M.J. Osborn

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The Way It Was

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Abstract

Mary Osborn was a native Californian. She was an undergraduate at the University of California, Berkeley, where she worked in the laboratory of I.L. Chaikoff. She received her PhD at the University of Washington, where her work on the role of folic acid coenzymes in one-carbon metabolism revealed the mechanism of action of methotrexate. After postdoctoral training with Bernard Horecker in the Department of Microbiology at New York University (NYU), she embarked on her research career as a faculty member in the NYU Department of Microbiology and in the Department of Molecular Biology at Albert Einstein College of Medicine. In 1968 she moved as one of the founding faculty of the new medical school of the University of Connecticut, where she remained until her retirement in 2014. Her research was focused on the biosynthesis of the endotoxin lipopolysaccharide (LPS) of gram-negative bacteria and on the assembly of the bacterial cell envelope. She made seminal contributions in these areas. She was the recipient of numerous honors and served as president of several important scientific organizations. Later in her career she served as chair of the National Research Council Committee on Space Biology and Medicine, advisory to the National Aeronautics and Space Administration (NASA), which produced an influential report that plotted the path for NASA's space biology research program in the first decade of the twenty-first century. Dr. Osborn died on Jan. 17, 2019.

Contents

1. THE LONG AND CIRCUITOUS ROAD TO MICROBIOLOGY

When I was about ten my father asked me what I wanted to do when I grew up. An avid reader, I was deep into a series of girls' novels about a nurse (Sue Barton). When I said I wanted to be a nurse, he replied "Why don't you want to be a doctor?" This was back in the 1930s but gender as an issue didn't exist for my parents. Achievement was the name of the game and perfection the goal. As an only child, the aim and the goal belonged to me.

I was born in Colorado, but the family moved to Southern California just in time for the devastating Long Beach earthquake in March 1933. We were at some distance, but I still remember the chandelier swaying and the dishes crashing from kitchen shelves. I grew up in West Hollywood and Beverly Hills, graduating from Beverly Hills High School. I majored in physiology at the University of California, Berkeley, and I still thought I wanted to be a doctor. That was until, just as I was about to apply to medical schools (too naive to know most of the best ones did not accept women), I suddenly realized there was no way in the world I wanted to spend my life treating sick people. Medicine no longer the goal, where would I find a new ambition? At complete loose ends, I spent a year as a graduate student in English; I had taken a number of literature courses as an undergraduate and was (and have remained) greatly interested in literature and poetry. No good—strong interest, yes; passion, not really; talent, minimal.

My entry into research was pure luck. Indeed, the role of luck will be a recurring theme in this article. I had two friends at Berkeley who had both become graduate students in the laboratory of I.L. Chaikoff in the physiology department. Between the two, I was essentially conned into

applying and was accepted. It took no time at all for me to realize that research was something I enjoyed greatly and was good at. I had found the goal, the passion, and perhaps the talent. My project had to do with fatty acid metabolism in the alloxan-diabetic rat, looking at incorporation of C^{14}-labeled glycolytic intermediates into fatty acids in rat liver slices. The research was satisfyingly productive—four *Journal of Biological Chemistry* papers in two years, with two first authorships. I'm not sure that I was the only female student or postdoc in the lab, but I know I was the first to have first authorship. However, on the other side of the luck scale, there was the very important discovery that I almost made, should have made but didn't make. For reasons that made sense at the time I asked the effect of malonate, an inhibitor of the citric acid cycle, on incorporation of my labeled substrates into fatty acids. Expecting inhibition, I was astonished to find an enormous stimulation. I even followed up with the right experiment. A young professor in chemistry had synthesized C^{14}-labeled malonate, but unfortunately the specific activity was very low and the label was in a carboxyl group. Negative result, end of story, until several years later when I heard a seminar by Salih Wakil, who had just discovered the essential role of malonyl-CoA in fatty acid biosynthesis. The goddess Fortuna is known to be fickle.

I stayed in this program only two years. The lab was enormous, over 40 students and postdocs, and the professor was essentially unavailable as mentor, at least to me. Further, research approaches were limited and already becoming old-fashioned. For example, the use of broken cell preparations was forbidden. If I were to become a biochemist, I needed to go elsewhere. My husband and I decided to take a year off to travel in Europe. When we returned, it was not to Berkeley but to Seattle and the University of Washington.

