ABSTRACT

Parents and pediatricians of children with autistic disorders know infectious fever is often accompanied by dramatic relief of autistic behavior. How this happens has not been detected, but a little-known clue suggests a promising line of inquiry – improvements sometimes begin hours before fever. Improvements before fever are also more likely to persist after fever breaks; improvements in most children last only as long as fever lasts. Is there a temperature-independent mechanism and a temperature-dependent one? The temperature-dependent mechanism may simply be fever increasing brain metabolism and blood flow – consistently low in these children. The temperature-independent mechanism may be release of free glutamine from skeletal muscles for anabolic responses to infection. Why would glutamine relieve autistic behavior? Glutamine is precursor (via citrulline) of arginine – required to detoxify ammonia in the liver, synthesize creatine, and only substrate for the vasodilator nitric oxide. Ammonia is often high in these children from intestinal bacteria and yeast, glutamine consistently low, yet urinary orotic acid (orotate) tests rarely detect chronic high ammonia. High plasma ammonia and low glutamine without high urinary orotate was reported in children with propionic acidemia. Brain glutamine facilitates entry of tryptophan (precursor of serotonin). Oral citrulline bypasses the liver, making arginine more available for brain nitric oxide and creatine. Evidence that Cuba – with an autism incidence a fraction of ours – requires vaccinations (especially against measles) should temper fears that vaccines induce autism, and confirm fears of acetaminophen (Tylenol), which depletes glutathione (thus glutamine/arginine) – and which Cuba prohibits without prescription.

INTRODUCTION

Though there is practically no mention of the high fever/improved behavior phenomenon in the entire autism literature, every knowledgeable person – both parent and professional – I approached for information knew of it. Sullivan 1980 [1]

Improvements were reported both during and prior to the onset of fever. Zimmerman et al. 2008 [2]

At the 7th International Meeting for Autism Research (IMFAR) pediatric neurologist Andrew Zimmerman and colleagues presented parents’ reports of dramatic improvements of their child’s autistic behavior during fever [2]. These reports were spontaneous responses to their study published the previous year (2007) confirming fever’s benefit in 30 children with autism spectrum disorders (ASD) [3]. A few parents reported improvements in a sauna, steam room, or hot tub/bath – rare events. A single sentence on their IMFAR poster lay dormant: “Improvements were reported both during and prior to the onset of fever.” The first formal report of improvements of autistic behavior before fever – a critical clue to the phenomenon.

Parents and practitioners have known for decades that fever often relieves a child’s autistic behavior dramatically, and rarely aggravates. It’s also known that improvements in some children persist days after fever breaks, although improvements in most children subside when fever does. Evidence of fever’s dramatic benefit was summarized [4], notably (a) personal accounts by parents and pediatricians published by Ruth Christ Sullivan in her column Parents Speak [1]; (b) personal observations by psychologist Gary Brown: “The changes that occur in these autistic children are . . . dramatic, more like a metamorphosis in which the autistic child suddenly becomes almost normal. These children experience increased alertness, a decrease in social isolation and self-injurious behavior, an increase in verbal behavior, and an attempt to reach out and communicate with adults.”[5]; (c) confirmation of fever’s benefit by Zimmerman and colleagues [3]; and (d) Martha Herbert’s conviction that fever’s dramatic benefit reveals autism is a “chronic dynamic encephalopathy” – not a permanent structural one [6,7].

Zimmerman summarized parents’ anecdotal reports of improvements before fever [8]: “My impression has been that those children who improve before the appearance of fever are those who also have the most striking improvements overall during fever (and are more likely to have enduring effects after fever subsides), possibly 10% of those who have the ‘fever effect’. The ‘fever effect’ may be more common than we realize because there are different gradations of the responses. Also, the improvements in social relatedness and language may then be obscured by sickness behavior during the illness and are subject to a ‘threshold effect’ (i.e. caregiver recognition).” Zimmerman suggested a low grade fever might explain early benefits. “It is usually a period of hours [up to 6–8] when benefits are seen before fever is recognized. . . . Unfortunately we do not have clinical data to support these observations.”

HOW DOES FEVER BEGIN?

We now propose that the set point for body temperature is localized within the posterior hypothalamus and is determined and maintained by the inherent ratio in the concentrations of two essential cations, Na+ and Ca2+. This concept is based on experiments in which the balance between Na+ and Ca2+ levels was selectively altered within specific regions of the hypothalamus. Myers & Veale 1971 [9]

Fever resembles the body’s response to cold [10] – skin blood vessels constrict to conserve heat, and heat is generated by muscle contractions (shivering) and acceleration of metabolism. The immune system orchestrates these responses by releasing signaling proteins (cytokines, e.g. interleukins) and hormone-like fatty acids (prostaglandins) that may also generate heat [11]. Body and brain metabolism accelerate from heat (13% per °C [12]) and from epinephrine released by the adrenal medulla, which mobilizes metabolic fuels (fatty acids, glucose from glycogen), accelerating
metabolism 5–10x more than norepinephrine [13]. Magnesium enters cells to support the energy requirements of accelerated metabolism [14].

Tang and Kiyatkin [11] found in rats that intravenous injection of bacterial tissue (LPS) caused fever to appear about 48 minutes later, in phases. The first phase was heat conservation and production in the periphery: “[T]he first phase of LPS-induced brain and body temperature elevation . . . coincides with vasconstriction, which is tonically maintained for many hours . . . . While peripheral heat production also appears to determine the first phase of brain and body temperature elevation . . . a further prolonged increase in brain-muscle differentials (onset at ~100 min) suggests metabolic brain activation as a factor contributing to brain and body hyperthermia.” This second phase involved “central mechanisms that increase body metabolism” – notably sympathetic nervous system release of epinephrine.

The temperature set point in the hypothalamus also rises, so that heat accumulates, not dissipates via vasodilation and sweating. Myers and Veale concluded the critical factor that determined the set point was the ratio of sodium ions to calcium ions in the posterior hypothalamus; they proposed that sodium ions move from cerebrospinal fluid (CSF) into the brain, displacing calcium ions, which raises the set point [9]. Frosini detected this sodium/calcium shift in rabbits in response to endogenous pyrogen (interleukin 1β) – heat increased CSF calcium, but fever increased it much more; only fever decreased CSF sodium [15].

