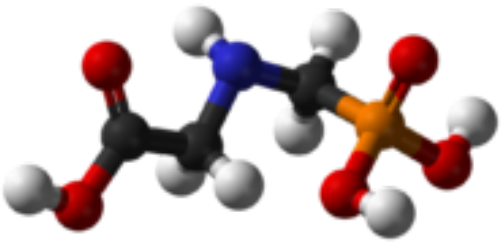
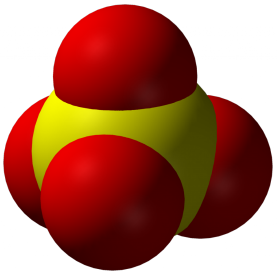
The Many Roles of Sulfate in the Body and its Disruption by Glyphosate

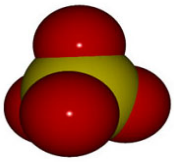


Stephanie Seneff
MIT CSAIL
September 7, 2019

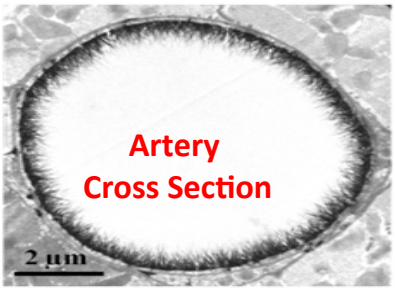


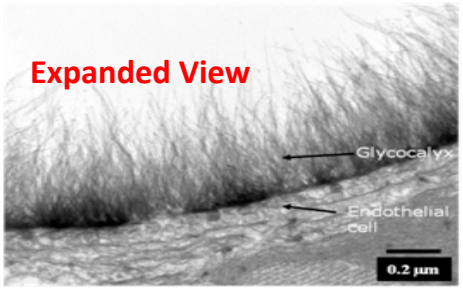
Outline

- Sulfate and the Glycocalyx
- Cholesterol Sulfate and Heart Disease
- Glyphosate as a Glycine Analogue
- Endothelial Nitric Oxide Synthase: a Moonlighting Enzyme
- Roundup, StAR and Sterol Homeostasis
- Glyphosate, Sulfate and Autism
- Summary



Sulfate and the Glycocalyx





It's All About the Blood

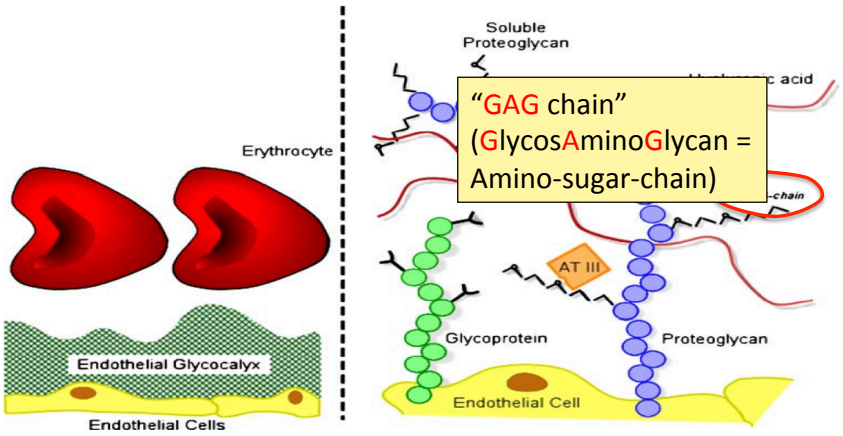
- We are 2/3 water by mass and 99% by molecule count
- Most of the water in the body is gelled
- The BIG EXCEPTION is the flowing blood!
- Blood delivers nutrients to and removes waste from all the tissues
- Gelled water lines the vessel wall and provides slick, frictionless passage of red blood cells through the capillaries

- Sulfate keeps the water gelled along the border
- Sulfate transport is problematic

Sulfate is Crucial to Maintain Structured Water

- Most cells in the body maintain an extracellular matrix formed from proteins with complex sugar chains attached to them
- Sulfate ions bind to the sugars at strategic locations in an irregular but non-random pattern
- The "glycocalyx" that lines the walls of all blood vessels is formed from these sugar-protein complexes

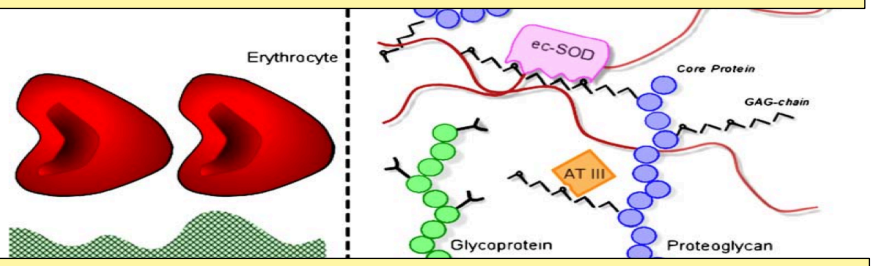
Endothelial Glycocalyx Schematic*



*S Reitsma et al., Pflugers Arch - Eur J Physiol (2007) 454:345–359

Endothelial Glycocalyx Schematic*

Gel layer prevents attack of vessel wall by blood sugars and oxidizing agents.



Cellular signaling mechanisms (e.g., *antioxidant* ec-SOD and *clot-protective* AT III) depend on sulfates to work.

*S Reitsma et al., Pflugers Arch - Eur J Physiol (2007) 454:345–359

Exclusion Zone: Model of Capillary*

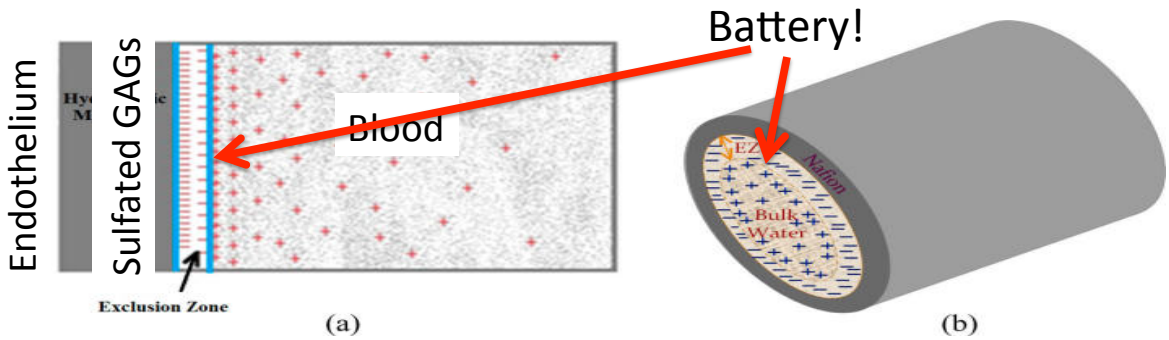
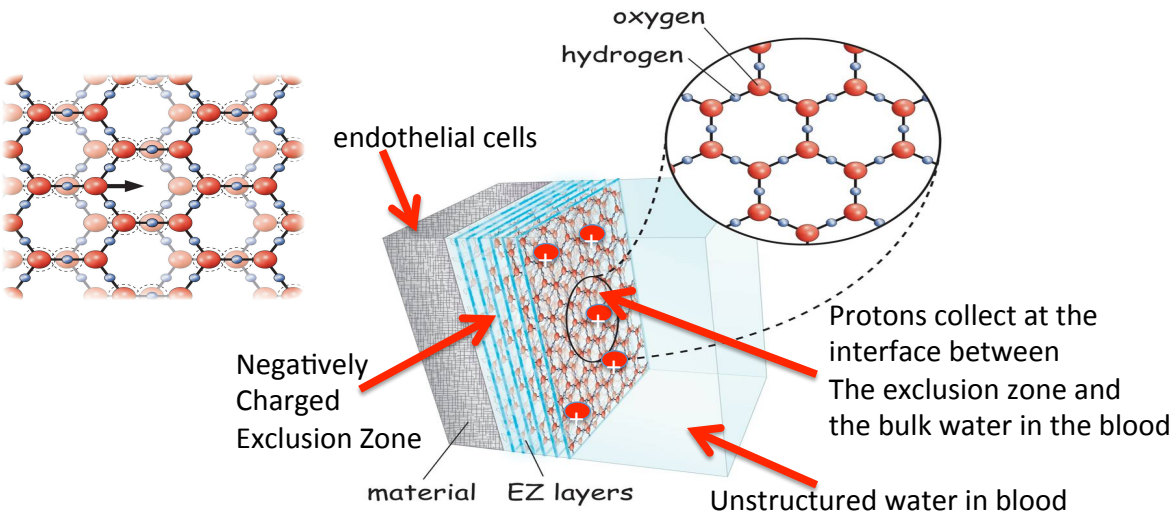


