

Top Ten Papers in Multiple Sclerosis

The first understandings of the disease



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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.¹ Here are our first three landmark papers, which chart the beginnings of understanding of pathology, immunology and treatment of multiple sclerosis.

1916: The pathological anatomy of the lesion in multiple sclerosis

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

James Dawson (1870-1927) left the greatest pathological account of multiple sclerosis in the English language (Dawson 1916). First he summarises 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,² Williamson^{3,4} and Marie,⁵ who suggested that infections initiate the changes in blood vessels. Bielschowsky⁶ considered that the vascular process is directed primarily at nerve fibres. Strumpell⁷ considered that exogenous insults act upon an 'intrinsically weak-

ened' system; and Bramwell⁸ also saw multiple sclerosis as primarily a developmental disturbance. Müller,⁹ 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 Charcot¹⁰ and most of his school. Redlich¹¹ and Huber¹² 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: Rindfleisch¹³ 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).

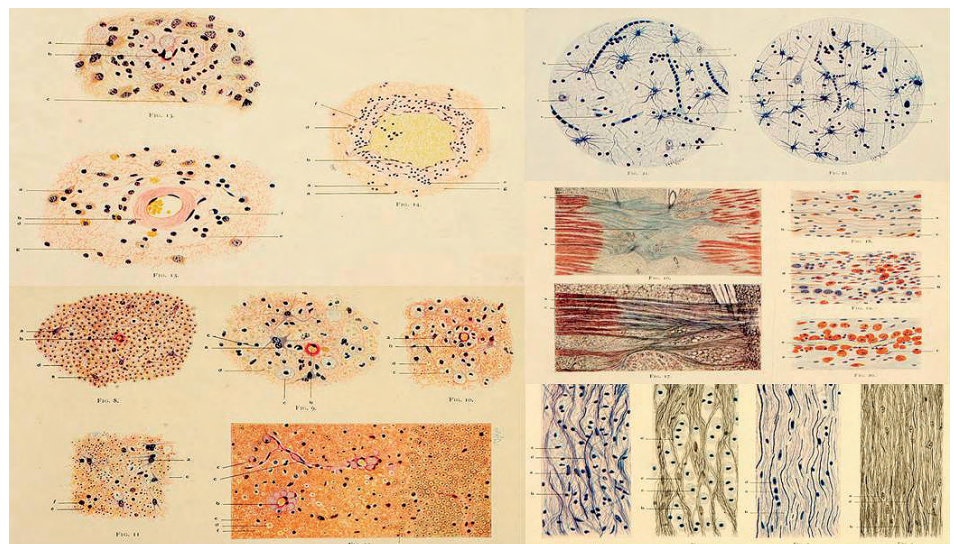


Figure 1 from 25. (A) [Figures 1-4] Successive stages in the evolution of a sclerotic area in the posterior columns of the cervical spinal cord. Sections cut in longitudinal direction of the nerve fibres show increasing glia fibril formation. a: Glia nuclei; b: glia fibrils; c: fat granule cells; d: persistent axis cylinders. [Figures 1 and 3] Ford-Robertson's methyl violet stain. [Figures 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. [Figure 8] Alterations in the glia cell and myelin. [Figure 9] Gitter cells. [Figure 10] Fat granule cells accumulated in blood vessels. [Figure 11] Glial fibrils increasing and axons intact. [Figure 12] Gliosis with few cells and preserved axons.

Reviewing the histology of nine personal cases (LW, a kitchen maid, aged 28; CS, aged 22; Mrs G, aged 30; JW; SS, a nurse aged 44; CG, a baker's shop-woman, aged 24; J McN, a cabinet maker, aged 42; MR, a typist, aged 33; and LH, aged 30), Dawson devotes the majority of his text to LW. 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 29th) 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 Markschatenherde (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 LW, 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 characterised 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 summarises his ideas on plaque

formation around brain inflammation to include a sequence of events that, although not disease-specific, produces recognisable 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.

1960: Evidence for an immune response within the central nervous system in multiple sclerosis

Lowenthal A, Vansande M, Karcher D.

The differential diagnosis of neurological diseases by fractionating electrophoretically the CSF proteins. J. Neurochem. 1960;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 their 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.¹⁴ 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.¹⁵ 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 (γ_1 , γ_2 and γ_3) in the cerebrospinal fluid of patients with multiple sclerosis, which were not present in normal individuals. And they distinguished these from the increased γ_4 and γ_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.

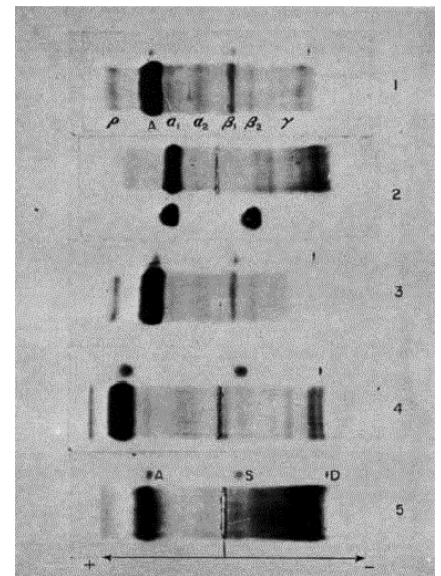


Figure 2 from 26. Lowenthal's agar gel microelectrophoresis pattern of CSF from: 1 Multiple sclerosis. 2 Subacute sclerosing leucoencephalitis (SSLE). 3 Normal. 4 Neurosyphilis. 5 African trypanosomiasis.