Does sodium/calcium exchange in the brain relieve autistic behavior? Salt cravings are common in these children [16], hypnatremia has been reported, fluid/salt diets improved behavior [17], and brain calcium accumulation has been detected [18]. Sodium cotransports glutamate into astrocytes for conversion to glutamine (Gln) [19] – an amino acid usually deficient in blood and brain of these children. Sodium also carries water (as does glutamine); are autistic brains dehydrated? But can sodium/calcium exchange persist after the set point returns to normal? And if sodium/calcium exchange raises the set point, can that happen hours before fever appears? What does happen hours before fever?

Release of muscle amino acids for anabolic responses to infection

In sand fly fever and other infections in man decreased values for plasma amino acids were observed to begin very early in the infectious process and before the onset of any recognizable symptoms. Wannemacher et al. 1972 [20]

Wannemacher and colleagues investigated the response to infection in eight healthy volunteers inoculated with sand fly virus to induce mild illness [20]. Fever appeared 56–70 hours after inoculation. Hours before fever’s onset, however, concentrations of most plasma amino acids (AA) fell: “[S]ignificant depression in the concentration of total amino acids and most individual amino acids was evident 9–23 hr before the onset of fever or symptoms of illness . . . .” The researchers concluded amino acids released from skeletal muscles by catabolism of their proteins were taken up avidly for anabolic responses to infection, especially by immune cells and the liver.

In a second paper Wannemacher named three “major visceral compartments” that competed for these amino acids [21]: “The first compartment includes the essential body proteins, such as those in the heart or brain, which are essential for the maintenance of homeostasis within the host. . . . The second compartment is the utilization of amino acids by leukocytes for synthesis of antibody, y-globulin, lymphokines, granulocytic proteins, and cell transformations which are associated with humoral and cell-mediated immunity . . . . The third compartment is the liver, where the amino acids are utilized for hepatic and plasma protein synthesis and for gluconeogenesis.” Wannemacher noted that although muscles release amino acids hours before the loss of appetite (anorexia) that accompanies fever, anorexia when it appears rapidly depletes glycogen stores throughout the body, provoking further release of amino acids as fuel. He also noted alanine and glutamine were the predominant amino acids released by human skeletal muscle, and thought branched-chain amino acids (BCAA) in muscle readily formed alanine and glutamine.

One exception to the general fall in plasma amino acids was tryptophan, which rose in plasma once fever appeared. Wannemacher explained that only the liver can degrade tryptophan (though other tissues use it) so muscles may have released more tryptophan than needed. Tryptophan supplements, however, have helped children with ASD (see Discussion). Another critical observation by Wannemacher may be that gluconeogenesis increases urea production, and febrile persons lose much nitrogen. In light of considerable evidence of high blood ammonia in ASD children, does loss of nitrogen before and during fever improve behavior? Fever also releases the free (nonprotein) amino acid taurine from muscles. Taurine helps detoxify ammonia and glutamate to glutamine – and was the amino acid most wasted or depleted in urine of ASD children [22,23].

AMMONIA AND GLUTAMINE IN ASD

The low level of plasma glutamine . . . is suggested as a screening test for detecting autism in children especially those with normal IQ. The decreased level has been reported before in all children with autism. Ghanizadeh 2013 [24]

High blood ammonia (NH₃) in children with autism was detected by the early 1980s [23] and more recently [25-27]. Wakefield and colleagues suspected their diseased intestines generate more ammonia than their impaired liver can clear at first pass, which enters the brain [28]. Ammonia is a consistent byproduct of amino acid metabolism (amino group: NH₂) and protein degradation, especially in the intestines. Ammonia produced in the large and small intestine moves via the portal vein to the liver for conversion to urea, most of which is excreted. Ammonia in the kidneys helps regulate pH. Although highly toxic to the brain, Souba pointed out ammonia is “an essential nutrient and a key component of all proteins, nucleic acids, and amino acids . . . [and] a vital and major source of interorgan nitrogen transfer.” [29] This transfer usually begins with the enzyme glutamine synthetase (GS) in tissues that generate (and trap) much ammonia (e.g. skeletal muscles, liver, and brain). Glutamine synthetase combines ammonia and excitatory amino acid glutamate to form nontoxic glutamine, which safely carries two molecules of ammonia in plasma to the intestines for conversion to urea by the liver – and nourishes many cells and tissues along the way.

Glutamine is normally the most abundant amino acid in blood [30], a primary brain osmolyte, alternative fuel for brain neurons and astrocytes, especially during hypoglycemia [31], and primary fuel in rapidly replicating cells, e.g. liver cells, blood vessel endo-
When plasma ammonia is high in hepatic encephalopathy (HE) from liver failure, or in children with inborn urea cycle disorders (UCD), plasma and brain glutamine are also high [36]. In ASD children, by contrast, plasma glutamine is consistently low [24, 37-40], even when ammonia is high [27] – although Pangborn reported infrequent cases of high plasma ammonia and glutamine [22]. Brain glutamate/glutamine (Glx) is often low [41-43] not always [44]. Wakefield and colleagues, finding serum glutamine low in ASD children, and knowing liver dysfunction impairs synthesis of astrocyte glutamate transporters, suspected brain glutamine was also low [28]. Corroborating that speculation, children with urea cycle disorders and high brain glutamine rarely show autistic behavior. Krivitzky and colleagues: “[C]hildren in this cohort [UCD] show other behavioral/emotional strengths, including a minimal percentage with previous diagnoses of Autism spectrum disorders, mood disorders, and other psychiatric disorders.” [45] A professor of Pediatrics confirmed this observation: “I see lots of kids with . . . UCD but few (perhaps none) have ASD.” [46] Further corroboration is high concentrations of myoinositol in ASD astrocytes [47] – a weak brain osmolyte that compensates the primary osmolytes taurine and glutamine. When astrocyte glutamine is high in HE or UCD, myoinositol is consistently low.

Horder and colleagues detected by magnetic resonance spectroscopy (MRS) significant reductions in combined glutamate/glutamine (Glx) in the basal ganglia (subcortex) of the brain in adults with ASD: “Taken together, these results demonstrate that, rather than being a ‘global’ neurobiological abnormality, Glx changes seen in ASD are highly regionally specific, suggesting that the underlying neurobiological cause(s) are also localized.” They concluded this could underpin some clinical symptoms [43].

