HISTORICAL BACKGROUND

Despite an ever-increasing international research effort, motor neurone disease (MND) remains one of the great enigmas of medical science. A strong commitment to MND has existed within the Department of Neurology at the Royal Preston Hospital for some time, and the momentum of this programme has been steadily increasing in recent years. It is particularly apposite that Preston should emerge as a centre of some importance in MND research in an historical context. The contribution of Sir Charles Bell, Professor of Surgery in the University of Edinburgh, to early clinical descriptions of MND in the early nineteenth century has been increasingly recognised. One of the patients mentioned by Bell had been referred to him in 1825 by one RWR Robinson, a physician who practised in Preston. Robinson was prominent in the medical life of the town at that time and was instrumental in establishing the Preston Dispensary in the early years of the nineteenth century. It is possible that his referral letter to Bell may be one of the earliest descriptions of the syndrome of progressive bulbar palsy in the medical literature.

TRACE ELEMENTS, FREE RADICALS & MND

There is still little understanding of the mechanisms which initiate the motor neuronal degeneration which is the essential feature of MND. In Preston we have been attracted for some time to the idea that free radical mechanisms may be important. This first came out of work done some years ago examining trace element distribution in MND. This produced evidence, to some extent supported by the work of others, that the distribution of manganese and selenium is altered in MND. There were indications that selenium levels might be increased in erythrocytes, cervical cord, liver and bone with evidence to suggest that spinal cord manganese levels were also increased. There was also a suggestion that hepatic manganese content was reduced in MND. Both these elements are closely involved with free radical mechanisms. Manganese can itself trigger free radical formation and is also a constituent of mitochondrial superoxide dismutase (MnSOD), an enzyme important in the inactivation of free radical species. Selenium is a structural component of glutathione peroxidase (GSHPX), an enzyme also implicated in the inactivation of free radicals.

In the light of this work it was thought that the increased spinal cord manganese and selenium levels could indicate that spinal cord GSHPX and MnSOD activities were increased in MND. If this were so, an obvious implication would have been that free radicals might be involved in the pathogenesis of MND.

At the same time one recalled previous work measuring nucleic acid concentrations in surviving motor neurones in MND. Reduced RNA levels had been found by two groups without concomitant loss of DNA. This was subsequently interpreted as possibly pointing to a defect of DNA transcription as expressed in the DNA hypothesis of Bradley and Krasin. This hypothesis was that the observed nucleic acid changes were the result of a defect of enzymatic DNA repair mechanisms and that MND arose as a direct result of this. Although this is a very elegant and attractive hypothesis it has been very difficult to test.

There are, however, other ways in which DNA can be damaged so as to disrupt transcription of the genetic code. One well known example of this is exposure to ionising radiation. Similar changes can also occur if the polynucleotide chain is damaged by free-radicals. The “Free Radical Hypothesis” (figure 1) was thus developed and this has formed the essential basis for the MND research programme which has developed in Preston and Lancaster in recent years.

![Figure 1 - Free Radical Hypothesis of ALS/MND](image)

We first tested this “free radical hypothesis” by examining CSF parameters of free radical activity and lipid peroxidation. No differences were found between MND patients and controls. On reflection this result was not considered surprising. Remembering that the blood brain barrier is generally preserved in MND, it was quite possible that evidence of abnormal free radical activity might not be expressed in CSF. This work has since been greatly extended not only in the context of a double blind placebo controlled crossover trial of seleagine in MND, but also a small number of measurements of liver and spinal cord GSHPX activity in patients who have died of MND.

In recent years there has been an indication that seleagine, a selective MAOB antagonist and a commonly used drug in...
treatment of Parkinson’s disease (PD), might actually slow the rate of progression of the neurodegenerative process in PD\(^{14}\). If this is so, the mechanism is unknown. This remains a controversial issue. Selegiline has been, perhaps rather loosely, referred to as an “antioxidant” in this context and one postulated mechanism for such an effect is that selegiline might modify the free radical-mediated reactions that have been implicated in the degenerative process of PD.

In the light of all these pieces of evidence we instituted a double blind placebo controlled crossover trial of selegiline in MND. This has now been completed and the results are awaited.

The protocol of this trial extended over an eleven month period for each participant and clinical and laboratory assessments were made every four weeks. The laboratory assessments consisted of measurements of various parameters of antioxidant activity including serum tocopherol and caeruloplasmin, erythrocyte GSHPX and superoxide dismutase (SOD) as well as serum and leucocyte ascorbic acid. CSF measurements of some of these parameters were also made in a few patients. These laboratory studies were primarily intended to determine whether or not selegiline modifies parameters of free radical and antioxidant activity.

An assessment of the role of selegiline must await the outcome of the final analysis of the results. An interim analysis of the laboratory studies did, however, show changes with possible implications not only for our general understanding of free radical defence mechanisms in the nervous system but also for understanding sequential changes in parameters of free radical activity as MND progresses. According to an independent monitoring group these changes were independent of any drug effect.