Lady Luck had intervened again. The only reason we went to Seattle was that my husband, in the navy in World War II, had docked in Seattle on one of its rare brilliant days and had fallen in love with the area. I knew little about the biochemistry department. The School of Medicine had been founded less than ten years earlier, but Hans Neurath had built a young and vigorous department (including two future Nobel laureates, the two Eds, Krebs and Fisher). My thesis advisor, Frank Huennekens, was an ideal mentor. His door was always open for advice; problem-solving, flights of fancy and fantasy, and even excited phone calls at 10 o'clock at night elicited equal enthusiasm. My thesis research was concerned with the role of folic acid coenzymes in one-carbon metabolism. A major emphasis was characterization of dihydrofolate reductase. My major finding was the discovery that aminopterin and amethopterin (known clinically as methotrexate) are quasi-irreversible inhibitors of dihydrofolate reductase (22), thereby revealing the mechanism of action of methotrexate, which is still used clinically in treatment of rheumatoid arthritis and in cancer chemotherapy.

It's important to note that all of this was a very long time ago; I entered the Chaikoff lab in 1950 and received my PhD in biochemistry in Seattle in 1958. The kinds of questions that could be asked with hope of answers were much simpler, and techniques and technologies were, by modern standards, primitive indeed. The use of radioisotopes was in its infancy. C^{14} was counted only after wet combustion as $BaCO_3$ with an efficiency of less than 5%. Metabolic pathways were still a major focus of biochemistry, and microbial genetics was just beginning to emerge. All this would change, it seemed almost overnight, in the next decade.

The reductase was also responsible for my first exposure to microbiology. The impetus was a collaboration with Helen Whiteley (later president of the American Society for Microbiology) on the dihydrofolate reductase of *Micrococcus aerogenes*. The exposure was entirely indirect—she simply provided cell extracts. I do still remember asking Helen some utterly idiotic question about the organism, betraying my total ignorance of the field of microbiology, and her look of pitying patience as she replied. My real introduction to bacteria came soon after, on the day I began my postdoctoral fellowship.

2. INTRODUCTION TO MICROBIOLOGY: TRAVAILS OF A POSTDOCTORAL FELLOW

I thought I was heading for the National Institutes of Health. I had applied to Bernard Horecker, whose work on pentose pathways interested me. However, he was in the process of moving to NYU Medical School as chair of the Department of Microbiology, and I found myself in New York before he had actually arrived. Furthermore, he had just returned from a sabbatical in Jacques Monod's laboratory at the Pasteur Institute, where nutrient active transport via newly discovered permease systems was a major focus of research. In Paris Horecker had initiated characterization of galactose transport in *Escherichia coli*, and my project was to investigate the energy requirement of the system. My first task, though, was to chemically synthesize β-methyl-galactoside to confirm that it was a substrate for the galactose system. The procedure seemed straightforward, but my product was α-, not β-methyl-galactoside. Over and over again. Bernie visited every two or three weeks and was sympathetic and supportive, but his suggestions were no more effective than my own. To make matters worse, my lab was adjacent to the new medical examiners building, then under construction. My dreams were haunted alternately by pile drivers and α-galactosides. Then one day, for no apparent reason, the method worked. The results strongly suggested that entry and efflux involved different systems, but neither concepts nor technologies were then in place to take the problem further. Nor was there any recognition that galactose could be transported by something like seven different systems, with different energy requirements.

I turned back to my original reason for joining the Horecker lab, his earlier work on pentose metabolism. In Seattle I had become interested in the possible role of vitamin B_{12} in deoxyribonucleotide synthesis. With Bernie's encouragement I submitted a grant application to the NIH to identify a B_{12}-dependent ribonucleotide reductase (RNR) in *E. coli*, and it was funded. Right idea, wrong organism. As the groundbreaking work of Peter Reichard and coworkers later established, the RNR of *E. coli* and most bacteria, as well as all eukaryotes, uses an iron-tyrosine center as reductant (26). Although archaea and a scattering of bacteria use a B_{12}-dependent RNR, the active coenzyme is the adenosylcobalamin derivative of B_{12}, unknown at the time. Needless to say, the project failed miserably.

3. BETTER DAYS: BIOGENESIS OF LIPOPOLYSACCHARIDE

I thought I had hit a stone wall. Once again, good fortune came to my rescue, indeed set the course of my future career. Luck was a departmental seminar given by Otto Westphal, Director of the Max Planck Institute for Immunobiology in Freiburg, Germany. Westphal was a leader in the structure and immunochemistry of the LPS endotoxin that is characteristic of the gram-negative bacterial cell surface (**Figure 1**). He described what was then known about the tripartite structure of the *Salmonella* LPS: the toxic lipid A moiety that somehow anchored the LPS to the cell surface, the terminal O-antigen polysaccharide that defined the serotype specificity of the strain, and the core oligosaccharide to which the O-antigen is attached and which in turn links the O-antigen polysaccharide to lipid A (**Figure 2**). This was 1961, the beginning of the era of macromolecular biosynthesis. Windows into mechanisms of RNA, DNA, and protein synthesis were beginning to open, but complex polysaccharides were still *terra incognita*. I remember looking across the room at Bernie and him looking back at me. The decision was made without a word spoken. I would investigate the enzymatic pathways of LPS synthesis.

