Electrospray Ionization

Electrospray Wings for Molecular Elephants (Nobel Lecture)**

*John B. Fenn**

With best wishes (and my blessings!) for what they may be worth! John Fenn

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From the Contents

Upto 1952

My father, Herbert Bennett Fenn, the eldest of three children was born and raised on a farm in northern Delaware which his father operated but did not own. I never saw that farm but I vividly remember my Grandmother's frequent reference to a single chestnut tree in the front yard, so large and prolific that the nuts from that one tree paid the taxes on the farm every year! My mother was the sixth of ten children in the family of John Clarence Dingman, a country doctor in Spring Valley NY whose three surviving sons also became physicians. Dad worked his way through college, graduating in electrical engineering from Rutgers in 1910, the same year that mother received a degree in home economics and nutrition from Columbia. They were both hired by the Presbyterian Mission Board to teach at the Sheldon Jackson Mission School in Sitka, Alaska, originally settled by the Russians and sometime capital (until replaced by Juneau in 1900). The Chairman of that Board told each one about the other and warned them both not to fall in love, but if they did and got married he would provide them with a house for as long as they stayed in Sitka! They did and he did, after mother's death in 1990 (Dad had died in 1944) we found in her papers a clipping from the Sitka paper with a photograph of the "Honeymoon Cottage".

During a cruise in 1996 I spent a day in Sitka. The Mission School is now Sheldon-Jackson University. I visited the library, discovered a complete file of student newspapers, and found several articles about my parents! Moreover, the present Librarian was then living in the "Honeymoon Cottage" and gave me a tour so I was actually able to look out through its front windows and see the magnificent view of Sitka Bay that my parents used to rave about.

The newlyweds were very happy in Alaska and no doubt would have remained there indefinitely had it not emerged that mother would be unable to bear children except by Caesarian Section, a procedure not then available in Sitka. Determined to have family she persuaded Dad to move back to the States where he became manager of the Metacloth Company, a small enterprise in Lodi, New Jersey whose main product was cotton duck treated by immersion in a concentrated ammoniacal solution of copper hydroxide followed by a wash in acid. The deep blue "cuprammonium" solution dissolved some of the cellulose which reprecipitated during passage through the succeeding acid bath, filling the pores of the cloth enough to make it "water resistant" but not "waterproof". Residual copper provided a characteristic blue-green color along with a high resistance to attack by microorganisms and white ants. For these reasons, "Metacloth" was in fairly steady demand for tents and tarpaulins in tropical latitudes. My abrupt introduction to "chemistry" occurred on one of the treasured Saturdays when Dad took me to the plant. Nosing around outside I lifted a small flap on the cover

[*] Prof. Dr. J. B. Fenn
Department of Chemistry
College of Humanities and Sciences, Box 2006
Virginia Commonwealth University
1001 West Main Street, Richmond VA 23284-2006 (USA)
Fax: (+1) 804-367-8599
E-mail: jbfenn@vcu.edu

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Our home was in Hackensack, N.J., next door to Lodi and County Seat of Bergen County. I was born in New York City in 1917 and three-plus years later my brother Norman arrived in Paterson, N.J. where two of mother's brothers were surgeons. The Metacloth company was sold in 1926 and Dad was unceremoniously dumped by the new owners. He was not a vindictive man but he got no little satisfaction out of the several occasions in the next two years when his help was needed in overcoming mistakes made by the new management. Meanwhile, approaching fifty and finding equivalent jobs scarce, he was paying the bills by working as a draftsman at the Fokker Aircraft Company in Teterboro, N.J. The value of having a trade as back-up for a profession was an enduring object lesson for a youngster on the eve of the Great Depression but there was for him a far more exciting consequence of the Fokker connection. When Lindbergh's "Spirit of St. Louis" was shipped back from Paris after its famous flight across the Atlantic, it was parked for a time in a hangar at the Teterboro airport. The thrill of a lifetime for a ten year old boy was when his Dad took him into that hangar where he was allowed to sit in the cockpit and move the controls, pretending to be pilot of that famous plane!

Meanwhile the family fortunes were going down hill, much further than my brother and I were aware. Just before Dad lost his job, he and mother had invested their life savings in a new (for us) house that everyone insisted was worth "every nickel" of the $15,000 that it cost! Unfortunately, after we had to move and before the house could be sold the great depression had begun. No prospective buyers had enough nickels so those savings disappeared in the meltdown of foreclosure. Meanwhile, a door of opportunity had opened in Berea, Kentucky, a small community of 3500 or so inhabitants at the edge of bluegrass country some 40 miles south of Lexington and roughly halfway between Cincinnati, Ohio, and Knoxville, Tennessee. The town of Berea was home for a remarkable institution known by the same name but officially entitled "Berea College and Allied Schools". Both the community and the school had their roots in a non-sectarian Union Church founded in 1848 by John G. Fee, on a ridge of land donated by Cassius Clay, a brother of Henry Clay, the famous orator and statesman from Lexington, Kentucky.

Fee was a Congregational Minister from Massachusetts and a fervent abolitionist determined to provide educational opportunities for needy students, whatever their race or creed. After decades of effort by himself and his followers his dream grew into what in 1928 comprised a coeducational student body of about 1700 divided among four schools: 1) The Foundation-Junior-High School with an ungraded program into which students with as little as two years of formal schooling could enroll and progress at their own rate up through the equivalent of eighth grade into a standard and accredited ninth-grade curriculum; 2) The Academy, with an accredited curriculum for grades 10 through 12, 3) the Normal School with a two-year program leading to a Teaching Certificate, and 4) the College which offered accredited curricula leading to BA degrees in the liberal arts and sciences as well as BS degrees in home economics and agriculture. Mother's sister was teaching in the College and knew that the Industrial Arts Department of the Foundation School and the Academy needed someone to teach auto mechanics and practical electricity. Dad was eminently qualified and got the job. We moved to Kentucky in time for me to enter the eighth grade in the "Training School" of Berea's Normal School in the fall of 1928. The next fall I entered ninth grade in the Foundation School and continued on through the Academy and College.

A lot of misgivings, pain, and confusion attended our passage through the newly opened door, including an automobile accident on the way to Kentucky, but life on the other side turned out to be rich and rewarding beyond what any of us had dreamed. In later years Dad and Mother both said time and again, that losing his "good" job in New Jersey turned out to be the greatest blessing that we could have received! To this day my brother and I share those sentiments and count ourselves especially privileged to have been reared in what was a truly remarkable community. Its soul was its President, William J. Hutchins, father of Robert Maynard Hutchins, the "boy wonder" of the American education scene who became Secretary of Yale at the age of 24, Dean of its Law School at 26 and President of the University of Chicago at 30! "William J", as the father was fondly known at his own institution, was truly one of nature's noblemen. A striking man of vision and patrician to the core, his Berea was a singular stage on which the play was always provocative and the message meaningful. Name an outstanding man or woman of letters, the arts, science, or religion, of those days and the odds are high that he or she came to Berea to talk at one of the thrice weekly "United Chapels" for students from all schools. Attendance was required of all, and resented by most, but at my 50th class reunion there was a remarkable consensus among my surviving classmates that the community experience of those United Chapel services were by far the most memorable and valuable components of their educational experience. Alas, that tradition has long since disappeared, one more victim of "students' rights" and television. Moreover, as the public education system improved in surrounding "Appalachia", over the years, the need for Berea's Foundation School, Academy, and Normal School faded so that the only survivor is a now a substantially larger Berea College.

A unique feature of Berea was and is a student-labor program that provided much of the manpower required to run the institution. Under the supervision of permanent staff, the dining halls, dormitories, offices, and yard work were all maintained and operated by student labor. In addition were several "industries" including a bakery, broom factory, dairy, large truck garden, sheep farm, piggery, a "woodwork department" that made fine furniture, and a weaving establishment. The normal work load was two hours a day by which a student could earn as much as half or two thirds of his or her out-of-pocket expenses that in the 1930s averaged only $250 to $300 per year because tuition was free! The system was very democratic because every student was

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required to work at least two hours a day. A fair fraction of the student body comprised so-called half-day students who worked four hours a day, earning enough to pay all expenses. I knew students who arrived at Berea with nothing but the clothes on their backs, having dropped out of school after the second grade, and worked their way through college! It is not surprising that competition to enter Berea, especially the college, was extremely keen. To avoid having to reject such a large fraction of applicants, the Trustees had established a policy requiring that 85 percent of the student body come from a region of Appalachia comprising some 500 or so counties in Virginia, West Virginia, Kentucky, Tennessee, and North Carolina. The remaining 15 percent of the students came from the rest of the world. Today's Berea is quite a different place because the growth of alternative educational opportunities in Berea's "territory", has decreased demand to the point that in 1987, at my 50th reunion, I learned there were only two applicants for every opening in the College compared to 20 or 30 in my day. Part of that decrease in demand is due to a higher level of average income in that "territory" so that fewer students can show that they cannot afford to go elsewhere, a requisite for admission.

When I graduated from the Academy in 1932 I was only 15, too young, my parents thought, to go to college, so I stayed in the Academy an extra year taking courses in mechanical drawing and shorthand. I also continued the piano lessons that I had started the year before and spent enough time practicing so that I actually gave a recital during my last year in college! Alas, whatever piano skills I had have faded until now I do well to play chopsticks with my grandchildren! When I entered College with the class of '38 I had not decided on a major but I leaned towards a science, probably because of my long affair with the "Book of Knowledge", an encyclopedia set for young people which my parents bought when I was around eight or nine. During the hours that I pored over them, its 20 volumes became a well-worn magic carpet to new and fascinating worlds. I have often quipped that "I got through college on the Book of Knowledge", a bit of rhyming hyperbole that contains an appreciable kernel of truth.

The College required at least one course in science for all students no matter what their major. Having enjoyed my chemistry course in the Academy I enrolled in "Introductory Chemistry" during my freshman year. It was taught by Professor Julian Capps, the senior of the two-member chemistry faculty. He was a wonderful teacher who made his subject live. I was so seduced that chemistry became my major even though gravimetric analysis gave me fits in my sophomore year. I repeated the lengthy phosphorous determination on three samples each of ten unknowns before I got one set of three results with acceptable agreement! It seems ironic that mass spectrometry, the center of my scientific life for the last 20 years, is really just an exercise in gravimetric analysis!

During that sophomore year an experiment in another kind of chemistry turned out to have a big effect on my chemical education. I fell in love with a girl in the junior class. Determined to catch up with her and graduate when she did, I went to summer school in 1936 at the University of Iowa because it had a 10-week session that allowed me to earn 12 semester hours of credit in organic chemistry under "Uncle Charlie" Raiford, and inorganic chemistry under Professors Jacob Cornog and Perry Bond. All in all it was a very hot and grueling summer. The romance that had blossomed in the spring and sent me off to summer school, withered the following fall. However, because I would be within two courses of finishing my degree requirements by end of that year, my third in college, I managed to get classified as a Senior and became a provisional member of Class of '37 at the June commencement, the objective inspired by a young man's fancy of the previous spring. My courses in chemistry thus far had required only some facility in simple algebra. Straight A's in all Professor Peck's courses in the Academy (plane geometry, advanced algebra, solid geometry, and trigonometry) had excused me from freshman math in college, all that was then required for a major in chemistry.

Fortunately for my future our next door neighbor, George Bent, was visited by his brother Henry, then an assistant professor of chemistry at Harvard. When Henry discovered that I was contemplating graduate work in chemistry, having had no college math, he was aghast and spent a couple of hours impressing me with the importance of mathematics in chemistry. As a result I spent much of my third and last year of college immersed in that subject. Fortune had tossed me another favor in the form of Berea's new curriculum based on four nine-week quarters instead of two 18-week semesters. So-called "intensive" courses met six hours a week for nine weeks while "running" courses met three hours a week for eighteen. Thus, a standard full load of 15 class hours per week comprised two intensive courses and one running course, an arrangement which I heartily endorse. One major advantage is that a student takes only three courses at any one time instead of five. It is much less distracting to be studying three subjects at a time rather than five. The particular advantage for me was that the calendar time for a three-credit-hour course was cut in half so that both Course B, and its prerequiste Course A, could be completed in two nine-week quarters instead of two eighteen week semesters. Thus, in my senior year I was able to take analytic geometry, differential calculus, integral calculus, and complex variable, all in that same year! It was pretty heavy going, but it enabled me to survive in graduate school where I took one more undergraduate course in advanced calculus. The record seems to say that I have also survived after graduate school but a lack of mathematical skills has been a great handicap throughout my life.

Though I had taken part in the June commencement exercises at Berea in 1937, I still had course requirements to complete before I could get my degree. I fulfilled those requirements at Purdue University that summer by taking physical chemistry with lectures by Roy Newton and laboratory under Hershel Hunt, along with a course in chemical microscopy under Ed F. Degering.

During that hectic last year in college I had applied to several universities for graduate study in chemistry. Yale and Northwestern responded with offers of teaching assistantships that would meet most of my expenses. I first leaned toward Northwestern but, for reasons I won't take space to enlarge

on, I finally decided on Yale. That decision delayed by half a century my meeting Malcolm Dole who in 1937 was a young assistant professor of chemistry at Northwestern. It was the experiments he reported much later (in 1968) that changed the course of my scientific life.

At Yale I found myself in a new and different world. I had done time in two much larger universities and one much smaller college but I was completely unprepared for, and awed by, the splendor of Yale's campus. Its architectural theme has been disdainfully dubbed "Fifth Avenue Gothic" by the self-styled experts who sneer at such expensive imitation of the old world. But a wide-eyed small-town youth from Kentucky found it all fascinating and loved to wander around and through the gardens, gargoyles, and gates that were scattered throughout the campus. To me it was rewarding recreation to discover and explore the little nooks and courtyards where one could sit in the shade of a tree completely oblivious to the cacaphony of a busy city, only a few yards away but completely shut out by the thickest walls and heaviest masonry I had ever seen. Sterling Library, then said to be the largest in the world with open stacks, became a favorite haunt where I spent many winter weekend afternoons with a good book and an apple or a candy bar. My room in the Hall of Graduate Studies looked out on a forest of chimneys, slate roofs, and Gothic towers that resembled a Hollywood version of medieval London. I ate in an elegant dining hall, complete with menus and waiters—twenty one meals per week for a now incredible eight dollars! Even so, to save money I took advantage of an option in the board contract and "signed out" every week for dinner on Friday night along with breakfast and lunch on Saturday. This "fasting" saved me $1.15 every week and probably was good for my figure! My assistantship paid a total of $850 for nine months, of which $350 went for tuition and fees. By such frugalities as the weekly "fast" I could make the remaining $500 cover most of my other expenses. My parents were horrified when they later learned of my miserly existence at Yale but I was determined to get along without their support.

On the appointed day in September the six new graduate students in physical chemistry met in Sterling Chemistry Laboratory with Professor Herbert Harned, the ranking faculty member in that subject whose colleagues included Lars Onsager, Benton B. Owen, Rodney Smith, George Murphy, John Vance, and Gosta "Gus" Akerlof. With little or no ceremony, no introductions, and no opportunity to ask questions or indicate preferences, we were assigned to advisors. I recall "Herbie" as he was known but not addressed, saying to me: "Fenn, you might as well go with Gus." Thus, began what blossomed into a close friendship that lasted until the day Gus died, many years later, in Princeton.

