Oxidative stress and aging: is intervention possible?

Simon Melov Ph.D.
Buck Institute for Age Research

The Buck Institute for Age Research

Aging research approaches and opinions of the past

- Grind & Find
- Correlations, correlations, correlations…..
- Too complex
- Programmed death
- Thousands of genes involved
- Dogma

1990's - THE decade of invertebrate aging research

- Johnson, T.E. Increased life-span of age-1 mutants in *Caenorhabditis elegans* and lower Gompertz rate of aging. *Science* 249, 908-12 (1990)
- Kenyon, C., Chang, J., Gensch, E., Rudner, A. & Tabtiang, R. A *C. elegans* mutant that lives twice as long as wild type. *Nature* 366, 461-4 (1993)
- Vanfleteren, J.R. Oxidative stress and ageing in *Caenorhabditis elegans*. *Biochem J* 292 (Pt 2), 605-8 (1993) ; Larsen, P.L. Aging and resistance to oxidative damage in Caenorhabditis elegans. *Proc Natl Acad Sci U S A* 90, 8905-9 (1993)
- Lithgow, G.J., White, T.M., Melov, S. & Johnson, T.E. Thermotolerance and extended life-span conferred by single-gene mutations and induced by thermal stress. *Proc Natl Acad Sci U S A* 92, 7540-4. (1995)
- Kimura, K.D., Tissenbaum, H.A., Liu, Y. & Ruvkun, G. daf-2, an insulin receptor-like gene that regulates longevity and diapause in *Caenorhabditis elegans* [see comments]. *Science* 277, 942-6 (1997)
- Lin, Y.J., Seroude, L. & Benzer, S. Extended life-span and stress resistance in the Drosophila mutant methuselah [see comments]. *Science* 282, 943-6 (1998)
- Sun, J. & Tower, J. FLP recombinase-mediated induction of Cu/Zn-superoxide dismutase transgene expression can extend the life span of adult *drosophila melanogaster* flies. *Mol Cell Biol* 19, 216-28 (1999)

Trivial to extend lifespan in invertebrates

WORM GERONTOGENES: black = published, red = unpublished				
gene	**chromosome**	**life span/WT**	**Longevity reference**	**Comment**
age-1	*II*	*1.6*	Klass 1983, Friedman & Johnson 1988	
age-2	*I*	*1.2*	Yang and Wilson, 1999	longer-lived at higher temperature
age-n			Keightley et al., 2000	
age-n(a)			2000 East Coast *C. elegans* Meeting abstract 136	suppressor of daf-16 shortivity
age-n(b)			2000 East Coast *C. elegans* Meeting abstract 136	suppressor of daf-16 shortivity
che-2	*X*	*1.4*	Apfeld & Kenyon 1999	
che-3	*I*	*2*	Apfeld & Kenyon 1999	
che-11	*V*	*1.4*	Apfeld & Kenyon 1999	
che-13	*I*	*1.3*	Apfeld & Kenyon 1999	
clk-1	*III*	*1.4*	Lakowski & Hekimi 1996	
clk-2	*III*	*1.1*	Lakowski & Hekimi 1996	
clk-3	*II*	*1.2*	Lakowski & Hekimi 1996	
ctl-1	*II*		Taub et al., 1999	required for age-1 Age but not for twp-1 Age
daf-2	*III*	*2*	Kenyon 1993	
daf-5	*II*		2000 East Coast *C. elegans* Meeting abstract 43	only one allele was Age
daf-6	*X*	*1.3*	Apfeld & Kenyon 1999	
daf-10	*IV*	*1.6*	Apfeld & Kenyon 1999	
daf-12;daf-2	*X*	*3.4*	Larsen et al., 1995	strongly allele-specific
daf-19	*II*	*1.3*	Apfeld & Kenyon 1999	SOMEONE is doing chip work on daf-19
daf-21[hsp90]	*V*	*1.5*	1999 International *C. elegans* Meeting abstract 524	hsp90
daf-28	*V*	*1.1*	Malone et al., 1996	
des-1		*1.6*	Herndon & Driscoll, 2000	
eat-1	*IV*	*1.3*	Lakowski and Hekimi 1998	
eat-2	*II*	*1.4*	Lakowski and Hekimi 1998	
eat-6	*V*	*1.4*	Lakowski and Hekimi 1998	
eat-13	*X*	*1.3*	Lakowski and Hekimi 1998	
eat-18	*I*	*1.4*	Lakowski and Hekimi 1998	
efk-1	*III*	*1.25*	1999 International *C. elegans* Meeting abstract 830	Elongation Factor 2 Kinase homolog
gro-1	*IV*	*1.2*	Lakowski & Hekimi 1996	
ins-18	*1*	*–1.2*	Kawano et al., 2000	T28B8.3 (authors also use "Ceinsulin-1")
itt-n(a)			Walker et al., 1998a,b	
itt-n(b)			Walker et al., 1998a,b	
itt-n/HG25		*1.2*	Yang and Wilson, 2000	
itt-n/HG96		*1.5*	Yang and Wilson, 2000	
itt-n/HG246		*1.3*	Yang and Wilson, 2000	
mec-8	*I*	*1.6*	Apfeld & Kenyon 1999	
old-1	*II*	*2*	Murakami and Johnson 1998	
osm-1	*X*	*1.4*	Apfeld & Kenyon 1999	
osm-3	*IV*	*1.6*	Apfeld & Kenyon 1999	
osm-5	*X*	*2.2*	Apfeld & Kenyon 1999	
osm-6	*V*	*1.6*	Apfeld & Kenyon 1999	
pdk-1	*X*	*1.6*	Paradis et al., 1999	Daf-c
rad-8	*I*	*1.3*	Ishii et al., 1994	Only at 16oC
spe-10	*V*	*1.4*	Cypser and Johnson, 1999	
spe-26	*IV*	*1.6*	Van Voorhies 1992	Supressible by daf-16, but no known Daf
tax-4	*III*	*1.9*	Apfeld & Kenyon 1999	
unc-4	*II*	*4*	Gems et al., 2000	male-specific, but JC finds herms strongly Itt
unc-13	*I*	*2.9*	Gems et al., 2000	male-specific
unc-26	*IV*	*1.5*	Lakowski and Hekimi 1998	Eat phenotype
unc-31	*IV*	*1.3*	Ailon et al., 1999	Daf-c
unc-32	*III*	*2.8*	Gems et al., 2000	male-specific
unc-64	*III*	*1.8,2.4*	Ailon et al., 1999, Gems et al., 2000	NOT male-specific
unc-76	*V*	*1.4*	Gems et al., 2000	male-specific

