MULTIPLE SCLEROSIS: PSYCHOSOMATIC ORIGINS AND THE ROLE OF NITRIC OXIDE

Peter Good

Summary

Biochemical evidence has revealed that multiple sclerosis myelin is developmentally immature and uncompacted, resembling the myelin of children. This implicates failure of the second stage of myelination--puberty and adolescence--when myelin sheaths normally compact and mature. Because the risk of multiple sclerosis is known to be acquired near puberty, evidence of psychosomatic factors in the family background of these persons should be seen with new eyes.

Evidence of suppressed puberty in multiple sclerosis patients acquires even greater significance in light of the recent discovery of inducible nitric oxide in the lesions. Because astrocytes are a primary source, this inducible nitric oxide may originate as a protective response to compensate deficiencies of endothelial and neuronal nitric oxide.

Two signs that endothelial nitric oxide may be chronically depleted in multiple sclerosis are that patients tend to be very heat-sensitive, and their platelets are sticky. Sensitivity to stress may reveal depletion of the parasympathetic transmitter neuronal nitric oxide. Other reasons to suspect endothelial nitric oxide depletion in multiple sclerosis are apparent deficiencies of sex hormones, magnesium, and zinc. Estrogen, testosterone via estrogen, and magnesium all utilize endothelial nitric oxide, the primary endogenous vasodilator, to relax vascular smooth muscle. Endothelial and neuronal nitric oxide synthases are zinc enzymes.

Another reason to suspect nitric oxide depletion is the likelihood that abnormal red blood cells--large, osmotically fragile, spiny, less deformable, and agglutinated--are hemolyzing in blood vessels. Free hemoglobin scavenges nitric oxide avidly, which may create deficiencies especially in the central nervous system, with its greater vasodilator tone.

If depletion of endothelial nitric oxide shifts blood from the arterial circulation to the venous circulation, as it does in diabetics, the most parsimonious explanation of multiple sclerosis might be that too little blood in arteries and arterioles leads to vasospastic symptoms, while too much blood in veins and venules leads to blood-brain barrier leakage and lesions.

Acute and chronic deficiencies of endothelial nitric oxide may explain why CNS vasodilators were so effective against acute and chronic multiple sclerosis in the 1950s. Trials of sex hormones show they improve lesions as well as symptoms. The potential of L-arginine, magnesium, and zinc to increase endothelial nitric oxide and decrease inducible nitric oxide should be investigated. Two tests for endothelial nitric oxide depletion in multiple sclerosis patients are proposed.

Introduction

Multiple sclerosis (MS) is so called because many firm lesions are found scattered throughout the brain and spinal cord at autopsy. These lesions (called plaques) are patches where the myelin sheaths that surround and insulate nerve fibers have disintegrated and been replaced by astroglial scar tissue. Demyelination is presumed to account for the great variety of multiple sclerosis symptoms, which may mimic blood vessel disorders (Matthews 1978), tumors, or poisonings.

'Multiple' also indicates that symptoms are commonly dispersed in time as well as space. Symptoms usually begin in attacks lasting from seconds to weeks, then remit for months or even years, although remyelination (when it occurs) is usually limited and incomplete (Lumsden 1961; Franklin 2002). Transient symptoms in the face of persistent lesions have led researchers to conclude that reversible "secondary factors" act on demyelinated nerves--factors like vasospasm (Namerow & Thompson 1969), edema (Halliday et al. 1977), or acidosis (Selhorst et al. 1981). Lumsden suggested that secondary factors might even explain permanent symptoms:

> *... given that the 'cause' of the disease primarily demyelinates the axon and does not necessarily destroy conduction nor prevent its return, it seems logical to ask whether the permanent loss of function when it finally supervenes may not be due to repeated exposures to secondary factors just as the temporary loss of function may be due to single exposures to such factors. (Lumsden 1961)*

With the ability of magnetic resonance imaging to closely monitor the progression of lesions, the frequent lack of correlation between symptoms and lesions in multiple sclerosis has been called the "clinico-radiological paradox" (Barkhoff 2002). Nevertheless, most researchers today consider demyelination the fundamental essential lesion in multiple sclerosis.

In the 1940s and 50s, however, neurologists thought the primary multiple sclerosis lesion was a reversible condition, which if not relieved led to irreversible demyelination. The best evidence for this view was the benefit of central nervous system vasodilators, pioneered by Bayard Horton of the Mayo Clinic, which not only relieved acute attacks promptly, but often prevented the progression (Jonez 1952; Brickner 1958; Hess 1959, 1962; Good 2002).

In our time, cells of the immune system are thought to be the proximate agents of demyelination in multiple sclerosis. Yet significant numbers of these cells are not always found in areas of active demyelination:

> *In the sorts of numbers in which lymphocytes are commonly present in the plaque tissue, and at plaque contours, they appear too few to account per se for the extent of the demyelination. (Lumsden 1972)*

> *... continuing demyelination of this type (i.e., edge activity in old plaques) can proceed with very few, if any, lymphocytes present outside the perivascular spaces in the affected areas. This must argue against cellular immunity directed at myelin or oligodendrocytes as an important pathogenetic mechanism in this type of disease activity. (Prineas & Connell 1978)*

> *Regarding the role of lymphoid cells in pathogenesis, their paucity or absence in many plaques suggests that cellular immunity directed at myelin or oligodendrocytes is not the primary pathogenetic mechanism. Instead, it seems that they are drawn to the scene by damage taking place primarily in CNS tissue. (Roizin et al. 1982)*

The possibility that the immune system is responding to an agent like a virus is countered by the reality that no such agent has ever been identified nor transmitted to any animal or human.

The pathogenesis of multiple sclerosis revisited

The most definitive challenge to immunological conceptions of multiple sclerosis was recently published by PO Behan and A Chaudhuri of Glasgow University, together with BO Roep of Leiden University (2002). *The pathogenesis of multiple sclerosis revisited* threw down the gauntlet to MS researchers everywhere by contending there is little support for contemporary views that multiple sclerosis is an immunological disease. Accordingly, there is little benefit from treatments based on this misconception:

> *In this review, we have suggested that MS is not an autoimmune disease due to its intrinsic clinical, immunological, radiological and histological differences with ADEM [acute disseminated encephalomyelitis] and its experimental model, EAE [experimental allergic encephalomyelitis]. In our opinion, MS has the characteristics of a metabolically determined neurodegenerative disorder with strong genetic influence....*
> *Multiple sclerosis remains a disease of unknown aetiology. In recent years, most researchers have uncritically accepted the hypothesis that it is an autoimmune disorder. An in-depth review of the literature failed to support this concept, and the immunological claims for this disease are tenuous and fragile....*
> *The proposition that MS is a prototype autoimmune disease is weak and open to question: MS is not a model of EAE or, indeed, of any known autoimmune disease. Its histology compares more favorably with other forms of demyelination known to be metabolic in aetiology .... Many scholars of MS in the past have argued strongly that the disease is toxic/metabolic in origin....*
>
> *Clinical trials are, however, in abundance: a rise from 50 such papers in 1965 to more than 300 by the year 2000. This has been described by some as reflecting 'the sense of excitement in the field of MS therapeutics', but is regarded by us as a sad reflection of the gullibility of researchers in accepting an unproven hypothesis....*
> *The enormous number of trials based on putative immunosuppression and immunoregulatory mechanisms has singularly failed to show a cure or to convey major benefits to MS patients in addition subjecting them to an increased morbidity and mortality.... We have analyzed the literature on immune-modifying therapy in MS and it is clear that none of these agents can qualify as a candidate therapy under scrutiny. (Behan et al. 2002)*

One compelling argument against immunosuppressive therapy is the evidence that multiple sclerosis patients who subsequently developed acquired immunodeficiency disease (HIV) continued to suffer relapses:

> *If MS is an autoimmune disease a particularly informative setting for studying this would be in patients who have MS and are concomitantly infected with HIV infection, a situation which is known to induce a severe state of immune deficiency. Berger et al. reported seven such patients, six of whom developed an immunodeficient state after HIV infection but who continued to have relapsing-remitting MS.... These data would argue strongly against any autoimmune aetiology, i.e. T-cell mediated autoimmune pathogenesis. (Behan et al. 2002)*

These writers proposed that multiple sclerosis is instead a metabolic disorder with environmental and genetic influences. One clue to the metabolic disturbance, according to Behan and colleagues, is the reduced anisotropy in multiple sclerosis myelin--the tendency for water to diffuse more freely across MS myelin than normal myelin:

Diffusion of water molecules in relation to the directional organization of the myelinated white matter can be measured by diffusion tensor MRI. In this form of neuroimaging, magnitude and translational motion of the water molecules ('anisotropy') with respect to the white matter tracts can be defined. Myelin and cell membranes provide natural barriers to diffusion across the white matter fibres. As a result, the water molecules can diffuse only along these fibres. Therefore, a high degree of anisotropy is expected in normal myelinated while matter fibres and this has been confirmed in histologic and imaging studies. On the other hand, decreases in diffusion anisotropy have been shown to occur in diseases that affect myelin or axonal integrity, such as MS, neurodegenerative diseases, cerebral ischaemia and leukodystrophies....
In MS, reduction of the functional anisotropy in diffusion tensor MRI precedes any other change in the conventional MRI.... There is a progressive gradient in the decline of anisotropy observed in MS from the NAWM [normal appearing white matter] to the peri-plaque and intra-plaque white matter with the latter showing the most extensive changes.... The anisotropic changes in the normal appearing brain tissue is always present in all clinical phenotypes of MS irrespective of their presentations.

This vulnerability of multiple sclerosis myelin to water diffusion is supported by biochemical investigations by Moscarello and colleagues (1994), who concluded that MS myelin is immature and uncompacted, resembling the myelin of children. Because the risk of multiple sclerosis is known to be acquired around the time of puberty, previous evidence of psychosomatic factors in the puberty and adolescence of these persons should be revisited.

Finally, the association of multiple sclerosis and puberty assumes even greater significance in light of the recent discovery of astrocytic inducible nitric oxide in the lesions. This report proposes that inducible nitric oxide in multiple sclerosis arises to compensate depletion of constitutive nitric oxide--due to sex hormone deficiencies, magnesium depletion, zinc displacement, and the avid binding of nitric oxide by free hemoglobin. The large, osmotically fragile, spiny, less deformable, agglutinated MS red blood cell may result from magnesium depletion, heavy metal poisoning, and other factors. Potential treatments deserving investigation are L-arginine (substrate of nitric oxide), estrogen, testosterone, magnesium, and zinc.

Why is the risk of multiple sclerosis acquired near puberty?

One mystery of the disease is why multiple sclerosis predominantly strikes persons in their prime of life. Infectious diseases usually appear in infancy or childhood, degenerative diseases toward the end of life. But multiple sclerosis attacks young adults between 20 and 40 years old:

... it is therefore broadly true that multiple sclerosis is a disease peculiar to the period of sexual life, the period of minimum morbidity .... (Lumsden 1961)

Another curiosity is that the risk of getting multiple sclerosis is usually acquired years before any symptoms appear. Studies of migration patterns have established that the risk is acquired by age 15, though the peak incidence of the disease is about age 30 (Rawson 1969). Kurtzke (1972) asserted:

... the causative agent of MS is acquired when the patient is near the age of puberty.

Wainerdi (1961) found a correlation between the course of the disease and the age at onset of puberty:

These data ... suggest a relationship between sexual maturation and multiple sclerosis. It would be premature to speculate on the nature of the relationship, but if further statistically valid studies corroborate this observation, it might be wise to focus on the development of myelin at puberty.

Fischman (1981) analyzed the data of Kurtzke and Hyllested on the outbreak of MS in the Faroe Islands years after their occupation by British troops during World War II:

The concept that the pathogenesis of multiple sclerosis is dependent on both an environmental factor and host factors associated with the puberty period was tested using the data for the Faroe Islands "outbreak." Although this "outbreak" affected only 25 individuals, its value may be unique since it is the only virgin outbreak described.... Although 10 cases were below the age of puberty at the time of the occupation, none of these developed disease prior to reaching puberty. The data suggest that onset of pathogenesis of MS is dependent on passing or having passed through the puberty period.... The fact that many aging curves such as those for estrogen, testosterone or 17-hydroxycorticosteroid show striking similarity to the MS incidence curves raises the question whether infection is the entire explanation for the age distribution. (Fischman 1981)

is multiple sclerosis myelin immature?

Moscarello and colleagues (1994) reported compelling biochemical evidence that the normal-appearing white matter of multiple sclerosis patients is unlike the myelin of other persons. Instead, it resembles the myelin of young children:

> *... we have determined that myelin obtained from victims of MS is arrested at the level of the first growth spurt (within the first 6 yr of life) and is therefore developmentally immature.... We postulate that this developmentally immature myelin is more susceptible to degradation .... The different techniques, isolation of charge isomers, x-ray diffraction, electron microscopy, and mass spectrometry do not agree on the exact developmental age. However, it must be in the first 6 yr of life, i.e., one of the two periods of active myelination (the second active period is during adolescence).*

Moscarello and colleagues characterized the myelin of MS patients as "developmentally immature" because its basic protein (MBP) had increased levels of a component their previous studies had found was associated with less compact, immature myelin:

> *These studies [in Jimpy mice] suggest that interference with the normal process of myelination and compaction results in the continued production of myelin proteins found in immature and poorly compacted myelin.*

Moscarello and colleagues discovered high levels of this component, called C-8, in MS myelin:

> *... the increased proportion found in MS samples was a reflection of greater amounts of less compact myelin in MS. From our studies we propose that the myelin sheath in multiple sclerosis is developmentally immature largely because of the continued synthesis of C-8 at high levels and the failure to synthesize sufficient quantities of C-1, thereby preventing the assembly of highly structured myelin.*

Subsequently, Moscarello and colleagues studied the histology and biochemistry of a person who died of acute multiple sclerosis (Marburg type):

> *Postmortem examination of the brain revealed extensive areas of gross rarefaction in the hemispheric white matter. Histologically, well-demarcated areas of demyelination with a large influx of macrophages and a subtle perivascular infiltration of lymphocytes were seen with relative preservation of the axis cylinders. Myelin basic protein (MBP) was slightly larger in molecular weight than MBP from normal brain or from chronic MS brain. When expressed as the ratio of least cationic form of MBP to the most cationic (C-8/C-1), the normal ratio was 0.82, chronic MS 2.5, and the patient in this study 6.7. Because the ratio of 6.7 was similar to 7.5 found for a 15-month-old infant, MBP was considered to be of the immature form. The data are consistent with a genetic factor influencing the charge microheterogeneity of MBP. The resulting less cationic MBP cannot carry out its normal function of compacting multilayers. (Wood et al. 1996)*

Myelin sheaths in the central nervous system are formed by oligodendrocytes wrapping their cell membranes around axons, creating spirals of fatty insulating layers between axon membranes and the extracellular fluid. Myelin sheaths in the CNS are not continuous along axons but are segmented, separated by brief unsheathed areas called nodes of Ranvier. Myelin sheaths accelerate conduction by forcing the ions of the nerve impulse to cross the axon membrane only at the nodes. Myelin sheaths reduce the leakage of ions across the axon membrane, and the attraction of ions inside the axoplasm to oppositely charged ions in the extracellular fluid. Thus, myelin sheaths accelerate conduction by increasing the resistance of the axon membrane and decreasing its capacitance (Woodbury & Patton 1960; Schauf 1983).

