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REVIEW ARTICLE

Misincorporation of the Proline Analog Azetidine-2-Carboxylic Acid in the Pathogenesis of Multiple Sclerosis: A Hypothesis

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Abstract

The misconstruction of proteins as a result of the displacement of one of more proline residues by their congener, azetidine-2-carboxylic acid (Aze), can result in various disorders. A number of lines of evidence suggest that multiple sclerosis may be among these. This concept adheres to the current view that multiple sclerosis lesions originate in the myelin sheath and that the underlying molecular abnormality involves the myelin basic protein. The Aze hypothesis posits that myelin basic protein and possibly other closely related molecules are misassembled in sites of lesion formation because of the substitution of Aze for one or more prolines within consensual epitopes. These include a highly conserved myelin basic protein hexapeptide sequence, PRTPPP, and an α helix bounded by prolyls. Recent studies have focused on the immunopathogenetic effects of posttranslational modification of this region. This hypothesis proposes that the domain is structurally, functionally, and antigenically altered by the intrusion of Aze in place of proline and that such misassembly may involve other proteins and adversely affect interactions with neighboring molecules. This report reviews evidence supporting the hypothesis that ingestion of Aze in the diet, in conjunction with genetic susceptibility, may predispose or contribute to the pathogenesis of multiple sclerosis.

Key Words: Azetidine-2-carboxylic acid (Aze), Ion channel, Multiple sclerosis, Teratogenesis, Translation

INTRODUCTION

There are hundreds of amino acids in nature. The 22 *protein* amino acids comprise a small subset, evolutionarily selected from the larger group for inclusion in proteins. Their location in the linear sequence of a protein is stipulated by a codon in messenger RNA.

The other amino acids, referred to as *nonprotein* amino acids, are found principally in plants and in lower marine forms (1, 2). Their biologic assignments are not ordained by codons, and thus they do not ordinarily become incorporated into proteins. They function instead as nitrogen storage compounds and as arsenals of poisons, which are deployed to establish botanical territoriality and to defend against marauding predators (1, 2). In addition, there is an ever-expanding class of *unnatural* amino acids synthesized in the laboratory (3).

Two nonprotein amino acid congeners of alanine, β-*N*-methyl-amino-L-alanine and β-*N*-oxalyl-L-alanine, have been implicated in the pathogenesis of neurological diseases. The disorder caused by β-*N*-methyl-amino-L-alanine is a form of amyotrophic lateral sclerosis that has been prevalent among native populations in Western Pacific Islands. The disorder caused by β-*N*-oxalyl-L-alanine is neurolathyrism, a form of spastic paraparesis that has afflicted certain indigenous groups in East Africa and southern Asia (2, 4).

The present communication calls attention to the pathogenetic potential of another naturally occurring nonprotein amino acid, azetidine-2-carboxylic acid (Aze), a congener of proline. To avoid its unwieldy name, the amino acid will be referred to in the form of its abbreviation, Aze. Recall that the word proline is used in place of the cumbersome terminology, 2-carboxypyrrolidine (Aze is pronounced by sounding each of its letters separately).

Azetidine-2-carboxylic acid is present in food and is capable of exerting noxious effects in many species, including humans, resulting in a wide range of toxic, teratogenic, inflammatory, protein catabolic, and degenerative abnormalities, many of which involve the nervous system (1, 5). These facts have led to the hypothesis that Aze may be involved in the pathogenesis of multiple sclerosis (MS).

PROLINE AND AZE

The harmful effects of Aze can be understood by comparing its structure to that of proline, the protein amino acid for which it masquerades (Fig. 1). Proline is unlike any of the other 22 protein amino acids in that its amino group is covalently locked within a 5-member ring (Fig. 1). Such bonding constrains the torsion angles of the peptide union between the nitrogen group of a proline and the carboxyl group of the adjacent amino acid. Such bonds may impart a local rigid flexure to a string of amino acids.

In the peptide link between amino acids, the hydrogen atom of the amino group of one and the oxygen atom of the carboxyl group of the other occupy opposite positions (*trans*) in the plane of the bond. Because of the absence of a second hydrogen atom on proline's nitrogen and the latter's unique electron environment, there is a strong tendency for the proline ring structure to drift to the same side (*cis*) of the

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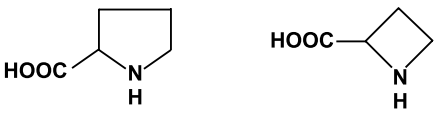

proline azetidine-2-carboxylic acid

FIGURE 1. The structures of proline and azetidine-2-carboxylic acid. The ring of proline has 5 members and the ring of azetidine-2-carboxylic acid has 4. Note that the nitrogen atoms of each molecule are covalently bound within the ring structure.

peptide plane as that occupied by the oxygen atom, a configuration that causes major changes in the shape of a protein as the bulk of the molecule rotates, markedly altering regional conformation. The process of *cis-trans* isomerization, accelerated by prolyl isomerases, converts subtle shifts in the electronic milieu into major biomechanical events and plays a key role in protein folding. Polyproline helices composed of *cis* isomers (poly-Pro I) form right-handed helices, whereas polyproline helices composed of *trans* isomers (poly-Pro II) form left-handed helices.

