

## Top ten papers in multiple sclerosis

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Having been recognised only since the late nineteenth century, there has been just over a hundred years of research on multiple sclerosis. Over this time, a picture has emerged of this disease as an inflammatory disorder of the central nervous system, caused by a complex interplay of multiple genetic susceptibility alleles and unknown environmental triggers. We have tried to illustrate this in our choice of landmark papers, at the same time being aware that strong cases could be pressed for other studies to be included. It is clear that many lines of scientific attack on the disease have benefited from increasingly potent weapons, and in many cases our papers reflect the application of the very latest technology of the day. Finally we note that three of our “top ten” were authored by Ian McDonald (1933-2006), testimony to his extraordinary contribution to understanding multiple sclerosis (McDonald 1999).

### **1. 1916: The pathological anatomy of the lesion in multiple sclerosis**

*Dawson JD 1916 The Histology of Disseminated Sclerosis. Transactions of the Royal Society of Edinburgh 50: 517-740*

James Dawson (1870–1927) left the greatest pathological account of multiple sclerosis in the English language (Dawson 1916). First he summarizes the literature. The issue (then as now for some contemporary logicians) is whether the disease is ‘inflammatory’ or ‘developmental’ (degenerative). The primary vascular, inflammatory, doctrine was espoused by Dejerine (Dejerine 1894), Williamson (Williamson 1894; Williamson 1908) and Marie (Marie 1884), who suggested that infections initiate the changes in blood vessels.

Bielschowsky (Bielschowsky 1903) considered that the vascular process is directed primarily at nerve fibres. Strumpell (Strumpell 1896) considered that exogenous insults act upon an ‘intrinsically weakened’ system; and Bramwell (Bramwell 1903) also saw multiple sclerosis

as primarily a developmental disturbance. Müller (Muller 1910), the most articulate teacher from the developmental school, proposed that any participation of the blood vessels within the lesion is secondary and his concept of 'multiple gliosis' as the essential process rehearses the final position taken by Charcot (Charcot 1868) and most of his school. Redlich (Redlich 1896) and Huber (Huber 1895) also saw the insult as a toxin- or microorganism-induced primary degeneration of the myelin sheath with secondary inflammation and blood vessel changes. But, as often is the case, the best account was the first: Rindfleisch (Rindfleisch 1863) assigned priority to the blood vessels, proposing a sequence in which a chronic irritative condition of the vessel wall alters the nutrition of nerve elements, leading to atrophy with metamorphosis of the connective tissue producing monster glia (Deiters or Rindfleisch cells).

Reviewing the histology of nine personal cases (L.W., a kitchen maid, aged 28; C.S., aged 22; Mrs G., aged 30; J.W.; S.S., a nurse aged 44; C.G., a baker's shop-woman, aged 24; J. McN., a cabinet maker, aged 42; M.R., a typist, aged 33; and L.H., aged 30), Dawson devotes the majority of his text to L.W. She was admitted to hospital in Edinburgh under the care of Dr Alexander Bruce on 4th April 1910 with a two year history of weakness and tremor in all four limbs, dysarthria and sphincter disturbance. In hospital (from May 29<sup>th</sup>) she has an episode of brainstem demyelination (deafness and tinnitus, right facial palsy, numb left arm, right lateral rectus weakness, tongue deviation to the left and dysphagia). In August, she loses vision in both eyes, develops increasing bulbar failure and dies from septicaemia on 5 September 1910. Dawson describes the features of early and established lesions in the spinal cord and cerebrum (**Figure 1**), offering an analysis of their evolution through stages of fat granule cell myelitis (in the cord) to glial hyperplasia. He devotes text to the unusual lesions, including Markschatthenherde (shadow plaques), and those appearing in grey matter and around the ventricles, optic nerve, peripheral nerves and roots which he considers to be evolving lesions, and he mentions three hyperacute cases with an accelerated clinical pattern of relapses, rapid accumulation of deficits and characteristic histological features. Curiously, he neglects Marburg's (1906) important monograph identifying shadow plaques which we now know to be indicative of remyelination not partial demyelination. Next, Dawson turns to an analysis of the changes to be observed in each cellular element of the nervous system – nerve cells and their axons, neuroglia, blood vessels and lymphatics. Form, symmetry and the distribution of lesions are all addressed. After listing the tragic accumulation of lesions throughout the brain and spinal cord of the unfortunate L.W., Dawson attempts a

clinicopathophysiological correlation. Weakness in the legs is consistent with the extensive spinal cord gliosis; intention tremor with lesions in the superior cerebellar peduncles and red nuclei; disordered eye movements with the periaqueductal plaques; and the several cranial nerve palsies with involvement of the pons and medulla. Dawson shows that old (sclerotic lesions) are characterized by complete absence of myelin (Weigert stain), dense fibrillary tissue (glial stain), persistence of axis cylinders (silver stain), numerous blood vessels (diffuse stains), no active myelin degeneration (Marchi stain) and an abrupt transition to normal tissue. In acute lesions, the differences are infiltrated blood vessels, active demyelination with fat granule cells, and transitional zones shading into normal tissue. He illustrates the text with 22 colour and 434 black-and-white figures in 78 plates.

Dawson summarizes his ideas on plaque formation around brain inflammation to include a sequence of events that, although not disease-specific, produces recognizable clinical characteristics when directed at glia, leading to degeneration of the myelin sheath with fat granule cell formation, and a reactive change in glia involving cell proliferation with fibril formation culminating in sclerosis. The whole process is triggered and modified by exogenous factors whose influences fluctuate, causing the characteristic relapses. Remissions depend more on rerouting of synaptic connections – for us, plasticity – than remyelination. Maybe he falls into the trap of believing that the pathologist can see the cause, effects and evolution of disease merely by observing snap-shots of its end-state.