As myelin matures, it becomes less hydrated, more compact:

> *... during maturation ... a compaction of the myelin sheath and a decrease in its water content occurs. Specific toxic agents have been shown to induce edema and vacuolization of myelin and this process has been shown to be reversible, suggesting that active mechanisms exist for maintaining the low water content of myelin. (Sapirstein et al. 1978)*

Characterizing various forms of toxic demyelination, Cammer (1980) described how the zinc enzyme carbonic anhydrase pumps ions and water from between myelin layers:

> *It is likely that, even in adult animals, the myelin sheath is not entirely inert; that is, components are slowly undergoing degradation and resynthesis. During these dynamic processes, compaction of the myelin lamellae would also be taking place continuously and one role of carbonic anhydrase would be its activity in pumping ions and water from between the myelin lamellae.*

myelination at puberty and adolescence

The observations of Moscarello and colleagues reveal not only that the myelin sheaths of multiple sclerosis patients are immature; they suggest that the myelination of childhood may take place normally in these persons. It is the second stage of myelination, the stage of puberty and adolescence, that fails.

Puberty and adolescence, like early childhood, are times of intense myelination required by the growth spurt. Adolescent myelination, in fact, appears to be necessary for myelin sheaths to fully mature:

> *Although myelination of all tracts in the human CNS has commenced by 2 years of age, histological studies show that fully mature myelin sheaths are not found until the end of the second decade. (Cuzner, Davison, & Thompson 1981)*

> *Postmortem studies suggest that axon diameter and myelin sheath undergo conspicuous growth during the first 2 years of life, but may not be fully mature before adolescence or even late adulthood. (Paus et al. 1999)*

A relationship between multiple sclerosis and maturation was pointed out by Brouwer in 1920. He contended that certain bodily functions are affected more than others in multiple sclerosis, although demyelination is usually widespread throughout the brain and spinal cord. Motor disturbances in the nerve tracts (pyramids) that connect the brain and spinal cord are common, appearing as ataxia and loss of spinal reflexes. Sensory disturbances, while also common, are more transitory and less intense. Functions of the optic nerve (a tract of the CNS) are affected far more often than functions of other cranial nerves. Usually only central vision is affected, although demyelination is generally diffuse along the optic nerves.

Brouwer pointed out that those functions of the optic nerve, brain, and spinal cord most affected in multiple sclerosis are functions that appeared latest in the evolution of our species. Because each individual's development reflects the evolution of the species (ontogeny recapitulates phylogeny), Brouwer argued, newer evolved functions appear later in individual development. Today, sensory complaints like numbness and tingling are considered common signs of multiple sclerosis, yet Kennedy (1936) too described "neglect of the sensory paths" in MS. If Brouwer was correct, those functions most disturbed in multiple sclerosis are functions which myelinate latest, i.e. during puberty and adolescence.

Grinker and Robbins (1954), on the other hand, pointed out that multiple sclerosis symptoms resemble regression to an infantile state, which suggests that childhood myelination may also be impaired:

> *From the physiological point of view all the symptoms of multiple sclerosis indicate a regression to a state of functional helpless infancy. Enuresis, defective coordination, slurred speech, dissociant eye movements, and spastic ataxic movements, are examples of extensive physiological regression to lower or more infantile levels of motor activity.*

is there a characteristic multiple sclerosis personality?

> *It has been pointed out that there is a difficulty in differentiating multiple sclerosis with psychiatric disturbances from essentially psychogenic illness with psychosomatic neurological symptoms. (Teitelbaum 1977)*

A distinctive multiple sclerosis personality was first described by Charcot in 1874, and subsequently observed by many physicians. Euphoria, stupor, unmotivated laughing and crying, and loss of memory have all been reported--usually assumed to result from the disease itself:

> *... it seems clear that intellectual deterioration, euphoria, personality change, and exaggeration of emotional expression are, like physical disability, symptomatic of the disease, and are directly due to damage to the CNS. (Surridge 1969)*

Multiple sclerosis patients are very susceptible to emotional and physical stress, especially anxiety and frustration. Stress has brought on first attacks as well as relapses (Brickner & Simons 1950; Grant et al. 1989). This characteristic emotionality and sensitivity to stress may result from lesions in the brain, as many assume. Yet researchers have found evidence that multiple sclerosis patients were emotionally disturbed long before their disease appeared:

> *Psychologically, the premorbid state of the multiple sclerotic is that of great immaturity since early infancy. He is emotionally abnormal long before he shows signs of his organic disease. The multiple sclerotic seems to have an excessive need for love and affection which was not gratified in childhood. The resulting frustrations evoke anger which must be repressed in order to preserve whatever gratifications are available. As a result the external personality is happy-go-lucky, with a paramount desire to please and to be approved. There seems to be an outward calm and a deeply concealed inner tension. (Grinker & Robbins 1954)*

> *The response of these patients to turbulent and/or cloying childhood influences is passive and docile and leads them to hide feelings behind a smiling or unsmiling mask.... These vignettes typify most of the features of MS: extreme emotional dependence, passivity, problems in separation from key figures, brought about by*

> *courtship, marriage, illness and death, and a giving-up response when attempts at separation threaten, or actuate, reprisals from a key figure .... (Paulley 1977)*

One clue to the immaturity and passivity of multiple sclerosis patients was uncovered by Groen and colleagues (1969):

> *The psychosomatic aspects of multiple sclerosis are discussed on the basis of continuous psychiatric observation of the life events, behavior and attitudes of patients during their remissions and relapses, and partly also on clinical psychological studies. The disease occurs preferentially in patients who stem from a family with a tyrannical father or a mother who was subservient to her husband. They have strong taboos about sex, which have produced in them at an early age strong feelings of guilt and reinforced their fears. The personality development is fixed at an infantile phase. In particular they have difficulty in expressing aggression. They try to disarm, even at an adult age, the feared authority figure by much subservient, innocently smiling behavior:* puérilisme mentale*. In addition the patients were found to be markedly compulsive.*

"Personality development fixed at an infantile phase." Did these persons miss puberty, and perhaps some childhood as well? Benedek and Rubenstein (1942) pointed out that sexual suppression can suppress the sex hormone releasing factors (gonadotropins) in the brain:

> *If the psychosexual development was such that the gonadotropic function was suppressed, the sexual cycle would not develop, that is, the hormone production would remain similar to that of children.*

Langworthy (1948) too noted the emotional and sexual immaturity of MS patients:

> *It has always been apparent that poor emotional adjustment was a problem in many cases of multiple sclerosis even before the symptoms of organic disease developed. In these instances the patients may be emotionally immature and show this immaturity in all their interpersonal relationships. It is often seen particularly in their sexual adjustment, which can be evaluated at a preadolescent level of emotional growth.*

Psychiatrist Wallace Ellerbroek observed (personal communication 1978) that multiple sclerosis patients

> *primarily have an excess of anger and a marked lack of being horny--and the latter is associated with increased estrogen levels, not a lack. [A] frequent initial complaint, not recognized as MS, is vaginal anaesthesia. [B]oth the males and females are primarily carefully concealed hysterical personality disorders .*

Increased estrogen levels *do* inhibit male sex drive by neutralizing testosterone, the primary libido hormone, but low sex drive is more often associated with low testosterone than with high estrogen. Because testosterone is the hormone of aggression (Mazur & Booth 1998) as well as libido, suppression of anger as well as suppression of libido presumably suppresses testosterone. And because the vagina is estrogen-sensitive (Sherfey 1972), "vaginal anaesthesia" seems more likely to be associated with low estrogen than with high estrogen.

Sex hormones in myelination

Testosterone at puberty is responsible for growth of the long bones in both sexes (Tanner 1967; Murad & Kuret 1991). Suppression of testosterone, therefore, presumably inhibits growth, though too much testosterone at an early age (like too much estrogen) fuses the epiphyses of the long bones and also arrests growth. Because estrogen deposits fat in both sexes during puberty, especially in the skin, we might wonder if estrogen helps form fatty myelin sheaths in the central nervous system.

The effects of testosterone, estrogen, and progesterone on myelination have been studied in rats. Progesterone is an immediate precursor of testosterone (the primary androgen), and testosterone an immediate precursor of estradiol (the primary estrogen) as well as dihydrotestosterone. Martini and Melcangi (1991) described these pathways in relation to myelin:

> *In the brain, androgens may be metabolized according to two different major pathways. The first one, known as the aromatase pathway, transforms testosterone into estradiol and androstenedione into estrone. The second one transforms testosterone into dihydrotestosterone through the action of 5a-reductase . Two new findings emerge from the present studies: (a) the existence of higher 5a-reductase activity in the white matter structures and (b) the association of the enzyme with the myelin sheath.*

In injured rats, Schumacher and Baulieu (1995) found that administering progesterone at the site of the lesion "promoted the formation of new myelin sheaths." Curry and Heim (1966), studying estrogen in rats,

concluded: "It is suggested that the effects of oestradiol on myelination are, at least in part, a result of its action on cholesterol metabolism."

Casper and colleagues (1967) investigated changes in newborn rat brains after administration of estradiol:

> *The increase in cerebrosides after estradiol and cortisol suggests that hormones may influence the process of myelination which is very active at this age period. Histological studies have also shown that myelin appears earlier in the rat brain after estradiol treatment during postnatal periods of development. It is suggested that precocious functional maturation of the central nervous system after neonatal estradiol and cortisol treatment may be partly a result of enhanced myelination.*

Estrogen may also assist myelination by augmenting calcium accumulation. Estrogen enhances calcium retention in bone (Riggs et al. 1998); frequent sex is said to modify a woman's bone structure significantly (Haley 1986). Goldberg (1974) analyzed the unusual geographic distribution of multiple sclerosis, and concluded that lack of vitamin D from sunlight and lack of dietary calcium were the significant variables. Vitamin D deficiency causes calcium deficiency, and calcium is needed by the enzymes that synthesize myelin, and by the myelin sheath itself. Calcium deficiencies could therefore cause the production of unstable myelin, Goldberg asserted. Commenting on this evidence, Sayetta (1986) concluded:

> *Because the usual age at onset of MS is postpubertal, the disease symptoms occur at a time of great elaboration of the anabolic sex steroids, Estrogens, in particular, have a strong influence on calcium retention by the body .... Puberty and early adulthood are generally times of positive calcium balance in the body due to the effect of the sex steroids (unless a vitamin D insufficiency is severe enough to be offsetting). The hypothesis that decreased vitamin D and calcium levels are promoters of MS at the (postpubertal) time of onset is inconsistent with this fact.*

But if the sex hormones of multiple sclerosis patients are *suppressed* at puberty, as we have reason to believe, calcium balance may be impaired as Goldberg described. A 1-2 year trial of calcium, magnesium, and vitamin D in 16 MS patients significantly decreased their relapse rate (Goldberg et al. 1989).

Sex hormones in multiple sclerosis

Clues that sex hormones influence multiple sclerosis are (a) women acquire the disease nearly 2x as often as men (Detels 1978); (b) women tend to have an early onset, men a later onset (Wainerdi 1961); (c) women usually have a relapsing-remitting course, men a progressive course (Leibowitz & Alter 1973); (d) relapses are less common during pregnancy, but increase postpartum:

> *It has long been suspected that hormonal factors contribute directly and indirectly to the etiology and pathogenesis of multiple sclerosis (MS). The susceptibility of MS is higher in women than in men and women are even more susceptible to hormonal influences when onset occurs at an early or delayed age. Pregnancy has a short-term favorable effect on the course of the disease but there is an increased rate of relapse during the post-partum period. In addition, women often report premenstrual exacerbation of their symptoms with remission during menses. These findings suggest that in women estrogens may exert a stabilizing effect on the clinical manifestations of MS. (Sandyk 1996)*

Jonez (1952) regularly administered combined male and female hormones to stabilize the emotions of patients at the Multiple Sclerosis Clinic of St. Joseph Hospital in Tacoma, Washington:

> *Within a few short hours after the proper injections of combined male and female sex hormones, sclerotics are in gratifying control of their emotions. [I]n a good many cases, I found that women got about as much lift from the androgenic hormones as from the estrogenic--without the distressing side effects of bleeding and tumor-stimulation, but there were undesirable results when women received large doses of androgens. Finding that neither female nor male sex hormones were exactly the answer I tried combining [them].*

The Clinic's last medical director, Dr. George Hess, wrote (1962):

> *Most multiple sclerosis patients have low hormone levels, and so we routinely prescribe the appropriate substance, either estrogen or testosterone.*

Studying the sex hormone levels of 52 patients with clinically definite multiple sclerosis, Wei and Lightman (1997) reported that 24% of the males had low serum testosterone, and 25% of the pre-menopausal females had low estrogen.

Zorgdrager and De Keyser (1997) reported that 26 of 60 women with relapsing-remitting MS "regularly experienced worsening of their MS symptoms in the period just before, or at the beginning of, the menstruation." Many of the women who reported no premenstrual change in symptoms were taking oral

contraceptives, "suggesting a protective effect," the researchers concluded. Interestingly, women with chronic progressive MS reported that the menstrual cycle had no effect on their symptoms.

