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Autism and Renal Sulfate Transport

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ABSTRACT

Sulfate is an important nutrient and enzyme cofactor. Blood sulfate is depressed for individuals with autism, partly due to poor resorption in the kidney. We model the kidney nephron using simple mathematics and examine flowrates, concentrations and resorption along the length of the proximal tubule. Three math experiments are performed using our kidney model. Assuming constant resorption, NaS1 transport protein density is examined. Blood levels of sulfite and thiosulfate inhibitors are increased to show their influence on neurotypicals. Then blood sulfate is varied to show how inhibitor levels may be decreased, potentially resulting in symptom relief and improvement of overall health for those on the spectrum. Expression of the NaS1 transport protein is linked to vitamin D and estrogen chemistry, suggesting feedback mechanisms for sulfate homeostasis. Finally, sulfate supplementation and sulfite avoidance are discussed as potential strategies for both the prevention and treatment of autism.

Keywords: Autism; Kidney; Sulfate; Sulfite; Vitamin D; Estrogen

Abbreviations: ASD: Autism Spectrum Disorders; VDR: Vitamin D Receptor; EST: Estrone Sulfotransferase; STS: Steroid Sulfatase; MSM: Methyl-Sulfonyl-Methane

Introduction

Autism Spectrum Disorders (ASD) affect social interaction, communication, behavior and the senses. In the United States, the prevalence is 1 in 54 for all children and 1 in 34 for boys based on data from the Centers for Disease Control and Prevention (Maenner, et al. [1]). One characteristic of autism is depressed resorption of sulfate in the kidney leading to high levels in urine and low levels in blood. In this paper, we model the kidney using simple mathematics to examine sulfate flowrates and concentrations. Then the model is used to investigate sulfate inhibitors, sulfate regulation and possible steps to correct imbalances. An important feature of autism is dysfunctional sulfur metabolism. In particular, the oxides of sulfur are implicated: sulfite, thiosulfate and sulfate (SO_3^{2-}, S-SO_3 and SO_4^{2-}). Sulfate may be ingested directly or it may be converted from the amino acid methionine by a series of enzymes including sulfite oxidase. An English study reports the urine of those with autism contains 50 times the sulfite, 7 times the thiosulfate and double the sulfate of neurotypicals (Waring, et al. [2]). An Arizona study found depressed levels of blood sulfate in those with autism, only 35% of normal in the case of free sulfate (Adams, et al. [3]). And a French study of nasal stem cells found 91% of those with autism had decreased expression of genes (MOCOS and AOX) within the molybdenum cofactor pathway (Feron, et al. [4]). This pathway is responsible for several important enzymes including sulfite oxidase. There are 5 upstream genes (MOCS1, MOCS2, MOCS3, NFS1 and GPHN) in this pathway, requiring several cofactors including bioactive vitamin B6 (PLP).

Interference with of any of these elements will impair sulfite oxidase enzyme and depress the conversion of sulfite to sulfate as indicated above. Sulfate is a common nutrient and is necessary for a variety of chemical processes including the development of tissue for important organs. During human pregnancy, maternal circulating sulfate levels double during the final trimester. This highlights the importance of sulfate in fetal development (Dawson, et al. [5]). In particular, heparan sulfate is essential for neuron regulation. In studies of mice with compromised heparan sulfate synthesis, symptoms similar to autism resulted, including impairments in social interaction, expression of repetitive behavior and difficulties with vocalization (Irie, et al. [6]). In humans, the examination of postmortem brain tissue in

young individuals showed reduced levels of heparan sulfate for those with autism (Pearson, et al. [7]). Finally, sulfate supports sulfonation and sulfotransferase enzymes which help to remove xenobiotics. Through a sulfonate intermediary, 3'-phosphoadenosine 5'-phosphosulfate (PAPS), sulfate is attached to unwanted chemicals increasing water solubility to facilitate removal (Gamage, et al. [8]). Without sufficient sulfate, children may be at heightened risk from environmental factors that require clearance via sulfonation. Sulfate within the kidney filtrate is returned to the blood via resorption through proximal tubule membrane cells. This is facilitated by two transport proteins: NaS1 (SLC13A1) sodium-sulfate co- transporter located at the brush border membrane and SAT1 (SLC26A1) anion exchanger located at the basolateral membrane. NaS1 moves sulfate from nephron lumen into kidney membrane cells and SAT1 moves sulfate from membrane cells back into the bloodstream. When operating properly, they help to maintain sulfate blood levels within a healthy range. For those with autism, kidney resorption is partially blocked resulting in urine levels that are double normal and blood levels that are one third normal as reported above.

Methods

We investigate renal sulfate resorption using a simple mathematical model. Available data for sulfate transport kinetics are presented and estimates made to more fully characterize the NaS1 transport protein. The kidney nephron is mathematically modeled to predict the sulfate concentration profile along the proximal tubule. Several math experiments highlight the renal characteristics of autism. Regulation and NaS1 expression are discussed with special attention to the vitamin D receptor and estrogen chemistry involving estrone sulfate. Finallly, strategies are suggested to improve sulfate levels and minimize interference from inhibitors.

What is Known

An Australian study (Lee, et al. [9]) investigated the NaS1 transport protein encoded by the mRNA of the human kidney. A sulfate rate constant was determined and thiosulfate was noted as the most potent inhibitor tested. A German experiment using rat mRNA (Krick, et al. [10]) investigated the SAT1 anion exchanger. A sulfate rate constant was reported along with inhibition constants for both sulfite and thiosulfate. This information has been summarized in (Table 1) along with blood and urine concentrations of sulfite, thiosulfate and sulfate with estimated data shown in red print.

Table 1: Published Blood, Urine and Sulfate Transport Data.