## **2. 1960: Evidence for an immune response within the central nervous system in multiple sclerosis**

*Lowenthal, A., Vansande, M., Karcher, D. 1960. The differential diagnosis of neurological diseases by fractionating electrophoretically the CSF proteins. J. Neurochem. 6, 51–60*

The most consistent laboratory abnormality in multiple sclerosis is the finding of a restricted number of 'oligoclonal' immunoglobulins within the cerebrospinal fluid. These are produced by B cells in the parenchyma of the central nervous system and drift into the cerebrospinal fluid like oil in the sump. However, their role in the pathogenesis of multiple sclerosis, if any, remains completely unknown. But their everyday importance is value as a biomarker that supports the diagnosis of multiple sclerosis, being found in 90-95% of people with the disease; but also in conditions having an inflammatory basis and, rarely, apparently by chance. The history of their discovery is intimately tied to technological advances.

In 1948, the Nobel Prize for chemistry was awarded to the Swede, Arne Tiselius, for his application of physical techniques to biological molecules, mainly electrophoresis of proteins. This work was soon taken up by medical researchers. For instance, Elvin Kabat and Harold Landow studied protein electrophoresis of cerebrospinal fluid from patients with a variety of conditions, including multiple sclerosis, at the Neurological Institute of the College of Physicians and Surgeons at Columbia University in New York. In their 1942 paper, submitted a few days after Landow's death, Kabat and Landow showed that the ratio of gamma-globulin to albumin in cerebrospinal fluid is normally identical to serum, except in patients with neurosyphilis (Kabat, Moore et al. 1942). Rather poetically, they conclude that 'the data would suggest that some formation of gamma globulin could take place within the tissues of the central nervous system and be poured into cerebrospinal fluid'. This was a new concept; up until then, there was little evidence in humans for an immune response confined to the central nervous system. The researchers commented in passing within the results section that, of five cases of multiple sclerosis, one had some evidence for intrathecal gamma-globulin synthesis. But they made no more of this.

The Tiselius technique is based on fluid boundaries, requires expensive bulky equipment, and is difficult to perform. From the 1940s onwards, 'zone' electrophoresis was developed, with filter paper used as a substrate. Then, in 1955, an English medical-school drop-out called Oliver Smithies developed gel-based electrophoresis (Smithies 1955). In 2007, he received the Nobel Prize with Mario Capecchi and Martin Evans 'for their discoveries of principles for introducing specific gene modifications in mice by the use of embryonic stem cells'.

The paper we have chosen comes from Lowenthal and colleagues, at the Neurochemical Research Laboratory of the Neurological Department, Antwerp, translated from the French by Charles Poser, author of another of our top ten papers. This group pioneered the application of agar electrophoresis to cerebrospinal fluid proteins. They saw, for the first time, multiple sharp gamma-globulin bands ( $\gamma_1$ ,  $\gamma_2$  and  $\gamma_3$ ) in the cerebrospinal fluid of patients with multiple sclerosis, which were not present in normal individuals. And they distinguished these from the increased  $\gamma_4$  and  $\gamma_5$  bands seen in subacute sclerosing panencephalitis (**Figure 2**). They made a point of saying that such bands were rarely seen in cases of African trypanosomiasis (although they confessed that the electrophoresis of these specimens had been delayed by one week because the lumbar punctures were performed in the Belgian Congo!). Now, cerebrospinal fluid electrophoresis was being promoted as a diagnostic aid for multiple sclerosis in clinical practice.

The next technological innovation was isoelectric focusing of agarose-gel electrophoresis, which improved sensitivity yet further. Hans Link and colleagues were early in exploiting this showing, as well as improved definition of the ‘oligoclonal bands’ (a term he coined), that these were largely due to the presence of IgG antibodies (Link 1967; Link 1972).

The scientific dividend from the discovery of cerebrospinal fluid oligoclonal bands has been frustratingly small. It seems that there is no consistent antigenic target for the antibodies and they are unaffected by most effective therapies. But the recent discovery of meningeal B cell lymphoid follicles and the moderate efficacy of B-cell depleting antibodies, has reawakened interest in the role of B cells and antibodies in multiple sclerosis (Cross and Wu 2010).

In clinical practice, the advent of magnetic resonance imaging has reduced the frequency with which it is necessary to test the cerebrospinal fluid in the diagnosis of multiple sclerosis. But in the tricky diagnostic case, the finding of cerebrospinal fluid oligoclonal bands can be an indispensable ally, for it remains the only direct clinical test of the pivotal disease process - active inflammation within the central nervous system.

### **3. 1970: An exemplary trial of steroid treatment of the acute relapse**

*Rose AS, Kuzma JW, Kurtzke JF et al 1970 Cooperative study in the evaluation of therapy in multiple sclerosis: ACTH vs placebo: Final report. Neurology 20: 1–19*

“In 1960, at a symposium concerned with the evaluation of drug therapy in neurologic and sensory diseases, the many particular difficulties involved in the clinical trials of therapy in multiple sclerosis were recognized, including those pertaining to the conduct of cooperative studies.”

So opens this massive, 59-page, report on a trial of ACTH as a treatment of multiple sclerosis relapse. The symposium mentioned led to an ad hoc committee which reported in 1965 on the ideal trial for a multiple sclerosis therapy (Schumacher, Beebe et al. 1965). And, five years later, the first application of its principles were published. It represents a landmark in trial rigour and quality, despite a rather unsatisfactory conclusion.

By 1965, there was agreement that ACTH did not influence multiple sclerosis in the long-term, but conflicting small-scale reports on its short-term effect on relapses. Rose and colleagues suspected that ACTH might have an effect, but of small magnitude, which would require careful trial design to reveal. So, they insisted on a placebo control, and on the use of 10 neurology centres, to maximise recruitment of the required number of patients (in the end

197). They described as a particular strength of the trial: “a statistical centre office and staff, backed by computer facilities, ensured randomization, diminished bias in data review, and provided opportunity for the multiple analyses that were required for the extensive clinical observations.”