3.1. Biosynthesis of the LPS Core Oligosaccharide

Mutants of *Salmonella enterica* Typhimurium were already known that produced incomplete polysaccharides due to blocks at specific stages of assembly. Among these were mutants

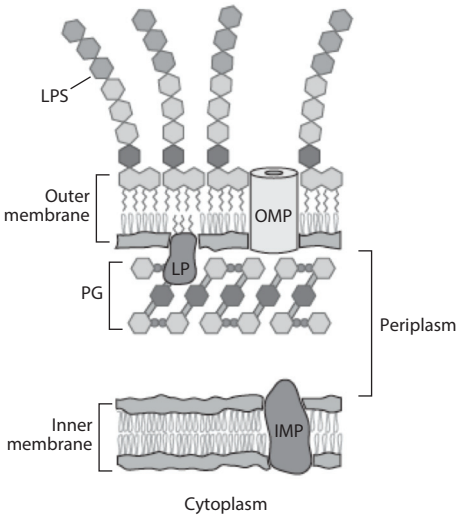

Figure 1

Cell envelope structure of gram-negative bacteria. The O-antigen chains of lipopolysaccharide molecules are shown extending outward from the outer leaflet of the outer membrane. Adapted with permission from Reference 31, copyright Cold Spring Harbor Laboratory Press. Abbreviations: IMP, inner membrane protein; LP, lipoprotein; LPS, lipopolysaccharide; OMP, outer membrane protein; PG, peptidoglycan.

conditionally defective in synthesis of the relevant nucleotide sugars that serve as sugar donors in polysaccharide biosynthetic pathways. The use of the mutants was absolutely the right idea, but we were not the first to have thought of it. Hiroshi Nikaido had already isolated a mutant deficient in synthesis of UDP-galactose and shown that it produced an incomplete LPS lacking part of the core oligosaccharide as well as the terminal O-antigen chain (18). Further, the particulate cell wall fraction catalyzed the incorporation of galactose from the nucleotide sugar donor into a product that appeared identical to LPS (19). Scooped but not daunted, we confirmed and extended Nikaido's results (24). At this point, Larry Rothfield arrived in the lab as a postdoc. We continued playing the nucleotide sugar-mutant game using a UDP-glucose-deficient mutant. Larry demonstrated the incorporation of glucose into the mutant LPS and showed that incorporation of galactose depended on prior addition of glucose to the incomplete polysaccharide core (29). Further, addition of a second glucose residue depended on prior addition of galactose, and, finally, *N*-acetylglucosamine could now be transferred from UDP-*N*-acetylglucosamine to complete the growing core (summarized in References 25 and 32). The sequence of the reconstructed oligosaccharide, together with the chemical analyses of Westphal and colleagues (32), established the *Salmonella* LPS core sequence as -*N*-acetylglucosaminyl-glucosyl-galactosyl-glucosyl-(X).

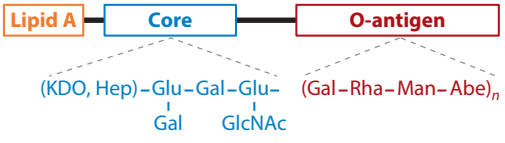

Figure 2

Lipopolysaccharide of *Salmonella enterica* Typhimurium. Abbreviations: Abe, abequose; Gal, galactose; GlcNAc, *N*-acetylglucosamine; Glu, glucose; Hep, heptose; KDO, ketodeoxyoctonate; Man, mannose; Rha, rhamnose.

X, the unknown innermost part of the core, turned out to have only two sugars, both unusual: an aldoheptose and ketodeoxyoctonate (KDO), soon named more accurately but less conveniently, as 2-keto-3-deoxyoctulosonate (**Figure 2**). KDO was apparently previously unknown. I was in a race with Ed Heath, strong competitor and good friend who was working on a similar system in *E. coli*, to identify this unknown sugar, and I again lost by a nose (6, 21).

Some years later we again used a combined genetic-biochemical approach to study incorporation of heptose (5) and KDO (17, 27, 28) into the LPS core; we isolated mutants in formation of the desired nucleotide sugar and looked in cell wall preparations for transfer of the substrate sugar from added nucleotide sugars into the incomplete mutant LPS.