The Yale curriculum for chemistry graduate students in those days was pretty cut and dried. During the first year we took four courses, spent 12 to 14 hours per week as assistants in undergraduate labs and the rest of the time getting our feet wet in the laboratory on what would become our dissertation research. At the end of that first year the faculty, on the basis of course grades and day to day observations, decided whether or not each student was Ph.D. material. Those for whom thumbs were down were given a Master's degree and dismissed. The others continued, almost always receiving a Ph.D. after two more years. The second year was much like the first except that we took only a couple of courses and spent much more time on our research projects. At the beginning of the third year we had to take a two day written comprehensive exam. While I was a student, all who took that exam passed it so I don't know what the consequences of failing would have been. One very big difference in the third year was that we could no longer be teaching assistants but were expected to spend full time on research. Thus, unless we were lucky enough to win one of the two fellowships available to third year students, we were on our own financially. My solution to this problem was to get married, not to my sophomore flame but to Margaret Wilson, who became supervisor of my student labor at Berea while I was working in the Registrar's Office. She was a beautiful woman by whom I (then in the tenth grade!) was smitten the first day she started work as Assistant Registrar. Always ten years older than I she seemed to be so far beyond my reach that I never dreamed she could ever be more than the close friend and confidante that she became. I had escorted her to several events while I was in college and when I was home after I graduated. Romance bloomed while I was at Yale and she finally agreed to become my bride at end of my second year. She also became my "fellowship" during that third year at Yale, supporting us both on the 50 cents per hour she earned at various odd jobs. To this day I marvel that the daughter of a very conservative minister had the courage to ignore the disapproval of her parents and the inevitable elevation of eyebrows by everybody, everywhere, at our flagrant departure from the social norm. One of my closest friends told us decades later that he had been sure our marriage would not last more than a year or so. In fact it was not until 1992 that I lost my bride of 53 years in a New Zealand car crash. She was then 85 but looked and acted ten years younger than the husband she had "snatched from the cradle."

The plain truth is that in matters of matrimony this society's norms are genetically out of joint. Women on average live about ten years longer than men and yet become the wives of husbands who are several years older. That is why widows greatly outnumber widowers. Some say that this imbalance in life expectancy will be redressed as women increasingly become exposed to the stresses of employment in the workplace. I am much more inclined to believe the geneticist who told me that the female of the species lived longer than the male because she is fundamentally tougher and more resistant to the ravages of age. In choosing a mate men should follow the advice of Ben Franklin in his letter of advice to a young man on choosing a mistress. His message? "be sure she is older than you are!"

Only in choosing a graduate school did I exert any influence on the research I would do for my thesis. That influence was unwitting because I knew nothing of the faculty research interests at either Yale or Northwestern. Indeed I had only the vaguest idea of what graduate study and research were all about. Thus, I felt neither joy nor apprehension when I was summarily assigned to Gus Akerlof's group. Most of the research by Harned and his colleagues was on the properties of electrolyte solutions, the more dilute the better. So narrow

was this focus that when I left Yale I halfway believed the old cliche that "physical chemistry was the study of slightly contaminated water." Gus was a bit of a maverick because he had an interest in concentrated solutions. In essence what I had to do was measure the potential difference between electrodes of silver/silver chloride and platinum/hydrogen in solutions of HCl with molalities from 0.01 to 10.0 in solvents comprising methanol in water at concentration intervals of 10 per cent from 0 to 90% methanol, at temperatures every ten degrees from zero to 50 Celsius. As I remember the total number of acceptable electromotive force (emf) measurements was around 3000, taking into account that each measurement was made in duplicate. Thus, my routine every afternoon for most of two years was to prepare three pairs of cells with both cells of each pair containing the same solution. The cells were suspended in a large water bath or "thermostat" and allowed to equilibrate over night (i.e. with hydrogen bubbling over the Pt electrodes.) If by the next morning the emfs in each pair of cells agreed to within some forgotten small fraction of a millivolt, then I would haul some 80 pounds or so of ice from the basement to lower the bath temperature to near 273 K. The rest of the day would be spent recording emf values for all six cells at every 10 K as the thermostat was heated to 313 or 323 K depending on the vapor pressure (i.e. the methanol content) of the cell solvent. All the results were fitted by least squares to a quadratic curve for the dependence of emf on concentration. The least-square calculations were all carried out on a hand-cranked Monroe calculator, except when I was lucky enough to grab the one electrically driven machine in the calculating room. Clearly, I was less than inspired by this introduction to "original research". The experiments were a boring chore with few redeeming features. The results contained no surprises and nothing of much interest to anyone, least of all me. It was some years later before any of them found their way into the literature and then only as part of a table in a review article that Gus had been asked to write. My dissertation attested to the sterility of that project, consisting as it did of 45 pages of tables with only three pages of text! Although this first experience in "original research" shattered some illusions, my three years at Yale were a rewarding experience. I made many long lasting friendships with both fellow students and faculty. The physical chemists were a very congenial group and Herbie Harned was really a very interesting man, except in the class room. He came by almost every lab almost every day to chat with us students about almost everything. Sometimes his visits were inconveniently long and would interfere with experiments. One of the older graduate students had told me that Herbie would run for cover if he were asked to help, for example, by holding a light or a tool during some adjustment. That ploy worked like a charm and saved me many hours. Actually, the Harneds were very kind and warm hearted people. They held an open house every Friday night which both students and faculty felt obliged to attend more or less regularly. More often than not we ended up around the piano in a group sing-along with Roger Bates, then a Sterling Fellow and accomplished musician, at the keyboard.

I also learned a lot about life and lore outside of chemistry, for example, to play bridge, to drink beer, and to smoke a pipe during the mostly dull seminars every Tuesday night. I had many interactions with many interesting people. The weekly university calendar was filled with provocative events ranging from seminars to sermons and concerts to contests. My perspectives were stretched by memorable if abstruse encounters with great minds, including lectures on resonance by Linus Pauling, and two required courses in statistical mechanics under Lars Onsager (known to the students as Norwegian I and II!). Gus and his wife Rosalie, who was the sister of Joe Hirschfelder, looked after his students with great care and affection, making their house our home away from home. Rosalie took my new bride under her wing, showering us newlyweds with almost too much attention. I was extremely fond of Gus and most grateful for his always calm demeanor and seeming imperturbability. He strongly disapproved of marriage for graduate students but when I told him I wanted to take a wife he protested not at all and simply said "OK, we take it from here." He and "Magee" as my wife was later known, became extremely fond of each other and he later told me many times that marrying her was the smartest thing I ever did, an opinion shared by everyone who has known us both. All in all, my time at Yale was happy and rewarding but I left with a much diminished interest in "scholarly research", and no desire to return. I often think about how surprised and pleased Gus would have been to know that six decades later his son George Akerlof would share the 2001 Nobel Prize in economics and his student John Fenn would share the 2002 Prize in Chemistry!

My immediate and exciting prospect after graduation from Yale was a job in the research department of the Phosphate Division of Monsanto Chemical Company in Anniston, Alabama. The starting salary was $2700 per year, then the going rate for new Ph.D.'s in Industry and a mind-boggling jump from the $1000 or so a year Magee and I had been living on. I couldn't understand why my mother laughed when I once remarked that I didn't know how we could spend so much money!

Anniston, the county seat of Calhoun county and "Cast Iron Pipe Capital of the World", was located halfway between Atlanta and Birmingham. Living there was my introduction to the deep south where Cotton still grew and the Confederate Flag still flew. It was the home of Fort McClellan, a long-time army base that was already gearing up for America's forthcoming participation in World War II. Two of my mother's brothers had been medical officers at McClellan during World War I and two of our closest friends from New Haven passed through there after being drafted in the wake of Pearl Harbor. We had the chance to compensate them a wee bit with an occasional home-cooked meal. I was more fortunate because the army was not yet drafting husbands.

Monsanto had recently acquired the Anniston Plant when it bought Swann Chemical Company started by a legendary character named Theodore Swann. He had pioneered the production of phosphoric acid from elemental phosphorous shipped in by tank car from a plant in Columbia, Tennessee where it was produced by smelting "phos rock" in an electric furnace. At Anniston the phosphorous was burned in air to produce its pentoxide which could be hydrated to form acid of any desired concentration. Major customers for the acid

per se included the soft-drink industry, especially the Coca Cola Company in nearby Atlanta. According to local folk lore, the first tank car of food-grade acid to be delivered was filtered through laboratory Buechner funnels because the filtration plant was not ready in time to meet the deadline on the sales contract! Much of the phosphoric acid left the plant in the form of sodium and potassium phosphates that among other applications were widely used as soap builders, water conditioners, and in food products, such as baking powders.

The other main product line, which some said was the main reason Monsanto bought Swann, comprised biphenyl and its chlorinated derivatives known as Arochlors, the now notorious PCBs (polychlorinated biphenyls). Biphenyl was produced by bubbling benzene vapor through large baths of molten lead at a temperature of around 800 °C, as I remember. Liquid metal as a heating agent provided a rapid rise and close control of the temperature, thus minimizing formation of byproducts which mostly comprised *ortho*, *meta*, and *para* diphenyl benzenes. The various Arochlors were characterized by their chlorine content which ranged from 10% to 60% by weight. One of their main uses was as transformer oils because they had very good heat-transfer characteristics, would not burn, and were very inert. Indeed, they were so inert and sticky that the chemists insisted that if Arochlor ever got into a laboratory some of it would remain forever! Because it was so inert we practically bathed in the stuff, never dreaming that it might be toxic. General Electric dumped PCB wastes into the Hudson River for many years and is now resisting Federal orders to dredge it all up. Recently, the press has reported growing anger in Anniston and threats of legal action as new surveys have shown the extent to which PCBs from the plant where I worked have contaminated the soil and waters around the town. My own substantial exposure seems to have had no ill effects, but I daresay that even today, sixty years later, the PCB content of my fatty tissue would horrify the EPA (environmental protection agency)! There is no doubt that PCBs in the environment accumulate in the fatty tissues of mammals and fish. The evidence of resulting toxicity is less convincing. I remember a paper by an Australian chemist claiming that the only documented evidence of significant toxicity stemmed from two cases in which PCBs, used as the heat-transfer medium in the heating coils of deep fat fryers, leaked into the fat. The resulting reaction produced highly toxic benzo compounds whose identity I have forgotten. I do know that sixty years ago I practically bathed in the stuff while operating a pilot plant in which PCB was produced in the vapor phase by reaction of biphenyl with HCl and air over a copper catalyst in a variation of the old Deacon process for producing chlorine by the oxidation of HCl. Occasionally I would get a slight skin rash, as did the other operators, but I have detected no lasting effects.

After I had been at Anniston for a year, James W. Mullen, II a young Ph.D. organic chemist from Princeton came aboard. He and I became good friends, sharing a growing dissatisfaction with the way things were being run at work. Jim insisted that someday he was going to start a research company and that when he did he wanted me to join him. Talking about what we might do was fun but it all seemed like pipe dreaming to me. Having become increasingly disenchanted with the work situation we both resigned and left Anniston on the same day in 1943. Jim went to Bell Labs in New Jersey. I took my family, which now included an 18 month old daughter, Marianne, to Wyandotte, Michigan where I had obtained a job in the research department of Sharples Chemicals, a relatively small producer of alcohols, amines, esters, and other derivatives of amyl chloride produced by the chlorination of pentane. One day in June of 1945 a letter came from Jim saying that he had finally started his company and wanted me to join him. I took a train down to Richmond to find out more. At Bell Labs he had become involved in Project Bumblebee, a large-scale effort by the Navy to develop a ramjet-powered anti-aircraft missile for the fleet. The ramjet depends upon ram pressure from high-speed-flight to compress air enough so that its expansion after heating by combustion could provide useful thrust. It was clear that supersonic flight velocities would be required to achieve substantial thrust but because there was little or no information on drag at such velocities, nobody knew whether that thrust could equal or exceed the drag. The Project name, Bumblebee, derived from a bit of aeronautical badinage to the effect that by all the laws of modern aerodynamics it is impossible for a bumblebee to fly, but the bumblebee doesn't know any aerodynamics so it goes ahead and flies anyway!

From Flames to Flying Elephants

Experiment, Inc., the name Jim Mullen gave his company, prospered over the next seven years. To watch and participate in its growth, from a total of three employees when I arrived to perhaps 45 or so when I left in 1952, was an exciting experience. Most of its business was based on research-and-development (R and D) contracts with government agencies for which the central theme was combustion and propulsion but we did pursue some commercial ventures. There were some important personal dividends from my investment of time at Experiment. One was that I learned a little bit about the dynamics and thermodynamics of compressible flow, a newly popular area of research spawned by the advent of jet propulsion and sometimes referred to as "aerothermodynamics", or "aerothermochemistry" if changes in chemical composition were involved. Another personal dividend was the opportunity to do some research in combustion, both applied and fundamental. Jim was a strong believer in publishing our results. Thus the first research publication of my life was a paper on ignition in high-speed flow, coauthored by Jim and a junior colleague, M. R. Irby. That paper appeared in 1949, almost a decade after I left graduate school.[1] Consequent to several more papers, visits to other labs, and attendance at various meetings, I began to get some recognition in the "combustion community". Moreover, in an about-face from my feelings at Yale, I found I enjoyed doing research and communicating with others about my findings and theirs.

My life in Berea had made me think that I would someday like to become a college professor. But I had also thought that I should get some of the real-world industrial experience that

had seemed to help make Julian Capps, my chemistry professor at Berea, such an effective teacher. That desire to join a college faculty had faded somewhat at Yale but was revived a bit during my experience at Experiment, Inc. We obtained a number of provocative results on some properties of flames, in particular on stabilizing them in high-velocity flows. Both Jim and the sponsors of our studies were happy to have us attend technical meetings and to publish results of our studies, (so long as they didn't involve classified information.) Consequently, I got to know, and become known by, a number of people in the combustion community. One result of this new and limited "notoriety" was an offer from Princeton University to serve as Director of Project SQUID, a program of pure and applied research in "those fields of science relating to jet propulsion" including combustion, fluid flow, and heat transfer. Financed by the Office of Naval Research (ONR) and administered by Princeton, Project SQUID was named after the jet-propelled decapod of the sea. (That name had additional but unpremeditated relevance in that both the animal and its namesake project secreted ink, the former in clouds that confused predators, the latter in print in reports that confused readers!) SQUID's organizational structure comprised a prime contract between ONR and Princeton, with subcontracts at any one time between Princeton and from 12 to 20 or so university and industrial laboratories. The overall purpose was to cultivate interest and activity by students, faculty, and other investigators in combustion, fluid flow, and heat transfer.

One reason for my interest in Princeton was that by then I had learned that research could be a lot more fun than it had been when I was a student at Yale. Another reason was that my Yale mentor, Gus Akerlof, had set up a laboratory in its newly established James Forrestal Center to develop, with Navy (but not SQUID) support, an electrical-discharge process for the production of hydrazine, an attractive rocket propellant. Gus had once visited me in Richmond and in fact had put my name in the pot at Princeton when he learned that the previous director of SQUID was leaving. One of the "other reasons" for my interest was that Jim Mullen and many of his friends, were Princeton graduates. After seven years among those fiercely loyal alumni I had been brainwashed to the point of believing that maybe Old Nassau really was the "best old place of all." Moreover, as I have already noted, my life in Berea had long made me think that I would enjoy becoming a teacher and living in a college community.