Demonstrable success in aging research

- Known mechanisms of action are key (bias)
- Worm, fly, mouse
- New mutants are no longer novel (caveats)
- Linkage of gene action to physiology
- Inferred therapeutics

What are the effects of oxidative stress?

Exogenous studies

- toxicological studies, dose response etc.
- correlative

Endogenous studies

- ROS produced as a result of normal metabolism

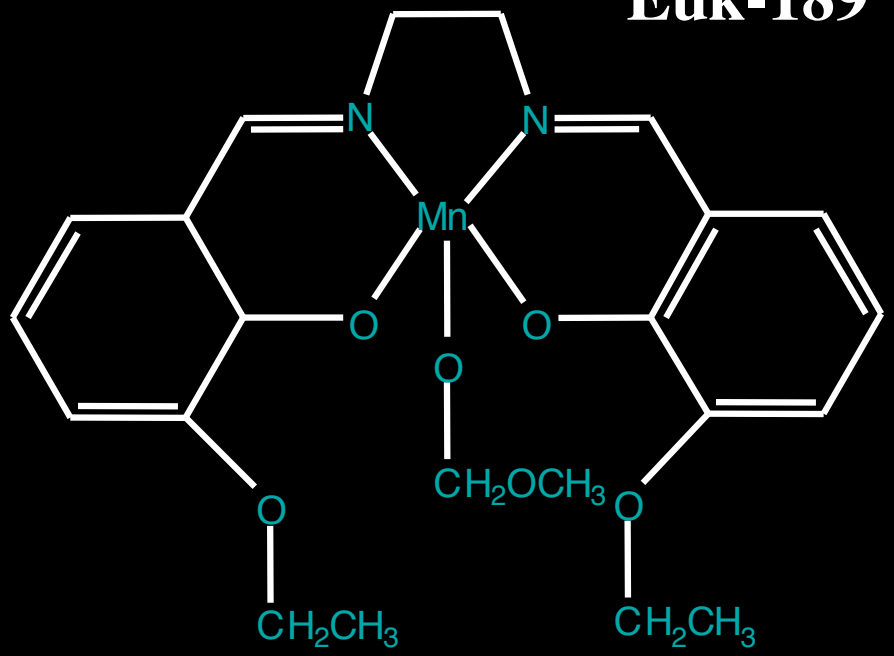
Genetics: transgenic as well as Knockouts

