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Title: The history of myelin

Author: Anne Isabelle Boullerne

Address: 835 South Wolcott Ave. M/C 513 room E720

University of Illinois at Chicago, Department of Anesthesiology

Chicago, IL 60612

Email: abouller@uic.edu

Cell: 312-714-0748

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Abstract

Andreas Vesalius can be attributed the discovery of white matter in the 16th century but Van Leeuwenhoek is arguably the first to have observed myelinated fibers in 1717. A globular myelin theory followed claiming all elements of the nervous system, except for Fontana primitive cylinder with outer sheath in 1781. Remak axon revolution in 1836 relegated myelin to the unknown. Ehrenberg described nerve tubes with double borders in 1833, and Schwann nuclei in 1839, but the medullary sheath acquired its name of *myelin* coined by Virchow only in 1854. Thanks to Schultze osmium specific staining in 1865, myelin designates the structure known today. The origin of myelin though was baffling. Only after Ranvier discovered a periodic segmentation, which came to us as *nodes of Ranvier*, did he venture suggesting in 1872 the nerve internode was a fatty cell secreting myelin in cytoplasm. Ranvier hypothesis was met with high skepticism, because nobody could see the cytoplasm, and *Schwann cell* very slowly emerged in the vocabulary with von Lenhossék in 1895. When Cajal finally admitted the concept of Schwann cell internode in 1912, he still firmly believed myelin was secreted by the axon. Río-Hortega re-discovered oligodendrocytes in 1919 (after Robertson in 1899) and named them *oligodendroglia* in 1921, thereby antagonizing Cajal for discovering a second cell type in his invisible third element. Penfield had to come to Río-Hortega rescue in 1924 for oligodendrocytes to exist. They jointly hypothesized myelin could be made by oligodendrocytes, considered the central equivalent of Schwann cells. Meanwhile myelin birefringence properties by Klebs in 1865 then Schmidt in 1924 confirmed its high fatty content, ascertained by biochemistry by Thudichum in 1884. The 20th century saw X-ray diffraction developed by Schmitt, who discovered in 1935 the crystal-like organization of this most peculiar structure, and devised the g-ratio in 1937. A revolution happened around the same time: saltatory conduction, the very reason of myelin existence, discovered by Tasaki in 1939 and confirmed by Huxley and Stämpfli in 1949. After the second world war, widely available electron microscope allowed Geren to finally discover the origin of myelin in 1954, exactly a century after Virchow coined myelin in 1854. Geren had the genial insight that Schwann cell wraps around the axon and generates a spiral of compacted membrane –myelin. The central origin of myelin took a little longer due to the special configuration of oligodendrocyte distanced from the axon, and in 1962 the Bunges established

the definite proof that oligodendrocyte secretes myelin. The era of myelin biology was launched. In 1973 Norton devised a method to purify myelin which launch the molecular era.

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1543 Vesalius first mention of white matter

The renaissance physician Andreas Vesalius (1514-1564), considered the father of modern anatomy, was the first to describe cerebral white matter and grey matter in the human brain in a monumental treatise known as '*La Fabrica*' illustrated with many detailed anatomical drawings (Vesalius, 1543). Vesalius, born Andries van Wesel in Brussels, was encouraged early by his family to become physician, his grandfather being the Royal Physician of Emperor Maximilian. Vesalius became Professor at the University of Padua and later himself Imperial physician at the court of Emperor Charles V. Since an early age Vesalius dissected cadavers he was stealing in cemeteries and from executed criminals. He published his tome *La Fabrica* at 28 year-old, thereby correcting many errors from the Greek Galen who had only access to animals, and stirred many disagreements among scholars at the time (Zalc and Rosier, 2016). Galen had described the corpus callosum in animals, but the white and grey matter were not distinguished from each other in antiquity. The renaissance also saw the emergence of *cerebrum* and *medulla* (Schmahmann and Pandya, 2007).

1717 Leeuwenhoek first description of myelinated fiber

The first to describe myelinated fibers is arguably Antoni van Leeuwenhoek (1632-1723), draper from Netherlands Delft (Figure 1). He was extremely gifted grinding his own lenses and endowed with avid curiosity, regularly publishing in the London *Philosophical Transactions of the Royal Society*. He described myelinated fibers in a letter dated 2 March 1717 (pages 310-313 in English by Clarke and O'Malley, 1968): "Often, and not without pleasure, I have observed the structure of the nerves to be composed of very slender vessels of an indescribable fineness, running lengthwise to form the nerve. The diameter of the vessels is such that if you compare it with its canal, it is a third larger than the canal." (Leeuwenhoek, 1719). Leeuwenhoek lamented further that in less than a minute the central

lumen of these minute tubes would collapse (Figure 1). He used a lovely image: “Just as if we were to perforate a piece of paper in certain places with a very fine needle and look at the sun shining through those holes”. Leeuwenhoek is credited for naming the nervous fiber a ‘tube’. Leeuwenhoek took the secret of his lenses to the grave. After him a globular theory of nerves prevailed (Ranvier, 1878, page 27): “Indeed, most anatomists of last century and the beginning of this century [19th] had quite other ideas on the constitution of nerves. Since they dissociated them in water, covered them with a thick slide and probably exerted a strong pressure, they saw under the microscope a quantity of granules or globules, which they surmised composed the medullar substance of the nerve. » The globular theory had the unintended consequence to reveal that the so-called globules were constituted by a birefringent substance analogous to fat. Hence 18th century investigators concluded myelin globules composed the integral part of nerves.

1781 Fontana primitive cylinder with outer sheath

Determining the nerve elementary structure was main concern for Felice Gaspar Fontana (1730-1805), director of the Physics and Natural Sciences Museum in Florence (Fontana, 1781, page 203): “whether it is composed of channels or of simple threads; whether it consists only of globules or whether it contains a non-organic, irregular, spongy substance”. Teasing a nerve with extremely fine needles in a drop of in water, Fontana finally obtained (page 204) “several very small, more or less transparent, cylinders … that I shall name *primitive nervous cylinders*”. Not yet convinced, Fontana used his most powerful lenses magnifying 800 fold that revealed the elementary cylinders had a rough less transparent outer envelope made of extremely fine threads. Surprised, Fontana noticed that, despite their extreme slenderness, the threads almost doubled the cylinder thickness through extreme accumulation of curling and coiling (Figure 1). Fontana concluded these cylinders were the elementary structure of nerves (page 207): “The nerve is composed of a large number of transparent, homogeneous, uniform, and very simple

cylinders… Each of these cylinders is endowed by an envelope in shape of outer sheath, which is composed of an immense number of winding threads… and are the simple and *basic organic elements* of nerves, because I could never succeed in dividing them further, no matter the sharpest needles." Fontana's observations were remarkable for his time, noting similarly as Leeuwenhoek that the sheath thickness was almost half the axon diameter. Fontana's observations were corroborated a century later by Ranvier who reported that myelin in water finely disheveled in a myriad of filaments (Ranvier, 1878).