Sandyk (1996) found that withdrawal of estrogen therapy affected the emotions of MS patients like the premenstrual fall of estrogen:

> *Thus, women with MS may be particularly sensitive to physiological fluctuations in estrogen levels and may develop dramatic alterations in mood and cognitive functions following abrupt changes in plasma estrogen levels such as during the premenstrual period or as reported in this case upon withdrawal of estrogen therapy.*

Ghezzi and colleagues (1979) studied the effects of oral contraceptives in multiple sclerosis:

> *The data point to a protective role on the part of oestrogens, while the picture for progesterone is less conclusive. It would seem that these biological and immunological findings justify the use of oral contraceptives in subjects with M.S., though due caution is imposed by the absence of clinical and epidemiological data.*

Because both multiple sclerosis and experimental autoimmune encephalomyelitis (EAE) improve during pregnancy, Kim and colleagues (1999) administered estriol to mice with EAE:

> *Estriol treatment reduced the severity of EAE significantly. Estriol doses that induced serum estriol levels that approximated estriol levels during late pregnancy were capable of ameliorating disease. Estriol as a hormone involved in immune changes during pregnancy may provide a basis for the novel therapeutic use of estriol for MS and other diseases that improve during late pregnancy.*

Sex hormone treatment for multiple sclerosis is presently being investigated by Voskuhl and colleagues. Preliminary studies in animal models of autoimmune diseases show that androgens protect females and males alike:

> *These data indicate that in order to ameliorate EAE in female mice, DHT [dihydrotestosterone] doses that induced androgen levels significantly higher than those present normally in male mice would be required. Implications for therapy of MS were that very high doses of androgens would be needed for effective treatment of women with MS. Such high levels would likely not be well tolerated in the long term in women due to masculinizing effects ....*
> *Further, because treatment with physiologic doses of testosterone reduced the severity of disease in young adult, hormonally intact male mice, this indicated that the beneficial effect of supplemental testosterone treatment was not dependent on having a low baseline testosterone level, and that hormonally intact men with MS should benefit from supplemental pharmacologic doses of testosterone. (Voskuhl 2002)*

A trial by Voskuhl's group of the pregnancy hormone estriol in women with relapsing-remitting multiple sclerosis showed promise (Sicotte et al. 2002):

> *We treated nonpregnant female multiple sclerosis patients with the pregnancy hormone estriol in an attempt to recapitulate the beneficial effect of pregnancy. As compared with pretreatment baseline, relapsing remitting patients treated with oral estriol (8 mg/day) demonstrated significant decreases in delayed type hypersensitivity responses to tetanus, interferon-gamma levels in peripheral blood mononuclear cells, and gadolinium enhancing lesion numbers and volumes on monthly cerebral magnetic resonance images. When estriol treatment was stopped, enhancing lesions increased to pretreatment levels. When estriol treatment was reinstituted, enhancing lesions again were significantly decreased.... This novel treatment strategy of using pregnancy doses of estriol in multiple sclerosis has relevance to other autoimmune diseases that also improve during pregnancy.*

On the other hand, Bansil and colleagues (1999) found increased lesion activity in 30 women with MS when their estrogen was high during the menstrual cycle. In the follicular phase before ovulation, with estrogen high and progesterone low, these women showed a greater number of active lesions by magnetic resonance imaging, and greater leakage across the blood-brain barrier:

> *Patients with high estradiol and low progesterone levels had a significantly greater number of enhancing lesions than those with low levels of both these hormones. Patients with a high estrogen to progesterone ratio had a significantly greater number of active MRI lesions than those with a low ratio.*

Nitric oxide in multiple sclerosis

Nitric oxide is a gaseous, readily diffusible molecule with a variety of functions throughout the body. Nitric oxide synthesized by the endothelial cell layer of blood vessels, especially arteries and arterioles, relaxes the smooth muscle surrounding the vessels, and inhibits the adhesion and aggregation of platelets. In the autonomic nervous system, nitric oxide is a parasympathetic neurotransmitter that relaxes smooth muscle in the cerebral circulation, the genitals, and the gastrointestinal and respiratory tracts (Faraci & Brian 1994; Gai & Blessing 1996; Bredt 1999; Tomita et al. 2000). In skeletal muscle, nitric oxide increases blood flow to active muscles and regulates their contractility (Bredt 1999).

Formerly called endothelium-derived relaxing factor (EDRF), nitric oxide is now known to be the primary endogenous vasodilator (Fleming & Busse 1999) as well as the active principle of therapeutic nitrovasodilators like amyl nitrite and nitroglycerin (Robertson & Robertson 1996).

> *... several vasodilators (acetylcholine, bradykinin, histamine, substance P) operate by stimulating endothelial NO formation.... Vasodilator mechanisms, physiological as well as pharmacological, may therefore be characterized as endothelium-dependent (i.e. NO-mediated), or endothelium-independent (i.e. not mediated by NO). (Wennmalm 1994)*

Nitric oxide is produced from the amino acid L-arginine by the enzyme nitric oxide synthase (NOS). Three forms of NOS have been identified: two kinds of *constitutive* NOS (cNOS) primarily in endothelial cells (eNOS) and neurons (nNOS), and an *inducible* form (iNOS) primarily in astrocytes and macrophages.

> *Activation of cNOS releases relatively low levels of NO for short periods of time whereas induction of iNOS releases high levels of NO for extended periods of time. (Brosnan et al. 1994)*

> *Nitric oxide synthases have been examined in whole animals, tissues, and cells for functional properties and recently three genes have been identified for the isoforms responsible for the activities in various tissues. Accordingly, the respective enzymes have been designated as neuronal (NOS-I), macrophage or induced (NOS-II), or endothelial (NOS-III). Any tissue or cell may contain more than one isoform of nitric oxide synthase, thus contributing to the production of NO under various physiological circumstances. (Okita & Masters 1997)*

In recent years, unusual elevations of nitric oxide and its inducible synthase (iNOS) have been detected in multiple sclerosis lesions:

> *Nitric oxide (NO) is a free radical found at higher than normal concentrations within inflammatory multiple sclerosis (MS) lesions. These high concentrations are due to the appearance of the inducible form of nitric oxide synthase (iNOS) in cells such as macrophages and astrocytes. Indeed, the concentrations of markers of NO production (eg, nitrate and nitrite) are raised in the CSF, blood, and urine of patients with MS. (Smith & Lassman 2002).*

> *Recent studies suggest that NO and its reactive derivative peroxynitrite are implicated in the pathogenesis of multiple sclerosis (MS). Patients dying with MS demonstrate increased astrocytic inducible nitric oxide synthase activity, as well as increased levels of iNOS mRNA. Peroxynitrite is a strong oxidant capable of damaging target tissues, particularly the brain, which is known to be endowed with poor antioxidant buffering capacity. (Calabrese et al. 2003)*

Increased blood volume has also been detected in acute MS plaques. Haselhorst and colleagues (2000) concluded: "Our results indicate that the acute phase in MS is accompanied by vasodilation. In later stages of gliosis, the perfusion decreases with increasing axonal injury."

The role of nitric oxide in multiple sclerosis was reviewed most lucidly by Smith and Lassman (2002):

> *Because NO is a reactive molecule, it does not exist in tissues only as a free radical; it also gives rise, sometimes reversibly, to several other related compounds. These compounds include the nitroxyl (NO-) ion, nitrous acid (HNO2), the nitrogen dioxide (NO2) radical, peroxynitrite (ONOO-; a product of the combination of superoxide and nitric oxide) and peroxynitrous acid (ONOOH).... NO has two major effects on cerebral vessels, both of which may be involved in the pathogenesis of MS lesions--namely, vasodilation and a disturbance of the BBB. NO can arise in lesions from various sources, including nerve terminals, the induction of iNOS, or the release of neurotransmitters, and it provokes profound vasodilation of the cerebral vasculature in both normal and pathological conditions....*
> *NO-mediated vasodilation generally occurs in conjunction with a disturbance of the permeability of the BBB, which will promote the passage of inflammatory cells and mediators into the CNS parenchyma. Indeed, the integrity of the BBB is well known to be compromised in MS at the sites of inflammatory lesions .... In agreement with this belief, the nitrite and nitrate concentrations in CSF of patients with MS are correlated with BBB breakdown, as measured by the leakage of albumin.*

Nitric oxide can also be toxic to oligodendrocytes, the myelin-producing cells of the central nervous system (Brosnan et al. 1994; Mitrovic et al. 1994).

Despite this evidence that nitric oxide and its metabolites are harmful in multiple sclerosis brains, efforts to reduce the activity of NO and NOS have not helped in animal models of MS--perhaps because NO has anti-inflammatory as well as proinflammatory actions:

> *NO has long been regarded as a major factor aggravating inflammation in the CNS, but recent studies aimed at treating experimental autoimmune encephalomyelitis by the administration of relatively specific inhibitors of NOS have provided confusing results. The findings indicate that NO may on the one hand exert a proinflammatory action ... but may on the other hand help to control the immune response via several immunoregulatory processes. Activated T cells can enter the CNS compartment in the process of immune surveillance and, when they encounter their specific antigen, they start the proinflammatory cascade by producing cytokines.... NO can interfere with this inflammatory cascade at several steps, thus assuming an anti-inflammatory role. (Smith & Lassman 2002)*

> *Nitric oxide, generated by the inducible isoform of nitric oxide synthase (iNOS), has been described to have beneficial microbicidal, antiviral, antiparasital, immunomodulatory, and antitumoral effects. However, aberrant iNOS induction at the wrong place or at the wrong time has detrimental consequences and seems to be involved in the pathophysiology of several human diseases. (Kleinert et al. 2003)*

> *One possibility worthy of further investigation is that astrocyte-induced NO might be beneficial, whereas microglial NO is involved in neurotoxicity. (Murphy et al. 1993)*

Smith and Lassman (2002) discussed evidence that nitric oxide may interfere with conduction along axons by blocking ion channels for sodium, potassium, and/or calcium. This observation is most intriguing, because it supports the convictions of many researchers over the years that demyelination is not the only cause of symptoms:

> *Demyelination has long been known to impair conduction, and the loss of function in MS was, for many years, primarily attributed to the associated and prominent demyelination. However, several observations have now emphasized that inflammation may also be important .... [Smith & Lassman 2002)*

Smith and Lassman suggested that inhibiting the formation of the toxic metabolite peroxynitrite might be more effective against multiple sclerosis than inhibiting the formation of nitric oxide:

> *From the information presented in this review, it might appear that an effective therapy for MS could result from the inhibition of NO production, especially the inhibition of iNOS. However, over the past 10 years around 40 investigations have examined the role of NO in experimental autoimmune encephalomyelitis, but no clear picture has emerged....*
> *To reduce the damage caused by NO, there may be an alternative to limitation of NO production. Several of the deleterious effects of NO may actually be mediated by peroxynitrite, which ... is formed from the combination of NO and superoxide. Therefore, it may be possible to limit peroxynitrite formation by restricting the production of superoxide rather than NO.*

inducible nitric oxide as a compensatory response

> *There are reports suggesting that both inhibitors of nitric oxide synthase and nitric oxide donors protect against some forms of injury. This is probably due to the dual nature of nitric oxide, which is on the one hand cytotoxic and on the other a vasodilator and thus potentially protective. (Moncada & Higgs 1993)*

One reason for suspecting that the inducible nitric oxide in multiple sclerosis lesions originates as a protective response is that inducible nitric oxide synthase appears when release of endothelial nitric oxide is impaired. Miller and colleagues (1996) administered the nitric oxide synthase inhibitor L-NAME to guinea pigs and rats, and observed a compensatory rise in inducible nitric oxide synthase:

> *We addressed the hypothesis that administration of nitric oxide synthase inhibitor ... L-NAME does not result in a sustained suppression of nitric oxide synthesis, because of a compensatory expression of inducible nitric oxide synthase (iNOS).... We conclude that iNOS expression is responsible for a compensatory increase or normalization of NO synthesis during sustained administration of L-NAME.*

Henningsson and colleagues (2000) reported that the pancreatic islets of mice given L-NAME in their drinking water showed a compensatory increase in inducible nitric oxide synthase:

No iNOS was found in freshly isolated islets of freely fed control mice, but there was a strong expression of iNOS in the islets of L-NAME-treated mice that contributed to an increase in total islet NO production. The compensatory increase in iNOS activity after chronic L-NAME drinking suggests the occurrence of a regulatory NO-producing system within the islets of Langerhans trying to assure adequate production of NO by inducing iNOS expression when cNOS activity is suppressed ....

Yan and colleagues (1996) found that nitric oxide synthase was induced in the smooth muscle cells of rat carotid arteries after the arterial endothelium was damaged by a balloon catheter:

Since vascular smooth muscle cells (VSMCs) produce NO in response to cytokine stimulation and after arterial injury, we speculated that NO produced by VSMCs could compensate for the loss of endothelium.... These results demonstrate that arterial injury triggers the expression of iNOS in the lesion and that NO produced by iNOS inhibits platelet adhesion and restores blood flow.... Thus, expression of iNOS in lesions may represent a protective mechanism that compensates for the loss of endothelium. (Yan et al. 1996)

Human studies confirm that inducible nitric oxide synthase appears in response to injury to the vascular endothelium:

Another isoform of NO synthase insensitive to changes in intracellular calcium may be induced following exposure to cytokines or under some pathological conditions such as sepsis, inflammation or after vessel wall injury. The massive and long-lasting release of NO caused by induction of NO synthase requires a latency period of several hours. The inducible NO synthase may compensate the dysfunction of the endothelial isoform after injury (angioplasty) or in atherosclerosis. However, uncontrolled regulation of inducible NO synthase expression may have deleterious consequences on the vascular wall. (Boulanger 1995)

Studies in rats have demonstrated that cardiac myocytes express iNOS after treatment with cytokines. This has led to the suggestion that induction of NO synthase in the heart may explain the specific cardiac dysfunction of endotoxin shock and that of some immunological conditions such as dilated cardiomyopathy (DCM).... Heart tissue from patients with DCM showed significant activity of iNOS ... while cNOS activity was 10-11-fold lower ... or not detectable.... Normal rat myocardium contains cNOS and expresses iNOS during endotoxaemia. Furthermore, isolated myocytes of the rat express iNOS only after stimulation with cytokines. If the same is true of man, our results suggest that iNOS is expressed in the myocardium only under pathological conditions. (de Belder et al. 1993)

Hecker and colleagues (1999) reviewed human studies of nitric oxide synthase induced in smooth muscle cells (VSMC) after vascular injury:

In vascular injury ... iNOS expression in VSMC may be beneficial as a compensatory mechanism for the lack of endothelial NO synthesis ....

evidence of endothelial cell damage

Microparticles released from endothelial cells of multiple sclerosis patients reveal acute and chronic damage to the endothelium, according to Minagar and colleagues (2001):

Endothelial cell dysfunction may contribute to the pathogenesis of MS. Elevations of soluble adhesion molecules intracellular adhesion molecule, vascular cell adhesion molecule, and platelet-endothelial cell adhesion molecule-1 (CD31) have been reported as markers of blood-brain barrier damage in MS .... Endothelial cells release microparticles ... (EMP) during activation or apoptosis.... Platelet-poor plasma from 50 patients with MS (30 in exacerbation and 20 in remission) and 48 controls were labeled with ... anti-CD31 and anti-CD51... antibodies, and two classes of EMP (CD31+ and CD51+) were assayed by flow cytometry.... Plasma from patients in exacerbation had 2.85-fold elevation of CD31+ EMP as compared with healthy controls, returning to near control value during remission. The CD31+ EMP concentration showed a positive association with gadolinium enhancement in patients with MS. In contrast, CD51+ EMP remained elevated in both exacerbation and remission. This suggests that CD31+ EMP is a marker of acute injury, whereas CD51+ EMP reflects chronic injury of endothelium. MS plasma induced release of both CD31+ and CD51+ EMP from MVEC culture in vitro.... We speculate that elevated CD51+ EMP reflect erosion of endothelium with exposure of the subendothelial matrix.... Our observation that ... filtered MS plasma, but not normal plasma ... induces release of CD31+ EMP indicates that MS plasma contains one or more soluble factors that induce release of EMP.