Autism Research Institute (ARI) practitioners reported giving ASD children oral glutamine to heal their intestines [4], from 250mg–8g/day, with few side effects (some hyperactivity) – although one neurologist reported seizures. One said 5g/day of glutamine was “fantastic” to heal intestines. Only two practitioners, however, reported improved behavior from glutamine. Franco Verzella (MD) in Bologna, Italy gives ASD children 5–7g/day of oral glutamine after cleansing their intestines of pathogens like bacteria and Candida: “Multifactorial and multisystemic is the condition, so that the improvement has different aspects in different children. Most common: sedation, less stereotypes, better sleep, more concentration.” [48] Pangborn warned that intestinal bacteria and yeast can degrade oral glutamine and other amino acids to toxins. He recommended cleansing the intestines before giving ASD children any amino acid except taurine, which helps turn ammonia to glutamine [23]. But Lands noted the importance of glutamine for immune functions of the gastrointestinal, respiratory, and genitourinary tracts, especially against Candida [32].

Shabert and Wilmore [49] concluded: “The concentration of glutamine exceeds all other amino acids and, in plasma, its concentration is at least four to five times greater than other amino acids, except alanine and valine. In the skeletal muscle-free amino acid pool, glutamine and taurine are the most abundant amino acids with all other amino acids comprising less than 30% of this compartment.” They cited evidence that glucocorticoids from the adrenal cortex responding to the stress of infection or injury stimulate release of 3–4x usual amounts of glutamine from muscles – “probably all free glutamine” Wilmore observed [50].

Rennie and colleagues [51] proposed that release of free glutamine (and increase of BCAA) in muscles induced by disease or injury was due to loss of sodium-dependent glutamine transport: “One clue was the unusually high distribution ratio (intracellular/extracellular concentration) for glutamine compared with that of other amino acids. Glutamine did not appear to be complexed, as was, for example, taurine, suggesting that glutamine was concentrated within muscle by a secondary active-transport mechanism involving cotransport of an ion, probably sodium . . . . These observations were consistent with the hypothesis that in injury or disease, when it is known that intramuscular concentrations of Na are elevated and membrane potential depressed, there would be effects on the activity of the transporter, such as altering the balance between accumulation and release of glutamine in the direction of increased release.” Does hyponatremia in ASD children [16] limit glutamine accumulation in muscles? Hyponatremia may also reduce brain glutamine by causing astrocytes to swell, then release osmolytes taurine and glutamine and their water to compensate. Enlarged astrocytes in ASD children thought to be part of an immune response may reveal osmotic imbalance.

Healthy humans produce 50–80g/day of glutamine, largely in muscles [52]. Skeletal muscle proteins contain 4–5% bound glutamine [49]. Bergström and colleagues biopsied muscle tissue of healthy adults to measure free amino acids: “The majority of the amino acids showed much higher concentration in intracellular water than in plasma. . . . For a normal man with a body weight of 70 kg and a muscle mass of 40% of the body weight, the total volume of intracellular muscle water is 18.2 liters and the total intracellular amino acid content in muscle is 86.5 g. Of this total pool of free amino acids the eight essential amino acids represent only 8.4%, whereas free glutamine constitutes 61%, glutamic acid 13.5%, and alanine 4.4%.” [53]

Munro concluded that glutamine synthesis in muscle requires BCAA from dietary protein: “After a meal containing abundant protein, the liver selectively degraded the excess of most essential amino acids, while allowing the three branched-chain essential amino acids to pass through, so that in consequence they account for more than 50 per cent of the amino acid outflow from the splanchnic area. During this period of absorption muscle takes up amino acids, over 70 per cent being the branched-chain amino acids where they are catabolised, the nitrogen becoming available for formation of non-essential amino acids for muscle protein synthesis. The release of large amounts of glutamine from muscle is dependent on branched-chain amino acid degradation, whereas the release of large amounts of alanine is regulated by availability of pyruvate from glucose.” [54] [my emphasis] ASD children resist eating protein (a sign of high ammonia) and prefer carbohydrates – one reason their plasma glutamine is consistently low?
How common is high blood/brain ammonia in ASD?

It’s hard to dispute observation. Ray Magliozzi (Car Talk) 2014

Pediatricians and specialists in metabolic disorders reported their observations and conclusions about ammonia in children and adults with autistic disorders:

Biochemist William Shaw (PhD) of The Great Plains Laboratory reported that about 250,000 tests of ASD children and adults found the organic acid orotate (orotic acid) in urine – a marker of chronic high blood ammonia – often slightly elevated: “The orotate is commonly abnormal in autism. The degree of abnormality is slight, usually 5–10% above reference range. I would estimate that perhaps 10–15% of cases of autism have this slight abnormality.”[55]

Kurt Woeller (DO): “I think the vast majority of kids on the spectrum have ammonia issues of some sort because first, their guts are full of yeast and bacteria which leads to a condition called dysbiosis. Second, the yeast and bacterial toxins can interfere with Kreb Cycle function, and third the methylation and urea cycles are impacted by infectious toxins because of underlying SNPs [single-nucleotide polymorphisms – genetic anomalies] that predispose them to problems. . . . I rarely see orotic acid high on the Organic Acids Test. Perhaps it takes a lot of imbalances to push this particular biochemical pathway to get it to appear elevated. When I do see it high it is usually a mild elevation. This experience comes after looking at hundreds and hundreds of organic acids tests from Great Plains Laboratory over the years.”[56]

John Green (MD): “I have measured blood ammonia in lots of [ASD] kids, and find it elevated more than occasionally, but much less than frequently. It’s hard to get a reliable measurement, as it has to be done in hospital (though Tapan Audhya developed a method of stabilizing it, so that we could send it overnight to his lab and get reproducible results). It is certainly an issue for a good many kids, those with mitochondrial problems, and those with significant bacterial dysbiosis.”[57]

Richard Lord (PhD): “Ammonia is highly toxic to the brain, thus we are endowed with multiple layers of protection that operate with high efficiency under a wide spectrum of conditions to prevent the rise of blood levels that reach the brain. . . . [T]here are 3 principal routes of ammonia clearance: (1) Conversion into urea by hepatic urea cycle activity. Collectively, the four enzymes of this pathway constitute the highest incidence for inborn errors of metabolism, with the result that there is wide variation of individual capacity for ammonia clearance by this mechanism. . . . [T]here is also the variable of arginine supply [required by the urea cycle], especially for those on low protein diets. (2) Direct excretion of ammonium salts (primarily with citrate, aconitate and isocitrate as counter ions) in urine. This may be detected as a pattern of elevations of those compounds in an organic acid profile. Dietary and other metabolic activities determine the availability of those tricarboxylic acids for binding ammonia in the kidney. (3) Increased rates of carbamoyl phosphate conversion to orotate that causes its spill into urine. This is the first step in the ubiquitous purine anabolic pathway present in most tissues, especially liver. . . .