Each patient was in hospital for two weeks, receiving twice daily injections of diminishing doses of ACTH or placebo. They were assessed each week for four weeks on several scales:

- A rather arbitrary “Estimate of Overall Condition”
- Kurtzke’s Disability Status Scale & Functional Systems Score
- The Standard Neurological Examination
- Seven Day Symptom Score, which attempted to capture what would now be called an “area under the curve” disability metric
- Quantitative examination of neurological function

52 pages of charts, tables and text describe the results of these analyses. Each outcome assessment is compared to another, and across centres, to see which was the most consistent, and which scales correlated with each other. The conclusion, which has been tested many times ever since and has yet to be upset, was “the Disability Status Scale, together with the Functional Systems, comprises an adequate system of evaluating change in a therapeutic trial of MS and, of all the measures used in this study, apparently is the most consistent indicator of change”. In contrast to the detail on outcome measures, there is none on the trials’ selection criteria, just a reference to the protocol, published in a previous issue of *Neurology*. And there is no discussion at all of statistical technique and power.

The primary outcome measure was comparison of patients’ disability at baseline with that at 4 weeks after starting treatment. There was a significant difference in favour of ACTH, but the authors were not impressed. Firstly, they noted that the size of benefit fell between week 3 and 4, suggesting that it might disappear altogether on extended follow-up. Secondly, they questioned whether the statistically significant difference was clinically significant: “the treatment results of the study as revealed by extensive analysis of a large mass of data may be considered noteworthy for, although the degree of improvement of the patients treated by ACTH attained statistical significance by each of the several methods of evaluation, at no time was the improvement particularly obvious or outstanding. Indeed, 69% of the patients who were treated by placebo attained improvement, a factor that will not be overlooked by thoughtful investigators. It is evident that the “placebo effect” of a well ordered, seriously applied therapeutic effort, although complex and difficult to define, provides a powerful

influence which may qualify treatment results. These observations should serve to temper the enthusiasm of those who would advocate a specific therapy for MS unless the therapeutic trial is adequately and appropriately controlled.”

Soon, clinicians moved to using synthetic corticosteroids, rather than using ACTH to promote release of endogenous steroids. The lack of extended follow-up in the Rose study was corrected by a study in Wales of 50 people with multiple sclerosis treated with placebo or intravenous methylprednisolone (Milligan, Newcombe et al. 1987). And more still was learnt from the effect of steroids on optic neuritis (Beck, Cleary et al. 1992; Beck, Cleary et al. 1993; Group. 2008; Keltner, Johnson et al. 2010). The conclusion of all of these studies are that steroids reduce the duration of a relapse of multiple sclerosis, but have no impact on the extent of residual disability nor of the subsequent disease course.

#### **4. 1972: the first clinical demonstration of demyelination**

*Halliday AM, McDonald WI, Mushin J 1972. Delayed visual evoked response in optic neuritis. Lancet i: 982–985*

Ian McDonald and Martin Halliday showed that visual evoked potentials can detect past episodes of optic neuritis, so introducing a non-invasive test to assist in the diagnosis of suspected multiple sclerosis.

Recording of electrical activity of the brain over the scalp had been pioneered by Hans Berger in the 1920s. The motivation behind his experiments, it seems, was to identify the mechanism underlying telepathic communication with his sister during an accident whilst Berger was serving with the cavalry. In 1924, he recorded the first human electroencephalogram (Berger 1929). Lord Adrian, who received the 1932 Nobel Prize in Physiology or Medicine with Sir Charles Sherrington, promoted Berger’s work and showed the value of EEG in neurological practice (Adrian 1934). It was a small step from there to measure scalp potentials over the parietal or occipital lobes following a sensory stimulus or flash of light: somatosensory and visual ‘evoked potentials’ respectively. George Dawson methodically solved the technical challenges, not the least by introducing a technique to reduce noise in small evoked potentials (Dawson 1954).

Martin Halliday (**Figure 4**) set to applying these new methods to people with multiple sclerosis, showing in 1963 people with multiple sclerosis have delayed somatosensory evoked potentials (Halliday and Wakefield 1963). But the changes were not robust and he soon

turned his attention to visual evoked potentials. In the meantime, Tom Sears and Ian McDonald had demonstrated the electrical consequences of central nervous system demyelination (McDonald and Sears 1970; McDonald and Sears 1970). They showed that direct micro-injection of diphtheria toxin into the spinal cord of the cat produces a highly circumscribed demyelinating lesion which leads to conduction block, or prolongation of the refractory period for transmission and an impaired ability to transmit high frequency trains of impulses.

Patients were trawled from Ian McDonald's clinic at the Moorfields Eye Hospital. Nineteen patients with unilateral optic neuritis, 17 in the acute phase, were studied with flash visual evoked potentials and a new technique, 'pattern' visual evoked potentials (an alternating black and white checkerboard"). In optic neuritis, the mean latency of visual evoked potentials in the affected eye was 155 msec, an increase of 30% over that from healthy people or the unaffected eye; and the peak amplitude was halved at 3.68 microV. In the 5 patients seen acutely with visual acuities of 6/60 or less, there was no evoked response at all; but, as their vision recovered over weeks, so their visual evoked potential reappeared, although much delayed. Evoked potentials remained delayed even when visual acuities had recovered to normal, for up to five years. Pattern-evoked responses elicited more reproducible and sensitive responses than a flash response. The authors concluded: 'since a persistently increased latency may be present with normal optic discs, fields, and fundi, the technique described here provides a useful objective test for previous damage to the optic nerve. Its potential usefulness in the diagnosis of multiple sclerosis when patients present with clinical evidence of only a single lesion not involving the visual system is obvious.'

They went on to test the 'obvious' in a group of unselected multiple sclerosis patients (Halliday, McDonald et al. 1973). In 24 individuals with a previous history of optic neuritis, nine had normal discs and all had abnormal visual evoked potentials; so abnormal visual evoked potentials are especially reliable indicators of past optic neuritis. Perhaps more usefully, in 27 patients with no history of optic neuritis, 25 had abnormal visual evoked potentials, of whom discs appeared normal in 12. So, it seems as though abnormal visual evoked potentials provide a sensitive means of identifying previous subclinical optic neuritis.

