IN MEMORIAM: BERNARD W. AGRANOFF (1926-2022)

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Disclosures: Stephen K. Fisher was previously a member of the Editorial Board of the Journal of Neurochemistry from 1990-1999 and served as Deputy Chief Editor from 2000-2012. Photograph courtesy of the Michigan Neuroscience Institute. The authors wish to thank Dr. Anne M. Heacock for her helpful comments.

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Bernard William Agranoff M.D., or 'Bernie' as he was affectionately known by friends and colleagues, died on October 21, 2022. One of the leading figures in the field of neuroscience, he studied the biochemistry of the brain and was the first to show that long-term memory formation required protein synthesis. He made numerous other seminal discoveries related to the fields of inositol lipids and cell signaling, neuroplasticity and brain imaging and mentored a large cadre of trainees. For 12 years, he directed and reshaped the Mental Health Research Institute (MHRI) at the University of Michigan (U-M) which has since been renamed the Michigan Neuroscience Institute (MNI).

Bernie was born in Detroit in 1926 and grew up in the same city. At a young age he demonstrated a keen interest in chemistry and maintained a laboratory in his basement. Coincidentally, Eugene Roberts, who elucidated the structure and function of the inhibitory neurotransmitter, γ-aminobutyric acid (GABA), lived in the same neighborhood as Bernie and also ran a chemistry laboratory in his basement (Agranoff, 2006)! Bernie attended Cass Technical High School in Detroit and although he elected to start as an art student, he quickly transferred to the science curriculum and graduated in 1944. Bernie then entered the Navy Premedical Officer Training Program and was assigned, by chance, to the University of Michigan. There he was able to further develop his interests in Chemistry and he took a heavy class load, allowing him to graduate in only 2 years. He could have continued his medical education at the University of Michigan but would have had to wait a year before entry-so he transferred to Wayne State University to pursue his MD. From his own accounts, Bernie found Medical School to be rather boring at times and seriously contemplated transferring to a PhD program at the University of Minnesota-but he was talked out of it. Following graduation from Medical School in 1950, Bernie did an internship at the Guthrie Clinic in Pennsylvania followed by a 1-year post-doctoral fellowship at MIT, working with Francis Schmitt who was one of the founders of the field of Neuroscience.

In 1952, the Navy recalled Bernie because of the Korean Conflict and he returned to the Naval Medical School in Bethesda where he ran a clinical Chemistry facility and taught some medical

biochemistry. After completing his tour of duty, Bernie joined the Section of Lipid Chemistry, NINDS that was run by Seymour Kety (a founding editor of the Journal of Neurochemistry). There he was given free rein to pursue his interests and it was here that he developed his long-standing interest in the role of lipids in cell signaling within the CNS. During these 4 years, Bernie made two key discoveries; first, along with Roscoe Brady, he identified cytidine diphosphodiacylglycerol as a liponucleotide precursor required for the synthesis of phosphatidylinositol (Agranoff et al., 1958). This was of importance because of the earlier observation made by Lowell and Mabel Hokin that the turnover of both phosphatidate and phosphatidylinositol was dramatically enhanced in brain and pancreas following the addition of neurotransmitters-but the metabolic relationship between these two lipids had not been established. It is noteworthy that Bernie's elucidation of this pathway occurred more than two decades before the importance of the role played by inositol lipids in signal transduction events within the CNS was fully recognized. Second, while working in Fedor Lynen's lab on a sabbatical at the Max-Planck Institute in Munich, Bernie elucidated the conversion of isopentenyl pyrophosphate to dimethylallyl pyrophosphate, a key step in the biosynthesis of cholesterol (Agranoff et al., 1960).

In 1961, Bernie moved to the U-M as an Associate Professor of Biological Chemistry and member of the newly formed MHRI (where his laboratory was located) and he was promoted to full professor in 1965. At that time Bernie was encouraged by Ralph Gerard, the then Director of the Laboratories of the Institute, to explore the biochemistry of learning and memory. In the mid 1960s, Bernie published groundbreaking studies demonstrating that protein synthesis was required for long-term memory formation in goldfish brain, one with his technician, Paul Klinger and the other with his post-doctoral fellow, Roger Davis (Agranoff and Klinger, 1964; Davis and Agranoff, 1966). These papers, which demonstrated that puromycin had no effect on the acquisition of a light coupled-to-shock avoidance task but could block the formation of long-term memory in the fish, attracted a great deal of interest and led to an invited article in Scientific American on memory formation and protein synthesis that was reprinted 100,000 times with copies distributed to Schools, Colleges, and Universities (Agranoff,

1967). This article was the stimulus for several prominent neuroscientists to enter the field of neuroscience. While Bernie was among the first to propose that neuroplasticity might be a prerequisite for learning and memory, he also recognized that his studies linking goldfish memory to protein synthesis could be considered as correlational rather than causative. Accordingly, he sought a model system in which the formation of proteins in an adult brain undergoing neuroplastic synaptogenesis, as he believed to also occur during memory formation, could be monitored. The regenerating teleost optic nerve, following prior crush, appeared to constitute an ideal model system. Following intraocular injection of radiolabeled proline (Neale et al. 1972), newly synthesized proteins were readily identified in both the ipsilateral retina and contralateral tectum following nerve crush. Two proteins of MW 68- and 70 KDa were of particular interest since they were induced in retinal ganglion cells following optic nerve regeneration (Heacock and Agranoff, 1976). These proteins were subsequently identified as members of a superfamily of proteins that includes 2',3'-cyclic nucleotide 3'-phosphodiesterase, but to this day their physiological significance remains unknown (Ballastero et al. 1995). In 1974, Bernie pursued a sabbatical with R.M. Gaze at the National Health Institute at Mill Hill in England, where he was introduced to the use of retinal explants from frogs or fish whose optic nerves had previously been crushed. When cultured, such retinal explants put out neurites and thereby provided Bernie with an in vitro system readily amenable for studying the biochemical requirements for regeneration. Unexpectedly, the neurites demonstrated a marked tendency to rotate clockwise, an effect attributed to the sliding of growing and spiraling helical fibers over the matrix (Heacock and Agranoff, 1977).