Plumb and colleagues (2002) used laser scanning microscopy to examine the tight junctions of endothelial cells in active lesions and normal-appearing white matter in MS brains:

Blood-brain barrier (BBB) breakdown, demonstrable in vivo by enhanced MRI is characteristic of new and expanding inflammatory lesions in relapsing-remitting and chronic progressive multiple sclerosis (MS). Subtle leakage may also occur in primary progressive MS.... We investigated the possible involvement of interendothelial tight junctions (TJ) by examining the expression of TJ proteins (occludin and ZO-1) in blood vessels in active MS lesions from 8 cases of MS and in normal-appearing white (NAWM) matter from 6 cases.... TJ abnormalities manifested as beading, interruption, absence or diffuse cytoplasmic localization of fluorescence, or separation of junctions (putative opening) were frequent (affecting 40% of vessels) in ... active plaques but less frequent in NAWM (15%), and in normal (< 2%) and neurological controls (6%). Putatively "open" junctions were seen in vessels in active lesions and in microscopically inflamed vessels in NAWM. Dual fluorescence revealed abnormal TJs in vessels with pre-mortem serum protein leakage. Abnormal or open TJs, associated with inflammation may contribute to BBB leakage in enhancing MRI lesions and may also be involved in subtle leakage in non-enhancing focal and diffuse lesions in NAWM. BBB disruption due to tight junctional pathology should be regarded as a significant form of tissue injury in MS, alongside demyelination and axonopathy.

If the vascular endothelium is damaged or impaired in multiple sclerosis brains, is the synthesis or release of endothelial nitric oxide likewise impaired? There is, in fact, evidence suggesting that endothelial nitric oxide may be chronically depleted in multiple sclerosis. Most patients are extremely heat-sensitive, and their platelets are sticky.

platelet adhesion and aggregation

A disturbance in platelet function in multiple sclerosis has been repeatedly demonstrated by independent groups of investigators, and this must be considered one of the few established systemic features of this disease.... In most of the other studies referred to above, a similar broad correlation was found to exist between clinical activity of multiple sclerosis and enhanced platelet stickiness .... (Prineas 1970).

There remains, nevertheless, the tantalising record of increased platelet stickiness in multiple sclerosis, which has never been very satisfactorily explained. (Adams et al. 1985)

Fog et al. (1955) measured platelet count in the blood of MS patients and found that during clinical activity there was a tendency to a low level followed by a rise during clinical improvement.... The results reported here demonstrate that MS platelets show hyperaggregability to [platelet activating factor], which supports earlier reports of increased adhesiveness, aggregability toward ADP and spontaneous aggregability.... The results suggest that the increased aggregability investigated by us is due to some defect in the platelets of MS patients, and not due to a plasma factor. (Khan et al. 1985)

Under normal conditions, endothelial nitric oxide inhibits the adhesion and aggregation of platelets:

NO is also released abluminally to interact with circulating platelets. Increases in cGMP in platelets are associated with a decreased adhesion and aggregation of cells. Thus, endothelium-derived NO, through its vasodilator and anti-aggregatory properties, prevents vasospasm and thrombus formation in the circulation and thereby helps to maintain blood flow to vital organs such as the heart. (Luscher 1991)

Endothelial nitric oxide, however, is not the only nitric oxide that normally inhibits platelet stickiness. Platelets express their own calcium-dependent, constitutive nitric oxide synthase similar to endothelial NOS (Radomski et al. 1990).

The generation of NO also acts as an autocrine regulatory system, for platelets do not seem to transfer NO to other platelets or cells but modulate their own ability to aggregate by generating NO. (Moncada et al. 1991)

This observation is especially interesting in light of a report by González-Fernández and colleagues (1998), who studied the expression of inducible nitric oxide synthase in rat carotid arteries denuded of endothelium:

In the present study we have shown that iNOS protein is present in the arterial wall several days after endothelial denudation. Early after arterial wall injury, iNOS protein expression is very weak. Platelets play a crucial role in preventing iNOS expression early after endothelial damage, and this negative modulatory effect can be prevented by GP [platelet glycoprotein] IIb/IIIa-blocking drugs.

Are platelets sticky in multiple sclerosis patients because they have been activated by damage to the vascular endothelium? Is inducible NOS expressed in multiple sclerosis because endothelial and platelet NOS is impaired? Because sticky platelets have been detected in a variety of other diseases, Prineas (1970)

concluded they are unlikely to be responsible for multiple sclerosis. Nevertheless, they may offer clues to the defect that *is* responsible.

heat sensitivity

A 1º C rise in body temperature usually suffices to bring on attacks and relapses of MS (Selhorst et al. 1981). Uhthoff's symptom--transient blurred vision from exercise--also resembles heat sensitivity. Andreeva and colleagues (2001) found that heating humans to a rectal temperature of 39.0-39.5º C caused blood cells to produce nitric oxide. Kellogg and colleagues (2003) measured NO concentrations in the skin of nine healthy subjects before and during whole-body heat stress:

> *NO does increase in skin during heat stress in humans, attendant to active vasodilation. This result suggests that NO has a role beyond that of a permissive factor in the process; rather, NO may well be an effector of cutaneous vasodilation during heat stress. (Kellogg et al. 2003)*

Thus, heat-sensitivity may reveal impaired release of endothelial nitric oxide, which limits the ability to respond to warming with cutaneous vasodilation. Yet not all MS patients are sensitive to heat or exercise, and those who are heat-sensitive are not always exercise-sensitive and vice-versa (Selhorst et al. 1981). Selhorst and colleagues suggested that acidosis might be the common denominator of heat stress and exercise.

Charkoudian and colleagues (1999) tested the effects of steroid contraceptives on skin blood flow in women:

> *These findings indicate that modifications in cutaneous vascular control by female steroid hormones include enhancement of the vasodilator response to local warming and are consistent with reports of the influence of estrogen to enhance nitric oxide-dependent vasodilator responses.*

This study seems especially relevant in light of the sex hormone deficiencies detected in multiple sclerosis patients, and the evidence of sexual suppression during their puberty and adolescence.

Sex hormones and nitric oxide

Sex hormones, notably estrogen, utilize nitric oxide to dilate pelvic blood vessels and engorge genital erectile tissues (Guyton & Hall 1996; Mendelsohn & Karas 1999). The vascular relaxation induced by estrogen is thought to underlie its protective effects on the heart and circulation, most obvious in the lesser incidence of heart disease in women (Orshal & Khalil 2004).

> *It is increasingly recognized that sex steroids have, among many other effects, the ability to cause vasodilation. The vasodilatory effects of estradiol have been the best documented and described. At low concentrations, estradiol has the ability to improve impaired endothelium dependent (nitric oxide mediated) relaxation in estrogen deficient subjects. At high concentrations, estradiol causes vasodilation principally by endothelium independent mechanisms, in a gender independent fashion, which appear to involve a number of pathways such as ATP-dependent K+ channels. Testosterone also has ability, at higher doses, to cause vasodilation of the coronary circulation, in a gender independent fashion. (Hutchison et al. 1997)*

> *Estrogen deficiency, hyperinsulinemia, type II diabetes, atherosclerosis, and a past history of elevated blood pressure may be associated with increased risk of Alzheimer's disease. Common to all of these risk factors is a diminished capacity of vascular endothelium to generate nitric oxide. (McCarty 1998)*

> *Estrogen has both rapid vasodilatory effects and longer-term effects on the vasculature. The longer-term effects of estrogen are produced, at least in part, by changes in vascular cell gene and protein expression that are mediated by the ligand-activated transcription factors, estrogen receptor (ER)-alpha and ER-beta. The rapid vasodilatory effects of estrogen do not require changes in gene expression and are produced by estrogen-stimulated increases in endothelial cell nitric oxide synthase activity. This results in nitric oxide-mediated increases in cyclic guanosine monophosphate in vascular smooth muscle cells, which mediate vasodilatation. (Mendelsohn 2002)*

Estrogens protect the brain in several ways, including stimulating the synthesis of nitric oxide:

> *Oestrogens can also dilate cerebral vessels, here acting through increased synthesis of nitric oxide and by stimulating such compounds as prostacycline and a potent vasodilator--epoxyeikosotrienoic acid. There is a body of recent evidence which suggest that during brain ischaemia the physiological estrogen stimulation, of both brain metabolism and cerebral blood flow, becomes biased towards increased release of*

> *vasodilating substances. As the metabolism is not spurred accordingly, the net effect of oestrogens is neuroprotection. Other protective properties of oestrogens within the brain are related to attenuation of the excitotoxic effects of glutamate and to the activation of enzymes scavenging free oxygen radicals. Moreover, oestrogens can diminish free radicals synthesis and act as free radicals scavengers themselves. (Rudzinski & Krejza 2002)*

Testosterone too may utilize nitric oxide to engorge erectile tissues. Rossi and colleagues (1998) measured testosterone, nitric oxide, and endothelin concentrations in men with psychogenic impotence:

> *[Testosterone] and NO were significantly lower in the penile venous blood, while [endothelin 1-2] showed no statistical difference. These data support the hypothesis of testosterone dependence of penile nitric oxide synthesis.*

Shabsigh (1997) noted that experimental castration decreased penile concentrations of nerves containing nitric oxide synthase:

> *Although androgen receptors in the penis decrease after puberty, they usually do not disappear completely. Animal data show that androgens support erectile function through a direct effect on the erectile tissue. Experimental castration results in impaired erectile response to central and peripheral stimulation and decrease in penile tissue concentration of nitric oxide synthase-containing nerves. Testosterone replacement reverses these abnormalities.*

On the other hand, Weiner and Thompson (1997) concluded: "Estradiol, but not progesterone or testosterone, increases CA(2+)-dependent NOS activity." Reviewing the evidence, Orshal and Khalil (2004) concluded:

> *... gender differences in vascular tone are less likely related to androgens and more likely related to estrogens.... [A]lthough most of the sex hormones tested appear to cause vascular relaxation and inhibit vascular smooth muscle contraction, the vascular relaxant effects of estrogen significantly surpass those of progesterone or testosterone.*

One explanation for this conflicting evidence was proposed by Singh and colleagues (2000), who studied the effects of castration and administered androgens on neuronal nitric oxide synthase (nNOS) in male rats. They suggested that tissues containing aromatase turn testosterone into estradiol, which stimulates nitric oxide production; tissues containing 5a-reductase turn testosterone into dihydrotestosterone, which does not stimulate NO production:

> *Nitric oxide synthesized by the enzyme neuronal nitric oxide synthase plays an important role in many brain functions. These include effects on long-term potentiation, gonadotropin secretion, and sexual behavior. NO functions as a neurotransmitter and nNOS is present near gonadotropin-releasing hormone terminals and in regions of the brain that appear to regulate emotional behaviors....*
> *[M]any of the actions of estradiol (E2) on the brain have been suggested to be through a NO-mediated mechanism....*
> *The effect of androgen is opposite to that of E2, which increases nNOS in various regions of the brain.... Testosterone (T) may act like E2 to increase nNOS in some areas of the body and the brain where aromatase is present, whereas it would decrease nNOS in other areas where 5a reductase is present. This could explain the discrepancies observed in the actions of T in regulating NOS activity in different organs of the body.*

nitric oxide stimulates gonadotropins

The interplay between sex hormones and nitric oxide appears reciprocal, inasmuch as nitric oxide stimulates the release of gonadotropin-releasing hormone from the hypothalamus, which triggers the release of luteinizing hormone and follicle-stimulating hormone from the pituitary:

> *The preovulatory surge of gonadotropin releasing hormone (GnRH) is essential for mammalian reproduction. Recent work has implicated the neurotransmitters glutamate and nitric oxide as having a key role in this process. Large concentrations of glutamate are found in several hypothalamic nuclei known to be important for GnRH release and glutamate receptors are also located in these key hypothalamic nuclei. Administration of glutamate agonists stimulate GnRH and LH release, while glutamate receptor antagonists attenuate the steroid-induced and preovulatory LH surge. Glutamate has also been implicated in the critical processes of puberty, hormone pulsatility, and sexual behavior. Glutamate is believed to elicit many of these effects by activating the release of the gaseous neurotransmitter, nitric oxide (NO). NO potently stimulates GnRH by activating a heme containing enzyme, guanylate cyclase, which in turn leads to increased production of cGMP and GnRH release. (Dhandapani & Brann 2000)*

sex hormones augment endothelial and inhibit inducible nitric oxide

Hayashi and colleagues (1998) reported that estradiol inhibited inducible nitric oxide synthase in the macrophages of rodents, in contrast to estradiol's reported enhancement of endothelial NO synthesis:

