In Memory of J. David Robertson

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J. David Robertson, the premier electron microscopist of cell membranes, died at his home August 11, 1995 of intractable leukemia. He was 72 years old. Until weeks before his death, he was still actively engaged in biomedical research and was a Professor Emeritus of Neurobiology at Duke University School of Medicine in Durham, N.C. He is survived by his wife of 49 years, Dody, and by his three children, Karen, an English professor at Vassar; Elizabeth, an English professor at University of Colorado; and James D., Jr., an artist in Cambridge, Massachusetts.

J. David Robertson, pioneering electron microscopist, photographed at his light-box while in his mid-50s.

Robertson was one of the very early members of The American Society for Cell Biology, joining just two years after it was founded in l961, and was a major early contributor to the *Journal of Cell Biology*, publishing seven papers in its first seven years, back when it was known as the *Journal of Biophysical and Biochemical Cytology*. He came to electron microscopy via a medical background.

Born and raised in Tuscaloosa, Alabama, the son of a motorcycle policeman and a grade school teacher, Robertson was educated at the state university in Montgomery. There, he reached his first year of medical school just as World War II began. He enlisted in the U.S. Navy and was sent to finish medical school at Harvard. After receiving his M.D. and training briefly at the Mallory Institute of Pathology in Boston, he returned to practice pathology at the University of Alabama as his military service.

After the war, Robertson chose to return to graduate school to learn electron microscopy in Frank Schmitt's outstanding laboratory at MIT. This new pursuit he attributed to the inspiration of H. Stanley Bennett in the late 40s. (H.S. Bennett, our members will recall, was one of the very first electron microscopists and would later co-found the *Journal of Biophysical and Biochemical Cytology* with Drs. Porter, Palade, and others.)

At that time, F.O. Schmitt's lab at MIT was the premier focus of efforts to combine electron microscopy with other emerging biophysical techniques including polarized light microscopy and x-ray diffraction. This combination of approaches allowed Schmitt and colleagues to elucidate the molecular architecture of the myelin sheath around nerve fibers. One of Robertson's early successes in applying electron microscopy to nervous tissue was to prove that myelin was in fact a spiral wrapping of the Schwann cell membrane around the axon (Fig. 1). This was an idea that Betty Geren had first espoused. (Interestingly, Dr. Geren also trained for her PhD in F.O. Schmitt's lab at MIT at roughly the same time as Robertson. She has gone on to become a well-known electron microscopist at Childrens Hospital in Boston.) Robertson remarked, in describing his reaction to obtaining images like Fig. 1: "I shall never forget the elation I felt when I examined this micrograph with a hand lens and realized what it meant. It showed exactly what would be predicted by Betty's theory." Thus, these illustrious early members of F.O. Schmitt's lab worked together to show that the molecular architecture of myelin could be used to elucidate the fundamental architecture of the cell membrane in general. Robertson chose to illustrate this approach in many memorable diagrams such as that shown in Fig. 2.

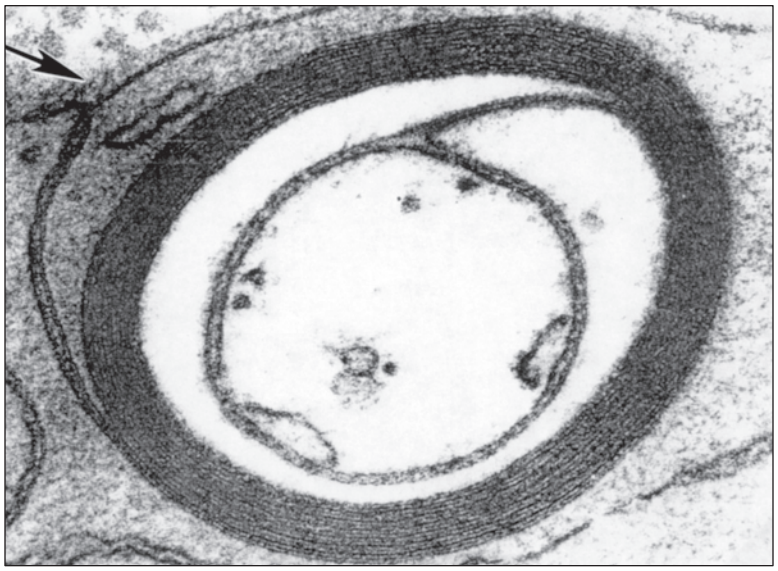

Figure 1. *Robertson's once-in-a-lifetime view of the myelin sheath around a small axon in a neonatal mouse, which showed in perfect cross-section both the inner and outer 'mesaxons' or connections of Schwann cell membrane to the compact dark lamellae of the myelin sheath itself. Such images of $KMnO_4$-fixed nerves proved definitely Betty Geren's original idea that myelin was a compact spiral wrapping of membrane, and hence that myelin would serve as a model for understanding membrane architecture in general. (From: Sci. Amer., 206: 64-72, 1962)*

