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John W. Daly – An Appreciation

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Abstract – John W. Daly was engaged in groundbreaking basic research for nearly 50 years at NIH in Bethesda, Maryland. A primary focus of his research included the discovery, structure elucidation, synthesis and pharmacology of alkaloids and other biologically active natural products. However, he earned further acclaim in other areas that included the investigation of the structure-activity relationships for agonists/antagonists at adenosine, adrenergic, histamine, serotonin, and acetylcholine receptors. In addition he was a pioneer in studies of the modulation and functional relationships for systems involving calcium, cyclic nucleotides, ion channels and phospholipids and in the mechanism of actions of caffeine and other xanthines.

INTRODUCTION

A memorial tribute entitled “A life dedicated to chemistry in nature” was held on June 12, 2008 at the NIH campus in Bethesda, MD to honor the scientific and personal legacy of the late John W. Daly. John was one of the most highly renowned scientists at NIH, who was nearing the mark of 50 years at the NIH at the time of his passing. He valiantly fought pancreatic cancer for roughly six months from the time of his diagnosis until his death on March 5, 2008. He was active professionally until the end of his life and was even planning to deliver an invited lecture on natural products at the National Meeting of the American Chemical Society in April, 2008. His optimism and desire to persevere with his science were intense – the same intensity that characterized his entire career. The program celebrated the life and spirit of John Daly, both his tireless pursuit of scientific goals and his human qualities, and was planned to coincide with what would have been John’s 75th birthday on June 8, 2008. Ten years earlier a celebration entitled “Roots of Chemistry at NIH” was held for his birthday in the same auditorium. Highlights of the 65th birthday symposium included the presentation of a South American poison blow dart gun to John by the late Nobel laureate Julius Axelrod of NIH (Figure 1). The presentation was accompanied by the reading of a laudatory

statement by Bernhard Witkop, who recruited John to NIH in 1958 and introduced him to the study of toxins. Some of the same people who spoke ten years ago again converged on Bethesda to honor John. We were filled with sadness that he was no longer with us to mark his 75th birthday. Unlike the 65th birthday symposium, which provided an opportunity for John's close associates and former trainees to display their own recent scientific accomplishments, the memorial tribute consisted of a series of short talks that allowed the speakers to reminisce about John and objectively assess his scientific impact. A full video of the symposium is available on the internet.¹



Figure 1. Photo of Dr. Julius Axelrod of the National Institute of Mental Health presenting an authentic poison dart blowgun to John W. Daly on the occasion of his 65th birthday symposium in Bethesda, MD (June, 1998).

Many messages of condolence had been received prior to the symposium. Alfred Bader, founder of the Aldrich Chemical Co. was very fond of John. In the past, beginning in the 1960s, Bader personally visited the NIH labs to find out what newly discovered chemical substances might be in demand and spur research if made available, and John was an important contact for him. Bader stated: "I was truly saddened reading in this week's *C&E News* that my old friend John Daly died of cancer in March. I have admired him for many years and he sent us one of our first really great articles for the *Aldrichimica Acta*".² At the end of the symposium, Koji Nakanishi of Columbia University stated: "When the structure of batrachotoxin, the first of the frog toxins, was published, its uniqueness surprised the natural products community. John continued to study the frog toxins and opened a new field of natural toxins. He almost monopolized this intriguing field. He was a very modest scientist, and the passing of this special natural products chemist was a great loss, particularly as such a 'young' age."

DALY'S STUDIES OF NATURAL PRODUCTS

John surrounded himself with complex molecules, many of which he discovered in nature and characterized both structurally and pharmacologically. He typically would isolate the substances, elucidate the structures, and then proceed to probe structure activity relationships with both naturally occurring and synthetic analogues. In other cases, John's group synthesized new analogues of known natural products. In this manner, both natural products chemistry and pharmacology became his focus. Figure 2 shows eight molecules from his treasure chest that were scientific "home runs" for John. They are important pharmacological probes that are used as tools in countless labs around the world. Four of the molecules are frog skin toxins that John characterized chemically and biologically and in three cases (2-4) discovered. John isolated these toxins from large numbers of specimens that he personally collected, often at great personal inconvenience, if not bodily risk. Batrachotoxin (1) is one of the most poisonous non-protein substances known to mankind; a lethal dose in mice is only 2 micrograms per kilogram – and John and his collaborators figured out why – because it causes persistent opening of sodium channels to paralyze nerve cells.³ Two other compounds shown also affect the flow of metal ions across the cell membrane. Pumiliotoxin B (2) allosterically stimulates sodium flux in nerves, and histrionicotoxin (3) blocks sodium channels and the action potential.^{4,5} The story of epibatidine (4) demonstrates John's perseverance in science. Isolated from an Ecuadoran frog in the 1970's in a sub-milligram quantity, John showed that it had potent analgetic activity in mice. However, this observation was initially not publishable because of a lack of structural and mechanistic data. It took 18 years for John to uncover the secrets of this molecule: the elusive chemical structure and its antinociceptive mechanism.⁶ Surprisingly, epibatidine (4) works by activating nicotinic receptors (cholinergic ion channels), not receptors for opioids, which was the obvious but incorrect first guess. This compound is now a key lead in the quest for future pain relief medicines that might have fewer side effects and not produce tolerance.⁷