1833 Ehrenberg double contour tube

The next significant contribution was made by Christian Gottfried Ehrenberg (1795-1876), a scientific hero to the Berlin school (Schwann, Schleiden, Kölliker, Virchow…), one of the last universal scientists who traveled to Siberia with Alexander von Humboldt (Prof. Kettenmann, Berlin Max Delbrück Center, personal communication). Ehrenberg was instrumental to the microscope revolution in Germany in the 1830's and felt compelled to mention the Berlin manufacturer: ''Since the year 1834 I use a new and the strongest microscope from Pistor and Schiek and have therefore frequently confirmed and extended my observations". Ehrenberg acknowledged Leeuwenhoek anatomy, and (re)established the fibrillary nature of the nervous system in teased preparations (Ehrenberg, 1833). Ehrenberg had to create a new terminology besides the Latin word *medulla* (*Medullar-Substanz)* simply designating white matter since 15th century. He differentiated varicose (structured as beads on a string) 'brain tubes' (*Hirnröhren*) from cylindrical 'nerve tubes' (*Nervenröhren*). Varicosities are well-known in freshly dissected central nervous system (CNS) fibers by lack of endoneurial sheath (Rosenbluth, 1999). The great merit of Ehrenberg was to suggest a continuity between gray and white matter, and between spine and nerves, but he conceived tubes similarly as Leeuwenhoek vessels (1833, pages 452-454): "The interior of varicose brain tubes is overall quite limpid, so that they could be held for transmitting mist or water… This milk color is absent in the cortical substance, which consists of the tips or beginnings of varicose brain tubes, and thus indeed possess the tube walls, but lack the voluminous contents thereof. From this it seems reasonable to conclude that the white color is inherent in particular to the content of the brain

tube…The cylindrical simple nerve tubes, however, possess an essential difference from the structured brain tubes, for they have a much larger internal cavity, and enclosed there is a very noticeable, less transparent content which has long been recognized. … By transverse section of each nerve its sinewy sheath… is of white color. This is marrow substance." Noticeably Ehrenberg reserved the term double wall (*doppelter Wandung*) for peripheral fibers and his drawing clearly showed four parallel lines (Figure 1). Ehrenberg great merit, in contrast to his predecessors, was to specify for the first time that nerve marrow meant strictly the sheath, and not the whole nerve fiber. His precise terminology for myelinated 'marrow tube' (*markführende Röhre*), composed of a sheath of 'nerve marrow' (*Nervenmark*) around a 'brain tube' (*Hirnröhre*) paved the way for Remak fundamental axon discovery.

1836 Remak primitive band

Robert Remak (1815-1865), embryologist who established the three fundamental embryonic layers, dabbed in neurology as student of Johannes Müller in Berlin University. Remak was allowed to use Müller's and Ehrenberg's excellent microscopes (Clarke and O'Malley, 1968). His first paper described that myelin sheath was acquired by nervous fibers in rabbit developing spinal roots using a new terminology (Remak 1836 first sentence): "We learned from Ehrenberg's investigations that … the brain is composed of varicose marrow-less primitive fibers (*marklosen Primitivfasern*), while nerves are composed of cylindrical marrow fibers (*markhaltigen Fasern*)." Remak described for the first time a fiber not conceived as hollow (*Primitivfasern*), in contrast to Ehrenberg's tubes, then coined the better term of primitive band (*Primitiv Band*), pointing out with candor (Remak, 1837, page 39): «it would be possible that the content of the primitive band was originally cylindrical and the flat form incurred by the pressure of the observation slide". Remak primitive band was made of a pale homogeneous substance, without double line at the edges, contained no marrow (myelin), and absolutely no wrinkle in contrast to the rough appearance of myelinated fibers. Remak concept of axon was breaking away profoundly with the old conception dating back from the Antiquity, viewing nerves as hollow channels transmitting animal spirits. Remak truly re-conceptualized the nervous elementary structure. His

medical dissertation in Latin (Remak 1838) further expanded on the pellucid primitive fiber denuded of sheath (*Fibra primitiva pellucida vaginâ denudata*) and named the sympathic unmyelinated fibers 'organic fiber' (*fibra organica*) (Figure 1). Interestingly, Remak may have borrowed his terms from Fontana's *primitive* nervous cylinder and basic *organic* element*,* acknowledging Fontana had seen the primitive band (Remak, 1838). It should be noted that Remak was the first to describe nuclei on unmyelinated organic fibers (Remak, 1838), but Schwann ended up credited for discovering them (Jacobson, 1993). Remak concept of axon was not immediately unanimously accepted because of the competing globular and animal spirit theories. Remak's bands or fibers persist to this day for unmyelinated pain peripheral nerve fibers.

1839 Schwann sheath and nuclei

Theodor Schwann (1810-1882) worked with Johannes Müller then moved to Belgium in 1839 for the chair of Anatomy at Louvain then Liège University. That same year Schwann and Schleiden published a textbook famous for enunciating the cell theory (Schwann, 1939). Regarding myelin, Schwann disliked the common term marrow and renamed it *white substance*. He described the nervous fibers as composed of two types: common white nervous fibers and gray so-called organic fibers, hence adopting Remak terminology. Schwann is credited for discovering two key elements: neurilemma and its nuclei (pages 146-147 in English translation, 1847): "The white substance [myelin] of each nerve is surrounded externally with a structureless and peculiar membrane, which appears to be minutely granulated. This membrane presents itself as a narrow, clear border, which is readily distinguished from the dark contours of the white substance." The sheath of Schwann should not be confused with the myelin sheath. In numerous instances, Kölliker and Ranvier stated that central myelin lacked the sheath of Schwann. Indeed, there is no cytoplasm lining CNS myelin since the oligodendrocyte plasmatic membrane fuses with myelin. The terms 'membrane of Schwann' and 'sheath of Schwann' were used quite interchangeably in the 19th century literature and is a source of ambiguity. Rudolf Albert von Kölliker (1817-1905), Swiss histologist in Würzburg University, very influential in 19th century across

Europe for demonstrating with brilliant clarity that axons arose from nerve cells, credited Schwann for naming myelin *white substance* and separately discovering a thin sheath, which was actually much more difficult to observe than myelin (Kölliker, 1855). In a subsequent edition of his manual for medical students he used the term "*Schwann´sche Scheide"* (Schwann's sheath), sealing the association of Schwann with neurilemma (Kölliker, 1863). Schwann noticed nuclei on nerve fibers particularly during development before myelination, and was quite surprised to still see nuclei once fibers were myelinated (Figure 1). Schwann proposed these nuclei were a remnant of an earlier stage when nuclei formed a continuous chain of cells that coalesced to form the nerve fibers, formulating the enduring cell-chain or catenary theory (Kettenmann and Ransom, 2005).

Myelin acquires a name

1854 Virchow coins myelin

The word *Myelin* was coined by German pathologist Rudolf Ludwig Virchow (1821-1902), author of the fame Neuroglia (Kettenmann and Ransom, 2005). There was much confusion mid-19th century regarding the medullary substance, and Virchow expressed the need for a better terminology in Virchow's Archiv, the journal he founded (Virchow, 1854, page 571): „das Bedürfniss, sie mit einem Worte bezeichnen zu können, vorliegt, so schlage ich vor, um jede Verwechselung mit anderen schon bezeichneten, aber noch problematischen Substanzen zu vermeiden, sie Markstoff, Myelin zu benennen. The necessity exists of being able to identify a word, so I suggest, to prevent any confusion already created by others and avoid more problematic substances, to name the marrow material *myelin." Myelin* derives from Greek *myelos* after bone marrow color and texture (*medulla* in Latin). Nevertheless, Virchow article entitled "On the disseminated occurrence of a substance analogue to nerve marrow in animal tissues" suggested myelin was quite a floating term, and discussed a substance found in sicken lung and other tissues. In his landmark textbook on cellular pathology Virchow (1858) gave *myelin* wide exposure, but did not strictly anchor it to the nervous system (pages 234-235 in English translation, 1860):" From blood-cells, from

pus-corpuscles, from the epithelial cells of the most various glandular parts, from the interior of the spleen and similar glands unprovided with excretory ducts, this substance can in every case be obtained by extraction. It is the same substance which forms the principal constituent of the yellow mass of yolk in the hen's egg, whence its taste and peculiarities, especially its peculiar tenacity and viscidity which are employed for the higher technical purposes of the kitchen, are familiar to every one. It is this substance, for which I have proposed the name of *medullary matter* (Markstoff), or *myeline,* that in extremely large quantity fills up the interval between the axis-cylinder and the sheath in primitive nerve-fibres." The following decade showed occasional use of *myelin* for equally various attributions.

