

**ROS - PEROXIDASE INTERACTION:  
Implications for Neurodegenerative Disease.**

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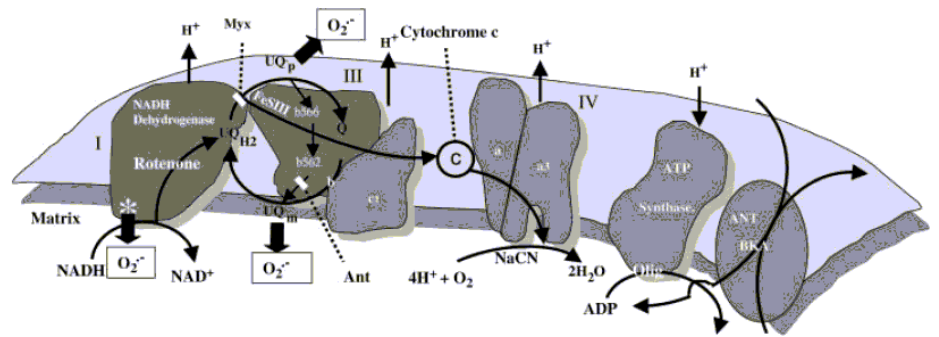
# ROS (Reactive Oxygen Species).

- Principal Focus This Talk: Hydrogen Peroxide ( $\text{H}_2\text{O}_2$ ) and superoxide anion radical ( $\text{O}_2^-$ ).<sup>1</sup>
- Harmful byproducts of oxidative metabolism.

1. Membrane / membrane-bound receptor destruction; propaga-

tion of lipid peroxidation – autooxidative and via

arachidonic cascade (inflammatory).



Production of  $\text{O}_2^-$  by electron transport chain (ETC) reactions.  $\text{H}_2\text{O}_2$  is produced by SOD-catalyzed dismutation. From Cortassa *et al.*, (2004).

2. DNA damage.

3. Release of excitatory amino acids, *e.g.*, glutamate => excitotoxicity.

4. Reduced cellular respiration and ATP production.

<sup>1</sup> Other biologically important ROS include nitric oxide (NO), singlet oxygen ( $^1\text{O}_2$ ), peroxy radical ( $\text{RO}_2^-$ ), hydroxyl radical ( $\text{HO}^\bullet$ ) and peroxynitrite ( $\text{ONOO}^\bullet$ ).

- Normal Physiologic Function: ROS also

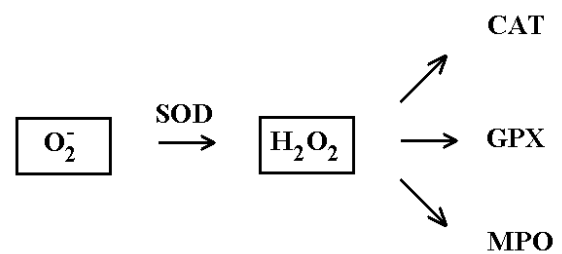
1. act as signaling molecules and secondary messengers;
2. activate signaling cascades;
3. induce and suppress gene expression.

## ROS Detoxification.

1.  $O_2^- \rightarrow H_2O_2$ : SOD

2.  $H_2O_2 \rightarrow H_2O$ : CAT, GPX, MPO, *etc.*

3. More accurately, peroxidase enzymes, such as EPO, LPO and MPO, can function as SOD and CAT – *i.e.*, they can detoxify both  $O_2^-$  and  $H_2O_2$ .

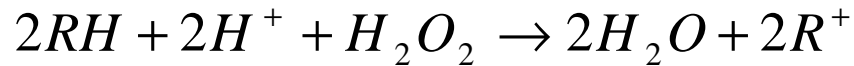


Sequential ROS detoxification by scavenger enzymes. CAT – catalase; GPX – glutathione peroxidase; MPO (myeloperoxidase); SOD – superoxide dismutase (three species).

# Peroxidase Enzymes.

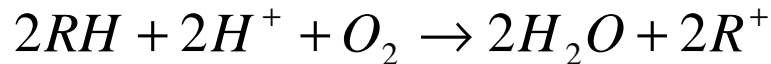
- Catalyze oxidation of a hydrogen donor.

1. Peroxidase reactions:



- a. Many substrates.
- b. Oxidation by  $H_2O_2$ .

2. Peroxidase-Oxidase reactions:



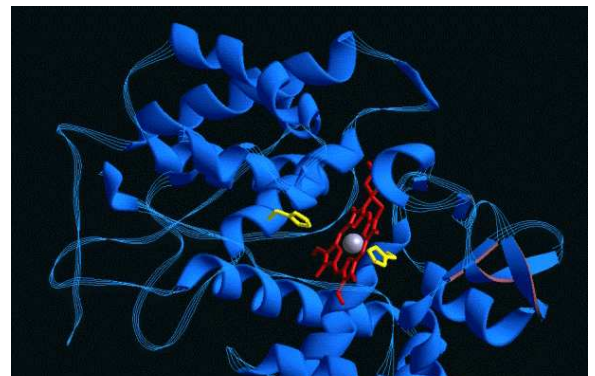
- a. Fewer substrates: Nad(P)H, IAA, DHF.
- b. Oxidation by  $H_2O_2$  and  $O_2$ .

- Structure.

1. Most organized about a heme group.

2. Active site of GPX contains Se  
– uses glutathione as electron donor

- Evolutionarily ancient.

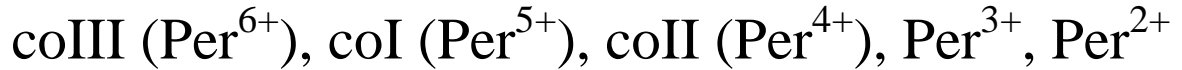


Cytochrome C peroxidase depicting the functional group, Protoporphyrin IX (red) with  $Fe^{3+}$  (gray), and histidine ligands (yellow). (Swiss Institute of Bioinformatics)

- Functions:
  1. Antioxidant scavenging (GPX, MPO)
  2. Host defense against infection (LPO, EPO, MPO)
  3. Mediate immune response (EPO)
  4. Attack tumors (MPO, EPO)
- Sources, as well as scavengers of ROS. MPO, EPO, *etc.* produce potent oxidants - hypochlorous acid (HOCl), hypobromous acid (HOBr), hydroxyl radical (HO<sup>•</sup>), singlet oxygen (<sup>1</sup>O<sub>2</sub>), peroxy radical (RO<sub>2</sub><sup>-</sup>), and peroxynitrite (ONOO<sup>•</sup>) that destroy pathogens via
  1. Halogenation and nitration of their proteins;
  2. Humoral immune response.
- Peroxidase-catalyzed reactions manifest complex dynamics in the lab and in living cells.