Not all of John's favorite molecules were toxins. The last four molecules shown in Figure 2, adenosine (5), AMP (6), caffeine (7), and forskolin (8), modulate G protein-coupled receptor signaling pathways, which are important mechanisms involved in the action of a large fraction of the pharmaceuticals in clinical use today. He was a pioneer in studies on intracellular signaling pathways that involved the examination of functional relationships and modulation of systems involving calcium,⁸ cyclic nucleotides,⁹ ion channels¹⁰ and phospholipids.¹¹ John established the relationship between certain commonly used pharmaceuticals, such as the antidiarrheal drug loperamide, and SOC (store-operated calcium) channels.^{10,12} Forskolin (8), perhaps the most widely used of John's treasured molecules, was isolated from the Indian plant *Coleus Forskohlii*.¹³⁻¹⁵ Thanks to John establishing its biochemical mechanism of action, forskolin has been an essential research tool for several decades in nearly every pharmacological lab in the world.

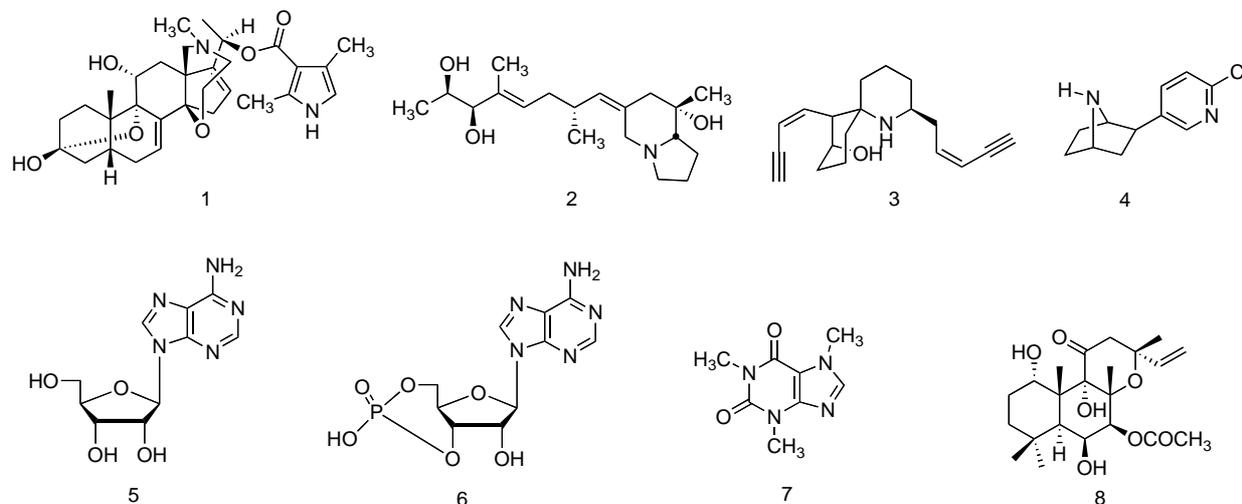
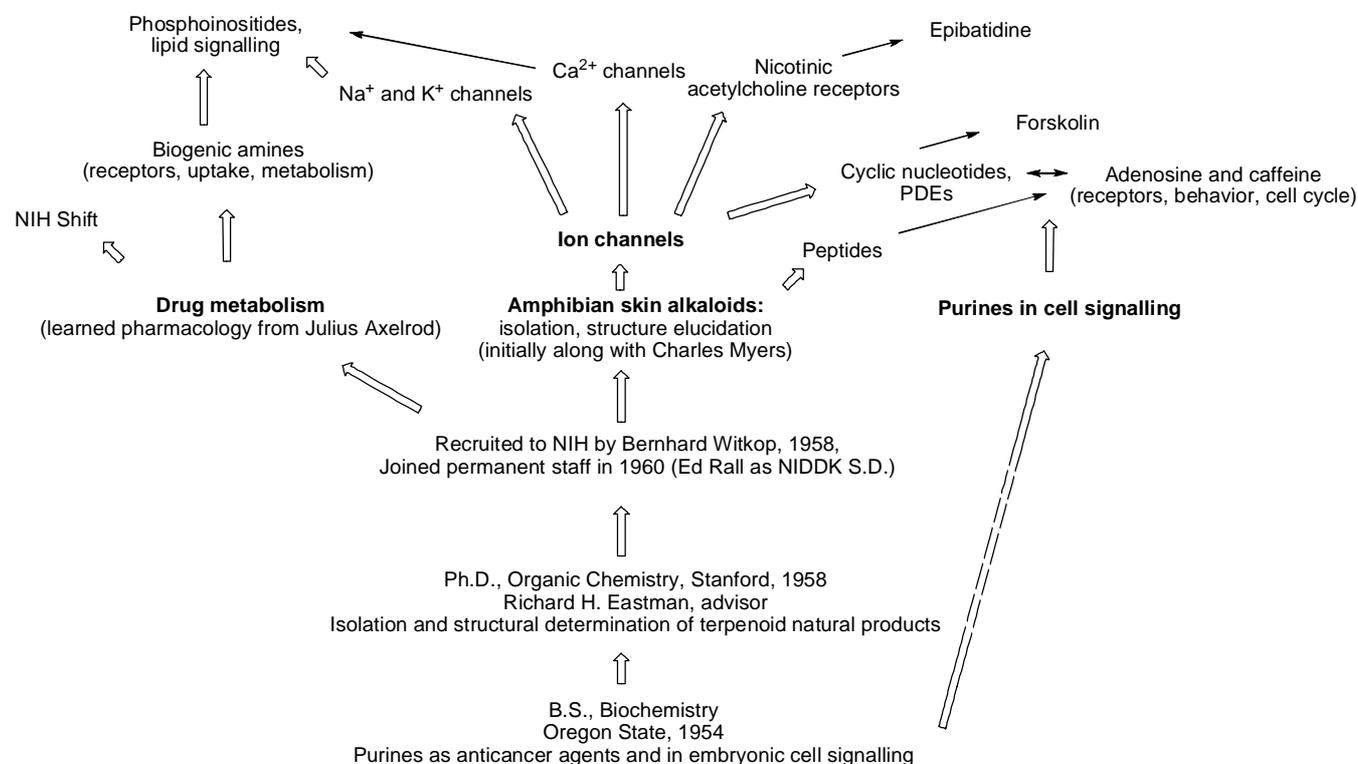


Figure 2. Structures of eight key natural products that were discovered or co-discovered by John Daly (frog toxins **1-4**) or whose mechanisms of action were investigated in depth (**1-8**) by John Daly.

John's world of science was both very diverse and unusually deep. He played a major role in revolutionizing thinking in multiple fields of pharmacology. Figure 3 shows a simplified depiction of the various research topics John pioneered. He was adept at making connections between divergent paths of inquiry to