1865 Schultze magic osmium stain

The discovery a decade later of osmium stain changed everything. Max Schultze (1825-1874), Professor in Bonn and friend of Otto Deiters (Kettenmann and Ransom, 2005), published in the first issue of his journal that a weak solution of perosmic acid was excellent to contrast and harden tissues (Schultze and Rudneff, 1865, pages 301-303): "Next after the fats is the nerve marrow, which takes exceptionally quickly osmium staining … A nerve cord freshly taken from the animal turns deep blue black …Bundles of medullated fibers, which are embedded in gray matter, or individual fibers that were previously hard to see, come forth now with great sharpness … It is obvious that with the help of this staining method a number of important questions will be solved, and that a great future lies ahead for the osmium staining in the anatomy of the brain and spinal cord". Osmium staining was disseminated further through a chapter in the wildly popular *Stricker Handbuch* (Schultze, 1871). A decade later, Ranvier in his seminal 1875 and 1878 books published crisp illustrations of osmium-stained myelinated axons (Figure 2). Ranvier, technological wizard who devoted several chapters to the study of osmic acid, "a reagent of paramount importance for the study of the nervous system" (1878), gave the key why myelin thereafter was not confused with other tissues (1875, page 108): "Osmic acid is not only used to harden tissues, but moreover to color certain elements in more or less intense black, with brown or blue hues. Hence

myelin is colored in blueish black and the fat in brownish black." Ranvier mentioned that despite osmic acid fumes were toxic and dissections carried behind a glass wall to avoid conjunctivitis, all histologists used it. Giving beautiful black and white renditions, osmium was far superior to carmine, and had another welcome property to retain the natural anatomy, preventing myelin 'coagulation' in globules. Obviously with a great future, osmium is used to this day in electron microscopy.

From chaos to organization

1868 Charcot myelin droplets in multiple sclerosis

In 1868, the neurologist Jean-Martin Charcot (1825-1893) used myelin (*myéline*) in what can be considered its first correct attribution. Charcot was an avid and respectful reader of German literature, until the 1870 French-German war dampened his enthusiasm (Guillain, 1955). Charcot established the clinical and histological criteria to diagnose multiple sclerosis in two articles. The histology article described demyelination in lesions (Charcot, 1868, Figure 2) expanded the following year by two Charcot residents (Bourneville and Guérard 1869, pages 46-48): "At the heart of the sclerotic lesion, we find almost constantly globules or granules with fatty element appearance…These myelin drops and fatty granules can infiltrate the mesh of the reticulum and spread wide; they never occupy the center of the sclerotic plaque, since there, the fibrillary metamorphosis and the destruction of the nervous tubes are completed; but, on the contrary, one finds them at the plaque edges, where the medullated cylinder disappears progressively… The nervous tube is thus finally reduced to the axis-cylinder. Accumulation of the medullary or fatty droplets coincides with the destruction of the myelin sheath, and ceases to happen when completed. We can hence conclude that the medullary and fatty corpuscles are only the rubbish from the disintegration of the nervous tubes." This surprisingly accurate description matches the multiple sclerosis lesion stages known today.

1871 Ranvier discovers the node

Louis-Antoine Ranvier (1835-1922), chair of Anatomy at the Collège de France, was interested as much in structure as in function, having trained with homeostasis physiologist Claude Bernard (Boullerne, 2011). He loved experimenting on myelin, studying its swelling and disheveling in water, and observed that water stopped nerve conduction. Ranvier (Figure 2) was struck by the beauty of fresh myelinated nerves he compared to silk having a wavy lustrous finish (1878 page 24):« white, more or less opaque, shimmering and sparkling as moire». Ranvier mastered many staining techniques, including classic carmine used by Ludwig von Mauthner (1860) who first reported the concentric organization of myelin (Figure 1) and his eponymous periaxonal sheath underneath myelin. Using carmine and silver nitrate in mouse thoracic and rabbit sciatic nerves, Ranvier noticed myelinated axons were stained only at regular intervals. He realized myelin had periodic gaps allowing stains to access the axon. Intrigued, Ranvier used a powerful lens to dismiss a possible artifact:" Indeed, under 800 diameter magnification, the nerve tube constriction seems determined by a narrow convex ring confounding itself with the Schwann membrane…that I shall designate by the name of constricting ring (*anneau constricteur*)" (Ranvier, 1871). Ranvier soon provided a much needed drawing (Figure 2), and especially used osmic acid to confirm that myelin was absent at the level of the node (Ranvier 1872). Osmium-blackened myelin glaringly revealed Schwann nuclei nested in the sheath. Surprised, Ranvier determined two important facts: 1) there is only one nucleus per internode, and 2) located at equal distance from each node. Several additional rules were internode proportional to the fiber diameter (the larger the axon, the longer the internode); protoplasm surrounding nucleus extends from node to node; internode elongates during development (Ranvier, 1872). He wondered why, considering the power of osmium, prominent histologists had not formerly observed nodes (1878, page 52): "we only see easily the facts on which one's attention is already attracted. " Ranvier mentioned that only a certain Johann Czermak (1849) from Breslau drew nodes with remarkable acuity as constriction (*Einschnürung*). At any rate nodes were vaguely represented sporadically but dismissed as artifacts. Remak actually provided the earliest node outline with the legend: "constriction, as seen very frequently on all fibers" (1836). It seems Ranvier's

nodes (*Ranvier'schen Schnürringe*) was universally accepted rapidly. Unfortunately Ranvier forcefully claimed the nodes did not exist in CNS, and easily dismissed contemporaries who reported them (Tourneux and Le Goff, 1875). Aggravating this fact, the other towering figure Kölliker also did not believe in CNS nodes. They both had such an enormous influence that doubt persisted well until Santiago Ramón y Cajal (1852-1934), Nobel Prize winner with Golgi in 1906 for the neuronal theory and founder of the Spanish school of neuroscience, imposed nodes centrally (pages 269-270 in Cajal monumental tome of 1909-1911 vol1). The topic was not contentious anymore with Kölliker passed away and Ranvier retired living in seclusion.

1874 Incisures of Schmidt and Lanterman

The sideways incisures present in thick myelin were discovered by two Americans since fallen back into obscurity. H. D. Schmidt from New Orleans reported incisures in fresh nerves after four years of observation, well aware of the skepticism he would meet (Schmidt, 1874). His drawing of incisures in fresh nerves were not forceful tough, and his discovery was rescued by A.J. Lanterman from Cleveland working in Strasbourg (Lanterman, 1877). Lanterman was intrigued by the "hitherto hardly noticed structure in myelinated nerve fibers" and used osmium, thereby producing striking rendition of the incisures. This immediately convinced the scientific community as 'Schmidt and/or Lanterman incisures'. Cajal described their complex shape (1909, page 258 vol1):" These incisures partition the myelin of the internode in a number of rings or cylindrical portions of highly variable volume, interleaved or standing against their basis…These incisures are veritable complete infundibuliform partitions, located all around the axon." The role of these incisures was baffling everybody, and were simply attributed a myelin partitioning, despite stain differences from nodes true 'cellular cement'. By the turn of the 20th century, the complete peripheral internode structure was shown by Cajal in his nervous system histology encyclopedia (1909, page 254 vol1) spelling *a*, Schwann sheath; *b*, transversal disc and Ranvier constriction; *c*, bands of Frommann; *d*, Mauthner sheath; *e*, cylindrical cones of myelin; *f*, Lanterman incisures; *g*, perinuclear protoplasm; *h*, nucleus; and *i,* axis-cylinder (Figure 2).

Guessing myelin origin

1872 Ranvier myelin made by internode adipocyte

Ranvier, a sagacious observer particularly tracking staining artifacts, realized both sheaths of Schwann (1839) and Mauthner (1860) were not simple envelopes but contained a sheet of cytoplasm running from node to node. The question of whether there was cytoplasm in these sheaths was not trivial, because there was no specific stain for cytoplasm at the time. Ranvier hence boldly envisioned the axon enveloped by a muff of cytoplasm with finite boundary at the nodes. All cellular elements being present: plasmatic membrane, single nucleus and cytoplasm containing myelin, allowed Ranvier to formulate the hypothesis an internode equated to a sort of elongated adipocyte secreting myelin inside its cytoplasm (Ranvier, 1872). In subsequent textbooks he greatly expanded the analogy with a fat cell pierced by the axon (Ranvier, 1875; 1878). He speculated further, to the disbelief of his peers, that Schmidt-Lanterman incisures were remnant cytoplasmic bridges between the Schwann and Mauthner sheaths after myelin secretion had completed and taken over most of the cytoplasm. Ranvier revolutionary hypothesis of a fat cell rolled around the axon paved the way to the Schwann cell concept. Ranvier himself never wrote 'Schwann cell' because his hypothesis was highly speculative. The term slowly emerged in the following decades. One of the first instances of *Schwann cell* (*Schwann'schen Zellen*) is by Michael von Lenhossék in the same textbook he coined *astrocyte* (Lenhossék, 1895) regarding cell proliferation during nerve regeneration. Jean Nageotte (1866-1948), successor of Ranvier at the Collège de France, adhered early to the prevailing concept that the 'cell of Schwann' was restricted to the nucleus surrounded by a heap of cytoplasm (Nageotte, 1910). Schwann cell at the beginning was viewed as a tiny flatten sheath cell apposed to myelin with various opinions on its cytoplasmic boundaries.