Published Blood, Urine and Sulfate Transport Data (Estimated Data Shown in Red Font)		
Kinetic Constants	**NaS1 (species, Source)**	**SAT1 (species, Source)**
Sulfate Transport		
Km(sulfate)	310 uM (human, Lee 2000)	162 uM (rat, Krick 2009)
Ki(sulfite)	103 uM (human estimate)	54 uM (rat, Krick 2009)
Ki(thiosulfate)	229 uM (human estimate)	102 uM (rat, Krick 2009)
Concentrations	**Urine (Source)**	**Blood (Source)**
Neurotypical		
[S] (sulfate)	3030 uM (Waring 2000)	300 uM (Markovich 2001)
[I] (sulfite)	2.1 uM (Waring 2000)	1.2 uM (Mitsuhashi 2004)
[I] (thiosulfate)	18.6 uM (Waring 2000)	5.5 uM (Farese 2011)
Autism		
[S] (sulfate)	6820 uM (Waring 2000)	105 uM (Adams 2011)
[I] (sulfite)	107 uM (Waring 2000)	61 uM (estimate)
[I] (thiosulfate)	131 uM (Waring 2000)	39 uM (estimate)

Estimates for Transport Kinetics

Sulfite and thiosulfate have been reported as significant competitive inhibitors of NaS1 sulfate transport but interference concentrations (Ki) have not been determined for humans (Markovich, et al. [9,11]). The most detailed study of sulfate transport to date is the German experiment (Krick, et al. [10]) that investigated the SAT1 anion exchanger cloned from rat liver. A sulfate rate constant was reported along with inhibition constants for both sulfite and thiosulfate. For our purposes, we estimate NaS1 inhibition constants for sulfite and thiosulfate to be in the same ratios (Ki/Km) as for rat SAT1. This results in human NaS1 estimates of sulfite (Ki=103uM) and thiosulfate (Ki=229uM).

Estimates for Fluid Concentrations

Blood levels of sulfite and thiosulfate for those with autism have not been published. Both blood and urine values are known for neurotypicals and the ratios of blood to urine concentrations can be calculated. Applying these same ratios to autistic urine results in a blood sulfite estimate of 61uM and a blood thiosulfate estimate of 39uM. These estimates are not intended as rigorous predictions, just starting points for further investigation.

Simple Model of the Kidney Nephron

The human kidney pair contains approximately one million small tubes called nephrons. As blood passes through tiny pores upon entry, red and white cells are blocked and only plasma passes into the nephron. As this filtrate moves along the small tubes, nutrients are returned to the bloodstream while waste and toxins flow into the urine. The front section of each tube is called the proximal tubule and this region is responsible for the resorption of 65% of the general filtrate and nearly all of the sulfate (Zhuo, et al. [12]). The inner brush border membrane of the proximal tubule includes NaS1 transport proteins that move sulfate from the filtrate into the cytoplasm of the cells lining the tube. The outer basolateral membrane includes SAT1 transport proteins which complete the task by moving sulfate from cytoplasm back into the blood. It is generally assumed that NaS1 proteins form the rate limiting step for sulfate transport, therefore this study will consider only flowrates and kinetics for NaS1 transport proteins (Lee, et al. [9]).

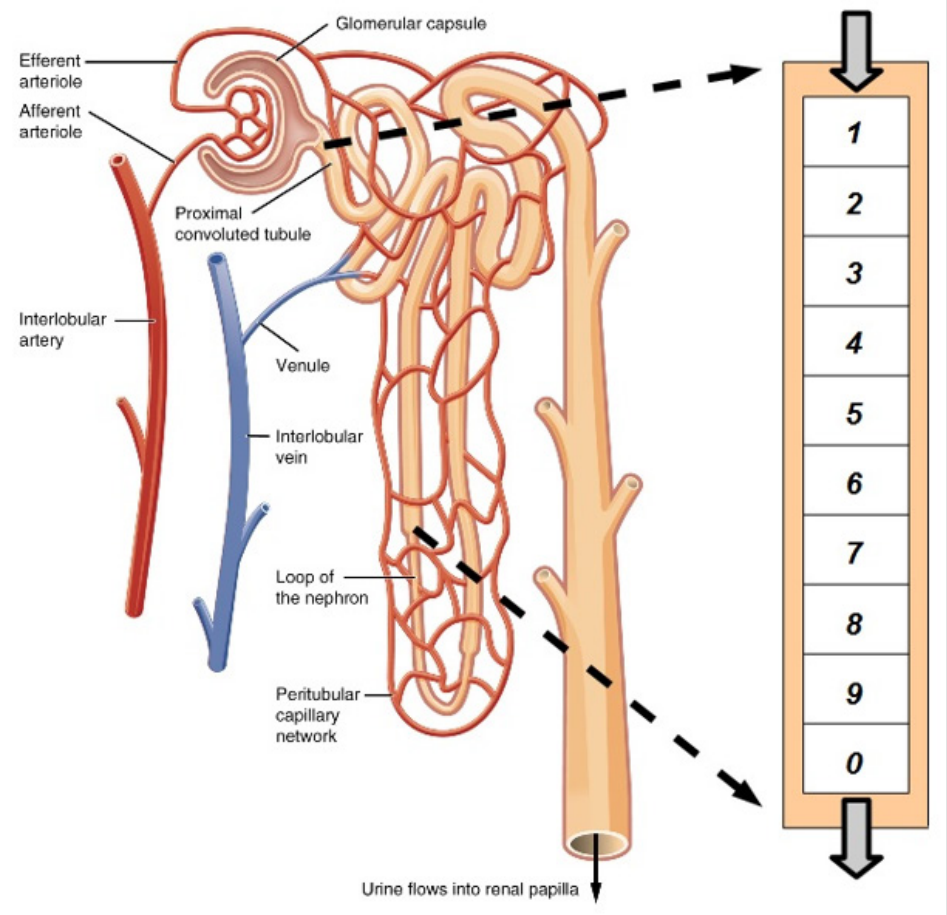

Figure 1: Simple Model of Segmented Proximal Tubule (Wikimedia 2013).

As shown in (Figure 1), we model the kidney nephron as a tube, beginning with the proximal tubule divided into 10 segments which are followed by an undifferentiated remainder. As filtrate flows down the tube, the concentration of sulfate and its inhibitors varies as they are reabsorbed along with water and other chemicals. The flowrate of chemicals in the filtrate can be specified at the entry and exit points by multiplying the appropriate concentrations by the flowrate of water. For a typical pair of human kidneys, the flowrate of the filtrate (which is mostly water) is 180L/day at the bloodstream entry and about 1.4 L/day at the urine exit. We define the following symbols.

Flowrate = Concentration times Water Rate

Sulfate Flowrate S = [S]W and Inhibitor Flowrate I=[I]W

S and I are flowrates of sulfate and its inhibitors (umol/day)

[S] and [I] are concentrations of sulfate and inhibitors (uM)

W is flowrate of water (Wentry=180L/day & Wexit=1.4L/day)

Using concentrations from our tables, the neurotypical flowrate of sulfate at bloodstream entry is (300umol/L) times (180L/day) which equals 54,000 umol/day. At urine exit, the sulfate flowrate is 4,240 umol/day. Note, this is a resorption percentage of nearly 92%. (Table 2) summarizes data for the other solutes. Autism values for sulfite and thiosulfate are not included since our blood entry concentrations are just rough estimates.