Another exceptional property of proline arises from the fact that its nitrogen atom is bonded to only 1 hydrogen atom. Therefore, proline cannot function as a hydrogen bond donor; it can, however, serve as a hydrogen bond acceptor (6, 7).

The presence of a proline residue may change the direction of a chain and result in a turn. Sharp (tight) turns at surfaces are often sites of posttranslational modifications and of epitope formation. Some proline-rich sequences weave a characteristic extended helix, which is the configuration of a strand in collagen. On the other hand, the intrusion of a proline within an α helical chain or within a β sheet disrupts the basic architecture. Therefore, prolyls are excluded from these regions, but they can serve as the initial amino acid of α helices and at the edges of β sheets.

Azetidine-2-carboxylic acid is identical to proline except that its ring contains 4 members instead of 5 (Fig. 1). This structural difference results in changes in torsion angles, the direction of turns, and in *cis-trans* isomerizations. Thus, the substitution of Aze for proline can change protein structure and function (8, 9). These effects take on significance because Aze is readily misincorporated into proteins in place of proline, and such misconstruction can eventuate in a wide range of clinical disorders (2). As already mentioned, Aze is present in the human diet (2, 5).

AZE SUBSTITUTION FOR PROLINE AND ACQUIRED PROTEIN MISASSEMBLY

Errors in protein synthesis, customarily attributable to alterations in DNA sequence, may lead to the accumulation of malformed molecules, the dysfunction of which eventuates in disease (10). A genetic missense mutation causes 1 amino acid in a specific protein to be substituted for another. The underlying disorder involves a mutation in a protein-coding region in DNA (10).

Amino acid substitution in proteins can occur for a different reason, a typesetting error arising from a faulty translational process in which transfer RNAs (tRNAs) fail to distinguish between their cognate protein amino acids and impostor nonprotein amino acids. The tRNAs, charged with alien molecules, deliver their cargo to the ribosomal stations through which the exotic molecules make their way into a nascent protein, displacing the authentic amino acids (1, 2). The failure of a tRNA to exclude a specific nonprotein amino acid is a translational error that may be shared by an entire species. This mechanism of disease, the substitution of a nonprotein amino acid for its protein amino acid analog, can eventuate in exceedingly diverse clinical manifestations because virtually any number of different proteins may be involved and each at one or more critical locations. Unlike hereditary homozygous missense mutations that deform all of the miscoded proteins, the misassembly of proteins owing to tRNA promiscuity affects only those proteins undergoing ribosomal synthesis at the time of the delivery of the culprit nonprotein amino acid. Thus, the misconstruction of proteins is likely to be spotty, and the manifestations, highly variable.

The failure of a tRNA to distinguish between a protein and a nonprotein amino acid is an imprecision in protein synthesis distinctive from the wobble mechanism (10). Wobble involves the tRNA anticodon, whereas the misincorporation of a nonprotein amino acid involves the tRNA amino acid attachment site and the aminoacyl-tRNA synthetase. Therefore, different molecular mechanisms are responsible for these 2 types of translational infidelity (10).

A factor influencing the pathogenicity of molecular misincorporation is the amino acid replaced by the impersonator. Substitution for proline may be a worst-case scenario. As previously indicated, proline is strikingly unlike any of the other 22 protein amino acids and its influence on protein architecture can in certain instances be critical. The displacement of a proline by a molecular analog may result in untoward effects on the folding conformation, charge distribution, immunogenicity, and function of a protein.

To understand why Aze may be a cause for concern, it is necessary to review the biology of the nonprotein amino acid and the history of the events that led to the sudden increase in human exposure to it.

AZE IN THE BIOSPHERE

Fowden discovered Aze more than 50 years ago in lilies, in the seeds of the Royal Poinciana tree, and in the root of the sugar beet (11, 12). He demonstrated its presence in 20 of 90 plants he tested (11–13). The nonprotein amino acid did not attract attention as an etiologic agent in human disease, presumably because humans do not consume lilies, poinciana seeds, or the roots of sugar beets. In 2006, Aze was reported to be present in garden or red beets, the vegetable that is a constituent of the human diet and a staple food in many regions (5).

