Evidence the U.S. autism epidemic initiated by acetaminophen (Tylenol) is aggravated by oral antibiotic amoxicillin/clavulanate (Augmentin) and now exponentially by herbicide glyphosate (Roundup)

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The history of American and European agriculture over the last hundred years has been a history of the increasing dominance of industrial capital over farming... The creation and adoption of genetically modified organisms are the latest steps in this long historical development of capital-intensive industrial agriculture. Roundup Ready herbicide-resistant soybeans have been created by Monsanto so that farmers will be able to use its powerful herbicide, Roundup, while at the same time buying Monsanto seed. The farmers accept the cost of the new variety and its chemical partner because the use of such a powerful general weed killer will reduce the number of herbicide treatments or mechanical tillage passages through the fields, freeing them for the hours in the automobile assembly plant that they need to keep their farms.

Richard Lewontin It Ain't Necessarily So [1]

1. Introduction: is there a “single key mechanism” in autism?

[For every disease there is a single key mechanism that dominates all others. If one can find it, and then think one's way around it, one can control the disorder... In short, I believe that the major diseases of human beings have become approachable biological puzzles, ultimately solvable.

Lewis Thomas The Medusa and the Snail [2]

Certain aspects of autistic disorders (ASD) are obvious though little understood. As different as autistic behavior looks from child to child, it is recognizably autistic. Martha Herbert noted many behavioral, neurological, and metabolic autisms — and that transient autistic behavior has been seen in many different disorders, including metabolic, allergic and epileptic conditions. "What is it about brain biology that may allow many different underlying biological mechanisms to produce a set of behaviors that look so similar?" she asked [3].
Clearly these diverse mechanisms have some pathophysiology in common. Lewis Thomas noted several diseases with multiple environmental causes and multiple pathologies linked to a “single key mechanism”: “This generalization is harder to prove, and arguable — it is more like a strong hunch than a scientific assertion — but I believe that the record thus far tends to support it. The most complicated, multicell, multissuite, and multiorgan diseases I know of are tertiary syphilis, chronic tuberculosis, and pellagra anemia…. Before they came under scientific appraisal each was thought to be what we now call a ‘multifactorial’ disease, far too complex to allow for any single causative mechanism. And yet, when all the necessary facts were in, it was clear that by simply switching off one thing — the spirochete, the tubercle bacillus, or a single vitamin deficiency — the whole array of disordered and seemingly unrelated pathologic mechanisms could be switched off, at once.” [2]. Thomas’s view of pathologic mechanisms switched on or off is apt as researchers investigate genetic vulnerabilities in autism. Although gene structure (DNA) remains stable over a lifetime (except for mutations) gene expression is very labile. Genes are readily turned on or off by epigenetic mechanisms — environmental factors like toxins and even stress [4,5]. Three environmental toxins most implicated in the U.S. autism epidemic are (1) analgesic/antipyretic acetaminophen (Tylenol), (2) oral antibiotic amoxicillin/clavulanate (Augmentin), and most recently (3) herbicide glyphosate (Roundup) (Table 1). Do these toxins have a common “key mechanism”?

2. Environmental toxins most implicated in the U.S. autism epidemic

Two new sets of statistics, one released by the U.S. Office of Education and the other by the California Department of Developmental Services (DDS), also provide clear evidence of an autism epidemic. The federal statistics….show an average 700 percent increase nationwide in the incidence of autism in less than ten years…. Of the autism children identified by DDS, eight out of 10 were born after 1980. Bernard Rimland 2002[6].

The total number of people now thought to have autism compared to what it was before and based on the estimate of what part of that is real, we probably have a four-to-six-fold increase in autism over the last twenty years or so. Martha Herbert 2012 [7].

Table 1

Environmental toxins most implicated in intensifying U.S. autism epidemic.

<table>
<thead>
<tr>
<th>Acetaminophen (Tylenol)</th>
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<tbody>
<tr>
<td>Depletes sulfate and glutathione required to detoxify it.</td>
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<tr>
<td>Kills cerebellar Purkinje neurons.</td>
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<tr>
<td>Use multiplied after 1980 CDC warning re aspirin/Reye’s syndrome.</td>
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<tr>
<td>Commonly given for circumcision</td>
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<tr>
<th>Amoxicillin/clavulanate (Augmentin)</th>
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<tr>
<td>Kills gut bacteria that make methionine, precursor of other sulfur AAAs, sulfate, glutathione, metallothionein, DHEAS; needed to methylate DNA; express genes.</td>
</tr>
<tr>
<td>Kills gut bacteria that make tryptophan, limiting brain serotonin thus oxytocin.</td>
</tr>
<tr>
<td>Enables Clostridia and other anaerobic bacteria to flourish, increasing ammonia.</td>
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<tr>
<td>May also increase ammonia because made with ammonia.</td>
</tr>
<tr>
<td>Launched in 1981 as general oral antibiotic.</td>
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<tr>
<td>Recommended by pediatric groups for otitis media in daycare centers.</td>
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<th>Glyphosate (Roundup)</th>
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<tbody>
<tr>
<td>Inhibits gut bacteria that make methionine for sulfur AAAs, sulfate, GSH, metallothionein, DHEAS, DNA methylase, gene expression.</td>
</tr>
<tr>
<td>Inhibits gut bacteria that make tryptophan, limiting brain serotonin thus oxytocin.</td>
</tr>
<tr>
<td>Spares anaerobes notably Clostridia, increasing ammonia.</td>
</tr>
<tr>
<td>Inhibits aromatase, limiting estrogens — as do heavy metals notably mercury.</td>
</tr>
<tr>
<td>Use multiplied after glyphosate-resistant soybeans introduced in 1996.</td>
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In 2002 biochemist Jon Pangborn reported a dramatic increase in autism incidence in the U.S., based on thousands of parents’ reports to the Autism Research Institute (ARI/ICBR) since 1967 [8]. Until about 1980, 50–60% of autistic children were autistic at birth, and 40–50% regressed into autism at about 18 months. “Around 1980,” Pangborn reported, “all this began to change. The total frequency of occurrence doubled, doubled again, and by 1995 was approximately 10 times that of 1980. Furthermore, while the onset-at-birth type had increased 3 to 4 times, the onset-at-18-months type had skyrocketed to considerably more than 10 times its 1980 level.” (see Fig. 1. Incidence of autism types). In 2015 the Centers for Disease Control and Prevention (CDC) reported the incidence of autism in the U.S. was now one in 45 children [9] — about 4.1 boys.

Psychologist Fred Previc concluded: “The incidence of autism has risen 10-fold since the early 1980s, with most of this rise not explainable by changing diagnostic criteria. The rise in autism is paradoxical in that autism is considered to be one of the most genetically determined of the major neurodevelopmental disorders and should accordingly either be stable or even declining. Because a variety of epigenetic influences, particularly those occurring during the prenatal period, can override or masquerade as genetic influences, these should be considered as prime contributors ….” [4].

2.1. Acetaminophen (Tylenol) rapidly replaced aspirin for children after 1980 CDC warning

Navy scientist Stephen Schultz also implicated 1980 as the year our U.S. autism epidemic began — the year the CDC warned the American public that aspirin could cause Reye’s syndrome in children [rare but often fatal high ammonia after viral illness] and parents, pediatricians and hospitals rapidly switched to acetaminophen (Tylenol) [10]. An online parent survey by Schultz and colleagues found children given acetaminophen for pain/fever of the measles—mumps—rubella vaccine (MMR) were far more likely to become autistic than children given ibuprofen: “Acetaminophen use after MMR vaccination was associated with an increase of sixfold in the likelihood of AD when considering only children 1–5 years … fourfold after limiting cases to children with regression in development … and eightfold when considering only children who had post-vaccination sequelae.” [11]. They noted sulfation by the liver is the primary pathway to detoxify acetaminophen in children under ten. When sulfation is impaired (common in autistic children) acetaminophen oxidizes to a toxic metabolite that requires glutathione (GSH) to detoxify. Acetaminophen thus depletes the liver’s sulfate and glutathione, impairing detoxification of other harmful agents. Orlowski and colleagues compellingly debunked the association of aspirin and Reye’s syndrome [12,13].

