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Review Article

Reflections on New England Nuclear (NEN)†

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Abstract: Selected vignettes from the author's personal experience with technology and colleagues at New England Nuclear are related. Copyright © 2007 John Wiley & Sons, Ltd.

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When I was first invited to contribute an article about New England Nuclear (NEN) for an issue of this journal I regarded it as a unique opportunity, especially since we are celebrating our 50th year in business. However, it seemed a daunting task to attempt to adequately tell the story of a company that has grown from a handful of people 50 years ago to a significant part of a large global organization with such diverse products and customers. I began to appreciate that within the constraints of a brief article it would not be possible to do justice to the history of our complex technical company. Rather, this discussion is a very personal recollection which should still provide the reader with a good idea of where NEN came from and where it is heading. At first privately held, NEN was at one time part of DuPont and since 2000, following several acquisitions and changing corporate titles, now part of PerkinElmer Life and Analytical Sciences. However, for simplicity sake and the obvious fact of brand recognition to isotope customers, I will refer to it here as NEN.

NEN was founded in March 1956 by two young and ambitious entrepreneurs, Edward Shapiro and Seymour Rothchild, who had met while working at the nearby firm Tracer Labs in Waltham, Massachusetts. Tracer Labs specialized entirely in instrumentation, but these two visionaries sensed the growing need for radiochemicals especially in the medical community and felt the time was right to respond to that need. Shapiro became the first President of NEN while Rothchild was named its first Technical Director, and NEN emerged as one of the first US companies to focus exclusively on the manufacture of radiochemicals. Laboratory space was acquired on Albany St in Boston's South End very close to the Boston University medical campus and their fledgling chemistry staff then included John L. Morgenthau, Charles A. Hainley and Felix R. DeLeo. Curiously, about a year ago I rediscovered Rothchild's first notebook with its first entry on 14 May 1956 and that page is reproduced in Figure 1. This important historical document represents the initial intellectual property of NEN. Over the years more staff were hired and the company expanded in size, scope and complexity, adding numerous compounds labeled with various radioactive isotopes to its growing catalogue portfolio. Because of the unique demand of isotopes like ^{125}I and others, separate and specialized laboratories were also established in Billerica, Massachusetts, about 25 miles north of Boston where they still function today.

I first learned of NEN while a chemistry graduate student at M. I. T., working for Glenn Berchtold on natural product synthesis. In 1972 I was visited there in my lab by a friend, Bill Kwoka, who like myself had recently graduated from Trinity College in Hartford. At that time he had just joined NEN and portrayed it as a dynamic and innovative company. Desiring to stay in the Boston area, I filed that chance encounter away for later reference. In the fall of 1977 after finishing up a postdoctoral program in brain cancer chemotherapy with John Neumeyer at Northeastern University, I decided to seek more permanent employment. I visited NEN and while I have forgotten much of that interview process, I vividly recall meeting with Larry Geller who at that time was Operations Manager for the Boston Site. He had first come to NEN as a steroid chemist,

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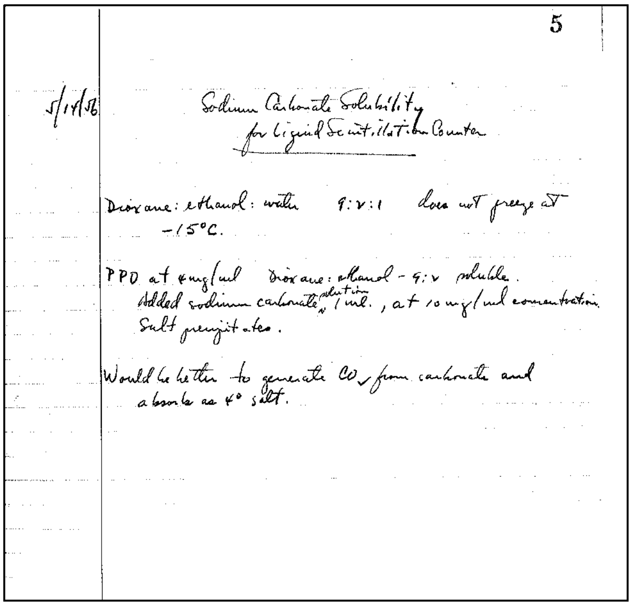

Figure 1 NEN's first notebook page.

earlier working for Sir Derek H. R. Barton on the famous Barton reaction as a co-author on the first announcement of that intriguing synthetic process in 1961.[1] It is probably forgotten by many that this well-known photochemical name reaction was discovered at the Research Institute for Medicine and Chemistry in Cambridge just across the Charles River from NEN. Soon after the interview I was hired as a chemist in the Custom Synthesis laboratory and first reported to Jennie Ahern. Jennie, along with her husband David, had joined NEN a number of years earlier and she was very instrumental in my early training in radiochemistry and later career at NEN.

