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Autism and *Clostridium tetani*

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Abstract — Autism is a severe developmental disability believed to have multiple etiologies. This paper outlines the possibility of a subacute, chronic tetanus infection of the intestinal tract as the underlying cause for symptoms of autism observed in some individuals.

A significant percentage of individuals with autism have a history of extensive antibiotic use. Oral antibiotics significantly disrupt protective intestinal microbiota, creating a favorable environment for colonization by opportunistic pathogens. *Clostridium tetani* is an ubiquitous anaerobic bacillus that produces a potent neurotoxin. Intestinal colonization by *C. tetani*, and subsequent neurotoxin release, have been demonstrated in laboratory animals which were fed vegetative cells. The vagus nerve is capable of transporting tetanus neurotoxin (TeNT) and provides a route of ascent from the intestinal tract to the CNS. This route bypasses TeNT's normal preferential binding sites in the spinal cord, and therefore the symptoms of a typical tetanus infection are not evident. Once in the brain, TeNT disrupts the release of neurotransmitters by the proteolytic cleavage of synaptobrevin, a synaptic vesicle membrane protein. This inhibition of neurotransmitter release would explain a wide variety of behavioral deficits apparent in autism. Lab animals injected in the brain with TeNT have exhibited many of these behaviors. Some children with autism have also shown a significant reduction in stereotyped behaviors when treated with antimicrobials effective against intestinal clostridia.

When viewed as sequelae to a subacute, chronic tetanus infection, many of the puzzling abnormalities of autism have a logical basis. A review of atypical tetanus cases, and strategies to test the validity of this paper's hypothesis, are included.

Introduction

Over five decades have passed since Dr Leo Kanner published his initial study on a rare and mysterious developmental disability which he called autism (1). Today, autism is still viewed by professionals as a complex medical and psychological puzzle. However, autism is no longer considered rare and is now the third most commonly diagnosed childhood developmental disorder (2). In April of 1995, the National Institutes of Health held a conference on autism, during which a Duke University researcher who has studied the epidemiology of autism stated the rate of autistic spectrum disorder as being in excess of 22/10 000 (3). A preliminary estimate of the cost of autism has just been completed, and though this study assumed a prevalence rate of only 15/10 000, the estimated cost was $13.3 billion per year. This figure reflects the direct and indirect economic burdens placed on society, but does not include costs of insurance, early intervention, special education or out-of-pocket expenses borne by families with autistic

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children (2). These enormous costs indicate the necessity for aggressive research.

Autism is a pervasive developmental disorder (PDD), affecting boys four times more often than girls, and the symptoms may become evident in infancy or early childhood. Frequently, parents report that their children appear to be developing normally, before experiencing a serious regression during the toddler or early preschool years (4,5). Autism is the result of a neurological disorder that affects functioning of the brain. The diagnosis of autism is given when a child exhibits significant deficits in three crucial areas of development: communication, social interaction, and behavior. Some of the most frequently observed symptoms are (6,7):

- limited or no eye contact
- apparent deafness
- difficulty playing with other children
- sustained inappropriate, repetitious, or odd play
- communication disorders.

The unusual behaviors of children with autism have received more focus than the abnormal medical findings. Many physicians are completely unaware of the numerous biological abnormalities consistently found in significant numbers of individuals diagnosed as autistic (6,8–22):

- elevated organic acid metabolites in urine
- low phenol-sulphotransferase-P enzyme
- elevated glial fibrillary acidic protein
- elevated catecholamines in serum and urine
- loss of Purkinje cells in the cerebellar cortex
- seizures and EEG abnormalities
- increased frequency of C4B null allele
- serum deficiency of C4B protein
- increased incidence of ear infections.

Parental observations that have been mentioned in medical literature but are not as extensively explored and documented include (23,24):

- abnormal stools — loose, visible mucous, bulky, odorous, etc.
- very limited diet, difficulty chewing, strong gag reflex, orally defensive
- sensitivity to many foods
- hyper/hypotonic muscle tone
- tactile system hypersensitivity
- significant problems with sensory integration
- pale appearance
- sleeping disorders — frequent awakenings and limited sleep requirements.

According to Dr Kanner, autism was 'evident from birth'. Today, a well-established period of normal development does not preclude a diagnosis of autism (4,5). In many cases, the parents of children with late-onset autism report that their child's regression was preceded by the use of multiple rounds of broad-spectrum antibiotics (25).

Hypothesis regarding pathogenesis in a subgroup of autistics

This paper's hypothesis is that a subgroup of children diagnosed with autism are suffering from *Clostridium tetani* colonization of the intestinal tract and that the neurological symptoms are the direct result of in-vivo production of tetanus neurotoxin (TeNT). Indigenous organisms that reside in a healthy intestinal tract, known as microflora or microbiota, provide an effective barrier against colonization by opportunistic pathogens. This protective microbiota is severely disrupted when multiple rounds of broad-spectrum antibiotics are administered during early childhood (26–28). The child, while in this vulnerable condition, is exposed to *C. tetani* spores or vegetative cells. *Clostridium tetani* is an obligatory anaerobic, spore-producing bacillus that is widely distributed in soil around the world. Toxigenic strains produce TeNT, which is one of the most potent neurotoxins known to man. Given the ubiquitous nature of *C. tetani*, there is no doubt that spores or vegetative cells frequently enter the body from the environment, but usually fall prey to the body's natural defenses, such as microbiota, before germination or colonization can take place (29,30).

Could *C. tetani* and TeNT be responsible for the behavioral and medical symptoms present in a subroup of individuals diagnosed with autism? Although it is a new idea that *C. tetani* could be causally related to the onset of autism, there has been previous speculation regarding the possibility of clostridia in general as an unrecognized cause of various diseases. This is evidenced by a quote from Borriello: 'What is more of a surprise than the fact that clostridia cause gut disease is that they do not cause more infections, especially because *Clostridium* species constitute one of the most potent collections of toxigenic bacteria in existence. It may well be that they do cause more infections but that these infections have gone undetected' (26).

Defining the subgroup

Autism is currently subgrouped on the basis of behavioral symptoms and cognitive abilities (31). Many children diagnosed with autism do not undergo any metabolic testing. The individuals with autism that would best fit the subgroup of this paper's hypothesis would meet the following criteria:

1. history of chronic infection treated with antibiotics: 67% probability (32);
2. elevated levels of phenolic metabolites present in urine: 65% probability (33);
3. history of persistent loose bowel movements (25).

The percentages stated above are based on small studies and are offered as an approximation of the frequency of these abnormalities within the autistic population. An estimation of the combined occurrence of the above criteria has not been established. Behavioral symptoms have no bearing on this kind of subgrouping and, theoretically, could vary greatly between individuals depending on the severity of infection and quantity of neurotoxin affecting the CNS.

