Degani, Hadassa: Career Notes

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MY INTRODUCTION TO CHEMISTRY, BIOLOGY, AND NMR

Until I reached high school, my favorite subjects were history and Hebrew literature. However, when, in the second year of high school, we started chemistry, I was immediately fascinated, thanks primarily to an outstanding teacher, who taught us with great passion and love for the field. Consequently, in 1963, after my military service, I began my undergraduate studies in chemistry, at the Hebrew University of Jerusalem. The curriculum included a short introduction to NMR and its use in analytical organic chemistry, but I had never seen an operating NMR spectrometer. It was only in the summer of 1966, when I came to the Weizmann Institute of Science to enroll as an M.Sc. student in the Feinberg Graduate School, that I first became acquainted with the homebuilt, original 0.7-T NMR spectrometer built by Saul Meiboom and Shlomo Alexander, in 1955, and with a Varian DP60 spectrometer. I joined the group of Daniel Fiat in the Isotope Research Department and was fascinated by the ability to employ a completely noninvasive method to quantify structural and dynamic parameters of molecules with biological activity. Through temperature-dependent relaxation and shift measurements of the protons and ^{17}O of the cofactor of hemoglobin, namely, the chelate of iron3+ with porphyrin, interacting at the axial positions with water and pyridine, two important processes were characterized: the expected exchange kinetics of the axial ligands and the unexpected exchange from high spin to low spin of the iron as a result of temperature-dependent changes in the crystal field.[1] As I look back on this period, I can appreciate the opportunity I had of learning NMR theory during the numerous group meetings devoted to the study of the NMR "bible," Abragam's book, as well as the experimental experience of working with spectrometers with very limited automated functions.

After finishing my M.Sc. degree, I worked for a year as a laboratory research assistant in the Institute for Arid Zone Research, Beer Sheva, on the conversion of light into chemical energy in chloroplasts, employing ionophores that facilitate ion transfer across membranes. This introduction to a functional biological system was the basis of my subsequent PhD thesis under the supervision of Harold Friedman in the Chemistry Department, SUNY at Stony Brook (1970–1974). Harold encouraged me to select my thesis subject independently, and I chose to continue working on the structure and solution chemistry of the ionophore X-537A and its various metal complexes. His dedicated and stimulating supervision helped me develop self critique, scientific thinking, and a drive to understand and explain the roots of problems. During this period, I also established my general goal as a researcher: problem-solving, rather than NMR technique development. However, the balance between these two directions varied throughout my career, and even during my PhD studies, after using a range of spectroscopic methods, I turned again to NMR kinetic studies of the binding of X-537A to the transition metal ions, despite the fact that it required taking the snail-paced Long Island Rail Road during the cold winter, in order to get to the 220-MHz spectrometers at Rockefeller University in Manhattan.

I returned to Israel after finishing my PhD thesis and was recruited in 1976 by Zeev Luz, to join the NMR group in the Isotope Research Department at the Weizmann Institute as an independent researcher. Under the influence of a strong NMR group, and with a new 270-MHz spectrometer just across from my office, I started moving from solution studies toward more complex biological systems. This entailed investigations of ionophore-mediated transport, and later on, other transport processes in lipid vesicles and, eventually, in live cells, developing NMR methods for characterizing the kinetics of transport across membranes.[2–4]

My search for biologically relevant problems amenable for NMR studies opened the way to new and exciting research endeavors along with stimulating cooperative efforts with various Institute colleagues in the life sciences. In collaboration with the late Mordehay Avron, I embarked on investigations of the halotolerant alga Dunaliella and the mechanism of osmoregulation in this alga, by introducing in vivo NMR as a new research tool. Osmoregulation in this alga presented a very special and interesting metabolic control of an adaptation to the environment. After several years of intensive research, developing and applying in vivo ^{31}P, ^{13}C and ^{1}H NMR measurements, a new scheme for the mechanism of the osmoregulation adaptation was constructed.[5] It was the first time in my independent career that, based on a range of experimental data, particularly, in vivo NMR kinetic studies, I was able to scribble the whole step-by-step mechanism on one page. Upon presenting this drawing to Mordehay, he responded saying, "It is so striking that it must be true." I think he was right.