1909 Cajal and Nageotte axon makes myelin

Cajal enunciated in 1909 the prevailing opinion (page 264 vol1): "Regarding myelin, far from being the content of a corpuscle the axon would cross, it would be a simple secretion product from the axon itself." He did not see any cell in peripheral internode, simply assuming the nucleus was integral part of the Schwann membrane, because it easily detaches from myelin like an orange peel. Cajal however shifted his view after investigating cytoplasm, thereby sealing his acceptation of the 'cell of Schwann' (Cajal, 1912, page 223): "Ranvier had a brilliant intuition when he considered the interannular segment of the nerve tube as a vast cellular unit, within which was the axis-cylinder [axon] and the inclusions of cylindrical cones of myelin." Cajal was seduced by the beautiful rendition of cytoplasm using methylene blue method (Nemiloff, 1910). Methylene blue stains nucleic acid of cytoplasm synthesis organelles, which allowed Cajal to visualize the entire layer of cytoplasm from node to node. Cajal dutifully listed all Schwann cell structural elements with the glaring omission of myelin. Cajal still believed axon secreted myelin, and that myelin 'possesses' Schwann corpuscles (*mielina posee corpúsculos de Schwann*) presumably for support (Cajal, 1912). A year later, after the ultimate gold sublimate impregnation stained virtually all astrocytes, Cajal grew frustrated by the adendritic and apolar cells he still could not see, dubbed 'third element' (Cajal, 1913). He ventured to compare the white matter apolars to Schwann cells, based on their mutual exclusion from peripheral nervous system (PNS) and CNS. He surmised their presence filled a physiological role, but not based on any capacity to make myelin. Cajal, like Nageotte and his contemporaries, could not see any physical link between axon and apolar cells so how could they make myelin? Cajal speculated apolar cells in satellite perineuronal location had a perfect physiological symbiosis with neurons (Cajal, 1913). The Schwann cell in Cajal model was doing the same: support the axon make myelin.

It is quite difficult to pinpoint when and who first formulated the false theory of myelin axonal origin, but is certainly got broadcasted in a few high profile books. Nageotte wrote a chapter in one of them (Nageotte, 1932), in which he clearly explained why axon made myelin in absence of physical link between myelin and glial cells (pages 200-201): "I shall say simply that the fibers of the central system

possess a sheath of myelin constructed exactly like that of the peripheral nerve fibers, with easily demonstrable nodes and incisures. Now, whatever relations may exist between the central fibers and the oligodendritic cells of the neuroglia, it is impossible to see in the myelin a territory belonging to these cells, which amounts to saying that it forms a part of the neurite itself. The topographic relations, then, between the segments of myelin and those of the Schwann sheath are purely physiological and everything indicates that in this association of heterogeneous segments, it is the myelin that takes the initiative. " The theory of an axonal origin was deeply engrained and endured well until the 1970's.

Of note another Ranvier fellow, William Vignal (1852-1893), the first to represent individual Schwann cell against the erroneous syncytium theory (Jacobson, 1993), was particularly interested by nerve development (Vignal, 1889). He used osmium to track myelin formation and elegantly showed that the first thin layer of myelin hugging the axon underneath the 'connective' or 'sheath' cell (Schwann cell) was weakly stained by osmium. He interpreted myelin was still mingled with albumin material inside the protoplasm from which it was not yet completely separated. As myelination progressed, it was stained darker by osmium in a more 'perfect' way. Unfortunately, Vignal attributed the cytoplasmic muff to the axon, thus also believing myelin was secreted by the axon with a possible involvement of the homogeneous substance of the axon itself.

1922 Río-Hortega oligodendroglia could make myelin

The question of who first suggested myelin is made by oligodendrocyte is not easy to answer. We should perhaps start by the context surrounding the discovery of oligodendrocytes. Oligodendrocyte were first stumbled upon by the Scottish William Ford Robertson (1867-1923) in 1899 using a lengthy platinum impregnation over several months which revealed small branched cells (Robertson, 1899). Because these new cells had a strikingly different morphology than neurons or classic neuroglia (astrocytes) and uniquely took platinum, Robertson referred to them as 'mesoglia' basing his belief on their mesodermal origin (Robertson, 1900). It was the first time ever cytoplasmic expansions of oligodendrocytes were

visible, but Robertson never made any connection between mesoglia cells and myelin that he considered separate entities. A controversy exists as to which exact cells Robertson stained, whether oligodendrocyte, microglia, or both. However, as pointed by Wilder Penfield: “the method was so unreliable that it never found its way into laboratories outside of Edinburgh” (Penfield, 1928). Nevertheless, to honor the memory of Robertson pioneering work, Río-Hortega, concurring with Penfield, named a subpopulation of oligodendrocytes the "Robertson cells" (Río-Hortega, 1928) .

The rightful discoverer of oligodendrocyte is Pío del Río-Hortega (1882-1945) who in 1912 moved to Madrid to join Cajal’s disciple Nicholás Achúcarro and study neuroglia learning precious metal impregnation techniques. The following years Río-Hortega perfected histology during stints abroad in Paris, Berlin and London that led him to develop the famous silver carbonate method, which succeeded to capture Cajal’s third element. He revealed not one, but two new cell types he named *microglia* and *interfascicular glia* (Río-Hortega, 1919). This publication triggered the ire of Cajal and, joined to the premature death of his protector Achúcarro, forced Río-Hortega to leave Cajal laboratory and establish another laboratory in the *Residencia de Estudiantes* some 2 km away (Río-Hortega, 2013) . The same year he formally introduced interfascicular glia as *oligodendroglia* (Río-Hortega, 1921). Silver carbonate revealed oligodendroglia cytoplasmic processes spiraling around unstained myelin (Figure 2) reminiscent of Schwann sheath. A year later he cautiously ventured comparing oligodendroglia to Schwann cells, while reverentially substantiating Cajal hypothesis (Río-Hortega, 1922) and published in French for international outreach (Río-Hortega, 1924). Axon wrapping by oligodendroglia and Schwann cell pointed to similar physiological functions, as stated in the last sentence (Río-Hortega 1922, English translation 2012): “We are inclined to believe, however, that both kinds of cells carry out identical functions of support, isolation, and nutrition connected with nerve conduction”. Río-Hortega was conflicted on the origin of myelin and presented pro and con arguments (Río-Hortega, 1922). His main argument against the prevailing Cajal and Nageotte belief of axonal origin was discovering centrally an ensheathing cell. However the evidence was only circumstantial since he could not establish a physical link between myelin and oligodendroglia.