Efficacy of EUK antioxidants in biology

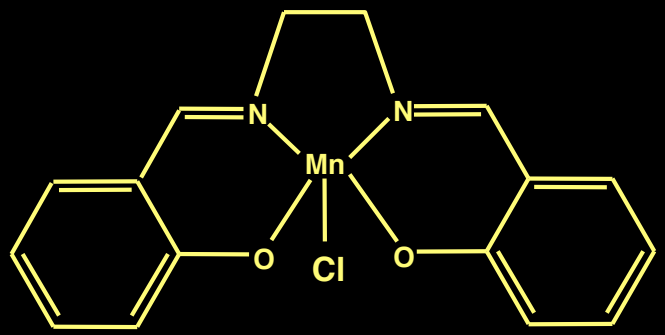
- Autoimmune disease
- Stroke
- Alzheimers disease
- Parkinsons disease
- ALS
- Apoptosis
- Mitochondrial dysfunction
- Radiation damage
- Aging

Catalytic antioxidants tested in *Sod2-/-* mice, and in aging paradigms

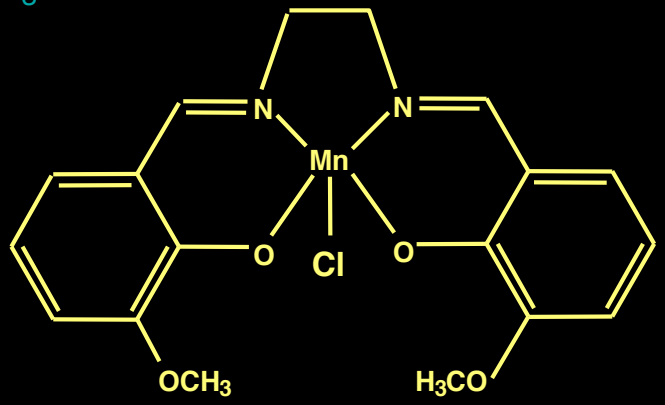
Euk-189



Euk-8

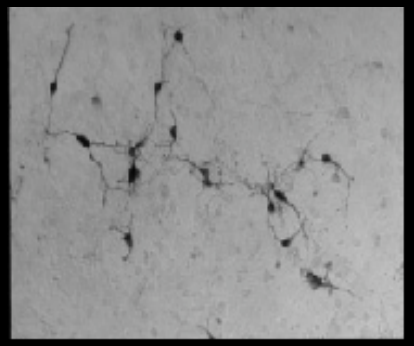


Euk-134



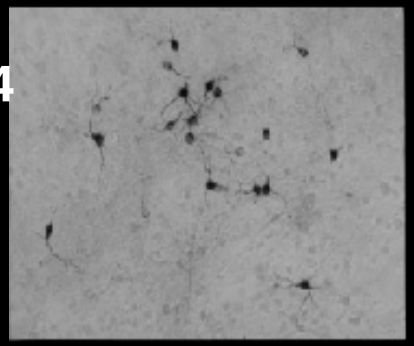
EUK-134 protects dopaminergic neurons from toxicity by MPP+

