ELSEVIER

Biophysical Chemistry 108 (2004) 17–22

Biophysical Chemistry

www.elsevier.com/locate/bpc

Graduate student days at MIT

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When the organizers of this issue of *Biophysical Chemistry*—which honors my good friend David Yphantis—asked me to write something about our early days as graduate students at MIT, I was happy to agree.[1] Nevertheless, as I began to write, doubts crept in. I realized that those days were 50 years ago and I had to ask myself how I could possibly know that my recollections were even moderately accurate. This is a valid question since—having heard old tales from others where I remembered the events quite differently—it is clear that memory is a faulty and selective device. Eventually, however, the comforting thought struck that anyone who might be in a position to argue with me was likely either to be dead or to have as faulty a memory as my own. So I have plowed ahead.

Given that disclaimer, I do remember that I met Dave's feet several weeks before I met his head. When I first came into the Waugh lab at MIT, Dave was already established as a beginning graduate student, and I certainly knew about him. However, he (or whoever it was) seemed to spend all his time lying underneath the Model E Analytical Ultracentrifuges—aligning the optics, replacing the drive, etc.—and as a consequence we were always tripping over his feet, but rarely saw the rest of him. I soon learned that our Ph.D. advisor, David Waugh, did not believe in service contracts. Rather he thought that graduate students should all be intimately familiar with the detailed workings of the laboratory's crown jewels, meaning its Spinco Model E Analytical Ultracentrifuges, and I soon assumed my position under a centrifuge as well.

Dave Yphantis graduated from Harvard College with a B.A. degree in June of 1952. I pursued a parallel track as an undergraduate at MIT, also in the class of 1952, but was enrolled in a combined 5-year B.S.–M.S. program in biophysics and obtained both of those degrees in 1953. During my last year in that program, I did my Master's thesis research in David Waugh's lab on the assembly and stability of the calcium caseinate micelles of skim milk, which involved much use of the analytical ultracentrifuge. Thus, both Dave Yphantis and I spent a lot of time working with these wonderfully well engineered and designed, but far from fool-proof, devices.

Dave was already the laboratory guru on ultracentrifuge techniques when I started my research since—as I recall—he had already been working many hours per week in the lab as an undergraduate researcher while enrolled at Harvard. From the beginning he had an amazingly strong grasp of both the mathematical basis and the technical aspects of ultracentrifugation, and even as a beginning graduate student he was the person whom everyone in the lab consulted when we had a question about the equipment, how to use it and how to interpret the results.

The population of the Waugh lab was an amusing and heterogeneous mixture of physical bio-

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[1] Please note that this brief and personal account should be viewed as supplementary to the more extensive history of Dave's career that the organizers have put together for this issue [1].

0301-4622/04/$ - see front matter
doi:10.1016/j.bpc.2003.10.006

chemists of varying interests, and David Waugh presided over the resulting chaos in a calm and genial fashion. He was good with people and gave us all our heads and the opportunity to develop our own interests, abilities and approaches. He was an excellent physical biochemist himself (one of the first who had an intuitive and well-developed grasp of proteins and how they might interact with one another), and the projects going on in his lab ranged from blood-clotting to the development of ultracentrifuge theory and instrumentation. I believe that most of his grants (probably in large part from the US Navy Research Office in those days, as well as from the fledgling basic research support agencies NIH and NSF) were intended to support blood-clotting research and ultracentrifuge development, and my work on casein micelles was justified as a model system for blood-clotting. This was not totally unreasonable, since I was studying the stability of casein micelles in solution and the mechanisms whereby they coagulated when certain components of them were cleaved by the proteolytic enzyme, rennin [2,3].

In addition to Dave Yphantis and me, the lab also contained John Fitzgerald, a genial Irishman, and his wife Marie, both of whom were graduate students, as well as their ~3-year-old daughter Molly, who roamed the labs getting underfoot and beaming brightly at us all.[2] Another interactive lab colleague was François Lamy, an exuberant Frenchman, who livened things up immensely with his unfettered pronouncements on every conceivable issue, all delivered in his version of French English. As I recall, the Fitzgeralds and François worked on various aspects of the blood-clotting program. Walter Koltun, who was more serious of mien and always seemed to be wearing a tie, was also in the lab. Walter subsequently gained some fame as the developer of the commercial version of the PCK (Pauling–Corey–Koltun) atomic models, which we all used to build molecular representations of proteins and their binding ligands and to test our ideas of how they might interact. I should remind the reader that our molecular models were often quite imaginary, since the first real globular protein structures were just starting to emerge from the crystallographic work of Kendrew and Perutz in England at the time.

