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EVIDENCE OF TOXICITY, OXIDATIVE STRESS, AND NEURONAL INSULT IN AUTISM

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According to the Autism Society of America, autism is now considered to be an epidemic. The increase in the rate of autism revealed by epidemiological studies and government reports implicates the importance of external or environmental factors that may be changing. This article discusses the evidence for the case that some children with autism may become autistic from neuronal cell death or brain damage sometime after birth as result of insult; and addresses the hypotheses that toxicity and oxidative stress may be a cause of neuronal insult in autism. The article first describes the Purkinje cell loss found in autism, Purkinje cell physiology and vulnerability, and the evidence for postnatal cell loss. Second, the article describes the increased brain volume in autism and how it may be related to the Purkinje cell loss. Third, the evidence for toxicity and oxidative stress is covered and the possible involvement of glutathione is discussed. Finally, the article discusses what may be happening over the course of development and the multiple factors that may interplay and make these children more vulnerable to toxicity, oxidative stress, and neuronal insult.

Autism is a neurological disorder that limits a person's ability to function normally. Behavioral abnormalities, social limitations, sensory processing abnormalities, and impaired ability to communicate are the main issues in this multifaceted disorder (Cohen & Volkmar, 1997). According to the Autism Society of America (ASA), autism or autism spectrum disorder (ASD) is now considered to be an epidemic. As many as 1.5 million Americans – children and adults – are thought to have autism/ASD today, and unfortunately, that number is increasing. Based on statistics from the U.S. Department of Education (USDE) and other governmental agencies, autism/ASD is growing at a rate of 10–17% per year (USDE, 2003).

Studies from the 1980s estimated autism to occur in 1 in 1000 children (Bryson et al., 1988; Sugiyama and Abe, 1989). However, a study in 1999 reported a prevalence rate in autism of 1 in 333 children (Baird et al., 2001); and another study in 2003 reported a prevalence rate in autism of 3.4 in 1000 children (Yeargin-Allsopp et al., 2003). The Center for Disease Control (CDC), as of November, 2004, reports prevalence rates ranging from 2 to 6 per 1,000 children (CDC, 2004). Reports from the California Department of Developmental Services also suggest that the rates in autism are increasing (Chakrabarti and Fombonne, 2001; California DDS, 1998). The increase in public awareness and broadening of the criteria may be possible contributing factors; however, the substantial increase in the rate of autism revealed by epidemiological studies and government reports implicates the importance of external or environmental factors that may be changing (USDE, 2003; Chakrabarti and Fombonne, 2001; Palmer et al., 2006).

The cause of autism, to date, is not known. In addition, it is unknown whether the neurological problems are primary in nature or if another system is malfunctioning and affecting the neurological system. Biomedical studies in autism/ASD disclose a variety of abnormalities, not only in the neurological system (Courchesne, 1991, 1995; Kemper & Bauman, 1993), but also in the immune (Cohly and Panja, 2005; Warren et al., 1990, 1992, 1995) and digestive systems (Horvath et al., 1998; Furlano et al., 2001; Molloy and Manning-Courtney, 2003). Abnormal metabolic indicators have been found, such as low plasma levels of inorganic sulfate and sulfur oxidation deficiencies (Waring and Klovrza, 2000; Waring and O'Reilly, 1990; Alberti et al., 1999). There have also been studies that suggest toxicity in this population, specifically phenolic precursors, including trimethylbenzene,

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ethylbenzene, xylene, toluene, and styrene (Edelson and Cantor, 1998), and various heavy metals, such as mercury, lead, bismuth, cadmium, and arsenic (Lonsdale, 2002; Filipek et al., 1999; Eppright et al., 1996; Holmes et al., 2003). Recently, decreased glutathione levels and increased oxidative stress were also shown in children with autism (James et al., 2004).Twin studies find a much higher concordance rate for monozygotic twins as compared to dizygotic twins, suggesting genetic inheritance as a causative agent (Bailey et al., 1995). Although studies suggest strong genetic influences, specific susceptibility genes still remain largely elusive (Trikalinos et al., 2006).

Many theories were postulated regarding the underlying cause of autism. For example, autism was implicated to be related to the measles, mumps, and rubella vaccine (Wakefield et al., 1998, 2000; Wakefield, 2003); mercury in vaccines (Bernard et al., 2002; McGinnis, 2001); immune system dysfunction (Cohly and Panja, 2005; Warren et al., 1990, 1992, 1995; Singh et al., 1993; Weizman et al., 1982); fungal infection (Shaw et al., 1994; Shaw, 1996), toxicity (McFadden, 1996); metabolic abnormalities (Waring and Klovrza, 2000; Waring and O'Reilly, 1990; Alberti *et al*., 1999); and "leaky gut syndrome"(Vantrappen and Geboes, 1993). Most recently, studies have suggested that decreased glutathione levels and increased oxidative stress may play a role in the pathology (James et al., 2004). Which of the many theories may be correct and/or how the various theories may fit together remains unclear. It is important to note that these theories are based on small studies and much more research is needed.