Effect of antibiotics on the intestinal tract

A high rate of frequent and prolonged ear infections in individuals with autism is a well-established fact (21,22). Ear infections in children are typically treated with a course of oral antibiotics. Antibiotics do not discriminate between harmful and helpful bacteria that are in the body. Therefore, an antibiotic taken to kill bacteria that is causing an infection will also be severely disruptive to the intestinal tract's protective microbiota, which provide a necessary and effective barrier against colonization from opportunistic pathogens (27,28). Direct evidence has been established that the use of oral antibiotics greatly increases the risk of invasion of the intestinal tract by pathogenic bacteria, especially opportunistic *Clostridium difficile* (26–28,34–38). Furthermore, the number of organisms required to produce disease is significantly reduced when the intestinal microbiota have been disrupted by oral antibiotics (38,39). For example, a healthy host with intact microbiota requires between one hundred thousand and ten million *Salmonella* organisms before the body's defenses are unable to prevent infection (40,41). In contrast, an intestinal tract disrupted by antibiotics can succumb to infection when exposed to less than fifty similar organisms (38,39). In one instance, thousands of people were exposed to *Salmonella newport* from ingestion of contaminated meat. Of those exposed, only 18 individuals, 12 of whom were currently or recently taking antibiotics, developed clinical symptoms of the disease (42). In young children, colonization by opportunistic pathogens can occur even in the absence of antibiotic use, because of the immaturity of gastrointestinal host defense mechanisms (37). Infant botulism is a serious illness that occurs when a young child's intestinal tract becomes colonized by *Clostridium botulinum* (43,44). The symptoms of the disease are directly related to the production of botulinum neurotoxin (BoNT) within the child's intestinal tract. However, after the first year of life, colonization of the intestinal tract by *C. botulinum* is rarely reported (44). When the combined risk factors of youth and antibiotic use are considered, it becomes evident that a young child taking an oral antibiotic is susceptible to pathogenic colonizations of his or her intestinal tract. It is also important to note that intestinal microbiota can be severely disrupted by diarrhea even in the absence of antibiotics (36). Such disruption could conceivably be enough to allow the entrance of opportunistic pathogens in such cases.

An assessment of other known clostridial infections of the gut can provide some insight into the possibility of *C. tetani* colonization. For example, clear evidence now exists for a causal relationship between antibiotic use and pseudomembranous colitis caused by *C. difficile*; this is sometimes referred to as antibiotic-associated pseudomembranous colitis (26,34,35). *Clostridium difficile* produces two exotoxins: toxin A, which is classified as an enterotoxin, and toxin B, which is classified as cytotoxic. Acting together, these toxins damage intestinal mucosa and cells and result in watery diarrhea, which is the primary clinical symptom of *C. difficile* infection (26,34,35). *Clostridium difficile* can lay dormant in its spore form for extended periods of time and can proliferate when a person's resident microbiota is disrupted by oral antibiotics. There are important similarities between *C. difficile* and *C. tetani*. Both are opportunistic pathogens that can lay dormant in spore form for long periods of time (35,45). Both species produce cytotoxins known to cause cell damage (46). Additionally, both *C. tetani* and *C. difficile* produce phenolic metabolites (36,47). Several significant differences should also be considered. *Clostridium tetani* does not produce an enterotoxin; therefore, watery diarrhea would not be a likely symptom of a colonized intestinal tract. *Clostridium difficile* does not produce indole (48), whereas *C. tetani* does; although production of indole is variable (29). Also quite significant is that toxigenic strains of *C. tetani* produce an extremely potent neurotoxin, whereas *C. difficile* does not.

Colonization in the intestines

In lab animals, colonization of the intestinal tract by pathogenic clostridia has been accomplished by preventing or disrupting (with antibiotics) the development of protective microbiota, followed by subsequent feeding with vegetative bacillus or spores (49,50). Continued life and apparent good health was observed in rats whose intestinal tracts had been

heavily colonized by *C. tetani*. Upon necropsy, biologically active TeNT and *C. tetani* spores were detected in the rats' cecum and colon. The amount of TeNT detected in the colon was significantly less than the amount detected in the cecum, which would indicate the toxin was either broken down by proteolytic enzymes and/or absorbed. The sera of the rats contained antibodies to TeNT in an amount greater than the established protective titer level of 0.01 IU/ml (50,51). This finding contradicts the commonly held belief that a tetanus infection does not produce enough neurotoxin to stimulate an immune response. Furthermore, the rats did not exhibit the clinical symptoms of tetanus; thus demonstrating that *C. tetani* can colonize a compromised intestinal tract and that colonization will not produce typical symptoms of tetanus infection despite the presence of biologically active TeNT. Additionally, a protectively high titer against the neurotoxin does not rule out the presence of the bacteria (50). A young child who is currently taking oral antibiotics has an increased risk of colonization of the intestinal tract by opportunistic pathogens. The accidental oral ingestion of *C. tetani* spores and/or vegetative cells can establish colonies in the intestinal tract of a weakened host (50). The circumstances for such ingestion could be as simple as mouthing a dirty finger or toy.

Although oral ingestion of *C. tetani* is the most obvious means by which a tetanus infection could develop in a compromised intestinal tract, other possible routes of infection should not be completely ruled out. Numerous experiments involving laboratory animals, and the clinical observations of wounded men, have shown that tetanus spores and vegetative bacillus are capable of creating infection at sites quite distant from the bacteria's point of entry into the body (45,52). Spores can remain dormant and escape phagocytosis for years and then germinate when growth conditions become favorable. Intramuscular or subcutaneous injections of washed *C. tetani* spores can germinate weeks or months later in bruised or irritated tissue (45,52). Young children are particularly prone to injury, and any break in the skin is an opportunity for *C. tetani* spores to enter the body. Additionally, natural openings in the body can also serve as points of entry. An example of this is otogenous tetanus, which is believed to occur as a secondary infection when purulent ear discharge is present and *C. tetani* is introduced into the ear via a dirty finger or cloth (53). In view of the known capabilities of these spores, it is conceivable that an ulcerated intestinal tract could become a favorable site for the germination and growth of spores traveling from the point of injury, even months after their initial entry into the body.

In light of published studies, the following points should be remembered: (i) *Clostridium tetani* spores or vegetative cells can travel from their point of entry into the body to find tissue capable of supporting germination, growth, and subsequent neurotoxin production (45,50,52). (ii) Toxigenic strains of *C. tetani* can colonize a compromised intestinal tract without inciting the typical symptoms of tetanus infection (50). (iii) Antibodies produced by the body against the neurotoxin are not effective against the bacterium and do not protect the intestinal tract from *C. tetani* colonization (50).

The primary danger of a tetanus infection is from TeNT. However, the action of tetanolysin, an oxygen-labile hemolysin produced by *C. tetani*, should not be overlooked. The role of tetanolysin in a tetanus infection remains unclear. However, it is thought to promote the growth of *C. tetani* by damaging otherwise healthy tissue in the vicinity of the infection (54). Tetanolysin lyses platelets, erythrocytes, and lysosomes, and is believed to directly damage membrane lipids (55–57). The possibility exists that tetanolysin's action in the intestinal tract could increase intestinal permeability in a manner akin to that of other bacteria. When intestinal permeability is increased, food molecules larger than those which would normally be absorbed from the intestinal tract enter the blood stream and can become antigenic (58). Children with autism frequently develop intolerance to foods containing gluten or casein proteins. Behavioral improvements and a reduction in autistic symptoms frequently follow the removal of foods containing gluten or casein proteins (59).