In relation to general aspects of free radical defence mechanisms in the nervous system, only very low CSF levels of caeruloplasmin, SOD and GSHPX were found. Other workers have previously reported reduced CSF SOD levels in MND\(^{13}\) but we have not so far been able to confirm this. Relatively high CSF concentrations of ascorbic acid have been previously reported\(^{13}\) and this has so far been confirmed by our data. The CSF/plasma ratio seems to range from 3 – 6 with a higher incidence of low ratios among the MND group (p=0.0172) (figure 2). The significance of this is uncertain at this stage. There is also apparently a strong correlation between CSF and plasma ascorbate levels (r=0.84) suggesting a specific transport mechanism across the choroid plexus (figure 3). Others have commented previously on the apparently low CSF levels of caeruloplasmin, SOD and GSHPX and suggested that CSF must possess some other means of free radical defence. It is possible in the context of these findings that ascorbate might fulfil this function in CSF.

![Figure 2 – CSF/Plasma Ascorbic Acid Ratios](image)

![Figure 3 – CSF and Plasma Ascorbate Levels](image)

Other trends in this preliminary analysis hinted at sequential changes in serum caeruloplasmin and blood GSHPX levels with disease progression. If these are confirmed in the final analysis and even if they do turn out to be epiphenomena, they may still have implications for our understanding of MND. Serum caeruloplasmin (figure 4) and blood GSHPX levels (figure 5) seemed to fall with disease progression. This analysis was based on data from 377 measurements of each parameter from the first 45 MND patients who were recruited into the trial. Twenty-two of these had completed the protocol at the time this data was reviewed. The apparent decline in serum caeruloplasmin content with disease progression was an unexpected finding. Caeruloplasmin does possess antioxidant activity and like manganese, copper is a constituent of SOD being present in the cytosolic form of the enzyme. There has been little work on copper in MND other than that it has been demonstrated in the liver of MND patients by scanning electron microscopy\(^{17}\) and also that copper can damage DNA in the presence of appropriate catalysts.

The decline in blood GSHPX seemed more striking (figure 5). Whole blood GSHPX activity is generally accepted to be the most reliable guide to body GSHPX status. Serum levels are lower and it was clear that there is no question of any serial decline in this parameter. What might be the implications of these findings? What factors might be involved? Duration of disease at trial entry did not seem to be a factor. An agonal effect however did seem to be a
Figure 4 – Serum Caeruloplasmin in MND

Figure 5 – Whole Blood GSHPX in MND
possibility. These observations could be of particular interest in relation to our other work in which GSHPX activity was measured in spinal cord and liver of patients dying of MND. Numbers are still small but some very low grey matter activities have been found in some of the MND patients as compared with controls.

In recent years the neurodegenerative diseases have increasingly been considered more as a group than as isolated disease entities. A reduction in nigral GSHPX activity has also been reported in PD. This is clearly relevant in the context of the possible reduction in spinal cord GSHPX activity in MND. Furthermore, it has more recently been reported that erythrocyte GSHPX activities are significantly lower in patients with advanced PD than in those suffering from early disease. We are clearly continuing to look into possible correlations between blood GSHPX status and clinical parameters in MND and it will be important to see if these impressions are confirmed in the final results from this trial. If there is a serial decline in blood GSHPX activity with disease progression an explanation will need to be found. Blood GSHPX activity is clearly rather remote from the anterior horn cell, but it is the most reliable index of general body GSHPX status. It is possible that these observations, linked with the possibly low spinal cord GSHPX activities in patients dying of MND, might represent a consumption effect. If the degenerative process in MND is associated with excessive free radical activity either as a primary or secondary phenomenon, it is likely that this would lead to a mobilisation of protective mechanisms. If this mobilisation involved GSHPX it is quite possible that as the disease reaches its final conclusion, there is progressive exhaustion of the capacity to synthesise GSHPX, resulting in a steady decline in whole blood level. It is also possible that this could be mirrored, at least in the terminal phase, by a reduction in spinal cord GSHPX levels. Although the present data are obviously not sufficiently clear cut to allow a conclusion that whole blood GSHPX measurements can be used as a biochemical marker of progression in MND, one is aware that different categories of GSHPX activity are now being recognised. It is possible that further refinements of the assay procedure addressing this point might lead us towards such a biochemical marker.

**EPIDEMIOLOGICAL ASPECTS**

Many health professionals working with MND sufferers have often gained the subjective impression that the disease tends to aggregate into geographical clusters. Until recent years this has been difficult to test objectively. Many previous reports addressing this issue have either been anecdotal or been based on relatively large areal population units.