The diversity of these findings and their variety of selection in different patients suggest that persons with autism/ASD comprise a heterogeneous population in regard to etiology. They also suggest that patients may respond to different sets of treatment (Kern et al., 2001, 2002). To date, treatments are varied and somewhat unpromising. The two most beneficial treatments, as reported by parents surveyed by the Autism Research Institute (ARI), are chelation therapy and a gluten and casein free diet (ARI, 2006). No treatments, to date, are considered curative.

The onset of autism is also not clear. The onset of the abnormal growth and development within the brain in autism is not known. Current thought by experts in autism, such as Bauman et al. (1997), is that the time of onset of the neurological problems is prenatal, occurring prior to 30 weeks gestation. However, autism comprises a heterogeneous population in that parents report either that their child was abnormal from birth, or that their child was developmentally normal until sometime after birth, typically 15–24 months, at which time the child began to regress or deteriorate (Filipek et al., 1999; Davidovitch et al., 2000; Tuchman, 1996; Kern, 2003). Typically reported is loss of verbal, nonverbal, and social abilities (Davidovitch et al., 2000; Kern et al., 2002; Goldberg et al., 2003). For example, a study by Goldberg et al. (2003) found that children that lost verbal skills did so at an average of 20.69 months; children that lost nonverbal skills did so at an average of 18.58 months; and children that lost both skills, lost verbal skills at an average of 21.2 months and nonverbal skills at an average of 18.9 months. Information provided by parents of children who were developmentally normal until a later onset does not fit with the current thought of the time of neurological onset of autism as being prenatal in all cases. It is conceivable that some of these children become autistic from neuronal cell death or brain damage sometime after birth as result of insult (Rice and Barone, 2000; Makri et al., 2004).

This article discusses the evidence for the case that some children with autism may become autistic from neuronal cell death or brain damage sometime after birth as result of insult; and addresses the hypotheses that toxicity and oxidative stress may be a cause of neuronal insult in autism. The article first describes the Purkinje cell loss found in autism, Purkinje cell physiology and vulnerability, and the evidence for postnatal cell loss. Second, the article describes the increased brain volume in autism and how it may be related to the Purkinje cell loss. Third, the evidence for toxicity and oxidative stress is covered and the possible involvement of glutathione is discussed. Finally, the article discusses what may be happening over the course of development and the multiple factors that may interplay and make these children more vulnerable to toxicity, oxidative stress, and neuronal insult.

Purkinje Cell Loss in Autism

One of the most consistent neurological abnormalities found in persons with autism is marked Purkinje cell loss in the cerebellum (as determined by histopathological post-mortem examination)

and atrophy of the cerebellar folia (as determined by in vivo neuroimaging) (Courchesne, 1991, 1995; Kemper and Bauman, 1993; Ritvo et al., 1986; Bailey et al., 1998). According to the Ritvo *et al*. (1986), the Purkinje cells in the vermis of the cerebellum were approximately 15 standard deviations below the mean, and approximately 8 standard deviations below the mean bilaterally in the cerebellar hemispheres in the subjects with autism, as compared to normal controls. Several animal studies showed that Purkinje cell loss results from insult, and ***in some cases the Purkinje cells are selectively vulnerable*** (Welsh et al., 2002; Kern, 2003). For example, Purkinje cells are selectively vulnerable to: (1) ischemia (inadequate blood supply) (Welsh et al., 2002; Fonnum and Lock, 2000); (2) hypoxia (inadequate oxygen supply) (Welsh et al., 2002; Cervos-Navarro and Diemer, 1991); (3) excitotoxicity, such as seizures, metabolic insufficiencies (Fonnum and Lock, 2000; Butterworth, 1993; O'Hearn and Molliver, 1997; Brorson et al., 1995; Kang et al., 2002); (4) G protein dysfunction (Reader and Senecal, 2001; Kish et al., 1993); (5) viral infections (Hornig et al., 2001); (6) vitamin deficiencies, such as thiamine (Butterworth, 1993); (7) heavy metals, such as mercury, lead, arsenic, cadmium, and bismuth (Ross et al., 1996; Sorensen et al., 2000; Kenntner et al., 2001; Stoev et al., 2003; Piao et al., 2005; Sakamoto et al., 2002; Warfvinge, 2000); (8) toxins, such as bilirubin, phenytoin, ethanol, alkaloids, toluene, and diptheria toxin (Crooks et al., 2000; Riedel et al., 1990; Fonnum and Lock, 2000; O'Hearn and Molliver, 1997; McDonald et al., 1998; Saavedra et al., 1996); (9) chronic malabsorption syndrome, such as celiac disease, inflammatory bowel disease (Bhatia et al., 1995; Tijssen et al., 2000; Hadjivassiliou et al., 2002); and (10) oxidative stress (Heaton et al., 2000; Chen et al., 2003; Yamashita et al., 2000; Barlow et al., 1999). Why the Purkinje cell may be selectively vulnerable is discussed in the next section.