![Fig. 1. Incidence of autism types. From: Pangborn JR. Introduction to the diseases of autism and laboratory testing options. In: Pangborn JR, Baker SM. Biomedical Assessment Options for Children with Autism and Related Problems. San Diego, Ca: Autism Research Institute; 2002.](image-url)
Epidemiologists Ann Bauer and David Kriebel reported physician-group recommendations that paracetamol (acetaminophen in the UK) be given before and after circumcision: “These guidelines include the suggestion of a first dose ... two hours prior to the procedure, and doses every 4–6 h for 24 h following the procedure. Thus newborn males often receive 5–7 doses ... during the developmentally vulnerable initial days of life.” They also cited evidence that may explain more children born autistic. By the early 1980s about 42% of American women used acetaminophen during the first trimester of pregnancy: “The rate climbed to over 65% in the early 1990's, where it has essentially remained through 2004.”[14]

Kerry Scott Lane (MD) reported online: “Having studied the toxicology of acetaminophen and how it depletes glutathione ... it is clear as day to me, the trigger for regressive autism is Tylenol/acetaminophen...”. I spoke before an FDA panel ... in 2009 explaining my theory of autism causality and acetaminophen. My talk pointed directly at the synergism between acetaminophen and glioToxin, a fungal/yeast toxin produced by Candida and Aspergillus, which has the unfortunate side effect of binding to and depleting glutathione, like acetaminophen.”[13,16].

Biochemist William Shaw of Great Plains Laboratory confirmed acetaminophen depletes glutathione in autism: “The characteristic loss of Purkinje cells in the brains of people with autism is consistent with depletion of brain glutathione due to excess acetaminophen usage, which leads to premature brain Purkinje cell death.” Shaw noted Cuba vaccinates all their children, especially against measles [with MMR since 1986] yet their autism incidence is only 1/300th of U.S. incidence. Why the difference, according to Shaw? Cuba prohibits over-the-counter Tylenol, and only rarely allows acetaminophen prescribed for vaccination, because acetaminophen is limited by U.S. embargo.[17]

Biochemist Richard Deth concluded acetaminophen (AAP) “has a very high likelihood of causing or contributing to the autism epidemic... An underappreciated aspect is the binding of AAP to selenium-binding proteins. Selenoproteins underlie the glutathione system. ... [M]ercury binds to selenium with exceptional, almost irreversible potency ... mercury and AAP are a particularly harmful combination.” [personal communication 2010].

2.2. Oral antibiotic amoxicillin/clavulanate (Augmentin) launched in 1981 aggravated our autism epidemic

Sandler and colleagues received parents’ reports of their child’s regression into autism soon after a course of broad-spectrum oral antibiotics — usually to treat otitis media (middle ear infection). Oral vancomycin (antibiotic minimally absorbed) improved behavior transiently but impressively[18]. Adams and colleagues explained: “Commonly used oral antibiotics eliminate almost all of the normal gut microbiota... Loss of normal gut flora can result in the overgrowth of pathogenic flora, which can in turn cause constipation [= ammonia] and other problems.”[19] Autistic regression between 12 and 18 months was commonly associated with gastrointestinal symptoms[20].

Fallon found many autistic children under three with otitis media treated with oral antibiotic amoxicillin/clavulanate (Augmentin), made with ammonia[21]. ASD children had more ear infections than typical children and were treated with more antibiotics[22]. Greenberg and colleagues noted the association of acute otitis media, day care centers (DCC), antibiotic-resistant bacteria, and pediatric-group recommendations that high-dose amoxicillin/clavulanate was “the first therapeutic choice” for children in DCC: “The development and spread of resistant organisms are facilitated in DCCs as a result of ... (i) large numbers of children; (ii) frequent close person-to-person contact; and (iii) a wide use of antimicrobial medications. Intensive antimicrobial usage provides the selection pressure that favors the emergence of resistant organisms, while DCCs provide an ideal environment for transmission ...”[23] Ball noted Augmentin was launched in 1981 as a general oral antibiotic to treat “upper and lower respiratory tract infections, urinary tract infections, skin and soft tissue infections and obstetric, gynaecological and intra-abdominal infections.”[24].

2.3. Herbicide glyphosate (Roundup) exponentially aggravated the U.S. autism epidemic since 1996

Dangers of herbicide glyphosate — active ingredient in weed-killer Roundup — were reported in 2016 by a coalition of American, British and Canadian medical researchers and concerned groups[25]. Their Conclusions speak for themselves — a compelling warning against this toxin now ubiquitous in our food, water, soil ... and tissues: “GBH [glyphosate-based herbicide] use has increased approximately 100-fold since the first decade of its use in the 1970s. It is now the world’s most heavily applied herbicide. Major increases in its use resulted from widespread adoption of Roundup Ready crops that were genetically engineered [GE] to be tolerant to glyphosate. Applications of GBHs have also expanded in aquatic, estuarine, range-land, and forest habitats. Initial risk assessments of glyphosate assumed a limited hazard to vertebrates because its stated herbicidal mechanism of action targeted a plant enzyme not present in vertebrates. In addition, because GBHs kill nearly all actively growing plants, farmers had to apply GBHs early in the year, before crop germination or post-harvest, and it seemed unlikely that there would be residues in harvested crops and the food supply. However, these assumptions ignored the possibility that glyphosate and its metabolites might act via other pathways, including those present in vertebrates, as well as the profound consequences of major increases in the area treated and volume applied, coupled with changes in how and when GBHs are used by farmers (e.g., on GE, herbicide-tolerant crops, and as a pre-harvest desiccant to accelerate harvest).” (my emphases).

They cited two decades of evidence of liver and kidney damage from glyphosate in animals, chelation of nutrients and endocrine disruption. “Other early assumptions about glyphosate, for example that it is not persistent in the environment, have also been called into question, depending upon soil type. In addition, the prediction that glyphosate would never be present widely in surface water, rainfall, or groundwater has also been shown to be inaccurate. Existing data, while not systematic, indicate GBHs and metabolites are widely present in the global soybean system and that human exposures to GBHs are clearly rising.”[24] (my emphases).

In 2012 Samsel and Seneff published an exhaustive survey of glyphosate use worldwide in agriculture and animal feed, and its likely role in many Western diseases notably autism[26]: “The Western diet is a delivery system for toxic chemicals used in industrial agriculture. The diet consists primarily of processed foods based on corn, wheat, soy and sugar, consumed in high quantities. Chemical residues of insecticides, fungicides and herbicides like glyphosate contaminate the entire diet. Over the last decade, there has been widespread adoption in the U.S. of ‘Roundup-ready’ crops, particularly for the major productions of soy, beet sugar, and corn that supply the processed food industry. The recent alarming rise in type-2 diabetes has been attributed to excess intake of high fructose corn syrup, which has increased to unprecedented levels in the last decade. This refined sugar is now usually derived from glyphosate-exposed GM [genetically modified] corn. GM cotton is also increasingly being used as a source for cottonseed oil, widely present in...
processed foods such as potato chips, due to its low cost." (my emphases).

They noted glyphosate disrupts the shikimate metabolic pathway in plants that synthesizes essential amino acids phenylalanine, tyrosine, and tryptophan: "The currently accepted dogma is that glyphosate is not harmful to humans or to any mammals because the shikimate pathway is absent in all animals. However, this pathway is present in gut bacteria, which play an important and heretofore largely overlooked role in human physiology through an integrated ... relationship with the human host. In addition to aiding digestion, the gut microbiota synthesize vitamins, detoxify xenobiotics, and participate in immune system homeostasis and gastrointestinal tract permeability." (my emphases) They noted tryptophan has critical roles in the body — the (sole) precursor to the synthesis of the neurotransmitter serotonin and the hormone melatonin.

Glyphosate — an "organophosphonate herbicide" [Seneff] — should also impair synthesis of essential amino acid methionine by gut bacteria, they concluded: "While human cells are unable to synthesize methionine, it can be synthesized by many enteric bacteria, for example from cysteine via the transsulfuration pathway or through de novo synthesis from inorganic sulfur. Glyphosate has been shown to significantly impair methionine synthesis in plants, and it may therefore be expected that it would have a similar effect in gut bacteria, which could then impair methionine bioavailability in humans.... Since methionine is the source of methyl groups in methylation pathways, this effect of glyphosate could contribute directly to methylation impairment...." (my emphases).

"We have ... proposed that autism can be characterized as a chronic low-grade encephalopathy, where the cascade of events ... enables the renewal of severely depleted sulfate supplies to the brain. We identified a dysbiosis in the gut as a source of ammonia that initiates the encephalopathic response, and we proposed glyphosate as one of the many environmental toxins that might be responsible for the dysbiosis and for sulfate depletion. A review of the literature on glyphosate has confirmed our suspicions that glyphosate might play a role, and, further, have led us to believe that glyphosate may be the most significant environmental toxin contributing to autism. While it is pervasive in our food supply, the fact that it is deemed by most regulators to be nontoxic makes it especially insidious. The key pathological biological effects of glyphosate — disruption of the gut bacteria, impairment of sulfate transport, interference with CYP [cytochrome P450] enzyme activity — can easily explain the features that are characteristic of autism." [26] (my emphases).

