

Remarks by Dr. Robert G. Spiro upon receiving the Rosalind Kornfeld Award

I wish to express my gratitude to the Society for selecting me as a co-recipient of the first Rosalind Kornfeld Lifetime Achievement Award. It is indeed a great honor to receive this award in memory of our esteemed colleague.

In accepting this recognition I would like to acknowledge that many of the studies in my glycobiology career were carried out in collaboration with my wife, Mary Jane, and of course also the contributions of many young scientists and students who received their training in our laboratory.

I also wish to say that it is great pleasure to share the award with Nathan Sharon as we come from the same vintage, now quite aged, of glycoscientists. It is an intriguing coincidence that we started our careers not only about the same time but in close geographic proximity. While I received my postdoctoral training in the laboratory of Baird Hastings at the Harvard Medical School (1956-58), Nathan was carrying out postdoctoral work at the Massachusetts General Hospital (MGH) in the laboratory of Roger Jeanloz. When I subsequently moved to the MGH for three years (1958-61) Nathan had already returned to Israel so that regretfully we never worked under the same roof although we were aware of each other's work through the literature. After my stay at the MGH I returned to Harvard where I devoted the remainder of my career to research and teaching.

I will now take the liberty of indulging in a few reminiscences of my years in the pursuit of the glycosciences. My entrance into this field was in retrospect quite unusual. Because of my medical training I developed a consuming interest in the nature of the deposits which accumulate in the glomeruli of diabetic kidneys. On the basis of histochemistry, which was a commonly used tool at that time, these deposits appeared to be replete with carbohydrate bound to or associated with protein.

Upon consulting the very sparse literature which existed in 1956 I essentially encountered a void with the exception of a 1938 paper by Albert Neuberger in which he showed that egg albumin contained a small amount of saccharide material. This was received with some skepticism until the paper of 1963 appeared in which he and his collaborators clearly showed that this association was covalent through the now well known *N*-glycosidic bond.

Neuberger's finding gave me courage to focus all my energy in attempting to find out as much as possible about such intriguing protein-glycoconjugates although I did not realize at that time that this would constitute a lifetime of study and that in contrast to other modifications which proteins can undergo that this was a most complex phenomenon which involved multiple enzyme systems and had impressive biological functions. To date the field of glycoproteins has given rise to 470 K citations listed in PubMed and in 2007 alone 26 K papers related to this subject appeared through the work of many talented scientists.

Due to the liberality, and in indeed encouragement, of Baird Hastings, one of the outstanding American biochemists of the first half of the 20th century, who at Harvard was renowned for encouraging M.D.'s to pursue basic sciences, I was given the freedom to embark on this journey in the second year of my postdoctoral training.

Our studies on the structure of peptide-linked carbohydrate units moved from secreted soluble glycoproteins such as fetuin and thyroglobulin to extracellular matrix components like the highly glycosylated collagens and proteoglycans of the kidney glomeruli and then of course to the components of the cell surface where the oligosaccharides play such an important biological role. The investigations which we carried out on the enzymatic assembly and processing of the carbohydrate units represented a fascinating endeavor as was the tracking of the journey of the proteins to which they were attached from the ER to the cell surface and in one remarkable case, we noted a translocation into the mitochondrion. Most exciting to us was our