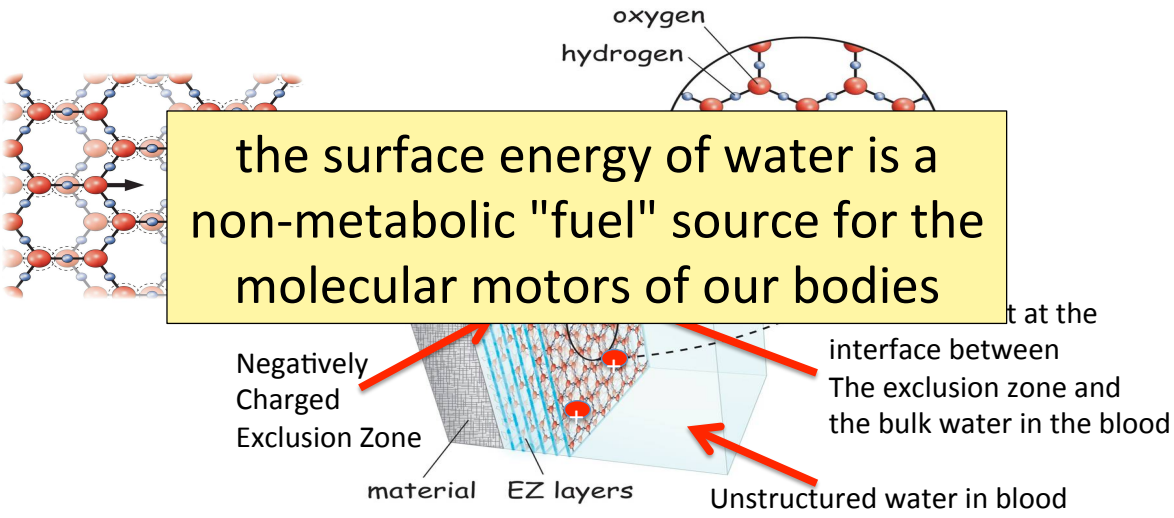
*Figure 9, M. Rohani and G.H. Pollack, Langmuir 2013, 29, 6556−6561

Exclusion Zone*



*http://doublehelixwater.eu/understanding-water-contents/exclusion-zone-form/

Exclusion Zone*



*http://doublehelixwater.eu/understanding-water-contents/exclusion-zone-form/

Cholesterol, Sulfate and Heart Disease

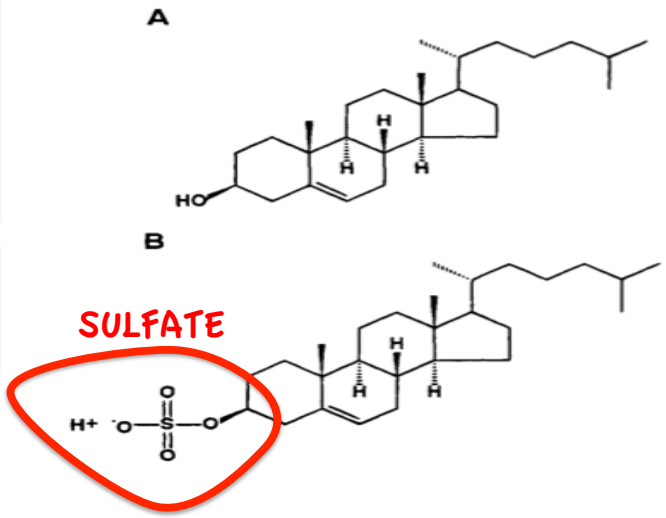
Cholesterol: Saint, Not Villain

- Cholesterol is to animals as chlorophyll is to plants: it gives us a brain and mobility
  - The brain makes up 5% of the body's weight and contains 25% of the body's cholesterol
- Why would cholesterol pile up in the arteries leading to the heart, if not for a good reason??

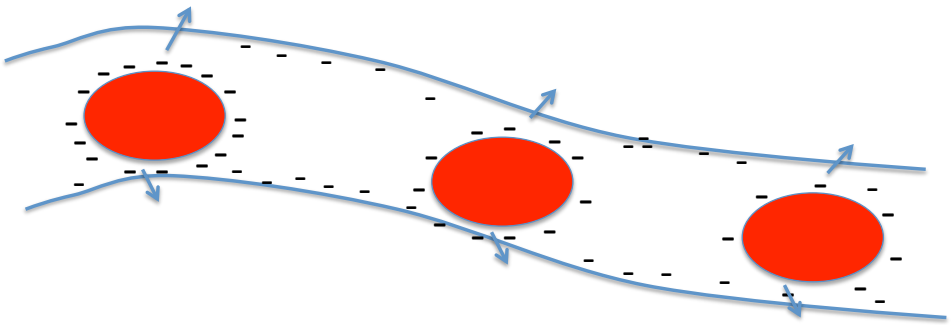
Cholesterol and Cholesterol Sulfate

Sulfation makes cholesterol water-soluble and therefore much easier to transport

Equally important is that cholesterol *carries sulfate*



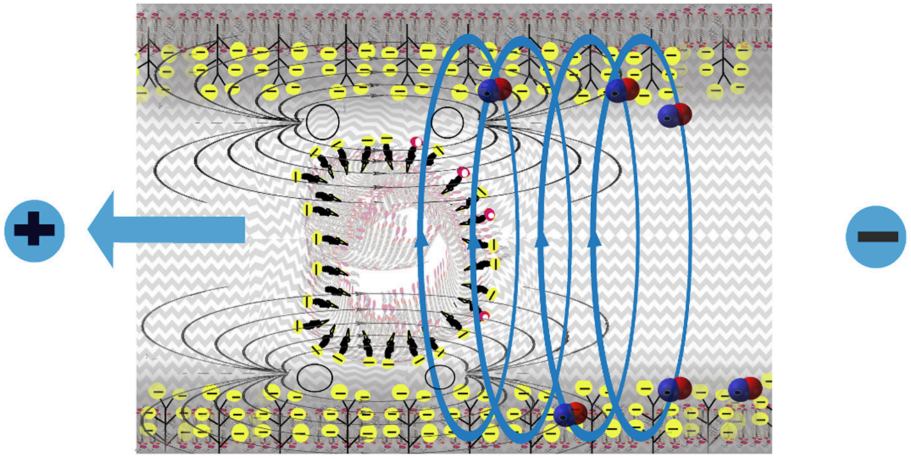
Cholesterol sulfate provides negative charge*



Red blood cells export cholesterol sulfate to the capillary wall, supplying it with cholesterol, sulfate, and negative charge

* Davidson and Seneff, Entropy 14, 1399-1442, 2012

The moving RBC creates an electromagnetic field that induces the release of nitric oxide from the capillary wall. This relaxes the vessel wall and promotes blood flow.



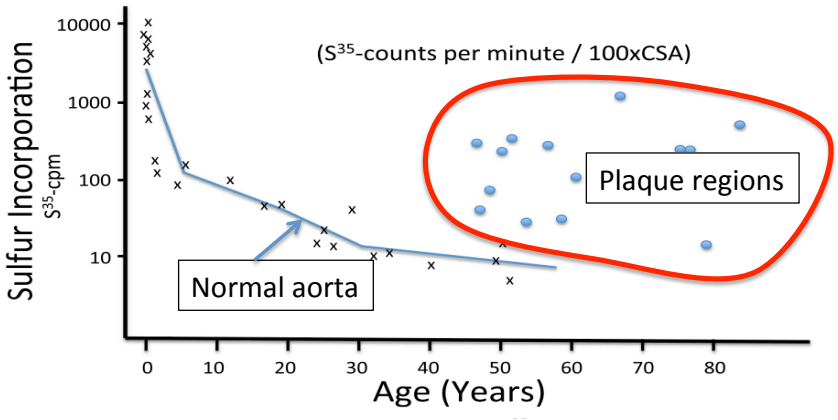
* Seneff et al. Theoretical Biology and Medical Modelling (2015) 12:9

They Knew a Long Time Ago*

- Article published in 1960
- Fed cholesterol to monkeys
  - Induced atherosclerosis
- If sulfur-containing nutrients are added, atherosclerosis is prevented
- These nutrients provide source of sulfate to enable cholesterol transport

* G.V. Mann et al., Am. J. Clin. Nutr. 8, 491-497, *1960*

Sulfur Incorporation into GAGs with Age*

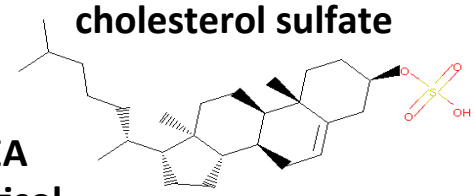


*WH Hauss et al.. J Atheroscler Res. 1962;2(1–2):50–61

Sulfation as Sulfate Transport

STEROLS

cholesterol sulfate



DHEA
Cortisol
Estrogen
Progesterone
Testosterone

Vitamin D
Vitamin C

NEUROTRANSMITTERS/HORMONES

Serotonin sulfate

Melatonin
Adrenalin
Dopamine
Thyroid hormone

- These are all sulfated for transport in the blood
- Is sulfate transport an important role that they play??