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.^{16,17}

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.¹⁸

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.

1970: An exemplary trial of steroid treatment of the acute relapse

Rose AS, Kuzma JW, Kurtzke JF et al.

Cooperative study in the evaluation of therapy in multiple sclerosis: ACTH vs placebo: Final report. Neurology 1970;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.¹⁹ 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 randomisation, 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

Fifty-two 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 four 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 three and four, 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.²⁰ And more still was learnt from the effect of steroids on optic neuritis.²¹⁻²⁴ The conclusion of all of these studies is 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. ♦

REFERENCES

1. McDonald WI. *Chance and design*. J Neurol, 1999;246(8):654-60.
2. Dejerine J. *Etude sur la sclérose en plaques cérébro-spinale. A forme de sclérose latérale amyotrophique*. Rev Med (Paris) 1894;iv:193-212.
3. Williamson RT. *The early pathological changes in disseminated sclerosis*. Med Chronicle (Manchester), 1894;19:373-9.
4. Williamson RT. *Diseases of the Spinal Cord*. 1908. Oxford: Oxford University Press and Hodder & Stoughton.
5. Marie P. *Sclérose en plaques et maladies infectieuses*. Progrès Med 1884;12:287-9, 305-7, 349-51, 365-6.
6. Bielschowsky M. *Zür Histologie der multiplen Sklerose*. Neurologisches Zentralblatt 1903;22:770-7.
7. Strumpell A. *Zür pathologie den multiplen Sklerose*. Neurologisches Zentralblatt, 1896;15:961-4.
8. Bramwell B. *On the relative frequency of disseminated sclerosis in this country (Scotland and the North of England and in America*. Rev Neurol Psych Edinb 1903;i:12-17.
9. Muller E. *Ueber sensible Reizerscheinungen bei beginnender multipler sklerose*. Neurologisch Centralblatt, 1910;29:17-20.
10. Charcot JM. *Histologie de la sclérose en plaques*. Gazette Hôpitaux 1868;41:554-8.
11. Redlich E. *Histologisches Detail zur grauen Degeneration von Gehirn und Rückenmark*. Neurologisches Zentralblatt 1896;15:961-4.
12. Huber O. *Zur pathologischen Anatomie der multiplen Sklerose der Rückenmarks*. Arch pathologische Anat, 1895;140:396-410.
13. Rindfleisch E. *Histologisches Detail zur grauen Degeneration von Gehirn und Rückenmark*. Arch Pathol Anat Physiol Klin Med (Virchow), 1863;26:474-83.
14. Kabat EA, Moore DH, and Landow H. *An Electrophoretic Study of the Protein Components in Cerebrospinal Fluid and Their Relationship to the Serum Proteins*. J Clin Invest, 1942;21(5):571-7.
15. Smithies O. *Zone electrophoresis in starch gels: group variations in the serum proteins of normal human adults*. Biochem J, 1955;61(4):629-41.
16. Link H. *Immunoglobulin G and low molecular weight proteins in human cerebrospinal fluid. Chemical and immunological characterization with special reference to multiple sclerosis*. Acta Neurol Scand, 1967;43:Suppl28:1-136.
17. Link H. *Oligoclonal immunoglobulin G in multiple sclerosis brains*. J Neurol Sci, 1972;16(1):103-14.
18. Cross AH, Wu GF. *Multiple sclerosis: oligoclonal bands still yield clues about multiple sclerosis*. Nat Rev Neurol, 2010;6(11):588-9.
19. Schumacher GA, et al. *Problems of Experimental Trials of Therapy in Multiple Sclerosis: Report by the Panel on the Evaluation of Experimental Trials of Therapy in Multiple Sclerosis*. Ann N Y Acad Sci, 1965;122:552-68.
20. Milligan NM, Newcombe R, Compston DA. *A double-blind controlled trial of high dose methylprednisolone in patients with multiple sclerosis: I. Clinical effects*. J Neurol Neurosurg Psychiatry, 1987;50(5):511-6.
21. Beck RW, et al. *A randomized, controlled trial of corticosteroids in the treatment of acute optic neuritis*. The Optic Neuritis Study Group. N Engl J Med, 1992;326(9):581-8.
22. Beck, R.W., et al., *The effect of corticosteroids for acute optic neuritis on the subsequent development of multiple sclerosis*. The Optic Neuritis Study Group. N Engl J Med, 1993. 329(24): p. 1764-9.
23. Group, ONS. *Visual function 15 years after optic neuritis: a final follow-up report from the Optic Neuritis Treatment Trial*. Ophthalmology, 2008;115(6):1079-82 e5.
24. Keltner JL, et al. *Visual field profile of optic neuritis: a final follow-up report from the optic neuritis treatment trial from baseline through 15 years*. Arch Ophthalmol, 2010;128(3):330-7.
25. Dawson JD. *The Histology of Disseminated Sclerosis*. Transactions of the Royal Society of Edinburgh, 1916;50:517-740.
26. Lowenthal A, Vansande M, Karcher D. *The differential diagnosis of neurological diseases by fractionating electrophoretically the CSF gamma-globulins*. J Neurochemistry, 1960;6:51-6.