At Princeton I would report directly to Sir Hugh Taylor, long-time Chairman of Princeton's Chemistry Department, then Dean of the Graduate School and Chairman of the University Research Board. The position was "with rank of Professor" and the Dean assured me that I would be welcomed as an active member of the academic community. And so, after much soul searching, Magee and I decided to leave the "Cradle of the Confederacy" for what was and is often called "the northernmost southern town in the country". That epithet stemmed from the substantial numbers of Princeton students that over the years have come from below the Mason Dixon line. Before the Civil War it had become customary for students from the south to celebrate graduation by freeing their personal servants. An interesting result is that Princeton has a much larger black population than do other towns of comparable size in that part of the country.

My new job turned out to be very interesting and highly educational. I had to travel a lot but was able to visit many laboratories and meet well-known scientists in many fields all over the country. I got to know the people in ONR pretty well and my circle of acquaintances included people in ARO (Army Research Office) and AFOSR (Air Force Office of Scientific Research) both of which were providing some support for Project SQUID and were represented on its Steering Committee. ONR had a branch in London (ONRL) that maintained a rotating corps of 12 to 15 Scientific Liaison Officers whose job was to promote interaction between scientists and engineers in Europe and America by visiting laboratories and attending scientific meetings. They would get to know the investigators, learn about what they were doing, help them establish contacts with their counterparts in America, arrange visits, find items of equipment, assist with travel arrangements, and the like. Research laboratories in Europe were beginning to recover from the trauma of World War II and they welcomed the communication links and the help with their needs that ONRL could provide. The SQUID office had occasionally been involved in making travel and visiting arrangements for some of the European scientists who came to America under ONRL's auspices. After I had been at Princeton for about three years I was invited to serve a year as an ONRL Liaison Officer in the areas of combustion and propulsion. My bosses in ONR Washington approved and I persuaded John Scott, who had just finished his Ph.D. in aeronautical engineering at Princeton, to mind the store at SQUID while I was gone. Thus, Magee and I and the children, then 9, 11, and 13, went to London for the calendar year of 1955. And what a wonderful year it was! The children still insist that living London was the high point in the Fenn family fortunes which have been going down hill ever since! I share some of those sentiments because that year was also a marvelous experience for the parents. The kids were in school so Magee was free to roam London during the day. I visited many laboratories in many countries, made many friends, and learned "more things than had been dreamt of in my philosophy."

Among these "things" was my stumbling on to something that was to set the stage for the rest of my scientific life. Up to that time flames and their behavior were the focal point of almost all efforts to elucidate the kinetics and mechanisms of the important reactions in high-temperature combustion. The general approach was mostly based on attempts to measure and interpret flame characteristics, such as propagation velocity, flammability limits, ignition temperatures and the like. I had become convinced that flames, with their extremely high gradients of temperature and composition, were much too complex to be useful as stages for the study of those reactions. I often recalled a comment by Philip Rudnick, Professor of Physics at Vanderbilt, who was active in the propulsion panel of Project Bumblebee. He said that the more he learned about flames the more he was convinced that they were organisms whose study properly belonged in the province of biology! I'm sure that view strikes a responsive note in many investigators who have been exasperated by the

vagaries of flame behavior that seem to give them a life of their own. Indeed, the analogy can be extended. Flames, like organisms, are born, need fuel and oxidant for nourishment, grow and multiply to the extent that those necessities are accessible, and are poisoned unto death when immersed in their own waste products! Not only is their structure and behavior complex but the conversion of reactants to products involves sequences of different reactions over such short distances that to determine how the composition and temperature change with distance or time in real flames, that is, the relation between their "fine structure" and their behavior, was not experimentally feasible with the tools and techniques then available.

Thus, for some time I had been somewhat naively musing about whether and how it might be possible to study chemical reactions by the same approach that physicists so successfully use in the study of nuclear reactions. They build accelerators to produce beams of atomic nuclei with energies of mega to giga electron volts with which they bombard stationary targets comprising collision partners of interest. They then characterize the products from reactive collisions by analyzing the post-collision trajectories of the product particles as measured by appropriate detectors, for example, bubble or cloud chambers. I had been introduced to the possible uses of molecular-beam scattering experiments in chemistry by Professor I. "Izzy" Amdur, a colleague of the Professor Frederick Keyes at MIT whose elegant measurements on the transport properties of gases were being supported by Project SQUID. Amdur was using beam scattering experiments to characterize the intermolecular potentials of molecules. His approach was based on the electrostatic acceleration of ions to relatively high energies and then neutralizing them by charge-exchange collisions with neutral molecules for which the cross-sections are much larger than those for momentum exchange. In this way he could produce beams of neutral molecules with translational energies equal to those of the incident ions. Space–charge effects give rise to the Childs–Langmuir law by which the maximum intensity (current density) of an ion beam is proportional to the square root of its energy. Consequently, neutral beams with intensities high enough to provide good signal-to-noise ratio in a scattering experiment, must perforce have energies substantially higher than the intermolecular potentials that Amdur was trying to probe. His solution to this problem was to locate his detector so that it would "see" only the post-collision beam molecules whose trajectories differed by only a very small amount from their precollision values. This "small-angle scattering" approach allowed him to exploit the high intensities obtainable with beams of high-energy molecules (in laboratory coordinates) in the study of molecule–molecule interactions at low energies in center-of-mass coordinates of the colliding partners. Unfortunately, that approach did not seem to lend itself to the study of reactive collisions.

When I went to ONR London in late 1954, nobody had successfully carried out reactive scattering experiments with molecular beams on any chemical reaction, even one with a negligibly small activation energy. Moreover, activation energies for many combustion reactions were known to be as high as 30 kcal mol^{-1} or more. The energies of beam molecules from conventional effusive sources are always limited by the temperature at which the source could operated. The average translational energy of an oxygen molecule from a source at 3000 K would be only about 10 kcal mol^{-1} (but that temperature is well above the melting point of most materials from which the source might be made!). Any polyatomic molecules of interest in combustion would completely decompose even at much lower temperatures. In sum, the collision energies that can be reached simply by raising beam-source temperatures are severely limited. Even so, the possibility of bringing about reactive collisions by colliding a beam of reactant molecules with other gaseous molecules had been contemplated since the late 1920s. In spite of numerous attempts, no really convincing results had been obtained when we left for London at the end of 1954. However, shortly after we returned to Princeton in early 1956, the age of reactive scattering experiments with molecular beams dawned when Datz and Taylor reported that intersecting beams of K atoms and HBr molecules produced detectable amounts of KBr.[2] These results gave some substance to my musings but there was a fundamental problem with the idea of applying the same method to combustion reactions. Activation energies for the K plus HBr reaction are negligibly small. For many of the most interesting reactions in combustion the activation energies were thought to range from 0.5 to 2.0 or more eV, much much higher than for the reaction studied by Datz and Taylor. Amdur's charge–exchange technique could easily produce beams with much higher energies but because of space–charge effects could not provide useful intensities at energies as low as a few eV. Nor did Amdur's ploy of looking only at small-angle scattering seem applicable in the study reactive scattering. To produce beam molecules from classical effusive sources with energies of even 0.5 eV would require source temperatures of 3000 K. Neither materials of construction for the source, nor reactant molecules other than atoms, could endure such temperatures. The situation thus seemed hopeless.

One day in London I was calculating rocket exhaust velocities achievable with various propellants and suddenly realized that many reactant molecules at the velocities attainable with some such propellants would have translational energies as high as two or more eV. I began to fantasize about using micro-rockets in vacuum as molecular-beam sources, anticipating by 30 years an experiment we actually carried out for another purpose! I then remembered that rocket visionaries had often touted hydrogen as an ideal propellant fluid for nuclear-powered rockets because of its low molecular weight. The limiting maximum convective velocity that can be reached by a gas expanding into vacuum is equal to $(C_p/M\,T_0)^{1/2}$ where C_p is its constant pressure heat capacity, M its molecular weight, and T_0 its source temperature. Thus the maximum velocity reachable by helium is about 3.33 times that for argon. If the helium is "seeded" with one or two per cent argon, and if those argon atoms are swept along like dust particles in a wind storm, the translational kinetic energy of the argon atoms after free jet expansion of the mixture would be almost 10 times that of the helium atoms, if the two reached the same terminal flow velocity.

A few days later I was browsing in ONRL's library and ran across the now famous paper by E. W. Becker and K. Bier reporting the production of intense beams of hydrogen molecules by expansion of the gas from high pressure through a small converging–diverging nozzle into vacuum.[3] That paper referenced the also now famous paper by Kantrowitz and Grey in the *Review of Scientific Instruments* which proposed the use of a converging–diverging nozzle to produce a jet of high-velocity molecules in a region of low pressure.[4] A small core portion of that jet would be admitted through a conical "skimmer" into a high-vacuum region to form a jet of molecules. The "static" temperature of the molecules (as measured by a thermometer traveling with the gas and at the same velocity) would be very low so that the divergence of the molecular trajectories downstream of the skimmer would be very small and the velocity distribution in the direction of flow would also be very narrow. In sum, the jet would comprise a very intense beam of molecules, all traveling at nearly the same velocity! Another paper in the same issue of that journal reported a not very successful attempt by Kistiakowsky and Schlichter to reduce the Kantrowitz–Grey idea to practice.[5] Both of those papers also happened to be in the ONRL library and when I read them I began to take my musings seriously. (I was told that Kistiakowsky and Schlicter had been so frustrated by troubles in their experiment that when Schlicter had suffered enough to deserve a degree, they vented their frustration by demolishing the apparatus with an axe!)

I later learned that 25 years earlier, T. H. Johnson, a Sterling Fellow at Yale, had produced intense beams of mercury atoms by expanding the vapor from a "boiler" at several hundred torr into vacuum.[6] Unfortunately this truly remarkable result had been ignored because it was at odds with the dogma from Otto Stern's lab in Hamburg that raising the source-gas pressure (density) would produce a sort of stagnant cloud of molecules at the exit of the source orifice. That mythical cloud would scatter a large fraction of the emerging molecules that might otherwise have contributed to beam intensity. Later in his career at the Franklin Institute Johnson found diffraction effects in the scattering of hydrogen from an LiF crystal, which confirmed the wave nature of particles much heavier than the electrons with which Davisson and Germer had earlier found diffraction effects. Unfortunately, some eight weeks earlier Estermann, Frisch, and Stern had found the same result in Goettingen[7] so Johnson's work again went unnoticed while Stern got the Nobel Prize! An able investigator who was 25 years too early with one landmark experiment and 8 weeks too late with another, Johnson had every right to feel frustrated by the fickleness of fate.

The results predicted by Kantrowitz and Grey in 1951, having been already achieved by Johnson in 1929, were confirmed in the above-mentioned paper by Becker and Bier in 1954. They showed that convective flow of gas through a small orifice from high pressure into vacuum could indeed produce molecular beams with much higher intensities than could the effusive sources introduced and exploited to great advantage by Otto Stern and his colleagues. Moreover, these "convective" beams had much narrower velocity distributions than did their effusive cousins. Consequently, the achievable intensity within a narrow velocity interval, a highly desirable feature, could be very much higher with convective beams. What had not yet been shown, nor apparently considered, was that heavy molecules could be accelerated to suprathermal translational energies by expansion of a light carrier gas, such as hydrogen or helium, in which those heavy molecules were present a low levels.

When I returned to Princeton after my year in London, I found that John Scott, who had run the SQUID Office during my absence, was going to the University of Virginia as an assistant professor of aeronautical engineering. He was wondering what research he ought to work on and I told him about my musings on molecular beams from high pressure sources. I also told him that if he would submit proposal on such work to Project SQUID I would try my best to get support for it. When he arrived at Virginia he found a group that was already using beam scattering experiments to characterize the exchange of energy and momentum between molecules and surfaces. Their goal was to understand and predict surface heat transfer and drag for aerodynamic bodies at very high altitudes where the particle flow was molecular rather than continuum. The high intensities achievable with beams from supersonic free jets (or "nozzle beams", as they came to be known) would be most useful in such surface scattering experiments. Moreover, the high velocities that seemed achievable with the seeded beams I was dreaming about, would make possible the study of drag and heat transfer in molecular flow at velocities much closer to those expected in ultrahigh-altitude flight. As a result of their enthusiasm and mine, the first nozzle-beam system in America was built in Charlottesville, Virginia[8] I should really say, the first to be built in America since the one which T. H. Johnson built at Yale in 1929 that produced high-intensity beams of mercury atoms but had been ignored. It now seems clear that the reason for Johnson's success was that the walls of his system were all water-cooled enough to condense most of the incident mercury atoms. The net result was an effective pumping speed, orders of magnitude higher than the nominal speed of his vacuum pumps or those of any of the other molecular-beam systems then in existence. (Fraser in his 1932 book on "Molecular Rays" refers to a system then being built with the "exorbitant" pumping speed of 100 liters per second.[9] The nominal speed of each of two 32-inch pumps that we bought "off the shelf" 45 years later was 30000 liters per second!) The real reason for the generally observed decrease in beam intensity with increasing source-gas density was scattering due not to the molecules in a "cloud" at the source-orifice exit but to those in the relatively high density of background gas throughout the system as a result of inadequate pumping speed.