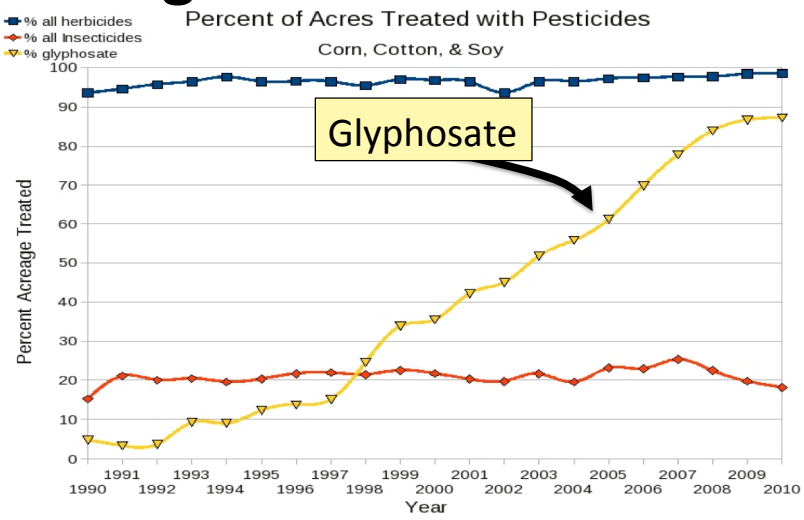
Glyphosate: A Glycine Analogue

"Glyphosate Now the Most-Used Agricultural Chemical Ever"*

- Glyphosate usage has increased 50-fold since 1996, when GMO glyphosate-resistant crops were introduced in the US.
- Today, 50 times more glyphosate is allowed by the EPA on corn grain than in 1996
- Half of the American farmers' fields have weeds that are resistant to glyphosate
- New GMO crops offer dual resistance to glyphosate & 2,4-D → Enlist Duo

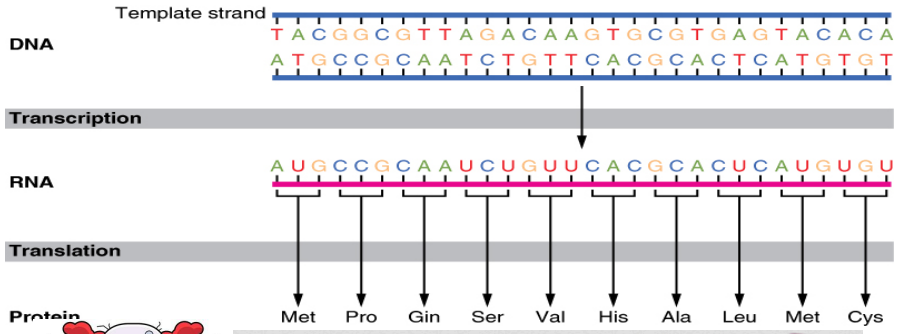
*Douglas Main, Feb 2, 2016 Newsweekwww.newsweek.com/glyphosate-now-most-used-agricultural-chemical-ever-422419

Glyphosate vs. Other Pesticides: Usage in the United States*



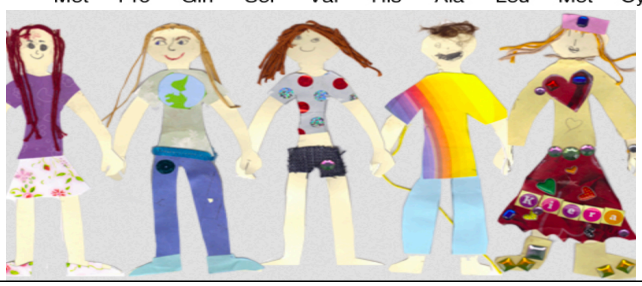
*http://sustainablepulse.com/wp-content/uploads/GMO-health.pdf

The Basics of Protein Synthesis

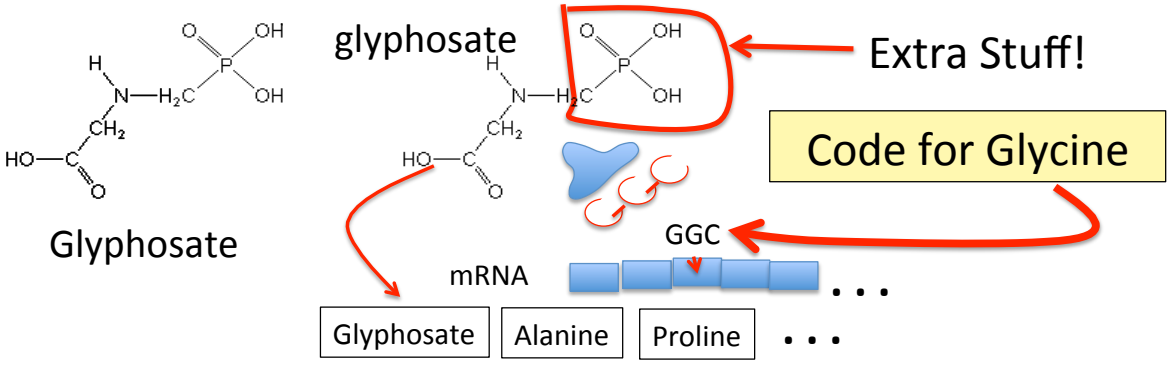


Glyphosate

© Can Stock Photo - csp23414190

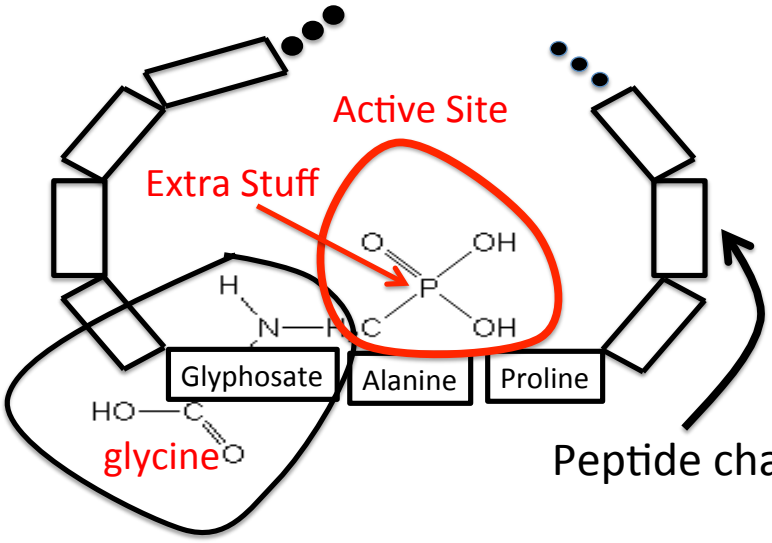


What if Glyphosate could Insert itself into Proteins during Synthesis???



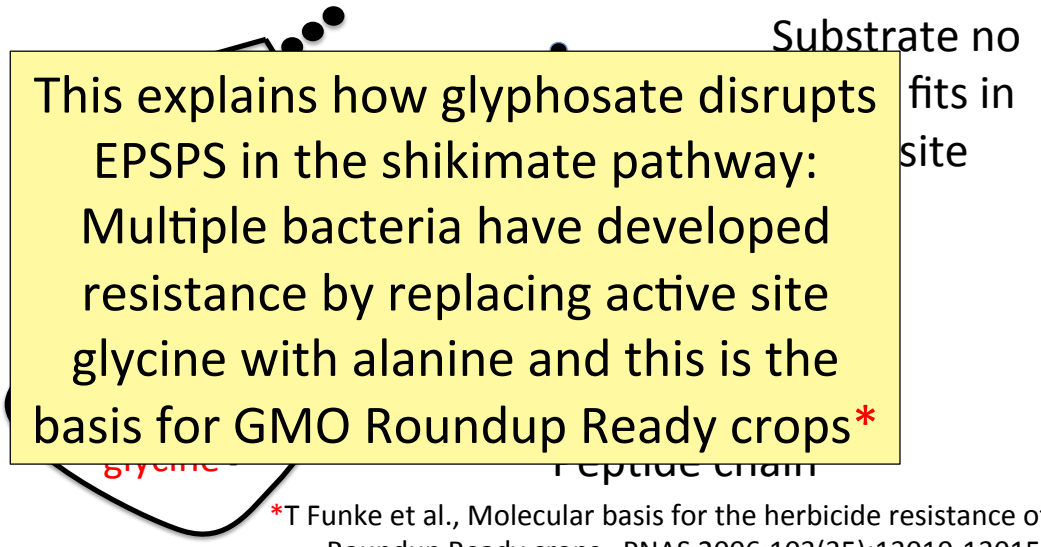
-- Any proteins with conserved glycine residues are likely to be affected in a major way

Extra Piece Sticks Out at Active Site



Substrate no longer fits in active site

Extra Piece Sticks Out at Active Site



This explains how glyphosate disrupts EPSPS in the shikimate pathway: Multiple bacteria have developed resistance by replacing active site glycine with alanine and this is the basis for GMO Roundup Ready crops*

*T Funke et al., Molecular basis for the herbicide resistance of Roundup Ready crops. PNAS 2006;103(35):13010-13015.