“Elevated urinary orotate is a biomarker of chronic ammonemia due to the orotate shunt pathway that takes over to clear ammonia (more specifically the ammonia condensation product carbamoyl phosphate) when the urea cycle fails to do so. . . . [W]hile ammonia is transient in various body fluids, orotate is quite stable, but it is only the spillover from the purine synthetic pathway that appears in urine and that varies in diurnal cycles. So blood ammonia is highly variable (brief excursions may cause mild episodes of ‘brain fog’) and urinary orotate is somewhat variable and may be undetectable in healthy individuals. But in chronic ammonemia there is relatively steady over-production of orotate. It’s easy to imagine ammonemia based on spot blood ammonia (famously transient) or other signs not solidly established as chronic ammonemia markers. . . . Release of ammonia is one of numerous nitrogen-metabolism activities found among multiple strains of colonic bacteria. They contribute to background levels of ammonia that flow directly to the liver for clearance.”[58]

Lockwood and colleagues injected radioactive nitrogen into healthy persons and patients with liver disease (HE) to determine relative clearance of ammonia by major organs [59]. They found most ammonia was trapped by the liver, skeletal muscles, and brain: “The importance of skeletal muscle in the maintenance of ammonia homeostasis is due to its large mass, over 40% of the body weight in normal humans. Although the liver is undoubtedly the most important organ in the maintenance of physiological blood ammonia levels in normal humans, in patients with advanced liver disease and portal systemic shunting, ammonia-laden portal blood bypasses the liver and is diverted into the systemic circulation. Under these circumstances, skeletal muscle may become the most important organ in ammonia homeostasis.”

At normal pH most ammonia in plasma is present as ammonium ion (NH\textsubscript{4}+), which enters the brain less readily than ammonia gas. Walker: “NH\textsubscript{3} is lipid soluble and enters the brain by diffusion. NH\textsubscript{4}+ enters through membrane ion channels and transporters.” [60] High brain ammonia may explain several prominent characteristics of autistic behavior – excitability, stupor, executive dysfunction, and loss of appetite. Acute ammonia toxicity stimulates brain neurons to release excitatory glutamate; chronic high brain ammonia releases inhibitory GABA [61]. Blaylock [62] implicated extracellular glutamate in autistic hyperactivity, as others have. Hindfelt concluded that accumulation of ammonia in hepatic encephalopathy increased subcortical brain blood flow at the expense of cortical flow, provoking a “frontal lobe syndrome.” [63] Felippo and Butterworth concluded likewise: “Accumulation of ammonia in brain results in a redistribution of cerebral blood flow and metabolism from cortical to sub-cortical structures.”[64] By binding alpha-ketoglutarate (key intermediate in the citric acid cycle) ammonia is thought to reduce ATP, but Hindfelt concluded that only happened in late stages of HE. Ammonia also causes astrocytes to swell from glutamine and other osmoletes; chronic ammonia accumulation reduces the capacity of astrocytes to metabolize ammonia. Aschner concluded: “Ammonia-intoxicated astrocytes lose their homeostatic control of the extracellular fluid, resulting in neuronal dysfunction secondary to altered astrocytic function.”[65]

Ammonia may also be high in children weaned before six months old [66] because solid food has much more protein than breast milk. Axelsson: “When other foods are introduced during the weaning period the protein intake increases remarkably to 3–4 g/kg/day in spite of the fact that the protein requirement is decreasing. . . . [T]he metabolic effects with high levels of urea in serum and urine, and the high levels of many amino acids may exceed the capacity of the hepatic and renal systems to metabolize
and excrete the excess of nitrogen."[67] Many infant formulas contain more protein than breast milk.

The situation in children with autistic disorders thus resembles what Wakefield and colleagues suspected [28] – more ammonia produced by the diseased intestines [and other factors] than the impaired liver can clear before it enters the brain. What limits their liver’s ability to detoxify ammonia? One explanation is glutamine deficiency; glutamine is precursor (via liver’s ability to detoxify ammonia? One explanation is glutamine paradox (high plasma ammonia, low glutamine) occurs in the inborn metabolic disorder propionic acidemia (PA) [68]. One explanation offered is that glutamine synthetase requires ATP -- low in PA [69]. Low concentrations of plasma ATP and precursors were detected in ASD children [70]. Other explanations for glutamine deficiency are lack of magnesium (Mg) and vitamin B6, cofactors for glutamine synthetase [31,70]. Rimland concluded from parents’ reports that high-dose Mg/B6 was most effective for children and adults with ASD [71]. But the most provocative explanation for glutamine deficiency in ASD may be depletion of glutathione by acetaminophen (Tylenol).

ACETAMINOPHEN AND THE AUTISM EPIDEMIC

The incidence of autism has risen 10-fold since the early 1980s, with most of this rise not explainable by changing diagnostic criteria. Previc 2007 [72]

Compelling evidence implicating an environmental cause or trigger of autism must be the number of normally developing children who gradually or abruptly regress into autism between 12 and 18 months of age. Parents implicated the diptheria-pertussis-tetanus vaccine (DPT) in their child’s regression in reports to Bernard Rimland even before he founded the Autism Research Institute (ARI) in 1967 [73]. Rimland [74] suspected the “autism explosion” in the early 1980s was due to accelerated efforts by the Centers for Disease Control and Prevention (CDC) beginning 1978 [75] to encourage wider use of the measles-mumps-rubella vaccine (MMR). Yet the MMR, often implicated by parents in their child’s regression, never used the mercury-based preservative thimerosal the DPT and other vaccines formerly used (but does contain 10% glutamate from gelatin to preserve the viruses [76]).

Schultz and colleagues asked whether autistic regression after the MMR might be provoked, not by the vaccine itself, but by acetaminophen (Tylenol) given for its pain and fever. An online survey of parents revealed that children given acetaminophen for adverse reactions to the MMR were significantly more likely to report fresh evidence that emphatically implicates acetaminophen paraacetamol as a cause of our autism epidemic, most notably that Cuba – with an autism incidence a fraction of ours (1/300th) – requires vaccinations (especially against measles), prohibits over-the-counter acetaminophen, and only rarely gives acetaminophen before vaccinations. Fevers persisting more than two days after vaccination are usually treated with prescription metamizole – a drug banned in the U.S. on questionable grounds.