> *Thus 17beta-estradiol inhibited the induction of iNOS by a classic receptor-mediated pathway. The inhibition of the NO release from iNOS by 17beta-estradiol is in contrast to the reported augmentation of continuous NO release from eNOS. These harmonious effects of estrogen on iNOS and eNOS may have some role in the antiatherosclerotic effects of 17beta-estradiol.*

Kauser and colleagues (1998) found that estradiol inhibited the expression of cytokine-induced nitric oxide synthase in the vascular smooth muscle of rats:

> *These results show that the ovarian sex steroid, 17beta-oestradiol is a modulator of cytokine-induced iNOS activity in rat vascular smooth muscle and its mechanism of action involves decrease of iNOS mRNA and protein.*

Tamura and colleagues (2000) found that ovariectomy enhanced and administered estradiol inhibited the production of inducible NOS in female rat aortas:

> *Two weeks after ovariectomy (OVX), rats (10-week-old) were treated with 17beta-estradiol (E2) and/or progesterone (P4) for 5 days, and aortae were obtained from these rats on the following day. OVX markedly increased the levels of iNOS protein in abdominal aorta, whereas treatment with E2 or a combination of E2 and P4 inhibited the induction of iNOS in the aorta. The present findings indicate that endogenous estrogen negatively regulates the expression of iNOS in abdominal aorta, and suggest that changes in the levels of circulating estrogen may affect vascular function.*

Wang and colleagues (2002) studied sexual changes associated with aging in the male Brown Norway rat:

> *The Brown Norway (BN) rat is an excellent model for male reproductive ageing. We and others have shown that with ageing, the BN rat exhibits low serum testosterone, low Leydig cell steroidogenic capacity, decreased Sertoli cell function and number, marked reduction in seminiferous tubule volume and sperm content, and accelerated germ cell apoptosis....*
> *We have further shown in the hypothalamus of ageing BN rats that while the excitatory amino acid receptor content is reduced, nitric oxide synthase (NOS) activity is increased which is due to increased inducible (iNOS) but not neuronal NOS (nNOS). The increased iNOS protein in the hypothalamus is associated with increased peroxynitrite formation and neuronal cell apoptosis.*

Thus sex hormones, particularly estrogen, augment the production of nitric oxide by endothelial nitric oxide synthase, whereas sex hormone deficiency leads to expression of inducible nitric oxide synthase.

Other deficiency conditions that induce nitric oxide synthase

Two other deficiency conditions known to be associated with multiple sclerosis--magnesium depletion and zinc deficiency--are also accompanied by elevations of inducible nitric oxide.

magnesium and nitric oxide

Like many vasodilating substances, magnesium apparently utilizes endothelial nitric oxide to relax vascular smooth muscle:

> *Our results suggest that 1) small blood vessels are very dependent on NO release for Mg(2+) dilatations and 2) the endothelium-dependent relaxation induced by extracellular Mg(2+) is mediated by release of endothelium-derived relaxing factor-NO from the endothelium, and requires Ca(2+) and formation of guanosine 3',5'-cyclic monophosphate. (Yang et al. 2000)*

At first glance this effect of magnesium seems paradoxical, because release of nitric oxide by endothelial cells depends on a rise in free ionized calcium within the cells, yet magnesium antagonizes the increase of free intracellular calcium (Satake et al. 2004). Furthermore, not all studies agree that magnesium stimulates nitric oxide:

> *... the data derived from intact vessels seem contradictory, with some investigators suggesting a direct relationship between magnesium concentration and [endothelial-*

derived relaxing factor] activity, others suggesting an inverse relationship, and still others suggesting an inverse relationship that is transient .... (Howard et al. 1995)

Pearson and colleagues (1998) studied the effects of magnesium deficiency on nitric oxide in the coronary arteries of dogs. "Hypomagnesemia selectively impaired the release of nitric oxide from the coronary endothelium," they concluded. On the other hand, Mak and colleagues (1996) measured plasma nitrate and nitrite of rats fed a magnesium-deficient diet for three weeks:

Plasma nitrate plus nitrite levels ... increased 1.7-fold during the 1st wk and increased 2- to 2.4-fold during the 2nd and 3rd wk on the Mg-deficient diet.

One explanation may be the activation of inducible nitric oxide synthase found by Rock and colleagues (1995) in magnesium-deficient rats:

Magnesium deficiency in rats leads to an oxidative stress involving an increased production of radical oxygen species. The present study was designed to examine the effect of experimental magnesium deficiency on plasma nitric oxide (NO) level and nitric oxide synthases (NOS) activities in rats. The data show that the concentration of NO is markedly increased in plasma of magnesium-deficient rats. This rise in plasma NO results from activation of inducible nitric oxide synthase (iNOS) rather than of the constitutive form (cNOS) of the enzyme. These data are in agreement with previous observations indicating that inflammation occurs during magnesium-deficiency and provide an additional cause of oxidative lesions through formation of peroxynitrite from nitric oxide and superoxide anion.

Mak and colleagues (1997) measured superoxide levels in the neutrophilic leukocytes of magnesium-deficient rats. Magnesium deficiency multiplied superoxide levels in the neutrophils, and a nitric oxide synthase inhibitor "significantly attenuated" this response:

The results suggest that neutrophils from Mg-deficient rats are activated endogenously to generate oxy-radicals which might directly mediate the in vivo peroxidative indices during Mg-deficiency. Furthermore, the neutrophil activity was lowered by NO-synthase inhibition suggesting that NO overproduction during Mg-deficiency participates in the neutrophil activation process.

Schooley and Franz (2002) studied the effects of magnesium deficiency on nitric oxide production and blood pressure in pregnant rats, a possible animal model of preeclampsia:

The highest plasma nitrite was associated with the lowest serum Mg levels. A low serum Mg would cause an increase in cellular calcium in vascular endothelial cells, which would promote increased nitric oxide synthesis.

Paradoxically, however, although plasma nitrite levels increased in the rats, blood pressure increased as well. The researchers suggested that the inflammatory state induced by magnesium deficiency may have inactivated some of the nitric oxide:

... Mg deficiency induces a proinflammatory, pro-oxidant state characterized by increased numbers of free radical-producing leukocytes, lymphocytes, and highly activated neutrophils, a situation that also occurs in preeclampsia. These activated leukocytes produce nitric oxide using inducible nitric oxide synthase.... Higher amounts of nitric oxide products, such as peroxynitrite, are found in Mg deficiency in animals and in human preeclampsia. Nitric oxide products can damage the vasculature and prevent nitric oxide from causing vasodilatation and lowering BP.... In conclusion, moderate Mg deficiency, during pregnancy in rats, leads to significantly increased BP and plasma nitrite.... [I]neffective nitric oxide may be due to Mg deficiency-enhanced vasoconstrictor tone, decreased nitric oxide bioavailability, or increased nitric oxide inactivation and scavenging by pro-oxidants formed in the Mg-deficient state. (Schooley & Franz 2002)

Thus, magnesium deficiency in preeclampsia may inactivate endothelial nitric oxide, and activate inducible nitric oxide synthase. Not all investigations have found nitric oxide elevated in preeclampsia, however, leading Benedetto and colleagues (2000) to propose this hypothesis:

... in preeclamptic women, reduced endothelial and platelet activity of NO occur early in pregnancy, representing one among several predisposing factors; vascular resistance increases in uteroplacental circulation and platelet aggregability is enhanced because of targeted reduction of NO release; and an increased NO production seems to be stimulated when overt preeclampsia develops. Such a finding could be interpreted as a compensatory phenomenon, although it does not necessarily represent improvement in the clinical condition. Indeed, the abundance of NO in the presence of superoxide produces peroxynitrite, which further impairs vascular function.

Another explanation for increased blood pressure despite elevated levels of nitric oxide in preeclampsia may be the paradox pointed out by Moncada and colleagues (1991):

> *... in the vessel wall, a paradoxical situation may exist whereby the Ca2+-dependent modulation of the release of NO from the endothelium may be antagonized by Ca2+ acting at the level of the smooth muscle to favour contraction.*

magnesium depletion in multiple sclerosis

Magnesium deficiency has been detected in the central nervous system and viscera of MS patients (Yasui et al. 1990), in red blood cells (Stelmasiak et al. 1995), and in serum (Altura et al. 1994):

> *... a significantly lower content of Mg was found in CNS tissues, as well as visceral organs, especially in CNS white matter including the demyelinated plaques of MS patients. Whether or not this low Mg content in tissues is related to demyelinating process remains uncertain. But it is noteworthy that Mg contents were significantly lower not only in the CNS tissues but also in visceral organs, suggesting an association of environments or a low intake of Mg with MS patients. (Yasui et al. 1990)*

> *[T]he MS patients exhibited low IMg2+ [ionized Mg] levels in serum despite having no change from normal in TMg [total Mg].... Patients with CHD [coronary heart disease], MI [myocardial infarction], rectal carcinoma and multiple sclerosis appear to exhibit a high incidence of serum ionized hypomagnesemia....*
> *[O]ur recent findings in patients with diabetes mellitus, which suggest strongly that the serum IMg2+ reflects red blood cell intracellular free ionized Mg levels, when taken together with the present findings, might be suggestive of intracellular deficits in cytosolic free Mg in CHD, MI, MS and some forms of cancer. (Altura et al. 1994)*

As we have seen, a 1-2 year trial of calcium, magnesium, and vitamin D in 16 MS patients significantly decreased their relapse rate (Goldberg et al. 1989). Earlier, Fouty (1978) commented on the resemblance of magnesium deficiency symptoms to MS:

> *The mechanisms of magnesium deficiency are numerous .... The fact that young, weight-conscious women appear more susceptible may be explained by the common use of diuretics, a known cause of deficiency, and birth control pills, a suspected cause. During the past 17 years, we have acquired considerable experience in recognizing and dealing with clinical magnesium deficiency.... In fact, some cases referred to us by the Rehabilitation Service are misdiagnosed as multiple sclerosis, only to respond completely to parenteral magnesium therapy.*

Yasui and Ota (1992) concluded:

> *Magnesium and zinc have been shown to be decreased in central nervous system tissues of MS patients, especially tissues such as white matter where pathological changes have been observed. The calcium content of white matter has also been found to be decreased in MS patients.... A magnesium deficit may induce dysfunction of nerve cells or lymphocytes directly and/or indirectly, and thus magnesium depletion may be implicated in the aetiology of MS.*

One explanation for magnesium depletion in multiple sclerosis was detected by Perger (1956), who discovered that various blood elements altered just before an attack. Most striking was a fall in plasma calcium accompanied by a reciprocal rise in plasma magnesium. In long-term patients severely deteriorated, these blood changes were chronic. Perger thought these changes resembled a stress response, and in chronic patients, exhaustion of that response.

This calcium-magnesium shift is now known to be a common response to stresses like cold, operations, hemorrhage, asphyxia, and anaphylactic shock (Terkildsen 1952; Platner & Hosko 1953; Seelig 1980). Calcium moving into cells may drive magnesium out, or magnesium leaving cells may allow calcium to enter. Over time, this shift might lead to intracellular magnesium depletion and calcium accumulation. Because more than 300 enzymes require intracellular magnesium, magnesium depletion inhibits metabolism (Aikawa 1971)--which might explain the "global reduction in brain metabolism" recently detected in MS patients (see Behan et al. 2002).
The hypometabolic state associated with high plasma magnesium is most obvious in hibernating animals:

> *The characteristic features of a hibernating animal are decreased physical activity, hypothermia, hypometabolism, and a reduction of other physiological functions to correspondingly minimal levels. The one characteristic biochemical finding in hibernation is an elevation in the serum concentration of magnesium. (Aikawa 1971)*

Huszák and colleagues (1962) detected a magnesium shift in the blood of multiple sclerosis patients subjected to artificial fever:

> *When the temperature reaches 38 degrees (100 F) the Mg++ level of the MS serum decreases, whilst that of the controls does not change at this temperature.... Simultaneously with the decrease of the Mg++ level of the serum the Mg++ content of the erythrocytes increases in MS patients.*

This observation is important for two reasons. First, it suggests that the increased magnesium Perger detected in MS plasma may have come from the red blood cells. Second, to cope with fever, MS patients apparently required additional intracellular magnesium. Huszák and colleagues pointed out the "energy requirements of the enhanced metabolism in fever."

If fever caused magnesium to leave the plasma and enter the red blood cells of MS patients, what stress in Perger's patients caused magnesium to leave the red cells and enter plasma? One possibility is cold, to which MS patients are known to be sensitive (Gowers, cited by McDonald 1986; Flammer et al. 2001). If MS patients are magnesium-depleted, they may be unable to respond to cold by increasing metabolism, and therefore respond by decreasing metabolism, i.e. hibernating.

zinc, nitric oxide, and multiple sclerosis

Zinc metabolism is known to be disturbed in multiple sclerosis. Significant elevations of cerebrospinal fluid zinc (Linke et al. 1977) and white matter zinc (Craelius et al. 1980) as well as reductions in plasma zinc (Wong et al. 1980), serum zinc, and possibly CSF zinc (Palm & Hallmans 1982) have been reported. Dore-Duffy's group found red cell zinc levels "dramatically increased during a clinically documented exacerbation of MS." (Ho et al. 1986).