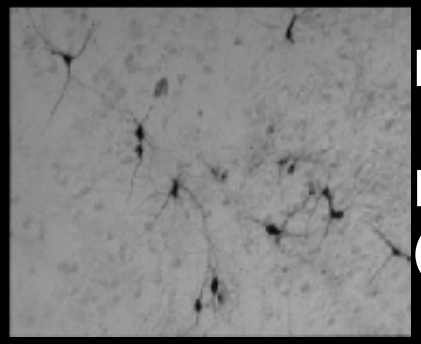
Control



MPP+

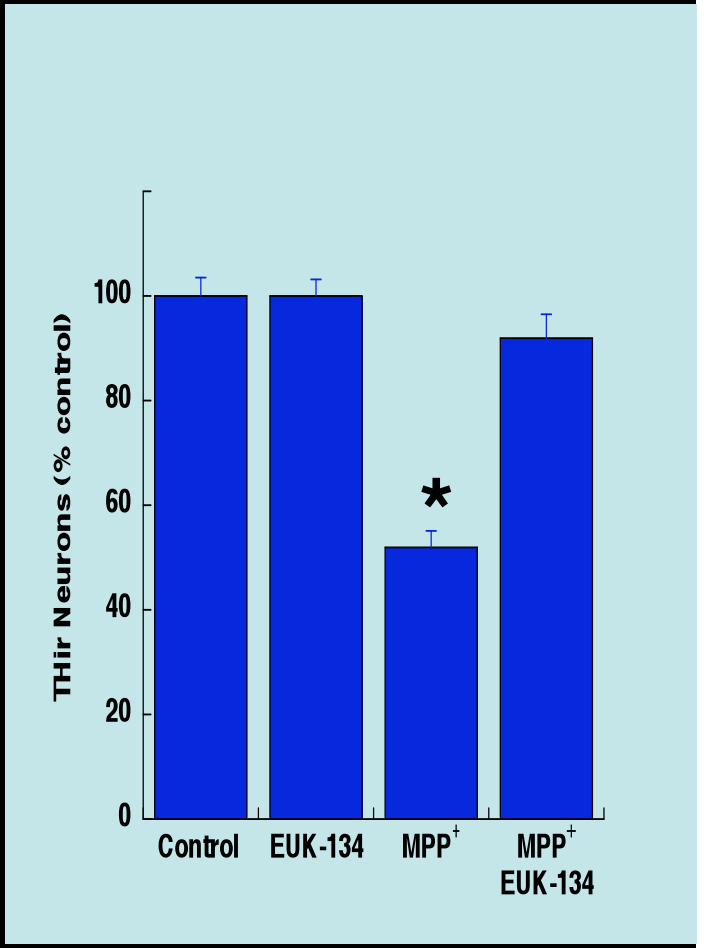
EUK-134 (0.5 μM)





MPP+

EUK-134 (0.5 μM)



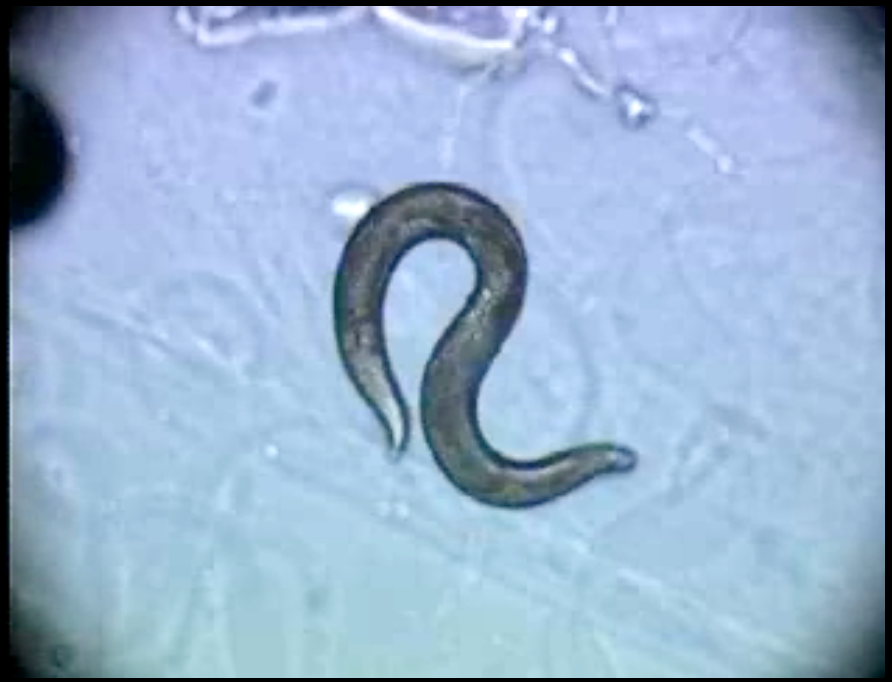
(MPP+ 10 μM)