Meanwhile, I was getting more and more intrigued with the problems and possibilities of beams from free jet sources and eager to become more directly involved. I had by this time learned that in spite of Dean Taylor's rosy assurances I could not become an active participant in academic research unless I had some direct association with a department. SQUID had been supporting research on two-phase flow by Shao Lee (Charlie) Soo, a young assistant professor of

mechanical engineering. During a visit to Soo's lab one day I met Robert Drake, the new chairman hired to refurbish that department's image by expanding its research activities. I described my wild ideas on free jet beams and said: "If I write a proposal and get some support will your department provide a home for the project?" Bob had done his doctoral research in rarefied gas dynamics at UC Berkeley. Familiar with the molecular perspective on gas dynamics he immediately said yes. I prepared a proposal on which I was listed as a "Consultant" because I was not on the faculty and under university rules could not be a Principal Investigator. That role was assumed jointly by Bob Drake and Michel Boudart, my next-door neighbor and close friend who was interested in chemical kinetics and catalysis and was on the faculty of chemical engineering. I had been discussing my beam ideas with him and he too found them intriguing. The proposal was submitted to the National Science Foundation (NSF) who sent it for review to Immanuel Estermann, a co-author with Stern and Frisch of the landmark paper on diffractive scattering of hydrogen from LiF, one of the two papers cited when Stern won the Nobel prize in 1943. Estermann had become Director of ONR's Material Sciences Division (whose "power branch" was responsible for Project SQUID) and was thus my ultimate boss. He took his reviewing seriously and spent a day in Princeton discussing the proposal with me. (The fact that his daughter was then living in Princeton was no doubt something of an inducement for him to make the trip from Washington.) He had visited Becker's laboratory in Marburg, Germany and was aware of the difficulties Becker had encountered in fabricating the tiny converging–diverging nozzles prescribed by Kantrowitz and Grey. As he took his leave he said: "John, I'm afraid this research may be too difficult to pursue with graduate students but I think it is important so I'm going to recommend support." He apparently was as good as his word because NSF gave us the money we had sought and we began work in the fall of 1960. As it turned out, Estermann's fears about the difficulties of making very small Laval (converging–diverging) nozzles were groundless. The Marburg group discovered empirically, that beam intensities became higher when the diverging section of the nozzle was cut off![10] The lower intensities obtained with a diverging nozzle are due to viscosity effects which Kantrowitz and Grey had ignored in their analysis. Dave Miller's group in San Diego, later showed experimentally that for most practical purposes the jets from a simple orificea (hole in a flat plate), a simple converging nozzle, or a long tube, had almost identical properties as long as the flow was "choked", that is, reached sonic velocity, at the exit plane of the nozzle, tube, or orifice.[11]

Michel Boudart was then hosting Jacques Deckers, a young postdoc who had done his doctoral research on ions in flames with Van Tiggelen at the University of Louvain. Jacques had had experience with vacuum systems and was very excited about our beam project so Michel turned him over to my tender love and care, (or me to his, I'm not sure which!). With Bob Drake and Michel looking over our shoulders we began designing an apparatus. One of the lessons from the experiments of the Kistiakowsky and Becker groups was the need for lots of pumping speed. When the salesman from NRC showed us the performance characteristics of its 32-inch (diameter) oil diffusion pumps, Jacques' enthusiasm overcame my reticence (or timidity) so the main chamber of our system became a long horizontal tank resting on and evacuated by two of those big pumps. In retrospect I think that in choosing such big pumps as the heart of our system we were either much wiser or more fortunate than we knew. Those who work with vacuum systems learn early that there is no such thing as too much pumping speed. Sooner or later they always wish they had more. It's nice not to have to worry about the vapor pressure of finger prints, even nicer to be able to sustain disasters. We arrived one Monday morning to find the apparatus completely filled with some 700 gallons of water from a failed cooling coil! We drained the system and easily decanted the water from the silicone pump oil. After cleaning, the interior surfaces of the pumps, pipes, and chamber, all of which were made of mild steel, sported a beautifully uniform beige coat of rust! We then closed up the chamber and ran the forepumps for a couple of days, passing a small flow of air through the system to help dry things out. With fingers crossed we then started the diffusion pumps and got the best vacuum we had ever obtained! Figure 1 shows a picture of that apparatus after it was moved to Yale.

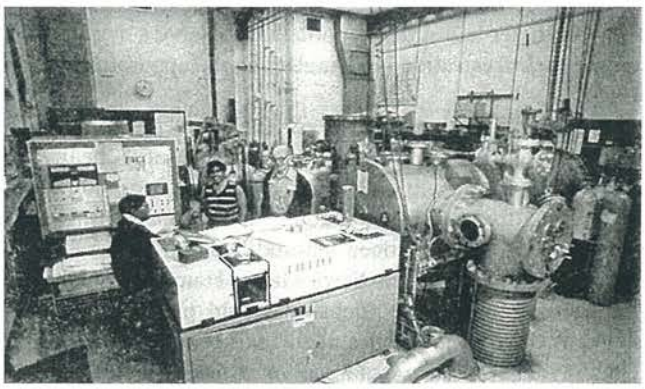


Figure 1. The "big" molecular-beam system, with two 32 inch diffusions pumps and one 16 inch "Jet Booster Pump", after it was moved from Princeton to Yale. The other people in the picture are S. P. Venkateshan (sitting) and S. B. Ryali (standing, center), both postdocs from India.

Meanwhile Dame Fortune had smiled at me in another way. Not long after the beam project started, Professor Soo, who had been teaching thermodynamics to the mechanical engineers, suddenly announced he would leave at the end of the first semester. Bob Drake, not knowing I had barely passed the only course I had taken in the subject, asked me if I would accept a temporary appointment as a lecturer and teach the sophomore course on thermodynamics in the second semester. After a deep breath and a small prayer I said yes. Things must have gone pretty well because at the end of the term he asked if I would consider a permanent position on the faculty. All of a sudden I had the chance to realize what I had long hoped for, the opportunity to become a first-class citizen in the academic community. Moreover, because my appointment as SQUID's director was "with rank of profes-

sor" I automatically became an instant full Professor of Mechanical Engineering, a transmutation that would be much more difficult in today's world. I also continued to run SQUID until 1962 when, by mutual agreement of all parties, its headquarters moved to the University of Virginia where John Scott became director.

After an extensive effort in design and construction we finally assembled the molecular-beam apparatus and got it working well. The first meaningful experiment was by Bob Koros, one of Michel Boudart's graduate students. He measured the sticking coefficient of water molecules on ice at liquid-nitrogen temperatures. The simplicity and success of the experimental design were strong evidence of both the achievability and utility of high beam intensities from free jet sources. Bob simply suspended a small target from a calibrated helical spring of glass and measured the time-dependent extension of the spring with a cathetometer viewing the target through a window in the vacuum system. Nearby baffles, filled with liquid nitrogen, maintained the target at low temperature. The beam flux of water molecules, from a source containing water vapor at pressure of a few torr, was sufficiently intense to deposit in a few hours enough mass of ice to extend the calibrated spring by measurable amounts. In a subsequent analogous experiment Jim Anderson, another Boudart student, found that oxygen molecules incident on a heated germanium wafer reacted to form a volatile GeO molecule. Above a threshold value that I've forgotten, the reaction probability of 0.04, was independent of the target temperature and the intensity of the incident beam. In that case we used the spring balance to measure the loss of mass in the germanium wafer. These preliminary experiments clearly demonstrated the utility of the apparatus whose design and performance were described and characterized in a 1963 paper.[12]

Over the next four years at Princeton and the following twenty at Yale this equipment gave rise to some 65 papers. We confirmed the high intensities of beam molecules predicted by Kantrowitz and Grey and first realized by Becker and Bier. Moreover, our hope that the seeding technique could produce beams of molecules with supra thermal translational energies was abundantly realized by ourselves and others.[13a]

An originally unanticipated virtue of free jet expansions was their ability to produce and maintain populations of molecules under non-equilibrium but steady-state conditions which some investigators have characterized as "new states of matter!"[14] For present purposes it will be useful to show the distribution of conditions encountered by molecules of a gas undergoing a free jet expansion from a simple orifice into vacuum. Such expansions are "self-similar" in that they scale in terms of the orifice diameter. That is to say, at any particular number of diameters along the jet axis, the equilibrium state of a particular gas will be the same, no matter what the diameter of the jet may be. Thus, at 5 mm downstream from an orifice 1 mm in diameter the equilibrium state of the jet gas is the same as in a jet of the same gas at 5 m downstream from an orifice 1 m in diameter. As it happens, however, the expansion rate from small-diameter orifices is so fast that the collision-induced processes and reactions involved in maintaining equilibrium in the jet gas may not be rapid enough for the gas to reach equilibrium before the gas density gets so low that the collision processes are arrested. The net result is that the terminal state of the jet gas may be far from equilibrium. This ability of free jet expansions from small nozzles to produce steady-state conditions in gases that are far removed from equilibrium is one of the reasons that these jets have proved to be such interesting research tools.

Figure 2 shows the axial distribution of density n/n_0 and temperature T/T_0 in supersonic free jets from a monatomic and a diatomic gas where subscript "0" refers to the value in the source when the gas is at rest. The curves show the values

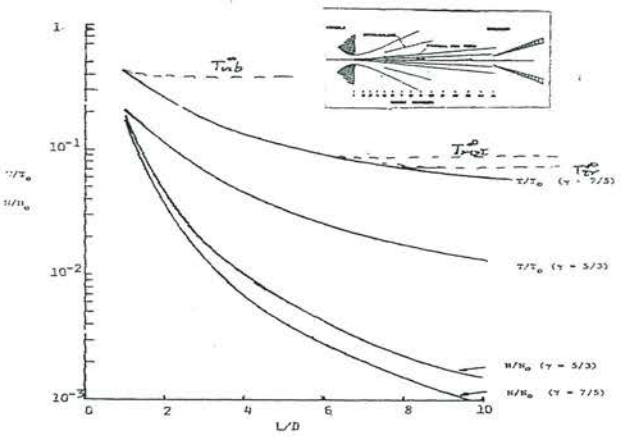


Figure 2. The axial distribution of gas properties in a supersonic free jet, normalized by their source values. The abscissa distance L from the nozzle is in terms of nozzle diameters D. The top two solid curves show the temperature distribution for a monatomic and a diatomic gas with rotational and vibrational degrees of freedom in equilibrium. Note that because the exchange of energy between translation and vibration is very slow, that is, requires many collisions per quantum, the "vibrational temperature" (for a diatomic gas such as nitrogen) "freezes" much earlier in the expansion than does the rotational temperature. The bottom pair of curves show the axial distribution of gas density for a monatomic and a diatomic gas.

first obtained by Owen and Thornhill from solutions of Euler's equation for monatomic species $C_p/C_v = {}^5/_3$ and for diatomic gases with rotational (but no vibrational) degrees of freedom $C_p/C_v = {}^7/_5$.[15] We had no means of measuring the gas density "n" but we could and did obtain values of the centerline temperature at any point by locating the skimmer at that point, and "chopping" the extracted beam into pulses with a rotating shutter. The signal-versus-time output from a fast-response detector located at a carefully measured distance downstream from the shutter was readily transformed into a velocity distribution and thus the translational temperature of the jet molecules at the skimmer entrance. From such velocity analysis at varying values of nozzle–skimmer distance we determined the axial distribution of translational temperatures of jet molecules along the jet axis. For monatomic and diatomic gases, for example, argon and nitrogen, the experimental values fell right on the theoretical curves out to distances at which the jet gas densities became so low that the collision frequency was not high enough to

maintain equilibrium. Because the jet expansion is adiabatic, and because vibrational relaxation times for many species are much longer than the sojourn time of the molecules in the jet, the vibrational energies for such molecules remain "frozen" at the source values. By means of an energy balance on the jet molecules it is thus possible to determine the relaxation rates for rotational degrees of freedom in polyatomic molecules.[16] It turns out that this approach is extremely effective for measurement of such rates at much higher gas temperatures than can be accommodated in the widely used instrument measurements based on the velocity of sound. In the present context the important result of our extensive studies of free jet expansion by velocity analysis is that the translational-temperature distributions obtained analytically by Owen and Thornhill and shown in Figure 2 have been experimentally confirmed by ourselves and others. It will emerge in what follows that these findings played a crucial role in the development of electrospray ionization mass spectrometry.

Having developed the ability to measure velocity distributions at any point in the jet we, and subsequently others, were able to verify our hope that heavy molecules seeded at low levels into a light carrier gas could be accelerated to high velocities and thus high translational energy.[13a] Figure 3 shows some early examples of the acceleration and the energies we obtained. Compargue and his group in France have since achieved translational energies as high as 45 eV.[13b] An important result of this seeding technique has been that studies of molecule–molecule and molecule–surface interactions, by ourselves and others, reached new levels of energy, utility, and sophistication.

Free jet expansions have also revolutionized the optical spectroscopy of polyatomic molecules. Our studies showed that such expansions cool rotational degrees of freedom without affecting the vibrational modes. Thus, by performing absorption or emission spectroscopy on polyatomic molecules in a free jet, one can eliminate the rotational broadening of vibrational lines. Unfortunately, we were not knowledgeable enough to realize the spectroscopic implications of rotational cooling so it was Wharton, Smalley, and Levy who provided the earliest demonstration of this effect, obtaining the first-ever spectrum of NO_2 in which the vibrational lines could be clearly distinguished and identified without rotational broadening.[17] Now the free jets used in optical spectroscopy far outnumber those used as molecular-beam sources in scattering experiments!

Because they can produce extremely low temperatures in gases at fairly high densities, free jet expansions can bring about supersaturation of almost any gaseous mixture for very short periods of time. Thus they have become sources of a wide variety of uncommon species, such as HeBr and ArI, and all kinds of aggregates, pure and mixed, even including helium. Consequently, by 1984 free jet expansions had began to play what has turned out to be a major role in the rapid development of so-called "cluster science and technology".[18] Indeed, the Scoles group at Princeton[19] and later the Toennies group at Goettingen[20] have found remarkable effects in the emission spectra of species contained in clusters of superfluid liquid helium where the absence of viscosity means that atoms can rotate freely!

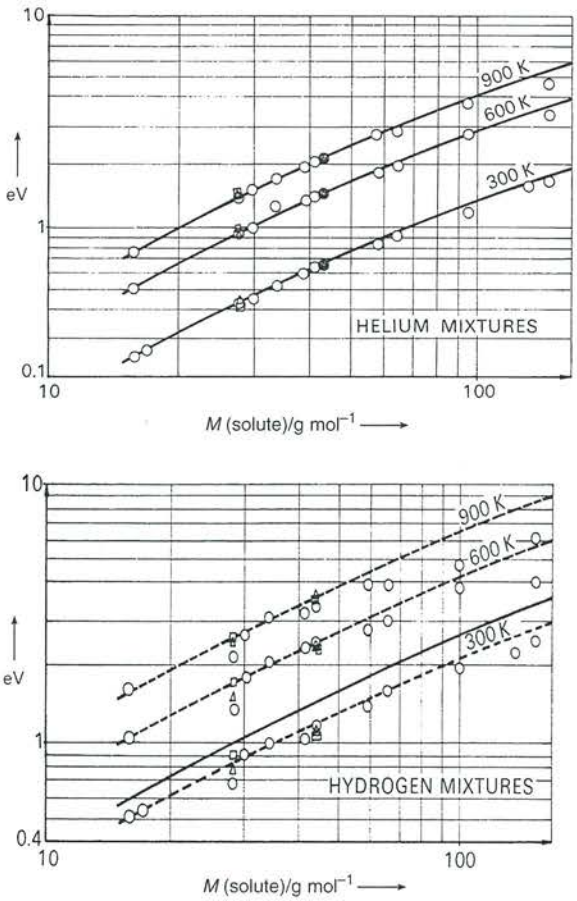


Figure 3. Some results on the acceleration of heavy molecules by a carrier gas comprising helium (top) and hydrogen (bottom). Note that, as is clear for hydrogen, with increasing difference in molecular weight between seed and carrier species there can be appreciable slip resulting in lower velocities for the seed species than would be the case if the velocities of the two species remained in velocity equilibrium. The dotted lines in the hydrogen data assume no relaxation of the rotational energy of hydrogen. The solid line at 300 K shows what would have happened if there were no slip and the rotational and translational "temperature" had remained in equilibrium. In Dole's experiments the differences in molecular weight between seed and carrier species were much greater than for any mixture in this Figure. We estimate that the actual velocities of some of his large polymer molecules were as much as 40% less than he thought they were.