Purkinje Cell Physiology and Selective Vulnerability

The basic nature of neurons in regard to location, function, and chemical makeup allows for a hierarchy of neuronal vulnerability of selective neuronal populations. Evidence suggests that the physiology of the Purkinje cell plays a role in its vulnerability. The Purkinje cell is an exceptionally large (50–80*u*m) inhibitory neuron in the cerebellum that receives extensive excitatory input from both parallel fibers (from granule cells) and climbing fibers (from the inferior olivary nucleus) (Ghez, 1991). Parallel fibers make about 200,000 connections on each Purkinje cell and input from these neurons trigger calcium influx (Sugimori and Llinas, 1990). Purkinje cells fire synchronously, forming one of the most powerful connections in the nervous system (Sugimori and Llinas, 1990; Ghez, 1991; Welsh et al., 2002). The response of the Purkinje cell is a large action potential followed by a high frequency of smaller action potentials (complex spikes) that are associated with a calcium influx that is unparalleled in the nervous system (Sugimori and Llinas, 1990; Ghez, 1991). As a result of the high level of excitatory amino acid synaptic connections and the response of the Purkinje cell that is mediated by voltage-gated and receptor-gated calcium channels, the Purkinje cell has an exceptionally high metabolic demand (Welsh et al., 2002). A high metabolic demand, combined with constant input from the inferior olive and large amounts of calcium stores and influx, makes the neuron exceptionally vulnerable to metabolic insufficiencies and excessive rises in calcium (Altman and Bayer, 1997; O'Hearn and Molliver, 1997). Excessive rises in intracellular calcium are associated with excitotoxicity and may produce cell death (Martin et al., 1998; Hoyal *et al*., 1998). Brorson et al. (1995) suggested that the Purkinje cell may be more vulnerable to excitotoxicity because they have alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors that undergo less complete desensitization. The AMPA receptors (which are in the glutamate receptor family), located at the synapses with the climbing and parallel fibers, are thought to mediate the selective vulnerability of the Purkinje cell to excitotoxicity (Sarna and Hawkes, 2003). Excessive glutamatergic neuronal stimulation increases the production of reactive oxygen species (ROS), which in turn induce oxidative stress, excitotoxicity, and neuronal insult (Savolainen et al., 1998; Nakaso et al., 2000). Very large neurons like the Purkinje cell have been shown to have differential expression of antioxidant proteins (Sarafian et al., 1999). Sarafian et al. (1999) suggested the antioxidant differences may also contribute to the selective vulnerability of these cells. A couple of studies found that the administration of antioxidants, such as vitamin E and isoindoline nitroxide, can reduce Purkinje cell death from oxidative stress (Chen et al., 2003; Heaton et al.,

2000). Evidence suggests there are interactions of excitatory neurotransmitters and xenobiotics in excitotoxicity and oxidative stress (Savolainen et al., 1998). Toxic metal exposure increases oxidative stress and glutamate excitotoxicity, and as mentioned, the Purkinje cells are known to be vulnerable to lead (Kang et al., 2004; Kenntner et al., 2001), cadmium (Stoev et al., 2003), mercury (Sakamoto et al., 2002; Sorensen et al., 2000; Warfvinge, 2000), arsenic (Piao et al., 2005), and bismuth (Liessens et al., 1978; Ross et al., 1996). Metal-induced oxidant stress can initiate a cascade of events including excitotoxicity, increased cytosolic free calcium, and cell death (Olanow and Arendash, 1994). It is apparent that multiple factors may contribute to the selective vulnerability of the Purkinje cell. The interplay between toxic metals and oxidative stress will be discussed further in a later section.

Evidence for Postnatal Cell Loss in Autism

Bauman et al. (1997) stated that the absence of gliosis found in their autopsy study suggests that the abnormalities occurred during early development (prior to 30 weeks gestation). Gliosis is proliferation of neuroglial tissue that follows neural damage (Vajda, 2002). Nervous system damage as a result of insult, such as metabolic insufficiencies, epilepsy, brain injury, toxins, infarction, viral infection, ischemia, or excitotoxicity, may lead to neuronal death; neuronal degeneration; apoptotic cell death, injury, cell loss; and gliosis (Vajda, 2002). Absence of gliosis suggests that cell count abnormalities are not due to neural damage.