Table 2: Solute Flowrates and Resorption Percentages.

Solute Flowrates and Resorption Percentages Resorption % = 100%*(blood input– urine output)/(blood input)				
Solute Flowrates	**Blood**	**Urine**	**Resorption**	**Resorption %**
Neurotypical:	(umol/day)	(umol/day)	(umol/day)	(%)
S (sulfate)	54,000	4,240	49,760	92.1
I (sulfite)	216	3	213	98.6
I (thiosulfate)	990	26	964	97.4
Autism: S (sulfate)	18,900	9,550	9,350	49.5

The efficiency of sulfate resorption is reduced to almost half for those on the autism spectrum. And neurotypical sulfite and thiosulfate are reabsorbed at percentages exceeding sulfate. This would seem to be consistent with kinetic constants reported for SAT1 by Krick (rat sulfate Km=162uM with competitive inhibitor rate constants Ki=54uM and 102uM). Noting that 65% of the filtrate is reabsorbed in the proximal tubules along with nearly 100% of sulfate, we can make a few assumptions. Since the filtrate is mostly water, the flowrate of water at the end of all the proximal tubules would be approximately 35% of the blood entry flowrate or 63L/day. Whereas for sulfate, the flowrate at the end of the proximal tubules would be the same as the urine flowrate. And the same would apply to the competitive inhibitors or their combination. (Figure 2) depicts this graphically assuming linear decreases in the flowrates.

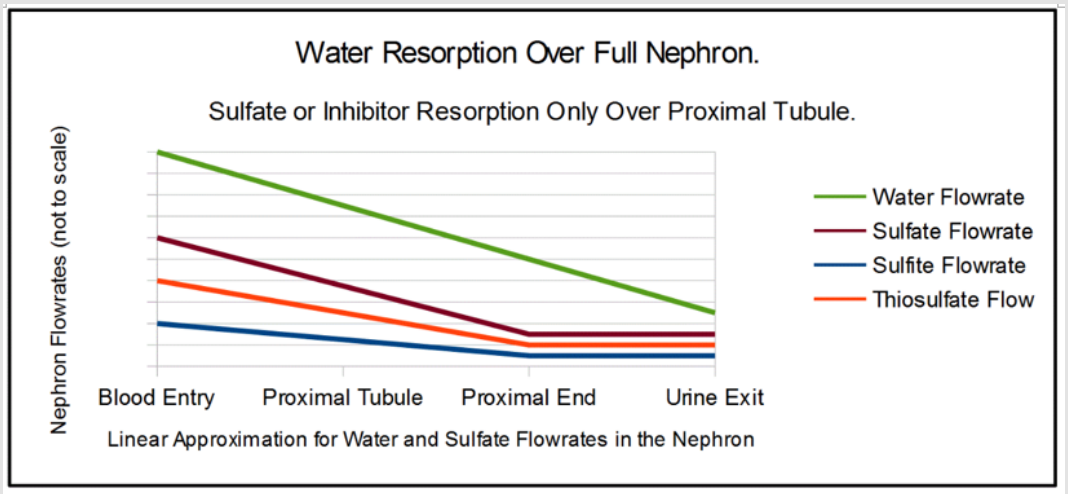

Figure 2: Linear Approximation for Nephron Flowrates.

As a first order approximation, assume resorption along the proximal tubule to be constant, resulting in a linear flowrate profile for sulfate, its inhibitors and water. Let z be the distance along the nephron and d the length of the proximal tubule. Then an independent variable may be defined as z/d, representing the normalized distance. The blood filtrate entry point becomes z/d=0 and the end of the proximal tubule (but not the entire nephron) becomes z/d=1. Simple linear equations can be written for all of the flowrates, using *to indicate multiplication.

Linear Flowrate:

Flowrate = b – m*(z/d)

b = entry flowrate

m = entry flowrate – proximal exit flowrate

For water, the entry flowrate is 180L/day and the proximal exit flowrate is 63 L/day as discussed previously. This makes m = 67L/day for water. For sulfate and inhibitors that are fully reabsorbed in the proximal tubule, the proximal exit flowrates are the same as those at urine exit.

Flowrate Calculation:

Sulfate Flowrate = S = [S]W

Inhibitor Flowrate = I = [I]W

where W = flowrate of water

Results

Using data from (Table 1), the linear formulas may be built into a spreadsheet (available upon request from rybett@aol.com) and concentrations calculated. (Figure 3) plots sulfate concentration along the z-axis of the nephron. The proximal tubule is represented by values of z/d < 1. The remainder of the nephron is depicted by values of z/d > 1 where concentrations rapidly increase to those of urine.

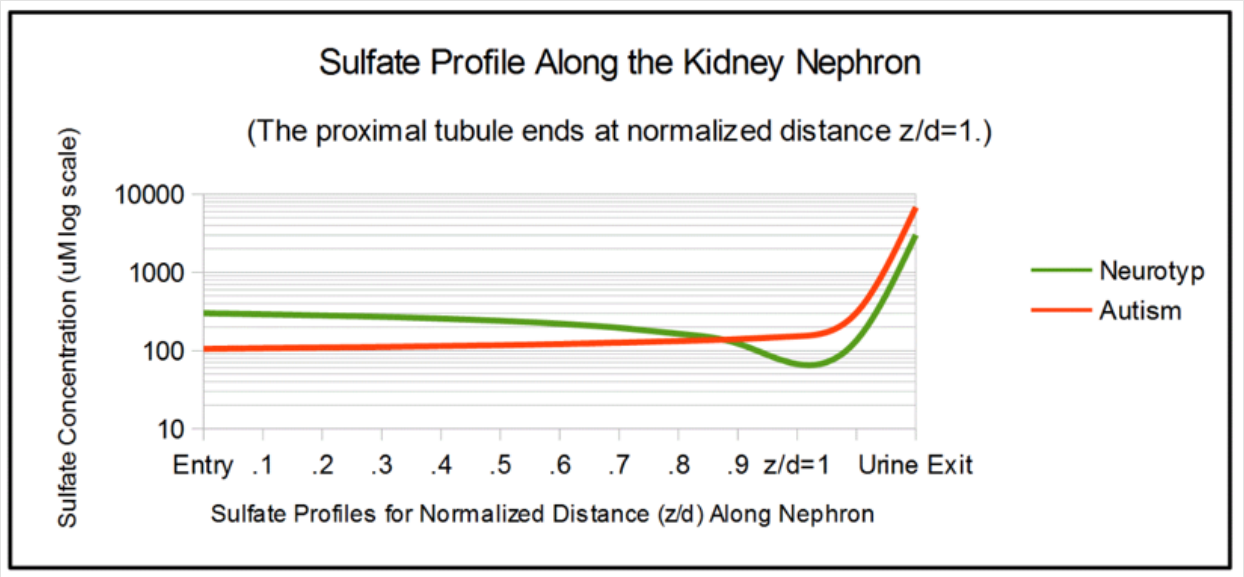

Figure 3: Sulfate Concentration along Z-Axis of the Nephron.