The implications of this evidence are obvious – and staggering. All children in Cuba are vaccinated, especially against measles. Yet our autism incidence is almost 300x theirs – mostly regressive autism about 18 months of age. We give Tylenol freely; Cuba requires a prescription because Tylenol is limited by embargo. But perhaps Cuba uses a single measles vaccine; some think triple vaccines like the MMR and DPT induce regression. A measles vaccine was introduced into Cuba in 1971 [87]; the MMR introduced in 1986 [88]. If the MMR hasn’t provoked an epidemic of autism in Cuba since 1986, why would it do so in the U.S?
Another challenge: The MMR is usually given at 12–15 months; why does regression usually happen about 18 months? Schultz pointed out regression often develops gradually, over months. Acetaminophen given with vaccines, he suggested, may weaken a child’s immune system, so the child gets sick again and takes more acetaminophen, thus “slowly regressing into autism.”[89]

Shaw cited evidence implicating acetaminophen in many other disorders, notably our epidemic of asthma: “Depletion of GSH as a consequence of acetaminophen toxicity to the liver has attracted the most attention in the medical scientific community, as it can frequently be fatal or require a liver transplant or emergency treatment to prevent liver failure . . . . However, acetaminophen toxicity has been implicated in a wide range of other disorders in humans and/or experimental animals including cancer, birth defects, asthma, allergies, and brain toxicity.”[86]

Shaw noted recent evidence associating prenatal and perinatal paracetamol (acetaminophen in the UK) with autism. Bauer and Kriebel reported recommendations that acetaminophen 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 hours for 24 hours following the procedure. Thus newborn males often receive 5–7 doses . . . during the developmentally vulnerable initial days of life.”[90] One reason boys are 4x more vulnerable to autism? Bauer and Kriebel also cited evidence that in 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.”[89]

Shaw reported that in recent years Johnson & Johnson has repeatedly run afoul of the Food and Drug Administration (FDA) for mislabeling children’s products (increasing risk of overdose) and poor quality control and contamination at manufacturing plants; millions of bottles of children’s medicines containing acetaminophen have been recalled. Johnson & Johnson also makes Risperdal (risperidone), the antipsychotic drug for ASD with serious side effects.

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-containing proteins. Selenoproteins underlie the glutathione system, and mercury binds to selenium with exceptional, almost irreversible potency . . . . It seems to me that mercury and AAP are a particularly harmful combination.”[91]

How does acetaminophen depleting glutathione deplete glutamine thus arginine? Hong and colleagues pointed out that glutamine enters cells more readily than glutamate, thus often provides glutamate to synthesize glutathione: “[G]lutamine-supplemented nutrition preserves hepatic glutathione, protects the liver, and improves survival during acetaminophen toxicity. . . . Glutathione is a tripeptide consisting of glutamate, cysteine, and glycine, and under various experimental conditions, the glutamate portion of the molecule is derived from glutamine. We hypothesized that the administration of glutamine-supplemented nutrition is protective after severe injury through the preservation of tissue glutathione stores.”[92]

As reported [4], Sandler and colleagues received parents’ reports of their child’s regression into autism after a course of broad-spectrum oral antibiotics – usually to treat otitis media (middle ear infection). Oral vancomycin (an antibiotic minimally absorbed) improved autistic behavior impressively short-term [93]. Horvath and Perman reported autistic regression at 12–18 months was associated with the onset of gastrointestinal symptoms [94]. As noted, Wakefield and colleagues suspected the diseased intestines of these children generate more ammonia than their impaired liver can clear [28]. Finegold and colleagues found ASD children had different species of intestinal bacteria than typical children [95]. 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 and other problems.”[96]

Fallon found many autistic children under the age of three with otitis media treated with the antibiotic amoxicillin/clavulanate (Augmentin). She noted that ammonia used in its manufacture might contaminate the drug, and ammonia may be caustic to the intestines [97]. Niehus and Lord concluded ASD children had more ear infections than typical children, and were treated with more antibiotics [98]. Greenberg and colleagues pointed out the association of acute otitis media with day care centers, antibiotic-resistant bacteria, and pediatricians’ recommendations that high-dose amoxicillin/clavulenate was “the first therapeutic choice” for children in day care centers [99]. Augmentin was launched in 1981 to treat “upper and lower respiratory tract infections, urinary tract infections, skin and soft tissue infections and obstetric, gynecological and intra-abdominal infections.”[100]

Richardson and colleagues investigated whether human colonic bacteria resemble bacteria in a cow’s rumen that digest only proteins, producing great amounts of ammonia. They found peptides produced the most ammonia, then casein, then amino acids. They detected bacteria that digested proteins when carbohydrates weren’t available – and bacteria that digested only proteins (“hyper-ammonia producing”) including species of Clostridia [101]. Finally, a study of plasma ammonia after exercise in healthy males and females led Esbjörnsson and colleagues to conclude: “The lower plasma ammonia after sprint exercise in females seems to be explained by a lower accumulation of ammonia in skeletal muscle and by a buffering of ammonia in the form of glutamine in females.”[102]

ARGININE, NITRIC OXIDE AND CREATINE IN ASD

When the L-Arginine took effect, it was like a light switch going on. He suddenly became calm, happy, social, and able to handle upsetting situations. Carrick & Carrick [103]

Adams asked whether supplemental arginine might help as much as fever, if fever relieves autistic behavior largely by increasing arginine [104]. He and his colleagues previously found plasma arginine normal in ASD children [70]. But Cynober stated: “[I]t is frequently asserted that plasma levels of AAs are difficult or even impossible to interpret. The grounds for this assertion are that the plasma pool of free AAs is very small compared with the intracellular pool of free AAs, which in turn is small compared with the protein-bound AA pool. . . .”[105] Pangborn found urinary arginine low in 25% of 61 ASD children [22,23].

