FACES OF MASS SPECTROMETRY

David Millington / Donald Chace

Anne Brenner and Hayes Simpson are sciences writers at Technica Editorial

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David Millington

Donald Chace

Wildcat Screeners: Part 1

When David Millington and Donald Chace get together to talk about their work, captivating stories unfold. A track record of developing mass spectrometry techniques together for more than three decades provides plenty of content. Their narratives will be shared in two parts. Part I focuses on how they got started in mass spec, overcame obstacles, and began to transform the field of newborn screening.

Collaboration between David and Don began at Duke University during the 1990s when they both began work on a new project to develop screening and tests primarily using mass spectrometry and dried blood specimens. This was during a time of transition from using magnetic sector instruments to the relatively simpler, more compact, and faster quadrupole systems. But there was still a common conception that mass spectrometers would never make it into clinical labs.

Looking back on their shared history, David and Don reflect on how it felt to get into newborn screening, which involved an area of analytical chemistry that was new and uncharted when it came to the application of mass spectrometry. They worked well together not only because of their energy and intellect, but also because they were renegades. Dubbed "Wildcat Screeners" by a peer who recognized their persistence, they were viewed as renegade scientists who chased their shared vision even when confronted with skepticism.

A key to the dynamic duo's success is the ability to find humor even in the face of opposition. They laughed about the nickname "Wildcat Screeners" then, and they still have a good chuckle about it now. They are compatible in a relaxed and easy way that comes from knowing each other for many years and sharing common memories. These are two good-natured people who are equally enthusiastic about their work and enjoy solid and lasting partnerships.

David is currently Professor Emeritus of Pediatrics at Duke University in North Carolina and Don is now in Massachusetts, the application and product specialist for Capitainer, specializing in quantitative dried blood sampling.

How did you get your start in the mass spec field?

David: In my case, I was first exposed to mass spectrometry when I was a final-year undergrad student at the University of Liverpool in 1966. In order to have a bachelor's degree, you had to do a research project during that final (honors) year. I was assigned at random to Dr. Bob Johnstone, who was interested in photochemistry and mass spectrometry and was in charge of the chemistry department's mass spectrometers. It so happened that when I synthesized or crystallized a new compound, as part of my project, he would take me up to the mass spec lab and show me how to generate a mass spectrum and perform accurate mass measurements, which were used to confirm the molecular formula. Of course, there was no computer in those days—all of this was done manually. Spectra were recorded on photographic paper and counted by hand. I was hooked right then and asked specifically if I could do a project for my PhD that involved mass spectrometry. That occurred from 1966 to 1969. If you remember, that was Beatlemania time in Liverpool, so it was good to be there—not only because of the science that was going on, but also because of the music scene in Liverpool, which was absolutely amazing.

Don: I got started in mass spectrometry as a PhD graduate student. I had a master's degree in forensic science and wanted to run a Forensic Toxicology lab so I needed a PhD. It made sense to pursue pharmacology and a project that had an abundance of analytical chemistry. I chose to study under Fred Abramson, who was developing a new mass spectrometry technique called CRIMS (chemical reaction interface mass spectrometry). It was a capillary GC MS but with a special interface between the GC effluent and the mass spec source. This device was a ceramic tube in microwave device that generated a plasma and thus combusted everything that entered it to its elements. Addition of a reactant (excess elemental gas like SO2 for oxygen) or hydrogen would combine with the elements forming small stable molecules like CO2 or NO and their isotopes 13CO2 or 15NO. If a compound was enriched with carbon 13 or nitrogen 15, you would detect an increase in the M+1 m/z value. With a

computer algorithm that subtracted out the known abundance of each isotope, you could produce a carbon 13 or nitrogen 15 selective chromatogram of just the compound that had the excess isotope. In this way, a labeled drug could have its metabolites identified easily in the chromatogram for retention time. Turn off the microwave and you could rerun and get a spectrum. We identified a new metabolite in canines of an anti-seizure drug in this way. What I remember most is how old the mass spec was (a Dupont 21-491 sector instrument), and it was mostly gauges, dials, and strip charts. It was at the time of the introduction of computer control and data interfaces, which was a unique experience. I really learned the ins and outs of mass spectrometry and drug metabolism, which ultimately paved the way to Duke. Duke was interested in looking for endogenous metabolites of rare disease by probing with isotope labeled precursors.