In 1967, for a variety of reasons, I decided to accept an invitation to join the chemical engineering group in a newly organized "Department of Engineering and Applied Science" at Yale. The "beam machine" was dismantled in Princeton and reassembled at Yale in a somewhat different configuration. The picture in Figure 1 was in fact taken at Yale. The very large size of the apparatus had turned out to provide an unexpected dividend. Because our published results had many interesting implications in chemistry, many chemists visited our laboratory in both Princeton and New Haven. Frequently intimidated by the very size of our apparatus, they would listen to our story and say "Very nice, but not for us." The net result was that for a long time we enjoyed a relative absence of competition! In time, of course, we and others learned how to achieve similar results with much smaller and

more compact gear so that free jet expansions have now become very commonplace tools in departments of chemistry and physics as well as engineering. It is perhaps appropriate to note that these supersonic free jets have played an important role in research that has helped at least five other investigators win Nobel prizes in Chemistry![21]

In 1968, the year after I arrived at Yale, Malcolm Dole published his now famous paper describing his attempt to measure the molecular weights of oligomers of synthetic polymers based on electrospray ionization.[22] He chose to look at polystyrene molecules because relatively monodisperse samples were readily available over a wide range of molecular weights. That paper caught the eye of Professor Seymour (Sandy) Lipsky in Yale's Medical School. With characteristic enthusiasm Sandy showed it to Csaba Horvath, one of my new Yale colleagues in chemical engineering who was working with Sandy on HPLC, then known as "High Pressure Liquid Chromatography." Sandy, who was also a mass spectrometrist, was quite excited because he thought that Dole's approach might make possible the mass-spectrometric analysis of proteins and other complex molecules of biochemical importance. This cherished but elusive goal had long seemed a nearly impossible dream because all the then common methods for ionizing any atom or molecule depended upon an appropriately energetic gas-phase encounter between a neutral molecule and an electron, photon, or ion of some other atom or molecule. But large polyatomic molecules, especially the complex and fragile species of biological interest, could not be vaporized without extensive fragmentation and decomposition. Noting the references to our Princeton papers Csaba told him that the John Fenn of those references had just moved to Yale so Sandy showed the paper to me. Always looking for new ways to apply our free jet technology I was readily seduced by Dole's results and Sandy's enthusiasm. Thus began a friendship with Sandy that lasted until his death and a romance with mass spectrometry that will doubtless last until mine. That romance led to the now popular Electrospray Ionization (ESI) which burst into prominence at about the same time as the other now most widely used method for ionizing large nonvolatile molecules, Matrix Assisted Laser Desorption Ionization (MALDI), began to show its potential. A brief history of each of these techniques will be summarized in the next two sections of this report. MALDI's story will be told first even though ESI's was the first to begin.

Ionization Based on "Energy-Sudden" Methods

One class of techniques for producing intact ions from complex and nonvolatile molecules was embodied in what might be called the "energy-sudden" approach, based on an idea originally proposed in 1974 by Beuhler et al.[23] On the grounds of rate theory for unimolecular decomposition they argued that sufficiently rapid heating could vaporize complex molecules before they had time to decompose. In some proof-of-principle experiments on the vaporization of small peptides they showed that decomposition indeed decreased with increasing heating rates but they never were able to heat rapidly enough to eliminate substantial fragmentation. The several techniques based on this idea include so-called "pyrolysis mass spectrometry" (PMS),[24] pre-MALDI versions of laser desorption (LD),[25] fast atom bombardment (FAB),[26] fast ion bombardment, (usually called SIMS for "secondary ionization mass spectrometry),[27] in which rapid energy addition is achieved by incidence of high-energy atoms, ions, or photons and plasma desorption (PD)[28] in which the sudden energy comes from the decay of a radioactive isotope, usually californium (^{252}Cf). Introduced by Macfarlane and Torgerson et al., PD was the first of these techniques to gain appreciable use.[28] Its name stems from the idea that the energy released by the nuclear disintegration formed a small blob of plasma on the sample-bearing surface. As many as 30 or more analyte ions are ejected from that plasma when the analyte is dispersed in an appropriate matrix, typically nitrocellulose. The ions are then accelerated through a drift region so that their masses can be determined by time of flight (TOF) analysis for which a convenient zero-time marker is provided by back-scattered decay products of the Cf disintegration.

All these energy-sudden methods depend on the nearly instantaneous achievement of high energy density in a sample dispersed in or on a solid or liquid surface. Thus they can be regarded as extensions of the rapid-heating idea of Beuhler et al. They all could produce intact ions from many complex molecules including peptides and small proteins. However, their procedures were complex and awkward, and because their processes were highly irreversible the yields of intact ions were very low. Even so, during the 1970s and 80s they provided substantial accumulations of mass-spectral data on a variety of complex, nonvolatile molecules, giving rise to a literature much too large for review or comment here.

By far the most important legacy of these energy-sudden methods is so-called matrix-assisted laser desorption ionization or MALDI introduced in 1987 by Tanaka[29] and expanded in 1988 by Karas and Hillenkamp.[30] In MALDI the analyte molecules are dispersed on a surface in a thin layer of matrix, usually an organic acid. The energy of an incident pulse of laser photons is absorbed mostly by the matrix to form a jet of matrix vapor that lifts analyte molecules from the surface and by mechanisms still not well understood, transforms some of them into ions that are mostly singly charged. Because those ions are all produced at a well-defined location in an exceedingly short time their mass analysis is most effectively achieved by time-of-flight methods, as in the case of PD. MALDI is one of the two ionization methods for biomolecules that promise to dominate the MS scene for the foreseeable future. Recently MALDI-TOF in vacuo has been supplemented by MALDI at atmospheric pressure with subsequent introduction of the resulting ions into any of several types of mass analyzers.

Ionization Based on Intense Electric Fields

In 1966, when Dole first had the idea that led to his 1968 paper, there was little evidence of any useful solution to the problem of vaporizing large polar molecules. However, in

1951 the first step had been taken along one of two paths that ultimately led to successful "soft" ionization methods for complex molecules, though it was not fully appreciated at the time. In that year Mueller discovered that when a sharp metal point was at a sufficiently high voltage in vacuo, the electric field at the tip of that point could be intense enough to extract an electron from a nearby gaseous atom or molecule to form a positive ion,[31] a discovery that led to his development of field ion microscopy.[32] The first use of such points as sources of ions for mass analysis was reported three years later by Inghram and Gomer,[33] but only with gaseous molecules. The ion currents were too small to stir up much interest but they were larger than could be accounted for by the flux of molecules from the ambient gas into the region occupied by the point. Beckey then suggested that this apparent excess in ion-formation rate could be accounted for by molecules that struck the surface of the wire at some distance from its tip and then arrived at the tip region by surface diffusion. In 1963 he showed that arrays of "whiskers" on thin wires could produce much higher currents than single needles and that nonvolatile species present on the surfaces of such whiskers could migrate to the tip by surface diffusion to be desorbed as intact ions. Beckey went on to develop this discovery into "field desorption ionization mass spectrometry" (FDI-MS) and in 1977 published a book on the subject.[34] Emitter electrodes comprising wires with dense arrays of whiskers are now available off-the-shelf at relatively low cost, but dosing them with sample and positioning them in the vacuum system are time-consuming and tedious tasks. Moreover, ion currents depend so strongly upon both the temperature of the emitter and the applied voltage that substantial manual skill is required to find and maintain the right combination for a particular sample. That control function might now be made much less tedious by computers but solving the control problem does not deal with an even more important difficulty. The ions from field desorption are released from high voltage into high vacuum. Consequently, their kinetic energies are so high that their mass analysis requires relatively large and expensive magnetic sector instruments. For these and other reasons FDI-MS a la Beckey has never been widely practiced. However, from the perspective of history's hindsight one can argue that the ability of intense fields at the surface of a tiny charged droplet, which play a vital role in Dole's ESI, is simply an obvious extension of the discoveries by Mueller and Beckey on the nature of field ionization at a sharp tip, that is, a surface with a very small radius of curvature. Maybe so, but it is also quite clear that nobody recognized these possibilities at the time, and that Dole arrived at the ESI approach by a logic that depended in no way on the field-ionization ideas of Mueller and Beckey. In other words Dole was a true pioneer in that his ESI ideas were breaking brand-new ground.

Fields intense enough to desorb solute ions at useful rates from a condensed phase can also be achieved without the array of very sharp needles or "whiskers" required by Beckey's approach. In 1972 Evans and Hendricks found that a high voltage applied to the surface of a nonvolatile conducting liquid in vacuo would produce a sharply pointed cone.[35] The field at the tip of such a cone was intense enough to desorb charge-bearing molecules and clusters of the liquid. If the liquid was a solution, the desorbed ions included solute species. These observations led to so-called "electrohydrodynamic ionization" (EHI) that was introduced to the mass spectrometry community in 1974 by Simons et al.[36] In EHI a sample solution is electrosprayed from a small-bore tube maintained at high potential relative to the surroundings, just as had been taught by Zeleny and adopted by Dole. The difference is that in Dole's ESI the droplets are dispersed into gas at near atmospheric pressure. In EHI the sample solution is dispersed into vacuum. In that case the solvent must have a very low vapor pressure to avoid "freeze drying". But as Dole had recognized, evaporation of solvent is the *sine qua non* (essential feature) for the formation of solute ions from charged droplets and such evaporation requires a source of enthalpy. This dilemma has never been completely resolved in EHI because no satisfactory method has yet been found by which the enthalpy needed to vaporize solvent from a droplet can be supplied to such a droplet in vacuo. Moreover, as in the case of FDI, any ions produced by EHI in vacuo have such high kinetic energies that their mass analysis generally requires magnetic sector instruments which are large, heavy, and expensive. For these reasons EHI-MS has never become widely used despite fairly extensive research that has been reviewed in detail by Cook.[37]

In sum, Malcolm Dole's paper in 1968 was the seed which, after an extended period of germination, ultimately blossomed into electrospray ionization (ESI), one of the two "soft" ionization methods that have made the precision, sensitivity, and elegance of mass spectrometry readily available for the study of biomolecules and their reactions. The germination and development of Dole's ESI will be briefly described in what follows.

Electrospray Ionization

The other of the two dominant methods for ionizing large molecules is electrospray ionization (ESI), the subject of the 1968 paper by Malcolm Dole and his colleagues[22] that Sandy Lipsky showed me in 1969. In fact Dole had first proposed his electrospray idea two years earlier (1966) at the International Symposium on Macromolecular Chemistry in Tokyo. Those two communications comprise a testament to Dole's remarkable acumen in realizing that the production and behavior of highly charged droplets from solutions of nonvolatile solute species in volatile solvents might produce intact gaseous ions of those species. Perhaps even more remarkable was the extent of his insight on the roles of ESI's several component processes. For example, he clearly recognized the need to disperse the charged droplets in a gas at relatively high (atmospheric) pressure. Such gas would provide the enthalpy required to vaporize solvent from those highly charged droplets. Moreover, collisions of the ions with molecules of that gas would reduce their initially high kinetic energies to those of the ambient neutral gas molecules, thereby avoiding the need for mass analyzers capable of accommodating high-energy ions, for example, magnetic sector instruments.

As most mass spectrometrists now know, the essence of Dole's idea was to take advantage of what happens during the

evaporation of solvent from a droplet that has a net electric charge. In 1882 Rayleigh had analyzed the behavior to be expected of such a droplet and found that as the solvent evaporated the density of charges on the droplet surface would increase to a critical value, now known as the "Rayleigh Limit", at which Coulomb repulsion would overcome surface tension. The resulting instability would cause the droplet to break up into a plurality of offspring droplets.[38] Thirty years later John Zeleny made the first reported observation of this "Rayleigh Instability" in pioneering experimental studies on the production and behavior of charged droplets carried out, first at the University of Minnesota and then at Yale.[39] He passed a stream of conducting volatile liquid through a small-bore thin-walled tube or "needle" (because it often comprised a short length of hypodermic needle tubing) maintained at a high potential relative to an opposing counter electrode. The resulting intense electric field at the tube tip dispersed the emerging liquid into a fine spray of charged droplets. Zeleny was able to see the break up of charged droplets as solvent evaporated, just as Rayleigh had predicted. Figure 4a is a schematic representation of the how droplets are formed. The emerging liquid at the tube exit forms a conical meniscus now known as a "Taylor Cone" because its formation was predicted theoretically in 1964 by G. I. Taylor.[41] In truth, Taylor's treatment applied to a nonconducting liquid of neutral molecules with dipole moments for which there is no electric current and no liquid flow. Even so, the Taylor name is still applied even when the liquid is a conductor because it contains anions and cations. In that case a thin jet emerges from the tip of the cone. As the result of another kind of instability, also first characterized by Rayleigh for uncharged liquids,[42] the interaction between viscosity and surface tension produces so-called "varicose waves" on the surface of such a liquid jet. Those waves grow in amplitude until they truncate the jet into a series of uniform droplets as illustrated in Figure 4 (top left). (That same kind of instability causes the break up into droplets of a solid jet of water issuing from a garden hose or, on a smaller scale, the tiny stream of water from a tap that is incompletely turned off.) In the electrospray

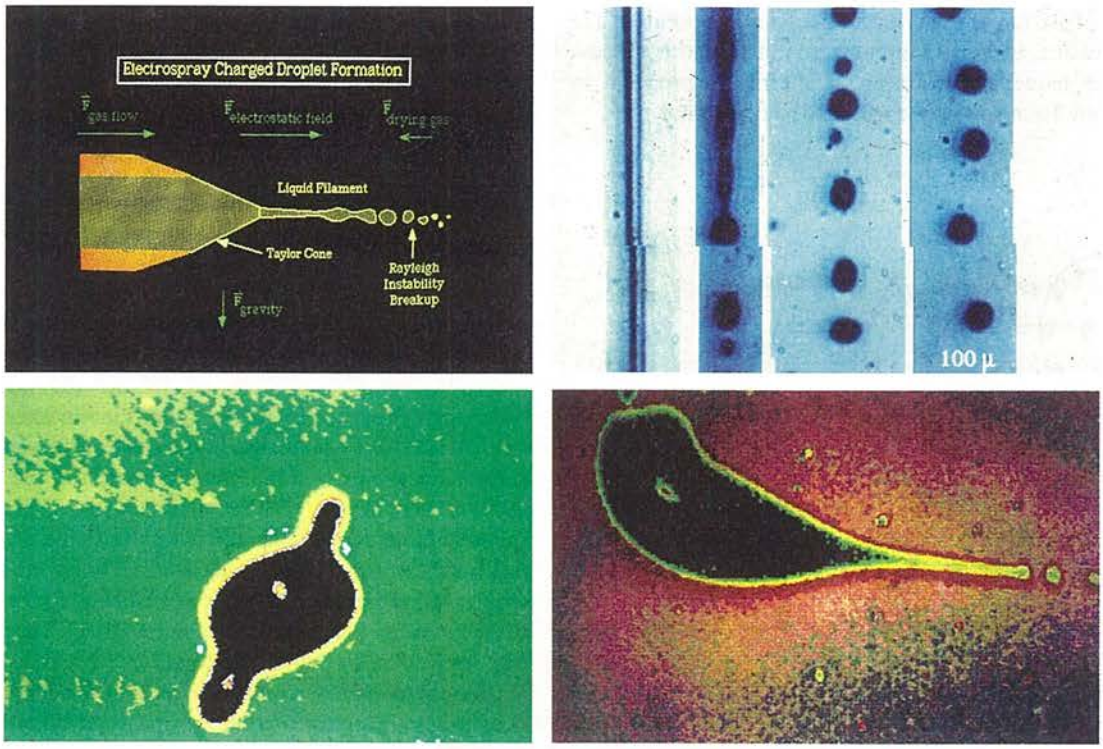


Figure 4. How a flow of conducting polar liquid is dispersed into highly charged droplets by an intense electric field at the exit tip of a small tube at high potential relative to an opposing electrode. Top left: schematic representation the conical meniscus formed by the emerging liquid as the result of competition between the surface tension of the liquid and forces owing to the interaction of dipoles in the liquid with the applied field. This meniscus is known as a "Taylor Cone".[41] When the liquid is electrically conducting, a small flow of the liquid emerges from the tip of the cone as a very narrow column or jet. Interaction between surface tension and viscosity produces so-called varicose waves which grow in amplitude until they truncate the jet into a sequence of segments of equal length that are molded by surface tension into droplets nearly uniform in size.[42] Because the droplets contain excess cations or anions on their surface, depending on the polarity of the field, they repel each other with the result that their trajectories diverge to form a conical "electrospray" of charged droplets. This sequence was captured in the photographs by Gomez and Tang (top right). If the liquid is volatile, evaporation shrinks the droplet thereby bringing the surface charges closer together until the resulting Coulomb repulsion overcomes surface tension and the droplets disrupt into a plurality of smaller droplets, a phenomenon characterized and predicted theoretically by Lord Raleigh in 1882. Two years more than a century later, Gomez and Tang captured on film, for the first time, the images in the lower two panels of Figure 4 showing a droplet in the throes of what is now known as a Rayleigh Instability, and sometimes referred to as a Coulomb explosion.

case the droplets all have charges of the same sign (excess anions or cations, depending on the polarity of the applied field). Coulomb repulsion thus results in a divergence of their trajectories to form a conical "electrospray" of charged droplets. Figure 4b is a series of four photographs made by Tang and Gomez[40] illustrating, from left to right, the undisturbed jet, the appearance and growth of the varicose waves, the resulting droplets, and the beginning of the divergence of the droplet trajectories (as a result of Coulomb repulsion) to form a conical spray. We believe the two lower pictures in Figure 4 are of historic significance as the first ever photographs showing droplets undergoing a Rayleigh instability when the Coulomb repulsion of the surface charges overcomes the surface tension that tries to contain the droplet liquid in a spherical shape.