Tetanus is not normally thought of as an intestinal tract infection, and therefore, exactly what happens to TeNT when it is exposed to the proteolytic enzymes in the human digestive tract (see Table) is not known. However, in laboratory experiments, TeNT has been shown to undergo limited proteolysis when exposed to the proteases trypsin, clostripain, endoproteinase Arg-C, chymotrypsin, endoproteinase Glu-C, and papain (60). The isolated TeNT fragments are at least a thousand times less toxic than the native di-chain toxin, which can be lethal even in very small doses (60). Nonetheless, with every enzyme tested, the limited proteolysis of TeNT produced TeNT fragments which retained the ability to inhibit the release of neurotransmitters under laboratory testing conditions (60). It is not unreasonable to assume, therefore, that TeNT produced in the intestinal tract as a result of *C. tetani* colonization would be similarly reduced to TeNT fragments and that these fragments would retain the ability to inhibit neurotransmitter release even at such a reduced potency.

Additional studies conducted on papain-digested TeNT show that it retains the ability to form channels

Table Proteases of the human digestive tract

Organ	Enzyme	Action
Stomach	Pepsin	Proteins → polypeptides
Pancreas	Trypsin	Proteins, polypeptides → polypeptides, dipeptides
	Chymotrypsin	Proteins, polypeptides → polypeptides, dipeptides
	Carboxypeptidase	Polypeptides → simpler peptides, dipeptides, amino acids
Intestines	Aminopeptidase	Polypeptides → peptides, dipeptides, amino acids
	Dipeptidase	Dipeptides → amino acids

in membranes but is no longer capable of producing the spastic paralysis associated with tetanus (61–63). This difference was attributed to the ability of TeNT fragments to block both excitatory and inhibitory neurotransmitters at approximately the same rate (61,62). During a typical tetanus infection, TeNT that has not been reduced to fragments by proteolysis preferentially blocks the release of inhibitory neurotransmitters from synaptic vesicles (54). This results in a reduction of inhibitory neurotransmitter signaling to motor neurons. This preferential blockage causes an imbalance between inhibitory and excitatory signaling to motor neurons. Consequently, the normal signal from inhibitory neurotransmitters during voluntary muscle movements and the signal to terminate reflexive contractions are both diminished. This results in muscle spasms, the most widely recognized symptom of tetanus infection (54).

Although many of the proteases used in these studies were different from those found in the human intestinal tract, others were identical. In light of this, it appears quite possible that TeNT from an ongoing intestinal infection could result in large quantities of digested toxin that would neither prove fatal to the host nor cause muscle spasms, but nonetheless would create an ongoing state of neurotransmitter disruption.

Tetanus within the central nervous system

Before behavior can be affected, TeNT must gain access to the central nervous system (CNS). Usually, TeNT reaches the CNS by retrograde intra-axonal transport (64,65). From the intestinal tract, the most direct path to the CNS is along the vagus nerve. When iodine-labeled TeNT is injected subperitoneally into the anterior wall of the stomach, TeNT's ascent up the vagus nerve to the medulla oblongata can be traced (66). In one such experiment, the animals were injected with a massive amount of highly toxic di-chain TeNT, and at no time prior to death were the muscle spasms symptomatic of tetanus observed. The study showed conclusively that TeNT can ascend to the CNS via nerve parts other than skeletomotor or fusimotor axons, and that the absence of classical tetanus symptoms does not preclude the possibility of such ascent (66). The vagus nerve provides a route of access to neuronal targets within the CNS, but because the vagus does not intersect with the innervation of the skeletal muscular system, the muscle spasms normally associated with tetanus would not be evident, even though other, more subtle effects might occur. These findings document that TeNT can be present in the body in the absence of clinical symptoms associated with tetanus.

Very small amounts of TeNT injected directly into the brains of laboratory animals create a stereotyped behavior syndrome. Reported behaviors include intensive licking, sniffing, gnawing, and repetitious movements such as to-and-fro runs or walks (67–70). These same words could be used in describing autistic behaviors in humans. Again, as in the study involving subperitoneal injections, the severe muscle spasms and rigidity associated with tetanus were not typically present. Suppression of stereotyped behavior in these same animals was observed following treatment with lithium, diazepam, haloperidol, fenfluramine, and valproic acid (70), all of which are drugs that have been used with some success to reduce the behavioral symptoms associated with autism (6,71).

Tetanus neurotoxin is also used as a research aid for creating an animal model of epilepsy. The selective blockage of inhibitory neurotransmitters produces an excitatory focus resembling human limbic epilepsy (72). Rat pups subjected to unilateral intrahippocampal injections of TeNT developed seizures (73–75). The incident rate of seizure in the autistic population is decidedly higher than that found in the typical population, and this association is undisputable (5,6,18). The risk of developing epilepsy appears to increase throughout the entire life span of an individual with autism (17); however, it is unknown whether this is an actual risk or a reflection

of more accurate diagnosis. Changes in behavior, learning ability, and memory have been observed in rats for as long as a year after active seizures stopped (75,76). Furthermore, as observed in animals injected with TeNT, most children with autism also have considerable difficulty in the areas of learning ability and memory (77,78).

Synaptic vesicles

All higher brain functions depend on the release of neurotransmitters, which are stored and released from synaptic vesicles. Various molecules span the membranes of the synaptic vesicles and serve as signaling agents when appropriate messages are received. Subsequent to such signaling, neurotransmitters released from the synaptic vesicles traverse the extracellular space, and many bind to appropriate receptors on the receiving neuron (79). During the last several years, researchers using TeNT have uncovered a tremendous amount of information regarding mechanisms of neuroexocytosis as well as TeNT's negative effects upon neurotransmitter release (80,81).

Synaptobrevin is a membrane-spanning protein involved in signaling to small synaptic vesicles and thus plays a crucial role in neuroexocytosis (80). Numerous studies have confirmed that TeNT induces cleavage of membrane-spanning synaptobrevin molecules and thereby causes sustained inhibition of neurotransmitters from synaptic vesicles (64,81–85). In contrast to other bacterial toxins such as diphtheria, pertussis, shiga, or cholera toxins, TeNT is not classified as necrotizing. In other words, TeNT-affected neurons typically do not die; instead, the toxin causes paralysis and degeneration of intoxicated synapses in neurons that tend otherwise to remain intact (82). It is now well-documented that TeNT and other clostridial neurotoxins induce long-term and severe inhibition of neurotransmitter release (70,73,75,76). Disturbances in synaptic mechanisms are believed to be central to many neurological and psychiatric disorders (64,80), and various drugs that modulate neurotransmitter signaling in the brain are commonly used to treat symptoms of autism (71,86).