Dismissing sugar beets as inedible may have been premature, inasmuch as their by-products can enter the human food chain (13). The mechanical harvesting and processing of a crop of sugar beets is followed by the extraction of sucrose by boiling and crystallization. The residue, consisting of beet molasses and fibrous plant tissues, containing minerals and

amino acids, is fed to meat-producing and dairy livestock. In this way, Aze can be carried into human food (14, 15).

A BRIEF HISTORY OF HUMAN CONSUMPTION OF AZE

The spread of Aze in the biosphere occurred as a consequence of geopolitical events in Europe and the Americas during the 19th and 20th centuries. Sugar beet agriculture is relatively new, an enterprise that first reached significance a little more than 150 years ago and now accounts for almost a third of the global sugar supply (16).

Humans have eaten beets throughout recorded history, but evidently in small amounts. Early documents suggest that the stalks and leaves were preferred. These do not contain Aze. The bulbous root was consumed for medicinal purposes (16). In 1747, a German apothecary chemist, Andreas Marggraf, discovered sucrose in the roots (bulbs) of sugar beet. The sugar was present in low concentrations, probably only a few percent, and therefore of little commercial interest (16). Franz Achard, Marggraf's student, pursued this discovery during the next 50 years, and by crossbreeding, he increased the sucrose content severalfold. It is now 16% to 18%. Achard established the first sugar beet–processing plant in Kunern, in the Silesian region of Germany in 1801–1802; it was destroyed in a fire 5 years later.

The blockades of Europe during the Napoleonic wars cut off the supply of sugar from the West Indies. Pressed by the disgruntled populace of an entire continent virtually deprived of sweets, Napoleon issued a decree in 1810 allotting some 32,000 hectares (about 79,000 acres) to sugar beet production. He funded scholarships, established special sugar beet schools, and directed the construction of 10 factories in northern France. Thereafter, sugar beet agriculture proliferated in France and Germany (16). Sugar beet agriculture, previously a small-scale activity, expanded gradually at first and then began to flourish by the middle third of the 19th century. It eventually surged into the third or fourth leading farming enterprise in Europe and became a major crop-growing endeavor in Canada and in the United States (16, 17). In this way, human populations may have become exposed only recently to this highly noxious nonprotein amino acid.

MS: THE AZE HYPOTHESIS

The cause or causes of MS remain enigmatic. It is widely believed that abnormalities of myelin basic protein (MBP), a major protein component of the central nervous system (CNS) myelin, play an important role in pathogenesis (18–25). A proline-rich sequence within a consensually identified epitope of MBP (residues 90–102) embraces a unique and highly conserved hexapeptide string containing 4 prolines, 3 of which are contiguous in the alignment of PRTPPP. The triple proline segment, residues 99–101, has been regarded as a keystone element in the overall architecture of the molecule (21). Numerous studies have focused on this region and on the effects of its posttranslational modification by phosphorylation, deamidation, and deimination (citrullination) adjacent to residues 99 to 101 (18). Similarly, an overlapping epitope (residues 85–96) is bounded by prolines and has been identified as an α helix that penetrates deeply into the phospholipid bilayer (22).

The present hypothesis calls attention to Aze substitution for prolines in the region. This could change charge distribution, protein structure, epitope conformation, induce immunogenicity, and exert tectonic stress because of the unique torsion angles and steric constraints of the proline analog (8, 9). Such effects would interfere with reciprocal nesting among adjacent lipids and proteins, especially with SH3 domains that bind proline-rich regions of proteins, as well as interfere with posttranslational modifications and interactions among the epitope, lymphocyte, and HLA receptors (18, 26–39). Studies of genetic susceptibility to MS have identified a variety of molecules including interleukins and HLA haplotypes that may play a role in its pathogenesis (39).

Central nervous system myelination in humans occurs principally in late gestation and during childhood. The gradual accumulation of misassembled molecules of MBP, as well as other related and interacting molecules, may reach a critical threshold in scattered locations. Thereafter, plaques may begin to form as a consequence of defective molecular conformation, intramembrane proteolysis, inflammation, and autoimmune responses. The initial lesions, which may be detectable with magnetic resonance imaging, are likely to be clinically silent. Such an unfolding of events is consistent with the observation that the geographic location of childhood residence influences both the age of onset and the lifetime risk of the disease (40). Most cases of adult-onset MS are diagnosed after the age of 25 years (40).