Inhibition of EPSPS by glyphosate: Resistant E coli mutant*

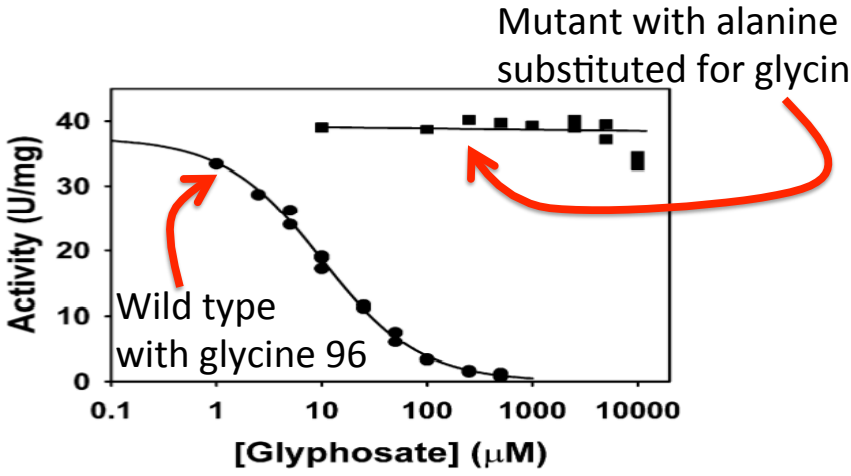


*Figure 3, S Eschenburg et al. Planta 2002;216:129-135.

Only Glyphosate Works!*

"More than 1,000 analogs of glyphosate have been produced and tested for inhibition of EPSP synthase, but minor structural alterations typically resulted in dramatically reduced potency, and no compound superior to glyphosate was identified."

Hypothesis:
These other molecules failed to work as an amino acid analogue of glycine, **because they were not amino acids.**

*T Funke et al. PNAS 2006; 103(35): 13010-13015.

Quote from Monsanto Study (1989)*

- Study exposed bluegill sunfish to carbon-14 radiolabelled glyphosate
- Measured radiolabel in tissues greatly exceeded measured glyphosate levels
- Proteolysis recovered more glyphosate
  - 20% yield → 70% yield

"Proteinase K hydrolyses proteins to amino acids and small oligopeptides, suggesting that a significant portion of the 14C activity residing in the bluegill sunfish tissue was tightly associated with or *incorporated into* protein."

*WP Ridley and KA Chott. Monsanto unpublished study. August, 1989.

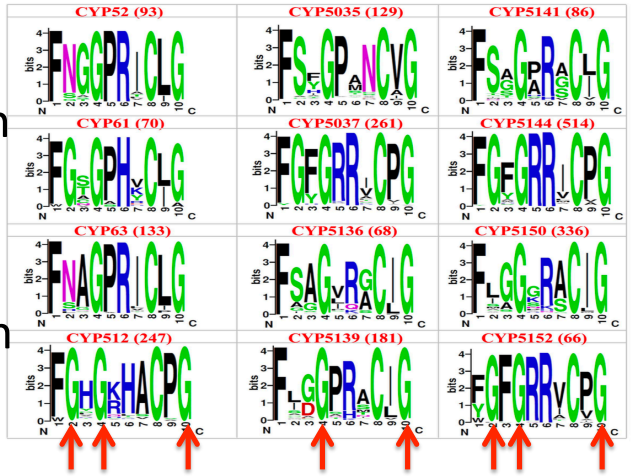
Some Predicted Consequences*

- Impaired cholesterol sulfate synthesis → heart disease
- Autism
- Impaired collagen → osteoarthritis
- Steatohepatitis (fatty liver disease)
- Obesity and adrenal insufficiency
- Hypothyroidism
- Impaired iron homeostasis and kidney failure
- Insulin resistance and diabetes
- Cancer

*A. Samsel and S. Seneff. Journal of Biological Physics and Chemistry 2016;16:9-46.

Glyphosate Disrupts Cytochrome P450 (CYP) Enzymes*

- Glyphosate has been shown to severely suppress CYP enzymes in rat liver
- CYP enzymes have a unique FGXGXRXCXG motif with two and often three critical glycine residues**



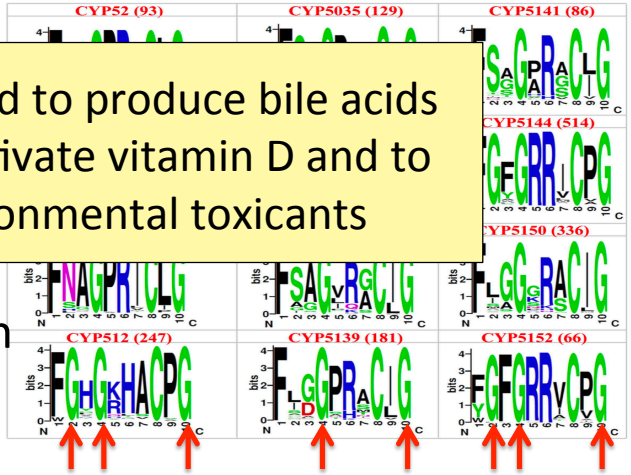
*A Samsel and S Seneff. Entropy 2013; 15: 1416-1463.
**K Syed and SS Mashele. PLOS ONE 2014; 9(4):| e95616.

Glyphosate Disrupts Cytochrome P450 (CYP) Enzymes*

- Glyphosate has been

CYP enzymes are needed to produce bile acids for digesting fats, to activate vitamin D and to detoxify many environmental toxicants

- unique FGXGXRXCXG motif with two and often three critical glycine residues**



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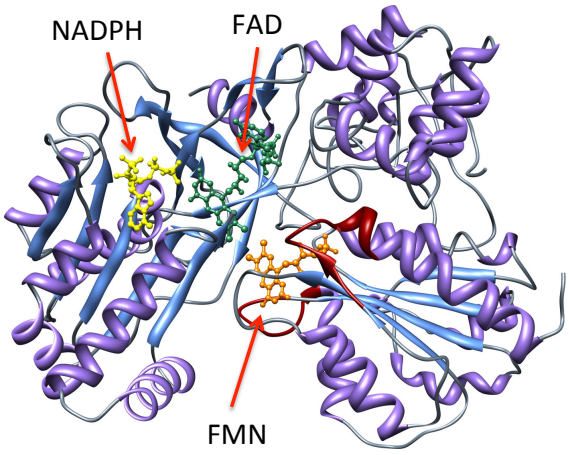
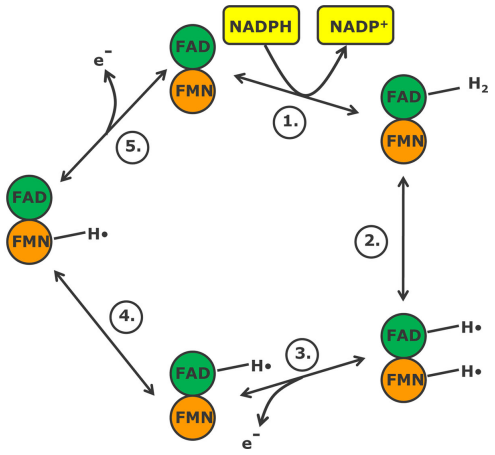
**K Syed and SS Mashele. PLOS ONE 2014; 9(4):| e95616.

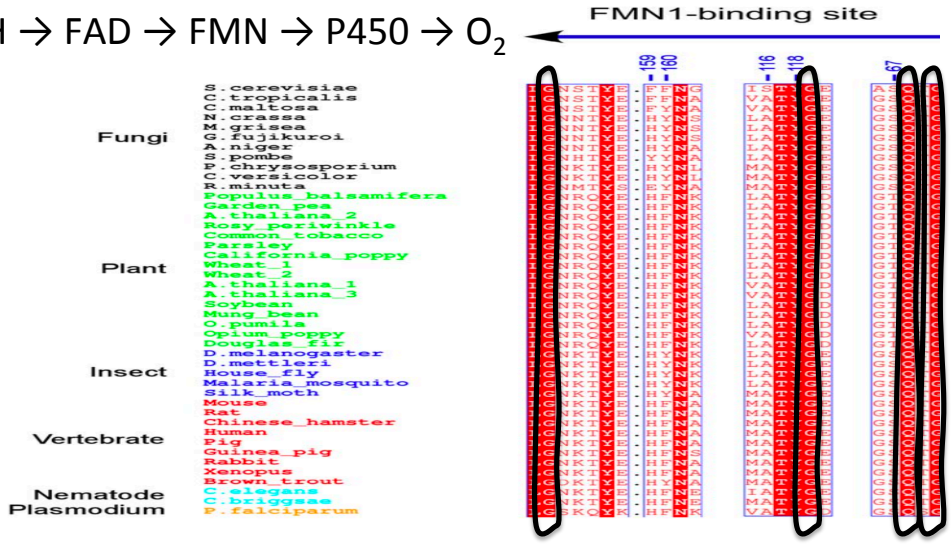
Cytochrome P450 Reductase





NADPH-cytochrome P450 reductases*

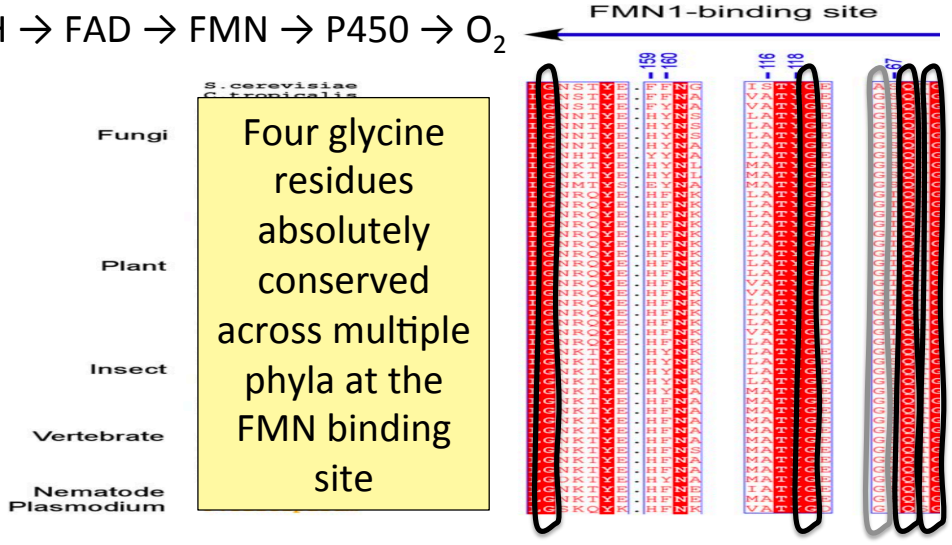
NADPH → FAD → FMN → P450 → O_2



*David C Lamb et al. Structure 2006; 14: 51-61.