The authors went on to propose diagnostic criteria for multiple sclerosis which incorporated visual evoked potentials (McDonald and Halliday 1977). To date, it remains true that the only clinical diagnostic test that can demonstrate that a central neurological lesion is *demyelinating*

is the cortical evoked potential, of which the pattern-evoked visual potential is by far the most sensitive and robust.

## **5. 1972: identifying the primary genetic association for multiple sclerosis**

*Naito S, Namerow N, Mickey MR, Terasaki PI. 1972. Multiple sclerosis: association with HL-A3. Tissue Antigens. 2:1-4*

Discoveries in the genetic basis for susceptibility to multiple sclerosis have followed each increment of technological and statistical innovation in genetics. But the most important association of the disease, with alleles of the human leukocyte antigen system, began to be uncovered in the early 1970s.

Human leukocyte antigens were first identified as serum factors in transplant recipients that reacted against a third party “tissue”, and which were associated with transplant rejection. A key figure was Paul Terasaki at UCLA who (having clawed his way into medicine from the low point of being interned as a schoolboy during the war because of his Japanese origins) developed the microcytotoxicity test, a tissue-typing test for organ transplant donors and recipients that required only 1 microliter each of antisera (Terasaki and McClelland 1964). (He founded a company to exploit the technology, One Lambda, which grew to generate sufficient income to enable him to make a \$50 million donation to UCLA in 2010).

In 1970, Terasaki organised the Fourth Histocompatibility Workshop in Los Angeles in 1970 which brought together fifteen different laboratories testing 116 highly selected antisera. The conclusion was that there were 11 official HL-A specificities (HL-A1, 2, 3, 5, 7, 8, 9, 10, 11, 12 and 13), and perhaps eight other specificities. Shortly after this workshop, Terasaki turned his attention to multiple sclerosis. His group concluded, from 94 patients and 871 controls, that HL-A3 was overrepresented (Naito, Namerow et al. 1972). Furthermore, they demonstrated that the geographical variation in prevalence of multiple sclerosis paralleled the prevalence of HL-A3. For instance, both are high in Scandinavian countries, middle range in America and low in “oriental”. They summarised some of the epidemiology suggesting an environmental cause for multiple sclerosis and concluded that “the evidence to date on MS, however, is still consistent with the idea that a genetic difference in susceptibility underlies some environmental influence.”

Soon after this paper, Casper Jersild and colleagues from Copenhagen wrote a brief letter to the Lancet, to make some generic points around HLA genetics (Jersild, Svejgaard et al.

1972). They described correction for multiple testing, the need for replication datasets and the usefulness of meta-analyses. Illustrating their argument, they announced the results of HLA serotyping in 36 Danish patients with multiple sclerosis followed by a replication set in 71 other patients. From these analyses, HL-A7 emerged as most associated with multiple sclerosis. However, when the Danes merged their data with that from the Terasaki study, and corrected for multiple testing, only HL-A3 retained significance. This set the tone for the years to follow of underpowered studies leading to false positive results and real associations revealed by combining datasets.

Paul Terasaki returned to multiple sclerosis with the discovery of the B-lymphocyte alloantigen serotypes. In 1976 both his group and one of the authors of this chapter discovered the association with what would come to be called the class II allele HLA-DR15 (Compston, Batchelor et al. 1976; Terasaki, Park et al. 1976). (This explains, at least in part, Naito et al's finding of an association of multiple sclerosis with HL-A3 serotype; in Western Eurasia, A3 is part of the longest known multigene haplotype, A3-B7-DR15-DQ6). HLA-DR15 remains the best characterized candidate susceptibility gene for multiple sclerosis. After 1976, several decades of increasingly large, expensive and sophisticated molecular genetic studies followed; all confirmed the association with DR15, but very little else. Only in the last few years has there been sufficient power in the techniques and cohorts, forged through large collaborations, to uncover the much smaller individual genetic contributions of a host of other alleles, especially studies from the International MS Genetics Consortium (De Jager, Jia et al. 2009).

The finding that multiple sclerosis is associated with the HLA system implicates the immune system in its pathogenesis; explains some of the geographical variation of the disease; provides a molecular substrate for the interaction of genetics and environment; and suggests treatment directed at the T-lymphocyte, the T cell receptor and the class II molecule.

## **6. 1973: remyelination is possible in the central nervous system**

*Gledhill RF, Harrison BM, McDonald WI. 1973. Pattern of remyelination in the CNS. Nature 244(5416):443-4*

A principal hope of people affected by multiple sclerosis is not only control of their disease but reversal of any damage already accrued: repair, possibly facilitated by therapy. Although not yet an everyday probability, trials of potential remyelinating therapies are being

conducted. Key steps that made such therapies possible were the demonstrations that remyelination was possible in the central nervous system; that this was mediated by the oligodendrocyte precursor; and that it was accompanied by functional improvement. We could have chosen one of several studies which contribute to this narrative. We have chosen the paper which definitively demonstrated remyelination in the adult mammalian central nervous system, and – perhaps most importantly- showed how to identify demyelinated fibres.

Critical work had already been done by Dick and Mary Bunge (Bunge, Bunge et al. 1961) who studied myelin repair in cats following demyelination induced by cerebrospinal fluid barbotage. They showed that the remyelinating cell differed from the mature oligodendrocyte and, proposed incorrectly as it turned out, that mature oligodendrocytes de-differentiated into a cell capable of remyelination. Perier and Gregoire (Perier and Gregoire 1965) showed, from electron microscopic multiple sclerosis plaques, that axons were surrounded by thin myelin lamellae, which they considered to be evidence for remyelination.