What now follows are four vignettes from my experience at NEN that are illustrative of both the company values and scientific contributions made by NEN scientists.

The growth of radioligands at NEN

Today, the awareness and exploitation of receptors and ion channels as targets for drug intervention is commonplace, but many forget just how recently their existence was demonstrated. It was only in 1973 that Solomon Snyder and co-workers at Johns Hopkins University first published in *Science* their landmark paper with the understated title 'Opiate Receptor: Demonstration in Nervous Tissue.'[2] In that paper Snyder used $[^3H]$naloxone, an opiate antagonist prepared at NEN, to demonstrate specific binding of this radioligand to an opiate receptor of guinea pig intestine and mammalian brain. This was certainly a very early (if not the first) description of a receptor binding assay and importantly Snyder showed using our $[^3H]$naloxone that competition for the opiate receptor by a number of opiate agonists and antagonists agreed nicely with their pharmacological strength. Although a profound and ground-breaking experiment, the $[^3H]$naloxone employed was of only modest (6.1 Ci/mmol) specific activity and the neurochemical community clamored for even higher specific activity receptor radioligands to fully exploit the receptor binding assay potential.

A second critical paper in this area appeared in 1977 and a key contributor was NEN chemist Malcolm Randall. Malcolm had joined NEN in 1969 after being trained as a chemist in England with postdoctoral work both in Australia as well as M. I. T. and was recruited primarily for his expertise in carbohydrate chemistry. By the mid-1970s his recognized management skills prompted a promotion to the level of Director with a number of groups under his supervision. Because of work by Snyder and others, Malcolm and colleagues at NEN were keenly aware of the emerging receptor binding assay technique and began reaching out to collaborate with thought leaders in the neurochemical area. One of these was Robert J. Lefkowitz at Duke University who alerted Malcolm to the critical need for $[^3H](-)$-dihydroalprenolol, a potent beta adrenergic antagonist, to study the beta adrenergic receptor system. That collaboration resulted in the synthesis and characterization of this important radioligand at high specific activity (33 Ci/mmol) along with the publication of its biological activity in receptor binding assay.[3] This seminal paper produced jointly from Duke and NEN laboratories demonstrated to neurochemical investigators that tools like $[^3H](-)$-dihydroalprenolol could be routinely prepared in a robust process with high specific activity and uniform quality. It also signaled that NEN was very eager to collaborate with them to discover new and valuable radioligands.

The discovery of $[^3H](-)$-dihydroalprenolol launched an amazing period of explosive growth for these products at just the time I came to NEN. In 1978 I was appointed as supervisor of the Ligands Group and our clearly understood mandate was to rapidly fill the NEN catalogue with valuable receptor radioligands. One of the first products that I personally introduced from the bench was $[^3H](-)$-apomorphine.[4] Later, in a collaboration with John Neumeyer, we reported on another potent dopaminergic agonist, $[^3H](-)$- *N*-propylnorapomorphine, in 1980[5] and it also became an NEN product soon thereafter. Not surprisingly, John, a recognized expert on the dopamine receptor, was also aware of the tremendous growth potential in the entire

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receptor area, especially the specific market to be served in supplying unlabeled neurochemicals to neurochemists. In the early 1980s John founded Research Biochemicals, Inc. (or simply 'RBI') which became the premier global supplier in that niche area. In the course of this work we at NEN also perfected the chemistry to prepare [^{3}H]naloxone at far higher specific activity than was originally used by Snyder[6] as well as [^{3}H] (–)-dihydroalprenolol at nearly triple its original specific activity. Another very important technical breakthrough emerging in the early 1970s at NEN also facilitated the rapid introduction of many tritiated radioligands. Norman Silberman and Robert O'Brien had cleverly worked out the preparation of [^{3}H]methyl iodide at greater than 80 Ci/mmol and first applied it to labeling steroids with tritium at high specific activity. It was soon recognized that many receptor radioligands were also decorated with *N*-methyl or *O*-methyl groups (or even methyl esters) that could be obtained in tritiated form at high specific activity by the action of [^{3}H]methyl iodide on the appropriate precursor. Also, using this reagent, tritiated methyl Grignard reagent as well as the Wittig reagent could be prepared and exploited. The latter assisted in our synthesis of [^{3}H]kainic acid[7] whose high specific activity tritium labeling would have been nearly impossible in any other way. Interestingly, it was during this work in preparing a number of [*N*-methyl-^{3}H] radioligands that we discovered some of the most dramatic examples of chromatographic isotopic fractionation ever reported.[8,9]