1924 Penfield support for oligodendroglia

The situation was frozen with Cajal, who refused to use Río-Hortega exact procedure out of pride (Cajal created excellent methods used to this day), and could never stain oligodendrocytes. Río-Hortega was embattled when in 1924 he welcomed American neurosurgeon Wilder Graves Penfield (1891-1976), who later founded the renowned Montreal Neurological Institute at McGill University. Penfield was fresh blood and Río-Hortega purposely wanted "a serious neurologist, hardly suggestible and a good technician to check our findings and give his valuable opinion on obscure points" (Río-Hortega, 1928). Penfield sojourn in Madrid lasted only 6 months but was extremely productive. He published a landmark article in Brain that put oligodendroglia on the map and helped overcome Cajal skepticism (Penfield, 1924). Although the silver carbonate method was temperamental, sometimes revealing also microglia and/or astrocytes, a selective stain had oligodendroglia prolongations beautifully clear-cut and much ramified (Penfield 1924). Penfield was more explicit regarding oligodendrocytes could make myelin (Penfield 1924, page 450): "The function of oligodendroglia cells is not settled, but their relation to nerve cells and medullary sheaths corresponds strikingly with the relation of the sheath of Schwann cells to peripheral nerves. Moreover, oligodendroglia cells appear in the CNS at the time of maximum myelinization, and contain unusually large cytoplasmic granules which suggest a secretory function. These facts, as well as the arrangement of the cells along the medullary tubes, make it probable that they have to do with the elaboration and maintenance of myelin."

Four years later Río-Hortega published a long memoir on oligodendroglia in which he classified 4 types according to phenotype: type I included "Robertson cell" (polydendrocyte) and cells wrapping multiple small axons; type II contacted parallel axons; type III dealt with few larger axons; and type IV had a one to one relationship with the largest axons similarly as Schwann cells (Río-Hortega, 1928). He noted the term *oligodendroglia* was immediately accepted by the scientific community, replacing their former dozen names. Five years later Penfield edited the first book in English on neuroglia, in which Río-

Hortega presented *microglia* (finally accepted by Cajal), while Penfield introduced *oligodendrocytes* (never accepted by Cajal). Penfield's genius was to compare oligodendrocytes alongside classical neuroglia, back then restricted to astrocytes, which facilitated their acceptance as a new cell type. Río-Hortega had already appropriated for himself the hypothesis that oligodendrocytes generate myelin (1928, page 11 in English translation 2013): « The American neurologist agreed with our hypothesis of possible involvement of the oligodendroglia in the formation and support of the myelin sheath." We will never know who, Río-Hortega or Penfield, first floated the idea, but we can be certain they discussed it.

Myelin functions: myths and reality

By mid-19th century, the concept of axon had replaced myelin as the essential and active component of nervous fiber. Myelin origin and function puzzled early investigators by appearing relatively late during development and not investing all fibers in adult. The following section, by no means exhaustive or comprehensive, presents the most remarkable theories.

1858 Virchow insulation of medullary sheath

Nerves were known to transmit electricity, which prompted Virchow to audaciously formulate the first insulation theory (Virchow 1858, pages 235-236 English translation, 1860): " The axis-cylinder [axon] would therefore seem to be the real *electrical substance* of natural philosophers, and we may certainly admit the hypothesis which has been advanced, that the medullary sheath rather serves as an isolating mass, which confines the electricity within the nerve itself, and allows its discharge to take place only at the non-medullated extremities of the fibres."

1878 Ranvier theories

Ranvier proposed several theories from his significant work on myelin (Ranvier, 1878). His elegant comparison with the transatlantic telegraph cables, operational in 1866, drew from Virchow hypothesis (page 131): «Myelin has perhaps another role; it is probably an insulating envelope. We know that electrical connections which are immersed in a conductive medium must be isolated by a non-conductive sleeve; construction of submarine cables rests on this principle. It is conceivable that transmission of the sensitive or motor impulses may have some analogy with the transmission of electricity, so perhaps each nervous tube must be isolated for this transmission to be more efficient." Ranvier also correctly suggested myelin confers an evolutionary advantage (page 133): «Nerve tubes with myelin do not exist in invertebrates. Therefore, they are not essential to the nervous system manifestations, since many animals possess all the nervous functions: sensitivity, motility, nutrition without having myelin tubes …The nervous tubes with myelin seem therefore constitute an improved transmission apparatus particular to the vertebrate nervous system." By far his most entertaining theory is about the nodes (page 34-35): "If liquid myelin was uninterrupted in the entire length of the nervous tube, in example in man sciatic nerve which stands vertical in our usual posture, it would glide to the lowest part by its own weight; there would not remain anymore myelin in the superior part of the nerve. But this is not the case; the myelin sheath is interrupted from distance to distance by transversal partitions which retains it." Ranvier more sensibly attributed nodes a nutritive role since myelin insulated the axon so well from dyes (page 132): «These observations allow us to conclude that the penetration of crystalloid materials or, if you prefer, the diffusible elements necessary for the axis-cylinder nutrition, which, as we know and as I will demonstrate, is the most important part of the nervous tube, would not easily happen if it was surrounded by myelin in its entire length…At any rate the penetration is much faster and much easier at the level of the nodes, and we can surmise, without over speculating, that it is through them that nutrition of the axis-cylinder occurs."

1914 Myelin as energetic fuel

Well before saltatory conduction was conceived, joined to a vastly unknown chemistry, the function of myelin unbridled imagination. The sixth edition of an American textbook for medical students is a telling example (Sajous, 1914, page 532): “May this supposed coating and insulating material, myelin, not be to the nerve what myosinogen is to muscle? The functioning nerve is the seat of *increased combustion*… This suggests that the nodes themselves ...may allow the blood-plasma to filter through them, thus bringing the oxidizing substance in immediate contact with the axis-cylinder… Indeed, if the various features enumerated are collectively considered, it will become apparent that *the myelin,* or *white substance of Schwann, when in contact with the oxidizing substance of the blood-plasma undergoes a reaction in which chemical energy is liberated.*" Recent developments have provided an interesting twist to this theory of myelin as energetic fuel. Two independent investigators, Klaus-Armin Nave based in Germany (Funfschilling et al., 2012) and Jeffrey Rothstein in the USA (Lee et al., 2012), have shown that myelin indeed is providing energetic support to the axon, which might explain why the axon eventually degenerates upon being stripped of myelin. The fuel currency has been found to be lactate, shuttled by the oligodendrocyte to the axon, in contrast to a self-combustion of the fatty sheath itself as enunciated a century earlier.

Biochemistry of myelin

1884 Thudichum biochemistry of myelin

It is beyond the scope of the present review to provide a detailed account of myelin chemical composition discovery. It was known by mid-19th century that in white matter the axons “consist of protein components very similar to muscular fibrin, the marrow sheath especially of fats from various

kinds" (Kölliker 1863, page 105). Toward the end of 19th century, Johann Ludwig Thudichum (1829-1901), considered the founder of neurochemistry, partially characterized many lipids of mylein, including its most characteristic galactocerebroside (Thudichum, 1884). Thudichum was in advance for his time and his 1884 book got widely criticized and rejected (Breathnach, 2001). A sample of galactolipid purified by Thudichum was discovered by chance in London and HPLC analysis determined a purity around 85%, not a minor feat for 19th century methods. At the turn of the 20th century, myelin was viewed as a semiliquid albumin-fatty substance, which chemical composition appeared one of the most complex and included "cholesterin, protagon, lecithin, cerebrin and neurokeratin" (Cajal, 1909). Speculation on the actual components began in the period 1914-1920 with the pioneering studies of Koch and Koch, MacArthur and Doisy, but more accurate inputs on the chemical nature of myelin came after World War I (reviewed in Norton and Cammer, 1984).

1973 Norton myelin purification method

A recurrent problem impairing the determination of myelin composition was the lack of a purification method until the 1970's. The earliest procedures were published in 1962 by Victor Whitaker in England and Eduardo de Robertis in Argentina, who isolated multiple brain membrane fractions including nerve endings and myelin with differential and density gradient centrifugation. William Norton (1929 -) at Albert Einstein College of Medicine in New York, one of the most prominent myelin biochemists of the 20th century, improved these procedures by devising a sucrose gradient for specifically isolating CNS myelin, which culminated in a landmark paper (Norton and Poduslo, 1973) effectively unlocking access to studying myelin chemistry. Norton went on to a seminal work that revealed isolated myelin contains 70-85% lipid and 15-30% protein depending on the source; no lipid is exclusively found in myelin but enriched; large differences in lipid composition exist between CNS and PNS; and myelin is remarkably conserved for both proteins and lipids across mammals and non-mammals (review in Norton and Cammer, 1984). Considering we are still mapping the proteins of myelin, the ultimate frontier may be differences by localization -spinal cord versus brain areas- and by oligodendrocyte subtype. Occasional investigations on myelin proteins (lipids are virtually ignored) have uncovered differences between small

and large axon myelin, but the majority of studies focus on Río-Hortega type II oligodendrocyte myelinating small and medium axons (Butt, 2013).