While continuing his studies on neuroplasticity, Bernie maintained an active interest in brain phospholipids. Along with Amiya Hajra, he identified a novel lipid intermediate-acyl dihydroxyacetone phosphate, which provided an alternative biosynthetic pathway for the synthesis of phosphatidate and for the synthesis of ether lipids in brain (Agranoff and Hajra 1971). He also continued his research into phosphoinositides and receptor-mediated cell signaling, making extensive use of a nerve ending (synaptosome) preparation (Fisher and Agranoff, 1987). Although it had previously been widely assumed that the increases in inositol

lipid turnover occurred within presynaptic structures, in a series of nerve lesion experiments, it was demonstrated that dendrite-derived (post-synaptic) structures in the preparation were in fact responsible, a finding consistent with other non-neural tissues (Fisher et al., 1981). Bernie also was one of the first to identify the formation of inositol trisphosphate following receptor-mediated activation of phosphatidylinositol 4,5-bisphosphate breakdown this time in platelets (his own!), using a high voltage electrophoretic technique pioneered in his laboratory several years earlier (Agranoff et al. 1983). In addition to inositol containing lipids, Bernie also had a long-standing interest in inositol itself, having worked on its biosynthesis with Harry Eagle while at NIH. There are nine possible stereoisomers of inositol (cyclitols), of which myo-inositol is the predominant form. Motivated by textbook errors in both the numbering and depiction of the Haworth projections of the six hydroxyl groups, Bernie devised a convenient mnemonic device to visualize the molecule three dimensionally by considering it as a turtle, in which the axial hydroxyl is its head and the five equatorial hydroxyls serve as forelimbs, hind limbs and the tail (Agranoff, 1978; 2009). The "Agranoff turtle" has been widely cited and in his autobiography, Bernie quipped "that it may well outlive his other contributions to science" (Agranoff, 2006)

Bernie also had an interest in applying imaging techniques to monitor neurochemical events in the brain *in vivo* (Frey et al., 1985), and fully appreciated the potential value of human brain neuroimaging in understanding the neurobiology of brain disorders. Consistent with this vision, Bernie played a seminal role in the establishment of a PET facility at the University of Michigan.

Bernie served as Director of the MHRI from 1983-1995, during which time he moved the Institute in a more molecular direction by actively recruiting several junior faculty members whose research programs emphasized molecular and genetic approaches to the study of the nervous system. In doing so, Bernie created a highly collaborative and successful group of neuroscientists that today remain the core of the MNI. In addition, Bernie was instrumental in recruiting Huda Akil and Stanley Watson, future co-Directors of MHRI, to the Institute before he himself became Director. The new and more molecular focus of the MHRI led to its renaming in

2005 as the Molecular and Behavioral Neuroscience Institute (MBNI) and subsequently, to the MNI in 2019.

In recognition of his many research accomplishments, Bernie was elected to the National Academy of Medicine (1991) and to the National Academy of Arts and Sciences (2002). In addition to his contribution to neuroscience research, Bernie cared deeply about the dissemination of neurochemical knowledge and was one of the founding editors (along with George Siegel, Robert Katzman, and Wayne Albers) of the textbook, Basic Neurochemistry, which is now in its 50th year and in preparation for the 9th Edition. He also co-edited Advances in Neurochemistry with Maurice Aprison and the Neurobiological Basis of Learning and Memory with Yasuzo Tsukada. Bernie was a very active participant in Scientific Societies and has served as a past President of the ASN (1973-1975), Chair of the ISN (1989-1991) and has served on the Society for Neuroscience Council. He has also served on several Editorial Boards of Journals including Journal of Biological Chemistry, Molecular Neurobiology, Neurochemical Research, Neuropsychopharmacology and as Deputy Chief Editor of the Journal of Neurochemistry (1982-1985).

The large number of graduate students and post-docs (60+) who trained with Bernie will most certainly be one of his most enduring legacies. Many have gone on to successful independent careers and all are greatly indebted to Bernie for his mentorship and guidance over the years. His trainees, colleagues and friends will always remember Bernie's sharp intellect, great friendship, and his unforgettable sense of humor. Outside of science, Bernie had interests in music, theater, art and above all, wine, and food. His interest in the latter led him to organize symposia on Molecular Gastronomy at Society for Neuroscience meetings and to publish an article on 'Brain Food' (Agranoff, 2008). Bernie's wife, the late Ricky Agranoff, was a warm and caring person who shared Bernie's interest in food. Indeed, Ricky was a gifted and accomplished chef and along with two colleagues, established and ran a renowned restaurant named the Moveable Feast in Ann Arbor for 20 years. Bernie is survived by his two sons, William and Adam Agranoff and their families. He will be greatly missed by all.

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