Dole's scenario further assumed that the "offspring" droplets resulting from a Rayleigh instability would continue to evaporate until they too would reach the Rayleigh limit and break up into still smaller droplets. If the original solution were sufficiently dilute, a succession of such Rayleigh instabilities would ultimately produce droplets so small that each one would contain only one solute molecule. That lone molecule would retain some of its droplet's charge to become a free gas-phase ion as the last of the solvent evaporated. The upper sequence in Figure 5 attempts to illustrate this process. (The lower sequence illustrates an alternative proposal by Iribarne and Thomson which will be described later.)

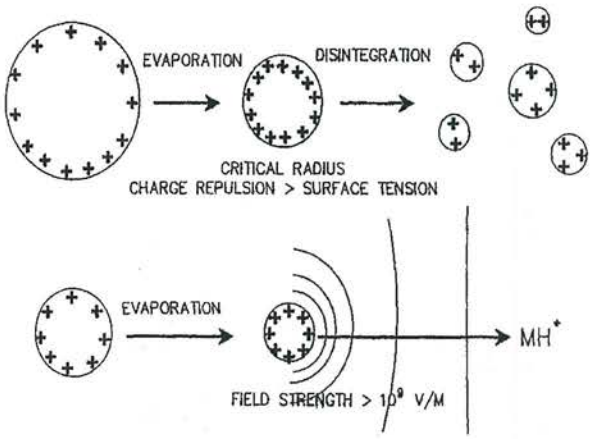


Figure 5. Top: Dole's idea of how solute ions are formed. He assumed that the offspring droplets resulting from a first Rayleigh Instability would continue to evaporate solvent so that they too would undergo a Rayleigh Instability and disrupt. If the original solution were sufficiently dilute, a sequence of such evaporation–disruption episodes would ultimately produce ultimate droplets so small that each would contain only one solute molecule. As the last of the solvent evaporated from the ultimate droplet the remaining solute molecules would retain some of the droplet charge and thus become a free gas-phase ion. This scenario embodies what is sometimes referred to as the "charged residue model" (CRM) for ES ion formation. The lower sequence of sketches portrays the so-called "ion evaporation model" scenario later proposed by Iribarne and Thomson[46]. It argues that before ultimate droplets containing only one solute molecules are formed, the field at a droplet's surface becomes sufficiently intense to lift a solute ion from the droplet surface into the ambient gas.

In the 1968 paper that described this scenario Dole also presented preliminary experimental results obtained in an attempt to reduce his idea to practice. As taught by Zeleny, a solution of polystyrene molecules was infused through a small-bore tube maintained at high potential relative to a counter electrode. Dole was always generous and conscientious in acknowledging the work of others so it is curious that though he refers to Rayleigh's analysis of droplet instability he never mentioned Zeleny in any of his papers, even though his method of producing charged droplets seems to derive directly from Zeleny's experiments. His account of the electrospray experiments in his autobiography simply says: "I got this idea from learning about the electrospraying of paint on to automobile bodies while working as a consultant for a paint company in Chicago".[43] Such "electrospraying" of paint consisted in maintaining a potential difference between the sprayer and the object being painted. The result was enough charge on the paint droplets to attract them toward the object being painted, thereby decreasing substantially the loss of paint to the surroundings by convection currents. Similar charging of droplets was practiced by "crop dusting" airplanes to decrease aggregation and increase adhesion of insecticides to the plants.

But Dole had another problem to solve. The mass spectrometers then available, even the most expensive magnetic sector instruments were unable to "weigh" singly charged ions with masses larger than about ten-thousand Daltons. The oligomer ions of interest to Dole could have molecular weights up to a million or more and he had apparently not taken into account the possibility of extensive multiple charging which then had not yet been discovered. The problem he thus faced was how to "weigh" such large ions after they had been produced. Moreover, as he also mentioned in his autobiography, he had found that the magnetic electron multiplier (MEM) on his Bendix mass spectrometer did not respond to his ES ions because "Although these ions had the same kinetic energy as light ions their velocity decreased in the ratio of the square root of their mass. We proved the mass limitation of the MEM in a separate experiment."[44] That comment strongly suggests that the ions Dole had produced from polystyrene oligomers did not have extensive multiple charging. Therefore, their mass/charge ratios were too high for available analyzers so he had to find another method for "weighing" them. That is how our free jet studies entered the picture.

As noted earlier, we had shown that during such free jet expansions of mixtures comprising a very dilute solution of heavy "seed" molecules in a light carrier gas, for example, argon in helium, the heavy seed species were accelerated like dust particles in a windstorm to almost the velocity that would be reached by the light carrier gas alone.[13] Consequently, the translational kinetic energy of the argon molecules after expansion was higher than would be achieved in a jet of pure argon by a factor roughly equal to the ratio of the molecular weights of the two species, that is, 10 in the case of argon accelerated by helium. This acceleration effect was important to Dole because it seemed to provide a way to weigh the large polymer ions he hoped to produce. As shown in the sketch of his apparatus in Figure 6 (from his 1968 paper) a variable-

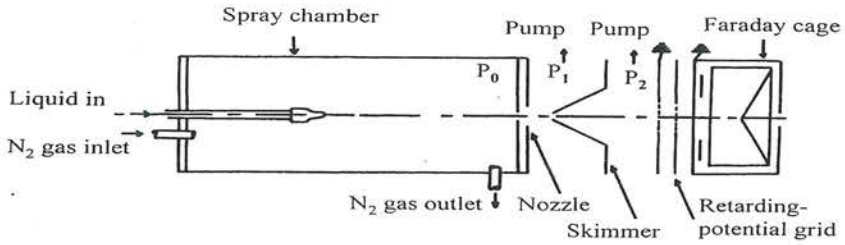


Figure 6. Schematic representation of Dole's original ESI apparatus. The electrospray forms at the tip of the tube through which liquid enters the spray chamber concurrent with the nitrogen bath gas. Most of that gas leaves the chamber near the end plate containing the nozzle through which a small portion of the mixture of bath gas and ions enters the vacuum system in the form of a supersonic free jet. A small fraction of the ion-bearing jet gas passes though a skimmer into a second vacuum stage, thence through a retarding-potential grid into a Faraday cage connected to a galvanometer.

potential grid was placed between the skimmer (that passed a collimated, ion-containing beam from the free jet source into a vacuum chamber) and a sensitive galvanometer in a Faraday cage contained in that chamber. The only ions that could pass through the grid and reach the galvanometer were those with a translational kinetic energy $mv^2/2$ exceeding the product of the charge on those ions and the potential on the grid. The translational velocity v of the stopped ions was equal to the readily calculated velocity reached by the carrier gas in the free jet. Thus, scanning the voltage applied to the grid should produce a current–voltage curve with a decrease in current to the galvanometer at every value of the voltage corresponding to the energy of an ion species in the beam. Figure 7 shows the curve Dole obtained with a fractionated sample of polystyrene having a nominal M_r of 51000 at a concentration of 0.01% by weight in 60:40 (v:v) benzene:acetone. Also reported were results with polystyrene having a nominal M_r of 411000 and with a mixture of the two. These results showed a certain consistency with expectations but, possibly for reasons to be described, were not convincing enough to persuade other investigators to confirm or extend them.

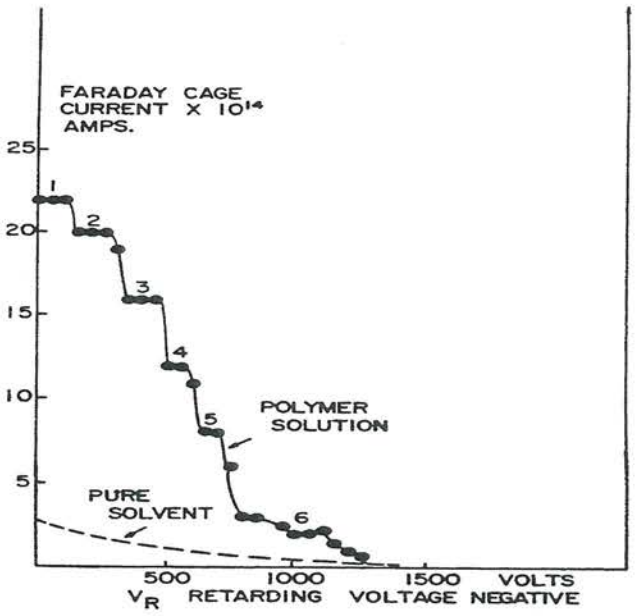


Figure 7. A current–voltage curve obtained by Dole when the electrosprayed solution comprised 0.005 wt% each of polystyrene samples having a molecular weight of 51000. The solvent comprised benzene:acetone (3:2 v/v). The distances from the needle to the first aperture, the first aperture to the second aperture, and the second aperture to the nozzle were respectively 2, 3, and 3 inches.

Electrospray Ionization at Yale

A year or so after Sandy showed me Dole's paper I persuaded a new graduate student, Mike Labowsky, to repeat Dole's experiments in our much bigger vacuum system with much faster pumps than Dole had. We also had a better understanding of free jet expansions and realized that Dole had overlooked two of their features which were crucial in his experiments: 1) For the very large differences in molecular weight between the carrier gas and the seed species that he was using, there would have been a substantial amount of "slip" as suggested by the results with hydrogen in Figure 3. Thus, the actual velocities of his heaviest ions could have been as much as 50% lower than he had reckoned. 2) During the adiabatic expansion that occurs in a free jet the temperature of the gas nose-dives. In the case of nitrogen, for example, at an axial distance downstream from the orifice of only ten nozzle diameters (ca. 1 mm in Dole's experiments) the absolute temperature of the gas drops to about 5% or less of its source value! Thus, the expanding gas rapidly becomes supersaturated with solvent vapor resulting from the evaporation of the ES droplets before the expansion. Ions are famously effective as condensation nuclei so that any ES ions in Dole's experiments must have been substantially resolvated to an unknown extent. Consequently, the actual masses of the unsolvated ions must have been much lower than Dole's measurements had indicated. To avoid such resolvation problems Mike modified the Dole arrangement so that the bath gas of dry nitrogen was introduced at the exit-nozzle end of the spray chamber. Consequently, drying gas flowed "upstream", counter-current to the flux of charged droplets and ions from the spray needle, leaving the spray chamber through an exit port at its "entrance end", that is, opposite to the end containing the nozzle leading into the vacuum system. Thus the solvent vapor from the evaporating droplets was carried out of the system so that the gas expanding into vacuum through the nozzle was free of solvent and any other "junk" that was not ionized. This counter-current flow of bath gas solved the problem of ion resolvation during the free jet expansion, greatly reduced system fouling, and has become a feature of the most successful ESI-MS systems. One can also avoid resolvation of ions during expansion by raising the temperature of the ion–gas mixture to a value high enough so that after free jet expansion the gas temperature remains above "the dew point" for the ions. This "fix" was demonstrated by Chowdhury et al.[45] and has been incorporated in some commercial instruments. It has had limited use because,

unlike a counter-current gas flow, it does not prevent uncharged "junk" from entering the vacuum system. Much more frequent cleaning is therefore required.

As already mentioned, Dole's paper apparently failed to persuade other investigators to confirm his experiments which though simple in principle were really very difficult and demanding in practice. For example, large macroions incident on the first dynode of a multiplier do not produce secondary electrons unless their incident velocities are very high, either because of multiple charging or high accelerating voltages. As implied by reference [44] it seems highly likely that Dole's ions did not have many charges. Consequently, in his later experiments he used mobility measurements to characterize his macroions.

In 1969 at the age of 66, just two years before mandatory retirement at Northwestern, Dole had accepted an offer to become Welch Professor of Chemistry at Baylor University in Waco, Texas where he was assured there would be no mandatory retirement as there was at Northwestern. At Baylor he continued his electrospray experiments using mobility measurements rather than mass analysis to characterize the ions. He presented a short paper on that work at the 33rd Annual Meeting of American Society of Mass Spectrometery (ASMS) in San Diego in 1985 where I also gave a paper that included a discussion of the cooling and resultant resolvation that occurs in free jets. Dole came up to me afterward to introduce himself and to express his gratitude for finally understanding why there didn't seem to be any reasonable relation in his experiments between the size of the molecules and the mobilities of their ES ions. He said "Now I realize that we have been measuring the mobilities of highly solvated ions!" That was our only face-to-face encounter but we subsequently exchanged several letters and had a cordial relationship. I was delighted when he was later able to attend the first ASMS Workshop on ESI-MS which was in Chicago. I didn't see him there because I had not been invited but by that time the "electrospray revolution" was well on its way so Dole had the satisfaction of realizing how fruitful his ideas had become before he passed on to his ultimate reward some months later. He would have been amazed if he had known that by 2001 the number of papers per year on ESI in the archival journals would reach 1500 and still be growing!