Saltatory conduction

1939 Tasaki shows saltatory conduction

The real function of myelin of course is saltatory transmission of nerve impulse, leaping from one node of Ranvier to the next. It was first suggested in 1925 by Canadian born Ralph Stayner Lillie (1875-1952), Professor of General Physiology at the University of Chicago (Lillie, 1925). Lillie experimented on an iron wire, considered a valid 'passive' nerve model. In a stroke of genius, he modeled the nodes by enclosing an iron wire in a glass insulant tube with periodic breaks, and noted that electric conduction occurred surprisingly faster in saltatory fashion. Pointedly noting the analogy with myelinated nerves transmitting impulse tenfold faster than when unmyelinated, he formulated the hypothesis that ions enter the axon at the nodes and generate saltatory conduction. This was not trivial to prove, tough. Another fundamental question was to understand how the impulse is propagated through long distance without fading. A theory had proposed local circuits propagated nerve impulse (Hermann, 1879). Sir Alan Lloyd Hodgkin (1914-1998), biophysicist in England Cambridge University, was primarily concerned with how an impulse in frog sciatic nerves blocked by cold or pressure summed with a subthreshold electric shock (Hodgkin, 1937). Hodgkin assumed that several internodes contributed to the potential which is recorded at any point in the axon, and further the node incurred a significant delay to nerve transmission. Hodgkin study did prove however the existence of local circuits producing the 'electrotonic' potential, developing an impressive array of mathematical formulae in a major step toward action potential mechanism.

Concurrently Ichiji Tasaki (1910-2009), biophysicist at Tokyo Keio University, was pursuing the study of anesthetics on isolated nerve fibers (Figure 3). At that time, there was ample evidence for an

outward flow of current at the nodes propagating the impulse. Tasaki observed that conduction was blocked when three or more nodes were anesthetized (Figure 3). He concluded that the flow of outward current directed at the neighboring nodes was so strong that, even after undergoing progressive attenuation in the anesthetized region over one or two nodes, it was still capable of exciting the first normal non-anesthetized node. In other words, the action potential jumps over the anesthetized region providing it does not exceed one or two nodes, and conduction block occurs because the current becomes *subthreshold* (too weak). Tasaki published these findings in the American Journal of Physiology just before World War II (Tasaki, 1939). During the following years, Tasaki extended his observations that the node was the only place where an inward current can be observed, but he could not publish anymore in the USA. He had to send his manuscripts to Germany via the Siberian railroad, rerouted after 1941 by submarine via South America and learned of their publication after the war (Tasaki and Takeuchi, 1941; 1942). Tasaki eventually had a long career at the U.S. National Institutes of Health.

1949 Huxley and Stämpffi prove saltatory conduction

Sir Andrew Fielding Huxley (1917-2012) shared the Nobel Prize with Hodgkin in 1963 for their discovery of the basis for action potential in the squid giant axon. The two brilliant English scientists carried out research at Cambridge University in the 1950's that laid down the foundation for modern electrophysiology. After World War II, Huxley collaborated with Robert Stämpfli (1914-2002) in Bern University Switzerland, who was elected in 1963 at the prestigious German Academy of Sciences Leopoldina. Huxley and Stämpfli experimental studies brought measurements of the resistance and capacitance of myelin, formerly assumed entirely insulant, and they developed complex mathematical equations to evaluate conduction velocity. Their work confirmed Tasaki's findings and culminated in a landmark publication strongly supporting the *saltatory* conduction of action potential from node to node in isolated frog nerves (Huxley and Stämpfli, 1949). Saltatory conduction was soon confirmed in the undissected (intact) myelinated frog fiber which sealed its acceptation (Frankenhaeuser, 1952). In summary, myelinated fibers act as receiving and transmitting stations at the nodes of Ranvier, receiving

the passive currents coming from the preceding node and generating a new action potential reaching the next node (Hodgkin's cycle). The conclusion of experimental research started in 1780 with Galvani and concluded in 1952 with Frankenhaeuser is that nerve conduction is electric but basically different from the current flow in a conductive cable.

Crystalline molecular organization

1924 Schmidt birefringence

Myelin optic property of birefringence was discovered only in the second part of the 19th century because of the technical aspect of microscopes. Polarized light is generated by a pair of perpendicular filters, generating positive and negative birefringence. Gabriel Gustav Valentin (1810-1883), German physiologist in Bern University, published the first study with polarized light (Valentin, 1861). When myelinated fibers were analyzed by Theodor Albrecht Klebs (1834-1913), assistant of Virchow in Berlin, a positive birefringence radiating from a neutral center (axon), and a negative birefringence when viewed laterally, suggested a high degree of molecular organization (Klebs, 1865). Much later, Gustaf Fredrik Göthlin (1874-1949) from Sweden Uppsala University, reported two birefringence patterns in nerve fibers: a protein-dependent, and a lipid-dependent that predominated only when myelin was present (Göthlin, 1913), thereby confirming chemistry that myelin is mainly composed of fatty material. Fascinated by the deep aesthetic of polarized exquisite colors, Wilhelm Joseph Schmidt (1884-1974) in Germany Jena University, made fundamental discoveries with polarizing microscope later confirmed by electron microscope, and laid the foundation for myelin ultrastructure. Schmidt established that the myelin sheath is constructed of lipoid fluid crystals with optic axes perpendicular to the axon direction and radially oriented (Schmidt, 1924). He then directed his attention to the protein elements of myelin by comparing fresh and alcohol-treated frog sciatic nerves. Alcohol extracted lipids from myelin and replaced the positive uniaxial birefringence of lipoids by a weak negative uniaxial birefringence, also

with radial optical axis, that signaled a protein fraction in myelin, not to be confused with axonal proteins orientated differently (Schmidt, 1936).

1935 Schmitt X-ray diffraction

Further elucidation of myelin molecular architecture awaited more powerful techniques. This was achieved by Francis Otto Schmitt (1903-1995) at the Massachusetts Institute of Technology (MIT), 20th century brain pioneer who mastered polarized light, spectroscopy, electron microscope, and especially developed X-ray diffraction (Figure 3). X-ray diffraction revealed a concentric pattern of myelin (Figure 3), and confirmed both CNS and PNS myelin across vertebrates is made of oriented fluid crystals, whose lipid long chains were "astonishingly well oriented radially" and perpendicular to the axon (Schmitt et al., 1935). A repetitive period was identified in fresh nerve myelin as a long fundamental spacing of 171 Å, which Schmitt accurately translated into eight lipid molecules for two fused membranes (1935, page 145): "If the myelin sheath is made up of concentric layers of these leaflet fluid crystals, each having a thickness of eight molecules, or 171 Å, there would be something less than two hundred such layers even in the thickest myelin sheath and perhaps only of the order of several dozen in thin sheaths." In a major step forward, Schmitt modelized the myelin fundamental unit (Figure 3), in which doublet of lipid bilayers were intercalated with protein sheets, the lipid polar groups facing aqueous interfaces and loosely bonding proteins (Schmitt et al., 1941). Of note, he lipid bilayer membrane concept was established in 1925 and generalized by Schmitt through polarized light. Interestingly, polarized light also showed the presence of protein oriented tangentially and concentrically in myelin (Schmidt, 1936; Chinn and Schmitt, 1937) impossible to detect by X-ray diffraction with accuracy. Schmitt determined the fundamental myelin period varied from 170 Å in amphibian to 186 Å in mammalian PNS (Schmitt et al., 1941). Later studies in CNS showed the myelin period is slightly shorter at 160 Å. In summary, mammalian nerve myelin in fresh state consists of two lipid bilayers, each about 5.5 nm (55 Å) thick, which alternate with 3 nm (30 Å) thick protein layers. An entire myelin lamella therefore measures about 17 nm across (170 Å) depending hydration. The lamellar structure of myelin was finally visualized

by the advent of electron microscope by Fernández-Morán (1950), then Sjöstrand (1953) at the Stockholm Karolinska Institute, who provided a typical image of stacked lamellae. The spacing by electron microscopy showed a typical 30% shrinking from fixation and dehydration, but maintained the difference between peripheral (119 Å) and central myelin (107 Å).