NADPH-cytochrome P450 reductases*

NADPH → FAD → FMN → P450 → O_2

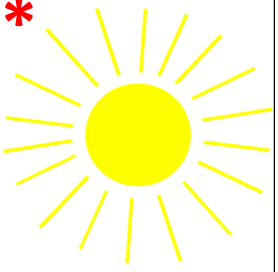


Four glycine residues absolutely conserved across multiple phyla at the FMN binding site

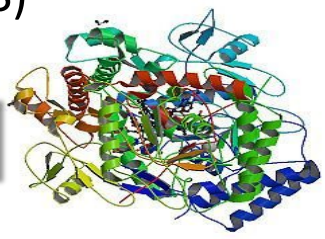
*David C Lamb et al. Structure 2006; 14: 51-61.

Endothelial Nitric Oxide Synthase: A Moonlighting Enzyme

A Provocative Proposal*



- Cholesterol sulfate supplies sulfur, oxygen, cholesterol, energy and negative charge to all the tissues
- Sulfate is synthesized from sulfide in skin and blood stream utilizing energy in sunlight
  - Protects from UV damage and keeps microbes out
- Endothelial Nitric Oxide Synthase (eNOS) performs the magic



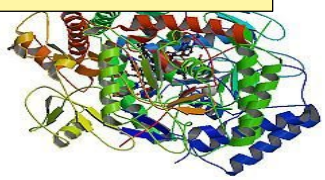
The skin is a solar powered battery!

*S Seneff et al. Theor Biol Med Model 2015; 12:9

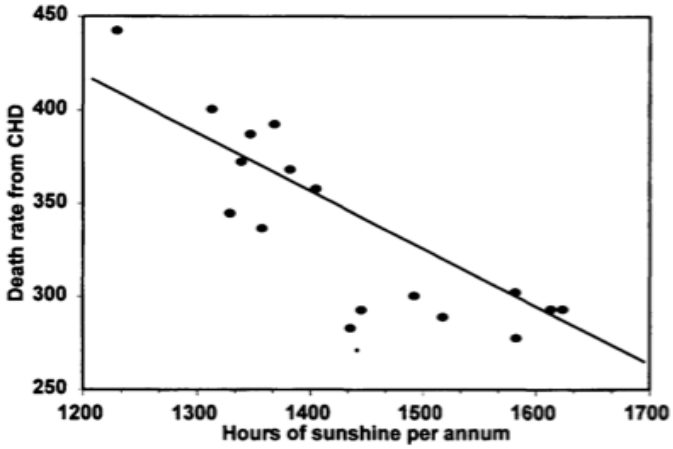
A Provocative Proposal*

BOLD CLAIM:

Deficiencies in cholesterol and sulfate supplies to the blood and to the tissues are the most important factors behind modern diseases



Heart Disease Mortality and Sunlight*



*Grimes et al., Q. J. Med. 1996; 89:579-589

Hypothesis: eNOS is a Moonlighting Enzyme*

- eNOS produces nitric oxide when it is phosphorylated and in the cytoplasm
- eNOS produces sulfur dioxide when it is attached to the membrane and unphosphorylated
- Electromagnetic signals from the flowing blood regulate the eNOS switch

*S. Seneff et al. Entropy 2012; 14: 2492-2530.

Maintaining Blood Homeostasis

- Sulfur dioxide → sulfite → sulfate

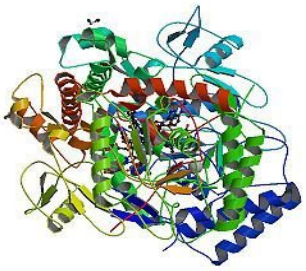
Rebuilds the matrix and thickens the blood

- Nitric oxide → nitrite → nitrate

Breaks down the matrix and thins the blood

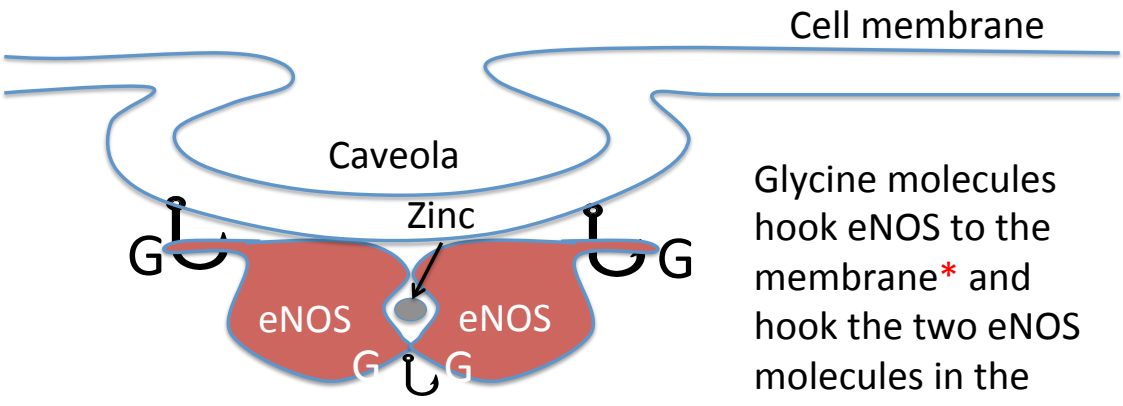
eNOS is Very Vulnerable*

- eNOS depends on:
  - Cobalamin (vitamin B12, cobalt)
  - Heme iron, sulfur, zinc, oxygen
  - NADPH
  - Sunlight
- eNOS is an orphan cytochrome P450 enzyme:
  - Highly susceptible to damage from various environmental toxicants like mercury, aluminum, glyphosate, etc.



*S. Seneff et al., Entropy 2012, 14, 2492-2530

Glycine is Essential to eNOS



Glycine molecules hook eNOS to the membrane* and hook the two eNOS molecules in the dimer together**

*WC Sessa et al. Circulation Res 1993; 72: 921-924.
**HJ Cho et al. Proc Natl Acad Sci USA 1995; 92: 11514-11518.

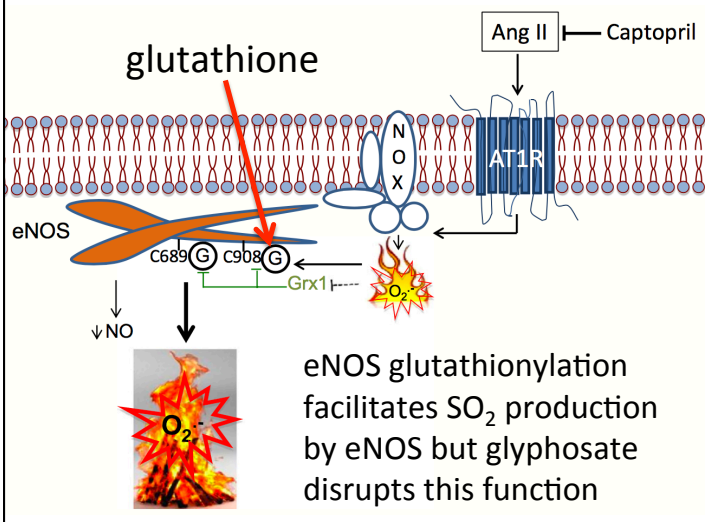
Glyphosate Disrupts eNOS*

- eNOS depends on *highly conserved glycines* both for dimer formation and for binding to the membrane
- eNOS is an NADPH CYP reductase homologue with FMN binding − more glycine dependencies
- Replacement of the glycines with glyphosate would disrupt all of these functions
  - This explains the "pathology" of superoxide release

eNOS cannot make sulfur dioxide AND it introduces damaging oxidizing agents that destroy cell tissues

*A Samsel and S Seneff, J Biol Phys Chem 2016;16:9-46.