It is more convenient to consider the proximal tubule divided into 10 equal segments. Segment concentrations and other metrics are defined as the average of values between the leading and trailing edges of each segment. For instance, segment n=3 would be the mean of data values at z/d=0.2 and z/d=0.3. (Figure 4) plots sulfate concentrations for segments n=1 to n=10. This graph is more revealing because it may be plotted on a linear scale with a restricted range. For neurotypicals, sulfate concentrations drop as water is removed more slowly than sulfate in the proximal tubule. Within autism, this effect is countered by the higher levels of sulfate in urine.

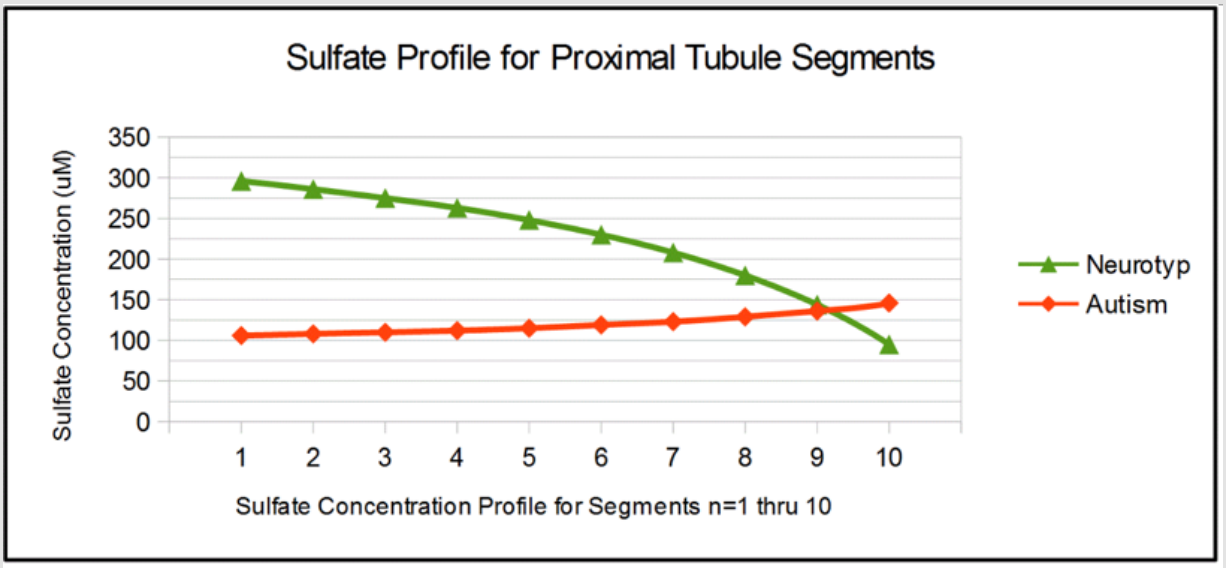

Figure 4: Sulfate Concentration for Proximal Tubule Segments.

Math Experiments

The sulfate profiles in (Figures 3 & 4) simply assume constant sulfate resorption along the proximal tubule. The experiments to follow employ Michaelis-Menten kinetics to gain further insight into kidney function within autism. For the NaS1 sulfate transporter, two inhibitors are significant, sulfite and thiosulfate. Velocity formulas for two inhibitors are complex, and in the case of sulfate transport, require unknown data describing interactions between the inhibitors. For simplicity, assume the inhibitors are independent and may be merged into a single combined inhibitor. To account for slight differences in affinity, form the ratio Ki(thiosulfate)/Ki(sulfite) using SAT1 data. Then upgrade the concentration of sulfite by this ratio before combining with thiosulfate. For the combined inhibitor, use the Ki of thiosulfate. These steps are summarized in the following formulas, where [I] in brackets references the combined inhibitor concentration and *denotes multiplication.

Combined Inhibitor:

A = Ki(thiosulfate)/Ki(sulfite)

[I] = A*[sulfite] + [thiosulfate]

Ki(combined) = Ki(thiosulfate)

For instance, if the Ki of sulfite is half that of thiosulfate, then the affinity is presumed double and sulfite should be doubled when forming a combined concentration. Referencing SAT1 data, the ratio becomes A = 102/54 = 1.89. Use this value of A for NaS1 calculations.

Math Experiment 1: Michaelis-Menten Transport Kinetics

As sulfate and inhibitor concentrations change, the effectiveness of transport proteins varies, following Michaelis-Menten kinetics. The NaS1 transport protein is the first step in moving substrate from the kidney lumen back into the bloodstream by making substrate available to its partner SAT1. Again, assume that NaS1 is the limiting step and ignore SAT1 in this analysis. Below are our definitions for segment flowrates, concentrations and resorption velocities:

Michaelis-Menten resorption velocity for segment n:

Vn = Vmax[S]/{(1 + [I]/Ki)Km + [S]}

where Vmax is the maximum possible sulfate velocity (units of umol per day)

[S] and [I] are the concentrations of sulfate and its combined inhibitors

Km is the sulfate rate constant (a concentration) yielding half max velocity

Ki is the inhibition constant (also a concentration) reducing the velocity

Without knowing the value of Vmax at this point, the ratio Vn/Vmax can be calculated for each segment. In our linear model, the resorption is assumed to be the same for each segment. Sulfate resorption for the full proximal tubule is known from previous calculations, so each segment would reabsorb one tenth. For neurotypicals, Vn = 4976 umol/day for each segment and for those with autism, Vn = 935 umol/day. This allows Vmax to be calculated and plotted in (Figure 5). Note that these curves rely on the assumption of constant resorption along the proximal tubule.