How did the two of you meet and start to collaborate with each other?

Don: It was at an ASMS meeting in Tucson, Arizona. My wife was pregnant at the time, and I was looking for my first job following a postdoc at the University of Maryland School of Pharmacy. It was a stressful time of course (job hunting, baby on way), and I met David after the job posting. We hit it off almost immediately with his British charm and interesting work in metabolism. That charm always persisted throughout my time at Duke, in addition to the creativity and passion we both shared.

David: I remember we were trying to expand our group at that time, toward the end of the 1980s. We had been doing tandem mass spectrometry in the clinical diagnostic lab for several years and established a test as one of the standard-of-care tests for inborn errors of metabolism. Then, Dr. Stephen Kahler, one of my colleagues, suggested we try this technique out on dried blood spots, and I didn't even know what a dried blood spot was. But that got us access to the state public health lab, where we were able to retrieve some of the dried blood spots from patients who were diagnosed at Duke later in life—obviously not in the newborn period. When we applied tandem mass spectrometry to the extracts of those stored samples, the signals from the expected diagnostic acylcarnitine species were clearly detected. This led to our first publication showing proof of principle for the new method. Subsequently Dr. Kahler and I received a grant from the state to develop the method for newborn screening, and then needed to expand. And at that meeting, I saw Don's name on the list of persons looking for new positions. Subsequently, he was hired as a faculty member in our division. So that's how we met.

Don: When I first got to Duke, the newborn screening of many of the diseases they were discovering was just starting. Although I was brought into the group to look for new metabolites using CRIMS, within a few weeks, I switched the project to newborn screening in part because of recent funding and an urgent need to get the program going. I remember we were in Wilmington, North Carolina, at an annual get-together of scientists and MDs. My daughter was due soon, and we were worried the birth would take place at the beach house. Charlie Roe, MD, who was division chief, said to me, "You don't have to worry, we have plenty of pediatricians around here." My thought was, "Great, but don't we need an obstetrician?" In any event, a few weeks later, my daughter was born, and I was asking for a dried blood spot from my daughter in the delivery room, because I was close to developing a new screening test. I never got the blood spot, but I did get the test developed.

David: Tracing the fate of metabolic compounds using stable isotopes was one of my interests, but it quickly got superseded with the idea of trying to get tandem mass spectrometry into newborn screening, because we knew that it was going to work—we knew that it was going to happen, because we'd done some research. So, it was all-hands-on-deck. Also, at that time, we had transitioned from using magnetic sector instruments for initial research work to using triple quadrupoles. That development took place in the late 1980s. By 1990, you could commercially obtain a triple quadrupole.

Don: A funny story: The first mass spec I used had a fast atom bombardment (FAB) beam, which ionized quite intensely. The sample would be gone in a couple of seconds, like a flash in the pan. Later, we got the new benchtop Quattro MS/MS that was fitted with a fast ion bombardment (FIB) source. This source was less intense, which resulted in a sample that stuck around longer, allowing more spectra to be obtained and improved results. Most would refer to the method that ultimately was developed as FAB MS/MS. I would always enjoy telling everyone that they were, in fact, FIB-bing.

David: You could say that the transition to the triple quadrupole was a very interesting time, because it became possible and practicable to analyze samples in an automated fashion. That didn't happen for some time. But we were still able to run samples manually at the rate of one every three minutes, and there was no possibility of carryover from one sample to the next.

When, and how, did you decide to focus specifically on mass spec as it relates to pediatric medicine?

David: That happened as a result of a chance meeting. I was at the School of Public Health at the University of North Carolina at Chapel Hill. There, I was introduced to a metabolic pediatrician by the name of Dr. Charlie Roe at Duke. He had just returned from a sabbatical in London, which had piqued his interest in mass spectrometry as it applied to the diagnosis of inherited metabolic disorders. In those days, diagnoses were made by detection of abnormal metabolites in the urine, using gas chromatography–mass spectrometry. That was pretty much the only type of mass spec you might find in any clinical laboratory, if it was there at all. During the meeting, we decided to collaborate. He was educating me about the biochemistry of metabolic diseases, and I educated him about tandem mass spectrometry. I joined his group at Duke in 1983, specifically to develop a method for the analysis of acylcarnitines, which we both knew would greatly improve the diagnosis of metabolic disorders. That's where the story really started.