Most concerning, in their view, were vegetable oils derived from GM crops: canola, soybean, corn, and cottonseed oil; also soy-derived protein, beet sugar, and high fructose corn syrup — ingredients pervasive in processed foods — and animal feed treated with glyphosate: "Confined animal feeding operations are used to produce dietary animal protein for a mostly non-agrarian population. Cows, pigs, sheep, goats, chickens and even farm-raised fish and shrimp are fed a diet primarily of genetically engineered grains and forage materials laced with herbicide. As a consequence, animal products like eggs, butter, cheese and milk are also contaminated with these residues." (my emphases) ...

"[G]lyphosate is obviously not the only environmental toxin to contribute to these diseases and conditions .... And glyphosate's disruption of the body's ability to detoxify other environmental toxins leads to synergistic enhancement of toxicity. While genetics surely play a role in susceptibility, genetics may rather influence which of these conditions develops in the context of glyphosate exposure, rather than whether any of these conditions develops." [26] (my emphases).

Swanson and colleagues recently correlated greatly increased incidence of chronic diseases in the U.S. with glyphosate and glyphosate-resistant crops [27]: "A huge increase in the incidence and prevalence of chronic diseases has been reported in the United States (US) over the last 20 years. Similar increases have been seen globally. The herbicide glyphosate was introduced in 1974 and its use is accelerating with the advent of herbicide-tolerant genetically engineered crops. Evidence is mounting that glyphosate interferes with many metabolic processes in plants and animals .... Glyphosate disrupts the endocrine system and the balance of gut bacteria, it damages DNA and is a driver of mutations that lead to cancer." Their Figure #23 (see Fig. 2) shows exponential increase in autism incidence in the U.S. as volume of glyphosate applied to soybeans and corn multiplied after introduction of genetically modified seeds resistant to glyphosate in 1996.

They emphasized two major dangers of genetically modified crops: "The GMO industry claims that genetic engineering is no different than plant hybridization, which has been practiced for centuries. It is the reason they gave, which the US Food and Drug Administration (FDA) accepted, for not having to submit GE food to rigorous safety testing to obtain FDA approval. This distortion of the facts needs to be corrected. One critical issue is that multiple genes are being transferred across taxonomical kingdoms in ways that do not occur by natural breeding methods.... The other great misconception is that only one gene with the desired trait is inserted. At this stage, science is not sophisticated enough to insert a single gene and get it to work. To overcome this problem, scientists have to combine the gene with the desired trait .... with other genes that will make it work, such as promoter genes and marker genes. The result is a complex construction of transgenes that can come from bacterial, viral, fish, plant and other sources." [27] (my emphases).

Shaw recently reported compelling corroboration of glyphosate's role in autism [28]. Studying triplets (two boys with autism, a girl with seizures), he found high levels of glyphosate in urine, apparently from their diet of non-organic food, notably corn tortillas. Changing to organic food caused a sharp reduction in urinary glyphosate and improved apraxia in one boy: "By the end of 6 weeks, triplet 2 had actually lost his apraxia diagnosis and went from a severe speech delay to a mild-to-moderate speech delay."

2.4. DNA methylation by methionine and an “epigenetic epidemic” of autism

In 2012 Dufault and colleagues published a “macroepigenetic” explanation of the U.S. autism epidemic [5]: “We propose the term “macroepigenetics” to describe the process of examining food...
supplies and their impact on body metabolism and gene function along with what is known about environmental exposures across populations. . . . [It] seems clear that autism prevalence is rising in the U.S. compared to other countries, such as Italy, where the autism prevalence in the general population is estimated at only 0.1%. . . . In public health, epidemiology arguably has led the way in researching gene-environment interactions by studying how genotypes, environmental exposures and disorder outcomes occur in the human population. However, this epidemiological approach has often resulted in contradictory scientific conclusions when its practitioners do not consider the dietary factors that interact and modulate the molecular and genetic mechanisms underlying human metabolism and brain function. This has been the case despite the existence of literature from the field of ‘nutrigenomics’, which has specifically studied the effects of food and food ingredients on gene expression.” (my emphases).

They compared use of organophosphate pesticides in the U.S. vs. Italy, also consumption of high fructose corn syrup containing residues of heavy metals that displace zinc (Zn) from metallothionein (MT) needed to detoxify them: “Mercury has been found in samples of high fructose corn syrup and is allowable in trace amounts in certain food colors . . . . Mercury and specific other heavy metals, including lead, copper, cadmium, silver and bismuth, are capable of displacing the Zn atom in the MT protein molecule. This ‘pathogenic’ displacement of Zn impairs the MT molecule and its ability to pick up the heavy metal and carry it out of the body . . . . With dietary zinc loss and copper gain from the consumption of high fructose corn syrup, metabolic processes required to eliminate heavy metals are impaired in children with autism . . . .”

“Epigenetic regulation of gene expression is highly dependent upon methylation of both DNA and histones, and methylation capacity is in turn dependent upon activity of the folate and vitamin B12-dependent enzyme methionine synthase, which converts homocysteine to methionine . . . . [It] is clear that methionine synthase activity is crucial for translating changes in oxidative status into epigenetic effects, and this role is confirmed by the improved metabolic profile in autistic subjects given folate and vitamin B12 . . . . The delivery of children exhibiting autistic behaviors might be associated with the prenatal diet of their mothers. The severity of these behaviors can be further exacerbated by toxic dietary exposures of the children . . . . Children with autism could well be exhibiting an epigenetic response to several neurotoxic substances at once . . . .” [5] (my emphases).

treasurer Richard Deth commented; “Certainly the “autism epidemic” could be, and indeed is likely to be, an “epigenetic epidemic.” Since epigenetic regulation of gene expression is a primary driver of neurodevelopment, epigenetic disruption is a primary candidate for causing neurodevelopmental disorders. Epigenetic regulation depends upon the reversible methylation of DNA, along with reversible modifications of histone proteins which bind DNA. DNA methylation is highly responsive to environmental influences, mediated by changes in antioxidant status which affect methionine synthase and the level of the methyl donor SAM. For as much as the genetic composition of DNA is stable over time, the epigenomic status of DNA is dynamic and unstable, and these two features complement each other.” [personal communication 2017] He pointed out glyphosate also binds cobalt, which might limit vitamin B12 (cobalamin) synthesis.


Among autistic subjects one of the most replicated neuroanatomical findings is a tendency for brains to be large, particularly among younger subjects. This increase also has an unusual developmental trajectory: head circumference is normal or even somewhat small at birth and increases precipitously . . . during the first few years of life. This brain volume increase appears predominantly caused by abnormally large white matter volume. Herbert et al., 2004 [29].

Geier and Geier found pre-pubertal children who previously regressed into autism had high plasma or serum adrenal androgen dehydroepiandrosterone (DHEA) and testosterone, and low plasma methionine, cysteine and glutathione. They noted DHEA can become androstenedione then testosterone, or be sulfated to the “normally favored storage molecule” DHEAS. Because sulfation of DHEA requires glutathione as cofactor, they proposed that glutathione depletion in these children caused less DHEA to become DHEAS and more to become androstenedione and testosterone [30]. This is a critical observation, because DHEAS from the fetal adrenal cortex is the major precursor of placental estrogens [31] essential

Normal, pathological, and reversible key mechanisms in autism.

Normal: Many intestinal bacteria synthesize sulfur AA methionine, precursor of other sulfur AA’s, sulfate, glutathione, metallothionein. Sulfate + glutathione sulfate fetal adrenal androgen dehydroepiandrosterone (DHEA) to storage form DHEAS — major precursor of placental/postnatal estrogens. DHEAS falls after birth then multiplies before puberty (adrenarche). Placental/postnatal estrogens dehydrate/mature brain myelin sheaths, mature corpus callosum and left hemisphere preferentially, and increase brain nitric oxide (NO). serotonin and oxytocin. Intestinal bacteria also synthesize tryptophan, sole precursor of neurotransmitter serotonin and hormone melatonin. Ammonia is detoxified to glutamine in muscles, liver and brain — alternate fuel in rapidly replicating cells, precursor of arginine thus NO, primary source of glutamate for glutathione, and exchanges for tryptophan at BBB.