Abou-Mohamed and colleagues (1998) concluded that the anti-inflammatory effect of administered zinc was due to its ability to suppress both inducible and constitutive NOS:

> *There is compelling evidence to indicate an anti-inflammatory action of Zn2+. Most inflammatory diseases are associated with an increase of the inducible form of nitric oxide (NO) synthase. Additionally, inflammatory mediators such as histamine or bradykinin stimulate the constitutive NO synthase. Thus, the present study was undertaken to investigate whether Zn2+ inhibits production of inducible NO synthase and/or constitutive NO synthase activity to produce NO.... Thus, our results indicate that Zn2+ is capable of inhibiting lipopolysaccharide- or interleukin-1beta-induced NO formation as well as NO formation by constitutive NO synthase basally or in response to bradykinin or A-23187, and may explain the reported anti-inflammatory activity of Zn2+.*

Yamaoka and colleagues (2000) reported that application of zinc ions inhibited the induction of nitric oxide synthase in rodent skin cells:

> *Zinc, an essential metal, is a critical component of zinc binding proteins such as zinc fingers, zinc enzymes and metallothioneins. Recently, evidence for its anti-inflammatory property in skin has been accumulating, as shown in the treatment of acne, alopecia and zinc deficiency. In cutaneous inflammations, a large amount of nitric oxide (NO) is produced through induction of inducible nitric oxide synthase (iNOS) under the influence of proinflammatory cytokines, resulting in tissue damages in skin, as clarified in other organs. Therefore, we asked if the effect of zinc on NO production and/or on iNOS expression in keratinocytes may explain the anti-inflammatory property of zinc in skin.... Ten microM of zinc ion remarkably suppressed cytokine-induced NO production in keratinocytes. Furthermore, zinc ion also suppressed cytokine-induced iNOS expression in the protein level as well as in the messenger RNA level. These results suggest the possibility that the suppressive effect of zinc ion on cytokine-induced NO production in keratinocytes may be in part implicated in the anti-inflammatory property of zinc in some skin disorders.*

If zinc administration inhibits inducible nitric oxide, does zinc deficiency stimulate it? Cui and Okada (2000) found that zinc deficiency in rats induced the formation of nitric oxide synthase:

> *Our series of research using a rat model demonstrated that inducible nitric oxide synthase in the intestine is upregulated by zinc deficiency when challenged ... and the systemic administration of nitric oxide synthase inhibits or attenuates both intestinal damage and inflammatory skin lesions induced by zinc deficiency. Evidence from both transcription and translation levels indicates that inducible nitric oxide synthase, one of three nitric oxide synthase isoforms, has already been induced in the skin and intestine of zinc-deficient animals, whereas it is not generally expressed in normal tissues. On the other hand, total nitric oxide synthase activity in the intestine of zinc-deficient animals is significantly lower than that in controls, indicating that zinc deficiency may induce a potential vulnerability to nitric oxide rather than an absolute increase of nitric oxide synthase activities. Tissue zinc and metallothionein levels are significantly decreased in zinc-deficient rats, suggesting lowered antioxidative capability. Whether nitric oxide is destructive in inflammation may depend on the status of homeostasis such as the zinc level of tissues and the balance between the three nitric oxide synthase components,*

Pinelli and colleagues (2001) studied the effects of an NOS inhibitor on the heart and vasculature of rabbits. As plasma levels of nitric oxide diminished, plasma zinc decreased and blood pressure and cardiac necrosis increased:

> *Nitric oxide plays a key role as a vasodilating agent and its deficiency is associated with ischemic heart diseases. The aim of this study was to induce biochemical alterations associated with ischemic heart lesions by blocking nitric oxide synthase.... L-NAME administration reduced the nitric oxide levels and consequently increased the diastolic blood pressure. It also caused small areas of myocardial coagulative necrosis, whose dispersed nature made it undetectable by electrocardiograph, and decreased the plasma levels of zinc, which is involved in the enzymatic activities that remove the peroxides damaging the myocardium.*

Some forms of nitric oxide synthase have zinc in their structure. Li and colleagues (1999) studied two kinds of human inducible NOS--one that required zinc, and a zinc-free form. The zinc-bound structure resembled endothelial NOS:

> *The crystal structures of the heme domain of human inducible nitric-oxide synthase (NOS-2) in zinc-free and -bound states have been solved. In the zinc-free structure, two symmetry-related cysteine residues form a disulfide bond. In the zinc-bound state, these same two cysteine residues form part of a zinc-tetrathiolate (ZnS(4)) center indistinguishable from that observed in the endothelial isoform (NOS-3).*

Zinc seems significant in multiple sclerosis in part because carbonic anhydrase, which compacts myelin as it matures, is a zinc enzyme. The heavy metals most implicated in multiple sclerosis--dental mercury (Baasch 1966; Huggins 1984), lead in gasoline (Ingalls 1983), industrial zinc (Stein et al. 1987), and copper (Downey 1992)--all displace endogenous zinc from enzymes (Schroeder 1956; Taniguchi et al. 1975; Brandao-Neto & Bell 1994). Zinc displacement may explain how zinc metabolism can be impaired in multiple sclerosis even though zinc levels are not always low, e.g. the dramatic increase in red blood cell zinc during an exacerbation detected by Ho and colleagues (1986).

> *While the separation of zinc from enzyme protein irreversibly destroys its activity, the inactivation of the enzyme does not necessarily liberate the metal. Under some circumstances, the presence of an excess of zinc may be due, therefore, to the presence of inactivated enzyme retaining its full complement of zinc. (Vallee et al. 1949)*

Free hemoglobin vs. nitric oxide

Because of its affinity for gas molecules like oxygen and carbon dioxide, hemoglobin is also an effective scavenger for free radical gases like nitric oxide:

> *... we have demonstrated that under hypoxic conditions, NO accelerates its own consumption by increasing its entry into RBCs.... The homeostasis of NO is attained through a balance between its production and consumption. In the vasculature, RBCs are the major scavenger of NO, because RBCs contain high concentrations of Hb, an effective NO scavenger.... Because Hb efficiently consumes NO at extremely high rates, the consumption of NO has generally been considered to be unregulated. However, when Hb is enclosed within RBCs, its consumption of NO decreases significantly. (Han et al. 2003)*

> *We and others have shown the NO reaction with RBCs is nearly 1,000-fold slower than the reaction with cell-free Hb. (Han et al. 2002)*

Thus, intravascular hemolysis may greatly exacerbate the depletion of endothelial nitric oxide. What makes this clue so compelling is the great body of evidence that red blood cells, platelets, and blood vessels are abnormal in multiple sclerosis.

Vascular anomalies in multiple sclerosis

> *The typical case of multiple sclerosis ... with its long clinical history of remitting and relapsing visual disturbances, paralyses and sensory disturbances referable to all and any levels of the central cerebrospinal axis, and with progressive accumulation of permanent disabilities therefrom ... is a museum of pathologies at autopsy. But whatever the stage at which complications terminate its course, there is no disease lesion in pathology more characteristic than the pattern of central nervous changes. This pattern lies in the combination of a multiplicity of lesions, the often surprisingly disproportionate extent of involvement of CNS tissue in contrast to the small amount of clinical disability and that whereas at any given time some of the lesions are tough, poorly vascularized and therefore apparently old, others (sometimes in myriads) are tiny, pink, vascular and apparently new. (Lumsden 1970a)*

abnormal red blood cells

Plum and Fog (1959) reported that the red blood cells of multiple sclerosis patients were larger in diameter than normal red cells. This observation was confirmed by Prineas (1968):

> *Thus it would appear that the red cell diameter in multiple sclerosis varies during the course of the disease, tending to increase at times of activity and decrease when the disease is quiescent, at least in so far as this can be said on clinical evidence.*

Plum and Fog also found reduced bone marrow activity, and concluded that "a developmental anomaly or inhibition of the bone marrow" was responsible for the larger red cells. Ohara and colleagues (1982) and Kam-Hansen and colleagues (1988) could not confirm this, however.

One clue to the size of MS red blood cells is their greater osmotic fragility, reported by Caspary and colleagues (1967) among others. Osmotic fragility measures a red cell's capacity to take up water before bursting. If the red cell is already swollen, its capacity for additional water is less. Thus, MS red blood cells may be larger because they are swollen.

> *A large number of enzymatic defects in carbohydrate metabolism have been described which result in shortening of red cell life span.... As summarized by Beutler these systems supply the red cell with the energy to maintain the biconcave form by (1) maintaining hemoglobin in reduced (Fe2+) form; (2) operating the sodium and potassium pumps; and (3) maintaining sulfhydryl groups of various substances in the reduced form. Without this source of energy a shortened red cell life span occurs because the cell becomes converted to a sphere that will not pass through the spleen. (Berlin & Berk 1975)*

Simpson and colleagues (1987) observed that the blood of 15 MS patients was more viscous, and their red blood cells less deformable:

> *Whole blood viscosity in MS females was higher than controls at 3 of 4 shear rates ... but in MS males blood viscosity was higher only at [the lowest] shear rate .... Samples from MS contained numerous examples of Type I and Type II echinocytes [crenated or spiny cells] ... whereas control group erythrocytes were predominantly discocytic.... [W]e have shown that MS blood is less filterable than controls. We believe that this is evidence of reduced erythrocyte deformability in MS....*
>
> *The key role of the blood and vasculature in the pathogenesis of MS plaques has been recognized for more than 100 years .... Even at the earliest stage, plaques of demyelination are centred on small veins, in which vessels the shear rates are lowest and blood viscosity is highest. In circumstances where impaired erythrocyte flexibility reduces flood flow rate in venules, the resulting diminution of shear rate will increase blood viscosity further because of the thixotropic nature of whole blood [viscosity increases as flow rate decreases]....*
>
> *These observations [by Macci and Roizin] of congestion, stasis and sludging confirm the existence of impaired microvascular blood flow in MS which could be expected if blood rheology was abnormal.... As a working hypothesis we postulate that the MS "lesion" involves a primary rheological abnormality with reduced flexibility of erythrocytes.... [W]hen blood rheology is abnormal, stasis can be expected to occur within the smallest veins where shear rate is lowest. (Simpson et al. 1987)*

red blood cell agglutination (sludging), hemorrhage, and hemolysis

> *Since most of the conditions which increase the adhesiveness of red cells have the same effect on platelets, it is likely that platelet aggregation accompanies agglutination of the erythrocytes. (Elliot 1973)*

Agglutination (sludging) means red blood cells adhering to each other in clumps that slow or block the circulation (Knisely et al. 1947; Bloch 1956). Slow flow allows plasma to leak out of vessels, further concentrating the red cells. Bloch found sludged blood in many human diseases, including multiple sclerosis:

> *The first change is the formation of erythrocytes aggregates, then a reduction in the flow rate, which is followed by hemoconcentration, later by the dilation of the vessel, and still later by tissue edema and vessel hypertrophy and tortuosity. (Bloch 1956)*

According to Prineas, Putnam (1935, 1937) detected agglutinated platelets or red blood cells in the small veins of multiple sclerosis brains:

> *Putnam described vessels partially occluded by organized platelet thrombi or containing agglutinated masses of platelets or disintegrating red cells. (Prineas 1970)*

Scheinker (1943), studying early lesion formation in 20 multiple sclerosis patients, frequently observed agglutinated red blood cells (some hemolysing) in the blood vessels within plaques:

> *Most of the blood vessels within the lesions revealed marked dilatation and enormous engorgement with blood. In some of the larger lesions the vascular engorgement and stasis were especially striking.... The presence of vascular occlusion and thrombus formation was not always easy to identify. Clots of agglutinated red blood cells were frequently observed.*

Roizin and colleagues (1953) observed sludged blood in vessels of the bulbar conjunctiva in most of 24 MS patients:

> *The findings suggest that, in multiple sclerosis, lumps of sludge may plug capillaries supplying nervous tissue and thus seriously interfere with its blood supply. In this way sludge may be of pathogenetic significance.... In view of these facts it is possible to speculate that if methods were found to disintegrate the sludge of multiple sclerosis prior to the production of irreversible pathological changes, some of the symptoms of this disease might likewise be mitigated or their progressive course arrested.*

Swank (1958) reported small subcutaneous hemorrhages on the legs and thighs of 48 of 62 female patients with MS:

> *Biopsies of five spontaneous hemorrhages, where trauma could be confidently ruled out, revealed extravasated red blood cells infiltrating the deeper layers of the derma and subcutaneous fat. There was nothing to distinguish these small hemorrhages histologically from extravasation occurring after trauma, spontaneous hemorrhage, or the small petechial hemorrhages which result from embolism.... These vascular phenomena ... appear periodically and frequently coincide with or precede activity of the disease as manifested by exacerbation of existing symptoms or the development of new symptoms.*

Courville (see Alvord 1968) observed pigment and masses of red cells within blood vessels in acute lesions of multiple sclerosis.

iron deposits surrounding plaques

Craelius and colleagues (1982) detected iron deposits at the periphery of plaques in the brains of five patients with multiple sclerosis of long duration:

> *The iron deposits in hemosiderosis of the CNS are believed to arise from small, chronic and subclinical extravasations of blood from subarachnoid blood vessels. Whether the iron around plaques arises from similar chronic extravasation of blood is unknown .... It is known ... that demyelination takes place outside the edges of old chronic lesions, without inflammation. Interestingly, myelin uptake occurs in these areas by an unusual microglial pinocytotic process, which resembles that used by reticuloendothelial cells in ingesting iron and other material.*

Adams and colleagues (1985) observed: "Plaques sometimes show small haemorrhages ... and areas of haemosiderin deposition are seen." In a follow-up study of 70 cases of multiple sclerosis and 70 controls, Adams (1988) confirmed the presence of perivenous hemosiderin in MS brains:

> *The multiple sclerosis cases showed venous intramural fibrinoid deposition (7%), recent haemorrhages (17%), old haemorrhages revealed by haemosiderin deposition (30%), thrombosis (6%) and thickened veins (19%). In all, 41% of all multiple sclerosis cases showed some evidence of vein damage. Occasional control cases showed haemosiderin deposition in the brain but, unlike the multiple sclerosis cases, these were diffuse and almost entirely related to coexistent cardiovascular or cerebrovascular disease.... It is concluded that the cerebral vein wall in multiple sclerosis is subject to chronic inflammatory damage, which promotes haemorrhage and increased permeability, and constitutes a form of vasculitis.*

Hemosiderin accumulation in the CNS usually indicates bleeding into the brain. If red cells are hemolysing within blood vessels, however, plasma leakage as well as overt bleeding might allow hemoglobin to pass into brain tissues. Lumsden noted the German writers who believed that "serous" or "plasmatic" exudation was

> *the pathological substrate of the transient encephalopathies that may follow acute infections.... [I]t seems likely that an element of ... 'serous inflammation' is present in all of the postinfectious encephalopathies. (Lumsden 1970b)*

The vulnerability of red blood cells to hemolysis in multiple sclerosis may explain why hemosiderosis of the spleen was one of the few "general visceral histopathological findings" in MS reported by Schaltenbrand,

according to Lumsden (1970a), although in his own cases Lumsden found it mild and not that common.