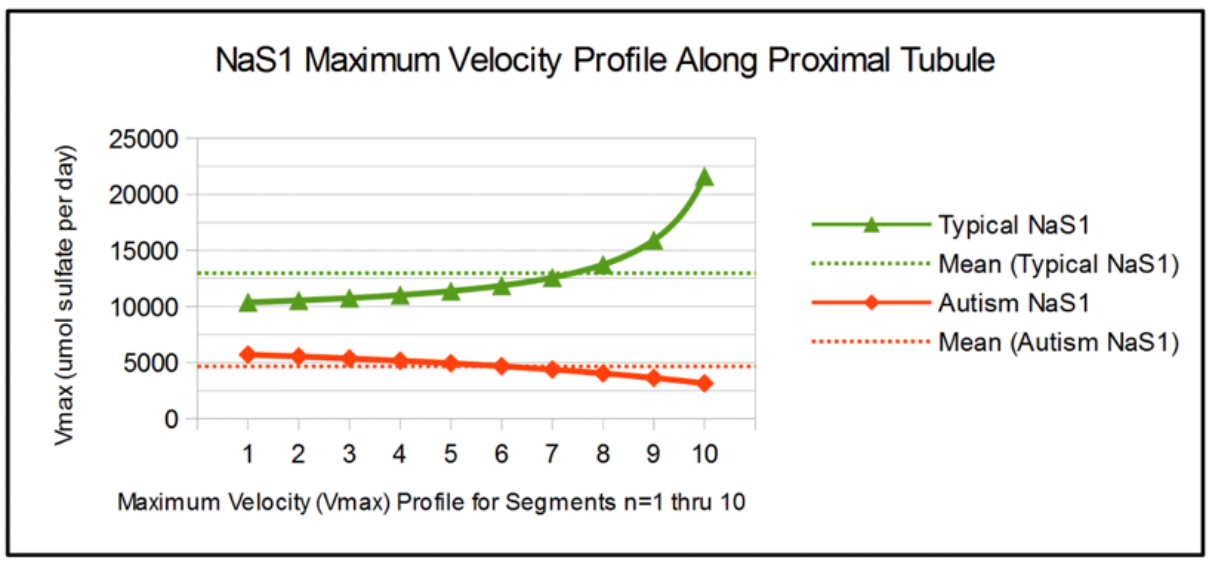

Figure 5: Maximum Velocity Profiles for Proximal Tubule Segments.

Velocity Calculations:

Vn/Vmax = [S]/{(1 + [I]/Ki)Km + [S]}

Vmax(neurotypical) = (4976 umol/day)*(Vmax/Vn)

Vmax(autism) = (935 umol/day)*(Vmax/Vn)

Math Experiment 2: Disrupting Neurotypical Sulfate

What would happen in a healthy kidney if neurotypical blood sulfite/thiosulfate combined concentrations (8uM) were raised to levels approaching autism (154uM)? This is a thought experiment which may be performed using our simple mathematical model. We start with a normal sulfate profile such as that shown in (Figure 4). Sulfate resorption in each segment is a flat 4976 umol/day per our linear model. Then inhibitor concentrations at blood entry are raised as high as 250uM. The resulting sulfate resorption velocities are calculated for each segment.

Summing the velocities for all segments yields total sulfate resorption for this experiment. Then this resorption can be compared to neurotypical values and a percentage calculated. The resulting decline may be plotted against inhibitor blood concentrations as shown in (Figure 6).

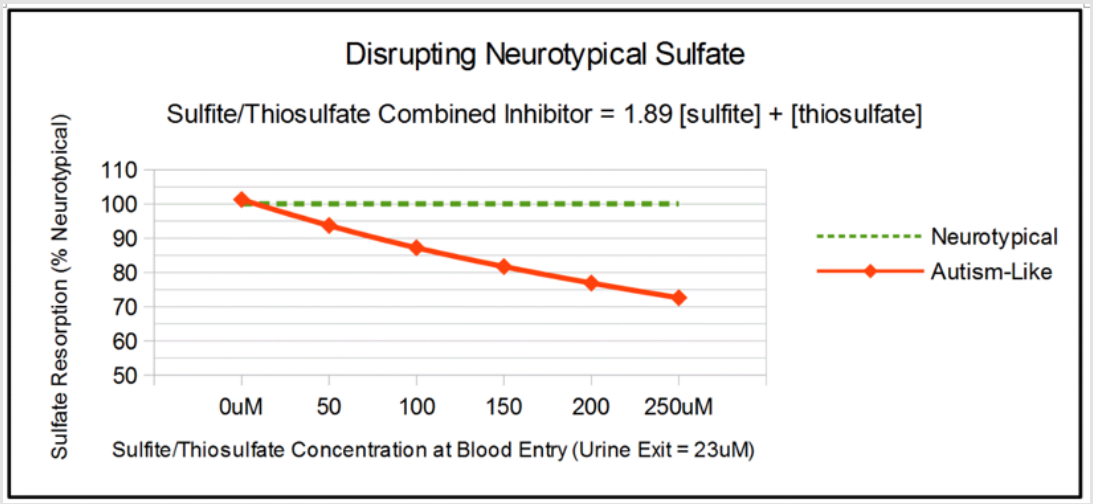

Figure 6: Disrupting Neurotypical Sulfate.

Experiment Formulas:

Vn = Vmax[S]/{(1 + [I]/Ki)Km + [S]}

Vexperiment = ∑Vn where n=1 thru 10

% Neurotypical = (100%)*(Vexperiment)/49760 umol/day

Math Experiment 3: Autism Improvement by Forced Sulfate

What would happen to a person with autism if blood sulfate was raised to a neurotypical level (300uM)? This could be an exercise similar to the first experiment if the roles of substrate and inhibitor were interchanged. Then sulfate becomes the competitive inhibitor and sulfite plus thiosulfate becomes the substrate. Since published data is thin, additional assumptions must be made. For NaS1 proteins, the sulfate Km value (310uM) must substitute for the needed Ki value. And likewise, the sulfite/thiosulfate Ki value (229uM) must substitute for the needed Km value. This is not as arbitrary as it may at first seem, as there is a precedent in the German study of rat SAT1. In that study of sulfate/oxalate co-transport, oxalate was treated as both a substrate and an inhibitor. The oxalate Ki as a sulfate inhibitor was measured as 64uM while the Km for oxalate transport became 52uM when sulfate acted as the inhibitor. Although these values are roughly equal, strict equality represents a source of potential error in our analysis. Keeping this in mind, we can proceed with calculations for forced blood sulfate concentrations over the range of 100 to 600uM. Then a percentage reduction in sulfite/thiosulfate resorption can be made when compared to typical autistic values as shown in (Figure 7). Since sulfite and thiosulfate are biological disruptors, reducing their blood concentrations should be beneficial to those on the autism spectrum.