3.2. Biosynthesis of O-Antigen

By 1965 the mechanism of assembly of the core oligosaccharide was pretty clear—successive transfer of single sugar residues to the growing chain with no template involved, only the unique specificities of the individual transferase enzymes. We now turned our attention to synthesis of the O-antigen polymer. I still remember driving to a meeting with Larry, talking as usual about research plans, when he interrupted to ask why I wanted to look at O-antigen, which would be merely cleanup of details since the mechanism was obviously going to be the same as we had already shown for the core polysaccharide. I responded with some heat that there was a good possibility of a twist since O-antigen chains are composed of a large number of repeating oligosaccharide units (in *S.* Typhimurium a tetrasaccharide: abequosyl-mannosyl-rhamnosyl-galactosyl) (**Figure 2**). A different kind of structure implying a different mechanism? In fact, a three-way race was already underway: Phil Robbins' lab versus mine on O-antigen, and Jack Strominger on the related problem of assembly of cell wall peptidoglycan (a polymer of repeated disaccharide-peptide units).

We began straightforwardly by showing the in vitro incorporation of mannose, rhamnose, and galactose into the cell wall fraction of a mutant deficient in formation of GDP-mannose (38). The product was a polymer of O-antigen repeating units (**Figure 2**) minus the abequose branches. The shock came when Ike Weiner joined the lab as a sabbatical visitor and realized that the polysaccharide products were not attached to LPS but were in phosphodiester linkage to some unknown carrier entity with properties of a lipid (34). We called the lipid moiety glycosyl carrier lipid.

Robbins' lab and ours had reached the same conclusion simultaneously, and the two papers were published side-by-side (34, 37). Strominger's group had also reported analogous intermediates in the peptidoglycan system (1). On the identification of the carrier lipid, we were thoroughly and doubly scooped: a year later, the Robbins (36) and Strominger (7) labs identified the carrier lipids in both systems as a C55-polyisoprenoid, undecaprenyl-P. We were still nowhere near identification of the carrier. This was one time when being at a medical school distant from a university campus was a significant disadvantage; we badly needed a chemistry department.

The final and greatest surprise was the later discovery by Bill Lennarz and others that the polyisoprene carrier lipid mechanism is not only ubiquitous in export of bacterial polysaccharides across the cytoplasmic membrane (see below) but is also universally employed by eukaryotic cells for synthesis of glycoprotein saccharide units in the rough endoplasmic reticulum. From little acorns….

4. LIPOPOLYSACCHARIDE TRANSLOCATION TO OUTER MEMBRANE: HOW TO TRAVERSE TWO MEMBRANES AND THE INTERVENING PERIPLASMIC SPACE

4.1. The Gram-Negative Cell Envelope

In the early days, LPS was routinely referred to as cell wall LPS, and the biosynthetic enzymes were studied in the crude cell wall fraction in broken cell preparations. In truth, the structural

organization of what we now call the cell envelope was little understood. It was not until improvements in thin-section electron microscopy arrived that the multilayered envelope structure became clear (2) (**Figure 1**). The cytoplasmic membrane (inner membrane) bounds the cell interior. Outside of the inner membrane is a variable gap (the periplasmic space) that separates the inner membrane from a thin peptidoglycan layer and a second structure, the outer membrane (**Figure 1**). There was strong biochemical evidence that this outer layer was a real, though unconventional, membrane since under appropriate conditions large amounts of material were found to bleb from the outer membrane as bilayer vesicles containing phospholipid, LPS, and a limited spectrum of proteins (30).

It seemed obvious from early on that synthesis of LPS must begin at the cytoplasmic side of the inner membrane, where nucleotide sugar substrates would be accessible to the biosynthetic enzymes. However, the final LPS product is limited to the outer membrane. It appeared likely that the molecule had first to be flipped from the inner to the outer leaflet of the inner membrane (how?) and then somehow released and moved across the periplasmic space to the outer membrane and integrated into the outer, surface-exposed leaflet of the membrane bilayer. We were able to show that translocation of LPS to the outer membrane was ATP dependent (15). However, the significance of that observation was clarified only much later with the identification by Raetz and colleagues of the ABC transporter, MsbA, as the flippase that carries lipid A and phospholipids across the inner membrane (39).

4.2. Separation of Inner and Outer Membranes

It was clear that unraveling the mechanism(s) of LPS translocation would require a reliable method for separation and isolation of inner and outer membranes. This proved tricky. After a good four years of frustration, we finally arrived at a technique that became more or less standard (23). It's a truism that publications describing new, widely useful methods turn up at the top of citation indices. The Osborn method for membrane separation is one of these in a limited way, but I was also sometimes incorrectly given citation credit for another much more important paper coauthored by a different Mary Osborn (33); the Weber and Osborn SDS gel electrophoresis method is one of the most widely employed techniques ever, but that M. Osborn is emphatically not me. We never met, although we did from time to time get each other's mail.