Evidence supporting the hypothesis that proline displacement by Aze might play a role in some instances of MS relates to geoepidemiology, chronoepidemiology, migrant epidemiology, and disease in animals. Each of these aspects is now considered.

MS EPIDEMIOLOGY AND THE AZE HYPOTHESIS

There is a tight fit between the worldwide prevalence of MS and the geography of beet agriculture. This correlation goes beyond the occurrence of MS in higher latitudes (41). Beets share this cartography, but their adaptability allows them to flourish under more temperate conditions, such as those of the Mediterranean coast. In each high-prevalence cluster of cases of MS, beets are a principal commodity or a dietary staple. This specification applies to Sardinia, the Orkney Islands, the Middle East, Finland, the Faroe Islands, Alberta, and the Tokachi province in Hokkaido, Japan (42–53). The Alberta prairie region is the center of Canadian sugar beet production and has the highest prevalence of MS in Canada (46, 53). Tokachi province has the highest rate of MS reported to date in Asians (47). Although it constitutes only 0.3% of the population of Japan, it produces 45% of Japan's sugar beets (54).

THE HISTORY OF MS AND THE AZE HYPOTHESIS

Multiple sclerosis may be a relatively new disease (55). Its beginnings in medicine are French. Jean Cruveilhier may

have recorded the first description of its pathology in 1835, and Jean-Martin Charcot provided the first account of its clinical features in 1868 (55). There seems to have been a progressive increase in its incidence during the late 19th and early 20th centuries. The extent to which the rising occurrence was real or was the result of improving medical sophistication is not known. In any case, there is a close correspondence in time (i.e. mid-19th century) and in geographic locale (northern France) between the growing recognition of MS and the sudden entry of unprecedented amounts of Aze in food.

DISEASE AMONG MIGRANTS

In addition to earlier well-known studies of the incidence of MS among migratory populations, a detailed epidemiological study has recently documented that an abrupt increase in incidence and prevalence of MS in the French West Indies during the late 1990s was attributable to the occurrence of the disease among returning West Indian natives who had emigrated to northern France for economic reasons during the 1950s and 1960s (56). The risk of developing the disease was highest for those who had moved to France before the age of 15 years. The mean duration of stay was 12.3 years. The mean interval between arrival in France and the development of the clinical disorder was 19.1 years (56). These features suggest that a slowly acting agent, present in the environment in northern France, plays a role in disease pathogenesis, especially during the period of CNS myelination. They are consistent with the Aze hypothesis, which proposes that the misincorporation of the nonprotein amino acid, a stochastic process, reaches a critical level and is followed by inflammation and the ignition of an autoimmune response. The chronology and geography of MS among the French West Indian migrants echo the chronology and geography of the first cases recognized in Europe more than a century earlier (55).

SWAYBACK AND THE AZE HYPOTHESIS

A natural experiment relevant to Aze substitution for proline took place in Alberta in 1972 (57). An outbreak of enzootic ataxia (swayback) occurred in a flock of newborn lambs; it killed more than 60 of 100 animals (57). The manifestations included prostration, head shaking, ataxia, trembling, swaying of the hind quarters, and collapse of the posterior trunk. Studies of the pathology of enzootic ataxia (Fig. 2) revealed widespread white matter damage resembling that seen in MS lesions (58–60). A salient observation about the Alberta outbreak led to the report that during pregnancy, lambing, and lactation, the ewes and the offspring had been fed an unusual diet containing large amounts of sugar beet silage (57).

Swayback has been attributed to viruses, copper deficiency, and autoimmune responses to myelin degeneration, but none of these proposals have been validated (61). Diseases resembling swayback have been reported in cattle, goats, and in various forms of wildlife, especially red deer; it is not clear whether swayback and enzootic ataxia should be considered the same disease (62, 63). In any case, swayback shows features of CNS myelin breakdown with parallels to MS in humans. Moreover, the studies of an outbreak indicate that the consumption of large amounts sugar beets early in life can be associated with a neurological disease and pathological alterations of CNS myelin.