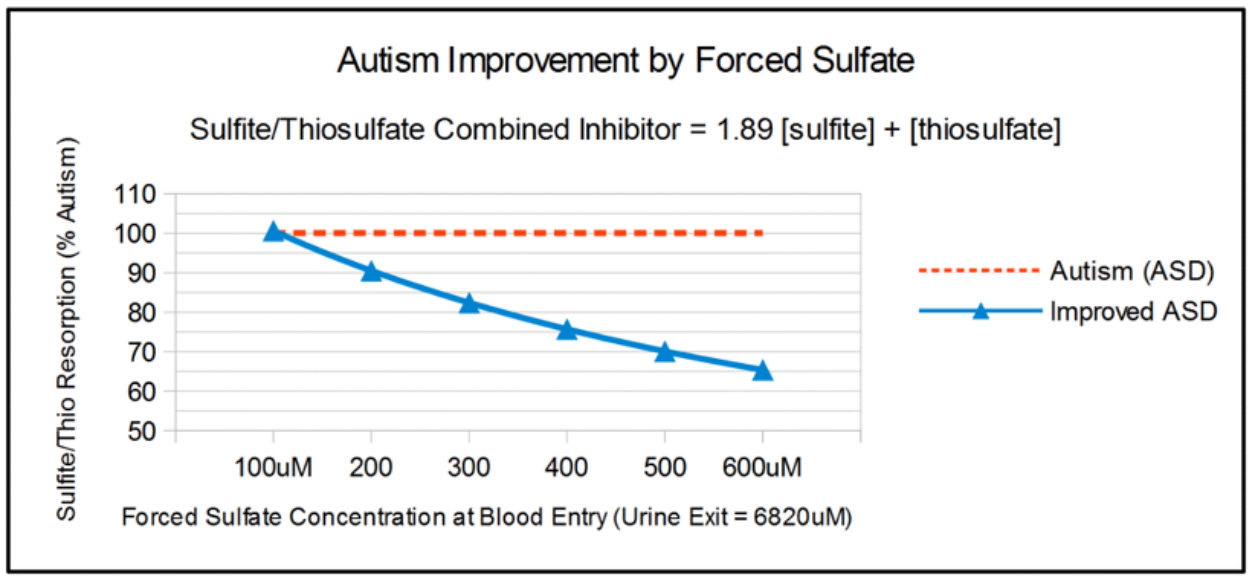

Figure 7: Autism Improvement by Forced Sulfate.

Discussion

The maximum velocity profiles in (Figure 5) exhibit quite a range for Vmax from 3,000 to 22,000 umol of sulfate per day. It seems logical to assume this range results from variations in the density of transporters embedded in the surface of the tubule membrane. And this suggests that the expression of NaS1 transport proteins may play an important role in sulfate regulation. If the expression of NaS1 can be properly linked to sulfate levels, a regulatory feedback loop may be established. Pathways relevant to this discussion are shown in (Figure 8) that follows.

Notes for Figure 8

- Dotted pathway lines indicate that genetic expression is promoted.
- CMO enzyme calcidiol 1-monooxygenase (EC 1.14.15.18)
- ETS enzyme estrone sulfotransferase (EC 2.8.2.4)
- STS enzyme steroid sulfatase (EC 3.1.6.2)
- HSD enzyme 17-beta-HSD (EC 1.1.1.62)
- ARM aromatase (EC 1.14.14.14)

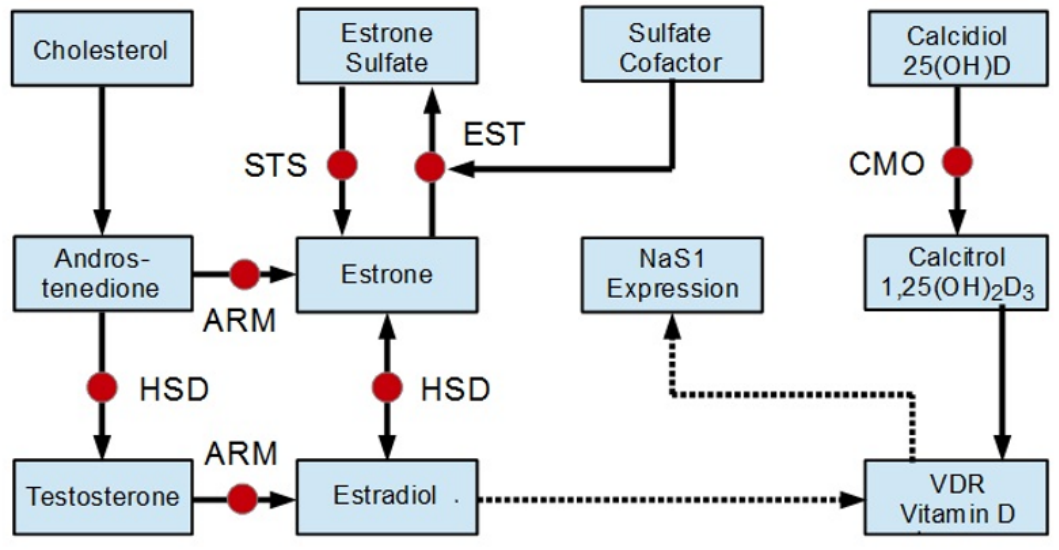

Figure 8: Simplified Metabolic Pathways for Vitamin D and Estrogen.

An Australian genetic analysis of NaS1 has identified a Vitamin D (1,25-(OH)2D3) responsive element in the promoter region of the gene (Dawson, et al. [13]). And a study of VDR knockout mice with diminished vitamin D receptor expression showed urinary sulfate increased by 42% while blood serum sulfate decreased by 50% (Bolt, et al. [14]). These studies confirm that repression of either vitamin D or its receptor interferes with the NaS1 transporter causing sulfate resorption to decrease. Studies of pregnant women in Sweden have noted Vitamin D (25OHD) deficiency increased autism risk by a factor of 1.58 (Lee B, et al. [15]). On the other hand, the Arizona study of blood sulfate previously referenced also tracked vitamins and minerals. For vitamin D, there was very little difference between neurotypicals and children with autism (Adams, et al. [3]). In fact, those on the spectrum measured about 2% higher. Perhaps this is a clue that the vitamin D receptor (VDR) may be a more likely candidate for regulation of NaS1 and sulfate.