There was a hiatus in ESI studies at Yale after 1975 when, for understandable reasons, Mike Labowsky got discouraged. The retarding-potential method of determining ion mass was most exasperating. The only available means for measuring the very small ion currents was an old vibrating-reed electrometer that on its best days was crankier than a model T Ford! Mike became interested in the evaporation behavior of the cloud of ES droplets and thus in the evaporation and combustion of a cloud of interacting fuel droplets, an important and challenging practical problem of interest to several of my colleagues in Chemical Engineering. He developed some unique computational techniques which together with several important papers on this classic problem comprised his Ph.D. thesis.

In 1982, Masamichi Yamashita, a young scientist I had met in Japan, came to my lab as a postdoc. When he arrived, "Gado" as he preferred to be called, asked for some suggestions about what he might work on. I mentioned that it might be interesting to take another look at ESI, but instead of trying to make ions out of big molecules, to explore whether Dole's idea would work with molecules of low-enough mass for their ions to be analyzed with a small quadrupole mass spectrometer that had been a detector in some of our molecular-beam experiments. It had an upper limit for m/z ratio of only 450. Gado liked the idea and off he went. Not only was he an extraordinary experimentalist, with sound instincts and magic hands, he knew everything from the biology of sea urchins to the practice of digital electronics (already in 1982!). In a relatively short time he assembled what was really the world's first ESI mass spectrometer from the bits and pieces of molecular-beam apparatus in our precious "junk pile". (My Scottish heritage of "Waste not, want not" makes it very difficult for me to throw anything away that might have some conceivable use!)

That first apparatus is shown schematically in Figure 8 and illustrates the arrangement for providing a counter-current flow of drying gas. We first tried electrospraying methanol–water mixtures and obtained spectra showing peaks corresponding, for example, to protons solvated by various combinations and numbers of methanol and water molecules that could be varied widely simply by adjusting the flow rate and/or temperature of the counter-current drying gas. We then added some solutes to the solvent and were rewarded with the spectrum shown Figure 9a. It was obtained with a solution of several tetra-alkyl ammonium (and one phosphonium) halides at concentrations of a few parts per million. These results were especially gratifying because those solutes cannot be vaporized without extensive, even catastrophic decomposition. Beginning to get excited, Gado dissolved a vitamin B tablet in methanol–water and obtained the spectrum in Figure 9b showing a clearly defined peak for intact ions of every species in that tablet (i.e. with an M_r value under 450.)

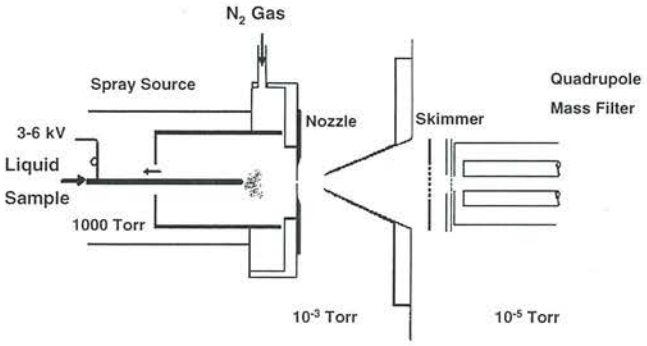


Figure 8. Schematic representation of the first electrospray mass spectrometer built at Yale. Sample solution was sprayed from the hypodermic needle into a counter-current flow of dry nitrogen. The needle was at high potential relative to the cylindrical electrode and the end plate containing the orifice into the vacuum system. A center portion of the resulting free jet passed through the skimmer into a second vacuum chamber containing a quadrupole analyzer.

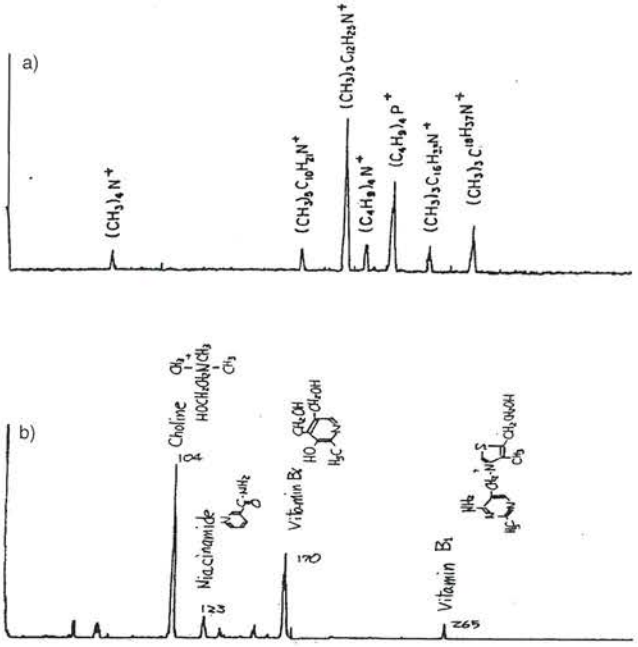


Figure 9. a) The ESI mass spectrum obtained with the apparatus of Figure 8 with a dilute solution (ppm) of quaternary ammonium halides and one quaternary phosphonium halide in methanol:water (50:50 v/v). b) The ESI mass spectrum obtained with a solution obtained by dissolving a vitamin B tablet in methanol:water (50:50 v/v).

The spectra in Figure 9a and Figure 9b were exciting to us because they clearly demonstrated that ESI could produce intact ions of each of several species in a plurality of fragile molecules in a dilute solution while avoiding both fragmentation and interference between them. The spectra also raised second thoughts about the mechanism of ionization. We had been tacitly assuming that Dole's "charged residue model" (CRM) accounted for the formation of the large ions he had found as well as the small ions that we had found in the results just described. In our early experiments, as well as in Dole's, there were always many more analyte (solute) molecules than charges in the ES flux. Consequently, as our results accumulated we had become increasingly uneasy about the adequacy of the CRM. The spectra in Figure 9 heightened that concern because we could not understand how the sequence of Rayleigh instabilities could produce, for each of several species in the same solution, singly charged ultimate droplets containing only one molecule of that species. Moreover, if the CRM model is to explain the results, the relative number of those droplets for each species would have had to be in direct proportion to the relative concentration of that species! How could the droplet know how to program its sequence of Rayleigh instabilities so as to produce a distribution of ultimate droplets such that the number of those ultimate droplets containing one molecule of any species X is always directly proportional to the concentration of species X in the original solution? Of course, that would happen if the droplet subdivision continued until all the droplets contained only one solute molecule. But that scenario somehow seems unlikely, especially when rapid evaporation from all droplets is occurring from the start. Moreover, if the subdivision process were to produce ultimate droplets for each ionizable species in a solution, one should then always find a spectral peak for every species in such a solution and that just doesn't happen.

These conceptual problems seemed to be resolved when we learned about some papers by Iribarne and Thomson, meteorologists at the University of Toronto.[46] Interested in the possibility that charged droplets of sea water might be a source of some of the ions found in the atmosphere, they had carried out experiments showing that evaporation of charged droplets could indeed produce gas-phase ions of solute species in those droplets. The charged droplets in those experiments were produced by pneumatically nebulizing a conducting liquid. In such nebulization the droplets become charged as a result of statistical fluctuations in the distribution of anions and cations among those droplets so that roughly equal numbers of positively and negatively charged droplets are formed. In their first experiments only aerodynamic forces were used to nebulize the liquid, as in a perfume atomizer. Then they found that 3.5 KV applied to an "induction electrode" (1 cm away from the nebulizing zone) would result in all the droplets, and the ions from their evaporation, having the same sign, positive or negative, depending on the polarity of that electrode. Iribarne and Thomson called their technique "atmospheric pressure ion evaporation" (APIE) a name which literally refers more to the mechanism of ion formation from charged droplets rather than to the method of producing the droplets. Moreover, both the earlier ESI of Dole and the later thermospray ionization (TSI) of Marvin Vestal,[47] also depend upon vaporization of charged droplets but their names do not identify any ion-forming mechanism. The essential differences between these three "spray" techniques consist mostly in the way the charged droplets are produced. Therefore, it seems more appropriate to give them names that relate to those characteristic differences, that is, "ESI" for electrospray ionization, "ASI" for aerospray ionization, and "TSI" for thermospray ionization. These names relate specifically to the way droplets are produced in terms of the kind of energy used to produce them, (i.e. electrical, aerodynamic, and thermal) and will be used hereafter.

The ASI technique of Iribarne and Thomson has never become as widely used as ESI. However, their ion evaporation mechanism (IEM) for ion formation from charged droplets, set forth in their papers, was a milestone contribution and has become a formidable competitor for Dole's charged residue model (CRM) with each one having its champions. Both models assume that a sequence of evaporation steps followed by Rayleigh instabilities leads to smaller and smaller droplets. In the CRM this sequence continues until the droplets become so small that each one contains a single solute molecule that becomes an ion by retaining some of its droplet's charge as the last of the solvent evaporates. The upper sequence of steps in Figure 5 attempts to illustrate this process. The IEM holds that before the droplets become small enough to contain only one solute molecule, the field strength at the droplet surface becomes intense enough to "lift" a surface ion from the droplet into the ambient gas. In other words the surface field can evaporate an ion from the

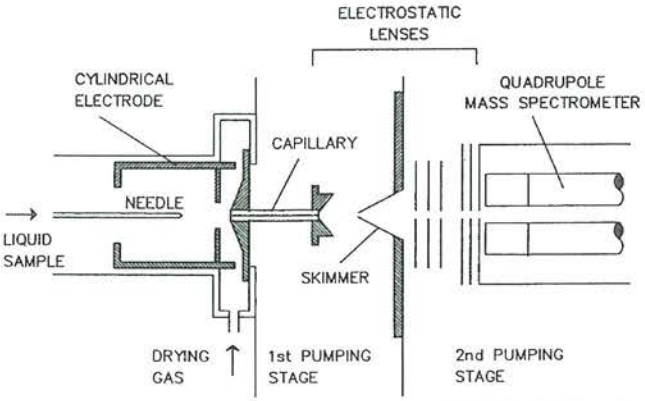
droplet surface. The lower sequence of steps in Figure 5 attempts to represent this mechanism. There is no unanimity in the ESI community as to which model is correct. The feeling in this corner is that under most circumstances the IEM is more consistent with more experimental observations than is the CRM. However, sometimes the CRM seems more likely to apply, especially in the case of very large analyte molecules.

A bit more needs to be said about TSI, the thermospray ionization technique introduced by Marvin Vestal and his colleagues beginning in 1978. The last of the charged-droplet sources to appear, TSI became the first to be widely used, in part because of its effectiveness and in part because it was the first to become available commercially as an add-on to existing mass spectrometers. This sequence of events would seem to fulfill that passage in the scripture which says "The last shall be first." (That passage continues by saying "And the first shall be last", which apparently is being fulfilled by ESI because it was the first to be introduced and now would seem likely to be the last of the spray techniques to be abandoned, if it ever is!)

TSI consists in passing analyte solution through a capillary tube whose walls are hot enough to vaporize 90% or more of the solvent. The resulting expansion produces the same kind of shear and acceleration forces on the liquid that occur in aerodynamic nebulization, for example, as in ASI or a perfume atomizer. The result is a dispersion of droplets in vapor that emerges from the exit of the capillary as a supersonic jet into ambient solvent vapor at a pressure of 10 to 15 Torr in a chamber whose walls are hot enough to maintain the vapor in a superheated state. The already-mentioned statistical fluctuations in the distribution of cations and anions give rise to equal numbers of positively and negatively charged droplets as in ASI. Evaporation of the droplets in the superheated vapor brings about the sequence of Rayleigh instabilities common to all the techniques based on charged droplets. That sequence leads to the formation of gaseous ions by either the CRM of Dole or the IEM of Iribarne and Thomson. The mixture of ions in vapor flows past an aperture through which ions of the desired polarity are driven by an applied field into the vacuum chamber housing a mass analyzer. For several years in the 1980s TSI sources became the preferred "hyphen" in LC-MS, but they have now been almost completely replaced by ESI sources.

Meanwhile we were achieving ever better results with ESI as we climbed the learning curve and Sandy Lipsky was keeping in close touch with our progress. He was a good friend of Brian Green of VG Analytics, a British manufacturer of mass spectrometers that has since evolved into what is now Micromass, Ltd. Brian would stop by New Haven and visit Sandy every few months and on most of those visits Sandy brought him by our laboratory. As our results with small molecules began to get better and better, Brian became more and more intrigued and asked us if the technique would work with larger molecules. We said we thought it might but couldn't be sure because our little quadrupole analyzer had an upper limit of about 450 for m/z ratio. Brian then arranged for VG to lend us a used quadrupole analyzer that could weigh ions with m/z ratios up to 1500 or so. That instrument was incorporated in a new system for which much of the design and assembly was done by Craig Whitehouse. Craig had worked in Sandy's lab after finishing college and Sandy urged me to take him on for graduate study in chemical engineering. The design of this new system is shown schematically in Figure 10 and has some novel features that deserve comment.

In light of the results obtained with Gado's machine, and with Csaba and Sandy hovering in the background, we increasingly viewed ESI as a possibly more potent hyphen in LC-MS than the already widely used TSI. An electric field, sufficiently intense to disperse analyte solution emerging from the spray needle into a fine spray, required a potential difference of up to several kilovolts between that tip and the



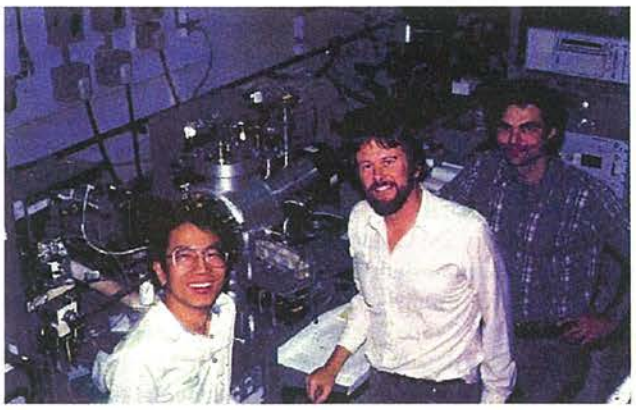


Figure 10. Top: Schematic diagram of the second ESI-MS apparatus. It features the same counter-current flow of drying gas shown in Figure 8 which excludes solvent vapor from the ion–gas mixture that enters the vacuum system by way of a free jet expansion. A novel feature of this apparatus is that the ion–gas mixture enters the free jet expansion at the exit of glass capillary rather than through the simple flat-plate orifice. Each end of that capillary is metallized so that it can be maintained at any desired potential. Thus the field required to "electrospray" the sample solution can be achieved with the spray needle while the metallized entrance of the capillary is at the required potential "below" ground. The ions entering the capillary are then in a potential well. The rapid flow of gas through the capillary then drags the ions out of that potential well to any desired potential at which the metallized exit end of the capillary is maintained. Thus, all external parts of the apparatus can be at ground, posing no hazard to an operator. Bottom: Photograph of the apparatus of Figure 10 showing (left to right) Chin Kai Meng (graduate student), Robert Dreyer (chromatographer from the Medical School) and Craig Whitehouse, (graduate student who did much of the design and assembly of the instrument).

orifice leading into the vacuum system. Thus, one was faced with a trilemma in LC-MS operation. Either 1) the LC system would have to be maintained at several KV above ground, 2) the MS at several KV "below" ground, or 3) the power supply would require enough capacity to maintain the required potential difference in spite of the leak to ground of current through the conducting path comprising the flow of liquid from the LC. Options (1) and (2) were not feasible but option (3) was being used in some systems. We then had a wild idea that turned out to be a very effective solution to this problem. We replaced the simple orifice into the vacuum system by a glass capillary tube (see Figure 10). That glass tube was metallized at each end to provide an electrical contact. The inlet end of the capillary would be maintained at the required potential below ground so the ions entering the tube would be in a potential well. We guessed, correctly as it turned out, that the fairly high-velocity flow of bath gas through the glass (dielectric) tube into the vacuum system could drag the ions out of that potential well up to whatever potential might be desired at the metallized exit end of that tube. It worked like a charm. Indeed, the gas flow could raise the ions to an exit potential of several tens of KV, sufficient to inject the ions into a magnetic sector analyzer. At the same time all exposed external parts and surfaces of the instrument could be maintained at ground potential, thereby posing no hazard to an operator.