David Waugh was himself basically interested in understanding the forces involved in protein folding and in protein–protein interactions, and actually came up with a mostly qualitative—but very prescient—view of what subsequently came to be called hydrophobic bonding, which he published in part in a huge review on protein interactions that appeared in Advances in Protein Chemistry in 1954 [4].[3] Waugh's ideas, like those being developed on the same subject by Kauzmann [5], were based on the results of Frank and Evans [6], Debye [7] and others on the anomalous thermodynamics of solutions of small nonpolar molecules in water. These results suggested that water might be differently structured around nonpolar solutes and led to the notion of minimizing nonpolar-water surface contacts, 'iceberg' formation and other aspects of what we call hydrophobic bonding today. While Kauzmann and then Tanford [8] were probably the first to put these ideas on a quantitative footing, the precursor notions were certainly floating around in the Waugh lab in the early 1950s and we all were very conscious of their importance in interpreting our micellar studies (including, in particular in my case, the temperature dependence of protein–protein interactions in forming micelles) and related issues in protein aggregation and blood-clotting. I have always felt that David Waugh did not receive the recognition that he deserved for his role in developing these early ideas.

The laboratories in the Department of Biology in those days functioned as pretty independent fiefdoms, but the graduate students mixed a lot with one another and those interactions were responsible for nucleating a number of formal and informal inter-laboratory collaborations. In those days the Department of Biology was very quantitatively ori-

[2] I note that, to my great surprise, I recently encountered Molly again as a charming and well-established Full Professor of Biochemistry at the University of Massachusetts!

[3] It is worth noting that the section that dealt with these issues was preceded by an acknowledgement that read: 'The author is indebted to Mr. David A. Yphantis for his co-operation in preparing particularly this section and the section on urea.' Clearly Dave Yphantis was ahead of his time in this area as well.

ented, and while it covered biochemistry pretty thoroughly with a strong group of biochemists and enzymologists (Bernie Gould, Irwin Sizer, Ed Herbert and John Buchanan, who came somewhat later), it was probably the nation's first relatively comprehensive biophysics department. The Department Chair (and unchallenged leader and driving force) was F.O. Schmitt who, shortly after Dave Yphantis and I graduated with our Ph.D. degrees, called together the nation's leading biophysicists and physical biochemists at the landmark Boulder Conference [9], which is generally viewed as the 'official' beginning of biophysics as a defined discipline on the national and international level.

The so-called biophysicists at MIT were divided into a 'Noah's Ark' of methodological specialties, including an X-ray diffraction lab presided over by Richard Bear, who worked on fibrous proteins (mostly collagen), and whose graduate students at the time included Carolyn Cohen and Paul Gallop; an electron microscopy laboratory led by Cecil Hall; a spectroscopy lab headed by John Loofburrow, whose graduate students included Allan Munck and Hal Wyckoff; and a bioelectronics lab under the direction of Kurt Lion.[4]

F.O. Schmitt was a cellular physiologist (in modern parlance he would probably be called a cell biologist). He was interested in all aspects of the molecular interactions that he reckoned must underlie cell and organelle function, and he tried to integrate his methodologists to work on common problems. The Department also included some adjunct scientists who were listed as Instructors, had M.D. degrees and were, in part, also affiliated with the Massachusetts General Hospital and Harvard Medical School. These included Jesse Scott, Bert Vallee, Jerome Gross and Betty-Ben Geren, and many of the Department's graduate students worked collaboratively with these individuals as well, since Prof. Schmitt clearly foresaw the potential of mixing medical issues with biophysical approaches to cellular problems. In addition, senior scientists from elsewhere wandered in and out, working loosely under F.O. Schmitt's jurisdiction on projects ranging from the function of nerve axons and membranes (Fritz Sjostrand) to muscle fibers (Hugh Huxley).

This was the background within which Dave Yphantis and I worked, and it was truly an exciting environment. As indicated above, the labs and projects tended to mix a lot at the graduate student level, and we organized our own graduate student seminar series in which we told one another about our work and about interesting articles that we had read.[5] Dave's work was of wide interest in this setting because a number of our graduate student colleagues saw the potential of putting their proteins into the analytical ultracentrifuge, and Dave was the portal through whom these collaborations generally flowed.