Then paper we have chosen comes from Richard Gledhill, Barry Harrison and Ian McDonald (Gledhill, Harrison et al. 1973). They compressed the spinal cord of three adult cats, which causes early demyelination with retained axons, and remyelination which starts three weeks later. Their main discovery, under the electron microscope, was that the remyelinated sheath is abnormally thin and has an internodal distance reduced by 50% compared to control fibres of the same diameter. Under the light microscope, they found no evidence for the presence of Schwann cells, so concluded that oligodendrocytes had been responsible for the remyelination. These ultrastructural characteristics – reduced internode distance and inappropriately thin myelin – have become the defining features used to recognise remyelinated axons (as opposed to the partially demyelinated axons) in experimental and human pathological studies.

The next important step was the demonstration that such remyelinated axons could restore function. This work was also supervised by Ian McDonald working with the electrophysiologist Ken Smith (Smith, Blakemore et al. 1979; Smith and McDonald 1980).

## **7. 1977: Plotting the epidemiology of multiple sclerosis**

*Kurtzke JF. 1977. Geography in multiple sclerosis. J Neurology 215(1):1-26*

Epidemiology is less dependent upon technological advances than other disciplines represented in this chapter, and more reliant on the steady accumulation of disparate data. So, it is less easy to identify one paper which has made a seminal impression on the field. We could have chosen something from the oeuvre of Geoffrey Dean, who published on multiple sclerosis from 1949 to 2002, focusing especially on the effect of migration on the risk of multiple sclerosis, initially on migration to South Africa, then from Asia, the Caribbean, and Africa to the United Kingdom (Dean and Kurtzke 1971; Elian, Nightingale et al. 1990; Dean and Elian 1997). We choose instead one of John Kurtzke's key papers. John Kurtzke saw action in World War Two as a pharmacist's mate (2nd class). On discharge, he went to medical school and spent most of his professional life as a neurologist in the Veteran's Administration service, remaining in the Naval Reserve and achieving the rank of Rear Admiral. He wrote his first paper on multiple sclerosis in 1953 (Berlin, Kurtzke et al. 1953) and is still publishing (McLeod, Hammond et al. 2011). He is responsible for producing the industry-standard 'Kurtzke Scale' of disability in multiple sclerosis (Kurtzke 1955; Kurtzke 1961; Kurtzke 1983) And he organised the first placebo-controlled clinical trial (of isoniazid) in multiple sclerosis (Berlin and Kurtzke 1957). But, his principal contribution has been the careful documentation and analysis of the varying prevalence of multiple sclerosis around the world and especially within the cohorts of US military personnel. Characteristic of his papers are a distrust of complex statistics and meticulously presented hand-drawn charts.

In this paper, which is part review and partly based on original data, Kurtzke lays out the big picture of multiple sclerosis epidemiology (**Figure 4**). He points out that the assertion of the day, that latitude determined multiple sclerosis prevalence, is incorrect. In Asia and the Pacific, latitude seemed not a factor at all and 'at 40 ° north, for example, MS is high in America, medium in Europe, and low in Asia'. In Europe and North America, there are zones of high frequency of multiple sclerosis between 65 ° and 45 ° north latitude. Neighbouring these (in Europe to the north, east, and south; in America for southern U.S.; and the remainder of Australia) are zones of medium frequency; everywhere else as of low frequency.

Measured serially in the same small region, Kurtzke asserts that the prevalence of multiple sclerosis appears stable over time although our experience in East Anglia, UK, for example, is different (Robertson, Deans et al. 1996). One area stands out as having a high prevalence of multiple sclerosis; this Fennoscandian focus .... 'from the waist and southeastern mountain plains of Norway eastward across the inland lake area of southern Sweden, then across the

Bay of Bothnia to southwestern Finland, and then back to Sweden in the region of Ume..... This clustering, as well as the broader geographic distributions already considered, mean to me that the occurrence of MS is intrinsically related to geography, and therefore that MS is an acquired, exogenous, environmental disease.' To determine when this disease might be acquired, Kurtzke turned to the migration studies, both his own and those of others. By comparing the age at which migration alters the risk of acquiring multiple sclerosis, he concluded that the key exposure occurs between the ages of 10 and 15 years, and that there is an 'incubation' period of some 20 years before the disease manifests. He then presents new data on the risk of multiple sclerosis in veterans by race and gender, showing that it is greatest in white women. Thus, he concludes 'MS is the white man's burden spread from western Europe'. Kurtzke argues that if multiple sclerosis is due to an infectious agent, rather than a toxin, transmissibility should be evident. This is why he is so keen to discuss possible 'epidemics' of multiple sclerosis. In 1977, he had just returned from a second visit to the Faroe Islands, where there seemed to be a cluster of new cases of multiple sclerosis following the stationing of British troops. He was to visit the Faroes many more times, and has just recently advanced the idea that gastrointestinal infections mediated the transmission between British troops and Faroese (Wallin, Heltberg et al. 2010).

Kurtzke's interpretation of the Faroese epidemic of multiple sclerosis has been the most controversial aspect of his work, with other commentators suggesting more prosaic explanations, for instance increased diagnostic vigilance resulting from improved medical services (Poser and Hibberd 1988; Poser, Hibberd et al. 1988). But that should not detract from the enormous service John Kurtzke has made in marshalling the huge and complex multiple sclerosis epidemiological dataset into digestible synopses, of which this paper is a prime example.

## **8. 1981: the first evidence showing that multiple sclerosis is treatable. The end of the beginning?**

*Jacobs L, O'Malley J, Freeman A, Ekes R. 1981. Intrathecal interferon reduces exacerbations of multiple sclerosis. Science 214: 1026-8*

'There is evidence that multiple sclerosis is caused (at least partially) by a viral infection of the central nervous system that acts as a "trigger" for repeated exacerbations of neurologic symptoms characteristic of the disease. Interferon is a naturally occurring biologic product with potent antiviral activities. It does not cross the blood-brain barrier in significant quantity

when administered systemically, but can be safely administered intrathecally'. So opens Larry Jacobs' landmark paper on the use of interferon as a treatment of multiple sclerosis.