reveal previously unrecognized synergistic relationships. This lateral thinking served him well, as evidenced by his publishing of over 600 papers, which have been cited collectively more than 20,000 times. His doctoral training was in organic chemistry, which continued to be the central foundation for his research on the complex interactions he discovered in nature. In an interview in 2002,¹⁶ John declared: "I still consider myself an organic chemist—that is the viewpoint that I bring to pharmacology". He strove to establish a detailed correspondence between each part of a molecule and its biological function, which was a novel approach at the time he was trained at Oregon State (B.S., Biochemistry, 1954; M.A., Organic Chemistry, 1955) and Stanford (Ph.D., Organic Chemistry, 1958¹⁷). His first papers after joining the Witkop laboratory at NIH dealt with pharmacological research on biogenic amine neurotransmitters.¹⁸

He was mentored in pharmacology by Julius Axelrod (Nobel Prize in Physiology or Medicine, 1970), a pioneer of neurotransmission whom John revered. Among the work that they published jointly was the



discovery that water is methylated in the brain to produce methanol.¹⁹ An offshoot of John's experiments--carried out with Gordon Guroff, Don Jerina, and Sidney Udenfriend--was the discovery of a hydrogen migration during enzymatic aromatic ring-oxidation. This became known as the "NIH shift". The arene oxide intermediate was the basis for Don Jerina's future discoveries in the field of carcinogenesis.^{20, 21}

Figure 3. Flowchart showing the development of various themes in the scientific career of John Daly.