VDR expression is regulated by the hormone estrogen (Schwartz, et al. [16,17]). Estrogen is a family name for several similar chemicals including estrone and estradiol which are the most abundant. Estrone and estradiol may interconvert as needed. Estrone may be removed by the enzyme estrone sulfotransferase (EST) to form a sulfate and returned via the enzyme steroid sulfatase (STS). Estrone sulfate acts as a reserve pool allowing regulation of overall estrogen. An important piece of this process is the cofactor sulfate. Without sufficient sulfate, estrone removal via EST is diminished which keeps overall estrogen levels high. This connection to sulfate completes a feedback loop that may play an important part in sulfate regulation.

Regulation of Sulfate via Negative Feedback

- Sulfate blood levels drop.
- EST is starved for its sulfate cofactor.
- Estrone rises which up-regulates VDR expression.
- This creates NaS1 proteins that bolster renal sulfate resorption.
- Increased resorption raises blood levels of sulfate to maintain homeostasis.

The feedback loop described above may offer insight into sulfate homeostasis which maintains normal serum concentrations in the vicinity of 300uM. Simply put, sulfate levels drop and this leads to enhanced NaS1 expression with increased sulfate resorption. Additionally, it suggests a mechanism for the distribution of transport proteins along the length of the proximal tubule. Our linear model with constant resorption is characterized by the sulfate concentrations in (Figure 4) and maximum velocities in (Figure 5). In the neurotypical case, as sulfate decreases along the length of the tubule, Vmax increases indicating that NaS1 protein density also increases. The feedback loop above could orchestrate such a density change along the z-axis of the nephron. Decreased sulfate near the end of the proximal tubule starves EST in membrane cells which up- regulates VDR expression to increase NaS1 density. However, simple logic suggests that regulatory feedback within autism must be compromised if overall sulfate resorption is so strongly depressed. For those on the spectrum, average values of sulfate, maximum velocities and protein density are all de-

pressed. Regulatory feedback would try to correct this but fails. Why?

The proposed feedback loop relies on estrone to adjust the density of sulfate transport proteins. When sulfate falls, EST reduces the sulfation of estrone and estrone levels should rise. Of course, this assumes that other paths also feeding estrone remain unaffected. A recent Chinese study of steroid sulfatase (STS) has shown sulfite to be an inhibitor of this enzyme (Zhang, et al. [18]). If sulfite inhbition is significant, STS conversion of estrone sulfate back to estrone would be reduced. This negates increases in estrone required by the sulfate feedback loop. A graph of STS inhibition by sulfite is shown in (Figure 9). Our analysis has estimated autism blood sulfite of 61 uM and this would result in a 30% inhibition of STS, disturbing regulatory feedback for those on the autism spectrum.

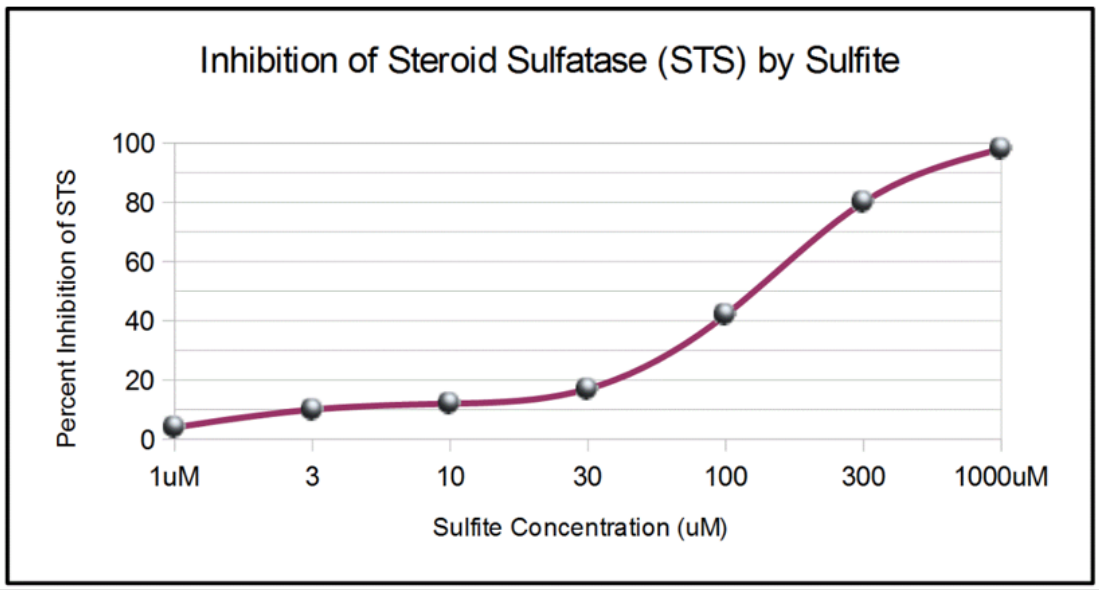

Figure 9: Inhibition of Steroid Sulfatase (STS) by Sulfite (from Zhang 2022).

Looking closely at our proposed regulatory mechanism, sulfate is an indirect cofactor of EST. Sulfate itself must first be converted to PAPS (adenosine 3'-phosphate 5'-phosphosulfate) by the PAPSS enzymes (ATP sulfurylase EC 2.7.7.4 and APS kinase EC 2.7.1.25) before becoming a cofactor. For sulfate to be properly regulated by negative feedback, PAPS concentration must correlate with sulfate levels and the PAPSS enzymes must operate in an unsaturated manner. Simply put, a change in sulfate from the homeostatic setpoint must produce a similar change in the output of the PAPSS enzymes. Enzyme kinetics defines Km as the substrate concentration resulting in half maximum output velocity. At concentrations well above Km, the output approaches saturation. Near Km and below, enzyme output is unsaturated and appropriate as a feedback element. So, PAPSS should be unsaturated, operating near or below the Km for sulfate. From the literature, the PAPSS Km is in the range 500-800 uM sulfate and typical operating concentrations are below these values keeping the enzymes unsaturated (Venkatachalam, et al. [19]).