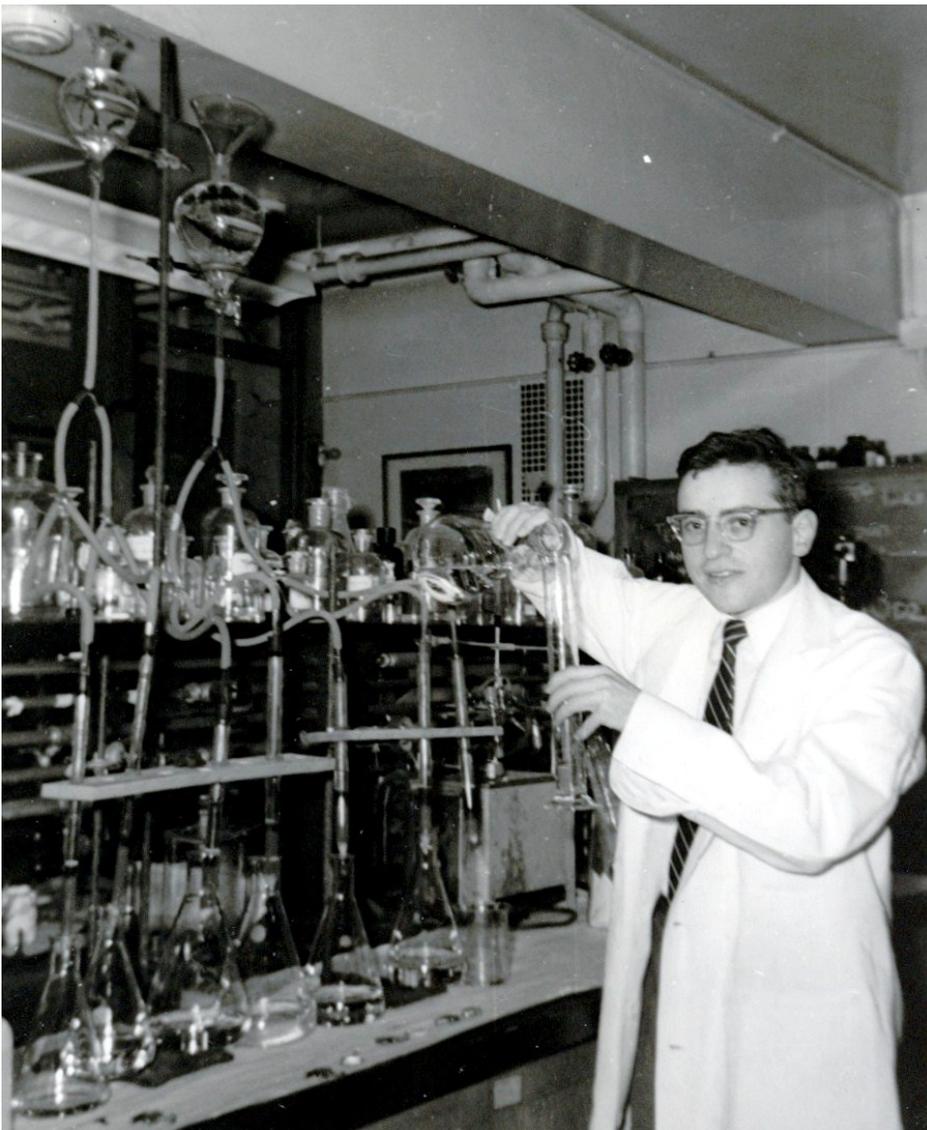
discovery of glucose on the polymannose portion of oligosaccharide-lipid donors and the nascent glycoproteins. Glucose is of course rarely found in mature glycoproteins and therefore serves as an ideal recognition signal for cotranslational *N*-glycosylation and recently it became evident, through our work and that of others, that it plays a major role in ER quality control. Furthermore we observed that during this process there is a substantial intracellular release of incompletely processed oligosaccharides.

The 1960's was the golden age of bio research funding; however only modest funds were needed as the thick catalogues of biological supply houses had not yet appeared. Accordingly we had to prepare many of our substrates like sugar nucleotides. Fetal serum could not be bought in neat vials but required, for the isolation of fetuin, trips to the slaughterhouse where we would wait for gravid cows and after opening the amniotic sac draw blood from the fetal calves therein by cardiac puncture. The tools available to us were by modern standards quite primitive. Scintillation counters were not generally available in 1956 and I remember distinctly when I wanted to measure the radioactivity in the glucosamine of serum glycoproteins I had to prepare phenylosazones of the sugar and plate this derivative on steel planchets which I placed into a Geiger counter one by one patiently waiting for each count for about half an hour.

Contrary to the relatively uniform methods of peptide sequencing, which was a major activity in these early years, the polyhydroxy nature of sugars gives rise to an almost infinite number of structural variations and this required the challenging development of numerous methods to uncover sequence, branching and inorganic substitutions. Moreover at the present time more than 13 distinct types of carbohydrate-peptide linkages have been identified and the *N*-glycosidic bond appears to be found throughout the phylogenetic range from mammals to *Archbacteria*. Clearly the oligosaccharides linked to the protein by this bond have proved to be by far the most complicated in their structure and biosynthesis.

In conclusion I would like to metaphorically compare the progression which we are witnessing in the field of glycobiology to a visit to one of the world's great museums of art. We enter the first room in our tour and are dazzled by the wonderful paintings and tapestries only to be confronted with the entrance to another room equally replete with visual riches and so we traverse the museum from room to room viewing and enjoying an expansion of the art each with its own distinctive beauty.

Indeed as the multitude of exciting abstracts for this meeting indicates we have not even come close to uncovering all the treasures of glycobiology although we have entered one more room in our long excursion.



Photograph of Robert G. Spiro in the laboratory of Baird Hastings at the Harvard Medical School during the second year (1957) of his postdoctoral fellowship. He is isolating the glucosamine from serum glycoproteins on a battery of Dowex 50 columns in order to determine its radioactivity as the phenylosazone derivative. This represented his first study in the field of glycobiology.