There are many problems with this paper. Its premise, that viral infections are the remedial cause of multiple sclerosis, is probably incorrect; its analysis is flawed; and, rightly, it met with considerable controversy. However, the paper deserves selection as a landmark because it introduced an intervention that does, to a degree, suppress disease activity in multiple sclerosis; and, in Larry Jacobs, it introduced one pioneer of the 'DMTs' (disease modifying therapies). But this was not the first study of interferons in multiple sclerosis; although not acknowledged in the paper, Verveken had used interferon-beta IM in 3 patients with "chronic progressive multiple sclerosis" (Verveken D 1979) and Fog tested interferon-alpha SC in 6 patients with similar disease-type (Fog 1980). Neither observed any benefit.

The "interferons" had been identified in 1975 by Isaacs and Lindenmann as products that interfere with viruses (Isaacs 1975). Human interferon could be made with difficulty in the laboratory by "superinduction" of human fibroblasts, and purified by affinity chromatography, to generate a "natural" interferon, so-called to distinguish it from the subsequent recombinant interferons. One such laboratory was the Roswell Park Memorial Institute (now Roswell Park Cancer Institute) in Buffalo, New York. From this unit came the first evidence that interferons can ameliorate chronic active hepatitis and kill tumour cells in vitro, both in 1979 (Dolen, Carter et al. 1979; Horoszewicz, Leong et al. 1979). At around that time, Larry Jacobs arrived as a young neurologist in Buffalo from his residency at Mount Sinai, to work at the Dent Neurologic Institute. With colleagues he initially contemplated using interferon from the Roswell Park Memorial Institute to treat amyotrophic lateral sclerosis, but their attention soon turned to multiple sclerosis.

Verveken suggested that interferon failed because it does not cross the blood-brain-barrier, and suggested that administration should be intrathecal. Larry Jacobs took up this suggestion, no doubt aware that a group at Roswell Park were using intrathecal interferon to treat meningeal leukaemia (Misset, Mathe et al. 1981). His study group consisted of 20 patients, four with relapsing-remitting disease, four with relapsing-progressive disease and 12 who were "stable with residua". 10 received natural interferon-beta by lumbar puncture, twice a week for four weeks then monthly for five months. 10 patients were used as unblinded controls. Patients were followed up for over a year. At the end of the study, 2 of the interferon-treated patients had experienced 4 relapses, compared to 10 relapses from 6

controls: for the first time, there was a hint that relapse rate in multiple sclerosis might be modified.

Jacobs' paper deserved some of the criticism that followed, for instance from Charles Berry from University of California San Francisco (Berry 1982). There are simple arithmetical errors in the tables and the primary outcome is not statistically significant, as was erroneously claimed. Jacobs' reliance on a change in relapse rate before and after treatment is potentially distorted by regression to mean. And, most oddly to modern readers, there is no explanation for the death of one patient receiving interferon in the first month of the study, other than to say it was unrelated to treatment.

However, the data were encouraging and more studies, led by Jacobs, followed. He went on to produce a much more rigorous trial, including placebo-injection lumbar punctures, in 69 patients with relapsing-remitting disease (Jacobs, Salazar et al. 1986) and did show a definite effect. However, a few years later a trial of natural interferon-beta had to be stopped early because it exacerbated rather than ameliorated multiple sclerosis disease activity (Milanese, Salmaggi et al. 1990). There was a sense of growing concern over the need for intrathecal injections and the biological variability of human-derived interferon. Thereafter, interferons derived from recombinant technology were given systemically. Still there were problems. Recombinant interferon-alpha was shown to have no efficacy in 1986 (Camenga, Johnson et al. 1986) and recombinant interferon-gamma (Immuneron, Biogen) provoked relapses (Panitch, Hirsch et al. 1987).

Larry Jacobs was undeterred. He set up the Multiple Sclerosis Collaborative Research Group to test Biogen's recombinant interferon-beta 1a. He designed a large trial, with some innovative features, which eventually led to a product licence for Avonex in 1996 in the US and in the EU from 1997. But he was pipped to the post by Ken Johnson, another key figure in the interferon story. With Berlex laboratories, Johnson had managed to get another recombinant, interferon-beta 1b (Betaseron), licensed in 1993 (Paty and Li 1993; The IFNB Multiple Sclerosis Study Group 1993)

In 1998, Larry Jacobs became the first holder of the Irvin and Rosemary Smith Chair in Neurology at Buffalo School of Medicine and Biomedical Sciences, which had been established through a \$1.5 million endowment by Biogen. He died in 2001, aged 63.

The introduction of the interferons as disease-modifying treatments of multiple sclerosis brought many benefits to people affected by the disease other than a modest reduction in

disease activity and an uncertain effect on the long term course of the disease; not the least by drawing the attention of the pharmaceutical companies to the potential marketplace for novel therapies, and also by requiring an infrastructure of neurological and nursing support, that improved the generic care of people affected by multiple sclerosis.

## **9. 1983: a step towards increased diagnostic accuracy**

*Poser CM, Paty DW, Scheinberg L, McDonald WI, Davis FA, Ebers GC, Johnson KP, Sibley WA, Silberberg DH, Tourtellotte WW. 1983. New diagnostic criteria for multiple sclerosis: guidelines for research protocols. Ann Neurol 13(3):227-31*

The first attempt at systematic criteria for the diagnosis of multiple sclerosis came from Allison and Millar (1954) who classified the disease as *early* (few physical signs but a recent history of remitting symptoms); *probable* (soon changed to early probable or latent: no reasonable doubt about the diagnosis); *possible* (findings suggesting the diagnosis and no other cause found but the history static or progressive and with insufficient evidence for scattered lesions); and *discarded* (Allison and Millar 1954; Millar and Allison 1954).