eNOS Uncoupling*

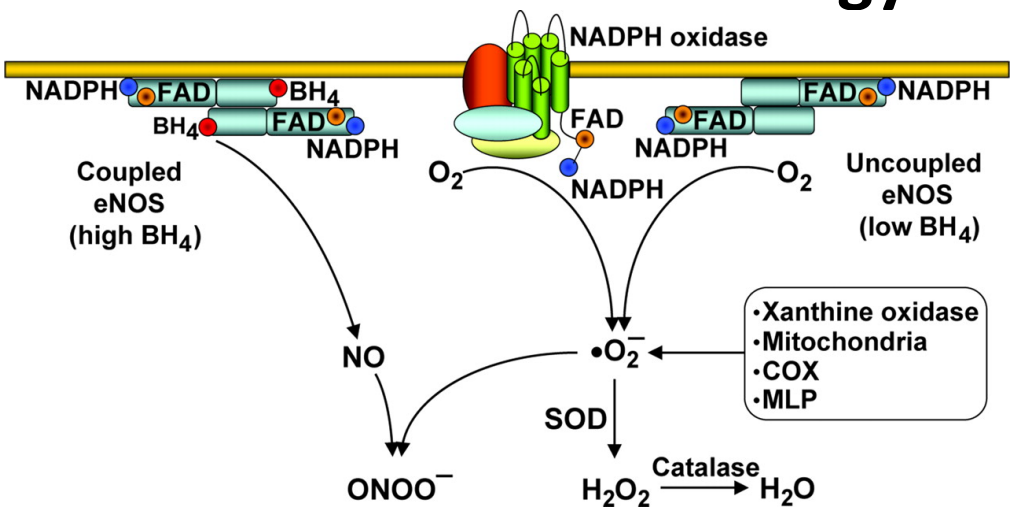


eNOS glutathionylation facilitates SO_2 production by eNOS but glyphosate disrupts this function

- Angiotensin II induces NOX
- NOX produces O_2^-
- NOX uncouples eNOS
- eNOS produces more O_2^-
- $ONOO^-$ "Oh No!"

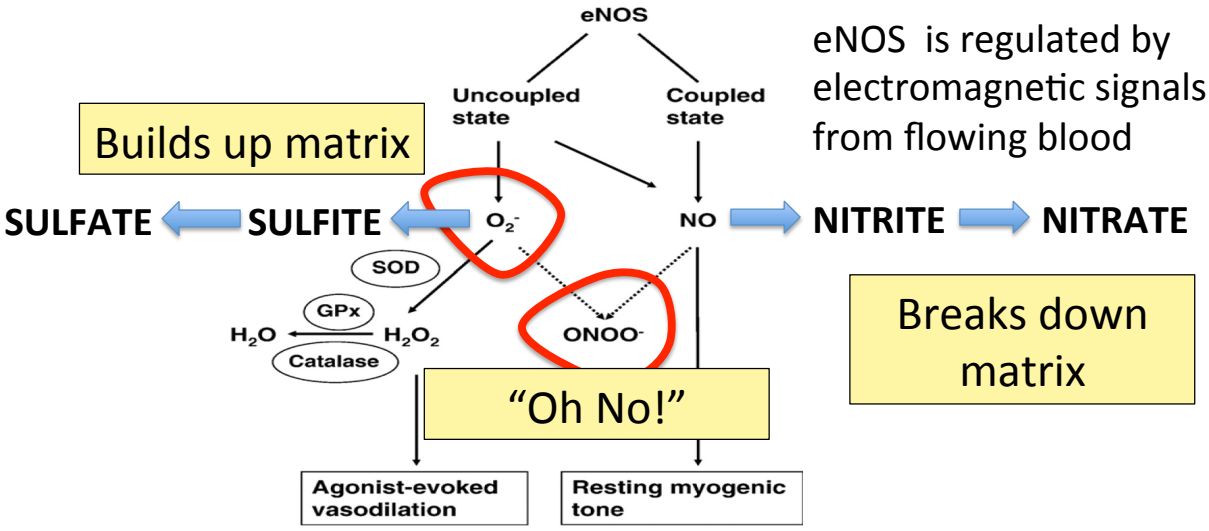
*Keyvan Karimi Galougahi et al. J Am Heart Assoc 2014;3:e000731.

eNOS and NOX Pathology*



*Ernesto L. Schiffrin. Coronary Circulation. Hypertension 2008; 51(1): 31-32.

eNOS Uncoupling*



eNOS is regulated by electromagnetic signals from flowing blood

*Figure 1, M Yokoyama and K-I Hirata Cardiovascular Research 73 (2007) 8–9

Roundup, StAR and Sterol Homeostasis

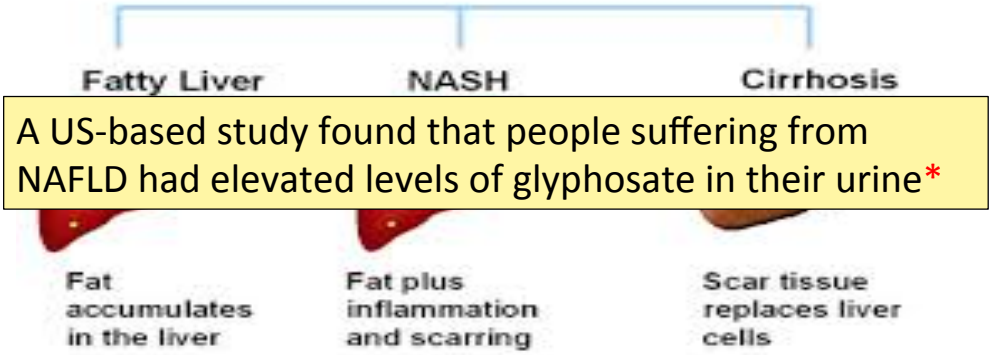
StAR:
Steroidogenic
Acute
Regulatory
protein

Non-alcoholic Fatty Liver Disease: An Epidemic in America

NAFLD affects almost one-quarter of the general U.S. population

Non-alcoholic Fatty Liver Disease: An Epidemic in America

NAFLD affects almost one-quarter of the general U.S. population

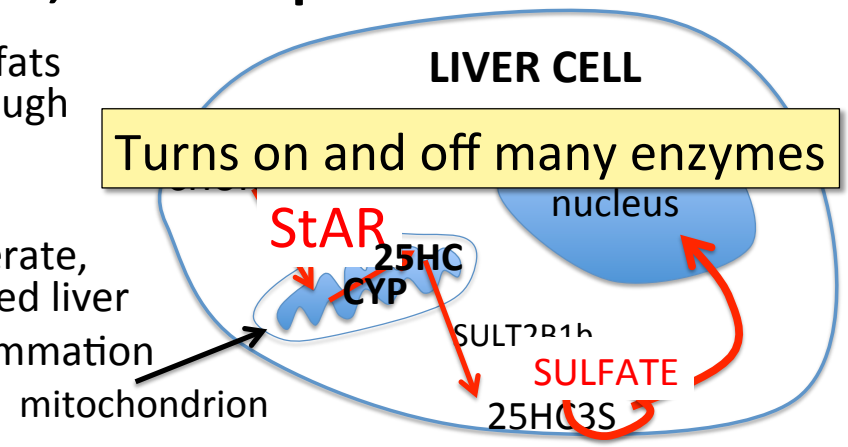


A US-based study found that people suffering from NAFLD had elevated levels of glyphosate in their urine*

*Paul J Mills et al. Clin Gastroenterol Hepatol. 2019 Apr 4. [Epub ahead of print]

"Sulfation of 25-hydroxycholesterol regulates lipid metabolism, inflammatory responses, and cell proliferation"*

- Cholesterol and fats shipped out through bile acids
- Fixes fatty liver
- Liver cells proliferate, restoring damaged liver
- Suppresses inflammation



*S Ren and Y Ying, *Am J Physiol Endocrinol Metab* 306: E123–E130, 2014

StAR is a Superstar!