Some years ago, it became apparent to us that an analysis based on the UK post code presented a powerful tool for a much more critical study of the distribution of MND over small areas. The post code lends itself to computer indexing and can in some cases cover a population of only thirty-eight and specify an address to within a hundred metres. In conjunction with the Department of Geography at the University of Lancaster we therefore undertook an analysis of a series of 171 MND patients presenting to the Department of Neurology at Royal Preston Hospital over the 10 year period 1976-1986. This department serves a population approaching 1.8 million resident in Lancashire and South Cumbria. Although some areas were found in which the rate of occurrence of MND seemed greater than would have been expected by chance, clear cut evidence of clustering was not detected.

The analysis of this data largely depended on classical statistical techniques such as the Chi-squared and Poisson distributions. Both of these methods have some theoretical disadvantages, but have been widely used in the past by other workers in this context. The other inherent difficulty with this approach is that any clusters might not necessarily respect the arbitrary boundaries of postcode sectors or administrative zones.

This series was subsequently extended to 235 MND patients presenting between 1976 and 1989. The main problem in undertaking this latter analysis was obtaining reference data on the population distribution within unit post codes with which the data from the MND patients could be compared. Unfortunately, validated age structured population data were not available for unit post codes in England at that time and we therefore used a random selection of 705 of the 40,090 unit post codes within the area covered by the study as a source of reference data for population density.

Although the actual location of the address of each patient and "control" thus depended on the postcode this latter analysis avoided any reference to arbitrary units such as post code sectors or local authority wards. This was made possible by the sophisticated mathematical facilities available within a Geographical Information Systems Environment making use of special FORTRAN programmes developed within the Department of Mathematics at the University of Lancaster where there is a well-established interest in examining methods of studying the distribution of point events as a function of spatial separation.

This work has provided a method which not only determines whether or not geographical clustering occurs, but also permits an assessment of its character in terms of the spatial level at which clustering might be occurring. It is also possible to take into account clustering in relation to a putative point source (eg nuclear installations in relation to childhood leukaemia). The distribution of the 235 MND patients was considered at distances of up to 10 km from each case at 200 metre intervals. Using this approach to the analysis of the data there was only a hint of clustering at a distance of about 6 km (p=0.014).

Since this time however, a prospective case control study has been instituted to address these questions further. The aim of this project is to identify all patients in Lancashire and South Cumbria presenting with MND over a five year period between 1989 and 1993 and from the data collected to determine whether or not MND does show any propensity to occur in geographical clusters. This work is well under way and such is the apparent success of ascertainment to date that the numbers of MND patients identified exceeds the number that would be expected from the literature by something of the order of 20%. It is hoped that definitive analysis of the rigorously controlled data set which is being obtained from this project will commence in the autumn of 1993. This data set takes into account the sufferer's entire life history and not just the place of residence at the onset of the disease. These are clearly critical issues in relation to suggestions that MND, and indeed other neurodegenerative disorders, might be related to exposure to environmental factors or toxins. We believe that the mathematical advances which have been an essential component of this work will lead to even more powerful techniques for studying the geographical distribution of disease and thus help to provide a much more critical approach to the study of possible environmental factors in disease.
GENERAL COMMENTS AND CONCLUSIONS

The aim of the research programme which has been described in this review has been to try to help piece together the jigsaw of mystery surrounding the mechanisms precipitating the neurodegenerative process in MND. It is hoped that the results obtained will eventually lead us closer to an understanding of the mechanisms underlying this degenerative process. Although these endeavours continue apace, MND remains one of the great mysteries of medical science. We must also not forget that MND is the cause of untold human suffering. It is hoped that this contribution will have helped to demonstrate the progress which is being made in this area and reinforce the hope that as the general effort in MND research continues to expand that substantial progress in this direction will become a realistic prospect as we move towards the twenty-first century.

ACKNOWLEDGEMENTS

The work described in this review has been generously supported by the Motor Neurone Disease Association (MND Association), Manchester Branch MND Association, George Barton Trust, Preston & Chorley Hospitals Research Fund, Motor Neurone Disease Research Endowment Fund of Preston Health Authority and Britannia Pharmaceuticals.

This programme would not have been possible without the collaboration and support of many individuals including Glynis Batterby and Enid Houghton, Research Sisters in the Department of Neurology; Ian Gatt, Glenis Rostron and Carol Bailey in the Department of Clinical Chemistry; Patrick Lynch, Neuropathologist; Malcolm Phillips, Pharmacist at the Royal Preston Hospital. I would also like to acknowledge the valued collaborations with Tony Gatrell of the Department of Geography at the University of Lancaster, Malcolm Jackson of the Department of Medicine at the University of Liverpool, Brian Pentland of the Astley Ainslie Hospital, Edinburgh and Bill East and Ian Harris of the Scottish Universities Research and Reactor Centre, East Kilbride.

This work would not have been possible without the support and encouragement of my neurological colleagues Edmund Critchley and Sarosh Vakil and I am particularly indebted to them for enabling this programme to develop and continue to expand.

The research programme described in this review was honoured by the presentation of the 1992 Lella Esposito Scarpone Randazzo International Award and Prize Lecture in MND Research to the author.

REFERENCES