However, there is evidence of gliosis associated with Purkinje cell loss in the cerebellum of some children with autism. An autopsy report by Bailey et al. (1998) found that the Purkinje cell loss was sometimes accompanied by gliosis and an increase in glial fibrillary acidic protein (GFAP). [GFAP is elevated in acute and chronic situations of nerve cell damage (Ahlsen et al., 1993).] Bailey et al. (1998) stated that the patchy glial cell hyperplasia found in their study suggests the possibility of postnatal loss of Purkinje cells. The authors also stated that some signs were suggestive of a developmental basis, yet other factors influencing neuronal cell survival also seemed to be important. In addition, in a study by Ahlsen et al. (1993) that examined the levels of GFAP in the cerebrospinal fluid of children with autism, GFAP was found to be three times higher than the level of the control group. Ahlsen et al. (1993) stated that the results could implicate gliosis and unspecified brain damage in children with autism. Recently, a study examined levels of GFAP in the frontal, parietal, and cerebellar cortices using age-matched autistic and control post-mortem specimens. GFAP was significantly elevated in all three brain areas (Laurence and Fatemi, 2005). The authors stated that the elevated GFAP confirms microglial and astroglial activation in autism and indicates gliosis, reactive injury, and perturbed neuronal migration processes.

Interestingly, Bauman and Kemper (1994) reported that Purkinje cells were enlarged in children, whereas the cells were small and pale in adults. The authors suggested that the cellular enlargement was a result of a compensatory mechanism. However, neuronal damage results in cell swelling (inflammatory reactive edema) (Vajda, 2002). Kiefer et al. (1989) found that phenytoin, which is toxic to the Purkinje cell, causes the Purkinje cell to swell.

Increased Brain Volume

Another consistent neurological abnormality found in persons with autism is increased brain volume. Many studies found that the brain in autism is enlarged (Courchesne et al., 2001; Hardan et al., 2001; Bailey et al., 1998; Aylward et al., 2002; Sears et al., 1999; Herbert et al., 2004; Piven et al., 1995, 1996; Sparks et al., 2002; Lotspeich et al., 2004). For example, Aylward et al. (2002) completed an MRI study of 67 non-mentally retarded children and adults with autism who were matched to 83 healthy community volunteers. ***The children with autism (up to age 12) had brains that were significantly larger than controls***. However, the persons with autism older than age 12 had brain volumes that were not different from controls. Interestingly, the head circumference in the persons with autism of all ages was increased, as compared to controls. The authors stated that this was suggestive that adolescents and adults with autism had a larger brain as children; and that while normal persons experienced a quantitative increase in brain size during the adolescent years, the persons with autism experienced a quantitative decrease in brain size during the adolescent

years. Aylward et al. (2002) called this apparent "normalization of the brain," and suggested after age 12 as the "cutoff." However, the authors said that a larger sample would be needed to determine this aspect clearly, especially since other studies suggested an earlier time when the brain stops increasing in size abnormally.

Evidence from an MRI study by Courchesne et al. (2001) suggests that children with autism have a normal head circumference at birth (which is indicative of normal brain volume), however, by 2–4 years of age the brain is enlarged. Courchesne and colleagues (2001) found that 90% of the boys in the study had 18% more cerebral white matter, 39% more cerebellar white matter, and 12% more cortical gray matter by 2–3 years of age. Courchesne and colleagues (2001) stated that there is an early overgrowth followed by later slowed growth.

Herbert et al. (2004) found an unexplained white matter enlargement in their imaging study that suggests an ongoing postnatal process. The authors state that the process suggests a nonaxonal component of white matter, possible myelin. Other have suggested that the volume increase is in the gray matter (Lotspeich et al., 2004; Courchesne et al., 2003).

Relationship Between Brain Volume and Head Circumference

Several studies reported that children with autism have statistically significantly larger head circumferences than typically-developing children (Courchesne et al. 2001, 2003; Aylward et al., 2002; Fidler et al., 2000; Fombonne, 1999; Deutsch and Joseph, 2003) beginning during the first year of life (Courchesne et al., 2003). Research indicates that head circumference is indicative of brain volume in children (Bartholomeusz et al., 2002; Endres and Cohen, 2001; Tramo et al., 1998; Hamano et al., 1990; Herbert et al., 2004). There is some debate as to at what age this relationship is not as correlative. For example, Bartholomeusz et al. (2002) stated that head circumference was an excellent predictor of brain volume in children 1.7 to 6 years of age, and only an adequate predictor after age 6.

Heavy Metals, Purkinje Cell Loss, and Increased Brain Volume

One aspect that Purkinje cell loss and increased brain volume have in common is that they both can be caused by environmental factors, specifically heavy metal toxicity (Ross et al., 1996; Sorensen et al., 2000; Kenntner et al., 2001; Hossain et al., 2004; Stoev et al., 2003; Piao et al., 2005). Heavy metals lodge in the brain, produce cellular degeneration, and decrease cellular function (Kenntner et al., 2001; Olson et al., 1984). A recent and important study conducted on rodents shows that lead induces the brain to swell (Hossain et al., 2004). This finding was associated with a 2-fold increase in vascular endothelial growth factor (VEGF). VEGF induces endothelial migration and proliferation, and vasogenic cerebral edema. It was also found that the cerebellum is preferentially susceptible to lead (the Purkinje cell in located only in the cerebellum). The authors state that this may be due to the delayed post maturation of the cerebellum as compared to the cerebrum (susceptibility to lead diminishes with maturity). An older study by Sundstrom and Kalimo (1987) also found the cerebellum was susceptible to lead, and interestingly, found the number of GFAP-positive cell bodies was increased in the cerebellar gray matter in lead-exposed newborn rats.