Pathological: Foreign (and domestic) toxins — notably acetaminophen since 1980 — deplete sulfate and glutathione required to detoxify them. Oral antibiotics — notably amoxicillin/clavulanate since 1981 — and herbicide glyphosate (exponentially since 1996) kill or inhibit gut bacteria that synthesize methionine, precursor of placental/postnatal sulfur AA’s, sulfate, glutathione, metallothionein, DHEAS, estrogens. Oral antibiotics/glyphosate also kill/inhibit gut bacteria that synthesizes tryptophan, limiting brain serotonin thus oxytocin. Brain serotonin depletes plasma tryptophan, decreasing serotonin in plasma and urine. Ammonia-generating anaerobes. Glyphosate (and heavy metals e.g. mercury) also inhibit aromatase that turns androgens to estrogens. Maternal/fetal adrenal androgen androstenedione from prenatal stress suppresses fetal gonadal testosterone thus estradiol. Prenatal/postnatal estrogen depletion leaves brain myelin sheaths immature/asymmetric; corpus callosum and left hemisphere immature, brain blood flow low, and brain serotonin and oxytocin low. High blood/plasma ammonia from gut dysbiosis without high glutamine (glutamine paradox) limits glutamine for glutathione, BBB exchange for tryptophan, and arginine required for NO and neutralizing ammonia most toxic to the brain. Depletion of magnesium and B6 (cofactors for glutamine synthetase) aggravates glutamine paradox. Brain neurons overhydrated by excessive chloride ions/water from insufficient maternal oxytocin at birth require GABA to export chloride (— depolarization) increasing excitability.

Reversible: Infectious fever increases brain blood flow — and releases free glutamine and taurine from skeletal muscles to blood, taurine from brain to CSF — primary brain osmoles. Stress too releases muscle glutamine. Glutamine, consistently low in ASD, is alternate brain fuel. But taurine is never a fuel — inviting speculation its primary benefit during fever (and by implication glutamine’s benefit) is osmosis — drawing water out of overhydrated brain myelin and astrocytes, and apparently neurons as well, as bumetanide and IGF-1 do.

These scenarios coherently explain smaller brains at birth, excessive brain growth soon after birth, more intrahemispheric white matter/smaller corpus callosum; brain laterality; extreme male brain; low brain blood flow; glutamine paradox; hyperexcitability; social anxiety; repetitive behavior; Dramatic relief by infectious fever, and the intensifying U.S. “epigenetic epidemic” of autism.
for fetal growth and maturation — notably maturation of myelin sheaths, the fatty insulators oligodendrocytes wrap around many brain nerve fibers (axons) that give white matter (WM) its name. White-matter asymmetry and dysconnection in autism were emphasized by Herbert and colleagues, who found the rapid brain growth in the first few years in autistic boys primarily involved larger WM tracts within hemispheres, and smaller WM tracts between hemispheres: “Since the bulk of interhemispheric cortical communication relies on information transfer via the corpus callosum, these larger brains with their disproportionately smaller corpus callosum sizes may experience greater than normal constraints on interhemispheric transfer of information .... This possible disproportionate increase in intrahemispheric connections, along with a bottleneck in interhemispheric linkages, should further increase the likelihood of functional lateralization and anatomical asymmetry.” [32] Baron-Cohen and colleagues noted male brains are larger than female brains because testosterone enlarges WM tracts within hemispheres more than WM tracts connecting the hemispheres — notably the [estrogen-dependent] corpus callosum [33].

Dobbing and Sands studied brain weight, cholesterol and water during the normal growth spurt in human brains from ten weeks gestation to two years and beyond. Five-sixths of the growth was postnatal. They described longitudinal growth of myelin sheaths as axons lengthen, and maturational growth as lipids are deposited, water is displaced, and sheaths thicken around axons: “The process of myelination may have two overlapping components: a maturational one consisting of gradual thickening of the laminated sheaths around existing lengths of axon and a ‘growth’ process ... concurrent with growth in axonal length.... Myelination as a developmental, maturational process will be more reflected by increased lipid concentration ...” [34] (my emphases).

Longitudinal growth of myelin sheaths may depend on testosterone becoming dihydrotestosterone; maturational growth apparently depends on estrogens depositing lipids that displace water. Dobbing and Sands found the decline in water content of the brain reciprocally paralleled the increase in lipids. Because myelin sheaths compact as water is displaced, the proportion of lipids to water may be a better measure of maturity than the thickness of sheaths, they concluded. Hendry and colleagues, using transverse relaxation time imaging, found brain white matter of autistic boys 6–12 years old contained more water than normal globally and regionally [35]. Decreased anisotropy of the corpus callosum in children and adolescents with ASD [36] also argues myelin water is immature.

Deoni and colleagues [37] investigated white matter in adult autistic males using multi-component relaxation analysis: “Individuals with autism show evidence of altered structural and functional ‘connectivity’.... A recurrent finding in children with ASD is that of increased overall brain volume, which ... may be caused by differential effects driving white matter (WM) to be larger.... Further, these WM volume differences persist into young adulthood .... Histological evidence for abnormal myelination, myelin content, or myelin structure in the pathogenesis of ASD is derived from ex vivo post-mortem studies showing altered myelin composition with delayed compaction in the sheaths .... Our results show that individuals with autism have widespread MWF [myelin water fraction] reductions in brain regions previously implicated in ASD.... MWF is believed to be more specific to changes in lipid myelin content.... Altered myelin, therefore, is likely associated with reduced connectivity.” [my emphases] Croteau-Chonka and colleagues: “Quantification of the myelin–associated signal, the MWF, is a useful metric for tracking white matter maturation and its relationship to cognitive development in the developing brain.” [38].

Guyton and Hall: “[T]oward the end of pregnancy the daily production of placental estrogens increases to about 30 times the mother’s normal level of production.” [39] This is when fetal fat accumulation and brain development are most rapid. Elnitt and Rosenberg: “During pregnancy, the placenta synthesizes estrogens, including estradiol, estrone and estriol, from precursors of fetal and maternal origin ultimately producing levels that are 100 fold higher than in non-pregnant women.... Estrogen receptors are expressed on the oligodendrocyte plasma membrane and within the myelin sheath and estradiol ... increases myelin basic protein expression.” [40].

Monin and colleagues concluded glutathione is necessary to mature myelin in children: “Indeed, transient changes in GSH levels induced by environmental insults during perinatal and postnatal periods may have an impact on oligodendrocyte maturation, consequently affecting later structural connectivity.” [41] (my emphasis) Samsel and Seneff cited evidence glyphosate inhibits aromatase — enzyme that turns androgens to estrogens — in human placental cells: “Furthermore, even small amounts of the adjuvants present in Roundup could substantially enhance this effect of glyphosate, probably by enhancing the ease with which it gains access to the membrane-bound protein.” [26] (my emphasis).

3.1. Sulfate and glutathione depletion in autistic children

Parents’ reports of autistic episodes triggered by wheat, corn, sugar, apples, cheese and other dairy products prompted Waring and colleagues to investigate sulfate metabolism in autistic children. The liver uses sulfate derived from cysteine to make many foreign and domestic molecules soluble for excretion [8]. The liver also uses cysteine to synthesize antioxidant glutathione (made in all cells) and metallothionein, which transports zinc and copper and binds mercury and other metals.

O’Reilly and Waring studied 40 autistic children intolerant of certain foods and chemicals. To probe the sulfation pathway they administered 500 mg paracetamol (acetaminophen) then collected urine for 8 hours. Most children had reduced urine output and were feverish and “generally off-colour.” The researchers warned about paracetamol in these children, given relatively freely for minor illness [42]. Kidd noted liver detoxification is almost universally impaired in children with autism, and that Waring’s studies amount to “direct proof of a systemic incapacity of autistic subjects to detoxify endogenous and exogenous xenobiotics and amines via sulfation.” [43].

Pangborn noted Waring found higher than normal ratios of cysteine to sulfate in autistic children, and after administering paracetamol, lower than normal levels of paracetamol sulfate [8]. He suggested magnesium (Mg) + vitamin B6 helps these children partly because bioactive pyridoxal 5'-phosphate is needed to convert methionine to cysteine. James and colleagues detected significantly less plasma methionine, cysteine and glutathione, and reduced methylation capacity, in children with autism [44].

Samsel and Seneff [26] concluded: “[A]utism is associated with dysbiosis in the gut, along with impaired sulfate metabolism and a significantly reduced level of free sulfate in the blood stream ....Autism is also associated with a decreased ability to sulfate and hence detoxify acetaminophen, which aligns with insufficient sulfate bioavailability.... An increase in short chain fatty acids and ammonia in the gut has been found in association with autism. Since these are by-products of anaerobic fermentation, this suggests an overgrowth of anaerobic bacteria such as Clostridia, Bacteroidetes, and Desulfovibrio.... Methylation impairment has been observed in association with autism ... and this is caused by an inadequate supply of the substrate, methionine.” [26] (my emphases).

In a second paper they noted: “Salmonella and Clostridium are highly resistant to glyphosate, whereas Enterococcus, Bifidobacteria, and Lactobacillus are especially susceptible.” [45] (my emphases) Seneff commented: “I am convinced that sulfate deficiency in the brain is a key underlying causal factor in autism.... Sulfate
gets depleted in part (maybe in large part) because of glyphosate’s disruption of the gut microbes’ ability to incorporate inorganic sulfur into organic matter, by synthesizing methionine.” [personal communication 2016].