The cerebrospinal fluid (CSF) of individuals with autism was compared with that of normal controls for concentration of a nervous-system-specific protein marker, glial fibrillary acidic (GFA) protein (12). GFA protein is considered to be highly sensitive for confirming organic CNS destructive pathology and is elevated in both acute and chronic nerve cell damage (87). The GFA protein concentration of individuals with autism was found to be three times higher than the level present in normal controls (12). The study's authors suggested that elevated GFA could be the result of gliosis or an increased rate of synaptic degradation and production. Neuropathological studies of individuals with autism have found no evidence of gliosis (88), rather, an increased turnover rate of synapses has emerged as the most likely explanation for the extreme elevation of GFA protein (89). The cleavage of synaptobrevin by TeNT is irreversible and causes degeneration of the affected synapse (82). Recovery from known clostridial neurotoxins produced by *C. tetani* and *C. botulinum* requires the creation of new synapses; this takes from 2 to 4 months (64,83). The findings of elevated GFA protein in the CSF of individuals with autism, in the absence of gliosis, is consistent with what might be expected during a chronic tetanus infection, as a result of synaptic turnover. Furthermore, TeNT-cleaved synaptobrevins and a continuous turnover of synapses would be disruptive to neuron signaling pathways (79). This is particularly relevant in regard to young children where activity-dependent development of additional neuronal interactions is critical. The lack of stability in these connections would most likely have a detrimental effect on information processing and memory, which is a severe problem for most people with autism (77–79).

Autism neuroanatomy

Autopsy studies have revealed a loss of Purkinje cells and granule cell neurons in the cerebellar cortex of individuals with autism (13,14). Although retrograde neuronal loss was not observed in the inferior olivary nucleus, changes were observed in the afferent neurons that project to the area of Purkinje cell loss. In autopsies of younger persons with autism, the neurons were unusually large, while in older individuals the neurons were unusually small (13). The significance of this difference is not yet known; however, there are some striking similarities in the neuronal damage described in these autopsy studies and those observed in neurons intoxicated with TeNT.

Changes in cells intoxicated with TeNT include swelling of the cell body, shrinking of the cell processes, and neuronophagia. Occasionally the entire cell is destroyed and disappears, leaving an empty vacuolated area (90). For instance, loss of granule cells of the dentate gyrus was observed to occur in rats following injection of TeNT into the hippocampus (91). Although TeNT is not classified as necrotizing, neuronal degeneration occurs in the presence of high quantities of TeNT or after prolonged exposure. Neuronal degeneration associated with TeNT is also thought to occur as a result of the unopposed action of glutamate-mediated excitatory transmission (72,91).

A comparison of tetanus of the intestinal tract versus deep wound tetanus, shows major differences in the TeNT's distribution patterns. In a typical deep wound infection, *C. tetani* proliferates in the surrounding tissue. Tetanus neurotoxin produced at the site of infection is taken into nerve endings at the neuromuscular junction but seldom acts at this site. The toxin moves transsynaptically out of the motor neurons, through the synaptic cleft, and into contiguous synaptic terminals. Tetanus neurotoxin is delivered along neuronal pathways to the spinal cord by way of retrograde axonal transport and transsynaptic movement (92). The toxin is routed to the spinal cord by way of retrograde intra-axonal transport of skeletomotor neurons (54,82). Tetanus neurotoxin accumulates in the spinal cord's anterior horn cells, and neuronal changes (as described above) occur (54,90). In an intestinal tract tetanus infection, the classic transport of TeNT by skeletomotor neurons is absent. Instead, the most likely path of ascent from the abdomen is the vagus (tenth cranial) nerve. Afferent fibers of the vagus nerve have been found capable of transporting TeNT to the nucleus solitarius (66). TeNT ascending via the vagus nerve would be routed past the toxin's preferential binding site of the spinal cord's anterior horn cells (66), and the classic muscle spasms typically associated with tetanus would be absent. Afferent pathways between the nucleus solitarius and the cerebellum have been neuroanatomically traced (93), and the possible significance to autism is evident. Inhibitory neurons that release GABA and glycine are a preferential target for TeNT (85,91). Purkinje cells are inhibitory neurons that release GABA (85,91). Furthermore, binding receptors for TeNT are expressed on both Purkinje cells and granular cells of the cerebellum (94–96). It appears entirely possible, therefore, that the loss of Purkinje and granular cells, conclusively documented to occur in autism (13), could be the direct result of TeNT affecting these cerebellar neuronal targets. This anatomical targeting could occur when the toxin (ascending via the vagus nerve versus skeletomotor transport) is unable to directly access the anterior horn cells of the spinal cord (66).

Autism metabolites

The formation of both phenolic and indolic compounds by intestinal bacteria is a well-documented fact, and clostridia are recognized as being particularly active in fermenting amino acids (36,47). Aromatic amino acids are metabolized by *C. tetani*, and byproducts include phenol, phenol alcohol, and propionic acid (47,97). Phenolic compounds, such as the ones produced by *Clostridium* species, are toxic to the body. The enzyme phenol-sulphotransferase-P (PST-P) metabolizes phenols into nontoxic, water-soluble metabolites which can then be excreted from the body. Studies conducted primarily on children with late-onset autism have revealed a severe deficiency in PST-P enzyme and sulfate, which is the substrate required by PST-P enzyme (10,11). When colonic mucosa has been damaged, sulfation of phenols is impaired (36). Tetanolysin would likely cause damage to the colonic mucosa (57). An ongoing intestinal tetanus infection would produce significant quantities of phenolic metabolites that would require detoxification by the body (47,97). Potentially, this could result in increased circulating levels of phenolic metabolites when the body is unable to properly detoxify them because of enzyme and substrate deficiency.

Diagnostic urinalyses have detected the metabolite dihydroxyphenylpropionic acid (DHPPA) in grossly elevated quantities in a significant percentage of individuals with autism (9,33). Interestingly, this is not the first time elevated levels of phenolic metabolites have been implicated in a neurological disorder. Phenolic compounds have previously been implicated in schizophrenia (98). Several children with autism, whose urine contained highly elevated quantities of DHPPA, were treated with antibiotics effective against *Clostridium* species. Urine samples were collected before, during and after treatment with either metronidazole or vancomycin. In every child treated, a dramatic drop was recorded in DHPPA (9). Parents reported clinical improvement of autistic symptoms only if the children were treated for several weeks. The improvements noted were decreased hyperactivity, decreased hypersensitivity, increased social interest, increased eye contact, and increased vocalization. Parents also noted that regression occurred very quickly after treatment with metronidazole or vancomycin was discontinued. In view of the rapid regression and the antimicrobial activity possessed by these antibiotics, a *Clostridium* species would likely be the pathogen responsible for the DHPPA production for the following reasons: (i) Clostridia are anaerobic gram-positive bacteria that would be killed by the use of metronidazole or vancomycin (99). (ii) Clostridia produce spores that would survive antibiotic treatment and thus would be capable of regerminating, explaining the rapid regression seen in the treated children. (iii) Some *Clostridium* species produce the most potent neurotoxins known to man, and create stereotyped behavior in laboratory animals similar to stereotypical behaviors of autism (5,70). (iv) Pure cultures of *Clostridium* species produce a variety of phenolic metabolites (36,47).

Atypical tetanus cases: insights for autism

A common belief is that the clinical picture of a disease is less severe if preexisting antibodies against the pathogen are present. This is the very basis of the immunization program currently in place throughout the world, yet tetanus occasionally occurs in immunized individuals (51,100–102). Furthermore, individuals with tetanus vary widely in actual symptoms and in degrees of severity, and many physicians feel that a preexisting titer to TeNT alters the clinical picture and lessens the severity of the disease (51). Perhaps as a result, physicians have become less confident in diagnosing tetanus (54). A study of tetanus in Finland reported that clinical symptoms were not recognized in 22% of infected patients (103). Clearly, individuals with autism do not manifest symptoms of acute tetanus, yet subacute tetanus is a well-known medical phenomenon and may be instructive in some cases of autism.