Plum (1961) found that iron was slightly increased in the CSF of MS patients:

> *... such an increase has hitherto been found only in patients with pernicious anaemia. This observation is interesting, for as mentioned by Plum and Fog, certain morphological changes can be found in the blood picture of patients with multiple sclerosis, changes which are identical with those also observed in pernicious anaemia ....*

vascular spasm

Vasospasm in multiple sclerosis patients was first reported decades ago by Franklin and Brickner (1947) and Grain and Jahsman (1950), and recently confirmed by Flammer and colleagues (2001). Grain and Jahsman observed many MS patients with pathological changes in toenail beds:

> *The outstanding characteristic of these abnormalities is the element of spasm which was demonstrated both in the capillaries of the nailbeds, observed microscopically, and in the behavior of the peripheral arteries of the feet, as tested physiologically. Associated abnormalities detected by capillaroscopy were tortuosity, presumably a structural abnormality, and dilatation, probably a sign of stasis. These latter changes are considered to be secondary to capillary spasm, the tortuosity, in its various degrees, probably representing progressive structural modification, resulting from the repeated occurrence of capillary spasm. Similarly, one may presume that the findings observed by dermathermometry represent varying degrees of arteriolar spasm, showing a progressive tendency towards occlusion....*
> *Our findings suggest that the peripheral changes occur as early as any clinical manifestation, and that they increase in severity with the progression of the disease. The findings in our present augmented series appears to give at least numerical support to our previous opinion that the lesions of multiple sclerosis develop as the result of recurrent episodes of anoxemia in circumscribed patches of the central nervous system, produced by occlusive ischemia on the arterial side of the capillary bed.*

Although Flammer and colleagues (2001) thought vasospasm in multiple sclerosis might be secondary to demyelination, their descriptions of a vasospastic predisposition and a "primary vasospastic syndrome" share obvious characteristics with multiple sclerosis, as they pointed out:

> *... we have observed that patients with MS suffer more frequently from vasospastic symptoms than the average population. They often have cold hands or decreased thirst. Moreover, patients with MS have significantly increased levels of endothelin-1 in the blood and in the cerebrospinal fluid.*

Flammer commented further (personal communication 2003):

> *... we got the impression that many patients with multiple sclerosis had a primary vasospastic syndrome before they acquired multiple sclerosis. They often mentioned typical symptoms of vasospastic syndrome (long onset of sleep, reduced feeling of thirst, low body mass index, cold hands, etc.) before they had the first symptoms of multiple sclerosis. It is therefore possible that patients with primary vasospastic syndrome have a higher chance to acquire multiple sclerosis.*

elevated endothelin-1

Endothelin-1 is a vasoconstrictor peptide normally produced by vascular endothelial cells. Elevated levels of endothelin-1 (a biochemical marker for the vasospastic syndrome, according to Flammer et al.) have been detected in the blood of multiple sclerosis patients by Haufschild and colleagues (2001) and in their cerebrospinal fluid by Speciale and colleagues (2000). Lerman and colleagues (1998) tested the effects of oral L-arginine (substrate of nitric oxide) on endothelin levels in humans with nonobstructive coronary artery disease. After six months of supplementation, coronary blood flow was greater and plasma endothelin concentrations were reduced. The researchers concluded:

> *Long-term oral L-arginine supplementation for 6 months in humans improves coronary small-vessel endothelial function in association with a significant improvement in symptoms and a decrease in plasma endothelin concentrations.*

Estrogen protected against the vasoconstrictions of endothelin-1 in the coronary vessels of dogs:

> *Thus 17 beta-estradiol attenuated constrictions of coronary microvessels to [endothelin-1] more than did similar concentrations of testosterone. The ability of 17*

beta-estradiol to modulate responses to endothelin may involve release of vasodilator prostaglandins and/or nitric oxide by 17 beta-estradiol. (Lamping & Nuno 1996)

CNS vasodilation--a forgotten effective treatment

Central nervous system vasodilation, called "relief by flush," was the treatment of choice for multiple sclerosis in this country in the 1950s (Current Therapy 1950-57, 1962). Between 1946 and 1959, more than 3000 patients with multiple sclerosis and other demyelinating diseases were treated with the CNS vasodilator histamine diphosphate at the Multiple Sclerosis Clinic of St. Joseph Hospital in Tacoma. Most of them improved, some quite dramatically (Jonez 1952). Because the benefit of CNS vasodilation was never proved in controlled trials, its effectiveness has been largely forgotten. Yet histamine vasodilation consistently relieved acute attacks and often the progression of a disease now considered virtually incurable (Good 2002).

Brickner (1958) conducted thousands of tests of CNS vasodilators against acute multiple sclerosis symptoms. Every kind of acute symptom could be relieved, Brickner asserted. He pointed out that (a) the transient improvement of vasodilation was easy to overlook; (b) this led physicians to think there was no lasting benefit, but it might only mean long-term vasodilation was necessary; (c) uncertainty due to the possibility of spontaneous remissions could be eliminated by looking for immediate effects; (d) the immediate relief of acute attacks by CNS vasodilators said something crucial about the nature of multiple sclerosis symptoms. Brickner made this last point succinctly:

This must mean that at the moment the drug is used, there is a potential for reversibility of the symptom which is not being realized spontaneously.

The influence of pressure on the form and location of lesions

One mystery of multiple sclerosis is why so many plaques form in close proximity to the "outer and inner surfaces of the brain" (Lumsden 1970b). These surfaces abut the cerebrospinal fluid spaces--the inner cerebral ventricles and the outer subarachnoid space.

One of the most striking features of multiple sclerosis is the presence of plaques in the periventricular white matter. It has been suggested that myelinotoxic substances enter the white matter from the cerebrospinal fluid and cause demyelination. However no such agent has been identified. Similar periventricular plaques are seen in animals with chronic relapsing experimental allergic encephalomyelitis, and it is proposed that trauma to the tissue from increased intracranial pressure may play a role in the induction of plaques at this site. Even in normal dogs, but especially in hydrocephalus, the ependyma and periventricular tissue in the lateral corners of the ventricles is damaged by raised intracranial pressure. If the same factors operate in man, tissue damage at the angles of the ventricles could be one factor in the predilection of periventricular regions for the development of multiple sclerosis plaques. (Weller l985)

The bilateral symmetry of plaques also suggests pressure effects. Brownell and Hughes (1962) found symmetry in number of plaques in every part of the brain they examined. Fog (1965) observed bilateral symmetry of plaques along the spinal cord. Lumsden (1970a) commented:

The evenness of bilateral distribution is perhaps the most striking statistical finding in multiple sclerosis.

Lumsden concluded that pressure effects might be implicated in the form and location of plaques, if not their cause:

An interesting observation is that amongst conditions which mimic plaques in shape the most striking are areas of malfixation--an observation that leads one to wonder whether tissue pressure affects the shape and development of plaques of multiple sclerosis. (Lumsden 1961)

... agencies like tissue drainage and tissue pressure (reflecting haemodynamic factors, however produced) which are not necessarily involved in initiating a demyelinating process, may nevertheless govern the localization and form of the lesions--as seems almost certainly to be the case in multiple sclerosis. (Lumsden 1970b)

Lumsden further suggested that venous and perivascular pressures might explain why plaques are more common along the optic nerves and spinal cord than in the brain:

The basis for this predilection of the multiple sclerosis process for the optic pathways and cord is still, in the writer's view, the major riddle in the pathology of the disease.... [T]here can no longer be any question that plaques arise in relation to the walls of small veins, and the form and distribution of plaques within the cord and cerebrum are determined in some manner by this relationship to veins.... But it will still remain a problem to explain, in terms of veins, the overall regional election of the multiple

sclerosis process for the optic pathways and cord in preference to brain--perhaps until more is known about the relative venous pressures in these regions and, by implication, about the tissue pressures and flow dynamics of the perivascular spaces .... (Lumsden 1970a)

The frequent enlargement of the cerebral ventricles in multiple sclerosis is commonly believed to be secondary to loss or shrinkage of the white matter, yet Barnard and Triggs (1974) concluded that neither white matter shrinkage nor obstruction of CSF flow could explain the ventricular enlargement they observed in most of 20 MS brains.

Cerebrospinal fluid pressure usually appears normal in MS patients, but Tourtellotte (1975) reported one unusual case "complicated by normal pressure hydrocephalus." Normal pressure hydrocephalus is characterized by dilated cerebral ventricles despite usually-normal CSF pressure (Morariu 1979). Because continuous monitoring has detected high-pressure waves in some cases, Gunasekera and Richardson (1977) proposed the term "idiopathic hydrocephalus".

Two decades ago, Schelling (1984, 2002) noticed a "striking" widening of the sigmoid venous sinuses where they join the internal jugular veins in multiple sclerosis brains postmortem. He concluded that excessive venous pressure transmitted to the cerebrospinal fluid could explain why plaques form near the ventricles and along the optic nerves and spinal cord:

Almost half a century ago it was stated: "Artificially produced perivenous extravasations of contrast-medium closely simulate the shape and the distribution of plaques in advanced stages of MS (solution had been forced into the great vein of Galen under heavy pressure."[Schlesinger 1939] Thus the uneven venous dilatation, leakage, and degeneration, as well as the retrograde perivenous spread of the patches, perivascular dilatations, and hemorrhages in the brain in MS may be due to intermittent retrograde venous hypertension. (Schelling 1986)

Gottlieb, Smith, and Neubauer (1990) proposed that leakage across the blood-brain barrier was caused by "focal hypertension," contending that multiple sclerosis patients, like persons with low tension glaucoma, may be unusually sensitive to pressures that seem normal.

Newman, Selzer, and Bell (1994) encountered three patients with multiple sclerosis together with idiopathic intracranial hypertension (pseudotumor cerebri):

... how might these two diseases be related? One explanation is that the disease process involved in multiple sclerosis somehow also causes an elevation of the intracranial pressure.... Although our three cases appear somewhat unique in the English literature in their association of multiple sclerosis and raised intracranial pressure, the Russian literature refers to cerebrospinal fluid hypertension in the setting of MS exacerbation as if it were a commonly accepted association [Skoromets et al. 1991]. A total of 40 patients, 29 women and 11 men, ages 24-44, were studied during an exacerbation of MS. All of them had raised intracranial pressure (220-380 mm of water) and symptoms of headache, nausea, and sometimes vomiting and changes in mentation. In 33 patients there was widening of the third ventricle by "echoencephalography."

Discussion

... it would be wrong to conclude that because the adverse and beneficial effects of NO are partly compensatory, NO has only a small role in MS. Rather, it seems that the role of NO in MS is substantial, and each of the compensatory forces is large. (Smith & Lassman 2002)

The recent discovery of inducible nitric oxide in multiple sclerosis lesions presents a genuine therapeutic dilemma. On the one hand, this nitric oxide could be entirely responsible for the central nervous system damage in multiple sclerosis, either directly, e.g. by opening the blood-brain barrier and/or poisoning oligodendrocytes, or indirectly, by forming toxic peroxynitrite. On the other hand, because the primary source of the inducible nitric oxide in the lesions appears to be the astrocytes (Broholm et al. 2004), the support cells of neurons, it may originate as a protective or compensatory response. Nitric oxide is a vasodilator, after all, and central nervous system vasodilators were once highly regarded in the treatment of multiple sclerosis.

As we have seen, inducible nitric oxide appears in response to endothelial cell damage, and/or impaired synthesis or release of endothelial nitric oxide. The cerebrovascular endothelium (blood-brain barrier) is clearly damaged in multiple sclerosis, and there are reasons to suspect that endothelial nitric oxide may be chronically deficient. Most multiple sclerosis patients are extremely heat-sensitive, and their platelets are sticky.

Sensitivity to stress in multiple sclerosis patients may reveal depletion of the parasympathetic transmitter neuronal nitric oxide. Furthermore, deficiencies of magnesium, zinc, and sex hormones--all known to be associated with multiple sclerosis--under experimental conditions show elevations of inducible nitric oxide. Endothelial and neuronal nitric oxide synthases are zinc enzymes.

How do these deficiencies arise in multiple sclerosis patients? If suppression of sexuality and aggression suppresses their pubertal sex hormones (to judge from their immature myelin) expression of endothelial and neuronal nitric oxide synthases may likewise be immature and/or impaired. Magnesium depletion may arise from the stress response Perger detected just before attacks, with calcium moving into cells and magnesium leaving. Zinc may be displaced by the heavy metals so often implicated in multiple sclerosis.

One challenge to this view is the beneficial effect of pregnancy on the incidence of relapses.

> *The concentration of Mg in the placental and fetal tissues increases during pregnancy. The requirements for this element in a pregnant woman's organism generally exceed its supply; hence, pregnancy should be considered a condition of 'physiological hypomagnesemia'. (Semczuk & Semczuk-Sikora 2001)*

If pregnancy increases the need for magnesium, why are relapses less common at this time? Decrease in disease activity is most obvious toward the end of pregnancy, when estrogen and progesterone levels are highest; disease activity increases when these hormones fall abruptly postpartum. This may mean that sex hormones protect more than magnesium deficiency harms.

Scavenging by free hemoglobin may further deplete endothelial nitric oxide in multiple sclerosis. Large, osmotically fragile, spiny, agglutinated red blood cells moving slowly through blood vessels narrowed by endothelial nitric oxide depletion must be very vulnerable to hemolysis, within vessels and without.