In parallel to the work on Dunaliella, I became fascinated by the sequential dramatic changes induced by estrogen on gene expression and metabolism in the female reproductive organs and related cancers, and started NMR investigations of this induction with the late Alvin Kaye from the Hormone Research Department and with Thomas Victor, a surgical pathologist from Evanston Hospital, Chicago, who joined my lab for a sabbatical. Since 1985, the main goal of my research has been focused on characterizing hormonal regulation of breast cancer and mechanisms of endocrine therapy. The handling of cell cultures and tumors in animal models by the lab technicians and the dedicated work of my students, all determined the work flow, success of experiments, and final productivity during the next 25 years of breast cancer research (see Refs 6–14). As a result of the increased complexity of the systems, from solution to live cell cultures, functioning tissues, tumors in animal models, and, eventually, in human patients, we developed experimental means to handle these systems inside magnets. Multinuclear NMR spectroscopy and MRI were the predominant experimental tools in these investigations; however, biochemical and molecular biology methods, as well as histopathology and immunohistochemistry became our routine adjunct tools. I owe to the late Mildred Cohn, who was a role model for me and for many other women scientists, my ability to focus on this project, work systematically, and search for the appropriate method to solve a question.

Although the main emphasis in my lab was on breast cancer research, there were a few other research projects that started as a result of the opportunity to employ NMR in new exciting areas. One such area was the melanotropin-induced signaling pathway in melanoma cells, done in collaboration with Yoram Salomon from the Hormone Research Department. In these experiments, we saw, for the first time in live cells, the rising of a ^{31}P signal of cyclic AMP and a new, unexpected phosphomonoester that turned out to be, after extensive biochemical analyses, fructose 1,6-bisphosphate.[15] Another project took us far into the field of cancer-related research: the development of in vivo functional sodium imaging of the kidney. It was well known that renal function is regulated by a sodium concentration gradient across the kidney; using in vivo sodium imaging was, therefore, an obvious target. We have demonstrated in a series of papers the potential capability of sodium imaging to monitor renal function in time and space. It was highly rewarding to discover that our sodium kidney image reached the cover page of *Kidney International.*[16] The translation of renal sodium imaging to humans was later demonstrated in the lab of my long-time collaborator and personal friend, Bob Lenkinski.[17]

IN VIVO ANIMAL STUDIES AND CLINICAL TRANSLATION

In 1983, I spent a sabbatical year in the laboratory of Bob Shulman, Yale University, working on the kinetics of creatine kinase and phosphate-ATP exchange in perfused rat heart and in rabbit brain in vivo, applying an inversion transfer protocol based on DANTE pulses.[18,19] I gained new experience in animal in vivo studies and also sufficient courage, to return to the Weizmann Institute and request a horizontal bore scanner for such studies. Luckily, the Institute Presidents at the time, believed in the potential of this new NMR field to advance biological research and obtained several donations that made possible the establishment of a biomedical research facility in the renovated laboratory of Weizmann, designed by the renowned architect Erich Mendelsohn, centered around a Bruker 4.7-T, 30-cm horizontal bore Biospec spectrometer, as well as 500-MHz high-resolution and 400-MHz microimaging spectrometers (Figure 1).