Chronic subacute tetanus infections

The idea that tetanus is always an acute infection is wrong; chronic infection can occur. The case history of a 39-year-old male, treated for 21 months for chronic tetanus, has been published (104). His diagnosis was partially based on the presence of a highly elevated titer to TeNT, even though he had not received any recent booster immunization. Stimulation of antibody production from immunization typically levels off and begins to decline within a few months. The result is a low, protective antibody titer; and the body is primed to respond quickly to a similar immunization or to stimulation by the actual antigen. Since there was no recent booster immunization in the above case, TeNT antibody levels were attributed to stimulation from the actual antigen, and the clinical diagnosis of tetanus was recorded.

Subacute tetanus illness

The idea that tetanus is always a severe illness is wrong. One variation of tetanus with a slow gradual evolution of symptoms has been studied and labeled 'subacute' (105). Symptoms presented differed greatly from one patient to another. Primary symptoms included very brief nocturnal muscle spasms lasting 2–5 s, generalized hypertonia, dysphagia, neck stiffness, and mild trismus (105). Given the mildness of the trismus exhibited in these patients, would this symptom have gone unrecognized by the physician if the patient was unable to describe the stiff feeling in the jaw? Children with autism frequently have no speech. They are unable to relate information as to what sensation might be inducing difficulty in chewing and swallowing food (23).

Tetanus despite immunizations

The idea that tetanus is completely preventable in immunized persons is wrong. Many published case histories document the occurrence of tetanus despite adequate immunization and prophylactic care of wounds (51,100–102). One interesting case history is that of a 29 year-old male who was hyperimmunized to produce tetanus immune globulin (106). Following multiple tetanus toxoid immunizations, he donated his plasma. He received emergency room treatment for left antecubital swelling and ecchymosis that developed 1 week after his plasma donation. One month later, he was diagnosed with severe (grade III) tetanus. The initial symptoms he presented were combativeness, bewilderment, muteness, low-grade fever, and sluggish response. His titer was 1:1280 upon subsequent admission to the hospital.

Further work: proposed research projects

The development of a diagnostic test for the presence of toxigenic strains of *C. tetani* in fecal samples would be of immeasurable value. The presence of other toxigenic bacterial strains such as *C. difficile*, *Vibrio cholerae* and *Shigella dysenteriae* in the intestines, can be rapidly detected by the use of polymerase chain reaction (PCR®) amplification, even when other methods have failed (107–109). *Clostridium tetani* is not a member of normal human intestinal tract flora. In one study, stool samples were examined from a wide variety of animals and from 201 humans, none of whom was reported as autistic. It is important to note that *C. tetani* was not isolated in a single human specimen (110). Another similar approach would be to analyze fecal samples from individuals with autism versus normal controls using random amplified polymorphic DNA (RAPD-PCR), in order to explore possible differences in the stool.

A large-scale sampling of tetanus titers in individuals with autism would be a relatively easy study to conduct. In a very small pilot study, three out of five children with autism had an unusually strong response to DTP booster immunization. While all of the normal controls and two of the children with autism averaged a two-fold increase in titers, up to an 80-fold increase in titer levels occurred in the other three autistic children (111). Although the study was very small in size, the percentage of children that responded abnormally would seem to indicate a need for further study. Additionally, the presence of abnormally low

titers may be equally significant, as children with these low titers may have had an extremely early exposure to *C. tetani* during the time the child's CD5+ B-cells were establishing their normal repertoire in regard to intestinal microflora (112). Commercial laboratories offer only an IgG assay for tetanus. An examination of IgM and IgA immunoglobulin levels to tetanus would perhaps provide additional useful information in light of this paper's hypothesis. If abnormal titer levels were present in a significant portion of individuals tested, this would not be proof of ongoing infection, but would further substantiate the need for more extensive laboratory studies.

In laboratory experiments, the cleavage of synaptobrevin in small synaptic vesicles can be measured with specific anti-synaptobrevin antibodies (113). It would seem possible to use this technique on the brain tissue of autopsied individuals with autism. The areas of brain tissue that show abnormalities could be tested for the presence of cleaved synaptobrevin. Tetanus neurotoxin and botulinum neurotoxin-B are the only two substances known to cleave synaptobrevin at exactly the same peptide bond (113). It must be noted that availability of autopsy tissue could severely restrict studies of this nature.

The greatly increased levels of the metabolites DHPPA, phenylcarboxylic, and indole compound in the urine of individuals with autism is also deserving of further evaluation. Testing on other phenol and indole compounds has shown them to have binding affinity to various receptors in the brain, which can result in the development of abnormal behavior (114–116). Phenol has also been shown to increase the permeability of the blood–brain barrier (117). Determining whether DHPPA, phenylcarboxylic and/or the indole compound possess binding affinity to neurotransmitter receptors could provide data as to just how relevant these metabolites are to the behavioral symptoms observed in autism.

Discussion

Mankind still has much to learn about the virulence and ability of bacteria to produce disease. It is only within the last decade that the causal role of *Helicobacter pylori* in peptic ulcer disease has been recognized. Currently, the widespread opinion among scientists is that genetic research will yield answers to the etiology of autism. On the surface, the 4:1 incidence rate of males to females would seem to support this view (7); however, without more evidence, it is premature to presume that most cases of autism have a genetic basis. Interestingly, tetanus infection is also documented to occur in males four times more often than in females (52). In other words, the high incidence rate of autism among males does not strengthen any case against an etiology of infectious origin.

Children with autism are sometimes described as having a 'fixed smile', hypertonia or rigidity, and difficulty in chewing and swallowing food. These symptoms might actually be mild manifestations of risus sardonicus, muscle spasms, dysphagia, and trismus — all of which are clinical symptoms of tetanus (54). Hypotonia/flaccid muscle tone is frequently associated with autism and can be a direct result of TeNT acting within the CNS (62). Elevated catecholamines (in plasma and urine), tachycardia, profuse sweating, and irregular breathing are all symptoms of autonomic nervous system dysfunction and are documented to occur in both autism and tetanus (6,15,16,24,54). In a tetanus infection, there is no doubt that autonomic nervous system dysfunction is a result of the neurotoxin's effect on the CNS (54). To date, no explanation is offered in medical literature for the presence of the autonomic nervous system dysfunction associated with autism, but a synaptobrevin-related dysregulation of sympathetic neurons by TeNT is a distinct possibility.

There are many myths surrounding both autism and tetanus. An individual unfamiliar with autism will often form a mental picture of a young child sitting alone in a corner avoiding all contact with the 'outside' world. The word tetanus often inspires images of a person lying rigidly contorted, jaw clenched and near death. In general, neither description is particularly accurate, considering the variety of symptoms which can be present in both tetanus and autism. It is time to dispel the myths, and ask some serious questions. Are certain cases of autism really an unrecognized manifestation of subacute, chronic tetanus infection? Can the use of antibiotics set up an environment in the human intestinal tract, similar to that in laboratory animals, allowing invasion by the ubiquitous pathogen *C. tetani*? If so, might some individuals with autism have contracted subacute tetanus during a period of antibiotic reduction of intestinal flora? During that time of reduced flora, might the *C. tetani* have entered the intestines via oral ingestion, or via otherwise insignificant cuts or punctures? Given the documentation in this paper, parents, doctors, and researchers must combine efforts to determine if some people diagnosed as autistic are actually suffering from unrecognized forms of subacute tetanus.