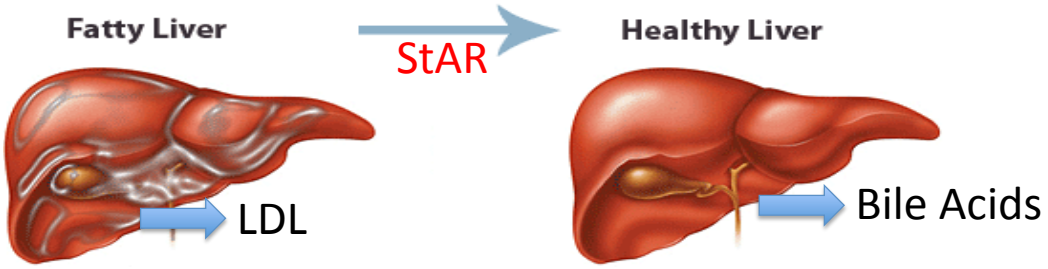
- StAR protects from fatty liver disease and elevated serum LDL by promoting bile flow
- StAR is essential for synthesis of cortisol, testosterone and estrogen by the adrenal glands and by the gonads
- StAR induces export of cholesterol from cardiovascular plaque into HDL

Roundup Inhibits Steroidogenesis by Disrupting StAR Protein Expression*

- In vitro study on testicular Leydig cells
- Roundup reduced testosterone synthesis *by 94%*
  - Effect due to both StAR suppression and CYP suppression
- Roundup reduced StAR protein levels by 90%
- Reduction in StAR expression in the adrenal gland disrupts synthesis of stress hormones and sex hormones

*LP Walsh et al., Environ Health Perspect 2000; 108:769-776

StAR Protein, Cholesterol Sulfate, and LDL*

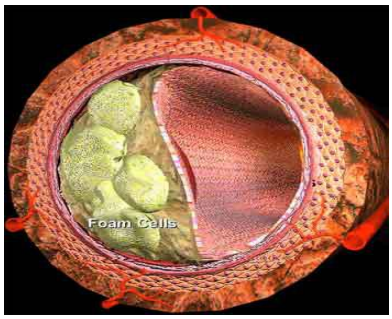


- 25HC3S is an important regulator of lipid biosynthesis
- Decreases liver accumulation of fat and cholesterol
- Increases bile acid production and decreases LDL export

*Q Bai et al., Metabolism. 2012 June ; 61(6): 836–845

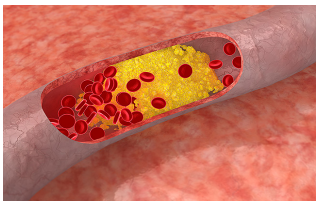
Overexpression of StAR increases macrophage cholesterol efflux to HDL-AI*

- *Atherosclerosis*: Macrophages infiltrate the artery wall and transform to foam cells, storing fat and cholesterol
- Macrophages export their cholesterol to HDL-A1, the so-called "good cholesterol"
- Overexpression of StAR in macrophages promotes cholesterol export



*JMW Taylor et al., Cardiovascular Research (2010) 86, 526–534

Why Does Cholesterol Accumulate in the Arteries Supplying the Heart?



- The heart is arguably the most important organ in the body
- It needs abundant cholesterol and sulfate to stay healthy
- When there are deficiencies, the artery wall stockpiles cholesterol waiting to become cholesterol sulfate
- Inflammation induces superoxide which oxidizes sulfur to sulfate (e.g. in homocysteine)
- Sulfate conjugation is needed for cholesterol export/delivery

Recapitulation

- Non-alcoholic fatty liver disease is an epidemic in America
  - Linked to glyphosate exposure
- Cholesterol sulfate protects from NAFLD
- StAR promotes healthy liver, bile flow, cholesterol efflux from plaque regions and steroid synthesis by adrenals and gonads
  - Glyphosate suppresses StAR synthesis
- Storage of cholesterol in cardiovascular plaque may be a mechanism to make cholesterol readily accessible for sulfation when sulfate becomes available

Glyphosate, Sulfate, Oxalate, Autism

Sulfate in Fetal Development*

- Fetus depends on mother for sulfate supply
- Sulfate is essential for transporting sterols (like estrogen and DHEA) and supplying extracellular matrix proteins everywhere with sufficient negative charge
- Sulfate detoxifies xenobiotics like acetaminophen (Tylenol) and is essential for excreting toxins like aluminum and mercury
- Sulfate is severely deficient in autistic children (1/3 the normal level of free sulfate in blood stream)

* PA Dawson, "Sulfate in Fetal Development," Semin Cell Dev Biol 2011;22(6): 653-9.

Thyroid and Sulfate

- Autism is associated with disrupted sulfate management → systemic sulfate deficiency*
- Glyphosate suppresses pituitary release of thyroid stimulating hormone (TSH) → hypothyroidism**
- Hypothyroidism in mom is linked to autism in child***
- Hypothyroidism causes sulfate loss in urine****

*RH Waring and LV Klovrza. J Nutr & Environ Med 2000; 10: 25-32.
**JS de Souza et al. Toxicology. 2017 Feb 15;377:25-37.
***GC Román, Ann Neurol 2013;74(5):733-42.
****K Sagawa et asl. Am J Physiol. 1999 Jan;276(1 Pt 2):F164-71.

Rosemary Waring on Autism (1990)*

"These results indicate that there may be a fault either in manufacture of sulphate or that sulphate is being used up dramatically on an unknown toxic substance these children may be producing ."

*p. 198, O'Reilly, B.A.; Waring, R.H. Enzyme and sulphur oxidation deficiencies in autistic children with known food/ chemical intolerances. *Xenobiotica.* 1990, *20*, 117–122.

Rosemary Waring Found Extremely Abnormal Urinary Sulfur Products in Autism*

TABLE 1. Excretion of urinary protein and anions in autism

	Autism ($n = 232$)	Controls ($n = 68$)
Age (years)	7.6 ± 2.4	8.5 ± 3.7
Protein μg ml^{-1}	103.2 ± 89.9*	64.5 ± 27.5
Sulphite	106.9 ± 162.9*	2.1 ± 6.3
Thiosulphate	130.8 ± 148.1*	18.6 ± 25.0
Thiocyanate	6.4 ± 16.9*	44.0 ± 101.0
Sulphate	6819.0 ± 6712.3*	3030.8 ± 1461.0

Anion excretion is given in nmol ml^{-1}, mean ± SD* $p < 0.001$ (Wilcoxon rank sum test).

* RH Waring and LV Klovrza. Journal of Nutritional & Environmental Medicine 2000; 10: 25-32.

Rosemary Waring Found Extremely Abnormal Urinary Sulfur Products in Autism*

> 50-fold increase in urinary sulfite suggests a deficiency in sulfite oxidase

Protein μg ml^{-1}	103.2 ± 89.9*	64.5 ± 27.5
Sulphite	106.9 ± 162.9*	2.1 ± 6.3
Thiosulphate	130.8 ± 148.1*	18.6 ± 25.0
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Anion excretion is given in nmol ml^{-1}, mean ± SD* $p < 0.001$ (Wilcoxon rank sum test).

* RH Waring and LV Klovrza. Journal of Nutritional & Environmental Medicine 2000; 10: 25-32.

Glyphosate Plausibly Disrupts Sulfur Enzymes

Sulfite oxidase (SuOx)*

- Depends on molybdenum as catalyst (glyphosate chelation could make it unavailable)
- Changing glycine at residue 473 with aspartate destroys enzyme activity
  - Leads to severe impairment in ability to bind sulfite and 5-fold reduction in catalysis
  - Aspartate has similar properties as glyphosate, being bulky and negatively charged
- Defective SuOx leads to severe birth defects and neurological problems resulting in early death

The sulfotransferases**

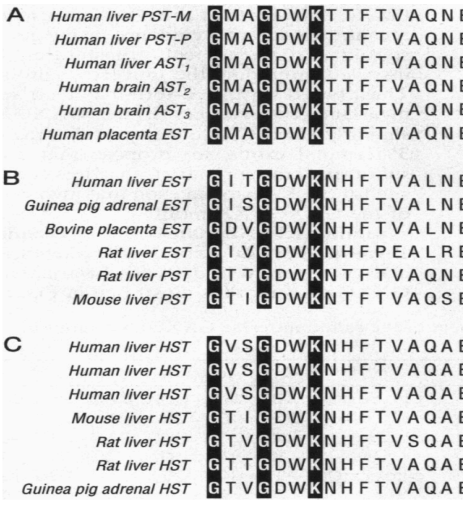
- GxxGxxK motif required for binding PAPS (activated sulfate)

*H.L. Wilson et al., Biochemistry 2006, 45, 2149-2160 2149.

**K. Komatsu et al., Biochemi and Biophys Res Comm 1994;204(3): 1178-1185.

GxxGxxK Motif in Sulfotransferases*

- Sulfotransferases are crucial to attach sulfate ions to multiple bioactive molecules
- **Steroids** (cholesterol, estrogen, testosterone, vitamin D, ...)
- **Glycosaminoglycans** (chondroitin sulfate, heparan sulfate, ...)
- **Polyphenols, aromatics** (curcumin, resveratrol, tryptophan, ...)
- **Neurotransmitters** (dopamine, serotonin, melatonin, ...)



*H Chiba et al. Proc. Natl. Acad. Sci. USA 1995; 92:8176-8179.

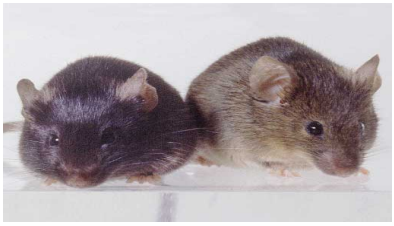
PCOS, Autism, PAPS Synthase

- PAPS synthase converts sulfate to an activated form
- It is essential for DHEA sulfate synthesis
- Defective PAPS synthase → polycystic ovary syndrome (PCOS) in women, high androgen*
  - Glycine 270 → aspartate mutation
- PCOS is a risk factor for autism in the woman and in her children**

*Cherskov et al. Translational Psychiatry 2018; 8:136.
**W Oostdijk et al. J Clin Endocrinol Metab. 2015;100(4):E672-80.

Autism-like socio-communicative deficits and stereotypies in mice lacking heparan sulfate*

- Experiment with "designer" mice: blocked heparan sulfate synthesis in brain ventricles
  - Mice exhibited all the classic features of autism – both cognitive and social



"Fractone-associated N-sulfated heparan sulfate shows reduced quantity in BTBR T+tf/J mice: a strong model of autism." **

* F. Irie et al., PNAS Mar. 27, 2012, 109(13), 5052-5056.
**KZ Meyza et al., Behav Brain Res 2012;228:247–53.