# Mechanism.

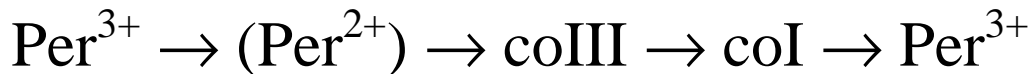
- Multiple enzyme states:



- Two redox loops:
  1. “Peroxidase” cycle:

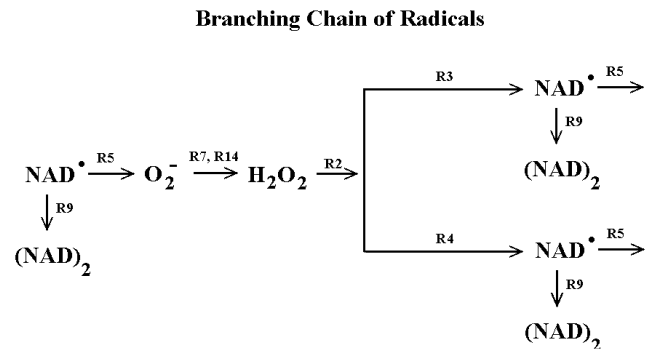


2. “Oxidase” cycle:



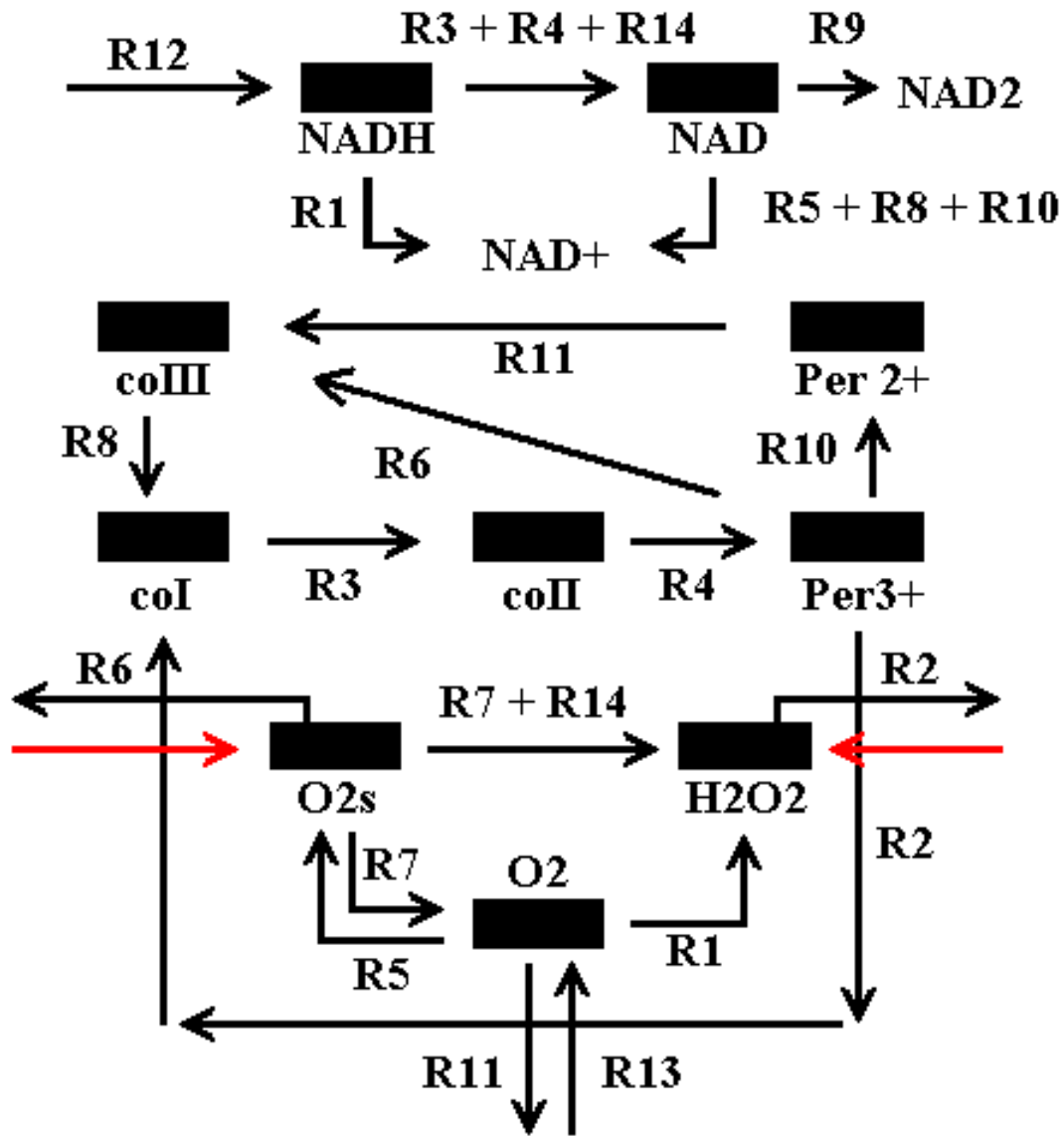
3. Coupled by reduction steps:  $\text{coI} \rightarrow \text{coII} \rightarrow \text{Per}^{3+}$ .

4. Non-enzymatic reactions. Include
  - a. “branching chain” of radicals.
  - b. radical removal by NAD dimerization.



Radical proliferation by the peroxidase cycle. Each NAD radical produces two new NAD radicals.

## Mechanism – II.



Peroxidase-oxidase reaction as modeled in [bso01] and as typically studied *in vitro*, *i.e.*, with oxygen input and NADH the substrate. Note the coupled redox loops referenced above. Red arrows indicate entry points of external ROS. For reactions and rate constants, see Table I.

## Modeling Peroxidase-Oxidase Reaction.

- Peroxidase models trace to Yamazaki [yy77] and the “FAB” group [fabb78] with subsequent workers adding and removing reactions (elementary steps).
- Detailed *vs.* “abstract” models.
- BFSO [bfso05] a detailed model developed for HRP.
  1. 10 Equations; 17 parameters.
  2. No explicit representation of cofactor concentrations / dynamics – subsumed into parameter values.
    - a. DCP (laboratory)
    - b. Melatonin, serotonin... (*in vivo*)
  3. Omits various reactions including



which was later added back in.