Another important recent finding is that mice that are susceptible (autoimmune disease-sensitive) and exposed to mercury after birth develop enlarged brains and autistic-like symptoms (Hornig *et al*., 2004). Another study found that the ethylmercury-containing preservative thimerosal disrupted in another growth factor, insulin-like growth factor 1 (IGF-1), and disruption in factor growth signaling (Waly et al., 2004). The metal ions disrupted normal IGF-1 activity and methionine synthase activity.

Studies suggest that toxic metals in the brain alter permeability, fluid balance, growth factors, and biochemical processes (Hornig et al., 2004; Quig, 1998; Hossain et al., 2004; Waly et al., 2004). The two studies mentioned earlier, Courchesne et al. (2001) and Herbert et al. (2004), stated that the enlargement of brain tissue in autism is suggestive of overgrowth. However, the possible role of altered growth factors, altered permeability, endothelial proliferation, or cerebral edema in the enlargement or "overgrowth" of the neurons of the tissue has not been parceled out. Bauman and Kemper (1994) found that neurons were enlarged in children with autism, which is suggestive of edema.

Evidence for Heavy Metal Toxicity

Examples of heavy metals include lead (Pb), mercury (Hg), cadmium (Cd), cobalt (Co), copper (Cu), and nickel (Ni) (Lopez-Artiguez and Repetto, 1993). Metals are environmental contaminants that are present in the environment ubiquitously; however, modern industry has brought about an increase (Soares et al., 2003). For example, a report from the Centers for Disease Control published in 1991 reported that 4 million preschool children in the United States have raised lead levels (CDC, 1991).

Anecdotal reports, as well as some studies, indicated that many children with autism possess abnormal/toxic levels of heavy metals (Holmes et al., 2003; Lonsdale, 2002; Filipek et al., 1999; Eppright et al., 1996; Accardo et al., 1988; Wecker et al., 1985; Shearer et al., 1982). For example, Filipek et al. (1999) found that 44% of autistic and psychotic children had blood lead concentrations greater than two standard deviations above the mean. Both Wecker et al. (1985) and Shearer et al. (1982) found lower levels of cadmium in the hair of children with autism/ASD. Lonsdale (2002) found children with autism had greater concentrations of arsenic in their urine than healthy controls. Typically reported are abnormal levels of mercury, lead, bismuth, cadmium, and arsenic (Lonsdale, 2002; Filipek et al., 1999; Eppright et al., 1996; Holmes et al., 2003; Fido and Al-Saad, 2005; Wecker et al., 1985; Shearer et al., 1982. Sulfhydryl-reactive metals, in particular, are found to be in high concentrations in autistic children (mercury, cadmium, lead, and arsenic are sulfhydryl-reactive metals) (Lonsdale, 2002; Quig, 1998).

Two pivotal studies, Holmes et al. (2003) and Bradstreet et al. (2003), influenced our understanding of toxic metals in autism. The study conducted by Holmes et al. (2003) of 94 children with autism who were gender- and age-matched to 45 controls, found that their first baby haircuts had mercury levels that were statistically significantly less than controls. The children with autism that were the most severe had the lowest levels and the children that were the least severe had the highest levels. The study also gathered information about the levels of Hg exposure (based on their fish consumption, exposure to mercury through childhood vaccines, and the mothers' amalgam fillings). The children with autism had higher levels of exposure than controls. This study suggests that children with autism may not be able to eliminate Hg and thus may accumulate it instead.

Bradstreet et al. (2003) found that when children with ASD and controls are treated with multiple doses of 2, 3-dimercaptosuccinic acid (DMSA) (an FDA approved chelating agent), the children with ASD excreted fives times as much mercury as controls. Evidence from the Holmes et al. (2003) study and the Bradstreet et al. (2003) study suggest that children with autism may be poor detoxifiers relative to normally developing children.

A recent study completed by Palmer et al. (2006) found that, in Texas, for every 1000 pounds of mercury released into the environment, there was a 61% increase in the rate of autism. This study was one of the first to show a correlation between environmentally released mercury and the rate of autism. It is important to note that inhaled mercury is almost completely absorbed by the lungs and crosses the placental and blood-brain barrier (Berlin et al., 1969; Yokel et al., 2006).