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References

1. Kanner L. Autistic disturbances of affective contact. Nervous Child 1943; 2: 217–250.
2. Geschwind S A. The economic burden of autism: a preliminary estimate of the cost of illness. Personal communication.
3. Costello E J. Epidemiology. J Autism Dev Disord 1996; 26(2): 126–129.
4. Diagnostic and Statistical Manual of Mental Disorders, 4th edn. Washington, DC: American Psychiatric Association, 1994: 65–77.
5. Rapin I. Autistic children: diagnosis and clinical features. Pediatrics 1991; 81(5 Pt 2): 751–760.
6. Gillberg C, Coleman M. The biology of the autistic syndromes, 2nd edn. New York: Cambridge University Press, 1992.
7. Freeman B J. The syndrome of autism: update and guidelines for diagnosis. In: Young Children 1993; 6(2): 1–11.
8. Shaw W, Kassen E, Chaves E. Increased urinary excretion of analogs of krebs cycle metabolites and arabinose in two brothers with autistic features. Clin Chem 1995; 41(8): 1094–1104.
9. Shaw W, Chaves E. Experience with organic acid testing to evaluate abnormal microbial metabolites in the urine of children with autism. Proceedings of 1996 ASA Conference, Milwaukee, WI, July 13, 1996.
10. O'Reilly B A, Waring R H. Enzyme and sulphur oxidation deficiencies in autistic children with known food/chemical intolerances. J Orthomolecular Med 1993; 8(4): 198–200.
11. Waring R H, Ngong J M. Sulphate metabolism in allergy-induced autism: relevance to the disease aetiology. Conference papers from 'Biological Perspectives in Autism' held at the University of Durham, April 1993. Published by Autism Research Unit, University of Sunderland, UK, pp: 25–33.
12. Ahlsen G, Rosengren L, Belfrage M et al. Gial fibrillary acid protein in the cerebrospinal fluid of children with autism and other neuropsychiatric disorders. Biol Psychiatry 1993; 33: 734–743.
13. Kemper T L, Bauman M L. The contribution of neuropathologic studies to the understanding of autism. Neurol Clin 1993; 11(1): 175–187.
14. Ritvo E R, Freeman B J, Scheibel A B et al. Lower Purkinje cell counts in the cerebella of four autistic subjects: initial findings of the UCLA-NSAC autopsy research report. Am J Psychiatry 1986; 143: 862–866.
15. Barthelemy C, Bruneau N, Cottet-Eymard J M et al. Urinary free and conjugated catecholamines and metabolites in autistic children. J Autism Dev Disorder 1988; 18(4): 583–591.
16. Launay J M, Bursztejn C, Ferrari P et al. Catecholamines metabolism in infantile autism: a controlled study of 22 autistic children. J Autism Dev Disorder 1987; 17(3): 333–347.
17. Gillberg C, Steffenburg S. Outcome and prognostic factors in infantile autism and similar conditions: a population-based study of 46 cases followed through puberty. J Autism Dev Disorder 1987; 17(2): 273–287.
18. Olsson I, Steffenburg S, Gillberg C. Epilepsy in autism and autistic-like conditions: a population-based study. Arch Neurol 1988; 45(6): 666–668.
19. Warren R, Singh V, Cole P et al. Increased frequency of the null allele at the complement C4b locus in autism. Clin Exp Immunol 1991; 83: 438–440.
20. Warren R, Burger R, Odell D, Torres A, Warren W. Decreased plasma concentrations of the C4B complement protein in autism. Arch Pediatr Adolesc Med 1994; 148: 180–183.
21. Konstantareas M, Homatidis S. Ear infections in autistic and normal children. J Autism Dev Disord 1987; 17(4): 585–594.
22. Gordon A. Ear disorders in autistic children. J Autism Dev Disord 1989; 19(3): 470–473.
23. Coleman M. Young children with autism or autistic-like behavior. In: Young Children 1989; 1(4): 22–31.
24. Wing L, Attwood A. Syndromes of autism and atypical development. In: Cohen D J, Donnellan A M, Paul R, eds. Handbook of Autism and Pervasive Developmental Disorders. New York: Wiley, 1987: 3–19.
25. Baker S M, Pangborn J. Clinical assessment options for children with autism and related disorders: a biomedical approach. A consensus report of the Defeat Autism Now (DAN) Conference, Dallas, Texas, Jan 1995. Published by Autism Research Institute, San Diego, CA.
26. Borriello S P. Clostridial disease of the gut. Clin Infect Dis 1995; 20(Suppl. 2): S242–S250.
27. Hentges D J. The protective function of the indigenous intestinal flora. Pediatr Infect Dis 1986; 5(1 Suppl.): S17–S20.
28. Hill H R, Meier F A. Host defense factors in the gastrointestinal tract. Pediatr Infect Dis 1986; 5(1 Suppl.): S144–S147.
29. Bizzini B. Tetanus: Bacterial Vaccines. San Diego, CA: Academic Press, 1984, Chapter 2: 37–68.
30. Freeman B A. Clostridium—the spore-forming anaerobes. Burrows Textbook of Microbiology, 21st edn. Philadelphia: Saunders, 1979, Chapter 27.
31. Eaves L, Ho H, Eaves D. Subtypes of autism by cluster analysis. J Autism Dev Disorder 1994; 24(1): 3–22.
32. Michael G. Chez, M. D., Pediatric Neurology, 900 N. Westmoreland Rd, Suite 123, Lake forest, IL 60045. Personal communication.
33. William Shaw, Ph.D., The Great Plains Laboratory, 9335 W. 75th Street, Overland Park, KS 66204. Personal communication.
34. Tsutaoka B, Hansen J, Johnson D, Holodniy M. Antibiotic-associated pseudomembranous enteritis due to *Clostridium difficile*. Clin Infect Dis 1994; 18(6): 982–984.
35. Fekety R, Shah A B. Diagnosis and treatment of *Clostridium difficile* colitis. J Am Med Assoc 1993; 269(1): 71–75.
36. Conway P L. Microbial ecology of the human large intestine (Chapter 1). Macfarlane S, Macfarlane G T. Proteolysis and amino acid fermentation (Chapter 4). In: Gibson & Macfarlane, eds. Human Colonic Bacteria. Boca Raton, FL: CRC Press, 1995.
37. Snyder J D. Bacterial diarrhea. In: Walker W A, ed. Pediatric Gastrointestinal Disease, Vol. 1, 2nd edn. St Louis, MO: Mosby-Year Book, 1996, Chapter 27, part 10.
38. Bohnhoff M, Miller C. Enhanced susceptibility to Salmonella infection in streptomycin-treated mice. J Infect Dis 1962; 111: 117–127.
39. Lipson A. Infecting dose of Salmonella. Lancet 1976; i(7966): 969.
40. McCullough N, Eisele C. Experimental human Salmonellosis. J Infect Dis 1951; 89: 209–213.
41. McCullough N, Eisele C. Experimental human Salmonellosis. J Infect Dis 1951; 88: 278–289.
42. Holmberg S, Osterholm M, Senger K, Cohen M. Drug-resistant Salmonella from animals fed antimicrobials. N Engl J Med 1984; 311(10): 617–622.
43. Hatheway C. Botulism: the present status of the disease. Curr Top Microbiol Immunol 1995; 195: 55–75.
44. Wigginton J, Thill P. Infant botulism: a review of the literature. Clin Pediatr (Philadelphia) 1993; 32(11): 669–674.