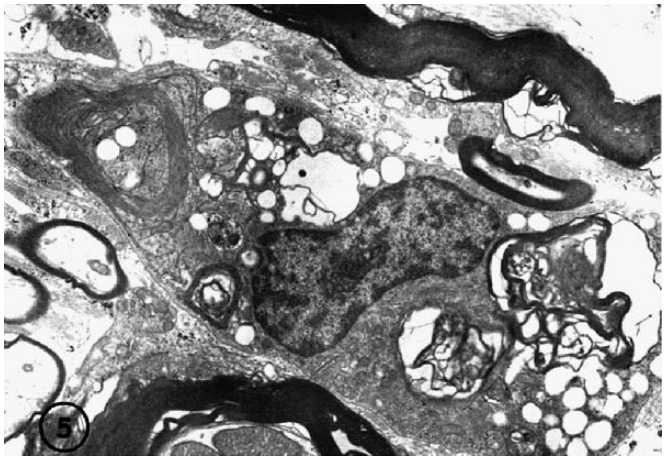

FIGURE 2. Electron micrograph demonstrating myelin breakdown and phagocytosis in the spinal cord of a lamb with swayback. The phagocytosed degenerating myelin at the upper left has irregular widened myelin lamellae. The myelin at the right shows greater degeneration and digestion. Intact and dense compact myelin is also seen in the upper and left lower portions of the field. Original magnification: 8,000×. Figure reproduced with permission from Springer (58).

OTHER NEUROPROTEINS RENDERED DYSFUNCTIONAL BY AZE

The hypothesis that Aze misincorporation in MBP may play a role in the pathogenesis of MS is supported by evidence that Aze-related misassembly exerts deleterious effects on other neuroproteins, including those involved in the gating of ion channels, the regulation of the hypoxia-inducible factor (HIF), and in embryogenesis. In investigations of ion channels and of HIF, Aze has been deliberately substituted for proline to demonstrate that a specific proline residue is critical to the function under study.

Cis-trans isomerization of a prolyl residue located between ion channel helical components is responsible for the opening and closing of the pore of a neurotransmitter gated cation-selective channel belonging to the Cys-loop receptor superfamily (64). Substitution of Aze for the proline interferes with normal regulation of the pore size and results in dysfunctional channels (64–66).

Another example of neuroproteins that are rendered dysfunctional by Aze misincorporation is the complex that constitutes HIF in which prolyl residues play a critical role in the protein functions (67). An entire repertoire of interacting molecules is involved in the tightly coordinated activities of the HIF complex that can be assigned to 3 general functions: 1) hypoxia sensing; 2) upregulation of hypoxia-correcting DNA-coding sequences, including the transcription of proteins involved in the production of endothelial growth

factor, erythropoietin, and the cascade of enzymes involved in glycolysis; and 3) the prompt proteolytic destruction of key components of the system after amelioration of local hypoxia. The HIF subunits that respond to hypoxia do so as a result of enzymatic hydroxylation of specific proline residues, and replacement of the prolyls by Aze markedly impairs this effect (68). Similarly, substitution of Aze for proline interferes with the degradation of the complex under normal oxygen conditions (68).

Hydroxylation of prolyl residues also regulates interactions with the von Hippel–Lindau suppressor (pVHL), the component of the ubiquitin-ligase complex that designates HIF subunits for proteosomal destruction. Among the numerous neurological disorders associated with the von Hippel–Lindau syndrome are angioblastic lesions, visual loss, and various neoplasms (69).

The collagens are long thin molecules composed of 3 helical chains that wrap around each other in a braided fashion (70). Proline constitutes about 15% of the amino acid residues. Because of the abundance of prolyls in collagen and their critical role in its conformation, collagen molecules are highly vulnerable to the effects of the misincorporation of Aze. This is especially true during the time span of embryonic biosynthesis and growth into adulthood. Numerous studies have demonstrated that experimental exposure to Aze causes severe impairment of collagen formation in chicks, mice, pigs, and human cells in vitro (2); these could result in a wide range of teratogenic abnormalities, including impaired formation of the neural crest and abnormal development of the CNS (2, 71–75). To date, however, there have been no reports of studies to determine the presence of Aze in naturally occurring tissues.

Beyond its deleterious effects on specific molecules, Aze misincorporation may add to the cellular burden of misfolded proteins; neurons appear to be especially vulnerable to such stress (76).

FUTURE STUDIES

The possible role of proline replacement by Aze in MS and in other diseases is hypothetical and based on circumstantial evidence. Further data are needed, such as the amount of Aze in meat, in dairy products, and other derivative foods as well as in untested vegetation; the antigenicity of Aze-substituted putative epitopes; the presence of Aze in MBP and other proteins derived from early MS lesions; the effects of Aze feeding in animal models, including lambs; and possibly, clinical trials of the effects of eliminating Aze from the diet and the use of proline washouts in susceptible individuals.

The many unanswered questions underscore the necessity for restraint in arriving at conclusions about the possible emergence of Aze as a cause of disease in humans and other animals. They suggest the need for research on many aspects and, in particular, argue for caution against premature conclusions about dietary hazards. Azetidine-2-carboxylic acid evolved as a weapon in the harsh struggle for survival among plants, their competitors, and their predators. That struggle is ongoing.