4. Omits halogenation reactions of EPO, LPO, MPO.

**Table I. Elementary Steps Used in the BFSO Model of the PO Reaction.**

Reaction	Rate Equation	Constant
1. $\text{NADH} + \text{O}_2 + \text{H}^+ \rightarrow \text{NAD}^+ + \text{H}_2\text{O}_2$	$k_1[\text{NADH}][\text{O}_2]$	$3.0 \text{ M}^{-1}\text{s}^{-1}$
2. $\text{H}_2\text{O}_2 + \text{Per}^{3+} \rightarrow \text{coI}$	$k_2[\text{H}_2\text{O}_2][\text{Per}^{3+}]$	$1.8 \times 10^7 \text{ M}^{-1}\text{s}^{-1}$
3. $\text{coI} + \text{NADH} \rightarrow \text{coII} + \text{NAD}^\bullet$	$k_3[\text{coI}][\text{NADH}]$	$4.0 \times 10^4 \text{ M}^{-1}\text{s}^{-1}$
4. $\text{coII} + \text{NADH} \rightarrow \text{Per}^{3+} + \text{NAD}^\bullet$	$k_4[\text{coII}][\text{NADH}]$	$2.6 \times 10^4 \text{ M}^{-1}\text{s}^{-1}$
5. $\text{NAD}^\bullet + \text{O}_2 \rightarrow \text{NAD}^+ + \text{O}_2^-$	$k_5[\text{NAD}^\bullet][\text{O}_2]$	$2.0 \times 10^7 \text{ M}^{-1}\text{s}^{-1}$
6. $\text{O}_2^- + \text{Per}^{3+} \rightarrow \text{coIII}$	$k_6[\text{O}_2^-][\text{Per}^{3+}]$	$1.7 \times 10^7 \text{ M}^{-1}\text{s}^{-1}$
7. $2\text{O}_2^- + 2\text{H}^+ \rightarrow \text{H}_2\text{O}_2 + \text{O}_2$	$k_7[\text{O}_2^-]^2$	$2.0 \times 10^7 \text{ M}^{-1}\text{s}^{-1}$
8. $\text{coIII} + \text{NAD}^\bullet \rightarrow \text{coI} + \text{NAD}^+$	$k_8[\text{coIII}][\text{NAD}^\bullet]$	Variable
9. $2\text{NAD}^\bullet \rightarrow (\text{NAD})_2$	$k_9[\text{NAD}^\bullet]^2$	$5.6 \times 10^7 \text{ M}^{-1}\text{s}^{-1}$
10. $\text{Per}^{3+} + \text{NAD}^\bullet \rightarrow \text{Per}^{2+} + \text{NAD}^+$	$k_{10}[\text{Per}^{3+}][\text{NAD}^\bullet]$	$1.8 \times 10^6 \text{ M}^{-1}\text{s}^{-1}$
11. $\text{Per}^{2+} + \text{O}_2 \rightarrow \text{coIII}$	$k_{11}[\text{Per}^{2+}][\text{O}_2]$	$1.0 \times 10^5 \text{ M}^{-1}\text{s}^{-1}$
12. $\text{NADH}(\text{stock}) \rightarrow \text{NADH}(\text{liquid})$	$k_{12}[\text{NADH}]_{\text{stock}}$	$1.143 \times 10^{-7} \text{ Ms}^{-1}$
13. $\text{O}_2(\text{gas}) \rightleftharpoons \text{O}_2(\text{liquid})$	$k_{13}[\text{O}_2]_{\text{eq}} - k_{-13}[\text{O}_2]$	$6.24 \times 10^{-8} \text{ Ms}^{-1}$
	$k_{-13}$	$3.73 \times 10^{-3} \text{ s}^{-1}$
14. $\text{NADH} + \text{H}^+ + \text{O}_2^- \rightarrow \text{NAD}^\bullet + \text{H}_2\text{O}_2$	$k_{14}[\text{NADH}][\text{O}_2^-]$	$3.0 \times 10^2 \text{ M}^{-1}\text{s}^{-1}$

- Converting Elementary Steps to ODEs.

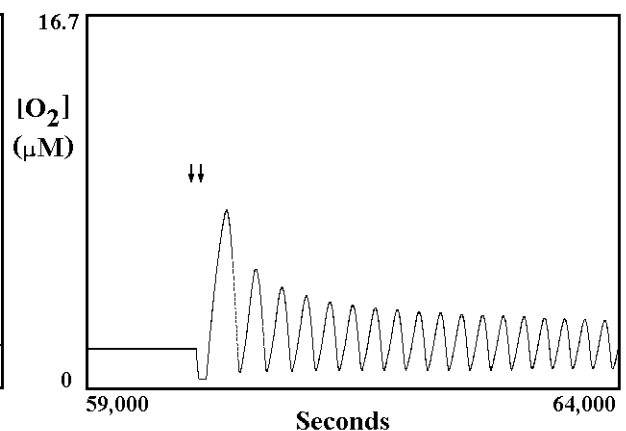
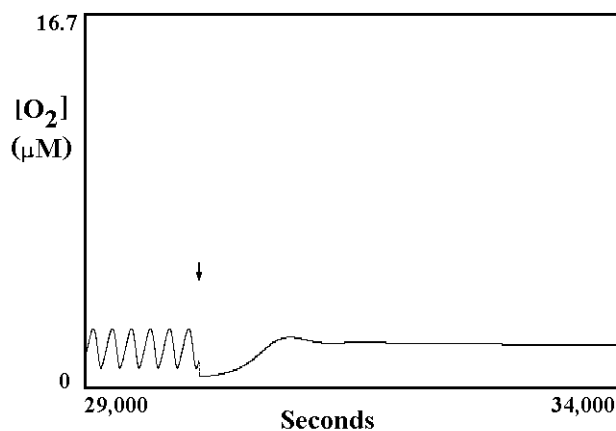
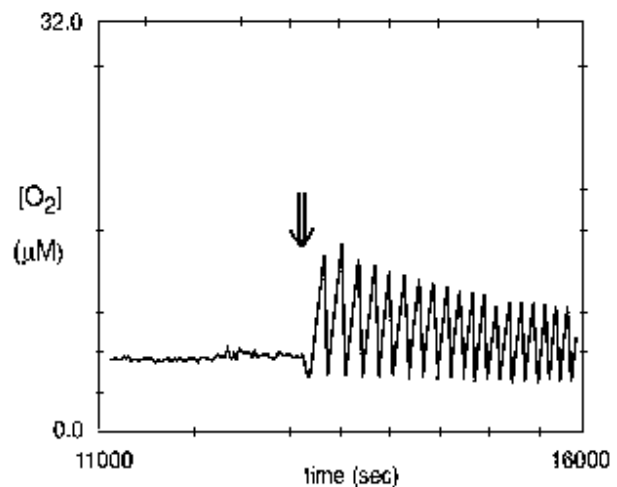
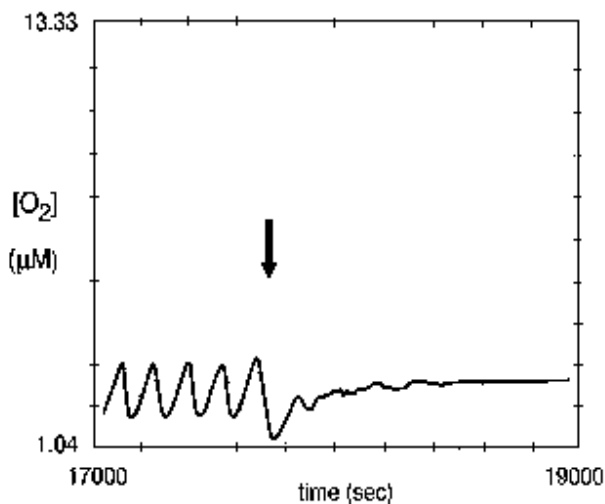
**Table 2. Differential Equations.**

$$\begin{aligned}
 d[\text{NADH}]/dt &= -R_1 - R_3 - R_4 + k_{12} [\text{NADH}]_{\text{at}} - R_{14} \\
 d[\text{O}_2]/dt &= -R_1 - R_5 + R_7 + k_{13} [\text{O}_2]_{\text{eq}} - k_{-13}[\text{O}_2] - R_{11} \\
 d[\text{NAD}^*]/dt &= R_3 + R_4 - R_5 - R_8 - 2R_9 - R_{10} + R_{14} \\
 d[\text{Per}^{3+}]/dt &= -R_2 + R_4 - R_6 - R_{10} \\
 d[\text{CoI}]/dt &= R_2 - R_3 + R_8 \\
 d[\text{CoII}]/dt &= R_3 - R_4 \\
 d[\text{CoIII}]/dt &= R_6 - R_8 + R_{11} \\
 d[\text{H}_2\text{O}_2]/dt &= R_1 - R_2 + R_7 + R_{14} \\
 d[\text{O}_2^-]/dt &= R_5 - R_6 - 2R_7 - R_{14} \\
 d[\text{Per}^{2+}]/dt &= R_{10} - R_{11}
 \end{aligned}
 \tag{II}$$

- BFSO first detailed model to replicate complex dynamics observed *in vitro* using plausible reactions and rate constants.
  1. Period-doubling to chaos in response to changing DCP concentrations [bfso95].
  2. Switch from period-doubling to period adding (in response to increased NADH input) occasioned by increasing pH – affects formation of NAD dimers. [hobs97, bsho98].
  3. Quasiperiodicity [sbo96] and period-doubled tori observed [obs02]. Latter observed experimentally subsequent to theoretical prediction [bsho98].
  4. Near heteroclinic and homoclinic behavior [sbo01].
- Addition of  $R_{14}$  allows BFSO to replicate experimentally observed bistability and bursting at low enzyme concentrations [bso01].
- Previously observed instances of model-experiment correspondence preserved / improved under the extended model [sbo01].