4.3. Events Across the Inner Membrane: Transmembrane Assembly of O-Antigen

Mature O-antigen chains are very long, 40 or more subunits in length (**Figure 2**), and the notion that the whole polymer might be dragged across the hydrophobic interior of the inner membrane did not appeal. We were more taken with an energetically simpler hypothesis—it was the lipid-linked oligosaccharide subunit that was flipped across the membrane to the periplasmic surface, where polymerization and transfer to the core LPS would take place.

We were able to show accumulation of lipid-linked O-polymer in vivo in a mutant conditionally defective in synthesis of the LPS core. The preformed polymer was then efficiently chased into LPS upon return to permissive conditions, confirming that the lipid-linked polymer was an intermediate in formation of the complete LPS molecule (11). Finally, my student Carol Mulford was able to establish by immunoelectron microscopy that the lipid-linked O-antigen was indeed exposed at the periplasmic face of the inner membrane and was rapidly chased into outer membrane LPS following return to conditions for normal core synthesis (16). The later identification by Reeves and coworkers of the *wzx* gene product as the subunit translocase/flippase (13) and more recent evidence that the Wzx protein encodes an O-subunit-proton antiporter (9) completed the

story nicely. As a footnote, we had earlier reported that the uncoupler, 2,4-dinitrophenol, inhibited synthesis of O-antigen (14), but at the time we had no real explanation. More recent advances in biosynthesis of lipid A and lipopolysaccharide are described in an excellent review by Whitfield & Trent (35).

4.4. Assembly of LPS into the Outer Membrane

Sites of close contact between the inner and outer membranes were first visualized by M.E. Bayer (3) in electron micrographs of plasmolyzed cells. Bayer named these structures zones of adhesion. In addition, there was always a fraction of intermediate density, containing markers of both the inner and outer membranes, in sucrose density gradient membrane separations (8, 23). The intermediate density fraction presumably represents the Bayer zones of adhesion. Pulse-chase experiments in the Rothfield lab later showed that this fraction contained newly synthesized LPS at an intermediate stage of its movement from inner membrane to outer membrane (8).

I tried, oh, how I tried and how long I tried, using every trick I could think of to isolate a mutant conditionally defective in translocation of LPS to the outer membrane. Zero. The problem was later solved when the two labs of Dan Kahne and Tom Silhavy unexpectedly found themselves at the same place at the same time, coming from quite different directions. Their finding that biogenesis of the outer membrane occurs via the parallel Lpt and Bam systems for LPS and outer membrane proteins, respectively, is a beautiful story, well told in recent reviews (12, 20). One more footnote: I was charmed to learn that the *lpt* system is localized in the presumed adhesion zone fraction between inner and outer membranes (4). Now, finally, the zones of inner membrane–outer membrane adhesion could be understood as sites of outer membrane assembly.

And incidentally, we now have an explanation of our findings of 30 years before (10) that phosphatidylethanolamine, introduced artificially into the outer membrane, was rapidly translocated back to the inner membrane. The middle band seemed to be the initial site of incorporation, suggesting that the zones of adhesion can probably support both the movement of proteins and LPS from inner to outer membrane and the two-way movement of phospholipid between the two membranes.

5. PEOPLE AND PLACES

5.1. The Horecker Years

Although my undergraduate experience in Chaikoff's lab in Berkeley suggested I could be good at research and my graduate years in the Huennekens lab gave me the confidence that this was so, Bernie Horecker was responsible for my maturation as a scientist. If you showed signs of talent the training in his lab was superb. There was no patience with sloppy thinking: Start with a clear hypothesis, and then test it to destruction. Consider alternative interpretations, and test those to destruction as well. Confirm results, yes, but also learn something new from every experiment. I well remember my first *PNAS* manuscript, which remained over the word limit despite my interminable cycles of rewriting. When I showed it to Bernie, he took out his red pencil: zip, zip, zip, and the paper was not only within the limit but much clearer and more readable. Lesson learned: I became ruthless in editing my own manuscripts.

I also learned how to present my findings with accuracy and clarity in publications and oral presentations. Rehearsals were obligatory for all talks at meetings, with critiques of slides a particularly important component. Don't clutter—one slide, one single point to be made. Tell the audience what the take-home message will be; tell it, and then tell again what the message was and why. The department was also very serious about the twice-weekly journal club.

After I finished my postdoctoral fellowship and remained in the department as an assistant professor, Bernie was very good about feeding me postdocs and also very good about promoting my visibility by facilitating opportunities for presentations at national meetings and symposia. On the other hand, his name stayed on my papers long past the time that I was fully independent. Why didn't I complain and confront? Though I have never been diffident about arguing a scientific point, I was at that time very uneasy about challenging authority in other respects. Though I would have denied it, most likely a residue of the woman-as-subordinate syndrome.

