, who joined LBC in 2005, is exploring new types of synthetic peptide nucleic acids (PNAs) and small molecules with unique biomedical applications. PNAs hybridize with natural nucleic acids and are stable to enzymes that degrade DNA and RNA. Key design features of the Appella PNAs are the incorporation of cyclopentyl and lysine moieties that impart greater stability to the hybridized structures as well as a chemical handle to build complex, multivalent structures at the nanometer scale (73). In other work, the Appella group is developing new classes of small molecules with anti-HIV and anti-cancer activities that interact with biological targets considered to be challenging for traditional methods of drug development. The goal of this work is to develop new types of therapeutic compounds that take advantage of unique mechanisms of action.

Acknowledgments

Regarding the research summaries, it would be impossible to give appropriate credit to all of the chemists that have contributed to the success of research in NIDDK. In particular, the invaluable contributions of countless postdoctoral fellows and students are only embedded in the summaries we have provided, and not specifically cited. Indeed, our research in NIDDK is driven by these young colleagues who come to NIDDK to further their training in chemistry, but who bring with them fresh energy and ideas. In general, space and time have limited us in our coverage of past and present research. We have used two main sources of information for the summary of early events. The first is an unpublished document composed by Elias Elvove in 1953, and we thank Paul Kovac who had been entrusted with this manuscript, for making it available to us. We also thank Kenner Rice and Tom Spande (NIDA) for helpful discussion. We acknowledge support from the Intramural Research Program of NIDDK, NIH, Bethesda, MD.

Supplemental Material

Page images of Ref. 3, an unpublished report by Elias Elvove, "History of the Laboratory of Chemistry from its Inception as the Division of Chemistry, Hygienic Laboratory, US Public Health Service, June 20, 1905, through the administration of Claude S. Hudson, 1950," can be found in the Supplemental Material for the *Bulletin for the History of Chemistry* at the journal's website,

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