- Low dimensional dynamics.
  1. Despite 10-D state space, all observed dynamics compatible with trajectorial evolution in a 3-D subspace.
  2. Attractor dimensions close to 2.
  3. Motivates continuing search for simplified surrogate models [sho06].

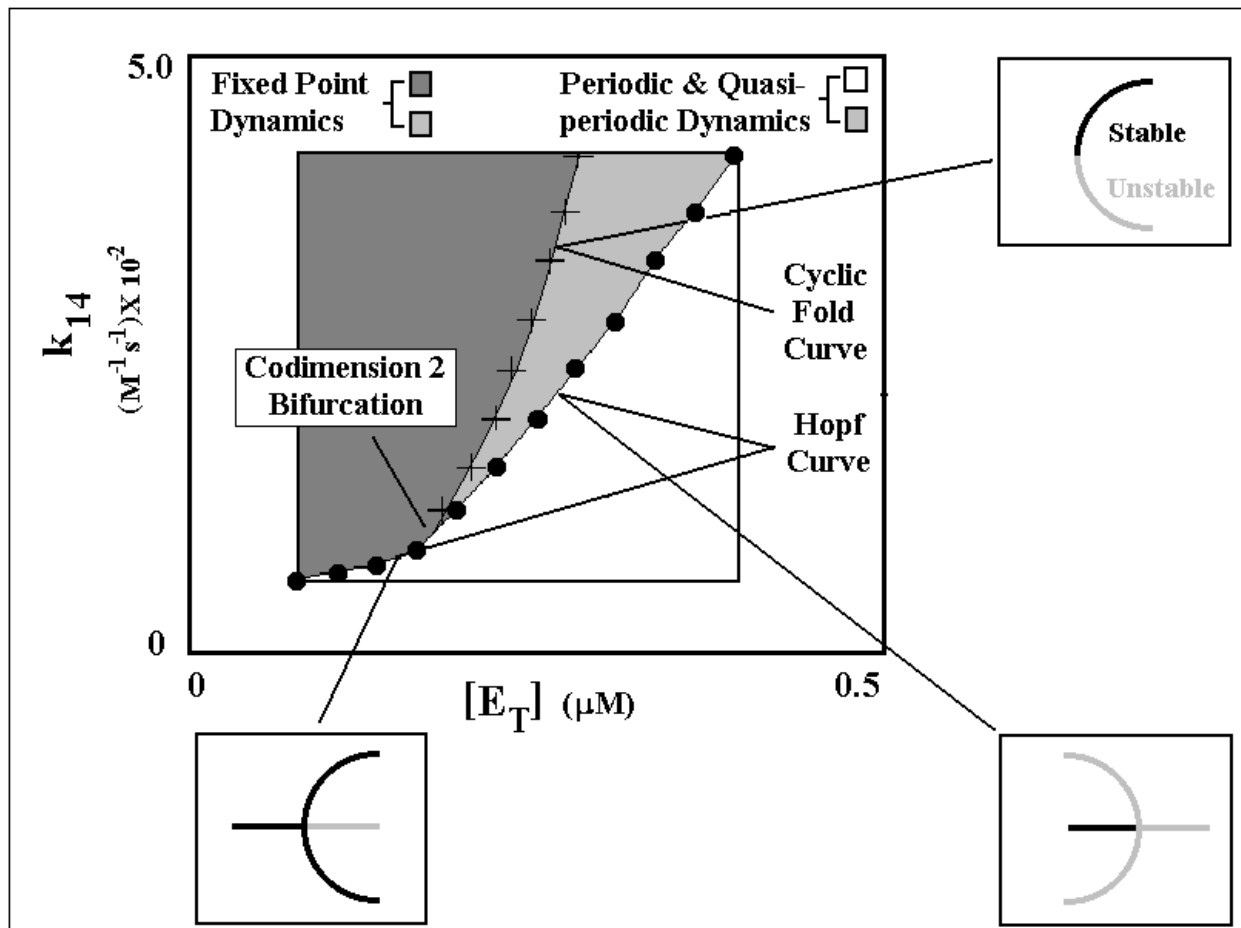
# Bistability.



Experimental [afo90] and theoretical [bso01] transitions between equilibrium and oscillatory dynamics. **Top Left.** Oscillatory behavior quenched by spiking (arrow) the PO reaction with 1.8 – 2 mMol  $H_2O_2$ . **Top Right.** Oscillations restored by 30-90 sec  $O_2$  input shutdown (double arrows). **Bottom Left.** Oscillations quenched by simulated addition of 6 mMol  $H_2O_2$ . **Bottom Right.** Oscillations restored by 90 sec simulated  $O_2$  input shutdown.

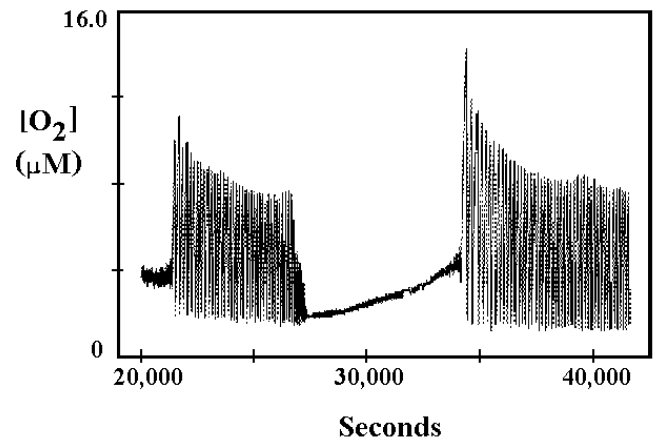
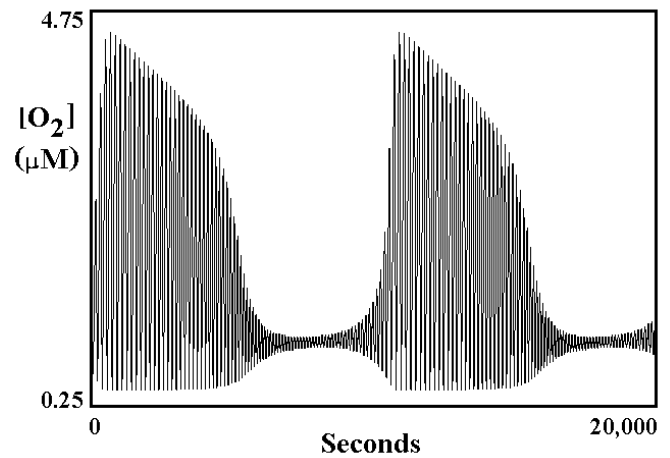
# Mechanism of Bistability.