Arginine is required to detoxify ammonia in the liver, synthesize

Ammonia and the autism epidemic
nitric oxide – primary dilator of blood vessels – and synthesize the ATP-transporter creatine. In a series of investigations since 1984, Naruse and colleagues reported that tetrahydrobiopterin (BH₄), a cofactor for synthesis of nitric oxide and neurotransmitters dopamine and serotonin, improved behavior in ASD children [citations in [107]]. In 2010 Frye and colleagues corroborated the benefit of sapropterin (synthetic BH₄) in ASD children [106]. Their 2013 study investigated whether sapropterin helped by increasing dopamine or serotonin (as Naruse et al. suspected) – or via effects on nitric oxide [107].

Frye and colleagues concluded improvements in communicative language in ASD children from sapropterin were due to restoration of NOS “coupling” disrupted by lack of BH₄, which dysregulated nitric oxide metabolism [107]. In support of their conclusion they cited evidence by Sweeten and colleagues [108] and others of high levels of nitric oxide metabolites nitrite and nitrate in blood of ASD children. In their previous study Frye and colleagues concluded: “[I]t is possible that BH₄ in ASD could be depleted by the overactivation of the immune system and inflammatory processes during an excessive production of nitric oxide.” [106]

There may, however, be more to this story [109]. A few months after publication of Frye et al. 2013, Staniewicz and colleagues reported that sapropterin increased reflex vasodilation in aging human skin by increasing synthesis of nitric oxide by endothelial and neuronal nitric oxide synthases [110]. Nitric oxide is produced by three different forms of nitric oxide synthase [NOS] – two constitutive forms present in blood vessel endothelial cells (eNOS) and neurons (nNOS), and a third form (iNOS) induced in brain microglia, astrocytes, other immune-system cells (and many others) in response to infections, toxins, and other agencies [111]. Endothelial nitric oxide maintains the vasodilator tone of blood vessels. Neuronal nitric oxide may be largely responsible for neurovascular coupling – dilation of nearby blood vessels when brain neurons fire. Faraci and Brian: “…NO appears to mediate cerebral vasodilatation in response to local neuronal activation.” [111] Koehler and colleagues: “…NO is required as a mediator of neurovascular coupling in the cerebellum, whereas NO acts as a modulator in the cerebral cortex.” [112] Induced nitric oxide is released in large quantities by iNOS to flush infective agents and toxins, and kill damaged cells.

If nitric oxide is too high in autistic disorders, Frye and colleagues concluded, induced nitric oxide is probably responsible. Sweeten et al. concluded likewise: “[I]t is reasonable to hypothesize that iNOS is involved in the elevated NO production in autism.” [108]

Yet induced nitric oxide is readily released to compensate deficiencies of constitutive nitric oxide [113,114]. One indication neuronal nitric oxide is depleted in ASD children is their failure of neurovascular coupling – their brains are often hyperexcitable, yet brain blood flow is consistently low [e.g. 115]. Nitrite and nitrate also serve as reservoir forms to deliver nitric oxide elsewhere [116]. Lundberg and Weitzberg: “[N]itrate and nitrite should probably be viewed as storage pools for NO rather than inert waste products.” [117]

Did sapropterin increase endothelial and neuronal nitric oxide in the brains of ASD children in Frye et al. 2013? Why would their constitutive nitric oxide be deficient? One explanation is BH₄ deficiency; another is deficiency of arginine – only substrate for nitric oxide [118]. Frye and colleagues found higher baseline levels of blood arginine in these children, and higher ratios of arginine to citrulline, were associated with greater improvements in language from sapropterin. They noted blood arginine and the arginine/citrulline ratio did not change significantly during sapropterin treatment – but also stated improvements in language were greater in children “with an attenuated increase in arginine.” [107]

Considerable evidence argues that arginine is depleted in ASD children: (a) high levels of induced nitric oxide; (b) consistently low brain creatine (arginine + glycine); (c) frequent high blood ammonia requiring arginine to detoxify; and (d) high levels of arginine vasopressin in autistic boys [119,120]. Furthermore, harmful oxidants superoxide and peroxynitrite are produced when BH₄ deficiency uncouples NOS – or when arginine is deficient [121]. Because most ingested arginine is taken up by the liver (thus unavailable to other tissues) [122], citrulline (arginine’s precursor) or glutamine (citrulline’s precursor) may be better sources of arginine for brain nitric oxide and creatine than arginine itself. Humming [e.g. the mantra Aum?] releases induced nitric oxide from nasal sinuses [123].

Creatine and phosphocreatine shuttles ATP from mitochondria to cytosol and the cell membrane, thus are vital for energy metabolism, especially in muscles and brain [124,125]. As reported [4] Minshew and colleagues detected by MRS less brain phosphocreatine in high-functioning autistic adolescents and young men, suggesting “increased utilization . . . to maintain brain ATP levels, or a hypermetabolic energy state.” [126] Friedman and colleagues found brain creatine reduced in ASD children 3–4 years old [127]; a follow-up study found less creatine and phosphocreatine in gray matter [128]. Hardan and colleagues found lower levels of NAA, phosphocreatine, and creatine in the left thalamus of ASD children [129]. Ipser and colleagues detected age-related changes in NAA and creatine: ASD adults had more creatine in temporal lobes; ASD children had less creatine in occipital lobes [130]. Abu Shmais and colleagues measured high plasma creatine in ASD boys suggesting low brain creatine [27]. Pangborn concluded only 5–10% of ASD children improve from oral creatine, perhaps because ATP is low or methylation of creatine impaired [23]. Woeller, however, reported online that oral creatine can be very effective in ASD children with low muscle tone, low metabolic energy, poor coordination, and difficulty with expressive language [131].

Wu and colleagues noted underproduction or overproduction of nitric oxide can harm the intestines, especially when inflamed: “Most studies have demonstrated beneficial effects of [arginine] on improving gastrointestinal function and gastric ulcer healing, accelerating intestinal mucosal regeneration, enhancing bacterial clearance, and reducing histological bowel necrosis.” [132] Large oral doses of arginine (~9g/day), however, have induced nausea and diarrhea. Wu et al. recommended divided doses of arginine throughout the day – or alternately, oral citrulline, which bypasses the liver (becoming arginine in the kidneys) and consumes ammonia in the conversion [133]. The Carricks give their adult son 4g/day of oral arginine, one every four hours [103]. Watermelon and its juice, rich in citrulline and arginine [133], may be a safe test – and remedy – for arginine depletion.