During this exciting time period I worked primarily for David Ahern, who had already established himself as an expert in radiolabeled lipid and prostaglandin chemistry at NEN, and who now took on the added responsibility of some of the receptor radioligands as well. The urgency to add new radioligands to our catalogue was so great that another group, the Drugs Group, was simultaneously formed with my colleague Steve Hurt as supervisor. Also a separate group dedicated to tritiated peptides was formed under Y. P. Wan. Steve and Y. P. reported to Richard Young who was not only a superb chemist but also tremendously informed about receptor biochemistry and excelled in locating and recruiting neurochemical consultants for us. All these groups reported through David and Richard to Malcolm Randall. We routinely contacted on a daily basis such neurochemical experts as Solomon Snyder, Henry Yamamura, Ian Creese, Nancy Zahniser, Philip Seeman and others as the need arose and their suggestions and evaluations of our prototype products proved invaluable. Both the Drugs, Ligands and Peptides groups along with other specialized groups at NEN were responsible for the introduction of scores of receptor radioligands as products that were critical to elucidating receptor function.

Tritium NMR at NEN

The first high-resolution tritium NMR experiment was described by Tiers and co-workers over 40 years ago.[10] Following that, some of the earliest and most meaningful work in tritium NMR was conducted by John R. Jones and colleagues at the University of Surrey, the contribution of which continues to this day. However, by the mid-1970s forward thinking scientists at NEN had also realized the important potential of tritium NMR, believing that it could also be a valuable quality tool in manufacturing tritiated products. In particular, Norman Silberman championed the early use of tritium NMR at NEN. Norman had joined NEN in 1964 as the 125th employee. He had worked in academia on steroid hormones and in those early years at NEN was asked to rapidly expand the catalogue offering, especially in the area of tritiated steroids. By the mid-1970s Norman became aware of the potential of tritium NMR and had forged a collaboration with Lawrence Altman who at the time was doing tritium NMR work at the State University of New York at Stony Brook. As a consequence of this fruitful collaboration, Altman and Silberman published several important papers using this technique.[11,12] Altman was also a co-author and important contributor to the paper on [^{3}H](–)-dihydroalprenolol which described its characterization by tritium NMR with a detailed explanation for the observed chemical shifts and complex tritium to tritium coupling constants.[3]

My first personal experience with tritium NMR at NEN occurred at about the same time these key papers were published. I was working for David Ahern on an NCI grant that NEN was awarded concerning the anticancer properties of retinoids and one of the initial goals I soon accomplished was to prepare [10-^{3}H] retinoic acid at high specific activity. The synthesis and characterization of that product were completed in the spring of 1978. David was aware of the ongoing NEN collaboration with Altman at Stony Brook and encouraged me to send him a sample for tritium NMR analysis. I had my misgivings but dutifully followed orders and sent it off to Stony Brook in a sealed NMR tube packed in dry ice. I will never forget the very excited phone call from David about a week later that the returned spectrum looked beautifully clean and documented the exclusive tritium labeling of the product in the tenth position.[13] From that point onward my respect and enthusiasm for tritium NMR and what it could demonstrate were firmly established.[14] Soon thereafter, NEN set up its own tritium

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NMR instrument in what was then the first floor of our 609 Albany St building and Altman was recruited to serve as a consultant and help us in the early stages. Afterward, Puliyer Srinivasan was hired to oversee the NMR laboratory. Over the years his skill at running and interpreting literally hundreds of tritium NMR spectra proved critical to our work. Srinivasan was especially creative in devising tritium NMR methods to determine specific activity when other conventional techniques (UV, MS) failed. It is very likely that our tritium NMR laboratory established in early 1978 was the first one in a US commercial operation.

The design and synthesis of [methyl-^{3}H] methyl tosylate and nosylate

One of the most intriguing stories to emerge from NEN in the past several years has been the brilliant design and efficient synthesis of [methyl-^{3}H] tosylate and nosylate by Scot Pounds. Scot came to NEN in 1986 after finishing up his Ph. D. at Boston University and quickly became an expert in tritium chemistry and a colleague you could easily turn to for sound technical advice. He had worked for many years with the reagent [^{3}H]methyl iodide that Silberman and O'Brien had designed decades before at NEN and which had proven so valuable to our catalogue expansion. However, Scot asked a simple question that apparently no one in our industry had yet considered. Could [^{3}H]methyl iodide be improved upon? Clearly, this reagent had been readily prepared by a number of methods for many years, but it was also unstable and not very convenient to work with on a small scale. Furthermore, it was volatile and could present environmental contamination issues if care was not taken. Scot wondered if an alternative reagent could be created that might be as versatile as [^{3}H]methyl iodide and as easy to prepare, but non-volatile, stable for long-term storage and able to be successfully employed on a small (10 mCi) scale as well?

