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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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SUMMARY

Because certain hereditary diseases show autistic behavior, and autism often runs in families, researchers seek genes underlying the pathophysiology of autism, thus core behaviors. Other researchers argue environmental factors are decisive, citing compelling evidence of an autism epidemic in the United States beginning about 1980. Recognition that environmental factors influence gene expression led to synthesis of these views – an 'epigenetic epidemic' provoked by pervasive environmental agents altering expression of vulnerable genes, inducing characteristic autistic biochemistries in many mothers and infants. Two toxins most implicated in the U.S. autism epidemic are analgesic/antipyretic acetaminophen (Tylenol) and oral antibiotic amoxicillin/clavulanate (Augmentin). Recently herbicide glyphosate (Roundup) was exponentially implicated. What do these toxins have in common? Acetaminophen depletes sulfate and glutathione required to detoxify it. Oral antibiotics kill and glyphosate inhibits intestinal bacteria that synthesize methionine (precursor of sulfate and glutathione, and required to methylate DNA), bacteria that synthesize tryptophan (sole precursor of neuroinhibitor serotonin), and bacteria that restrain ammonia-generating anaerobes. Sulfate plus glutathione normally sulfate fetal adrenal androgen dehydroepiandrosterone to DHEAS – major precursor of placental/postnatal estrogens. Glyphosate (and heavy metals) also inhibit aromatase that turns androgens to estrogens. Placental/postnatal estrogens dehydrate/mature brain myelin sheaths, mature corpus callosum and left hemisphere preferentially, dilate brain blood vessels, and elevate brain serotonin and oxytocin. Stress-induced weak androgens and estrogen depletion coherently explain white matter asymmetry and disconnection in autism, extreme male brain, low brain blood flow, hyperexcitability, social anxiety, and insufficient maternal oxytocin at birth to limit fetal brain chloride/water and mature GABA.

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?

[F]or 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].

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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, multitissue, and multiorgan diseases I know of are tertiary syphilis, chronic tuberculosis, and pernicious 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 pathological 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 autistic 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.

Acetaminophen *(Tylenol)*
- Depletes *sulfate* and *glutathione* required to detoxify it.
- Kills cerebellar *Purkinje* neurons.
- Use multiplied after 1980 CDC warning *re* aspirin/Reye's syndrome.
- Commonly given for *circumcision*

Amoxicillin/clavulanate *(Augmentin)*
- Kills gut bacteria that make *methionine*, precursor of other *sulfur AAs*, *sulfate*, *glutathione*, *metallothionein*, *DHEAS*; needed to *methylate DNA*/*express genes*.
- Kills gut bacteria that make *tryptophan*, limiting brain *serotonin* thus *oxytocin*.
- Enables *Clostridia* and other *anaerobic* bacteria to flourish, increasing *ammonia*.
- May also increase ammonia because made with ammonia.
- Launched in 1981 as *general oral antibiotic*.
- Recommended by pediatric groups for *otitis media* in *daycare centers*.

Glyphosate *(Roundup)*
- Inhibits gut bacteria that make *methionine* for *sulfur AAs*, *sulfate*, *GSH*, *metallothionein*, *DHEAS*, *DNA methylation*, *gene expression*.
- Inhibits gut bacteria that make *tryptophan,* limiting brain *serotonin* thus *oxytocin*.
- Spares *anaerobes* notably *Clostridia*, increasing *ammonia*.
- Inhibits *aromatase*, limiting *estrogens* – as do *heavy metals* notably *mercury*.
- Use multiplied after glyphosate-resistant *soybeans* introduced in 1996.

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].

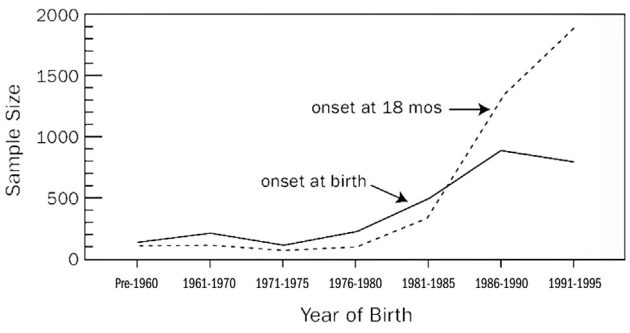

Fig. 1. Incidence of autism types. From: Pangborn JB. Introduction to the diseases of autism and laboratory testing options. In: Pangborn JB, Baker SM. Biomedical Assessment Options for Children with Autism and Related Problems. San Diego, Ca: Autism Research Institute; 2002.

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/paracetamol …. With limited or near zero glutathione in the brain at time of vaccination, the body is unable to detoxify the metals in the vaccines via the metallothionein system…. 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." [15,16].