45. Smith L D S. Clostridium tetani. In: Introduction to the Pathogenic Anaerobes. Chicago: University of Chicago Press, 1955.
46. Stevens D L, Bryant A E. Role of theta toxin, a sulfhydryl-activated cytolysin, in the pathogenesis of clostridial gas gangrene. Clin Infect Dis 1993; 16(Suppl. 4): S195–S199.
47. Elsden S R, Hilton M J, Waller J M. The end products of the metabolism of aromatic amino acids by clostridia. Arch Microbiol 1976; 107(3): 283–288.
48. Bittner J, Macovei A, Lemeni D, Arboreanu D, Potorac E. Contributions to the taxonomy and biology of *Clostridium difficile*. Roum Arch Microbiol Immunol 1992; 51(4): 253–262.
49. Sugiyama H, Mills D C. Intraintestinal toxin in infant mice challenged intragastrically with *Clostridium botulinum* spores. Infect Immun 1978; 21: 59–63.
50. Wells C L, Balish E. *Clostridium tetani* growth and toxin production in the intestines of germfree rats. Infect Immun 1983; 41(2): 826–828.
51. Passen E L, Andersen B R. Clinical tetanus despite a 'protective' level of toxin-neutralizing antibody. J Am Med Assoc 1986; 255(9): 1171–1173.
52. Wilson Sir G S, Miles A A. Tetanus. In: Topley and Wilson's Principles of Bacteriology and Immunity, 5th edn. Baltimore, MD: Williams & Wilkens, 1964, Chapter 76.
53. Fischer G W, Sunakorn P, Duangman C. Otogenous tetanus. Am J Dis Child 1977; 131: 445–446
54. Bleck T P. Tetanus: pathophysiology, management and prophylaxis. Dis Mon 1991; 37(9): 545–603.
55. Hardegree M C, Palmer A E, Duffin N. Tetanolysin: in-vivo effects in animals. J Infect Dis 1971; 123(1): 51–60.
56. Cox C B, Hardegree C, Fornwald R. Effect of tetanolysin on platelets and lysosomes. Infect Immun 1974; 9(4): 696–701.
57. Matsunaga T, Miyamoto K, Koshiura R. Cytotoxic effect of the culture supernatant of *Clostridium tetani*. Chem Pharm Bull 1982; 30(2): 702–707.
58. Udall J N, Pang K, Fritze I, Kleinman R, Walker A. Development of gastrointestinal mucosal barrier, I: The effect of age on intestinal permeability to macromolecules. Pediatr Res 1981; 15: 241–244.
59. Knivsberg A, Wiig K, Lind G, Nodland M, Reichelt K. Dietary intervention in autistic syndromes. Brain Dysfunct 1990; 3: 315–327.
60. Krieglstein K G, Henschen A H, Weller U, Habermann E. Limited proteolysis of tetanus toxin: relation to activity and identification of cleavage sites. Eur J Biochem 1991; 202: 41–51.
61. Helting T B, Ronneberger H J, Vollerthun R, Neubauer V. Toxicity of papain-digested tetanus toxin: pathological effect of fragment B in the absence of spastic paralysis. J Biol Chem 1978; 253(1): 125–129.
62. Takano K, Kirchner F, Gremmelt A, Matsuda M, Ozutsumi N, Sugimoto N. Blocking effects of tetanus toxin and fragment [A-B] on the excitatory and inhibitory synapses of the spinal motoneurone of the cat. Toxicon 1989; 27(3): 385–392.
63. Simpson L L, Hoch D H. Neuropharmacological characterization of fragment B from tetanus toxin. J Pharm Exp Ther 1985; 232(1): 223–227.
64. Ahnert-Hilger G, Bigalke H. Molecular aspects of tetanus and botulinum neurotoxin poisoning. Prog Neurobiol 1995; 46: 83–96.
65. Manning K A, Erichsen J T, Evinger C. Retrograde transneuronal transport properties of fragment C of tetanus toxin. Neuroscience 1990; 34(1): 251–263.
66. Hensel B, Seib U C, Wellhoner H H. Vagal ascent and distribution of 125 I-tetanus toxin after injection into the anterior wall of the stomach. Naunyn Schmiedebergs Arch Pharmacol 1973; 276(3): 395–402
67. Kryzhanovskii G N, Aliev M N. The pathogenesis of stereotypic behavior. Neurosci Behav Physiol 1984; 14(3): 232–240.
68. Foca A, Rotiroti D, Mastroeni P, Nistico G. Effects of tetanus toxin after intracerebral microinjection are antagonized by drugs enhancing GABAergic transmission in adult fowls. Neuropharmacology 1984; 23(2A): 155–158.
69. James T A, Collingridge G L. Rapid behavioral and biochemical effects of tetanus toxin microinjected into the substantia nigra: a dual role for GABA? Neurosci Lett 1979; 11: 205–208.
70. Kryzhanovsky G N, Aliev M N. The stereotyped behavior syndrome: a new model and proposed therapy. Pharmacol Biochem Behav 1981; 14(3): 273–281.
71. Gilman J T, Tuchman R F. Autism and associated behavioral disorders: pharmacotherapeutic intervention. Ann Pharmacother 1995; 29(1): 47–56.
72. Bagetta G, Nistico G. Glutamate transmission is involved in the mechanisms of neuronal degeneration produced by intrahippocampal tetanus toxin in rats. Toxicol Lett 1992; 64–65 Spec No: 447–453.
73. Lee C L, Hrachovy R A, Smith K L, Frost J D Jr, Swann J W. Tetanus toxin-induced seizures in infant rats and their effects on hippocampal excitability in adulthood. Brain Res 1995; 677: 97–109.
74. Britton P, Whitton P S, Bowery N G. Effect of tetanus toxin on basal and evoked release of 5-hydroxytryptamine and dopamine in rat hippocampus in vivo. Brain Res 1995; 673(2): 331–334.
75. Francis P T, Lowe S L, Bowen D M, Jefferys J G R. Lack of change in neurochemical markers during the postepileptic phase of intrahippocampal Tetanus Toxin Syndrome in Rats. Epilepsia 1990; 31(6): 697–701.
76. Brace H M, Jefferys J G R, Mellanby J. Long-term changes in hippocampal physiology and learning ability of rats after intrahippocampal tetanus toxin. J Physiol 1985; 368: 343–357.
77. Beitchman J H, Inglis A, Schachter D. Child psychiatry and early intervention, III: The developmental disorders. Can J Psychiatry 1992; 37(4): 241–244.
78. Boucher J, Lewis V. Memory impairments and communication in relatively able autistic children. J Child Psychol Psychiatry 1989; 30(1): 99–122.
79. Smith C U M. Elements of Molecular Neurobiology, 2nd edn. Chichester, UK: Wiley, 1996, Chapter 1.
80. Fried G. Synaptic vesicles and release of transmitters: new insights at the molecular level. Acta Physiol Scand 1995; 154: 1–15.
81. Hohne-Zell B, Ecker A, Weller U, Gratzl M. Synaptobrevin cleavage by the tetanus toxin light chain is linked to the inhibition of exocytosis in chromaffin cells. FEBS Lett 1994; 355(2): 131–134.
82. Montecucco C, Schiavo G. Mechanism of action of tetanus and botulinum neurotoxins. Mol Microbiol 1994; 13(1): 1–8.
83. Menestrina G, Schiavo G, Montecucco C. Molecular mechanisms of action of bacterial protein toxins. Molec Aspects Med 1994; 15: 79–193.
84. Schiavo G, Rossetto O, Benfenati R, Poulain B, Monecucco C. Tetanus and botulinum neurotoxins are zinc proteases specific for components of the neuroexocytosis apparatus. Ann NY Acad Sci 1994; 710: 65–75.
85. Poulain B. Molecular mechanism of action of tetanus toxin and botulinum neurotoxins. Pathol Biol 1994; 42(2): 173–182.
86. Cook E H. Autism: review of neurochemical investigation. Synapse 1990; 6: 292–308.
87. Noppe M, Crois R, Andries D, Lowenthal A. Determination in human cerebrospinal fluid of glial fibrillary acidic protein, S-100 and myelin basic protein as indices on non-specific or