What advancements in information technology support interpretation, reporting, tracking, and outcome evaluation of mass spec testing?

David: In the early days, the methodology required you to acquire a tandem mass spectrometer; there was no triple quadrupole, so we had to get a magnetic sector instrument. We call them

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dinosaurs now, but in those days, it really was quite a beast of an instrument—it was all hands-on, with no computer. Getting results out of it was challenging, but that's all we had. I had been fortunate enough to work for five years in a company that produced these instruments, so I already knew the solution to this problem, having worked for a company developing these methods. We were able to get a secondhand instrument and put it in the lab, sort of surreptitiously. People didn't like it—I can remember going to meetings and showing results from this mass spectrometer. My colleagues in clinical chemistry, especially in Europe, said, "David, you shouldn't be doing this. There's no way this kind of instrument is ever going to make it into clinical lab. It's too complicated and difficult to use." But I said to them, "Well, I know what's coming down the pike, and it's going to be a computer-controlled instrument that can do this."

Early on, were there barriers that impeded the implementation of mass spectrometry in screening for newborns? How did you overcome them?

David: Absolutely, there were barriers. The hands-on operation was a barrier not only in the lab, but also among clinicians who viewed anything new in the clinical field with great skepticism. So, Don and I overcame this by persistently showing up at meetings—we made sure it was on the agenda, and we discussed it there. We also published a lot of papers, especially in the early 1990s, out of our desire to inform people. It took almost 10 years from inception to reality, which is how long it takes for any new idea to become reality in this area. State funding was also a great help, because you have to get the samples to do this research. That means a good collaboration with the state public health lab. In that regard we were fortunate the lab stored leftover samples for up to 10 years, allowed the use of such samples for research, and supported our research plans to expand the newborn screening program to include more conditions.

Don: In terms of the mass spectrometer, we put neutral loss and precursor ion scans on the map. The other thing was, we were in pediatrics at a university with a good reputation, and Dave, Charlie Roe, and all of these folks got a lot of attention at the metabolic conferences they attended. Public health was very resistant to this, except for in North Carolina. They were progressive, but ultimately, we had to prove this was possible. That's where a private lab in Pittsburgh came in—Ed Naylor was doing supplemental screening. We did all of the pioneering work, but he did the first real use of it in a commercial sense. And that's what started to really get the attention of public health. Because originally, people were very resistant, even calling us "Wildcat Screeners." Remember, Dave? That was in France, right?

David: Yes, we got invited to this meeting in France, and the topic was newborn screening. Anybody who was anybody in the field was there. We had a poster describing initial results from samples that we obtained from a colleague in Australia. We had 60 dried blood spots collected over a period of 20 years, which Don analyzed for both acylcarnitines and amino acids. Our results were very convincing that tandem mass spectrometry was going to be able to pick up these disorders in the newborn. One of the platform chairmen was one of the organizers of the meeting, and to describe us, he used the term "Wildcat Screeners." Don and I just laughed about the use of that phrase, and we even considered having T-shirts made. The attitude toward us was, "What are these guys doing in our field with this technology?" But we had the audacity to come into their field with this technology—not by ramming it down anyone's throats, but simply by presenting the evidence.

Don: The reception was very negative. People said it would never happen—no one would ever be able to use mass spec with newborn screening, because they said it was just too complex, too sophisticated, and too expensive.

David: Yes, the resistance was definitely there, and yet because of that, it just made us all the more determined to succeed. I realized that you must persist—keep showing up at meetings, keep presenting information, and keep publishing, until you're past that barrier of resistance. That happened in 1997, when the state of North Carolina agreed to do a pilot study in which babies would be screened, using a contract with NeoGen Screening for a year or 18 months. The results after six months were so compelling that they decided they were definitely going into this. In that sense, basically, we changed the field of newborn screening completely.

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