"Heparan sulfate deficiency in autistic postmortem brain tissue from the subventricular zone of the lateral ventricles"*

"Aberrant extracellular matrix glycosaminoglycan function localized to the subventricular zone of the *lateral ventricles* may be a biomarker for autism, and potentially involved in the etiology of the disorder."

New neurons develop from stem cells in this zone through the action of "fractones" composed of heparan sulfate proteoglycans**

*BL Pearson et al., Behav Brain Res. 2013;243:138-45
**F. Mercier et al., Neuroscience Letters 506 (2012) 208–213

Is Encephalopathy a Mechanism to Renew Sulfate in Autism?*

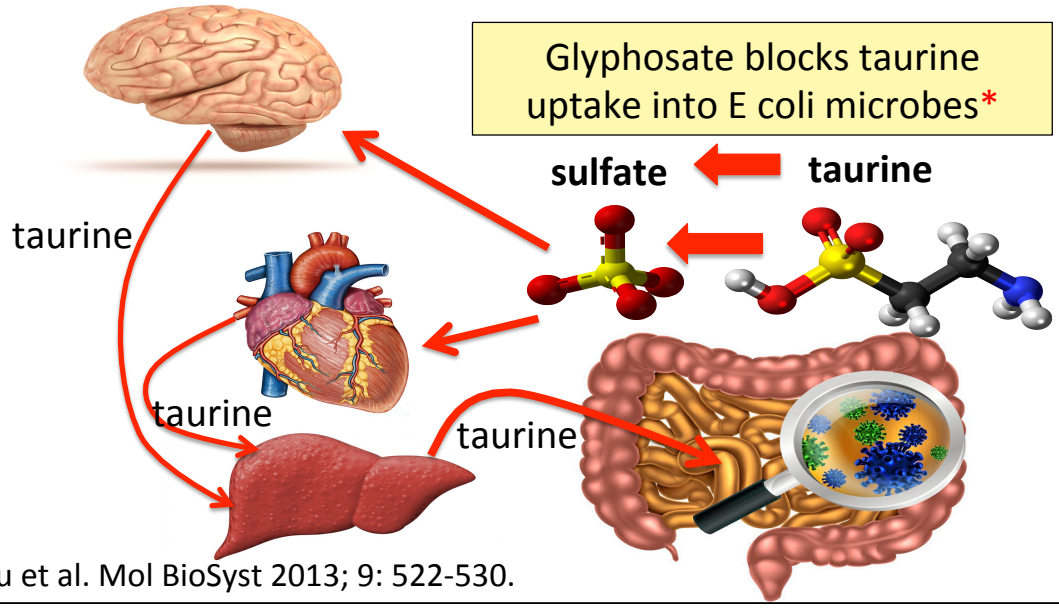
Abstract: "This paper makes two claims:

(1) Autism can be characterized as a chronic low- grade encephalopathy, associated with excess exposure to nitric oxide, ammonia and glutamate in the central nervous system, which leads to hippocampal pathologies and resulting cognitive impairment, and

(2) Encephalitis is provoked by a systemic deficiency in sulfate, but associated seizures and fever support sulfate restoration. …"

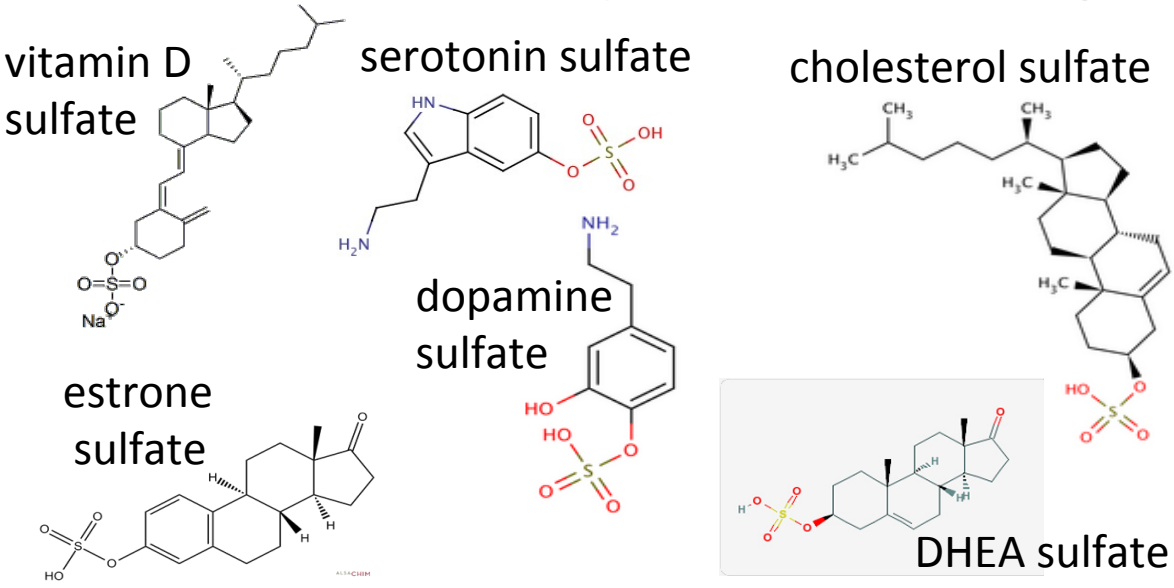
*S Seneff et al., Entropy 2013; 15: 372-406.

Gut Microbes to the Rescue!

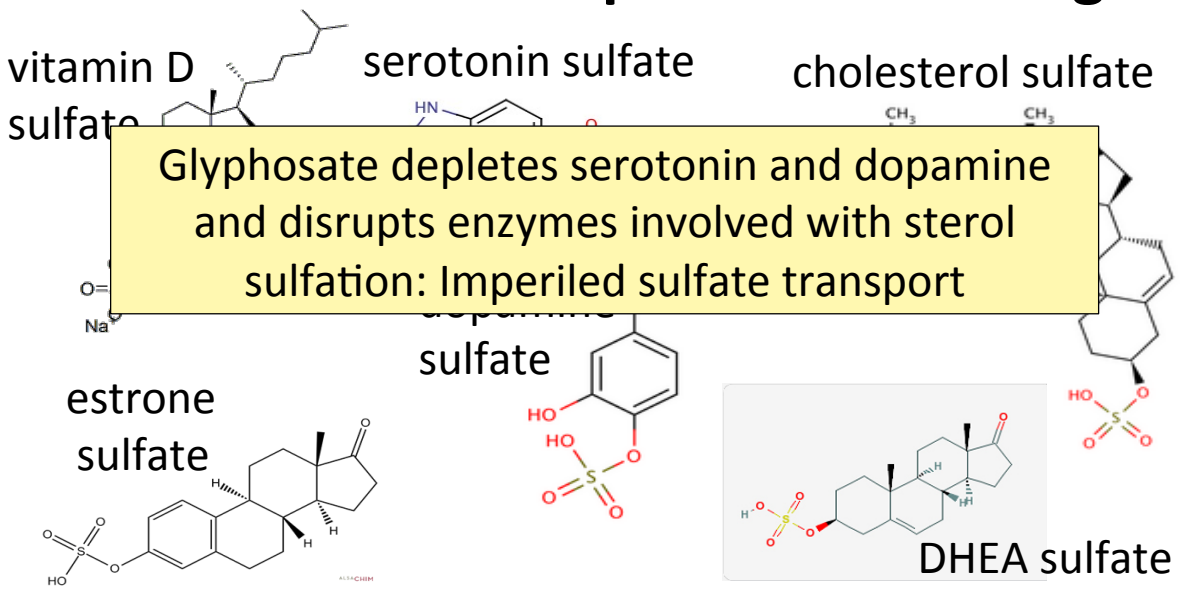


*W Lu et al. Mol BioSyst 2013; 9: 522-530.

Safe Sulfate Transport: Carbon Rings



Safe Sulfate Transport: Carbon Rings



Recapitulation

- Sulfate plays many essential roles in the body
  - Sulfate deficiency is a core feature of autism
- Sulfate synthesis and transfer depend critically on both glycine residues and molybdenum
- PCOS due to glycine mutation is a risk factor for autism
- Heparan sulfate deficiency in the brain is associated with autism in both humans and mouse models
- A low grade encephalopathy characterizes autism and may reflect the need to synthesize sulfate

Summary

- Sulfate's many essential roles in the body are under-appreciated and under-recognized
- eNOS is a moonlighting enzyme that switches between sulfur and nitrogen oxidation in response to electromagnetic signals
- An important role for many biologically active molecules is sulfate transport
- Glyphosate's insidious mechanism of toxicity is through substitution for glycine during protein synthesis (theory)
- Glyphosate can be predicted to disrupt sulfate synthesis, sulfate transport and sulfate attachment to substrates
- Glyphosate's effects on sulfate lead to heart disease, fatty liver disease, adrenal insufficiency, autism, PCOS and many other diseases