REFERENCES

1. Rosenthal GA, Bell EA. Naturally occurring toxic protein amino acids. In: Rosenthal GA, Janzen DH, eds. *Herbivores: Their Interaction With Secondary Plant Metabolites*. New York, NY: Academic Press, 1979: 361–63
2. Rubenstein E. Biologic effects of and clinical disorders caused by nonprotein amino acids. Medicine (Baltimore). 2000;79:80–89
3. Hartman MC, Josephson K, Lin CW, Szostak JW. An expanded set of amino acid analogs for the ribosomal translation of unnatural peptides. PloS ONE 2007;3:e972
4. Cox PA, Banack SA, Murch SJ. Biomagnification of cyanobacterial neurotoxins and neurodegenerative disease among the Chamorro people of Guam. Proc Natl Acad Sci U S A 2003;100:13380–83
5. Rubenstein E, Zhou H, Krasinska KM, et al. Azetidine-2-carboxylic acid in garden beets (*Beta vulgaris*). Phytochemistry 2006;67:898–903
6. MacArthur MW, Thornton JM. Influence of proline residues on protein conformation. J Mol Biol 1991;218:397–412
7. Pielak GJ. Woes of proline: A cautionary tale. Protein Sci. 2006;15:393–94
8. Tsai FH, Overberger CG, Zand R. Synthesis and peptide bond orientation in tetrapeptides containing L-azetidine-2-carboxylic acid and L-proline. Biopolymers 1990;30:1039–49
9. Baeza JL, Gerona-Navarro G, Vega MJ, et al. Azetidine-derived amino acids versus proline derivatives. Alternative trends in reverse turn induction. J Org Chem 2008;73:1704–15
10. Stryer L. *Biochemistry*. New York, NY: W. H. Freeman and Co, 1995
11. Fowden L. Nonprotein amino acids. In: Conn EE, ed. *The Biochemistry of Plants*. New York, NY: Academic Press, 1981
12. Fowden L. Azetidine-2-carboxylic acid: A new cyclic amino acid occurring in plants. Biochem J 1956;64:323–32
13. Couty F, Evano G. Azetidine-2-carboxylic acid, from Lily of the Valley to key pharmaceuticals. A jubilee review. Org Prep Proced Int 2006;38: 427–65
14. Stanton TL. *Feed Composition of Cattle and Sheep. Livestock Series Management*. Fort Collins, CO: Colorado State University, Number 1615, 1999
15. Harland JJ, Jones CK, Hufford C. Co-products. In: Draycott AP. ed. *Sugar Beet*. Oxford, United Kingdom: Blackwell Publishing, Ltd, 2006
16. Francis SA. Development of sugar beet. In: Draycott AP. ed. *Sugar Beet*. Oxford, United Kingdom: Blackwell Publishing, Ltd, 2006
17. Central Intelligence Ageny. *The World Factbook, Field Listing, Agriculture Products*, Washington DC: Central Intelligence Agency, 2006
18. Boggs JM. Myelin basic protein: A multifunctional protein. Cell Mol Life Sci 2006;63:1945–61
19. Sobel RA, Tuohy VK, Lu ZA, et al. Acute experimental allergic encephalomyelitis in SJL/J mice induced by a synthetic peptide of myelin proteolipid protein. J Neuropathol Exp Neurol 1990;49:468–79
20. Fraser PE, Deber CM. Structure and function of the proline-rich region of myelin basic protein. Biochemistry 1985;245:4593–98
21. Ridsdale RA, Beniac DR, Tompkins TA, et al. Three-dimensional structure of myelin basic protein. II. Molecular modeling and considerations of predicted structures in multiple sclerosis. J Biol Chem 1997;272: 4269–75
22. Bates IR, Feix JB, Boggs JM, et al. An immunodominant epitope of myelin basic protein is an amphipathic α-helix. J Biol Chem 2004;279: 5757–64
23. Nye SH, Pelfrey CM, Burkwit JJ, et al. Purification of immunologically active recombinant 21.5 kDa isoform of human myelin basic protein. Mol Immunol 1995;32:1131–41
24. Eylar RH, Brostoff S, Hashim G, et al. Basic A1 protein of the myelin membrane. The complete amino acid sequence. J Biol Chem 1971;246: 5770–84
25. Carnegie PR. Amino acid sequence of the encephalitogenic basic protein from human myelin. Biochem J 1971;123:57–67
26. Hauser SL, Waubant E, Arnold DL, et al. B-cell depletion with rituximab in relapsing-remitting multiple sclerosis. N Engl J Med 2008;358: 676–88
27. Boggs JM, Rangaraj G, Gao W, et al. Effect of phosphorylation of myelin basic protein by MAPK on its interactions with actin and actin binding to a lipid membrane in vitro. Biochemistry 2006;45:391–401
28. Musse AA, Boggs JM, Haraux G. Deimination of membrane-bound