Scot was also aware that we at NEN had worked out the preparation of [^{3}H]dimethyl sulfate as a catalogue item years before, although at low specific activity.[15] With this as some precedent, Scot considered whether [methyl-^{3}H] methyl tosylate could be a possible candidate. In unlabeled form it was also a good methylating reagent and in tritiated form it might possess all of the improved and upgraded features desired. No one had reported the preparation of [methyl-^{3}H]methyl tosylate (or related analogues) before, but Scot was able to efficiently prepare it in nearly quantitative yield from the reaction of silver tosylate and [^{3}H]methyl iodide in refluxing acetonitrile overnight with a non-radioactive literature procedure as guidance.[16] The product obtained was very pure even in its crude state but could be further purified by flash chromatography.

As hoped, [methyl-^{3}H] methyl tosylate was at least as effective as [methyl-^{3}H] methyl iodide in alkylating the usual alcohols and amines, and its non-volatility brought added advantages. Reactions could be performed in conventional glassware and monitored by TLC. If a reaction appeared to be sluggish or completely stalled, more [methyl-^{3}H] methyl tosylate could be sequentially added to coax it to completion. Also, the reaction could be heated or the reaction solvent changed by evaporation without loss of radioactivity or concern of radioactive contamination. Perhaps the most remarkable feature of this new reagent was its stability, since it was found to maintain its radiochemical purity at a concentration of about 600 mCi/ml in hexane:ethyl acetate at 4°C for at least 20 days. This encouraging result emboldened Scot to also prepare [methyl-^{3}H] nosylate (methyl-4-nitrophenylsulfonate) in similar fashion. Not surprisingly, this was an even more reactive reagent which alkylated even weak nucleophiles like carboxylic acids in hindered environments. Furthermore, in a comparison study of its reactivity versus added unlabeled methyl iodide in alkylating a carboxylic acid potassium salt, Scot found no evidence of isotopic dilution resulting from competing methyl iodide alkylation.[17] Again, it was surprising and gratifying to learn how stable this second reagent also was for long-term storage, showing little if any evidence of decomposition after being stored for at least 14 weeks at a concentration of about 39 mCi/ml in hexane:ethyl acetate at 4°C. Scot's ingenious efforts soon resulted in introducing [methyl-^{3}H] methyl nosylate as a new product for NEN.[18,]

Involvement with the International Isotope Society

From its founding NEN has had a rich and consistent history of technical innovation and also supported professional meetings and societies whose goal is to foster better understanding of isotope research, educating the public on the benefits of radioactivity. In the early 1960s NEN was perhaps the most significant organizer and supporter of a number of meetings entitled 'Symposia on Tracer Methodology.' The collected papers from these symposia were also published in a series named 'Advances in Tracer Methodology' for which Seymour Rothchild, our first Technical Director, served as editor.

With the founding of the International Isotope Society (IIS) in 1986 we were also eager to be part of its activities and mission. In 1985 I supervised the Custom

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Synthesis group and reported to Jeanne Krieger who had also come to NEN in the late 1970s after working for George Whitesides at M. I. T. From the very beginning Jeanne strongly felt that as many of us at NEN as possible should join the IIS, giving it our very best support by attendance at chapter and international meetings and working hard to accomplish its goals. Since 1985 in Kansas City we have participated in every international meeting of the IIS and a majority of its chapter meetings. Jennie Ahern also provided an especially strong example of IIS involvement, serving for a number of years on the IIS Board of Trustees as Treasurer and continuing to this day as Treasurer of the Northeast US IIS Chapter. NEN colleague Kennedy O'Brien in our sales organization has been an IIS member since 1993 and served in many capacities for the IIS, most notably as Secretary and President of the Central US IIS Chapter and as a member of the important Low Level Radioactive Waste subcommittee since its formation in 1998. With these examples as inspiration I have also had the pleasure to serve in the IIS in many ways since its founding. Certainly, one of our most memorable privileges at NEN was the opportunity to co-host the successful Boston 2003 IIS Meeting along with Keith McCarthy of Pfizer and Dennis Dean of Merck. Clearly, the legacy of such professional involvement initiated by our early NEN founders and colleagues will continue.

Conclusion

After nearly 30 years at NEN I reflect on the fact that it is indeed rare these days for someone to work so long at a company in the technical sector. These years have been rewardingly spent working with diverse scientists on many different topics. My enjoyable collaboration with these very creative and motivated colleagues recounted here should provide the reader with useful insight into NEN.

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