5.2. Microbiology at Last: The University of Connecticut School of Medicine

Among the excellent postdocs Bernie assigned to my lab, the most consequential for my future career was Larry Rothfield, sometime collaborator, occasional competitor, colleague, and friend for well over 50 years. Our professional relationship over those years consisted of a round-robin of reciprocal exchanges of authority. Initially, I was his boss. An MD who loved medicine but disliked private practice, Larry revived student interests in research and applied to Bernie for a postdoctoral fellowship to learn biochemistry. He joined my lab in 1962 just as the LPS work was beginning to take off. He did indeed learn biochemistry and soon became independent, doing pioneering studies on protein-lipid interactions. Five years later, he was offered the chairmanship of the new Department of Microbiology at the not yet opened University of Connecticut School of Medicine in Farmington. I was the first person he recruited. We moved in 1968, and he forthwith became my boss. Larry stepped down as chair in 1980 and I took over. I thus became, again, his boss. Larry had the last laugh, however—after I resigned as chair and closed my lab I continued as a senior postdoc in his lab, and he again became my boss until we both retired in 2014.

When we moved to Farmington in 1968, the medical school was just a big hole in the ground and we were given lab space at the main University of Connecticut campus in Storrs some 40 miles away. This was not fun, despite the incredible hospitality of the University of Connecticut faculty. The interstate highway through Hartford was not yet finished, and the commute was long and slow throughout a very snowy winter. Next spring, we moved to prefabricated buildings on the Farmington campus. These temporary structures remained for nearly 50 years, until the last one was demolished when construction began on the new Jackson Laboratories building. The prefabs were in fact very efficient, with a wide central corridor that accommodated all the major equipment and provided constant opportunity for discussions while standing at the centrifuges. There was one minor disadvantage—recognized when a field mouse built her nest in my technician's pocketbook. There was also one important, though unanticipated, advantage—the biochemistry department occupied the adjacent building, and we quickly discovered there was just the right space between for a volleyball court. Every noon, Microbiology competed with Biochemistry, with some very competent players on both sides. And then there was me, probably the second-worst player in the world. But I played. This continued until the main building was finally ready, over budget and behind schedule, some three years later.

We were ambitious in our recruiting goals; indeed, overambitious given a brand new institution with no history of achievement and limitations in space and institutional support. The list of candidates who declined our offers comprises a Who's Who of molecular microbiology, including at least two future Nobel Prize winners. However, we succeeded in building a fine, though small department including Henry Wu, Bob Poyton, Steve Pfeiffer, and a young virologist named Tom Shenk. Tom's lab was across the hall from mine, and we spent a good deal of time at the nearby hall blackboard talking science and ideas and plans. One more story—Nic Jones, an outstanding postdoc, eventually found adenovirus more interesting than *Salmonella*, and with my blessing (outwardly gracious, inwardly seething) he moved across the hall to Shenk's domain. But when I poked

my head into my lab the first morning he was gone, not only he but also his desk had disappeared. I stormed across the hall, "Steal my postdoc if you must, but give me back my damn desk!" Tom and I have each told the story many times.

5.3. Friends and Competitors

Early on, Salva Luria initiated a series of workshops on the just-beginning field of bacterial membranes, cell walls, and cell surfaces. These small weekend meetings were instrumental in welding the community into a congenial and highly interactive whole. They led to many life-long friendships that included Phil Robbins, a direct competitor who became a good friend. I felt particularly close to the large German contingent of key contributors to this research: from Freiburg, Germany, the two Ottos, Westphal, and Luderitz; and Bob and Barbara Jann; and from Tübingen, Germany, Ulf Henning, Volkmar Braun, Uli Schwarz, and Peter Overath.

6. WHAT IT WAS TO BE A WOMAN IN SCIENCE

I was most certainly not in the first generation of women to achieve prominence in biochemistry and microbiology, but I did spend a substantial fraction of my career as the only woman in the room. I think I was fortunate, perhaps unusually so, in being exposed only rarely to situations of overt gender discrimination. So rarely that I was shocked speechless when, after being flown to New York for a postdoctoral fellowship interview, I was asked first and foremost why I wanted a fellowship rather than going out and getting a (real) job. That foundation did offer me a one-year (!) fellowship, which I was pleased to decline in favor of one from the NIH for two years and a higher stipend. However, I was outraged again by the policy then in effect that only male fellows received an additional allowance for their spouses. I suppose it was expected that females were to be supported by highly paid husbands. And there was the time that the chair of a biochemistry department whose faculty had pushed him to offer me a position as associate professor responded: "There never has been a woman in this department above the rank of assistant professor and there never will be!" It's also true that I received fewer job offers than a man with a comparable record might have expected. However, among colleagues and within my research field I never felt less than fully accepted and respected.