The main transition involved developing know-how in animal studies using MRI and localized MRS. The former studies were primarily aimed at investigating breast cancer angiogenesis through the development of dynamic contrast-enhanced (DCE) methods. Since prior knowledge based on histological studies indicated high heterogeneity of the tumor microvessels' density, we emphasized high spatial resolution. The dynamic datasets were analyzed at pixel resolution, fitting the data to a two-compartment physiological model and obtaining parametric maps of perfusion. The results confirmed the high heterogeneity of the vascular distribution and function in breast cancer and revealed the changes in the vascular permeability under the influence of estrogen or treatment with tamoxifen.[20] As frequently occurs in the studies of biological systems, the extension of DCE to new tumor models indicated that in certain kinds of tumors the two-compartment model fails to fit the data. This discrepancy was resolved by discovering that these tumors exhibit high interstitial fluid pressure, which

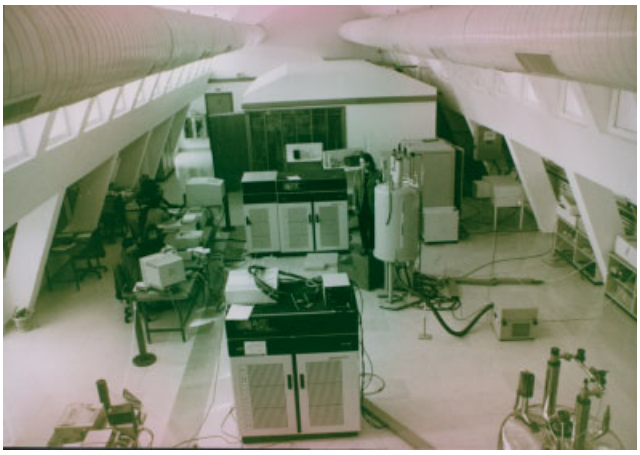

Figure 1 The Ilse and Maurice Katz Magnetic Resonance Laboratory for Biomedical Research. (Photography by Alex Agor, 1998)

prompted us to develop a contrast-enhanced MRI method for noninvasively mapping this distinct physical parameter.[21]

Overall, the extensive DCE investigations in the animal models directed us to the first clinical translation of mapping pathophysiological features of breast tumors in patients.[22] The need for high spatial resolution dictated a simulation rather than a fitting approach, on the basis of a model. The dynamics of the wash-in and wash-out patterns in breast tumors were simulated with the aid of a color-coded calibration map, using the minimum essential three time points (one precontrast and two postcontrast, hence termed the *three time-point*, or the *3TP* method). Designing an optimal scanning protocol and a user-friendly color-coded output for diagnosing cancer and follow response to therapy, enabled clinical tests of this method, meticulously managed by Edna Furman-Haran, a staff scientist in my lab, in collaboration with expert radiologists in the United States and Taiwan.[23,24]

Recently, we extended the analysis of the dynamic datasets applying principal component analysis adjusted with the 3TP method.[25] Moreover, in our search for a completely noninvasive method for breast cancer detection, we have recently applied diffusion tensor imaging and showed that it can track the mammary ductal trees and detect malignant growth. Today, all clinical examinations are performed on the Weizmann Institute campus in a special facility housing a 3 T human scanner. Overall, the intimate relation with the patients, the ability to optimize the scanner capabilities, and the keen collaboration with Myra Shapiro-Feinberg, head of the breast imaging unit at Meir Medical Center, make this part of our research highly exciting and gratifying. "Politically correct" in our concern for gender equality, we have extended the clinical translation of our methods to men by improving the ability to detect prostate cancer.[26]

In summary, every career is an evolutionary process, and mine is, in many ways, typical of the general shift in emphasis that has occurred in science during the last decades, starting from NMR in chemistry and ending with MRI/MRS in medical imaging. Throughout all stages of my career, the quest for reaching end points required total devotion, ability to constantly innovate, and acceptance of the fact that progress is achieved through a sequence of very small steps, some of them encouraging, many of them disappointing. Finally, everyday work in my lab was always based on a team effort and free

exchange of ideas and experience. The flow of bright young students presented a challenge as well as a reward. Many innovations stemmed from their ideas and desire to explore and discover.