Further evidence for heavy metal toxicity is from the Autism Research Institute. The Autism Research Institute collected data from over 22,300 parents of children with autism on the behavioral effects of biomedical interventions. The survey includes a list of 45 medications, 23 non-drug supplements or biomedical treatments, and 9 special diets. The parents were asked to rate the treatment on a 6-point scale. Of these 77 choices, parents rated chelation therapy (or the removal of heavy metals) as the highest. Seventy-six% of parents said that their child "got better" on this treatment. The next most effective treatment was a gluten and casein free diet at 65% (ARI, 2006).

Heavy Metals and Oxidative Stress

There is a particularly negative correlation between glutathione (GSH) levels and oxidative stress associated with toxic metal exposure. GSH is found in almost every cell of the body and is responsible for the removal of toxic metals; GSH will be described in more detail in a later section. Exposure to heavy metals exerts detrimental affects on glutathione levels.

Arsenic exposure decreases GSH levels and increases lipid peroxidation in rats and subsequent damage results from oxidative stress. Interestingly, rats that are pretreated with GSH precursors

prior to the exposure to arsenic, perform better in regards to maintaining GSH levels and reducing lipid peroxidation (Osbaldo et al., 1995). Mercury and cadmium have high affinities for GSH. These metals bind irreversibly with GSH, and then the conjugated metal-GSH molecule is excreted (Quig, 1998; Zalups and Lash, 1996). ***When the GSH antioxidant system is compromised, the metals sequester in the brain*** (Quig, 1998).

Oxidative Stress and Lipid Peroxidation

Cells use nutrients and oxygen to produce energy. Reactive oxygen species (ROS) are a natural byproduct of the normal metabolism of oxygen. ROS are unstable atoms and are harmful because they possess an unpaired electron which will pair by "stealing" an electron. This produces disruption to other molecules and damage to cells (Gutman, 2002).

The relationship between lipid peroxidation and ROS is well established (Efe et al., 1999). Excessive ROS results in lipid peroxidation in membranes and this, in turn, results in loss of membrane integrity and fluidity which ultimately leads to cell death (Esterbauer et al., 1991). Neurons are particularly vulnerable to free radical attack. Excessive exposure to free radicals or an inadequate response to free radicals induces neuronal cell death (Jesberger and Richardson, 1991). Maugeri et al. (2004) found a negative correlation between free radical levels and cognitive ability in elderly persons.

Both increased lipid peroxidation (Bilici et al., 2001; Ozcan et al., 2004) and oxidative stress (Khanzode et al., 2003) are damaging to cells, particularly cell membranes. Glutathione is the component that protects against lipid peroxidation and oxidative stress (Lenzi et al., 1994; Shi et al., 1998).

A study by Lenzi and colleagues (1994) found that glutathione not only reduced lipid peroxidation and oxidative stress (Roy et al., 2000), but also reversed some of the damage of the cell membranes (Lenzi et al., 1994). Another more recent study showed that glutathione exerts neuroprotective properties and reduces neuropathy (Casinu et al., 2002). A study by Smyth et al. (1997) also found that raising glutathione levels improved concentration abilities in cancer patients.

As mentioned, Purkinje cells (the cells that are found to be reduced in children with autism) are vulnerable to oxidative stress. Oxidative stress produces Purkinje cell death and reduction in numbers (Heaton et al., 2000; Chen et al., 2003; Yamashita et al., 2000; Barlow et al., 1999). ***Importantly, research has found that administration of an antioxidant protects Purkinje cell survival against oxidative stress*** (Chen et al., 2003; Heaton et al., 2000).

Evidence for a Glutathione Deficiency and Oxidative Stress in Children with Autism

Five recent studies showed that oxidative stress and/or lipid peroxidation are increased in autism (Yorbik et al., 2002; Chauhan et al., 2004; Zoroglu et al., 2004, James et al., 2004; Sogut *et al*., 2003). Sogut and colleagues (2003) and Zoroglu and colleagues (2004), found increased oxidative stress and enzymatic antioxidants in children with autism compared to gender- and age-matched normal controls. Sogut and colleagues (2003) found children with autism had increased red blood cell nitric oxide levels and increased glutathione peroxidase levels; Zoroglu and colleagues (2004) found increased red blood cell nitric oxide levels and increased thiobarbituric acid-reactive substances levels. Chauhan and colleagues (2004) found that lipid peroxidation was increased and antioxidant proteins were decreased in children with autism. In addition, and most ***importantly, there was a correlation between the decreased antioxidant proteins and the loss of previously acquired skills***. Specifically, antioxidant protein levels (serum ceruloplasmin and transferrin) were reduced most strongly in the children who had lost previously acquired skills. Children with autism who had not regressed and the normal controls had similar levels. This finding implies a possible role of oxidative stress in the development of clinical symptoms in regressive autism. ***James et al. (2004) found lower total glutathione plasma levels*** and higher concentrations of oxidized glutathione in children with autism as compared to normal controls. The lower redox ratio of reduced glutathione to oxidized glutathione indicates increased oxidative stress. James et al. (2004) also found that plasma cysteine levels were lower in children with autism and, as mentioned, cysteine is the rate limiting precursor for glutathione. In the James et al. (2004) study, 19 of the 20 children had loss of previously required skills. James et al. (2004) stated that the increased vulnerability of oxidative stress (environment, intracellular, or both) and impaired methylation capacity may play a role in the development of clinical symptoms in regressive autism.