I was never subjected to sexual harassment or offensive behavior, and I can think of only one rather amusing incident of inappropriate curiosity. It happened at a Gordon Research Conference while I was still in the Horecker department. The usual after-the-evening-meeting gathering with beer, conversation, music, and dancing was in progress. I was dancing with an eminent senior biochemist whom I didn't really know, when he suddenly asked if my boss took me with him on his trips to Europe. Relatively shockproof by that time, I just said, "No," and stepped on his toes. That ended both the dance and the conversation. There may have been, and probably were, other trivialities that either passed over me or are long forgotten. But I never felt that gender imposed second-class citizenship in the scientific community. Well, no, that's not quite true—my publications are all under the name, "M.J. Osborn." Intended very deliberately in the beginning to mask gender but continued later just because I had begun that way.

In fact, I think I was again lucky in that I arrived on the scene just at the time that recognition was beginning to grow in some fields that women might also have brains and talent. As I said above, my thesis advisor, Huennekens, was a fine mentor and builder of confidence. The Horecker lab was quite different—much larger and the boss more distant. It was very much sink or swim. If you sank, you sank without trace, but if you swam the training and support were superb and, if not gender-blind, at least gender-tolerant. A striking number of women who went on to

important careers were trained in his lab and department during this time. I was the first, but the roster included Ora Rosen, Ann Skalka, and Lucille Shapiro. One thing we had in common was confidence—in an era in which women were assumed to be self-effacing, uncomfortable, and unsure of their reception, we wrote well and confidently and spoke with confidence and were thereby visible.

Two of the most difficult and potentially contentious issues facing professional couples are the two-career family problem and how to be a parent while working 12-hour days. Back in those days, the question of who takes priority in the search for the best job didn't arise. It was, with few exceptions, taken for granted that the wife followed the husband. I faced neither problem. We had no children, and my husband was an artist, his work entirely independent of place. My career was a given and had his complete support.

7. ON THE NATIONAL SCENE

7.1. Beginnings that Grew

In the 1970s and 1980s national organizations were actively seeking women to serve on committees and boards and to run for office in professional societies. My calendar and curriculum vitae soon became ridiculously overstuffed. As mentor I have always advised young faculty, especially women, to learn to say, "No!", but I never quite managed to take my own advice. It began with a totally unexpected invitation to join the research committee of the American Heart Association (AHA). Needless to say, my research had nothing whatever to do with the cardiovascular system, and I still don't know how or why I came to their attention. However, the committee had a broad mandate to support basic biomedical research across boundaries of field. I was not the first woman to serve on the committee. At least two had preceded me, the cardiologist Harriet Dustan and the physical biochemist Mildred Cohn. I ended by chairing the committee and at the end of my tenure was presented with a Lalique glass bird that I still cherish.

The AHA Research Committee, again altogether unexpectedly, served as springboard to an advisory role to the National Aeronautics and Space Administration (NASA) that lasted off and on for more than 30 years. In the early seventies, the National Academy of Sciences convened a two-week workshop in Woods Hole, Massachusetts, to plan for the upcoming Apollo space shuttle missions. One of the other members of the AHA committee recommended me for the study group on space biology and medicine. It was a fascinating experience—about 15 biomedical types swimming in a sea of 100 or so physicists, astronomers, astrophysicists, and engineers. This led in turn to a term on the National Research Council (NRC) Committee on Space Biology and Medicine, advisory to NASA. The highlight of this experience was attending the initial presentation of research results by the astronauts of the first Skylab mission, the groundbreaking first long-term human exposure to microgravity. Finally, some twenty years later I again joined the Committee on Space Biology and Medicine as chair.

I was elected to the National Academy of Sciences (NAS) in 1978 and became President of the American Society for Biological Chemistry (ASBC; now the American Society for Biochemistry and Molecular Biology) in 1981. I was not the first woman to be elected—Mildred Cohn had preceded me by three years. After us came first a trickle and finally a steady stream of female presidents. It's a pleasing thing to be a pioneer of sorts, but the reward came in the evolution that followed, including a month-long distinguished lectureship (designed to raise awareness of women in science) at the University of California, Berkeley, hosted by the biochemistry department. I had several friends in the department and enjoyed the visit immensely.

There's a small story attached to the NAS election. The annual meeting of the NAS is always held in late April, and the election of new members is always on Tuesday morning. That year I was

at a Dahlem conference in Berlin during the relevant time, and I was egotistic enough to wonder if…. Tuesday came and went and I was cast down. On Thursday night, however, returning late to the hotel from a superb performance of the B-minor Mass (and still hearing the music in my head), I was given a message to call Connecticut. It turned out that the election notification had gone to, and vanished in, the main campus of the university rather than the medical school and had just resurfaced. I didn't sleep much that night. However, some ten years passed before I became active in these organizations.