1937 Donaldson and Schmitt g ratio

Around the turn of the 20th century, Henry Donaldson at the University of Chicago took a mathematical approach on myelin, calculating the surface areas of cross-sectioned axon and sheath. The invariability of their ratio prompted Donaldson and his student Hoke to systematically measure thousands of nerve fibers from 27 vertebrates representing the five main classes (fish, amphibians, reptiles, birds, and mammals including human). Their tedious observation showed that despite a large variation in axon size, from minute rat axons to the largest skate axons, the myelin sheath remained strikingly proportional (Donaldson and Hoke, 1905). They inferred that axon volume equaled that of the sheath, which was a point of remarkable similarity in vertebrates. Schmitt in 1937 took a quantitative approach but on myelin birefringence, formerly essentially qualitative. Schmitt explained that polarized light entering anisotropic myelin splits the initial beam into two perpendicular refractive indices, n_1 and n_2, with one retarded with respect to the other by a distance Γ (Greek gamma letter g). This relative delay obviously depends on myelin thickness. The chief difficulty of quantifying birefringence resulted from the radial orientation of myelin lipids (Schmitt and Bear, 1937). Schmitt solved the problem by developing a mathematical equation including Γ, the fiber diameter d_1 and the axon diameter d_2 (Figure 3). Schmitt was then struck by the continuity of the sheath birefringence curve plotted against the fiber diameter. In other words, the g ratio (axon diameter/fiber diameter) variation had great continuity and a tendency to decrease with axon diameter. Schmitt also noted the sheath disappeared altogether for axons under 2 micrometer diameter. Because Donaldson and Hoke (1905) studies were designed primarily to test constancy across species, but not across fiber range, Schmitt rightfully did not find his findings in conflict with Donaldson's. Further studies by Claes Hildebrand at the Karolinska Institute in

Sweden would show that the g ratio in CNS, similarly as in PNS, increases with axon diameter then reaches a plateau (Hildebrand and Hahn, 1978). Hildebrand noted further species differences with a linear increase with axon diameter in mouse, while in frog the number of lamellae increased very slowly and was markedly curvilinear. These observations launched the complex biology of myelin thickness regulation still being deciphered.

Mystery solved

1954 Geren myelin spiral of Schwann cell

The origin of such an exceptionally organized structure remained a mystery. The key person who solved it was Betty Ben Geren (1922-), neuropathologist who graduated MD in 1945 at St. Louis Washington University followed by a pathology internship at Boston's Children's Hospital (Figure 4). Geren received an American Cancer Society Research postdoctoral Fellowship to study with renowned Schmitt, and first addressed the question of whether myelin built by 'crystallizing out' droplets of lipid-protein, or started out as oriented layers. Geren observed by electron microscope a daily addition of layers inside the Schwann cell cytoplasm of chick embryo nerves (Geren and Raskind, 1953), but Geren was still reasoning with the prevailing concept of *concentric* layers. During frequent visits to MIT, Herbert Gasser, Nobel Prize winner in 1944 with Joseph Erlanger for physiological functions in single nerve fiber), made Geren familiar with Schwann cell *mesaxon* surrounding unmyelinated fibers (Gasser, 1952). A year later, while waiting for the microscope to cool off at 2 a.m., Geren had a luminous insight: "I picked up the chicken sciatic prints for just another look when all of a sudden I saw Gasser's mesaxon, and then it was longer and then another picture it was spiraled- no wonder that this couldn't be appreciated by light microscopy. And then I knew that this was myelin beginning to form." (Geren personal communication). It explained why the myelin unit was composed of two membranes instead of one, which Schmitt could

not fathom, and specified the layers were not concentric but *spiraling* (Figure 4). This paper is arguably the most important ever published on myelin (Geren, 1954). The discussion speculated on possible compaction mechanisms, and Geren correctly suggested the drive of the inner mesaxon to extrude cytoplasm from myelin. Geren hypothesis initiated one of the most challenging 3-D problem to solve in biology, followed by decades of speculation on how Schwann cell can spiral such a high number of myelin layers. Unfortunately, Geren images were spotty in this early age of electron microscope, and her publication was taken as a bold hypothesis rather than solid proof. It did not trigger immediate acclaim, although Nobel Prize Gasser did send a congratulation letter for her breakthrough discovery. Fate had Geren cross the path of same age MD biophysicist James David Robertson (1922-1995) while he graduated for PhD with Schmitt (Figure 4). Robertson, then a faculty at Kansas University, set to prove Geren hypothesis, and obtained an early success by proving that myelin is really a spiral around the axon inside Schwann cell cytoplasm (Robertson, 1955). Robertson was absolutely elated when he examined the micrograph with a hand lens and realized it showed exactly what Betty theory had predicted (Figure 4). Robertson would pursue understanding membrane architecture in a series of outstanding ultrastructural papers during the 1950's-1960's. He eventually devised the unifying theory of "unit membrane" that all cell membranes consist of a bimolecular lipid leaflet with protein adsorbed to the surface (Robertson, 1962).

1962 Bunge oligodendrocytes also spiral myelin

The studies of Geren and Robertson in the PNS triggered a rush by several laboratories to find a myelin spiral in CNS, but some prominent investigators concluded it did not exist (Luse, 1956; de Robertis, 1958). Luse (1956) provided evidence that oligodendroglia participate in the production of CNS myelin, but she held open the possibility that myelin is the product of other glial cells, while at the same time doubting the very existence of nodes. The case of de Robertis and collaborators (1958) is interesting because he was the first to show by electron microscopy a physical link between myelin and oligodendrocyte. He hence rightly believed CNS myelin was produced by oligodendroglia, but

unfortunately rejected the concept of spiral, and instead interpreted that myelin layers were formed concentrically within oligodendrocyte cytoplasm. Technical problems of electron microscopy were very difficult to overcome in CNS. Introduction of fixation by osmium tetroxide (Palay et al., 1962) launched routine electron microscope study of CNS that made possible the analysis of the structural relations between cells to an extent not formerly possible. As these techniques were mastered, the laboratory of Richard Paul Bunge (1932-1996) and his wife Mary Bartlett Bunge (Figure 4) in New York Columbia University finally identified a spiral of myelin in CNS. Interestingly, they published the classic sophisticated model of CNS myelination (Bunge et al., 1961) before they could actually produce a convincing electron micrograph after years of hard labor (Bunge et al., 1962; Bunge, 1968). The Bunges determined CNS myelin is formed by oligodendrocyte processes extending as much as 12µm, a long distance at the ultrastructure scale. Stunningly, the Bunge laboratory discovered that a single oligodendrocyte form multiple myelin internodes wrapped around different axons (Figure 4). The 3-D problem of myelin wrapping in CNS was simply daunting when compared to myelin PNS. The Bunges eventually made seminal contributions in Schwann cell biology, teaming with Patrick Wood, through tissue culture and transplant models. This led to Richard scientific direction of the Miami Project to Cure Paralysis. Mary Bunge is presently pursuing active research to promote spine functional regeneration.

1960's Invertebrate diversity of ensheathment

Only a cursory survey of the literature is presented here. In 19th century, the compact sheaths of invertebrate axons were referred to as *myelin* based on osmium staining and birefringence (Friedländer, 1889). Invertebrates glia were largely ignored until the advent of electron microscopy in the 1950's. In 1965, Bullock and Horridge compiled the knowledge on glia in all invertebrate phyla in a monumental book that revealed axons are always surrounded by glial cells. Betty Roots (1927-) based in Toronto, Canada, spent her career studying invertebrate sheaths. Often, this is only a single layer of cells as in

squid giant axons, but in many cases there are several overlapping layers referred to as *loose-myelin*. In annelids, phoronids and arthropods, multiple highly compacted layers resembling vertebrate myelin are found. One of the striking findings in invertebrates is their diversity. Sheaths of annelids differ radically from crustacea, as they are spirally wrapped whereas those of crustacea consist of concentric lamellae, implying radically different mechanism and regulation of myelination (reviewed in Roots, 2008).