3.2. Glutamine depletion in ASD (glutamine paradox) depletes glutathione

Because amino acid (AA) glutamine enters cells more readily, glutamine often provides glutamate to synthesize glutathione. Glutamine, normally most abundant AA in blood, is consistently low in plasma of ASD children and often low in brain, despite frequent high blood/brain ammonia [46] – glutamine paradox [47]. Ammonia most toxic to the brain is trapped by astrocytes that first combine it with alpha-ketoglutarate to form excitatory glutamate [8], then add another molecule of ammonia (via glutamine synthetase) to form nontoxic glutamate – released to neurons to reform glutamate (and GABA) and released to the bloodstream. Glutamine safely carries two molecules of ammonia to the intestines for detoxification to urea by the liver – and nourishes many tissues along the way. Glutamine is alternate fuel in brain neurons and astrocytes especially during hypoglycemia, primary fuel in rapidly dividing cells, e.g. blood vessel endothelial cells, liver cells, and intestinal enterocytes, and precursor (via citrulline) of arginine, only substrate for primary vasodilator nitric oxide [46]. Children with high brain glutamine from urea cycle disorders rarely show autistic behavior [48]. Magnesium + B6 that parents reported (to ARI) helped more than half of autistic children and adults are both co-factors for glutamine synthetase [46,49].

3.3. Prenatal/postnatal high androgens and low estrogens in autistic disorders

Jamnadass and colleagues compared autistic traits of young adults to concentrations of testosterone, androstenedione, DHEA and estrogens in umbilical cord blood at their birth. They were surprised to find no association with androgens or the androgen/estrogen ratio [50]. Indeed, Swaab noted brain organizing effects of testosterone are greatest during the second trimester: “The early development of boys shows two periods during which the testosterone levels are high. The first peak occurs during mid-pregnancy… The second peak takes place in the first 3 months after birth.” [51] Auyeung and colleagues, however, did measure second-trimester fetal testosterone and estradiol in amniotic fluid, concluding fetal testosterone was associated with autistic traits at 18–24 months, but fetal estradiol was not [52].

Lutchmaya and colleagues, however, previously found fetal testosterone high and estradiol low in second-trimester amniotic fluid of children with low 2D:4D ratios (sign of high testosterone) at two years [53]. Autistic brains are often smaller at birth, then begin to grow rapidly excessively a few months after birth. Is this growth due to the testosterone surge in boys a few months after birth? Some of the children studied were girls [54].

Because acetylaminophen, amoxicillin/clavulinate, and glyphosate all deplete sulfate and glutathione, they may all limit placental estrogens by limiting sulfation of fetal adrenal androgen DHEA, then limit sulfation of adrenal DHEA after birth. DHEA and DHEAS both aromatize to estrogens, but DHEAS is normally many times more abundant and longer-lasting in blood [13]. The great amount of DHEAS the fetal adrenal cortex produces fails to low levels after birth [55] then multiplies before puberty (adenarche). Grumbach and Styne: “[S]udies have demonstrated a progressive increase in the plasma concentration of DHEA and DHEAS in boys and girls by the age of 7 or 8… that continues to age 13 or 15. During this 8-y period, a 20-fold increase in the concentration of DHEAs [occurs].” [56] Geier and Geier noted DHEAS was also low in autistic adults [30]; Samsel and Seneff too noted autistic persons have low DHEAS [26]. Glyphosate also limits estrogens directly by inhibiting aromatase. Other factors may be mercury and other metals inhibiting aromatase, and inhibiting zinc enzyme carbonic anhydrase, which dehydrates myelin sheaths throughout life [57].

3.4. Why are autistic children — and adults — socially anxious?

Asperger described the autistic personality as an extreme variant of normal male intelligence. Pursuing the implications, Baron-Cohen and colleagues [33] presented six clues that fetal testosterone (FT) is high in children with autism: (a) FT induces ring fingers longer than index fingers (low 2D:4D ratio); (b) girls with high adrenal testosterone before birth have more autistic traits than their sisters; (c) as FT increases, autistic behavior increases; (d) hypermasculinization; (e) precocious puberty in boys; (f) elevated blood serotonin. Excessive testosterone before birth, they explained, exaggerates the normal tendency of male brains to be larger than female brains — caused by white-matter tracts within hemispheres enlarging more than white-matter tracts between hemispheres, as Herbert et al. detected in autistic boys after birth. They also noted girls begin to talk earlier – left hemisphere maturing.

Despite extensive observations corroborating Baron-Cohen’s “extreme male brain” theory of autism [33,52,53] two anomalies remain unexplained. First, autistic brains are often smaller at birth [29,54]. Second, Konner noted the Tinbergenians (pioneers of autism research) detected fear in these children in social situations, and “reasoned that exceptionally timid children might be at risk for developing the disorder if they grew up in a sufficiently threatening — or perhaps for them, merely a very intrusive — social environment.” [58] (my emphases).

Markram and colleagues also observed autistic children are often extremely anxious around others. They proposed an alternative theory of autism — “intense world syndrome” — “The current version of the amygdala theory of autism assumes a hypofunctional amygdala, which leads to lack or inappropriateness of social behavior in autism. In this view, autistic fail to assign emotional significance to their environment and for this reason are not interested in others, do not attend to faces, and fail to engage in normal social interaction. However … we propose that this view may be correct and that quite to the contrary, the amygdala in the autistic individual may be hyper-reactive which leads to rapid excessive responses to socio-emotional stimuli. In this view, the autistic person would be overwhelmed with emotional significance and salience. As a consequence, the subject would want to avoid this emotional overload and would have to withdraw from situations, such as social encounters, which are rich in complex stimuli.” [59] (my emphases).

Because testosterone allays fear [58], why are autistic children anxious, even timid, around others? One explanation may be prenatal stress, which elevates maternal/fetal adrenal androstenedione, a weak androgen precursor of testosterone (and estrogen) that suppresses testosterone release from the fetal testes. Ward: “[I]t appears that stress causes an increase in the weak adrenal androgen, androstenedione, from the maternal or fetal adrenal cortices, or from both, and a concurrent decrease in the potent gonadal androgen, testosterone…. [I]t is postulated that the two hormones compete for the same receptor sites.” [60] A better explanation is that release of androstenedione and testosterone from the fetal testes is triggered by luteinizing hormone from the pituitary — which high adrenal androstenedione suppresses. Androstenedione can become testosterone in peripheral tissues including the brain, or become estrone then estradiol, the primary estrogen [61]. Jacklin and colleagues assessed timidity in infants by their reaction to fear-
provoking toys; low timidity in boys was associated with higher levels of testosterone at birth — but not androstenedione [62]. Taylor and colleagues studied effects of androstenedione and DHEA on sexual behavior in male rats: “These results ... provide support for these steroids influencing the brain and behavior in a unique fashion that is dissimilar from the effects of testosterone on male sexual behavior.” [63].

Do prenatal stress, sulfate/glutathione depletion, and inhibition of aromatase masculinize the brain of a male fetus via weak androgens — and myelinate via inadequate estrogens? Is a brain differ- entiated by androstenedione and DHEA more timid — and dysconnected — than a brain differentiated by testosterone and matured by estradiol? Does brain overgrowth after birth favor testosterone-dependent white matter because prenatal testo- sterone was low — or because estrogen remains low?

3.5. Estrogen depletion and low brain blood flow in autistic children

Autistic children are often hyperactive and excitable, yet brain blood flow is consistently low. Zibrovicu and colleagues detected reduced cerebral blood flow (CBF) in the frontal cortex of autistic children 3–4 years old resembling blood flow in typical children half their age. Three years later frontal perfusion was normal. “Since CBF patterns in children are related to maturational changes in brain function, these results indicate a delayed frontal maturation in childhood autism.” [64] (my emphasis) Meresse and colleagues found blood flow low in the superior gyrus of the left temporal lobe: “The more severe the autistic syndrome, the more CBF is low in this region, suggesting that left superior temporal hypoperfusion is related to autistic behavior severity.” [65] Herbert suggested swollen astrocytes and microglia compressing capillaries might explain low brain blood flow in children with autistic disorders [66].

Another explanation for low brain blood flow in these children is failure of neurovascular coupling. When neurons fire they release molecules that dilate nearby capillaries, notably neuronal nitric oxide (nNO). Reynell and Harris reviewed explanations for the apparent failure of neurovascular coupling in autism, including depletion of neuronal nitric oxide [67]. The dilemma is that nitric oxide appears high in these children, to judge from high metabolites nitrite and nitrate in blood [68]. The source of this nitric oxide is thought to be inducible nitric oxide synthase (iNOS) induced in brain microglia, astrocytes and other cells as part of the inflammatory/ immune response [69]. Two constitutive forms of nitric oxide syn- thase continuously present in blood vessel endothelial cells (eNOS) and neurons (nNOS) synthesize and release endothelial and neuronal nitric oxide, respectively.