However, then as now, neurologists have not felt the need to be constrained by criteria when making the diagnosis of multiple sclerosis. As Charles Poser wrote in 1965, “many clinicians thus insist that there is, in arriving at any diagnosis, and certainly in diagnosing MS, an intangible, unpredictable, highly personal and almost mystic diagnostic item frequently referred to as the “feel” or the “smell” of the patient, and which can best be characterized by the almost classical, pontifical pronouncement: “Don’t ask me why I think that this patient has MS, I just know!”.” (Poser 1965)

Poser was not impressed. In his huge multiple sclerosis practice, he frequently encountered misdiagnosis, against which he battled all his life. He died in November 2010, at the age of 86. After escaping Nazi-occupied Belgium with his family, he grew up in New York City and attended George Washington High School and City College. After returning from Army service in World War II, he trained at the New York Neurological Institute under Dr. H. Houston Merrit.

Poser’s motivation to introduce diagnostic criteria for multiple sclerosis was to improve research, in particular the quality of epidemiological studies. He set out his stall in a classic paper in 1965 (Poser 1965). He asked 190 neurologists in 53 countries to read 30 case records and decide if they had “probable”, “possible” or “unlikely” multiple sclerosis. In fact, the

cases had all come to post mortem and included 25 have with pathologically proven multiple sclerosis, 3 cases with other conditions mimicking multiple sclerosis and in 2 cases, MS coexisting with other conditions. 108 neurologists replied (only two from England, Dr Acheson from Oxford and Dr Garland from Leeds). There was a consistent 2/3 diagnostic accuracy, across the board of geography and experience (except that the Swedes and those trained in Sweden, were less confident in making a diagnosis of “probable” multiple sclerosis). Somewhat embarrassingly, people regarded as multiple sclerosis experts performed rather worse than general neurologists. However, between individual diagnosticians, there was a great deal of variety. So Poser analysed symptoms and signs that neurologists find helpful in making the diagnosis of multiple sclerosis, both in negative and positive terms, from which he derived a rather complex scoring system to refine the clinician’s suspicion of multiple sclerosis. Immediately he recognised that his scoring system could be fooled by non-multiple sclerosis conditions such as brainstem glioma, so he mandated at least two years since the onset of symptoms before the diagnosis of multiple sclerosis could be made.

Ultimately, Poser’s scoring system was just too complex and it never took off. In the US, neurologists continued to use the Schumacher 1965 criteria; however this focused just on the “probable” group and did not incorporate the growing literature on paraclinical tests or imaging (Schumacher, Beebe et al. 1965). In the UK, the McDonald and Halliday (1977) criteria gained favour, as they recognised the value of, for instance evoked potentials (McDonald and Halliday 1977). Poser was not satisfied, so he set out in 1982 to come up with comprehensive diagnostic criteria for research: “The main reason for establishing these criteria is to restrict therapeutic trials and other research protocols to patients with definite MS; the category of probable is designed for the purpose of prospectively evaluating new diagnostic methods” So, Poser gathered at Washington the luminaries of multiple sclerosis, including George Ebers, Ian MacDonal and Donald Paty. They proposed four categories of multiple sclerosis: “clinically definite, laboratory-supported definite, clinically probable and laboratory-supported probable”. At last “paraclinical” evidence of a lesion could be substituted for clinical evidence. For instance, typical abnormalities on CT or “NMR” imaging, evoked potentials and induced hyperthermia (the “Hot bath test”). So, laboratory-supported definite multiple sclerosis could be diagnosed after one attack only, with paraclinical evidence of a subsequent new lesion affected (for instance a CEP that becomes abnormal) AND oligoclonal bands. *Clinically probable* was required two attacks with clinical

evidence of one lesion, or one attack and clinical or paraclinical evidence of two separate lesions, separated in time. *Laboratory supported* probable was two attacks and oligoclonal bands.

Poser's criteria lasted nearly two decades until replaced by the 2001 McDonald criteria, which were themselves modified in 2005 and, most recently, in 2010 (McDonald, Compston et al. 2001; Polman, Reingold et al. 2005; Polman, Reingold et al. 2011). Much of Poser's thinking remains. But he did not agree with the elevation in importance of MRI; "one of the big problems I see now is the numbers of patients who have minimal symptoms, and maybe some abnormal MRI findings, who have been treated for MS for years and who have never had it. I see people like this every week in my office" (Poser 2006). Of critical importance for the writers of the new McDonald is the ability to make the diagnosis of multiple sclerosis as early as possible, to allow the introduction of therapies. So, the absolute requirement for a second clinical (or paraclinical) attack has been dropped; instead any new MRI disease activity after a clinically isolated syndrome now fulfils the criteria to diagnose multiple sclerosis. This process has reached its apotheosis under the 2010, where it is proposed that evidence of dissemination *in time* can be derived from a single MRI scan *during* a clinically isolated syndrome; if it shows the simultaneous presence of asymptomatic gadolinium-enhancing lesions and non enhancing lesions at any time.

### **10.1988: surrogate markers in life for disease activity in multiple sclerosis**

*Miller DH, Rudge P, Johnson G, Kendall BE, Macmanus DG, Moseley IF, Barnes D, McDonald WI. 1988. Serial gadolinium enhanced magnetic resonance imaging in multiple sclerosis. Brain 111: 927-39*

Magnetic resonance imaging of the brain has become an invaluable technique for the diagnosis and management of people with multiple sclerosis, as well as into research of its pathogenesis and treatment. The paper we have selected is not the first study of multiple sclerosis using MRI. But it is, in our view, the first MRI study to bring new understanding of the pathogenesis of multiple sclerosis.

In 1973 a paper appeared in *Nature*, having been previously rejected as of insufficient general interest by the editor, entitled "Image formation by induced local interaction; examples employing magnetic resonance" (Lauterbur 1973). The author was Paul Lauterbur, a chemist at the State University of New York at Stony Brook. Peter Mansfield, a physicist from

Nottingham University, systematically solved the many problems of transforming this observation to a medical imaging system and produced, in 1976, the first “nuclear magnetic resonance” image of a human part (a cross-section of the finger) (Mansfield and Maudsley 1976). Thus arrived the definitive method for studying human tissue structure and function in health and disease for which the two received the Nobel Prize for Physiology or Medicine in 2003.