Eager to flex our muscles with the new apparatus we tried it out with a couple of cyclic peptide samples from friends in the Medical School, cyclosporin A and gramicidin S with molecular weights of 1184 and 1141 $g\,mol^{-1}$, respectively. Figure 11a shows the spectrum obtained with a solution of 1.0 $g\,L^{-1}$ of cyclosporin A in 85:15 (v:v) acetonitrile:water. The dominant peak at 1203 corresponds to singly protonated parent molecules M with one water of hydration $[M+H+H_2O]^+$. The small satellite peaks on either side are due to ions with one more and one less water molecule. The small triplet peak near m/z 600 is from doubly protonated ions of the same species $[M+2H]^{2+}$. (The right to left increase in m/z ratio is the opposite of custom owing to a quirk of the available chart recorder.) Figure 11b shows the spectrum for 1.0 $g\,L^{-1}$ of gramicidin S in 50:50 (v:v) methanol:water. Here the peak is much larger for the ions that are doubly rather than singly protonated. These differences can be understood in terms of the IEM mechanism and the greater hydrophobicity of cyclosporin. A hydrophobic molecule can be more easily removed from an aqueous environment than a hydrophilic one. Consequently, one charge can provide enough "lift" to remove the ion from the droplet surface. This observation seems to support the IEM of Iribarne and Thomson. As the droplet shrinks, the charges get close enough together so that two of them can attach to a single gramicidin molecule thus providing more lift, in part because of the two charges and in part because the surface field is stronger, owing to the higher surface charge density.

Peaks for doubly charged ions also dominated in similar spectra for the peptides bleomycin and "substance P". In the spectra for renin substrate and insulin chain B we found evidence of triple protonation. This propensity for multiple charging was quite provocative because the effective mass

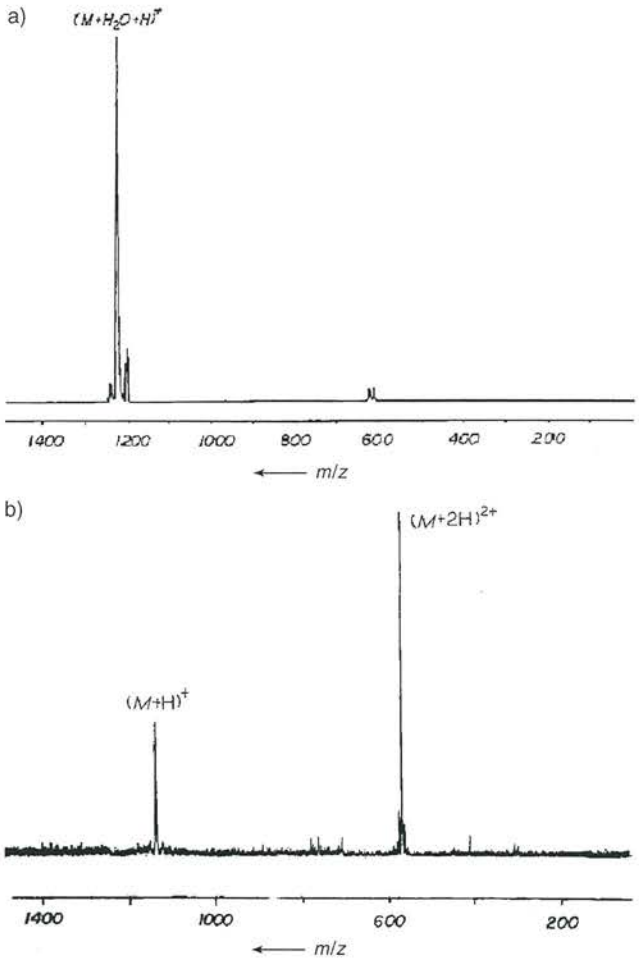


Figure 11. a) shows a mass spectrum obtained with a solution of the cyclic peptide, cyclosporin A, in methanol:water (50:50 v/v). The dominant peak is for a singly charged molecule with one adduct water molecule $[M+H+H_2O]^+$. The small peak on the right is due to the bare ion $[M+H]^+$. The tiny peak on the left is due to the parent molecule with two adduct water molecules $[M+H+2H_2O]^+$. b) The spectrum obtained with a solution of gramicidin S.

range of any analyzer increases by a factor equal to the number of charges per ion. To explore the extent to which such multiple charging can occur, and to identify and evaluate the controlling factors, we carried out an extensive study of ES ion formation with poly(ethylene glycol) (PEG) oligomers. Samples with a range of nominal molecular weights (M_r values) were kindly provided by Union Carbide and Chemical Corp. Each one comprised a mixture of oligomers with a Gaussian-like distribution of M_r values having a half width (FWHM) roughly 15% of the nominal M_r value of the most abundant oligomer. These species retain their chemical and structural similarity over a wide range of M_r values. Na^+ ions bind to the oxygen atoms in the oligomer chain with an energy of 2.05 eV and there is always enough sodium around so that spectra of samples from the manufacturer (Union Carbide and Chemical Corp) comprised oligomers with varying numbers of Na^+ adducts that increased with increasing molecular weight. (However, one can change these adducts by appropriate additions to the sample, for example, to K^+ by

adding KOH or KCl) Figure 12 shows mass spectra for PEG samples having nominal M_r values from 400 to 3350 for which the individual peaks are clearly resolvable in the original spectra and correspond to oligomers with varying numbers of

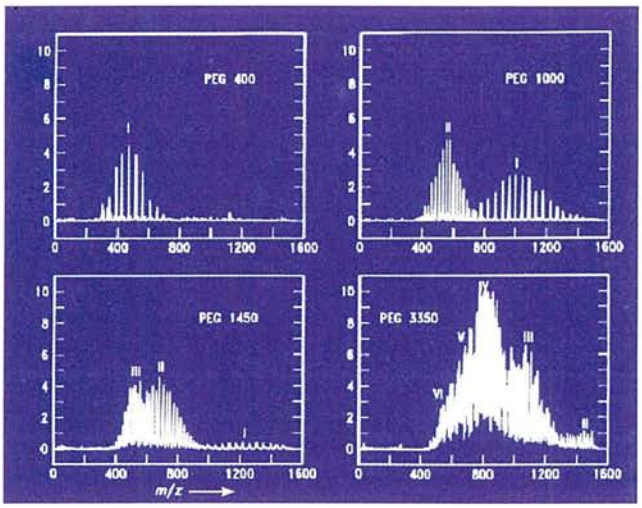


Figure 12. a) ESI spectra obtained with the apparatus of Figure 10 for poly(ethylene glycol) oligomers with nominal molecular weights of 400, 1000, 1450, and 3350.

Na^+ adducts. Figure 13 shows spectra for samples with nominal M_r values of 8000 and 17500 wherein the peaks are much too close together to be resolved (with our analyzer) because of the superposition of a wide distribution of oligomer sizes on a wide distribution of charge states for each oligomer.[48] A reviewer of that first paper on these results dismissed them outright, saying that the spectra in Figure 13 were "not mass spectra but were due to dirt in the system!" However, we have found over the years that for polymers like these one can correctly and usefully assume that the m/z values at the maximum intensity of the envelope of the overlapping peaks is a reasonably reliable estimate of the most probable m/z value for the most abundant oligomer in the sample. We used this assumption to great advantage in a later study which showed that intact ions could be obtained from PEG oligomers with M_r values of at least 5000000![49] Others have since shown that ESI can produce intact ions of species with molecular weights of over 100 million![50]

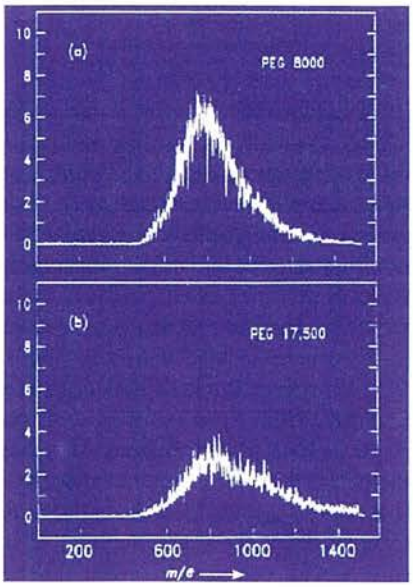


Figure 13. ESI spectra obtained with the apparatus of Figure 10 for poly(ethylene glycol) oligomers with nominal molecular weights of a) 8000 and b) 17500.

The message of these PEG experiments was clearly that to study the ESI-MS behavior of large molecules we needed samples of pure compounds in which all the molecules had the same molecular weight. Obvious examples of such compounds are natural peptides and proteins which for many other reasons, of course, are far more interesting and rewarding subjects of study than synthetic polymers. We began to get promising results with such compounds in late 1987 and presented the results in June 1988 at the 36th ASMS Conference on Mass Spectrometry and Allied Topics in San Francisco. Inspection of those spectra showed that each peak in the spectrum for any one species differs from an adjacent peak only by one adduct charge, usually a proton in the case of these peptides and proteins. The first reviewer of a preliminary paper on these results asserted that they showed the method to be worthless for two reasons: 1) The total ion current was divided among so many peaks that the signal/noise ratio for any one peak would inevitably be low and 2) the multiplicity of peaks for each species in a mixture would inevitably make the spectra too congested to be interpreted.

As I have often done, and still do, that reviewer underestimated the power of a computer. One day I commented to my then student, Matthias Mann, that each peak in one of these multiple peak spectra was really an independent measure of the parent ion mass. Therefore, there should be some way of averaging those independent values to get a more reliable and accurate measure of the parent molecule mass than any single-peak spectrum could provide. Two days later he had worked out a computer algorithm that transformed the multiple peaks into a single peak that would be obtained if all the ions had a single massless charge. The m/z value for that peak is thus the M_r value for that species. Figure 14 shows the ESI mass spectrum for cytochrome C.

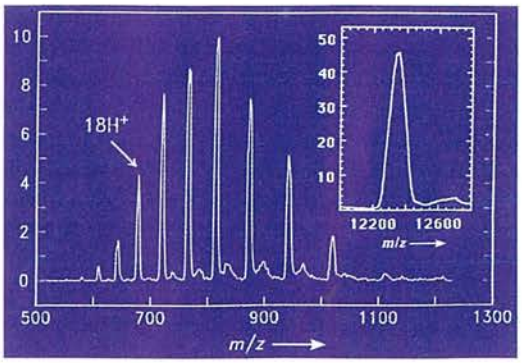


Figure 14. Spectrum for Cytochrome C. The insert shows the result of applying Matthias Mann's deconvolution algorithm.

The insert shows the result of the deconvolution by the algorithm Matthias had worked out, that is, the peak that would be obtained if all the ions had a single massless charge. The m/z value on the abscissa for the apex of that peak is thus the true molecular weight of the parent neutral molecule. It is noteworthy that the scale for the ordinate value, which represents the relative abundance of the ions in any peak, is the same on the insert as in the multipeak spectrum. Clearly, values for both total signal and signal-to-noise ratio are much higher in the deconvoluted spectrum than in the parent spectrum as measured. It is now taken for granted that computers can deconvolute what seem to be hopelessly complex mass spectra to obtain precise and accurate M_r values for very large molecules in an extremely complex mixture of species.

Funding agencies were also generally somewhat less than enthusiastic in response to our proposals. For example, when we submitted one for research to extend the results shown in Figure 9 the reviewer simply said: "It is impossible for this man to obtain these results with his equipment!" I was reminded of the slogan once promoted by a company which said: "Difficult tasks we can finish quickly. The impossible ones take a little longer!"

There were only a handful of people at the session of the ASMS meeting at which we presented these results. Nor were there many questions or comments from the floor. However, when those results were published in 1989 (the first complete paper on the method and results appeared in the 10/6/89 issue of Science and is by far the most cited publication that ever came out of our lab[51]) they began what has become a "flood" of papers and ignited what has been referred to as the "electrospray revolution!" Some idea of the dimensions of that flood can be gained from Figure 15 which was prepared by my good friend and former colleague, Professor Juan de la Mora at Yale. It shows how the publications on ESI-MS have been increasing with time. The sudden rise began in 1990, a year or so after we first presented the protein results and reaching over 1500 per year in 2001 and still climbing! Moreover those numbers reflect only a small fraction of the total ESI-MS activity, most of which is being carried out by the pharmaceutical companies and is not published.

In view of the flood of papers on ESI-MS revealed in Figure 15, even a rudimentary summary of what they have taught would clearly take much more than a little longer.

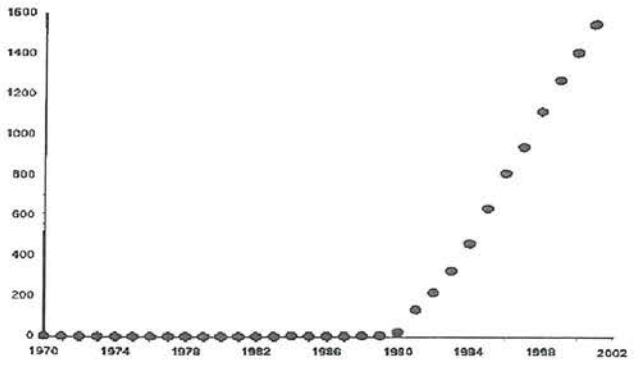


Figure 15. The number of publications each year on ESI-MS.

Consequently, I will stop here with a final word of appreciation to the many who have made possible the preparation of this paper. I would especially emphasize the extent of my gratitude and thanks to my eager and able colleagues without whose efforts I would not be here today (see Figures 10 and 16). Nor could I be writing this had I not had the support of numerous sponsors who over the years have provided the funds (albeit sometimes unwittingly!) without which we could have done nothing. They include the Department of Energy, the National Science Foundation, the National Institutes of Health, the Army Research Office, the American Cancer Society, the duPont Company, and the Jeffress Foundation. Finally, of course, I want to express my eternal gratitude to the Nobel Foundation and its marvelously helpful and gracious staff. They, along with what must have been generous and abundant approval from my peers and colleagues in the fellowship of scientists, have transformed my wildest dreams into a reality even more unbelievable than "flying elephants!"

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Figure 16. The colleagues, other than those shown in Figure 10, who played the most important roles in the development of electrospray ionization; from left: Mike Labowsky, Masamachi Yamashita, Matthias Mann, Shek Fu Wong

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