Robertson pursued his interest in understanding membrane architecture in a series of outstanding early ultrastructural papers, which led soon to his famous "unit membrane" hypothesis. This he first articulated at an American Physiological Society meeting in 1957 (*J. Physiol. Lond.* 140:58-59). His hypothesis held that all cell membranes, those that form intracellular organelles as well as plasma membranes, are composed of bimolecular leaflets of phospholipid with monolayers of protein adsorbed to their surfaces. To support

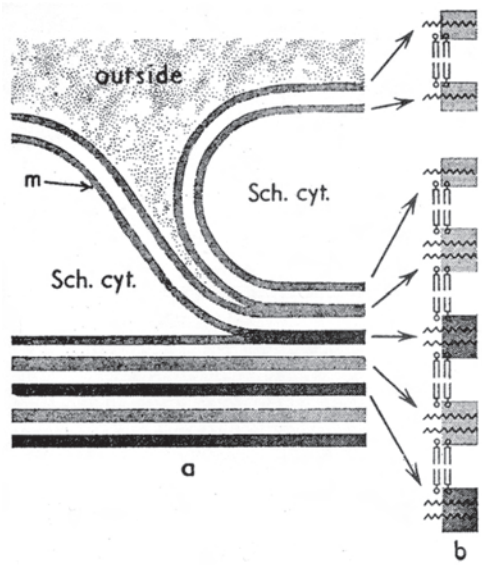

Figure 2: One of Robertson's typically attractive and unambiguous summary-diagrams, in this case of the outer 'mesaxon' seen at the arrow in Figure 1. It showed the various light and dark lines that appeared in optimal thin sections of $KMnO_4$-fixed and expoxy-embedded nerves, and showed how they could be interpreted in light of then-prevailing views about protein and lipid orientation in the myelin sheath. (From: Biochem. Soc. Symp., 16: 3-43, 1959)

this theory, Robertson relied heavily on a particular type of tissue preparation for electron microscopy— $KMnO_4$ fixation, acetone dehydration, and epoxy resin embedding — which invariably made all biological membranes look like ***pairs*** of dark lines in thin sections. This 'double membrane' image he interpreted as resulting from $KMnO_4$ staining of protein monolayers on either side of an unstained central 'core' comprising the bimolecular leaflet of lipids. His original diagram of this interpretation is shown in Fig. 3.

Unfortunately, though Robertson's electron micrographs of cell membranes were exceptional for their day, they failed to illustrate that many proteins penetrate the membrane bilayer, and since his images were necessarily static views, they gave no indication of the remarkable ability of proteins to diffuse laterally within the membrane. Subsequent recognition of such membrane dynamics led Jonathan Singer to propose in 1972 the "fluid mosaic" model of the cell membrane. This view abruptly eclipsed Robertson's "unit membrane"concept and is still the accepted paradigm today.

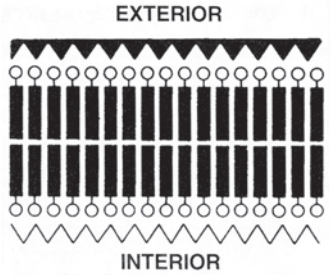

Figure 3: Robertson's original and often-republished diagram of his 'unit membrane' model, which showed the bilayer core of phospholipids as a string of dark bars (representing their acyl chains) with attached balls (representing their head groups), outside of which were zigzags meant to represent the outer layers of adsorbed protein and carbohydrates. Note that this was basically an enlargement of the single Schwann cell membrane shown at the very top of Figure 2. (From: J. Cell Biol., 91: 189s-204s, 1981)

Nevertheless, the basic elements of Robertson's theory (and certain features of his exceptional images of $KMnO_4$ fixed membranes) remain pertinent to still unsolved problems in membrane ultrastructure. For instance, Robertson argued that there could be an exceptional degree of membrane continuity between the plasma membrane and internal organelles, as well as amongst internal organelles (Fig. 4). However, this view was gradually discredited over the next two decades, largely by biochemical work on membrane fusion/fission events in vitro, as well as by subsequent electron microscopy of cells and organelles fixed with glutaraldehyde and OsO_4, the more contemporary fixative, which suggested that membrane organelles are **discontinuous** elements that generally communicate **indirectly** via shuttle-vesicles. Recently, though, the pendulum has begun to swing back in Robertson's favor, largely due to the development of drugs like brefeldin A, which block vesicle shuttling **without** interrupting membrane exchange, both in vitro and in vivo. Such drugs, as well as certain protein mutations that have been engineered very recently, have been found to produce a remarkable degree of membrane tubulation and organelle continuity within living cells, very reminiscent of Robertson's dramatic diagrams like the one in Fig. 4.