Other evidence for a glutathione deficiency is in the pathology seen in autism. Low glutathione levels may underlie many of the systemic abnormalities associated with autism. In autism, there is evidence for: (1) oxidative stress and lipid peroxidation; (2) toxicity, such as phenolic compounds (Edelson and Cantor, 1998) and toxic metals (Lonsdale, 2002; Filipek et al., 1999; Eppright et al., 1996; Holmes et al., 2003); (3) immune dysfunction, such as impaired or altered immune response and dysregulation of inflammatory cytokines (Cohly and Panja, 2005; Warren et al., 1990, 1992, 1995); and (4) impaired gastrointestinal integrity, such as epithelial pathology and increase gut permeability (D'Eufemia et al., 1996; Wakefield et al., 1998, 2000; Furlano et al., 2001; Horvath et al. 1999). GSH is important in each of these physiological processes. The following section describes what GSH is and how GSH is involved in these physiological processes.

Glutathione Structure and Function

Glutathione (GSH), or 2-amino-5-{[2-[(carboxymethyl)amino]-1-(mercaptomethyl)-2-oxoethyl]amino}-5-oxopentanoic acid, is a small protein made up of three amino acids: glycine, cysteine, and glutamic acid. Glutathione is a general term for glutathione sulhydryl, hence the abbreviation GSH. GSH is a thiol, and thus it containes sulfur (Sen, 1997). The side-chain sulfhydryl residue (-SH; sulfur and hydrogen) that is on the cysteine part of the molecule is responsible for most of its physiological properties (Sen, 1997). [A side chain is a part of a molecule attached to a core structure.] The sulfhydryl residue it is the critically active part of the molecule (Gutman, 2002). Importantly, sulfhydryl-reactive metals (mercury, arsenic, lead, and cadmium) bind with high affinity to sulfhydryl groups (Quig, 1998).

The main functions of GSH can be placed into three basic categories: (1) antioxidant, (2) detoxifier, and (3) immune system enhancer (Gutman, 2002; Bounous et al., 1993). GSH is called the master antioxidant because it is responsible not only for the metabolism of hydroperoxides and the direct scavenging of reactive oxygen species, it also is needed for the regeneration of other antioxidants such as vitamins C and E (Sen, 1997; Baruchel et al., 1998; Gutman, 2002). GSH is important in the reduction of oxidative stress and works by donating an electron to the free radical, neutralizing the free radical (Gutman, 2002). It is the principal protective factor in the cell. GSH is important in detoxification of xenobiotics (chemical substances that are foreign to the biological system, such as heavy metals). For example, Keith et al. (1997) examined the effectiveness of chelators in the removal of mercury from the rabbit kidney. GSH was almost as effective as the most powerful chelators (2, 3-dimercaptosuccinic acid (DMSA) and 2, 3-Dimercaptopropane- 1 – sulfonate (DMPS). At 3 hours, DMPS removed 95%, DMSA removed 87%, and GSH removed 75%. In addition, GSH removed the mercury without the negative side effect of zinc removal exhibited by the other chelators. GSH is imperative for the regulation, response, and maintenance of the immune system (Bounous and Molsen, 2003). GSH modulates the effect of inflammatory cytokines (Ho and Douglas, 1992). GSH is also important for maintaining gastrointestinal integrity and in the regulation of cell proliferation (Sen, 1997). Please see Table 1 which summarizes the relationship between GSH and the systemic abnormalities associated with autism.

Negative Cycle Can Result From Diseases or Disorders

As mentioned earlier, deficiencies in glutathione may be attributed to toxic metal exposure that increase the need for glutathione or inhibit glutathione formation (White et al., 1995). In addition, infection, pollution, stress, and a poor diet also deplete GSH (Gutman, 2002). Unfortunately, as the body becomes deleted in glutathione, the damage and insult that occurs consequently increase the need for glutathione and a negative cycle results.