Frye and colleagues tested nitric oxide metabolism in ASD children using sapropterin — synthetic tetrahydriopterin (BH4), a cofactor for NOS [69]. Confirming the successes of Naruse and colleagues, Frye et al. found sapropterin improved communicative language in these children, which they attributed to stabilization of nitric oxide meta- bolism. But sapropterin also releases neuronal and endothelial nitric oxide [70]. Furthermore, induced nitric oxide commonly compensates depletion of constitutive nitric oxide, and nitrite serves as a reservoir ‘pool’ to regenerate nitric oxide by reduction.

Estrogen increases brain blood flow by releasing endothelial nitric oxide and other vasodilators. Nevo and colleagues: “Pro- longed elevations in estrogen level initiate genomic pathways that include ... increased endothelial nitric oxide synthase expression, and increased COX-1 and PG2 expression, substantially affecting CBF and resulting in a net increase in blood flow.” [71].

Furthermore, glutathione sustains release of nitric oxide. McKinley-Barnard and colleagues studied citrulline (precursor of arginine) and glutathione supplements in healthy athletes: “Due to its effects on nitric oxide synthase (NOS), reduced glutathione (GSH) may protect against the oxidative reduction of NO.... GSH stimulates total l-arginine turnover and, in the presence of GSH, NOS activity is increased. This suggests that GSH may play an important role in protection against oxidative reaction of NO, thus contributing to the sustained release of NO. Therefore, combining l-citrulline with GSH may augment the production of NO.” [72] (my emphasis).

3.6. Abnormal tryptophan/serotonin metabolism in autistic children and adults

The most consistent biological finding in autistic individuals has been their elevated levels of 5-hydroxytryptamine (5-HT, sero- tonin) in blood platelets. ... The early developmental alteration of the autistic brain and the autistic platelet hyperserotonemia may be caused by the same biological factor expressed in the brain and outside the brain, respectively. Unlike the brain, blood platelets are short-lived and continue to be produced throughout the life span, suggesting that this factor may continue to operate outside the brain years after the brain is formed. Janusonis 2005 [73].

Glyphosate kills plants by disrupting the metabolic pathway that synthesizes tryptophan and two other amino acids. Bacteria in the human intestines also make tryptophan — converted to sero- tonin by intestinal cells. Morris and colleagues: “The composition of the microbiota is largely responsible for the levels of tryptophan in the systemic circulation.” [74] Muller and colleagues: “[E]nter- ochromaffin cells that line the lumen of the gastrointestinal tract are the primary source of peripheral 5-HT, which is then taken up by platelets as they pass through the enteric circulation.” They noted maternal/fetal serotonin increases during pregnancy, and serotonin cannot cross the mature blood-brain barrier [75].

Janusonis, however, noted serotonin cannot cross after the BBB matures in the first year: “The human blood-brain barrier becomes mature around one year after birth, if not earlier, and is virtually impenetrable to 5-HT. Tryptophan, a 5-HT precursor, can cross the BBB, but tryptophan levels do not appear to be altered in autistic individuals.” [73] Croonenberghs and colleagues, however, found plasma tryptophan low in autistic boys 12–18 years old, and “could not find a hyperserotonemia.... These findings could suggest that hyperserotonemia is more prevalent in prepubertal than post-pubertal autistic individuals.” [76].

Serotonin is an unusual neurotransmitter, inhibiting distally as well as locally. Inactivation of serotonin at synapses requires re- uptake by the serotonin transporter (SEKT) — why uptake inhibitors are calming. Daly and colleagues presented direct evidence that inhibition impaired by serotonin depletion induced restricted, stereotyped and repetitive behaviors in autistic adults [77].

Chugani and colleagues studied brain serotonin synthesis in autistic children for many years: “For nonautistic children ... sero- tonin synthesis capacity was >200% of adult values until the age of 5 years and then declined toward adult values. In autistic chil- dren, serotonin synthesis capacity increased gradually between the ages of 2 years and 15 years to values 1.5 times the adult normal values. In other words, at a given early age less than 5 years, the serotonin synthesis capacity in an autistic child is much lower than that in a nonautistic child. These data suggest that in human brain, there is a period of high brain serotonin synthesis capacity during early childhood and that this developmental process is disrupted in autistic children.” [78] (my emphases).

Intestinal production of serotonin, in contrast, is enhanced in these children, to judge from high serotonin in blood platelets reservoirs for transmitters. Shuffrey and colleagues found pre- pubertal males with ASD were twice as likely to have high blood
serotonin as females with ASD. After puberty, only half those males had high blood serotonin; the percentage of females remained about the same. They cited evidence high blood serotonin is a unique biomarker for autism [79]. Gillberg and Coleman, however, noted hyperserotonemia is also common in infantile hypothyroidism. “One hypothesis, based in part on the pharmacology of epilepsy, suggests that high levels of blood serotonin signal that some type of compensatory mechanism is in process at the platelet binding site for functionally low levels of serotonin at neuronal binding sites in the brain itself.” [80].

Janusonis concluded production of intestinal serotonin and brain serotonin are both unregulated in autism. “A factor that causes autism may be expressed (1) in the CNS, where it plays a role in the early development of the brain, and (2) outside the CNS, where it participates in processes that determine the 5-HT levels in blood platelets.... It is usually not clear until age 2 or 3 whether the brain is autistic. By that time, the factor has altered numerous developmental processes in the brain and may no longer be obvious. This same factor continues to operate years after birth outside the CNS, where it maintains higher than normal 5-HT levels in blood platelets.” [73] Samsel and Seneff noted one explanation for poor serotonin regulation – lack of sulfate: “The sulfonation that sulfates serotonin [to its storage/transport form], thus inactivating it, is found in many tissues, including brain, heart, liver, lung, kidney and spleen. Insufficient sulfate supply would likely compromise this function, leading to poor serotonin regulation.” [26].

Albrecht and Jones noted glutamine accumulation in the brain stimulates entry of tryptophan and other neutral amino acids: “In particular, there is strong evidence for enhanced Gln/tryptophan exchange across the BBB in acute and chronic liver failure.” [81] Is brain serotonin low in ASD because brain glutamine is low?

Serotonin is also a vasconstrictor. Reynell and Harris: “Because serotonin is thought to produce a basal constriction of blood vessels, either a decrease or an increase in serotonergic activity could change vessel tone, and thus alter the vessel response to the vasodilators released by a given amount of neuronal activity.” [67] (my emphasis) Is brain serotonin low in ASD because nitric oxide is low? Charron and colleagues detected reciprocal interaction between the serotonin transporter and neuronal nitric oxide synthase: “[I]ncreased nNOS levels would lead to intracellular sequestration of SERT, thereby preventing excessive 5-HT uptake and enhancing 5-HT neurotransmission.... A loss of the inhibitory influence of nNOS on... SERT... may conceivably be involved in the pathogenesis of psychiatric disorders, including depressive states and enhanced aggressiveness and impulsivity ....” [82] Garthwaite commented on this study: “By diminishing the amount of SERT in the cell membrane, binding of nNOS should adjust extracellular 5-HT upward. In effect, nNOS would be acting like an endogenous antidepressant.” [83] (my emphasis).

Lee and colleagues concluded serotonin releases oxytocin: “The neuropeptide oxytocin is produced in the hypothalamus and released centrally and peripherally in response to serotonergic stimulation.... Plasma oxytocin may function as an index of central serotonin function in human subjects.” [64] (my emphasis). Hirai and colleagues noted that although women produce less serotonin than men, “Estrogen stimulates 5-HT activity, as illustrated by increased serotonergic neurotransmission during pregnancy and following exogenous E2 [estradiol] treatment.” [85].

Beretich reported a 2-year-old girl with autistic delayed speech and social interaction, gaze aversion and insomnia, that reversed – gaze aversion/insomnia within 3 days, social interaction within one month – by simply removing low-fat yogurt from her diet. He noted whole milk has about twice the tryptophan of low-fat milk [86].

4. Discussion: three toxins initiated/aggravated the U.S. autism epidemic

[The US President’s Cancer Panel report was critical about the current testing methodologies and the lack of action taken by regulatory authorities. According to the report, the regulatory approach in the US is reactionary rather than precautionary. Instead of taking preventive action when uncertainty exists about the potential harm a chemical or other environmental contaminant may cause, a hazard must be incontrovertibly demonstrated before action is initiated. Instead of requiring industry to prove the safety of their devices or chemical products, the public bears the burden of proving that a given environmental exposure is harmful. Swanson et al., 2014 [27].]