John was offered a permanent position as an NIH researcher in 1960, which in those days required only the informal agreement of Bernhard Witkop, as Chief of the Laboratory of Chemistry, and the consent of the Scientific Director of the Institute, Ed Rall.¹⁶ John found Rall's leadership very conducive to research excellence. He considered Rall a compatriot in the quest for scientific truth. John was soon promoted to Chief of the Pharmacodynamics Section and later Chief of the new Laboratory of Bioorganic Chemistry (1978), an offshoot of the Laboratory of Chemistry.

The body of work that has brought John the most acclaim is the study of frog skin alkaloids. He was active in the discovery, structure elucidation, synthesis and pharmacology of alkaloids and other biologically active natural products. John was accompanied by herpetologist Charles W. Myers, then Curator of the

American Museum of Natural History in New York, on many of the early collecting trips beginning in the 1960s to Panama, Costa Rica, Ecuador, Venezuela, Colombia, and other countries. Some of the trips required spending longer than one month in a tropical rainforest, often collecting nocturnal species at night. During this time they were threatened by hazards, including piranha, coral snakes, poisonous scorpions, spiders, and insects, nearby plane crash and fire, infectious disease and injury, tropical storms that devastated their camp, accusation of being witches, police arrests, and violent political revolutions. John continued the collecting trips into his last years, to Madagascar, Australia, and elsewhere, and became almost a cult figure to frog devotees, with the publication in the popular press of his legendary explorations.²²⁻²⁵ How many NIH Intramural Research scientists have the distinction of being accosted, like John was, by autograph seekers at public forums? John's group characterized, and in some cases isolated and even synthesized, around 800 alkaloids from amphibian sources. This facilitated greatly his extensive study of ion channels, nicotinic receptors, and various pharmacological processes. For years John was the sole source in the world for many important and definitive neuroactive chemical probes, such as batrachotoxin. John's collection of hundreds of frog skin extracts, which still contain untold secrets, is irreplaceable.²⁶

John, his group and collaborators astonished the world of herpetology in the early 1990s by demonstrating that the alkaloids found in frog skin were *not* synthesized but were sequestered from an arthropod diet, chiefly ants, small beetles, millipedes and most importantly, mites. John's painstaking feeding experiments, following up years of clues provided by frog stomach content analyses and the overlaps provided by gas chromatography-mass spectrometry where many known ant venoms or trail markers were found in frog skin, finally convinced skeptical biologists that one animal could indeed sequester venoms or toxic alkaloids from another animal, the first instance of this phenomenon. The frog-arthropod trophic relationship is now generally accepted.³² John and his group also showed that frogs could metabolize one sequestered alkaloid, producing an even more toxic substance and could in one Australian genus, synthesize an entire alkaloid class, the pseudophrynamines, *de novo*. The consequences of these discoveries for chemical ecology continue to expand, as attempts are being made to link the aposematic coloration of the toxic frogs to toxicity of specific dietary prey and to determine whether the sequestered alkaloids serve functions beyond deterring predation. The discovery of many classic insect alkaloids in mites is forcing a re-examination of whether the ultimate sources are mites or even a symbiont. John expended a good deal of time with students around the world advising them in their studies on the dietary link to toxicity in these species. He also became embroiled in disputes, sometimes heated, with biologists who were careless in interpreting John's toxicity data for alkaloid classes. The sequestration of alkaloids by anurans is the exception rather than the rule as only four

families of frogs or toads out of approximately seventy examined by John, sequester alkaloids. These are stored in special skin glands. The uptake and storage mechanisms intrigued John, who considered them possibly linked with drug transport into or out of human cells, and in particular the pathway whereby cells remove chemotherapy agents.

Table 1. John W. Daly's major scientific awards.