- Result of a co-dimension 2 bifurcation whereby a curve of Hopf bifurcations emits a curve of cyclic folds.
1. At the bifurcation point, the Hopf bifurcation changes criticality – super to sub.
  2. Between the CF and H curves a stable point coexists with two cycles, one of which is stable.
  3. Equilibrium destabilized via subcritical H.



# BURSTING.

- The stable cycle produced by CF bifurcation can undergo torus bifurcation.
- With further parameter tuning, torus approaches heteroclinicity.
- Result in the time domain is bursting.
- Qualitatively similar behavior observed in the laboratory by [afo90].
- Interpreted as “autonomous,” *i.e.*, noise-induced, switching between coexisting attractors.



Bursting behavior *in simulo* and *in vitro*. **Top.** As predicted by [bso01]. **Bottom.** As observed experimentally by [afo90].

# H<sub>2</sub>O<sub>2</sub> Response to Continuing H<sub>2</sub>O<sub>2</sub> Input.

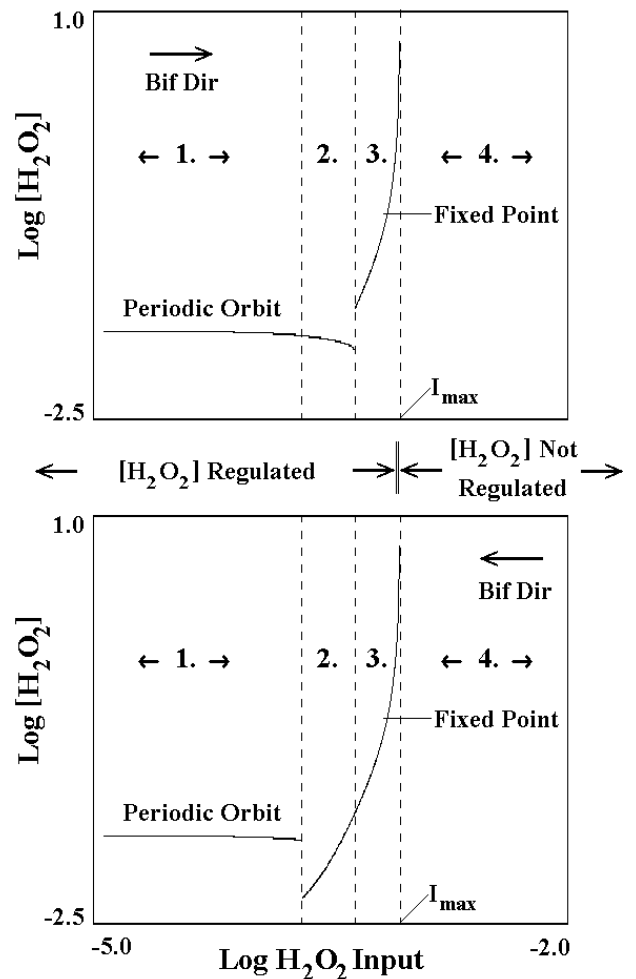
- Bistability no longer a low enzyme phenomenon. Persists up to at 1.5 μM at least.
- Four different dynamical regimes observed as one increases rate of input.

1. Inputs transduced into oscillations of ~constant amplitude.
  - a. Low E<sub>T</sub>, simple cycles.
  - b. Higher E<sub>T</sub>, complex cycles or chaos.

2. Oscillations and stable equilibria coexist. [H<sub>2</sub>O<sub>2</sub>]<sub>eq</sub> depends on input rate.

3. Equilibria only.

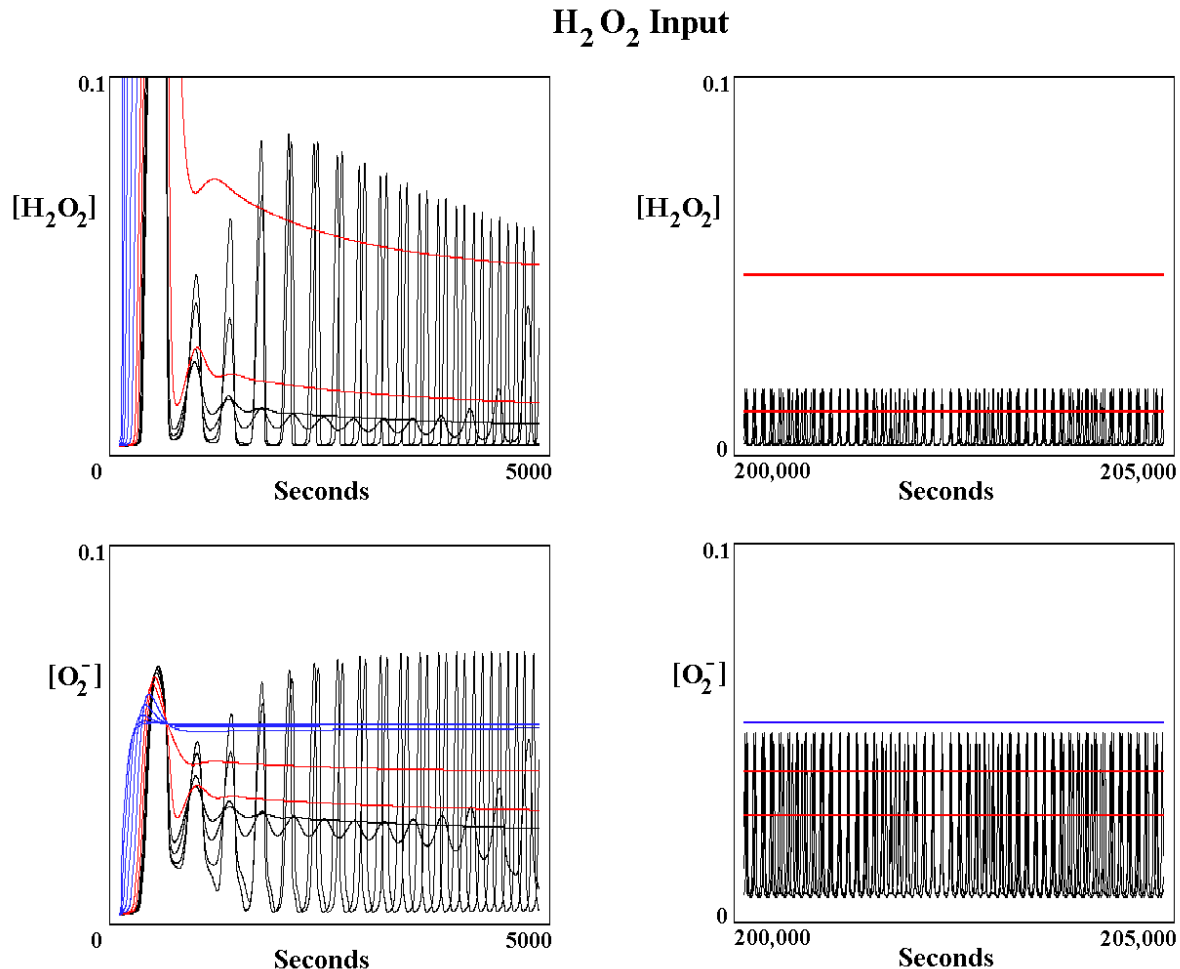
4. Blow-up: [H<sub>2</sub>O<sub>2</sub>] increases without bound.