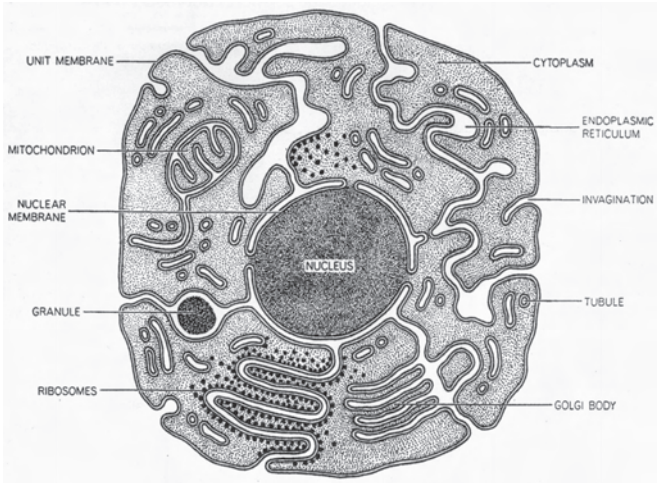

Figure 4: Robertson's now-infamous diagram of a hypothetical cell, illustrating, its legend said noncomittally, "relationships of the cell membrane to various cell organelles." In the original text relating to this figure, Robertson spoke more boldly, proposing that "it would not be unreasonable to conceive of an intracellular 'circulatory system' directly connected to the outside world in some places." Given what is now known about endo- and exocytosis, as well as what is known about membrane trafficking inside the cell, such an extreme degree of membrane continuity is considered unlikely; but Robertson's basic point was that because all cellular membranes are constructed along the same architectural principles, they are at least potentially capable of becoming continuous with each other. (From: Sci. Amer., 206: 64-72, 1962)

These now rather infamous diagrams seemed to suggest that every membrane could be connected to every other membrane inside the cell, even to the point of imagining that the lumen of the nuclear envelope could be continuous with the extracellular space! Today, such diagrams are considered to be dated and misleading (as well as wholly devoid of any recognition of the important role of the cytoskeleton in determining membrane configuration in living cells, since the cytoskeleton was not preserved at all by $KMnO_4$ fixation). Still, adaptations of Fig. 4 continue to appear in many introductory biology textbooks and other lay treatises. By so doing, they can serve to remind us of Robertson's great enthusiasm for his discipline and his determination to maximize the significance of whatever he saw in the electron microscope. Dan Tosteson, the Dean of Harvard Medical School and a long-standing personal friend of Robertson, called him "the most passionate scientist" he had ever known. One particular quote of Robertson's, occasioned by his early observation of the T-tubules in muscle cells, exemplifies this: "When I first saw these structures, everything suddenly clicked in my mind as I realized what they meant, and I got that intense frisson that comes so rarely to a scientist when he makes a truly new and important discovery." (From his reminiscences in the *Int. Rev. Cytol.* 100:129-196, l987. For those who wonder, the OED defines 'frisson' as a brief moment of emotional excitement; a shudder or thrill.)

Robertson's intense intellectual enthusiasm, combined with his wonderful personal charm, led to rapid career advancement. After starting up an outstanding electron microscopy unit in J. Z. Young's world-famous Anatomy Department at University College in London, where Robertson's "unit membrane" concept became — as J.Z. Young once dubbed it — his personal "dilemma," Robertson returned to Harvard Medical School to set up what became perhaps the most technically advanced of the new generation of electron microscope 'application' labs in the U.S. There, he was the first to discover the structural basis of 'electrical' synaptic transmission: namely, the close apposition of adjacent membrane channels that would come to be known as the 'gap junction' (Fig. 5).

Having unraveled myelin by age 30, developed the unit membrane concept by age 37, and discovered the structural basis of electrical synaptic transmission by age 41, Robertson had clearly established himself as a rapidly rising star in the field, a fact acknowledged by his recruitment to Duke Medical School to head its Anatomy Department when he was only 42. There he remained for the rest of his career, building a department of such depth and breadth that, when he retired in 1988, it had to be divided into three separate departments with three heads (Neurobiology, Cell Biology, and Physical Anthropology).