myelin basic protein in multiple sclerosis exposes an immunodominant epitope. Proc Natl Acad Sci U S A 2006;103:4422–27
29. Musse AA, Harauz G. Molecular "negativity" may underlie multiple sclerosis: Role of the myelin basic protein family in the pathogenesis of MS. Int Rev Neurobiol 2007;79:149–72
30. Mathey EK, Derfuss T, Storch MK, et al. Neurofascin as a novel target for autoantibody-mediated axonal injury. J Exp Med 2007;204:2362–72
31. Hedstrom KL, Xu X, Ogawa Y, et al. Neurofascin assembles a specialized extracellular matrix at the axon initial segment. Cell Biol 2007; 178:875–76
32. Howell OW, Palser A, Polito A, et al. Disruption of neurofascin localization reveals early changes preceding demyelination and remyelination in multiple sclerosis. Brain 2006;129:3173–85
33. Coman I, Aigrot MS, Seilhean D, et al. Nodal, paranodal and juxtaparanodal axonal proteins during demyelination and remyelination in multiple sclerosis. Brain 2006;129:3186–95
34. Dzhashiashvili Y, Zhang Y, Galinska J, et al. Nodes of Ranvier and axon initial segments are ankyrin G-dependent domains that assemble by distinct mechanism. J Cell Biol 2007;177:857–70
35. Schafer DP, Rasband MN. Glial regulation of the axonal membrane at nodes of Ranvier. Curr Opin Neurobiol 2006;16:508–14
36. Yang Y, Ogawa Y, Hedstrom KL, et al. Beta IV spectrin is recruited to axon initial segments and nodes of Ranvier by ankyrin G. J Cell Biol 2007;176:509–19
37. Solomaha E, Szeto FL, Yousef MA, et al. Kinetics of Src homology 3 domain association with the proline-rich domain of dynamins: Specificity, occlusion, and the effects of phosphorylation. J Biol Chem 2005; 280:23147–56
38. Mantzourani ED, Blokar K, Tselios TV, et al. A combined NMR and molecular dynamics simulation study to determine the conformational properties of agonists and antagonists against experimental autoimmune encephalomyelitis. Bioorg Med Chem 2008;16:2171–82
39. International Multiple Sclerosis Genetics Consortium. Risk alleles for multiple sclerosis identified by a genomewide study. N Engl J Med 2007;357:851–62
40. Kennedy J, O'Connor P, Sadovnick AD, et al. Age of onset of multiple sclerosis may be influenced by place of residence during childhood rather than ancestry. Neuroepidemiology 2006;26:162–67
41. Pugliatti M, Sotgiu S, Solinas G, et al. Multiple sclerosis prevalence among Sardinians: Further evidence against the latitude gradient theory. Neurol Sci 2002;22:163–65
42. Poskanzer DC, Prenney LB, Sheridan JL, et al. Multiple sclerosis in the Orkney and Shetland Islands. I: Epidemiology, clinical factors, and methodology. J Epidemiol Community Health 1980;34:229–39
43. Karni A, Kahana E, Zilber N, et al. The frequency of multiple sclerosis in Jewish and Arab populations in Greater Jerusalem. Neuroepidemiology 2003;22:82–86
44. Sarasoja T, Wikstrom J, Paltamaa J, et al. Occurrence of multiple sclerosis in central Finland: A regional and temporal comparison during 30 years. Acta Neurol Scand 2004;110:331–36
45. Kurtzke JF, Heltberg A. Multiple sclerosis in the Faroe Islands: An epitome. J Clin Epidemiol 2001;54:1–22
46. Beck CA, Metz LM, Svenson LW, et al. Regional variation of multiple sclerosis prevalence in Canada. Mult Scler 2005;11:516–19
47. Houzen H, Niino M, Kikuchi S, et al. The prevalence and clinical expression of MS in northern Japan. J Neurol Sci 2003;211:49–53
48. http://www.docushare.it/mediasoft/italy/sardegna/sardegna_en.htm. Accessed March 1, 2006
49. http://www.newadvent.org/cathen/11316c.htm. Accessed February 21, 2006
50. http://www.mfa.gov.il/MFA/MFAArchive/2000_2009/2001/9/Flora%20and%20Fauna%2. Accessed March 16, 2006
51. http://www.gastrolab.net/c1gfinle.htm. Accessed March 1, 2006