Possible Treatment Strategies

Sulfate is an important nutrient that is depressed within autism. With reference to the values in (Table 1), autistic urine contains 6820uM sulfate compared to 3030 for neurotypicals. Assuming daily urine discharge at 1.4 liters, the average extra sulfate in urine for those with autism may be calculated as 510 mg per day. This suggests that tissue of those with autism may be starved for sulfate, due to poor conversion of sulfite and poor resorption in the kidney. These problems may be the result of mutations or other aberrations within the molybdenum cofactor pathway, depressing the enzyme sulfite oxidase. It would seem probable that increasing blood sulfate and avoiding sulfite/thiosulfate would be beneficial to both the prevention and treatment of autism. Forced sulfate is exactly the strategy employed in the third math experiment which predicts nearly an 80% reduction in sulfite and thiosulfate resorption when blood sulfate is raised to 300uM in an individual with autism. Such an increase can be achieved by supplementing with Epsom salts (MgSO4 heptahydrate) purchased as saline laxative in any drugstore. Mixing ¼ teaspoon (1.33 g) of Epsom salts into a liter of purified water creates a mineralized water with a sulfate concentration of 518 mg/L. Drinking ¼ liter portions 4 times each day, would jump blood sulfate levels from 105 to 375uM, assuming an adult volume of 5 liters of blood. For the full day, 518 mg sulfate would be added to an otherwise normal diet. This would make up for the sulfate lost to autistic urine each day and bump

blood concentrations into the range of the math experiment. Missing sulfate would be replaced while sulfite and thiosulfate inhibitors would be reduced via altered resorption in the kidney. Of course, this is theoretical and would need to be tested for safety and effectiveness.

Supplementation with MSM (methyl-sulfonyl-methane) is often promoted as another way to increase sulfate levels. How does it compare to the mineralized water above? MSM must be metabolized to release sulfur dioxide which is converted to hydrogen sulfite in an aqueous environment and then oxidized by sulfite oxidase enzyme to become sulfate (Wedzicha, et al. [20]). Unfortunately within autism, sulfite oxidase enzyme is likely depressed resulting in only a partial conversion to sulfate. This means not all of the released sulfite is processed which adds to sulfite circulating in the blood. In turn, extra sulfite further depresses the resorption of sulfate in the kidney. For those with autism, MSM may be more of a burden than an effective means of increasing sulfate.

No matter the method of sulfate supplementation, it would seem prudent to minimize inhibitors such as sulfite. Sulfite is a common preservative used in many foods and beverages, including wine, white grape juice, molasses, lemon juice concentrate, potato flakes, scallops, pickled peppers, sauerkraut and many others. Sulfur dioxide may digest to produce hydrogen sulfite as noted above. Sulfur dioxide is used to preserve dried fruit and to process starch, gelatin and caramel color. Sulfa drugs contain a sulfur dioxide moiety that may partially metabolize to sulfite in the same way as MSM. Bactrim is a strong sulfa drug that is commonly prescribed for children with ear infections. For children with autism, it may be wise to consider alternatives to foods, beverages and drugs containing significant amounts of sulfite or sulfur dioxide.

Is there experimental evidence for sulfate supplementation? Two previous studies hint strongly at the need for additional sulfate during pregnancy for women at risk of autism. The first study looks at beverages consumed during pregnancy by mothers of children with autism. It reports a correlation between low sulfate and the severity of autism ($r=-0.32$, $n=86$, $p<0.01$) in a group of 86 mothers recruited on Facebook (Williams, et al. [21]). The second study examines the geographical distribution of autism per the New Jersey Autism Registry. A strong correlation between low sulfate and high rates of autism ($r=-0.94$, $n=10$, $p<0.001$) is demonstrated by comparing data from over 600 water systems grouped into 5 prevalence zones (Williams, et al. [22]).

Conclusion

Metabolism of sulfur is quite disturbed within autism. Sulfite in urine is 50 times normal while thiosulfate is increased 7 fold. Free sulfate is double in urine and only one third normal in blood. Dysfunctional levels of these oxides of sulfur may be explained by abnormalities within the molybdenum cofactor pathway, which are present in 9 out of 10 children with autism. In turn, these pathway abnormalities interfere with the creation of sulfite oxidase enzyme, necessary for the conversion of sulfite into sulfate. Low sulfate in conjunction with high sulfite and thiosulfate reduces renal resorption, further lowering blood sulfate. Sulfate regulation would help to correct this shortfall but may be compromised by inadequate vitamin D or its receptor (VDR). Higher testosterone and lower estrogen typical in males would reduce the expression of VDR, possibly explaining why boys are more strongly affected by autism than girls. In this paper, incomplete published data was augmented by estimates to more fully characterize blood levels and transport properties of sulfate in the kidney. A simple model of the kidney nephron was built assuming constant sulfate resorption along the length of the proximal tubule. Flowrates and concentrations were calculated and plotted, demonstrating how elevated sulfite and thiosulfate could interfere with renal sulfate resorption even in neurotypicals. A feedback mechanism was proposed to explain the regulation of sulfate via vitamin D and estrogen chemistry. And math experiments were performed on the kidney model, suggesting a protocol to improve sulfate levels and reduce inhibitors by drinking water enhanced with magnesium sulfate. It is hoped that this study expands the understanding of sulfur metabolism, leading to autism strategies that increase sulfate, lower sulfite, reduce prevalence and improve treatment.

Compliance with Ethical Standards

Conflict of Interest

Open access publication of this work was funded by Rybett Controls, Inc. per an award letter dated May 10, 2019. The author is an officer and shareholder of Rybett Controls. He donated his time to this project, receiving no compensation for his involvement. The author has some mild characteristics of autism which has led to his interest in this subject. He is an electrical engineer by training with degrees from Caltech. Rybett Controls has no products related to autism except an Amazon book titled "Autism, Enzymes and the Brimstone Demons" (Williams, et al. [23]). The book includes an economical recipe for sulfated water which we whimsically call Brimstone Water. For legal protection, Rybett Controls owns the trademark for Brimstone Water although the recipe is unprotected and freely available for all to use. Neither Rybett Controls nor the author have patents, commercial affiliations or business interests relating to autism and declare no conflicts of interest with this research study other than the book and trademark [24-26].

Ethical Approval

The study did not include human participants or animal experiments. It was simply a mathematical exercise using previously published data from a variety of sources. Therefore, informed consent or the approval of an institutional review board was not appropriate. The mineralized water protocol is a suggestion for further research and not a prescription for the general population.

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