> *During hemolysis ... cell-free ferrous hemoglobin ... in the plasma rapidly destroys nitric oxide by being oxidized to methemoglobin (FeIII) and nitrate ions. Owing to its low molecular weight, cell-free hemoglobin ... may diffuse into extravascular spaces. Limited nitric oxide bioavailability under these conditions promotes systemic vasoconstriction and organ dysfunction. (Schechter & Gladwin 2003)*

Poser (1969) concluded that the myelopathies following infections and vaccinations had elements in common, including vascular lesions and hemorrhage:

> *Diapedesis of red blood cells forming perivascular hemorrhages not only frequently occurs but may be so intensive as to obscure other pathologic changes.*

Bhasin and colleagues (2002) simulated intracerebral hemorrhage (ICH) in rats by infusing them with lysed red cells, and injecting their brains with packed red cells. The lysed red cells increased BBB permeability and induced brain edema within 24 hours, the packed red cells within 3 days:

> *Significant brain edema developed after 24 hours in rats infused with lysed RBCs and this was associated with a 3-fold increase [in] blood brain barrier (BBB) permeability to alpha-aminoisobutyric acid. We have previously shown that intracerebral injection of packed RBCs does not cause edema formation at one day but does at three days. In this study, we found that packed RBCs did not cause significant BBB disruption at one day but produced a 4-fold increase in BBB permeability at three days. These studies show that following ICH the leakage of substances inside the RBC, facilitated by cellular lysis, results in delayed edema.*

But if free hemoglobin in plasma can open the blood-brain barrier, leak into CNS tissues, and initiate demyelination, why hasn't multiple sclerosis been transmitted to animals via plasma? One explanation is that most of these experiments were short-term, as Prineas (1970) noted:

> *Many ... instances were reported in which a variety of experimental animals, including monkeys, developed neurological symptoms following the inoculation of blood or spinal fluid from patients with multiple sclerosis. Usually these were short-term experiments and the histological changes described were, for the most part, unlike those seen in multiple sclerosis.... Innes and Kurland (1952), in reviewing these studies and the equally numerous unsuccessful attempts to transmit multiple sclerosis to experimental animals, felt that both the positive and negative findings reported were equally inconclusive.*

Furthermore, if multiple sclerosis myelin is immature and uncompacted, the myelin of the experimental animals may simply have been more resistant to demyelination.

Craelius and colleagues (1982) detected iron deposits surrounding multiple sclerosis plaques. Adams (1988) found iron deposits in perivenous tissues in multiple sclerosis brains. Iron leaking into the brain may overwhelm oligodendrocytes, which store iron. On the other hand, these iron deposits may only mark the death of oligodendrocytes known to occur in multiple sclerosis. Spectrophotometric analysis of multiple sclerosis cerebrospinal fluid for the presence of the blood pigments oxyhemoglobin, methemoglobin, and bilirubin showed nothing unusual (Müller et al. 1989). More recently, however, LeVine (1997) reported:

> *The localization of iron in multiple sclerosis (MS) and Alzheimer's disease (AD) brains was investigated to further the understanding of its pathogenic role in these disease states. Earlier studies, utilizing a standard Perls' stain, yielded conflicting reports*

> *regarding the distribution of iron deposits in MS brains .... In the present study, a modified version of the ... stain was used .... This modified method can reveal iron deposits that are missed by the Perls' or DAB-enhanced Perls' stains. In addition to its normal deposition in oligodendrocytes and myelin, iron was detected in reactive microglia, ameboid microglia and macrophages in MS brains.*

The recent report by Barnett and Prineas (2004) of oligodendrocytes in acute lesions dying of apoptosis before any signs of inflammation appear raises the question whether iron overload in these myelin-producing cells might be responsible:

> *MS is a disease of the oligodendroglia-myelin system. Since iron is predominantly found in the oligodendroglia, dysfunction may result in an excessive accumulation of iron in structures that normally have relatively high iron stores. (Drayer et al. 1987)*

Overproduction of nitric oxide also poisons oligodendrocytes, perhaps leading to apoptosis:

> *Experiments both in vivo and in vitro have shown that NO is the mediator of glutamate toxicity, and is toxic for glial cells, particularly oligodendrocytes. (Brosnan et al. 1994)*

> *Cytotoxicity as a result of long-lasting NO generation is now established to initiate apoptosis. (Brune et al. 1998)*

How might nitric oxide depletion bring on multiple sclerosis symptoms? Smith and Lassman (2002) concluded that elevated concentrations of inducible nitric oxide could block movements of sodium, potassium, and/or calcium ions across nerve cell membranes. Inducible nitric oxide opening the blood-brain barrier could lead to edema, as has been proposed. James (1982) pointed out the resemblance of multiple sclerosis to symptoms of decompression sickness:

> *Because the commonest neurological symptoms of decompression sickness are dizziness (often accompanied by nystagmus), visual disturbances, impairment of micturition, and weakness of the legs, the condition invites comparison with multiple sclerosis. (James 1982)*

> *Hemoconcentration, with hematocrit as high as 60 to 70 percent, is a characteristic finding [in decompression sickness] that presumably results from widespread vascular injury and loss of plasma into tissues. (Johnson 1971)*

Nevertheless, endothelial nitric oxide depletion itself could be directly responsible for many multiple sclerosis symptoms. Deficiencies of the major endogenous vasodilator must increase susceptibility to vasospasm. Retinal vasospasm has been observed during attacks of optic neuritis, and CNS vasodilators have relieved all varieties of acute multiple sclerosis symptoms.

Why does nitric oxide depletion affect the CNS preferentially? Toda and Okamura (1998) pointed out that the peripheral circulation maintains a vasoconstrictor tone, while the cerebral circulation maintains a vasodilator tone:

> *The vascular tone, vascular resistance and blood flow in the brain are regulated by neural and humoral factors in quite a different way from those of peripheral organs and tissues. In contrast to the dominant vasoconstrictor control in the periphery, the intracranial vascular tone is predominantly influenced by vasodilator mediators over vasoconstrictor ones.*

In conclusion, impaired synthesis and/or release as well as scavenging of endothelial (and neuronal) nitric oxide in multiple sclerosis may induce vasospastic symptoms, and lead to compensatory overproduction of inducible nitric oxide, which damages the CNS directly by opening the blood-brain barrier and/or poisoning oligodendrocytes, and indirectly by forming toxic peroxynitrite.

One challenge to this hypothesis is the recent finding of significant immunoreactivity (IR) to endothelial nitric oxide synthase (eNOS) in vascular endothelial cells of multiple sclerosis brains:

> *Almost all sections from the three MS brains expressed moderate to strong IR to eNOS in the intraparenchymal vascular endothelial cells of capillaries and venules. This was in contrast to sections from control brains that contained only sparse amounts of eNOS IR in the vascular structures. (Broholm et al. 2004)*

Curiously, no immunoreactivity to eNOS in arteries and arterioles was described, although these vessels normally produce more endothelial nitric oxide than the veins and venules. Furthermore, elevated levels of endothelial nitric oxide synthase do not guarantee that endothelial nitric oxide is sufficient. Endothelial NOS is a zinc enzyme; zinc is known to be deficient in multiple sclerosis, and suspected to be displaced by heavy metals. Yet zinc levels can be high although (or even because) displacement has rendered zinc enzymes inactive. Ho and colleagues (1986) observed a dramatic rise in red cell zinc during exacerbations. Thus,

endothelial nitric oxide may be low even though its synthase is elevated.

Why are MS red blood cells large, osmotically fragile, spiny, agglutinated, and their membranes less deformable than normal red cells? Part of the reason may be magnesium depletion. Magnesium is needed by the (Na+-K+) ATPase that pumps calcium and other ions out of membranes and cells. In blood vessels, magnesium deficiency allows calcium to increase in smooth muscle, constricting the vessels--a scenario called "magnesium ischaemia" by Newman and Amarasingham (1993) who implicated it in eclampsia:

> *'Magnesium ischaemia' is a term used to denote the functional impairment of the ATP-dependent sodium/potassium and calcium pumps in the cell membranes and within the cell itself. The production of ATP and the functioning of these pumps is magnesium-dependent and is critically sensitive to acidosis. Zinc and iron deficiencies may secondarily impair these pumps and thus contribute to 'magnesium ischaemia' (as does acidosis). This term is two-dimensional at its simplest; it refers to a functional magnesium deficiency, whether actual or induced. It is argued that chronic acidosis is the most common inducing factor.*

Unich and Barabanchik (1986) detected low blood pH and compensated acidosis in multiple sclerosis patients that varied with the course of the disease.

According to Eaton and colleagues (1973), calcium entering red blood cells in sickle cell disease hardens the red cell membranes. Thus, magnesium depletion and calcium accumulation in multiple sclerosis may lead to swollen red cells with less deformable membranes.
Heavy metals implicated in MS may damage red blood cells by agglutination, corrosion, and hemolysis. Jandl and Simmons (1957) found that more mercury was needed to agglutinate red blood cells than copper, iron, or zinc, but lower concentrations of mercury were sufficient to hemolyze red blood cells:

> *Since the red cell is in effect a huge polyanionic molecule, with an overall negative charge at physiological pH levels, it is tempting to believe that agglutination supervened when the potential difference between the red cell and the medium of suspension was sufficiently reduced by 'electric adsorption' of positive ions.... Thus, it may be postulated that the reaction of multivalent cations with red cells may initially reduce their negative charge and permit aggregation; but it is probable that at some point direct cross-linkages develop between the cells.*

Jung (1947) found that mercuric chloride corroded red cell membranes, coagulated cell proteins, and precipitated hemoglobin inside the cells:

> *With lesser concentrations, we saw ultramicroscopically all degrees of progressive coagulation of the cell protein from outside to inside. The smallest amounts led to a precipitation of hemoglobin in the cell interior with a simultaneous increase in osmotic resistance, greater amounts to a gradual hemolysis, which bordered on a denaturing of the membrane.*

Siblerud and Kienholz (1994) compared red blood cell concentrations and hemoglobin levels of MS patients who had their mercury amalgam dental fillings removed against blood values of MS patients who retained their amalgam fillings:

> *MS subjects with amalgams were found to have significantly lower levels of red blood cells, hemoglobin and hematocrit compared to MS subjects with amalgam removal.... The MS amalgam group had significantly higher blood urea nitrogen and lower serum IgG.... A health questionnaire found that MS subjects with amalgams had significantly more (33.7%) exacerbations during the past 12 months compared to the MS volunteers with amalgam removal.*

Finally, we must inquire why pressure has such great influence on the form and location of the lesions. Recently, Ubels and colleagues (2001) reported an unusual redistribution of blood in insulin-dependent (Type I) diabetics. More blood was detected in the veins of these persons than in their arteries. The researchers explained this anomaly in terms of nitric oxide:

> *Impaired activity of endothelium-derived nitric oxide in Type I (insulin-dependent) diabetes mellitus will cause an increased vascular tone. Considering the lower production of nitric oxide in veins than in arteries, an impaired activity would have less vasoconstrictive effect in veins. The reported minimally changed total plasma volume in diabetes might, therefore, indicate a redistribution of blood volumes from the arterial to the venous side of the circulation.... The increase in venous blood volume and myogenic response and the decrease in distensibility of the large arteries in the upper arm are in agreement with the expected shift towards venous blood volume distribution in Type I diabetes with and without microalbuminuria.*

Is venous pressure (thus CSF pressure) elevated in multiple sclerosis patients because endothelial nitric oxide depletion has shifted blood from their arteries to their veins? If so, the most parsimonious explanation of multiple sclerosis might be that too little blood in arteries and arterioles leads to vasospastic symptoms, while too much blood in veins and venules leads to blood-brain barrier leakage and lesions.

Thoughts on therapy

> *Children usually cannot make enough histidine and arginine to support growth, especially during periods of stress, hence these two [amino] acids are at times essential to children. An undersupply of arginine causes animals to become sterile and brings about a decrease in the formation and mobility of sperm in men .... (Davis 1970)*

A curious aspect of L-arginine, called the "arginine paradox," is that supplementation stimulates nitric oxide production even though the tissues appear already saturated with substrate:

> *L-Arginine, the substrate of nitric oxide (NO) synthases, is found in the mammalian organism at concentrations by far exceeding K(M) values of these enzymes. Therefore, additional L-arginine should not enhance NO formation. In vivo, however, increasing L-arginine concentration in plasma has been shown repeatedly to increase NO production. This phenomenon has been named the L-arginine paradox; it has found no satisfactory explanation so far. (Tsikas et al. 2000)*

The dilemma of L-arginine as treatment for multiple sclerosis, however, lies in the present uncertainty whether the inducible nitric oxide in the lesions is entirely the problem, or whether it originates as the body's attempt at a solution. If inducible nitric oxide is a compensatory response that turns deadly, repletion of endothelial nitric oxide may eliminate the need for this overcompensation. Furthermore, studies show that L-arginine *deficiency* increases levels of peroxynitrite, the toxic NO metabolite most implicated in multiple sclerosis. Xia and colleagues used electron paramagnetic resonance spin-trapping to monitor the formation of nitric oxide and superoxide in human kidney cells. They reported that superoxide concentrations increased as arginine concentrations decreased, resulting in increased formation of peroxynitrite:

> *Thus, with reduced L-Arg availability NOS elicits cytotoxicity by generating O2- and NO that interact to form the potent oxidant peroxynitrite. (Xia et al. 1996)*

> *Among the toxic agents released in brain tissues by activated cells, we focus attention ... on peroxynitrite, the product of the reaction between nitric oxide (NO) and superoxide. Peroxynitrite is a strong oxidizing and nitrating agent which can react with all classes of biomolecules. In the CNS it can be generated by microglial cells activated by pro-inflammatory cytokines or beta-amyloid peptide (beta-A) and by neurons in three different situations: hyperactivity of glutamate neurotransmission, mitochondrial dysfunction and depletion of L-arginine .... (Torreilles et al. 1999)*

One way to circumvent the uncertainty about L-arginine until more is known may be to treat patients with sex hormones, zinc, and magnesium, known to be deficient in multiple sclerosis. These treatments may be safe ways to restore endothelial nitric oxide indirectly. The rapid relief of acute multiple sclerosis symptoms by inhalation of amyl nitrite (Brickner 1958), a nitrovasodilator, raises the question whether the nitric oxide inhalation now being tested against sickle cell symptoms (Reiter & Gladwin 2003) might be useful against multiple sclerosis.

In any case, it is by no means certain that too much nitric oxide, and not too little, is the fundamental problem in multiple sclerosis. Answering this question definitively would appear to be a first priority of multiple sclerosis research.

Testing this hypothesis

At this time, biopsy and Western blot analysis appears to be the only way to determine which form of nitric oxide synthase is present in any tissue. Blood platelets, however, apparently express the same constitutive nitric oxide synthase found in endothelial cells. Thus, platelets are a readily available tissue that can be tested for endothelial NOS depletion. Because platelets have a short life span, this test may reveal bone marrow anomalies already suspected to exist in multiple sclerosis. On the other hand, eNOS is a zinc enzyme, and therefore can be inactivated by zinc displacement even when present in normal concentrations.

The non-invasive method used by Ubels et al. (2001) to determine that diabetics have more blood in veins and venules than in arteries and arterioles may reveal a similar shift of blood due to endothelial nitric oxide depletion in multiple sclerosis patients.

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