52. http://go.hrw.com/atlas/norm_htm/sweden.htm. Accessed March 15, 2006
53. http://www.agr.gc.ca/misb/spec/index_e.php?s1=beta&page&intro. Accessed March 8, 2006
54. Statistics Bureau Ministry of Internal Affairs and Communications. *Japan Statistical Yearbook*, Japan, 2006:242
55. Talley CL. The emergence of multiple sclerosis, 1870–1950: A puzzle of historical epidemiology. Perspect Biol Med 2005;48:383–95
56. Cabre P, Signate A, Olindo S, et al. Role of return migration in the emergence of multiple sclerosis in the French West Indies. Brain 2005; 128:2899–910
57. Chalmers GA. Swayback (enzootic ataxia) in Alberta lambs. Can J Com Med 1974;38:111–17
58. Cancilla PA, Barlow RM. Structural changes of the central nervous system in swayback (enzootic ataxia) of lambs. IV. Electron microscopy of the white matter of the spinal cord. Acta Neuropathol (Berl) 1968;11: 294–300
59. Cancilla PA, Barlow RM. Structural changes of the central nervous system in swayback (enzootic ataxia) of lambs. V. Electron microscopic observations of the corpus callosum. Acta Neuropathol (Berl) 1969;12: 307–13
60. McC Howell J. Observations on the lesions in the white matter of the spinal cord of swayback sheep. Acta Neuropathol (Berl) 1969;12: 33–44
61. Symonds CP. Multiple sclerosis and the swayback story. Lancet 1975;1: 155–56
62. Cordy DR, Knight HD. California goats with disease resembling enzootic ataxia of swayback. Vet Pathol 1978;15:179–85
63. Richards RB, Edwards JR. A progressive spinal myelinopathy in beef cattle. Vet Pathol 1986;23:35–41
64. Lummis SC, Beene DL, Lee LW, et al. Cis-trans isomerization at a proline opens the pore of a neuro-transmitter-gated ion channel. Nature 2005;438:248–52
65. Andreotti AH. Opening the pore hinges on proline. Nat Chem Biol 2006; 2:13–14
66. Labro AJ, Grottesi A, Sansom MS, et al. A Kv channel with altered activation gate sequence displays both "fast" and "slow" activation kinetics. Am J Physiol Cell Physiol 2008;294:C1476–84
67. Schofield CJ, Ratcliffe PJ. Oxygen sensing by HIF hydroxylases. Nat Rev Mol Cell Biol 2004;5:343–54
68. Li D, Hirsilä M, Koivunen P, et al. Many amino acid substitutions in hypoxia-inducible transcription factor (HIF)-1α–like peptide cause only minor changes in its hydroxylation by the HIF prolyl 4-hydroxylases. Substitution of 3,4 dehydroproline or azetidine-2-carboxylic acid for the proline leads to a high rate of uncoupled 2-oxoglutarate decarboxylation. J Biol Chem 2004;279:55051–59
69. OMIM, Online Mendelian Inheritance in Man. Johns Hopkins University, Baltimore, MD, MIM Number 19330, 4/14/2008
70. Okuyama K, Okuyama K, Arnott S, et al. Crystal and molecular structure of a collagen-like polypeptide (Pro-Pro-Gly)10. J Mol Biol 1981; 152:427–32
71. Sanders EF, Pradad S, Cheung E. Extracellular matrix synthesis is required for the movement of sclerotome and neural crest cells on collagen. Differentiation 1988;39:34–41
72. Bradamante Z, Hall BK. The role of epithelial collagen and proteolgycan in the initiation of osteogenesis by avian neural crest cells. Anat Rec 1980;197:305–15
73. Bilozur ME, Hay ED. Neural crest migration in 3D extracellular matrix utilizes laminin, fibronectin, or collagen. Dev Biol 1988;125:19–33
74. Perris K, Kuo HJ, Glanville RW, Bronner-Fraser M. Collagen type VI in neural crest development: Distribution in situ and interaction with cells in vitro. Dev Dyn 1993;198:135–49
75. Loring JF, Erickson CA. Neural crest migratory pathways in the trunk of the chick embryo. Dev Biol 1987;121:220–36
76. Drummond DA, Wilke CO. Mistranslation-induced protein misfolding as a dominant constraint on coding-sequence evolution. Cell 2008;134: 341–52