During his tenure as Chairman of Anatomy at Duke, Robertson continued to play a pivotal role in electron microscopy, skillfully bridging the gap between purely technical people who were interested in developing new instrumentation and new preparative protocols, while also supporting a variety of medical people who wanted simply to apply the electron microscope to problems in cell biology and pathophysiology. Meanwhile, Robertson himself continued to explore the forefront of electron microscopy, using emerging techniques such as freeze fracture and Fourier analysis to further advance our understanding of membrane architecture.

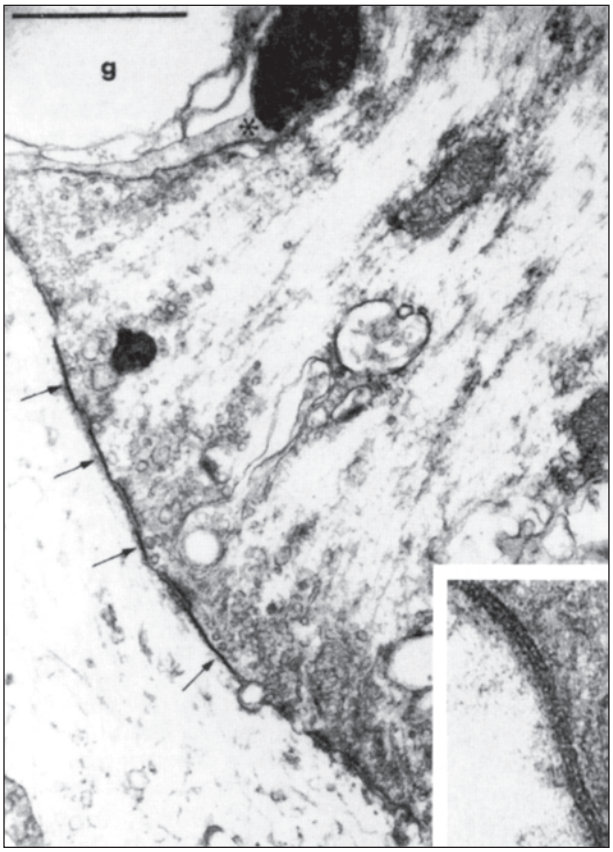

Figure 5: *Ultrastructure of the electrical synapse formed by the 'club ending' of an axon onto a 'Mauthner' motor nerve cell in the goldfish brain, illustrating one of Robertson's first views of the zones of close membrane approximation that later came to be called 'gap junctions'. His original view of the substructure that exists in such junctions, later understood to be close registration of pores composed of 'connexins' in the apposed membranes, is shown at higher magnification in the inset. (From:* J. Cell Biol.*, 19: 201-221, 1963)*

Describing this period from a more personal viewpoint, one of Robertson's illustrious recruits to Duke, Dr. Mike Reedy, recently said: "The Anatomy Department at Duke enjoyed remarkable harmony among the diversity of scholars who flourished under J. David Robertson's chairmanship. His storytelling charm was well known, his talent based on a sense of drama and a twinkle of diffident humor." He added; "Dave and Dody Robertson were a close and supportive couple throughout their 49 years of married life. They remain famous for great personal hospitality to colleagues, and for wonderful parties at Naples, Boston, and Duke that splendidly lubricated the social gears of science."

Upon retiring in 1988, Robertson reactivated an old collaboration he had started with J.Z. Young in London and had continued for many years at the Zoological Station in Naples, Italy, which he enjoyed visiting with his family each summer. This collaboration involved a search for the structural basis of learning and memory in the octopus brain. Robertson began to feel that such neural plasticity was basically due to synaptic "growth" or expansion of synaptic contact areas, and he worked diligently to try to demonstrate that this expansion came about by the induction and extension of actin-rich "microspikes" within the octopus neuropil. To test this idea, he attempted to block octopus learning by applying drugs like cytochalasin D directly onto its brain. This was the type of experiment that continued to fascinate Robertson till the end, despite the fact that it seemed to interest NIH study sections progressively less so.

Still, as was also true for the other great pioneers of electron microscopy, Robertson trained a number of individuals who went on to develop outstanding careers of their own in academic medicine or in industry, and some who even went on to become active members of the current generation of electron microscopists. All of these individuals have publicly and privately expressed their tremendous admiration and affection for him as a scientist and as a mentor. Thus, both by his personal scientific accomplishments and by his successful nurturing of these scientific offspring, J. David Robertson's legacy to the field of electron microscopy will be deep and abiding.

This obituary was originally published in the Newsletter of the American Society for Cell Biology in December of 1995, where it appeared on pages 11 through 13. Unfortunately, it did not include a proper byline and the photograph of Dr. Robertson in his laboratory was cropped too closely.