Year	Award	Source
2002	Ernest Guenther Award for Achievements in the Chemistry of Natural Products	American Chemical Society
1999	Karl Wilhelm Scheele Award	Swedish Academy of Pharmaceutical Sciences
1998	Presidential Rank Meritorious Award	United States Government
1997	Research Achievement Award	American Society Pharmacognosy
1997	Election as member	National Academy of Sciences, United States of America
1997	Election as Corresponding Member	Argentine Academia Nacional de Ciencias Exactas, Fisicas y Naturales
1996	Award for Outstanding Achievement	Washington Academy of Sciences in the Biological Sciences
1991	Elected Fellow	American Association for the Advancement of Science
1989	Award for Pioneering Research on the Biology and Chemistry of Adenosine	International Conference on Purine Nucleosides and Nucleotides in Cell Signalling
1978	Hillebrand Award	American Chemical Society, Washington DC Section

The third main stream of John's scientific exploration concerned the many biological actions of purines.^{27,28} His investigation of cyclic AMP signaling pathways led to probing the structure-activity relationships for agonists/antagonists at various G protein-coupled receptors, such as those for adenosine and biogenic amines. John was also an expert on the chemistry and biology of the stimulant caffeine and other alkylxanthines, which he found to act mainly as adenosine antagonists.²⁷ John pioneered the identification of the adenosine receptors as the most relevant target of consumed caffeine in the body and is credited with discovering one of the receptor subtypes. Prior to John's work, medical school textbooks

incorrectly ascribed the effects of caffeine to the inhibition of phosphodiesterases (PDEs) inside the cell, rather than antagonism of the effects of adenosine at the cell surface, the body's endogenous and protective quieting agent. John's interest in purines stemmed from his undergraduate work on their syntheses and anticancer properties.²⁹ John correctly predicted that purine derivatives would be important in cell differentiation, the cell cycle,³⁰ and other signaling functions,²⁸ but the ideas lay dormant until the 1960's when John tackled their role in intracellular signaling. On his first research paper he was lead author--in the prestigious *Journal of Organic Chemistry*--on purine work done as an undergraduate.²⁹ The compounds he synthesized then could have been fed directly into a study of adenosine receptors, except these receptors were not discovered until nearly two decades later. In 1976, he wrote the definitive book at the time on cyclic nucleotides in the nervous system.⁹ Thus, John's scientific insights and sense of the direction of science proved to be far ahead of his time.³¹

In 1997, John was inducted into the National Academy not as a chemist, but rather as a pharmacologist. Other honors earned by John Daly are listed in Table 1. Six papers authored by John Daly have been cited more than 750 times as of May, 2008, according to the Web of Science (Thomson Reuters). These are references: 13 (1563 times), 28 (1321 times), 14 (1074 times), 21 (1072 times), 15 (803 times), and 31 (753 times).

CONCLUSIONS

John's research was curiosity driven, as well as hypothesis driven. It was well suited for the NIH environment: as a creative scientific think tank in which one has the academic freedom to explore new, intriguing findings. He also took his role as mentor seriously and had an open door policy for meeting with junior researchers and students to share fresh exciting results. His friendly, inquisitive, and usually mild mannered nature--along with the aura of this unassuming genius, who roughed it in the rain forests or undeveloped areas around the world in order to extract nature's secrets--endeared him to staff and collaborators alike. John Daly's human side was beautifully described by Jack Cover, Curator, National Aquarium, Baltimore, MD: "...after reading this obituary the tears are flowing down. ... I can't tell you how many mutual friends contacted me ... to say what a positive influence John had on their lives. I consider myself very fortunate to have known him and to have spent time in Costa Rica with him. He had a routine - he would first find a suitable walking stick at the beginning of every trip. Next off, he would go straight up the steep hill with his walking stick and a supply of plastic collecting bags under his belt. We would usually split up and later meet back at the streambed. On some occasions I and other "young" collectors would manage to find just a few frogs. Then John would come back down the hill with his bags full of frogs! 'Where did you find them?' we'd ask. 'Oh just over that third ridge.' Always humble he

never bragged or boasted about any of his many accomplishments. And I do not think I have ever met anyone else as friendly and kind as John. Oh how I'll miss his occasional visits, research updates, and yes that Jeep. I always told him that I wanted to join him on one of his Costa Rican fishing expeditions. My desire to do this was more about wanting to witness John's joy of fishing and life in general than to enjoy the fishing myself."

In the months following the death of John Daly were heard a chorus of voices praising his vision, intellect, and accomplishments. It is striking that his interests were so broad that each of his associates was able to see clearly only a fraction of the scientific world that John Daly commanded. The success of his approach to science emphasizes the value of the way the NIH Intramural Program operates and the intellectual freedom offered. He thrived in this nurturing environment. John created a cadre of followers who are continuing his vision in one form or another, in industry, in academia, and in government.

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