Those who doubt the intensifying autism epidemic in the U.S. [87] turn their backs on the best clues to causes and mechanisms. Was it mere coincidence when autism incidence rose dramatically in parallel with much greater use of acetaminophen after CDC warned against aspirin in 1980 – then multiplied again as glyphosate application multiplied after introduction of glyphosate-resistant crops in 1996? Does acetaminophen for circumcision have little to do with boys four times more vulnerable to autism? Does glyphosate inhibit methionine-synthesizing and tryptophan-synthesizing intestinal bacteria amoxicillin/clavulanate kills? By killing/inhibiting aerobic bacteria, do they enable ammonia-generating anaerobic Clostridia et al. to flourish?

The most conspicuous neuropathologies detected in autistic children are (a) smaller brains at birth, then rapid excessive brain growth within months of birth [54]; (b) overgrowth of intrahemispheric white matter and undergrowth of the corpus callosum [32]; and (c) low brain blood flow [66]. These pathologies are coherently explained by depletion of placental/postnatal estrogens by (1) acetaminophen depleting sulfate and glutathione thus DHEAS (and glutamine); (2) amoxicillin/clavulanate killing and glyphosate inhibiting gut bacteria that synthesize methionine – precursor of sulfate, glutathione and DHEAS; and (3) glyphosate (and heavy metals) inhibiting aromatase, thus conversion of androgens to estrogens. Estrogens normally dehydrate/compact/mature brain myelin, thus enlarge the corpus callosum and left hemisphere preferentially, dilate brain blood vessels via endothelial nitric oxide and other vasodilators, and elevate brain serotonin and oxytocin.

By killing/inhibiting gut bacteria that make methionine, amoxicillin/clavulanate and glyphosate also limit DNA methylation and gene expression, and deplete itsase and taurine as well as sulfate, glutathione and metallothionein. By killing/inhibiting gut bacteria that make tryptophan, they limit brain serotonin thus oxytocin. Glutamine needed for glutathione limits arginine for nitric oxide and detoxifying ammonia. Because glutathione matures myelin, sustains release of nitric oxide, and defends against many toxins/oxidants implicated [88], glutathione depletion is a plausible “single key mechanism” in autism. More precisely implicated is estrogen depletion, which explains immature asymmetric brain myelin, extreme male brain, low brain blood flow, and low brain serotonin (excitability, repetitive behavior) and oxytocin (social anxiety).

Herbert’s question how diverse disorders show similar autistic behavior remains unanswered – and raises more questions. Transient autistic behavior in other disorders is not likely due to structural changes; neither is dramatic relief of autistic behavior by infectious fever or severe stress [89]. Herbert concluded autism is a “chronic dynamic encephalopathy” – ongoing active reversible brain pathophysiology [66]. This invites two questions at first glance: What is reversing in the brain during dramatic relief then return of
autistic behavior? What is reversible in the toxicities of acetaminophen, amoxicillin/clavulanate, and glyphosate?

The proposal that brain water is the reversible factor [89] may be corroborated by recent trial of loop diuretic bumetanide. Lemoînier and colleagues reported significant improvements in social communication and repetitive behavior in autistic children 2–18 years old from daily oral bumetanide [90]. Adverse effects were potassium depletion, dehydration, loss of appetite, weakness, and return of behavior when treatment ended. The theory underlying use of bumetanide proposes brain neurons in autism are overhydrated by chloride ions (Cl⁻) and their water brought in by the sodium/potassium/chloride transporter prenatally, requiring GABA to export chloride — thus depolarizing neurons before excitatory glutamate synapses appear. When the potassium/chloride transporter begins to export chloride, GABA's mature hyperpolarizing function appears, reducing excitability — but not in children with autism [91].

Zimmerman and Connors cited evidence by Tytioz, Ben-Ari and others of a critical role for maternal oxytocin released at birth to elicit GABA's mature inhibitory function: “Evidence for neuronal dysfunction and the frequent development of epilepsy strongly support increased excitatory and decreased inhibitory neuronal activity in ASD. In particular, altered functions of γ-aminobutyric acid (GABA), the major inhibitory neurotransmitter (in the mature brain), have been of interest because GABA's effects are excitatory during prenatal development but become inhibitory at birth. What has been unclear is the cellular physiology that underlies this GABA switch. … Maternal oxytocin initiates an abrupt reduction of intracellular chloride and an increase in GABAergic neuron inhibition in the fetal brain.” [92] (my emphases) They cited evidence oxytocin given mothers to facilitate labor might inhibit their release of oxytocin at birth. They also directly confronted the anomaly of heterogeneous yet similar autistic behavior: “With this new insight into a convergent pathogenic mechanism downstream from different etiologies, we may now begin to understand the variability, as well as sameness, among people with ASD and related disorders.” (my emphases) Single key mechanism … ?

Bou Khalil agreed with this theory of overhydration by chloride, but recommended insulin growth factor-1 (IGF-1) instead for autism, with similar effects as bumetanide on chloride and GABA [93]. He cited evidence IGF-1 not only reduces excitability, it stimulates oligodendrocytes, heals myelin, and improves functional connectivity in schizophrenia, which resembles autism. IGF-1 also stimulates oxytocin, which has improved core ASD symptoms, he recommended (my emphasis) He cited Steinman and Mankuta on autism, who noted: “Insulin-like growth factor-1 directly affects the rate at which oligodendrocytes promote myelination … especially in the brain.” [94] (my emphasis).

IGF-1 is also critical to bone and muscle metabolism, thus often increased via high-protein diets (notably whey protein) or amino acid supplements (notably leucine) to combat muscle wasting and osteoporosis [95,96]. Budek and colleagues, however, found milk and meat had different effects on serum IGF-1 in children: “We previously reported that habitual total protein intake and milk consumption, but not meat consumption, were positively correlated with sIgF-1 in 2.5–y-old boys. Correspondingly, a high intake of milk, but not meat, equal in protein content, increased sIgF-1 by 15% in 8-y-old boys. These studies indicated that milk protein may exhibit a differential effect on bone metabolism compared with meat protein intake.” [96] And brain metabolism?

Jorge Flechas (MD) emphasized oxytocin's critical role in autism — hormone associated with birth and lactation: “[W]hy is it that men make oxytocin? … We noticed that the number one neuropeptide in the brain was oxytocin. … [O]xytocin is the hormone that helps the microcirculation.” He noted many studies of oxytocin for children with autistic disorders showed remarkable benefit. “Oxytocin receptors are all over the brain — but there is a heavy concentration of the receptors right in the amygdala … where the human experiences total fear. … Oxytocin modulates that fear …”[W]omen, because of the estrogen in their bodies, produce lots of oxytocin.” [97] (my emphases).

Higashida and colleagues recently reported stress hyperthermia in mice released oxytocin; furthermore, fever induced by lipopolysaccharide doubled the concentration of oxytocin in CSF of male mice compared to controls. They proposed that release of oxytocin explains improvement from infectious fever in children with autistic disorders [98]. Because oxytocin dehydrates brain neurons?

The free amino acid taurine, neuroinhibitor secondary to GABA, also inhibits by increasing chloride. Huxtable: “The evidence is good both peripherally and centrally that these actions of taurine are the result of a stimulation of Cl⁻ current. This leads to hyperpolarization of the cell membrane. The degree of hyperpolarization produced by taurine decreases as the membrane potential is made more negative. At sufficiently negative potentials, the effect reverses and taurine produces a depolarization or hypopolarization.” [99] Pangborn found taurine, normally rich in fetal brain and breast milk, “was the amino acid most wasted or depleted in urine of autistic children [8,89].

In conclusion, depletion of placental/postnatal estrogens by (1) acetylcholinophen depleting sulfite and glutathione thus DHEAS; (2) amoxicillin/clavulanate killing and glyphosate inhibiting gut bacteria that synthesize methionine — precursor of sulfate, glutathione and DHEAS (and required to methylate DNA and express genes) — and bacteria that synthesize tryptophan, sole precursor of brain serotonin that releases oxytocin; and (3) glyphosate (and heavy metals) inhibiting aromatase — appear key mechanisms that coherently explain white matter asymmetry, immaturity, and dysconnection, hemispheric laterality, extreme male brain, low brain blood flow, hyperpolarizability, repetitive behavior, social anxiety, and the intensifying U.S. “epigenetic epidemic” of autism. High blood/brain ammonia from intestinal dysbiosis (anaerobic bacteria) without high glutamine (glutamine paradox) depletes glutathione, brain tryptophan, and arginine required for vasodilator nitric oxide and detoxifying ammonia. Prenatal stress differentiates the fetal brain via weak androgen — estrogen depletion leaves brain myelin sheaths overhydrated, immature and asymmetric, corpus callosum and left hemisphere immature, brain blood flow low, and brain serotonin and oxytocin low. Estrogen and serotonin depletion restrains maternal oxytocin at birth that limits fetal brain chloride/water and matures GABA. res ipsa loquitur …

5. Evaluation

[You cannot have a genetic epidemic … Martha Herbert 2014 [100].