One of the highlights of the year for the Department of Biology was our annual 'open house', when each laboratory displayed its instruments and methods for the edification of the local community. The ultracentrifuges were the major show-pieces of our lab, of course, and Dave Yphantis presided over these demonstrations and explanations with elan and enthusiasm. I remember particularly being involved in one of these events just days after I had joined the lab. Since I did not know much, Prof. Waugh assigned me to simply running the chamber hoist up and down on one of the ultracentrifuges—making a loud and satisfying noise—and explaining how fast the rotor would spin and the astonishing number of 'g's' that could be generated. I entered into the spirit of the thing with enthusiasm and when (in my view at the time) an aged and obviously interested gentleman wandered over to my station I started regaling him with my meager collection of facts and cautioning him not to be alarmed by the noise when I lowered the chamber. My listener was very amiable about all this, and when I noticed Dave Waugh waving his arms at me from the other side of the room I took these gestures to represent encouragement, and so redoubled my explanatory efforts. When finally my listener escaped from my clutches Dr Waugh came

[4] Professors Bear and Lion shared an office, which caused much merriment among undergraduates who wandered past and read the names on their door.

[5] I remember that one of my early talks in this forum dealt with John Schellman's thermodynamic treatment of the helix–coil transition, which had just appeared in the Carlsberg journal [10].

over to tell me that the person I had been thus regaling was, in fact, John Edsall, who hardly needed such explanations! Prof. Edsall and I subsequently became acquainted and he very kindly invited me to give my very first extramural seminar on my work at Harvard. He did, apparently, remember the 'open house' incident, and we had a good laugh over it.

At this time Dave Yphantis was working hard on attempting to devise theoretical solutions of the Lamm equation with boundary conditions appropriate for the centrifuge [11], but his primary delight—then as ever after—was practical work (including sophisticated machining) to make the ultracentrifuge easier to use and to permit elegant (and simplified) experiments. The multiple-channel short-column centerpiece for the ultracentrifuge cell, which took advantage of the earlier demonstration by Van Holde and Baldwin [12] that short columns of solution would reach equilibrium much faster and thus permit equilibrium runs to be done in much less time, was one ultimate product of these early efforts [13], and the notion of using radiative energy transfer to measure the temperature of the rotor without direct contact was another [14].

This temperature measurement issue had been an ongoing problem, because if you wanted to get accurate sedimentation data you had to know the temperature of the spinning rotor. The procedure that had been devised by the Spinco engineers at the time was to mount the equivalent of a phonograph needle vertically on the bottom of the rotor. This needle spun in a pool of liquid mercury at the bottom of the rotor vacuum chamber to achieve electrical contact of the temperature measuring circuits with the rotor. The technique worked well but was fraught with difficulties—including the problem that if the needle on the bottom of the rotor became even slightly bent it would splash mercury all over the inside of the rotor chamber, doubtless releasing toxic mercury fumes into the laboratory through the output of the oil diffusion vacuum pump. (In those days we were relatively unaware of the dangers of low levels of mercury vapors, and spent lots of time with our heads in the rotor chamber just above the open mercury pool.)

Dave's solution to the temperature problem was, in concept, more elegant. He suspended a thin black metallic disk at a fixed distance between the bottom of the rotor (also painted black) and a temperature reference block at the bottom of the chamber, and by measuring the temperature of the disk and of the block using thermocouples, and knowing the geometry of the system, he was able to calculate the temperature of the rotor with great precision and without physical contact. A related problem, however, was then to provide a method of controlling a heater to maintain the rotor at a constant temperature against the cooling induced by adiabatic expansion and by the refrigeration coils that were built into the walls of the rotor chamber. Dave solved this by winding thin wires around a frame that was suspended inside the chamber and running a current through them when heat was required. This gadget, which looked rather like a king's crown, operated on the same principle as a toaster, but—unfortunately—it was very difficult to control the thermal expansion of the wires when they were heated. Thus, one turn of wire would inevitably sag down and short-circuit the next, and the whole thing would vaporize with a 'poof', sending Dave back to the drawing board to find wire with more desirable coefficients of thermal expansion and to design a better winding pattern for the wire around the crown-shaped frame. Dave was generally the soul of calm and equanimity, but I do remember strings of uncharacteristic curses erupting when his heating device would short-circuit for the umpteenth time.