7.2. Back and Forth to Washington, DC

So began a period of almost two decades of absurdly overextended commitments. This began in 1980 with a six-year term as member of the National Science Board (NSB), the board of directors of the National Science Foundation. I was appointed by Jimmy Carter, but the term actually began with the Reagan administration, not a great time for basic science, with ever increasing pressure on funding. But the concentrated exposure to the physical sciences was fascinating, and those interests are with me still. Given six NSB meetings a year plus the ASBC presidency, I was on a plane to or from Washington at least nine times a year. Then in 1983 Ruth Kirschstein all but coerced me to join the Director's Advisory Council of the NIH's National Institute of General Medical Sciences (NIGMS). So now I was in Washington a dozen times a year in addition to attending to a growing list of other commitments. And, oh yes, in 1980 I became head of the Department of Microbiology and served during a period of significant faculty turnover and recruitment.

The 1990s were a bit less frenetic, but only a bit. My major and most satisfying activity was a return to the NRC's Committee on Space Biology and Medicine as chair and member of the parent Space Studies Board. I loved it. The committee produced a decadal report that set priorities for microgravity research in the era of the International Space Station and beyond. Then in the late nineties, NASA gave the Space Studies Board a new charge, extraterrestrial life (astrobiology) and the origins of life on earth. In 1999 as part of this initiative, Vice President Al Gore convened an afternoon workshop on the origins of life. It was an extraordinary experience. The 20 or so participants ranged from leaders in origins research through members of other relevant scientific disciplines, including social science and religion. As Chair, Gore was extraordinarily impressive, on top of all the issues and directing the discussion to elicit sometimes contentious exchanges. Consensus was approached on the question of possible origins and evolution, but opinions about extraterrestrial life seemed more dependent on world view than science. It's different now—the evidence grows more intriguing and the probability more compelling year by year.

I had never been more than peripherally involved in issues related to the status of women in science until, near the end of my career, I was both surprised and puzzled by an invitation to join a study on the status of European women in science. The European Union had charged a think tank in Barcelona to carry out the study, and who could resist the lure of three trips to that delightful city for a good cause? But why me? There is, of course, a story. As we all suddenly realized at the first meeting, they had invited the wrong Mary Osborn. The other Mary Osborn had participated in a previous similar study. How our addresses became confused remains a mystery. However, I was already there and not unqualified, and I may even have contributed in some small measure to the proceedings.

8. THROUGH THE RETROSPECTOSCOPE

The retrospectoscope is a fictitious instrument used facetiously by medical professionals to look back with 20/20 hindsight on past successes and blunders. At this point in time, I am sufficiently

ancient to look back at my long-ago self, my strengths and my weaknesses, with some degree of objectivity. In short, what contributed to success; what led me astray? How did this extremely shy young woman develop into a competent scientist?

I was smart, yes, and my mind worked quickly, but what gave me the self-confidence to believe in myself was psychological support and research success as a graduate student with Huennekens. By the time Bernie Horecker had licked me into shape as a postdoc by being both tough and supportive, I realized that I knew how to do research and how to present research findings with conviction. When I entered science it was all too common for women to present their research hesitantly and almost apologetically. This is much less common now, but often continues to be a real barrier to respect and recognition. One of the greatest compliments I ever received came in a 2010 email from a woman who told me that she had been inspired to go into science by hearing a seminar by me in 1973, just after she graduated from college. As a woman scientist I found this both gratifying and humbling.

As a scientist I don't see myself as an innovator. I had a few innovative ideas that paid off handsomely, but I have always worked step-by-step, and giant strides into the unknown are not my thing. This was probably an advantage overall but limited my willingness to move out of my comfort zone and into new directions. On the other hand, I have always been good at framing issues and options and building consensus. This made me a better department head and was invaluable in board and committee work as I became more and more committed to activities at the national level. I greatly enjoyed these, their variety and scope, and I was good at it. But there is an old song about the sad fate of a girl who couldn't say no. All too apt, I fear. During the last several decades of my career I found myself away from the lab more and more and thinking seriously and creatively about my own research less and less.

Do I regret this? Nothing ever matched the thrill of making new scientific discoveries, but in retrospect, I don't regret this change in direction. My focus had shifted; I had found a different path, and the satisfaction of contributing meaningfully to science policy issues gave me a satisfaction that never faded.

DISCLOSURE STATEMENT

Dr. Osborn left a near-final draft when she died in January 2019. Finishing touches were added by Larry Rothfield and Tom Silhavy, who are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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