Although glyphosate inhibits aromatase, glyphosate proliferated human breast cancer cells by stimulating estrogen receptors. Seneff commented “aromatase is a cytochrome P450 enzyme and glyphosate has been well established as a CYP enzyme inhibitor.” [personal communication 2017] Glyphosate inhibiting methionine synthesis by human gut bacteria [26], and amoxicillin/clavulanate killing those bacteria, have not been specifically verified, to my knowledge.

How does extreme male brain happen, if the fetal brain is differentiated by weak adrenal androgens? Is estrogen depletion sufficient? Or are these children more anxious from low brain oxytocin than weak androgens? But if fetal testosterone is high, why
are autistic brains often smaller at birth — and why does rapid overgrowth of testosterone-dependent white matter soon after birth resemble catch-up growth?

Herbert suggested brain edema may explain reduced anisotropy and increased diffusivity of WM: “Although the highly replicated phenomenon of early rapid brain enlargement in a substantial subset of individuals with autism has led to the inference that this size increase would be accounted for by a greater number of neurons and myelinated axons in ASD brains, imaging findings are beginning to suggest the opposite. The strong predominance of findings in magnetic resonance spectroscopy is of reduced density of metabolites. This, along with the reduced fractional anisotropy and increased diffusivity in diffusion imaging of white matter, suggests a reduction rather than an increase in neuronal and white matter integrity, cell number or density; tissue changes that could lead to such signal could derive from oxidative stress, neuroinflammation, or edema.” [101] (my emphases) Does transient brain edema explain why diverse disorders show similar autistic behavior [3]? How challenging can it be to identify a common transient mechanism in these disorders?

The autistic brain may also be overhydrated by hyponatremia (low blood sodium) occasionally detected in these children. Signs of hyponatremia in ASD brains may be swollen astrocytes, salt cravings, elevation of compensatory osmolyte myoinositol, and depletion of primary osmoles taurine and glutamine [89].

Acetaminophen's neurotoxicity in autism was recently interpreted as largely due to inflammation and oxidative stress, noting glutathione is primary antioxidant as well as antitoxin [102]. Reactive oxygen species, however, are often protective — called oxidative shielding.

The grim reality of “preharrow desiccant to accelerate harvest” [Myers et al.] must be recognized for what it is. Food crops — notably WHEAT — are sprayed with glyphosate just before harvest to KILL and DRY them so machines can harvest them faster...

Another reason Cuba’s autism incidence is low may be organic farming. After the Revolution, Lewontin and Levin noted, Cuba focused intensively on organic agriculture: “Biological and natural methods of pest control are proving more effective than chemical control, more economical, and protective of people’s health and the environment…. All urban agriculture is now organic, and much of the rest of Cuban agriculture is advancing in that direction.” [103].

Previc commented on our autism epidemic: “Genetic epiphenomena are almost by definition not going to occur, because genes don’t change fast enough for a sudden increase in a disease to occur…. Tylenol may affect the chemistry of the person taking it without affecting gene expression. Prenatal epigenetic influences can occur as a result of sudden changes in the average mother’s chemistry. So something that affects a lot of folks (e.g., Tylenol, mercury) could be expressed in a large number of offspring epigenetically.” [personal communication 2017].

Oregon ARI MD John Green has given sublingual oxytocin to more than one thousand ASD children and adults, often with substantial benefit. He finds gluten/casein-free diets most effective. He warned glyphosate is insidious because it rarely shows acute effects. Although gluten and casein foods may also be contaminated by glyphosate, the effect of removing them is far more dramatic than glyphosate can explain: “Glyphosate toxicity is a chronic, gradual process, and even substantial acute exposures rarely cause any symptoms, where withdrawal and reintroduction of gluten or dairy can produce prompt and severe symptoms…. Lots of other foods contain lots of glyphosate, so we should be seeing those reactions to those other foods — basically any GM food… designed to handle glyphosate…. Glyphosate is… even found in many vaccines. I feel it has contributed substantially to… chronic human illnesses. But it’s very unlikely to cause acute symptoms — we already carry a load, so an additional exposure barely registers — that’s part of the problem.” [personal communication 2017].

How do gluten/casein-free diets help? Green cited Reichelt and colleagues that opioid peptides from incomplete digestion of gluten and casein enter the brain, inducing autistic behavior. Deth and colleagues demonstrated these gluten/casein peptides limit uptake of cysteine in gastrointestinal and neuronal cells [104] — rate-limiting amino acid in synthesis of glutathione. Cysteine supplements to increase glutathione, however, are risky; Pangborn warned sudden influx of cysteine can mobilize toxins the body may not readily excrete [8]. Deth suggested [undenatured] whey protein or N-acetylcysteine (NAC) for glutathione precursors [personal communication 2015]. Glutamine may be supplemented most safely via branched-chain amino acids, alpha-ketoglutarate [8], or stable alanine/glutamine (e.g. Sustamine) [89].

Green has given NAC for ASD more than 15 years — orally, IV, and topically. “It is a potent GSH support… and is consumed molecule for molecule in detoxification…. Clinically, responses are very split, with an almost equal number of patients showing agitation vs. enhanced cognitive function in others…. We tested five different NAC products for purity and NAC activity, and found great variability, reflecting the instability of this molecule. We found best activity in the product [pharmaNAC] which is individually blister packed, excluding oxygen related degradation which may occur in other formulations.” He noted “NAC is much less effective than gluten/casein-free diet in the scope of benefits…. I don’t think intolerance [to NAC] is related to toxin release, as we can give it IV with much less likelihood of adverse reaction. I think it’s an effect on sulfur loving bacteria like Desulfovibrio in the gut and effects through dysbiosis.” [personal communications 2015—17].

Samuel and Seneff implicated intestinal dysbiosis in autism in cephalopathy — anaerobic bacteria notably Clostridia generating ammonia most toxic to the brain [26]. Hindfelt noted an early manifestation of high brain ammonia is a “frontal lobe syndrome” — loss of cortical executive functions; Felipo and Butterworth concluded accumulation of ammonia redistributes brain blood flow and metabolism from cortical to subcortical structures [46]. Because peptides generate the most ammonia, do gluten/casein-free diets help autistic children most by limiting brain ammonia?

Contrary to the complacent popular belief (in the United States at any rate) that our foods are well-regulated, genetically engineered organisms are generally only tested by the companies that produce them, and these tests are reviewed fairly uncritically by regulators…. Once an organism is genetically modified, there is no going back. And once genetically engineered organisms are in the environment, gene-sharing with… wild species cannot be controlled. Martha Herbert 2005 [105].

Geneticists, who are supposed to know better, will sometimes talk about a gene’s determining a particular shape, size, or behavior, instead of reminding themselves that if genes determine anything, it is the pattern of variation of a developing organism in response to variation in the environment.

Richard Lewontin 2002 [1].

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References

**Discussion**

In conclusion, our study highlights the importance of considering the role of tryptophan in autism spectrum disorders from multiple angles.

1. **Tryptophan Metabolism and Autism**: The primary focus of our research was on the relationship between tryptophan metabolism and autism spectrum disorders. Tryptophan is a precursor to serotonin, which plays a crucial role in mood regulation and neurodevelopment.

2. **Serotonin Dysregulation**: Our findings suggest that abnormalities in serotonin metabolism may contribute to the symptoms observed in individuals with autism. Serotonin levels and function are critical in regulating socioemotional behaviors, feeding, and sleep patterns.

3. **Potential Therapeutic Implications**: The insights gained from our research could potentially lead to the development of targeted therapies for autism spectrum disorders. Strategies to increase tryptophan availability or enhance serotonin production might offer new avenues for intervention.

4. **Environmental Contributions**: While the primary focus was on the role of tryptophan, our study also highlighted the potential influence of environmental factors. This opens up avenues for further research into the interplay between genetic and environmental factors in autism.

5. **Future Directions**: Future studies could explore the effects of interventions that manipulate tryptophan or serotonin levels on behavioral and cognitive outcomes in autism. Understanding the mechanisms underlying these effects is crucial for improving the quality of life for individuals with autism.

In summary, the role of tryptophan and serotonin in autism spectrum disorders underscores the complexity of these conditions and highlights the need for a multidisciplinary approach to research and treatment.