One of the highlights of those years in the Waugh lab were periodic visits from Howard Schachman, our chief (but, of course, delightfully enthusiastic and interactive) competitor in the development of ultracentrifugation methods.[6] Everyone loved Howie; he was a 'pied piper' and many of the younger generation of physical biochemists (including me) followed him back to his lair in Berkeley, where we did collaborative experiments with his equipment and enjoyed wonderful interactions with him, his students and also his

[6] His classic book, written in 1959, is still the 'bible' of the field as far as straight-forward descriptions of centrifugation techniques are concerned [15].

wife Ethel. In fact one of my fondest memories is of the 2-week period I spent in Howie's lab as a postdoc, living with Howie and Ethel and their (then young) sons. I spent the days running two Model E machines at once, including during a mild earthquake when the rotors wobbled and the machines swayed. My heart was in my throat—how would I explain to Howie that I had destroyed both of his centrifuges in one swoop—although obviously with a bit of help from Mother Nature! Fortunately, however, the shaking subsided, the machines ran on and disaster did not strike. We got terrific data and were able to establish a remarkably accurate value for the molecular weight of the monomers of muscle myosin [16]; partially—as we found out later—because the concentration dependence and the excluded volume of the molecule happened to cancel one another in a most obliging manner.

Howard and Dave Waugh were good friends, and Howie early recognized that Dave Yphantis represented a 'rising star' in this field. In fact, Howie and Dave Yphantis (in his subsequent positions) were principally responsible for keeping analytical ultracentrifugation alive during the long hiatus between the functional demise of Spinco (by then a part of the Beckman Instrument Company) in the late 1970s and the development and marketing of the new analytical ultracentrifuges (the XL-A and later the XL-I) by Beckman in the 1980s and 1990s. During that difficult era, the field of analytical ultracentrifugation was maintained by an informal coalition of academic scientists lead by Howie and Dave (which also included Tom Laue, Walter Stafford and other 'Yphantis descendants'), and some former Beckman ultracentrifuge field service people (including Floyd Vickers and others), who worked as freelance consultants and ultracentrifuge repair experts. These workers, again led by Dave Yphantis, who kept huge stocks of Model E components piled up in his lab and generously gave them away to any of his friends who needed them, made it possible for the old Model Es to keep running (and churning out great data) in many laboratories. And this, in turn, made it possible for many of us to continue to use these instruments to obtain solution data on macromolecules and macromolecular complexes of biological interest during those years.

Dave Yphantis also started another initiative that was crucial to the perpetuation of the ultracentrifuge field, in that he organized and led analytical ultracentrifuge workshops at his home base at the University of Connecticut for many years. At these workshops he gathered together the younger academics in the field to teach ultracentrifuge theory and practice to interested graduate students and postdocs, helping in this way also to maintain the national expertise in analytical ultracentrifugation, which otherwise could easily have withered away. These workshops, sponsored by the NSF, clearly played (and continue to play) a major role in holding the ultracentrifuge community together and helped keep the field alive until modern molecular biologists began to appreciate the value of actually knowing the frictional properties and molecular weights of the conformationally labile and oligomerization-prone macromolecules that comprise our present-day macromolecular machines and signaling networks.

Dave and I worked hard, and both graduated from MIT with Ph.D. degrees in 1955. They were three wonderful years for both of us, and we have both probably irritated our respective graduate students on more than one occasion since by pointing out that in the 'good old days' it did not take 5–7 years to get a Ph.D. degree. Of course, those were also simpler days, there was not that much background information to accumulate, grants were easy to obtain, the field was much smaller and—at least in the areas in which we worked—everyone was extremely friendly, helpful and noncompetitive.

After graduation Dave and I both stayed on for a year in the Waugh lab as postdoctoral fellows, and then Dave went off to become a biophysical scientist at the Argonne National Laboratory and eventually an assistant professor at Rockefeller University, while I headed down to Bethesda to work as a postdoc with Manuel Morales on myosin (hence the sojourn with Howie Schachman described above).

Dave and I remember our graduate school days as being extremely happy and liberating, and obviously our experiences during that time set us both

on life-long tracks of doing and appreciating research in what was then called ‘quantitative biology’ at MIT and is now more commonly referred to as physical biochemistry or perhaps ‘solution biophysics’. Although we ended up on opposite sides of the country and in relatively different research specialties, and thus have obviously seen one another over the years much less than we would have wished, we have remained close friends and I have had many occasions to be grateful to Dave for his good advice and generous help when our lab was hung up on some ultracentrifuge problem. Dave has obviously played a similar role in the scientific and personal lives of many others, thus accounting, in part, for the enthusiastic participation of so many in the present ‘festshrift’ in his honor. I hope that he will enjoy this personal account of our early years together and will forgive any inaccuracies that may have slipped in. I join Dave’s many other friends, former students and collaborators in wishing him and Lorna many more happy and productive years in Connecticut, in Crete and wherever else their travels happen to take them.

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