REFERENCES

1. H. Asman-Degani and D. Fiat, *J. Am. Chem. Soc.*, 1971, **93**, 4281.
2. H. Degani and G. A. Elgavish, *FEBS Lett.*, 1978, **90**, 357.
3. C. Lipschitz-Farber and H. Degani, *Bioch. Biophys. Acta*, 1981, **600**, 291.
4. F. F. Brown, I. Sussman, M. Avron, and H. Degani, *Biochim. Biophys. Acta*, 1982, **690**, 165.
5. M. Bental, U. Pick, M. Avron, and H. Degani, *J. Biochem.*, 1990, **188**, 117.
6. M. Neeman and H. Degani, *Proc. Natl. Acad. Sci. U.S.A.*, 1989, **86**, 5585.
7. S. M. Ronen and H. Degani, *Biochem. Biophys. Acta*, 1991, **1095**, 5.
8. Y. T. Ting, D. Sherr, and H. Degani, *Anticancer Res.*, 1996, **16**, 1381.
9. E. Furman-Haran, R. Margalit, D. Grobgeld, and H. Degani, *Proc. Natl. Acad. Sci. U.S.A.*, 1996, **93**, 6247.
10. R. Katz-Brull, D. Seger, D. Rivenzon-Segal, E. Rushkin, and H. Degani, *Cancer Res.*, 2002, **62**, 1966.
11. L. Bogin, R. Margalit, J. Mispelter, and H. Degani, *J. Magn. Reson. Imaging*, 2002, **16**, 289.
12. D. Rivenzon-Segal, S. Boldin-Adamsky, D. Seger, R. Seger, and H. Degani, *Int. J. Cancer*, 2003, **107**, 177.
13. G. Eliyahu, T. Kreizman, and H. Degani, *Int. J. Cancer*, 2007, **120**, 1721.
14. E. Dadiani, D. Seger, T. Kreizman, D. Badikhi, R. Margalit, R. Eilam, and H. Degani, *Endocr. Relat. Cancer*, 2009, **16**, 819.
15. H. Degani, J. O. DeJordy, and Y. Salomon, *Proc. Natl. Acad. Sci. U.S.A.*, 1991, **88**, 1506.
16. N. Maril, R. Margalit, J. Mispelter, and H. Degani, *Kidney Int.*, 2004, **65**, 927.
17. N. Maril, Y. Rosen, G. H. Reynolds, A. Ivanishev, L. Ngo, and R. E. Lenkinsky, *Magn. Reson. Med.*, 2006, **56**, 1229.
18. H. Degani, M. Laughlin, S. Campbell, and R. G. Shulman, *Biochemistry*, 1985, **24**, 5510.
19. H. Degani, J. R. Alger, R. G. Shulman, O. A. C. Petroff, and J. W. Prichard, *Magn. Reson. Med.*, 1987, **5**, 1.
20. E. Furman Haran, D. Grobgeld, and H. Degani, *J. Magn. Reson.*, 1997, **128**, 161.
21. Y. Hassid, E. Furman Haran, R. Margalit, R. Eilam, and H. Degani, *Cancer Res.*, 2006, **66**, 4159.
22. H. Degani, V. Gusis, D. Weinstein, S. Fields, and S. Strano, *Nat. Med.*, 1997, **3**, 780.
23. F. Kelcz, E. Furman-Haran, D. Grobgeld, and H. Degani, *Am. J. Roentgenol.*, 2002, **179**, 1485.
24. C. P. Chou, M. T. Wu, H. T. Chang, Y. S. Lo, H. B. Pan, H. Degani, and E. Furman Haran, *Acad. Radio*, 2007, **14**, 561.
25. E. Eyal, D. Badikhi, E. Furman-Haran, F. Kelcz, K. J. Kirshenbaum, and H. Degani, *J. Magn. Reson. Imaging*, 2009, **30**, 989.
26. E. Eyal, B. N. Bloch, N. M. Rofsky, E. Furman-Haran, E. M. Genega, R. E. Lenkinski, and H. Degani, *Invest. Radiol.*, 2010, **45**, 174.

Biographical Sketch

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