Peroxidase-oxidase reaction subject to continuous H<sub>2</sub>O<sub>2</sub> input. Numbers refer to the four dynamical regimes. **Top.** H<sub>2</sub>O<sub>2</sub> input increasing (forward bifurcation diagram). **Bottom.** Input decreasing (backwards diagram). Total enzyme concentration, [E<sub>T</sub>] = 0.4 μM. Bistability is also observed at higher values of [E<sub>T</sub>]. In this case, the oscillatory dynamics can be complex cycles or chaos.

## $O_2^-$ response to continuing input of $H_2O_2$ .

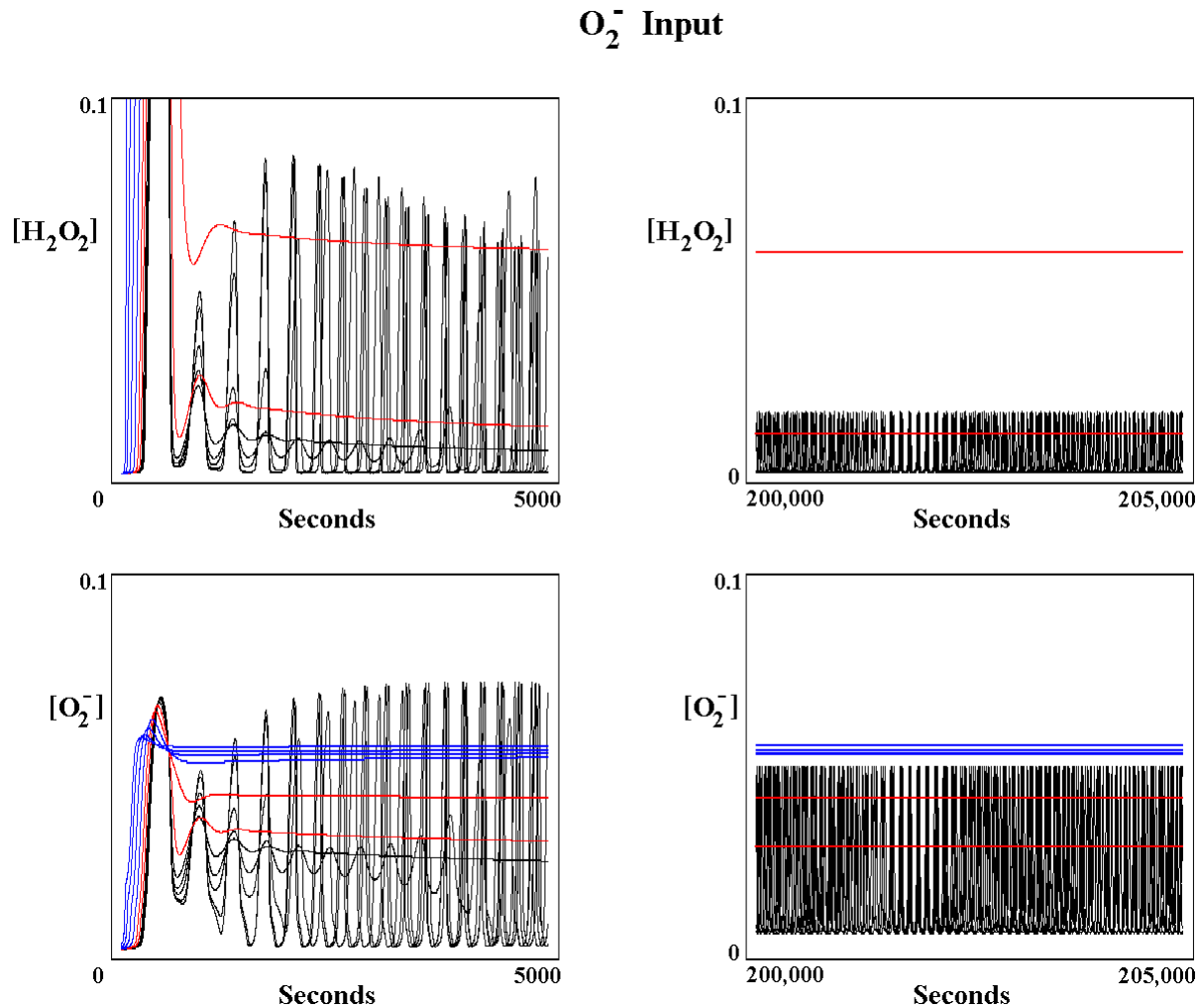
- The same, but  $[O_2^-]$  remains regulated at input rates for which  $[H_2O_2] \rightarrow \infty$ .



Response to increasing rates of  $H_2O_2$  input. **Left.** First 5000 s. **Right.** Final 5000 s. **Top.**  $[H_2O_2]$ . **Bottom.**  $[O_2^-]$ . Color-coding by dynamics. Black (oscillations) –  $\text{Log } I = -5.00 - -3.75 \mu\text{M}/\text{sec}$ . **Red.** (Input-dependent equilibria) –  $\text{Log } I = -3.5 - -3.25 \mu\text{M}/\text{sec}$ . **Blue.**  $[H_2O_2]$  unregulated;  $[O_2^-]$  regulated) –  $\text{Log } I = -3.0 - -2.0 \mu\text{M}/\text{sec}$ .

# Response to continuing input of $O_2^-$ .

- The same as response to  $H_2O_2$  input!

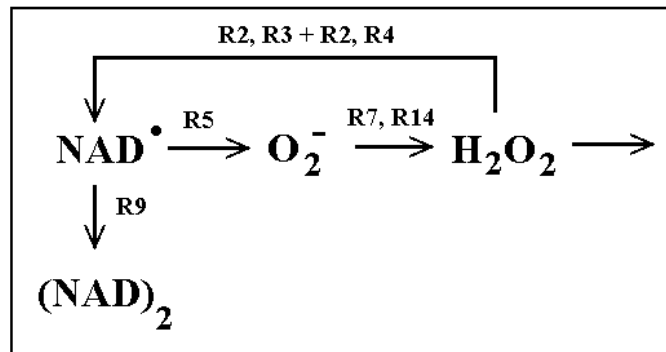


Response to increasing  $O_2^-$  input, I. **Left.** First 5000 s. **Right.** Final 5000 s. **Top.**  $[H_2O_2]$ . **Bottom.**  $[O_2^-]$ . Color-coding by dynamics. Black (oscillations) –  $\text{Log } I = -5.00 - -3.75 \mu\text{M}/\text{sec}$ . **Red.** (Input-dependent equilibria) –  $\text{Log } I = -3.5 - -3.25 \mu\text{M}/\text{sec}$ . **Blue.**  $[H_2O_2]$  unregulated;  $[O_2^-]$  regulated –  $\text{Log } I = -3.0 - -2.0 \mu\text{M}/\text{sec}$ .