The sheaths around giant fibers in the earthworms (Annelida), and around fibers in the eye stalk of the crab (Arthropoda) are remarkably similar to vertebrate sheaths with closely compacted lamellae held together by desmosome-like structures (McAlear et al., 1958; Hama, 1959), although lacking the characteristic specialization of desmosomes holding two cell membranes together very strongly (Roots and Lane, 1983).

Structures resembling Nodes of Ranvier were described in crustacea in early light microscopical studies (Retzius, 1890; Nageotte, 1916) and later by electron microscopy (McAlear et al., 1958; Heuser and Doggenweiler, 1966). In the earth worm, circular pores which act as nodes are found in the sheath (Günther, 1973). A detailed comparison between invertebrate and vertebrate nodes was carried out by Roots (1984).

Strikingly, the conduction velocity is increased, similarly as in vertebrates, in annelid (earth worm) and crustacean (prawn and shrimp). Not only do the sheaths increase the conduction velocity in axons, but saltatory conduction of action potentials is allowed by Node of Ranvier-like structures in crustacea and circular pores in the sheath in annelids. In the earthworm the sheath is not as efficient as vertebrate myelin (Günther, 1976), however, in shrimps the conduction velocity is over twice as fast as the fastest known vertebrate axon (Kusano, 1966). After a burst of interest in the 1960s-1970s no further studies were carried out on axon conduction velocity modulation by invertebrate glial sheaths.

Regarding the composition of invertebrate sheath membranes, it markedly differs from vertebrate myelin based on the little we know. Proteins in the earthworm and the pink shrimp are very different both from each other, and from vertebrate myelin (Pereyra and Roots, 1988; Okamura et al., 1986). The lipid composition is also very different from that of vertebrates (Okamura et al., 1986). According to Roots (2008) these differences in composition are not a justification for dismissing

invertebrate sheaths as myelin, based on the differences found in vertebrates between CNS and PNS myelin, and between mammals and fishes and amphibia.

Conclusion

Myelin can be conceived as a gigantic 3-D puzzle in time and space. The best scientists across the world have studied this structure directly or incidentally since the 18th century. Each of them brought a piece to the puzzle. Myelin was first reported as white matter inside the brain since the Renaissance. At the dawn of neuroscience early 19th century, myelin was considered the most important element of the nervous system. After axon was discovered, myelin went overlooked for decades by sheer ignorance of its function, but is now coming back full circle. Myelin has been tantalizingly difficult to understand, no doubt because of its tremendous structural and chemical complexity. It took three centuries to decipher its genesis and function. Several structural elements were rediscovered, illustrating the timeless rule of who and how new findings are published. We are getting close to fully understand the molecular mechanisms of this amazing biological structure, which allows us (our brain) to exist. Shall we say that the story of myelin has not yet ended and the last episode -myelin repair- is yet to be completed?

List of abbreviations

CNS: central nervous system

MIT: Massachusetts Institute of Technology

PNS: peripheral nervous system

Declaration of interests

The author declares no conflict of interest and assumes all translations where not specified. Accessing original documents generated discrepancies with their formerly cited references.

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Legend to figures

Figure 1. Early descriptions of myelin. A: Antoni van Leeuwenhoek by Verkolje painter in 1686 (courtesy London Wellcome Library). B: drawing of fresh spinal nerve section from cow or sheep (fig.2, Leeuwenhoek, 1719) with detail in C showing the regular pattern of myelinated fibers. D: fresh *primitive nervous cylinder* partly stripped of its rough sheath on the right (fig.6 plate 4, Fontana, 1781). E: fresh frog spinal myelinated fiber: "a *nerve tube* with double wall containing the actual *nerve marrow* and the transparent structured *brain tube* which is quite transparent, emerging in β" (fig.11, Ehrenberg, 1833). F: two fresh myelinated fibers (*tubuli primitivi*) with axon emerging in right and slender organic fibers (*fibrae organicae)* with nuclei (fig.6 plate 1, Remak, 1838). G: bovine myelinated fibers freed by pressure from its nerve showing *primitive bands with rough sheath* (axon with myelin) and *pellucid primitive bands denuded of sheath* (axon, gray threads); note the mushrooms of myelin at sections (fig.1 plate 1, Remak, 1838). H: bovine spinal cord section stained by carmine showing concentric myelin; *t* is *nervous tube*; *c* is *axis-cylinder* (fig.364, Ranvier, 1875). I: Calf nerve fiber showing a nucleus in the Schwann sheath (fig. 9 plate 4, Schwann, 1939).

Figure 2. From chaos to order. A: drawing by Charcot (fig.1, 1868) of a fresh multiple sclerosis lesion showing disseminated myelin and fatty droplets. B: first representation of nodes at 300x and 600x from rabbit sciatic nerve stained by carmine; *a* is node; *cy* is *axis-cylinder* (axon); *m* is myelin (fig.1, Ranvier, 1872). C: wood etching of Louis-Antoine Ranvier (courtesy Paris Descartes University Library). D: osmium blackening of myelin revealed the periodic segmentation of nodes across species from frog to rabbit; left fig.9 shows a complete internode with *e* for *étranglement* (node), *n* for nucleus and *p* for protoplasm; Schmidt-Lanterman incisures are visible figs.5-6; top right corner are myelin balls from a fresh sciatic nerve dissociated in water, reminiscent of Charcot myelin globules; fig.3 shows a fresh nerve with white wrinkled myelin sheath (plate 1, Ranvier, 1878). E: complete schematic of a peripheral internode (fig.88, Cajal, 1909-1911). F: third type oligodendrocytes stained by silver carbonate whose processes spiraled around invisible myelin (fig. 58, Río-Hortega, 1928).

Figure 3. Crystalline organization and saltatory conduction. A: Francis Schmitt (courtesy American Philosophical Society), founding father of neuroscience, pioneer of X-ray diffraction and creator of g ratio. B: mathematical equation using the Greek capital letter g (Γ) and schematic of g-ratio (fig.1, Schmitt and Bear, 1937, reproduced from Wiley). C: X-ray diffraction of mouse nerve myelin showing concentric diffraction pattern (fig.1, Kirschner et al., 1979, reproduced from Rockefeller University Press). D: 4 models of myelin unit structure (fig.1, Schmitt et al., 1941, reproduced from Wiley). E: schematic of saltatory conduction from anesthetized nodes N_0 and N_1 to normal node N_2 after repeated stimulation of the axon in *E* (fig.9, Tasaki and Takeuchi, 1942, reproduced from Springer). F: Ichiji Tasaki circa 1984 (courtesy Scholarpedia).

Figure 4. The origin of myelin: spiral from glia. A: Betty Geren at her electron microscope in the MIT basement circa 1950 (Geren courtesy) conceptualized the spiral of myelin. B: schematic of Schwann cell (*SC*) mesaxon spiraling myelin around the axon (*Ax*) (fig.5, Geren, 1954, reproduced from Elsevier). C: James Robertson at his light box (courtesy American Society for Cell Biology), creator of the unifying membrane theory. D: schematic of myelin condensation (shaded) by extrusion of cytoplasm in *D* and collapse of extracellular space in *E* (Robertson, 1962, reproduced from the Scientific American). E: Mary and Richard Bunge in their laboratory circa 1975 (courtesy Miami Project to Cure Paralysis) were the first to show oligodendrocytes spiral myelin in F (fig.7, Bunge, 1968, reproduced from the American Physiological Society). G: Bunges' hypothetical accurate schematic (fig.18, Bunge et al., 1961, reproduced from Rockefeller University Press). H: iconic electron microscopy image of oligodendrocyte making myelin (fig.2, Hirano, 1968, reproduced from Rockefeller University Press).