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 real 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, rangeland, 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 so 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 anticipated 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 encephalytic 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*

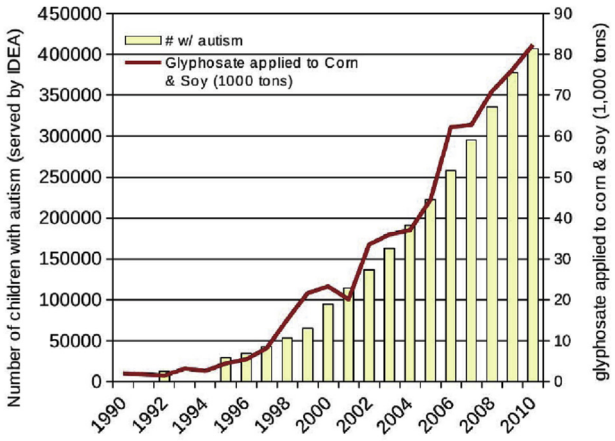

Fig. 2. Number of children (6–21 yrs) with autism served by Individuals with Disabilities Education Act (IDEA) plotted against glyphosate use on corn & soy (R = 0.9893, p <=3.629e–07) Sources: USDA:NASS; USDE:IDEA. From: Swanson NL, Leu A, Abrahamson J, Wallet B. Genetically engineered crops, glyphosate and the deterioration of health in the United States of America. Journal of Organic Systems 2014;9(2):6–37.

supplies and *their impact on body metabolism and gene function* along with what is known about environmental exposures across populations…. [I]t 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*…. [I]t 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).

Coauthor 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.

3. Key mechanisms in autism: acetaminophen, amoxicillin/clavulanate, and glyphosate deplete placental/postnatal estrogens – leaving brain myelin sheaths overhydrated/immature/asymmetric, brain blood flow low, and brain serotonin and oxytocin low

> *[A]mong 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 AAs, 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 neuroinhibitor *serotonin* and hormone *melatonin*. *Ammonia* is detoxified to *glutamine* in muscles, liver and brain – alternate fuel in brain neurons/astrocytes, primary 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 AAs*, *sulfate*, *glutathione, metallothionein, DHEAS, estrogens*. Oral antibiotics/glyphosate also kill/inhibit gut bacteria that synthesize *tryptophan*, limiting brain *serotonin* thus *oxytocin*, and kill/inhibit bacteria that restrain 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/overhydrated/asymmetric*, *corpus callosum and left hemisphere immature*, *brain blood flow low, and brain serotonin and oxytocin low*. High blood/brain *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*) aggravate *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 osmolytes*. *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. Elitt 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 pre-, peri- 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 antitoxin/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 phenols 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*, *Bacteriodetes*, 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 *glutamine* – 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 replicating 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 acetaminophen, amoxicillin/clavulanate, 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 falls to low levels after birth [55] then multiplies before puberty (*adrenarche*). Grumbach and Styne: "[S]tudies 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 *Tinbergens* (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, autists 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 not 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 differentiated 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 testosterone 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. Zilbovicius 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 rCBF 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 synthase 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 *tetrahydrobiopterin* (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 metabolism. *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: "Prolonged elevations in estrogen level initiate genomic pathways that include … increased endothelial nitric oxide synthase expression, and increased COX-1 and PGI^2 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, serotonin) 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.* Janušonis 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 *serotonin* 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]nterochromaffin 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].

Janušonis, 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 postpubertal autistic individuals." [76].

Serotonin is an unusual neurotransmitter, inhibiting *distally* as well as locally. Inactivation of serotonin at synapses requires reuptake by the *serotonin transporter* (SERT) – 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 … serotonin synthesis capacity was >200% of adult values until the age of 5 years and then declined toward adult values. In autistic children, 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 prepubertal 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].

Janušonis 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 sulfotransferase 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 *vasoconstrictor*. 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 emphases) *Is brain serotonin low in ASD because nitric oxide is low*? Chanrion 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*." [84] (my emphasis). Hiroi 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

> *[T]he 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 sheaths, mature 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 *cysteine* 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*. Lemonnier 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 Tyzio, 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 main 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 noted. Because *cannabis* also stimulates oxytocin, he recommended study of *cannabidiol* for ASD. He concluded: "… IGF-1 should be studied as a potential treatment of *ASD and other mental disorders characterized [by] brain dysconnectivity* …." (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-I in 2.5-y-old boys. Correspondingly, a high intake of milk, but not meat, equal in protein content, increased sIGF-I by 19% 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) *acetaminophen* depleting *sulfate* 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*, *hyperexcitability, 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 androgens*; *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 restrain maternal oxytocin at birth that limits fetal brain chloride/water and matures GABA. res ipsa loquitur* …

5. Evaluation

> *[Y]ou 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 are swollen astrocytes, salt cravings, elevation of compensatory osmolyte *myoinositol*, and depletion of primary osmolytes *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 "*preharvest 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 Levins 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 epidemics 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 moms (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].

Samsel and Seneff implicated *intestinal dysbiosis* in *autism encephalopathy – 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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