- Why?

1. Recall radical proliferation in peroxidase cycle.

- $\text{NAD}^\bullet \rightarrow \text{O}_2^-$
- $\text{O}_2^- \rightarrow \text{H}_2\text{O}_2$ .
- $\text{H}_2\text{O}_2 \rightarrow 2 \text{NAD}^\bullet$   
(coI, coII formation).
- $\text{NAD}^\bullet$  removed by dimerization,  $\text{R}_9$ , and by the oxidase cycle.



Radical proliferation. NAD radicals are produced auto-catalytically in the peroxidase cycle and removed by  $\text{NAD}^\bullet$  dimerization.

2.  $\text{H}_2\text{O}_2$  vs.  $\text{O}_2^-$ .

- Crudely, adding  $\text{O}_2^-$  the same as adding  $\text{H}_2\text{O}_2$  save that  $\text{O}_2^-$  gets passed along, whereas  $\text{H}_2\text{O}_2$  is either consumed by  $\text{R}_2$  or accumulates.
- $\text{H}_2\text{O}_2$  will accumulate if  $[\text{E}_T]$  limiting, *i.e.*,  $\text{H}_2\text{O}_2$  blows up, while  $\text{O}_2^-$  continues to be regulated.
- $\text{H}_2\text{O}_2$  accumulation promoted by reducing the rate of  $\text{NAD}^\bullet$  dimerization as results, for example, from increasing pH – affects  $k_9$  [hobs97].

## Consequences of Elevated $[H_2O_2]$ and $[O_2^-]$ .

- Direct effects of  $H_2O_2$ .

1. Increases  $[O_2^-]$ .

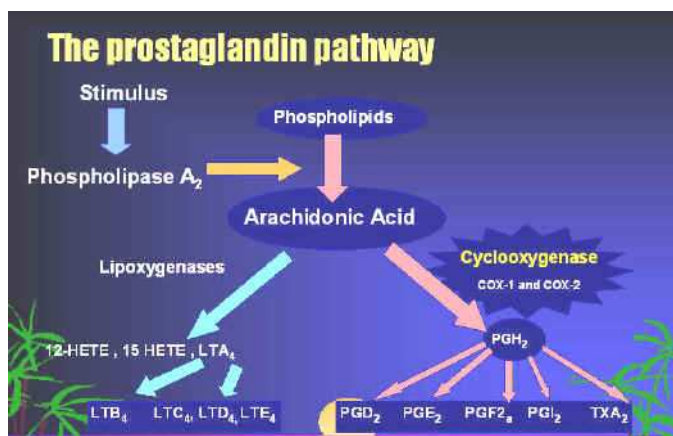
2. Impairs TCA cycle throughput via  $\alpha$ -KGDH impairment.

3. Impairs Mitochondrial function (Complex I).

4. Up-regulates NO production.

5. Inflammatory MPO: Up-regulates MPO production.

6. Inflammatory other: Activates microglia and arachidonic cascade via  $PLA_2$  induced production of arachidonic acid.



$H_2O_2$  triggers arachidonic cascade via  $PLA_2$ .

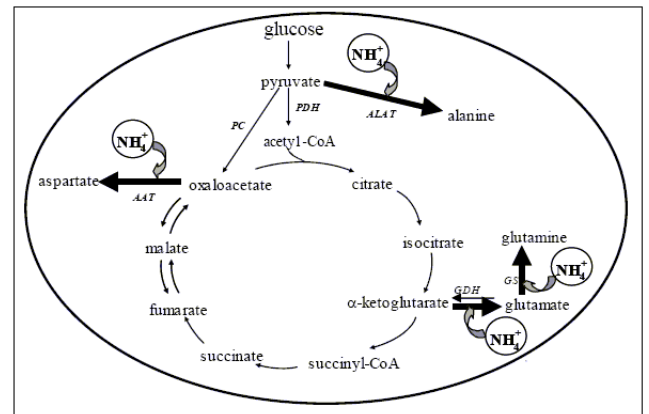
- Direct Effects of  $[O_2^-]$ .

1. Increases  $[H_2O_2]$ .

2. Increases production of halogenous acids by MPO.

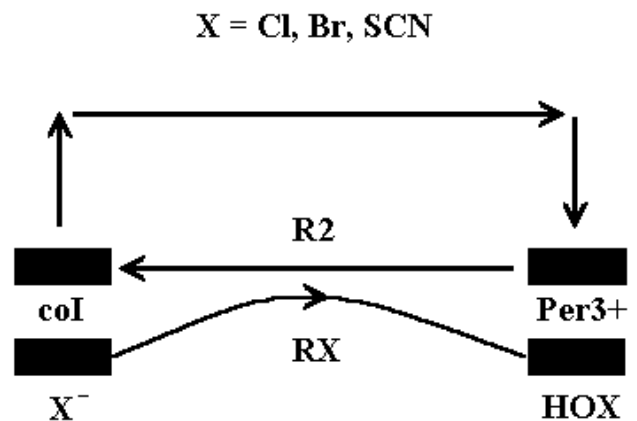
# Indirect Effects.

- TCA related.
  1. Energy production.
    - a. Inhibition of  $\alpha$ -KGDH reduces NADH produced by  $\alpha$ -ketoglutarate  $\rightarrow$  succinyl CoA.
    - b. Reduces overall cycle throughput.
  2. Reduced TCA cycle throughput
    - a.  $\Rightarrow$  Reduced high energy compounds.
    - b.  $\Rightarrow$  Reduced GSH (reduced glutathione) – indicator of oxidative stress.
    - c.  $\Rightarrow$  Reduced functioning of GPX (principal peroxidase in normal brain)
    - d.  $\Rightarrow$  More  $H_2O_2$ .
  3. Reduced TCA cycle compromises ammonia detoxification in brain.
    - a. No urea cycle in CNS.
    - b. Ammonia detoxification accomplished by TCA.
  4. Reduced TCA cycle throughput increases  $NAD^+ / NADH$ .