**DISCUSSION**
There are more explanations for fever’s benefit than people in this room – moderator at Autism Research Institute ‘think tank’ Baltimore, April 2013

You spoke of improvements persisting days after fever subsides. That hasn’t been my experience. In my experience improvements last only as long as fever lasts. Are there two mechanisms here? – pediatrician at ARI think tank Baltimore

Improvements of autistic behavior hours before fever – unless due to low grade fever – suggest a mechanism independent of temperature and its effects on brain metabolism and blood flow. By contrast, improvements that subside when fever does suggest a mechanism that does depend on high brain temperature or its effects in these children, whose brain metabolism [127] and blood flow [6,115,134] are consistently low. Improvements persisting after fever breaks need not be temperature-independent, but merely lasting effects of fever. Kiyatkin noted fever increases human brain temperature much more than ambient heat or stress, especially in children [135]. Zimmerman thought low grade fever might explain early benefits – increased brain temperature, metabolism, and blood flow too slight to notice but enough to improve behavior. But if low grade fever is sufficient for improvements, why doesn’t a sauna or hot bath help more often? Yet sometimes they do help, a few parents reported.

Zimmerman noted 80% of parents in Curran et al. 2007 [3] reported their child improved during fever on one or more Autism Behavior Checklist categories [8]. “In clinical care, approximately 30% of parents report that their children with ASD improved dramatically during fever . . . their symptomss are so obvious the family recognize them immediately. . . . The ‘range’ of effects therefore varies. When you ask parents to consider whether improvements occur, they often are uncertain or say ‘no,’ only to reply weeks, months (or even years) later that they have observed such improvements but had not been aware of them previously. In other words, it’s not an ‘all-or-nothing’ phenomenon.”[8]

Temperature-related events that might explain improvements from fever include (a) sodium/calcium exchange, (b) release of epinephrine, (c) increased brain metabolism/blood flow, (d) heat killing gut bacteria, (e) magnesium entering cells, and (f) catabolism and anorexia releasing muscle amino acids. Fever may also reduce CSF GABA – primary inhibitory brain transmitter and cryogen – to allow elevation of the set point [15]. Cohen noted high plasma ammonia is consistently accompanied by high plasma GABA [25]. Dhossche and colleagues suspected high plasma GABA they detected in autistic children explained mood disorders and stupor [136]. Burrell pointed out that ammonia generated by intestinal yeast may combine with propionic acid to produce a molecule structurally very similar to GABA, which may block GABA receptors, elevating GABA concentrations [137]. Taurine is a secondary inhibitory transmitter and cryogen, GABA primary.

Fever may also reduce brain calcium via epinephrine stimulating sympathetic beta-receptors. Under stress, β-stimulation shifts taurine and calcium into cells [138], e.g. in heart muscle calcium strengthens contractions, and taurine stores calcium in intracellular structures to regulate its active concentration in cytosol [139]. Intense β-stimulation, however, reverses this taurine shift. Durlach and Durlach: “[[Intense β-stimulation produces reverse effects on cell [taurine] influx: instead of an increase, a decrease is observed.”[140] Does the intense β-stimulation of fever flush calcium from the brain? And does this utilize the sodium/calcium exchanger [139], so that sodium enters as calcium leaves? Does intense β-stimulation also explain how severe stress (e.g. panic, blood drawing) improves autistic behavior dramatically [1,141]?

Until now the complexity of fever and its risks have restrained researchers from investigating the decisive factor(s) that improve autistic behavior so dramatically. We don’t know why improvements in some children persist days after fever breaks, yet improvements in most children subside when fever does. We don’t know why improvements sometimes appear hours before fever.

One telling clue here is that the same behaviors improve – primarily social relating and language. In other words, the early temperature-independent mechanism improves the same behaviors that fever improves – and that persist after fever. This might mean the early mechanism is not independent of temperature – e.g. low grade fever. It might mean the early mechanism and fever have common effect. But it might also reveal a single mechanism that can begin before fever, is strengthened by fever, and can persist after fever.

Increased brain blood flow is one obvious candidate, if early peripheral vasoconstriction shifts blood to the brain, or early induction of nitric oxide by infection dilates brain capillaries as Whitlock proposed [142]. Helt and colleagues pointed out enlarged astrocytes in ASD may compress brain capillaries [141]. Yet Frye et al. suspected induced nitric oxide is already too high in autistic brains [106,107].

Another mechanism that begins hours before fever and is strengthened by fever (and anorexia) is liver gluconeogenesis multiplying nitrogen lost as urea [21]. More nitrogen is presumably lost when glutamine released from muscles becomes arginine. Deutz noted about 30% of glutamine reaching the intestines becomes citrulline which the kidneys convert to arginine, all in one pass of blood through the circulation – about 30 seconds [143]. If arginine is decisive in ASD, free glutamine becoming arginine may explain improvements before fever, and protein-bound glutamine and arginine explain improvements during and after fever.

Another candidate may be tryptophan, precursor of the inhibitory brain transmitter serotonin. Carrick and Carrick reported their autistic son was prescribed tryptophan at age 4: “It helped calm him down and sleep better at night. He went from repeated awakenings, screaming, and insomnia, to a full night of sleep with the first dose. He is now 25, and we currently give him this as needed to reduce anxiety and stress. It works on the serotonin levels in the brain much the same way anti-depressants do. . . . When L-Tryptophan does work, it usually is a good indication that his serotonin levels are low.” [103] Yet blood serotonin is often high in ASD – which may mean brain serotonin is low [23].

Tryptophan is protein-bound, so not released until muscle proteins begin to break down. Albrecht and Jones, however, reported that glutamine accumulation in the brain stimulates transport of tryptophan and other neutral amino acids (NAA) across the blood–brain barrier: “In particular, there is strong evidence for enhanced Gln/tryptophan exchange across the BBB in acute and chronic liver failure.”[61] They cited studies by Cangiano and colleagues, who concluded: “High intracellular concentrations of Gln within brain microvessels stimulated uptake of the other NAA.s.”[144] Does low brain glutamine in ASD limit brain serotonin? Which pathologies appear most decisive in ASD – high brain
ammonia, low brain glutamine, low brain arginine (thus low nitric oxide and creatine), or low brain serotonin? Woeller’s observation [56] that the “vast majority” of these children make too much ammonia (largely by intestinal yeast and bacteria), yet rarely show high urinary orotate, suggests chronic/repeated high blood ammonia – and accumulation in the brain – may often go undetected. Arginine required to detoxify ammonia may deplete arginine for brain nitric oxide and creatine. Acetaminophen depleting glutathione may effectively deplete glutathione (thus arginine) by requiring it to synthesize more glutathione. Glutamine depletion in the brain may limit brain serotonin. Do these pathologies cascade in autism?