specific central nervous tissue pathology. Clin Chim Acta 1986; 155: 143–150.
88. Bauman M L, Kemper T L. Histoanatomic observations of the brain in early infantile autism. Neurology 1985; 35: 866–874.
89. Minshew N J, Goldstein G, Dombrowski S M, Panchalingam K, Pettegrew J W. A preliminary 31P MRS study of autism: evidence for undersynthesis and increased degradation of brain membranes. Biol Psychiatry 1993; 33(11–12): 762–773.
90. Baker A B. The central nervous system in tetanus. J Neuropathol Exp Neurol 1942; 1: 394–405.
91. Bagetta G, Nistico G. Tetanus toxin as a neurobiological tool to study mechanisms of neuronal cell death in the mammalian brain. Pharmac Ther 1994; 62: 29–39.
92. Halpern J L, Neale E A. Neurospecific binding, internalization, and retrograde axonal transport. Curr Top Microbiol Immunol 1995; 195: 221–241.
93. Crossman A R, Neary D. Neuroanatomy. New York: Churchill Livingstone, 1995.
94. Schnitzer J, Schachner M. Developmental expression of cell type-specific markers in mouse cerebellar cells in vitro. J Neuroimmunol 1981; 1(4): 471–487.
95. Weber A, Schachner M. Maintenance of immunocytologically identified purkinje cells from mouse cerebellum in monolayer culture. Brain Res 1984; 311(1): 119–130.
96. Fields K L, Currie D N, Dutton G R. Development of Thy-1 antigen on cerebellar neurons in culture. J Neurosci 1982; 2(6): 663–673.
97. Brooks J B, Moore W E C. Gas chromatographic analysis of amines and other compounds produced by several species of *Clostridium*. Can J Microbiol 1969; 15: 1433–1447.
98. Dangliesh C E, Kelley W, Horning E C. Excretion of a sulphatoxyl derivative of skatole in pathological states in man. Biochem J 1958; 70: 13P.
99. Ahmadsyah I, Salim A. Treatment of tetanus: an open study to compare the efficacy of procaine penicillin and metronidazole. Br Med J 1985; 291: 648–650.
100. Luisto M, Iivanainen M. Tetanus of immunized children. Dev Med Child Neurol 1993; 35: 346–358.
101. Peterson H. A case of tetanus in spite of active toxoid prophylaxis. Acta Chir Scand 1965; 129: 235–237.
102. Berger S A, Cherubin C E, Nelson S, Levine L. Tetanus despite preexisting antitetanus antibody. J Am Med Assoc 1978; 240(8): 769–770.
103. Middlebrook J L, Brown J E. Immunodiagnosis and immunotherapy of tetanus and botulinum neurotoxins. Curr Top Microbiol Immunol 1995; 195: 89–122.
104. Risk W S, Bosch E P, Kimura J, Cancilla P A, Fischbeck K H, Layzer R B. Chronic tetanus: clinical report and histochemistry of muscle. Muscle Nerve 1981; 4: 363–366.
105. Ogunyemi A O. The clinical recognition of subacute tetanus. J Trop Med Hyg 1986; 89(3): 131–135.
106. Crone N E, Reder A T. Severe tetanus in immunized patients with high anti-tetanus titers. Neurology 1992; 42: 761–764.
107. Kuhl S J, Tang Y J, Navarro L, Gumerlock P H, Silva J Jr. Diagnosis and monitoring of *Clostridium difficile* infections with the polymerase chain reaction. Clin Infect Dis 1993; 16(Suppl. 4): S234–S238.
108. Ramamurthy T, Pal A, Bag P K et al. Detection of cholera toxin gene in stool specimens by polymerase chain reaction: comparison with bead enzyme-linked immunosorbent assay and culture method for laboratory diagnosis of cholera. J Clin Microbiol 1993; 31(11): 3068–3070.
109. Begum D, Strockbine N A, Sowers E G, Jackson M P. Evaluation of a technique for identification of shiga-like toxin-producing *Escherichia coli* by using polymerase chain reaction and digoxigenin-labeled probes. J Clin Microbiol 1993; 31(12): 3153–3156.
110. Ebisawa I, Takayanagi M, Kigawa M. Some factors affecting isolation of *Clostridium tetani* from human and animal stools. J Exp Med (Japan) 1988; 58(6): 233–241.
111. Tsaltas M O. A pilot study on allergic responses. J Autism Dev Disord 1986; 16(1): 91–92.
112. Roitt I. Essential Immunology, 8th edn. Oxford: Blackwell Scientific, 1994: 228–229.
113. Schiavo G, Montecuco C. Tetanus and botulism neurotoxins: isolation and assay. Methods Enzymol 1995; 248: 643–652 .
114. Kanai Y, Wada O, Manabe S. Antagonism of gamma-aminobutyric acidA receptor-mediated responses by amino-gamma-carbolines. J Pharmacol Exp Ther 1990; 252(3): 1269–1276.
115. Jackson H, Kitchen I. Behavioural profiles of putative 5-hydroxytryptamine receptor agonists and antagonists in developing rats. Neuropharmacology 1989; 28(6): 635–642.
116. Goodwin G, Green A. A behavioural and biochemical study in mice and rats of putative selective agonists and antagonists for 5-HT1 and 5-HT2 receptors. Br J Pharmacol 1985; 84(3): 743–753.
117. Zaki A, Wardle E, Canalese J, Ede R, Williams R. Potential toxins of acute liver failure and their effects on blood–brain barrier permeability. Experientia 1983; 39(9): 988–991.