MRI was first explored in multiple sclerosis through a collaboration between the Hammersmith Hospital in London and the Central Research Laboratories, Thorn-EMI Ltd in Hayes, Middlesex. Their Lancet report from 1981 exudes excitement at the vastly improved ability to visualise multiple sclerosis lesions compared to computed tomography. The new technique “demonstrates abnormalities in MS on a scale not previously seen except at necropsy although the specificity of these abnormalities is uncertain at present” (Young, Hall et al. 1981). Other investigators soon picked up on the technique, and early work confirmed and extended its role in supplementing clinical evidence for the diagnosis of multiple sclerosis.

Enthusiasm for the technique soon spread beyond the academic world. The first commercial MR scanner in Europe (from Picker Ltd.) was installed in 1983 at the University of Manchester Medical School. In the same year, the Multiple Sclerosis of Great Britain and Northern Ireland funded the first MRI scanner in the world to be solely dedicated to multiple sclerosis research, at the National Hospital for Neurology and Neurosurgery at Queen Square, London. Ian McDonald led the group and their early work emphasised the number of “silent lesions” visible on MRI scans at presentation in multiple sclerosis and in clinically isolated syndromes (Ormerod, Miller et al. 1987).

The paper we have chosen comes from Ian McDonald’s group. Its importance lies in the insights it gave to the natural history of multiple sclerosis, particularly to the realisation that there is continued disease activity even during periods of clinical stability. The problem that David Miller and colleagues sought to solve was how to judge the age of an individual MRI lesion. They argued that distinguishing between new and old lesions would help in two contexts: first, in the assessment of the patient with a clinically isolated syndrome (where lesions of different age would suggest dissemination in time and hence the probability of multiple sclerosis); and, secondly, in therapeutic trials. They turned to the paramagnetic agent, gadolinium DTPA, which Donald Silberberg’s group at the University of Pennsylvania

had shown more frequently to demonstrate abnormalities in patients with clinical disease activity than unenhanced scans (Gonzalez-Scarano, Grossman et al. 1987).

10 patients with multiple sclerosis were scanned initially, eight of whom were experiencing a relapse at the time. Fifty six contrast enhancing lesions were observed in total compared to none in the two non-relapsing patients. In six of eight patients, an enhancing lesion was seen which was anatomically congruent with the relapse phenotype. A second scan was performed between three and five weeks later in nine of these patients. Of the previous 54 enhancing lesions, only 12 persisted. But 12 new lesions had appeared (including four previous lesions where enhancement extended into previously unaffected brain areas). Six months later, eight patients were rescanned and 15 new lesions seen on unenhanced scans, of which eight showed enhancement. In passing, the authors note that some enhancing lesions were seen in the cortex, and one enhancing spinal cord lesion is shown.

For the first time, the dynamics of plaque formation could be studied and some of the controversies arising from static pathological studies resolved. The observation that enhancement was seen as the first abnormality in every new lesion which appeared on interval scans placed breakdown of the blood-brain barrier as an initiating event in the evolution of the plaque. David Miller and colleagues suggested that the elevated T1/T2 ratio of enhancing lesions reflected the increased intracellular water associated with acute inflammation; and the low T1/T2 ratio of old non-enhancing lesions might reflect increased extracellular water from leakage of an incompletely repaired blood brain barrier. Cortical plaques, which were known from pathological studies but had not been seen on unenhanced scans could now be visualised with the use of gadolinium.

For most contemporary readers the big news was the revelations on the frequency of new lesions in people apparently with stable multiple sclerosis. This had several implications. For research, MRI provided a sensitive measure of brain inflammation: Don Paty, at the University of British Columbia in Vancouver, was the first to correlate active lesions with changes in peripheral immune function (Oger, O'Gorman et al. 1988). But the most obvious conclusion was that gadolinium- enhancing lesions could be used to reduce the duration and cohort sizes of clinical trials.

The findings of this paper were soon ratified. Henry McFarland at the National Institutes of Health (Bethesda) produced a study of six patients with “early, mild, relapsing-remitting multiple sclerosis” scanned monthly for 8-11 months and showed that “numerous enhancing

lesions were observed irrespective of clinical activity”; and, again, suggested that these lesions be used as an outcome measure in clinical trials (Harris, Frank et al. 1991).

## 11. Figure legends

- Figure 1 from (Dawson 1916). (A) [Figures 1-4] Successive stages in the evolution of a sclerotic area in the posterior columns of the cervical spinal cord. Sections cut in longitudinal direction of the nerve fibres show increasing glia fibril formation. a: Glia nuclei; b: glia fibrils; c: fat granule cells; d: persistent axis cylinders. Figs 1 and 3 Ford-Robertson's methyl violet stain. Figs 2 and 4 palladium methyl violet. (B) [Figures 16-17] Persistence of axis cylinders across a demyelinated area in the pons. [Figures 18-20] Stages in the demyelination of an area and in the evolution of the fat granule cell. a: Small glial nuclei; b: transition forms between a and b; c: fat granule cell; d: nerve fibre; e: blood vessel; f: proliferated glia nuclei. (C) [Figures 8-12] Successive stages in the evolution of a sclerotic area in the posterior columns of the cervical cord. a: Glia nuclei; b: blood vessel; c: fat granule cell; d: myelinated nerve fibre; e: finely granular glia tissue; f: naked axis cylinder; g: transition to normal tissue. Fig. 8: Alterations in the glia cell and myelin. Fig. 9: Gitter cells. Fig. 10: Fat granule cells accumulated in blood vessels. Fig. 11: Glial fibrils increasing and axons intact. Fig. 12: Gliosis with few cells and preserved axons.
- Figure 2 from (Lowenthal, Vansande et al. 1960). Lowenthal's agar gel micro-electrophoresis pattern of CSF from: 1 Multiple sclerosis. 2 Subacute sclerosing leucoencephalitis (SSLE). 3 Normal. 4 Neurosyphilis. 5 African trypanosomiasis.
- Figure 3: Martin Halliday and his equipment for measuring visual evoked potentials
- Figure 4: John Kurtzke's map of multiple sclerosis prevalence

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