Acta Microscópica Vol. 10 N° 1, 54-56, 2001
ISSN 0798-4545 / Depósito legal pp. 199202ZU873

Contribution of Humberto Fernández-Morán to the Electron Microscopy

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Humberto Fernández-Morán, was born in Maracaibo (Venezuela) on February 18, 1924 and died in Stockholm (Sweden) on March 17, 1999. He was an outstanding scientist that contributed in a prominent way to the development of the electron microscopy techniques[1] as well as for its applications in biology and medicine.

Fernández-Morán was a pioneer in introducing the concept of cryo-ultramicrotomy[2]; the diamond knife[3] and its applications for the ultrathin sectioning of biological samples and metals[4]; the technique of ultrarapid cryo-fixation with liquid-helium II[5-6]; the freeze-substitution technique for electron microscopy[6-7]; the concept of cryo-electron microscopy[8] and the cryo-electron microscope[9]; the use of superconducting lenses operating at liquid–helium temperature in cryo-electron microscopes[10] and the cryo-ultramicrotome operated at liquid-helium temperature[11]. Fernández-Morán also contributed to the modification of ultramicromes[12]; to the development of pointed filaments and single-crystal tip filaments[13-14] to provide intense microbeam illumination of high coherence used to enhance constrast, to improve resolution and for registering low dose electron micrographs with reduced specimen damage by irradiation; and for the development of specimen cryo-holders for liquid nitrogen or helium[5-6] .

The interest of Fernández-Morán on the cryo-fixation technique by ultrarapid freezing and on the structure of ice crystals[13] led him to become an avid proponent of the direct observation of of unstained biological specimens embedded in ice in a fully hydrated state (*frozen-hydrated* state) and to build in 1966 the first cryo-electron microscope with superconducting lenses operated at liquid-helium temperatures[8]. In his pioneering studies, Fernández-Morán[8-9] examined at 4.2° K several inorganic, organic and biological materials with 1 nm resolution, showing for the first time that the specimen damage could be diminished when low temperatures were used. In spite of this pionering work, which in practice set everything required for the general use of the low-temperature transmission electron microscopy (generally known as cryo-electron microscopy), very little happened for the next decade. The full potential of cryo-electron microscopy became apparent in 1971 after the pioneering work of R. M. Glaeser and coworkers[15-16] that demonstrated that the extent of the beam damage in the transmission electron microscope was diminished at low temperatures; and in 1975 with the seminal work of P. N. T. Unwin and R. Henderson[17] who obtained images at 1 nm resolution of unstained bacteriorhodopsin embedded in glucose at room temperature. Finally it was the work of K. A. Taylor and R. M. Glaeser[18-19] that in 1974 opened the way to obtain high resolution images of unstained

Humberto Fernández-Morán in the occasion of the dedication ceremony of the Venezuelan Institute for Neurology and Brain Research (IVNIC) on April 29th, 1954; which was the precursor of the present Venezuelan Institute for Scientific Research (IVIC). Photography courtesy of the Department of Scientific Photography of IVIC.
(see http://cbe.ivic.ve/hfm.html).

frozen-hydrated biological specimens, the *raison d´être* of cryo-electron microscopy as Fernández-Morán envisaged one decade before[20].

The contributions of Fernández-Morán in biology and medicine are many and very diverse, and I will restrict myself to refer here to only one of them. In the cover of the journal *Molecular Biology of the Cell*[21] published in the June issue of 1999 there were reproduced two electron micrographs taken in the laboratory of Fernández-Morán that were among the first to reveal the complexity of the structure of the mitochondrial membranes. These images were from a study in which Fernández-Morán and coworkers[22] correlated electron microscopic and biochemical data to define submitochondrial "elementary particles" on the surface of the cristae membranes. These particles, known as *Fernández-Morán particles*, consisted of three parts: a spherical or polyhedral head piece (diameter 8 - 10 nm), a cylindrical stalk (~ 5 nm long and 3 - 4 nm wide) and a base piece (4 x 11 nm). Studies by E. Racker and colleagues later showed that the stalk and base of these particles includes a transmembrane domain (F0), which carries protons across the membranes of the cristae; and that the head (F1) comprises an ATPase, which synthesizes ATP when protons pass through F0 down an electrochemical gradient. Both portions F0 and F1 are composed of multiple protein subunits. These images which were published by Fernández-Morán and coworkers clearly demonstrated the asymmetric orientation of membrane proteins and initiated a detailed biochemical research effort that produced a deeper understanding of how chemo-osmosis is coupled to the ATP synthesis to produce the oxidative phosphorylation in different cells. The structure of Fernández-Morán particles initiated the study of oligomeric protein complexes in the membranes in terms of its complexity and assymetry. This pioneering work of Fernández-Morán and his colleagues was seminal to the work done later by P. D. Boyer (University of California, Los Angeles, U.S.A.) and J. E. Walker (MRC Laboratory of Molecular Biology, Cambridge, U.K.) on the elucidation of the enzymatic mechanism underlying the synthesis of adenosine triphosphate (ATP). This work of P. D. Boyer and J. E. Walker was acknowledged when they shared the Nobel Prize of Chemistry of 1997 with J. C. Skou (Aarhus University, Denmark), for his first discovery of an ion-transporting enzyme, Na^+,K^+-ATPase.

Fernández-Morán was the founder of the *Instituto Venezolano de Neurología e Investigaciones Cerebrales* (IVNIC) (Venezuelan Institute for Neurology and Brain Research), precursor of the present *Instituto Venezolano de Investigaciones Científicas* (IVIC) (Venezuelan Institute for Scientific Research) which became a pivotal and exemplar institution for the development of science in Venezuela and in Latin America. To acknowledge the many contributions done by Fernández-Morán to the electron microscopy techniques as well as their applications to biology and medicine, the Venezuelan Institute for Scientific Research (IVIC) designated the Department of Structural Biology (http://cbe.ivic.ve), founded in 1997, in his honor.

"The research of Raúl Padrón was supported in part by an international research grant from the Howard Hughes Medical Institute"

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