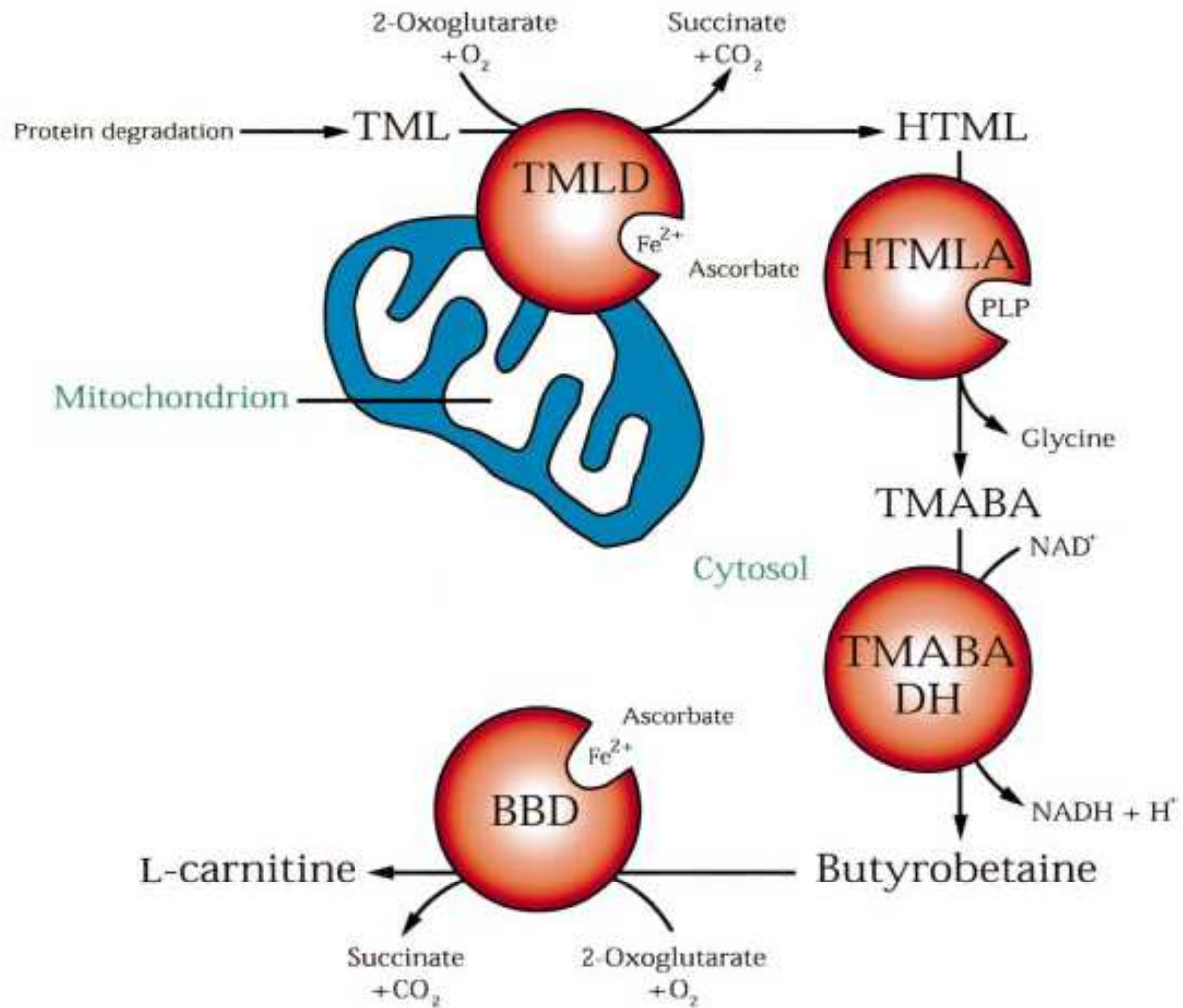
TCA cycle and ammonia detoxification in brain cells. In the presence of excess ammonia,  $\alpha$ -ketoglutarate is converted to glutamate, glutamate to glutamine (astrocytes only), oxaloacetate to aspartate and pyruvate to alanine. From [s05].

- Mitochondria.
  1.  $\text{H}_2\text{O}_2$  impairs mitochondrial function (complex I deficiency).
  2. Impaired mit function  $\Rightarrow$  more  $\text{H}_2\text{O}_2$ ,  $\text{O}_2^-$ .
- $\text{H}_2\text{O}_2$  up-regulates NO.
  1. Impairs CAT
  2.  $\Rightarrow$  more  $\text{H}_2\text{O}_2$
- $\text{H}_2\text{O}_2$  up-regulates MPO synthesis.
  1. MPO produces potent oxidants associated with inflammation.
  2.  $\text{H}_2\text{O}_2$  accumulation  $\Rightarrow$  IR.
  3. Inflammatory episodes in brain  $\Rightarrow$  neuronal damage / death.



Production of halogenous acids by EPO and MPO.

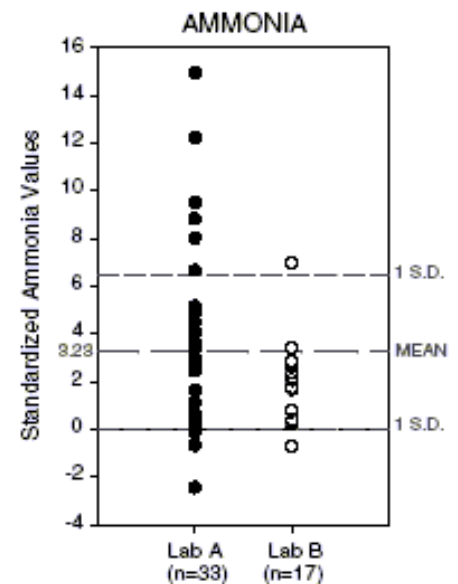
- Hyperammonemia (HA).
  1. Retarded TCA cycling => energetic deficits in brain.
  2. Impedes LTP via reduced cGMP.
  3. Increases  $\alpha$ -KG  $\rightarrow$  glutamate  $\rightarrow$  glutamine; reduces GABA
    - a. => seizures.
    - b. => glutamate mediated excitotoxicity via NMDA metabotropic receptors.
  4. Reduces SOD, CAT, GPX activity => more  $O_2^-$ ,  $H_2O_2$ .
  5. Reduced carnitine (via reduced  $\alpha$ -KG  $\rightarrow$  succinate conversion ) => reduced acetyl CoA => reduced TCA => increased HA.
- **Foregoing reaction network contains numerous sources of positive feedback.**
- **Positive feedback plus hysteresis (PO dynamics) => potential for long-lasting inflammatory episodes that persist after elevated ROS concentrations subside.**



Biosynthesis of carnitine requires direct conversion of  $\alpha$ -KG to succinate, thereby competing with TCA cycle and ammonia-induced conversion of  $\alpha$ -KG to glutamate.

## Neurodegenerative Diseases (AD, ALS, ASD, MS, PD).

- Inflammation.
  1. Overexpressed MPO.
  2. Elevated PLA<sub>2</sub>; elevated 3-chlorotyrosine (biomarker of chlorination by MPO).
  3. Activated microglia.
- Overexpressed NO (MS, ASD).
- HA and expected metabolic correlates (ASD)
  1. Abnormal TCA intermediates
  2. Reduced carnitine (consequence of HA);
  3. Fatty acid /  $\beta$  oxidation abnormalities.
  4. Digestive / gastric abnormalities (ASD).
- Decreased SOD, CAT, GPX (AD, PD, ASD)
- Reduced GABA (adult ASD); enhanced GLU (AD), GLN.



Transformed plasma ammonia concentrations of 100 autistic candidates for VPA therapy. Similar results observed for pyruvate and carnitine (reduced) and lactate and alanine (elevated). From [fjncg04].

- Abnormal melatonin / serotonin – natural peroxidase co-factors (ASD).
- Reduced  $\alpha$ -KGDH (AD, PD).
- Developmental Disorders (ASD, PDD-NOS, *etc.*).
  1. Consistent with above:
    - a. Abnormal brain structure.
    - b. Receptor deficits.
    - c. Impaired cognitive function.
    - d. Fatty acid abnormalities.
    - e. TCA species abnormalities.
    - f. Presumed mitochondrial dysfunction.
    - g. Comorbid seizure disorders.
    - h. Comorbid gastric, digestive disorders.
  2. Variable manifestation of metabolic abnormalities in ASD may reflect non-equilibrium dynamics as opposed to heterogeneous nature of the disease.
  3. Large number of interacting systems / reactions plus substantial positive feedback support this conjecture.

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