Factors That May Interplay

After a child is born, heavy metal exposure and exposure to xenobiotics in general may result in accumulation if a child has limited or compromised detoxification ability. These levels may accumulate and reach "critical mass" and result in oxidative stress, decompensation, and neurological damage. This would result in cell loss and loss of previously acquired skills. A question that may be

TABLE 1. The relationship between GSH function and the systemic abnormalities associated with autism

Glutathione Function	Pathology Found in Autism
Responsible for the metabolism of hydroperoxides and the direct scavenging of ROS (Sen, 1997; Baruchel et al., 1998).	Increased lipid peroxidation (Chauhan et al., 2004)
Antioxidant; reduces oxidative stress (Bounous et al., 1993; James et al., 2004)	Increased oxidative stress (James et al., 2004; Chauhan et al., 2004; Zoroglu et al., 2004)
Detoxification of heavy metals (Sen, 1997)	Abnormal heavy metal levels (Holmes et al., 2003; Lonsdale, 2002; Filipek et al., 1999; Eppright et al., 1996; Accardo et al., 1988)
Detoxification of phenolic compounds (Sen, 1997)	Abnormal phenolic compounds levels (Edelson and Cantor, 1998)
Detoxification of acetominophen (Sen, 1997)	Impaired acetominophen metabolism (Waring and O'Reilly, 1990)
Helps maintain gastrointestinal integrity (Sen, 1997)	Impaired gastrointestinal integrity (Wakefield et al., 1998, 2000; D'Eufemia et al. 1996; Horvath et al., 1998, 1999; Furlano *et al*., 2001; Kern et al., 2002; Molloy and Manning-Courtney, 2003)
Essential for immune function and response (Sen, 1997); includes modulating the effect of inflammatory cytokines (Ho and Douglas, 1992).	Impaired immune function and response (Cohly and Panja, 2005; Weizman et al., 1982; Warren et al., 1990, 1992, 1995; Singh et al., 1993); including dysregualtion of pro-inflammatory cytokines (Cohly and Panja, 2005)

asked is why some children are more vulnerable to neurological insults than other children. It may be that many factors are involved and how these factors combine is important.

First, age at time of insult is a factor. The impact of environmental compounds in the body is a function of developmental age (Makri et al., 2004). Infant and fetal tissue appears to be less resistant to some toxic effects than older children and adults (Graeter and Mortesen, 1996). For example, a higher amount of lead is absorbed through the gastrointestinal tract by young children than adults. In rats, for example, main route of elimination of methylmercury is by secreting the toxin into bile. In neonatal rats, this ability to secrete mercury into bile develops between 2 and 4 weeks of age and is correlated with the increasing ability of the developing liver to secrete glutathione into bile. Prior to 2 and 4 weeks of age, they are more vulnerable to the mercury toxin (Ballatori and Clarkson, 1982).

Genetic predisposition is an important factor in vulnerability to insult. One of the best examples of this is Gulf War Syndrome. Some soldiers during the first Gulf War were exposed to neurotoxins and the effects were extreme in some cases. Evidence from the work of Haley and colleagues (1999) showed that one difference was in detoxification abilities of different soldiers related to enzyme activity, involving the paraoxonase/arylesterase 1 (PON1) gene. Another example is a study by Ghosh et al. (2005) that found that even though everyone is susceptible to arsenic, some people have more genetic susceptibility than others depending on the presence or absence of a gene called GSTM1.

Stress and other illnesses may play a role in the vulnerability to insult. For example, children who are on antibiotics at the time of heavy metal exposure are thought to make the children more susceptible to damage; Rowland et al. (1980) showed that oral antibiotics noticeably inhibit mercury excretion to 1/10 of normal in rats. Stress on the body, such as infection, produces additional metabolic demands that may result in increased vulnerability (Morton et al., 1991; Ulrich, 1997). A combination of a genetic predisposition and a stressor (e.g., infection) may increase the impact of insult. One example of this is the metabolic disorder glutaric aciduria, type I. Children with this metabolic insufficiency (typically seen in the Amish) develop normally until some insult or stress occurs, such as infection, fever, etc. During the time of stress, the underlying metabolic insufficiency cannot be compensated for, and the child begins to incur neurological damage as a result of

toxic buildup at that point. The neurological damage manifests itself in mental retardation, cerebral palsy, spastic paralysis, and/or encephalopathy (Morton et al., 1991; Ulrich, 1997).

Environment also plays an important role. For example, the study mentioned earlier by Palmer and colleagues (2006) found that the more mercury in the air, the greater the incidence of autism. It is known that children who live in older homes painted with lead-based paint are more likely to be lead toxic (CDC, 1991).

Diet is another relevant factor. Kidd (2002) found that glutamine (a building block for GSH) is low in some children with autism, especially in those with an aversion to meat and/or poultry. Thus, diet may play a role in making children more vulnerable to insult and stress.

CONCLUSION

The diversity of the biomedical findings and their variety of selection in different persons with autism suggest that they comprise a heterogeneous population in regard to etiology. The evidence presented in this article suggests that some of these children may be experiencing neuronal cell damage or death sometime after birth as result of insult. The evidence also suggests that these children may be selectively vulnerable to the impact from external or environmental factors. Some children with autism may be like the canary in the coal mine, exposing policy and/or environmental issues that need to be addressed.

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