How does fever help? Muscle catabolism releases ammonia, but gluconeogenesis multiplies its conversion to urea [21]. Heat may kill intestinal bacteria that generate ammonia. Increased brain blood flow may flush accumulated ammonia and restore cortical flow. Free taurine released from muscles may help turn ammonia to glutamine [23]. Glutamine released from muscles may serve as substrate for alpha-ketoglutarate [145], move tryptophan into the brain, and become arginine to detoxify ammonia, synthesize creatine, and as only substrate for nitric oxide. Muscle catabolism releases protein-bound arginine.

Why do children with autistic disorders often have conditions that elevate blood/brain ammonia (early weaning, gut bacteria and yeast, constipation, mitochondrial dysfunction) and conditions that limit detoxification of ammonia (low glutamine/arginine, low-protein diet, low cofactors for glutamine synthetase, low ATP) – yet rarely show notably elevated urinary orotic acid (marker of chronic/repeated high blood ammonia)? A professor of Pediatrics reported that several tests of children with propionic acidemia (high plasma ammonia, low glutamine) did not show high urinary orotate; he thought relative deficiency of ATP in liver mitochondria in PA might limit formation of carbamyl phosphate [46]. Walker: “Orotic acid . . . is normally produced from carbamyl phosphate synthetized by cytosolic CPS II from glutamine and bicarbonate using ATP.” [60] If low ATP and glutamine in ASD (as in PA) keep carbamyl phosphate low, urinary orotate may look deceptively normal despite repeated high blood/brain ammonia.

Testing the hypotheses

Zimmerman reported that improvements before fever were more likely to persist after fever. If a single mechanism is responsible for early and persisting improvements, it may best be detected after fever, when risks of overheating and contagion are minimal. A brain scan by MRS days after fever breaks in a child whose dramatic improvements persist may reveal metabolites that vary with behavior, not temperature. Muscle scans may reveal more free glutamine in children who improve before fever. Children whose improvements persist may have more free + protein-bound glutamine, protein-bound arginine, and BCAA in muscles. Is high blood ammonia detectable in exhaled air? [146]

EVALUATION

Wilmore commented [50]: “The early release is most probably from the intracellular pool but as skeletal muscle protein starts to break down this is the main source of the gln [glutamine]. The free pool can probably be regulated by factors such as acidosis and dehydration but it seems it is followed by protein catabolism. . . . Blood glutamine is the balance between production – primarily from skeletal muscle – and uptake – primarily by liver, bowel and kidney. Turnover (flow through the plasma pool) may be quite high but amino acid concentrations may be quite low. After injury or infection gln concentrations fall but turnover or flux (from muscle to visceral organs and lymphatic tissue) increases. With time, intracellular levels of gln in skeletal muscle decrease and turnover falls.”

Are early improvements independent of temperature – or merely effects of low grade fever? Parents aware that fever helps their child presumably check for fever when they notice suddenly improved behavior; are they likely to miss low grade fever? Improvements persisting after fever breaks may merely be lasting effects of fever. The question then becomes: why don’t they last more often? If they depend on protein-bound glutamine, arginine, and BCAA in muscles, some children may have more than others. Because early improvements tend to persist after fever, these children may have more free glutamine as well. Children whose improvements subside when fever breaks may have less glutamine, arginine, and BCAA in muscles, and more alanine and pyruvate. We might also ask whether accelerated brain metabolism (if not blood flow) explains early improvements. But what might accelerate brain metabolism hours before temperature or epinephrine – and wouldn’t that look like fever?

We might also ask why implicate acetaminophen in the autism epidemic, if amoxicillin-clavulanate may be equally responsible? First, their harmful effects converge. By depleting glutathione, acetaminophen effectively depletes glutamine – precursor of arginine required to detoxify ammonia, and synthesize nitric oxide and creatine. Amoxicillin-clavulanate enables intestinal colonization by bacteria and yeast that generate ammonia. Second, the close association of regression with vaccinations is more likely due to an analgesic/antipyretic than an antibiotic. Jones recently reported that paracetamol induced release of nitric oxide in rat livers [147].

Do vaccines ever cause autism? Parents implicated vaccination in their child’s regression years before the CDC’s 1980 warning against aspirin. Yet vaccines alone cannot explain our autism epidemic. Cuba vaccinates all their children – especially against measles. Our autism incidence is now estimated at 1 in 68 children, mostly boys; Cuba’s autism incidence is 1 in 20,000!

Finally, because glutamine is the primary molecule that carries ammonia as nutrient throughout the body, what happens when plasma glutamine is deficient? Stevens and colleagues found that protein-deficient rats preserved ammonia to form amino acids [148]. One organ that ‘preserves’ ammonia is the brain [63-65].

Postscript

Oral citrulline has significant advantages over oral arginine: (a) it provides arginine for moderate production of nitric oxide by constitutive NOS, but not excessive production by inducible NOS [122,149]; (b) it stimulates protein synthesis when dietary protein is low (e.g. gluten-free/casein-free diets) [122]; (c) high-dose citrulline is safer than high-dose arginine [122,149,150].

Oral arginine is largely taken up by the liver (detoxifying ammonia); oral citrulline bypasses the liver and becomes arginine in the kidneys – thus presumably more available for brain nitric oxide and creatine. A therapeutic trial of oral citrulline vs. arginine may reveal which pathology is more decisive in ASD – high brain ammonia, or low brain nitric oxide/creatine.

Peter Good
Autism Studies
La Pine, OR
www.autismstudies.net
autismstudies1@gmail.com

acknowledgments

I’m most grateful to:

William Ellis of St. John’s Cathedral, Spokane WA – for generous support and encouragement
James Harduve of the Deschutes County Library, Bend OR – for resourceful retrieval of the literature over many years
the ARI pediatrician who suggested two mechanisms of fever’s benefit
James Adams – for a critical resonating question
Ann Bauer – for stoking the fire, and PubMed Commons
Helen Emily Couch (in memoriam) – for making this possible

references
104. Adams JB. Personal communication 2015.
142. Whitlock DR. Consider NO from iNOS. eLetter. Pediatrics 2007; [accessed 02.28.12].