K. Pong et al., Brain Res. (2000)

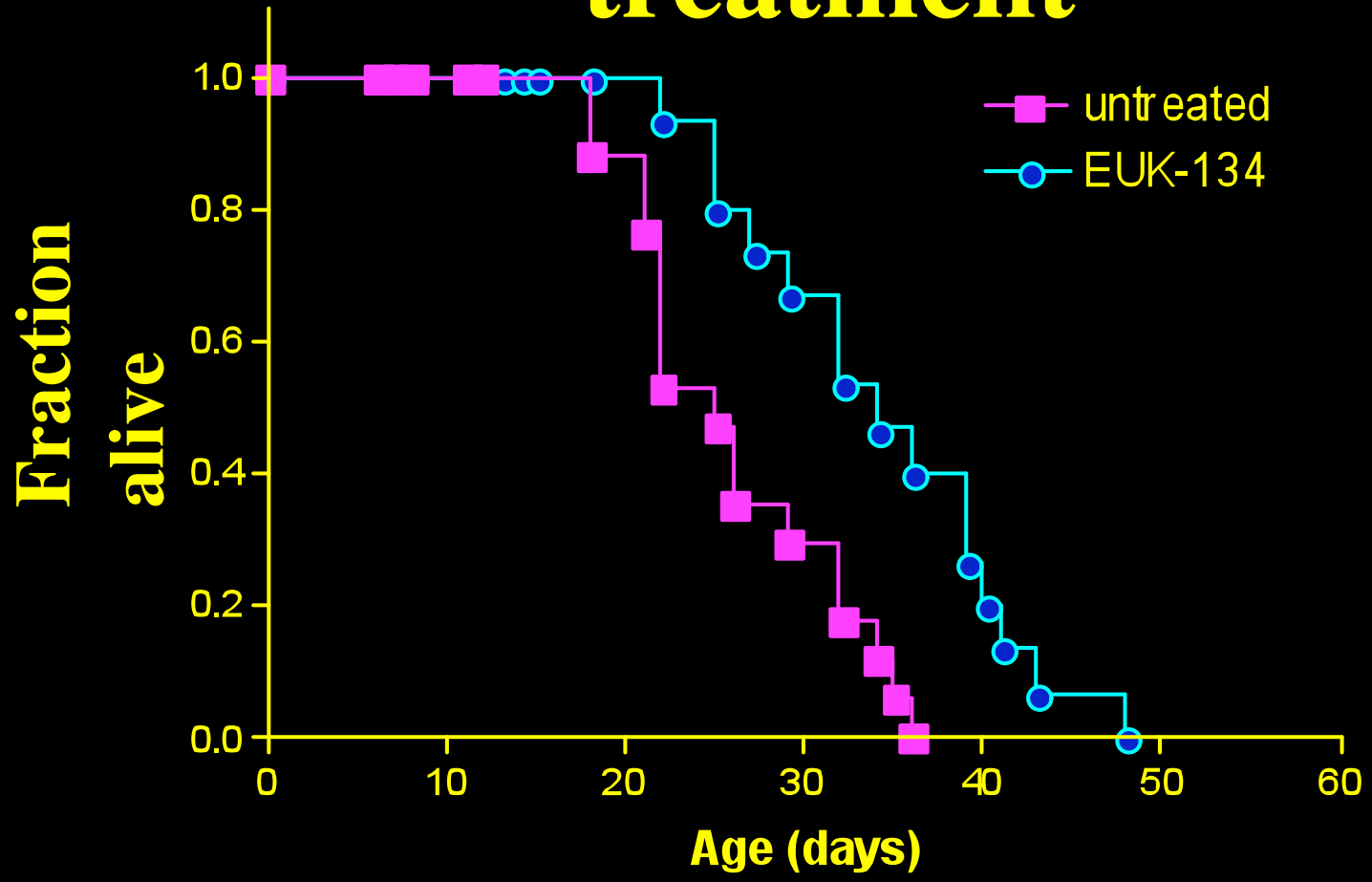
Caenorhabditis elegans

- **Advantages as an aging model**
  - **Small size, complete genome, AGE mutants (e.g. *age-1*), short lifespan**
  - **Pharmacological screening**
    - **(disadvantage - pharmacological screening)**
- **Advantages as a mitochondrial model**

Metabolic mutants
respiratory chain
increased lifespan
mev-1
Oxidative damage, shortened lifespan

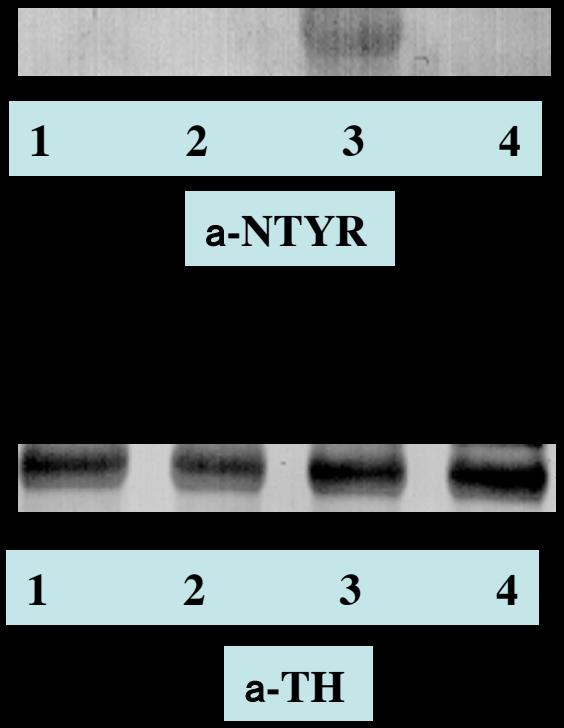


Extension of lifespan in *C.elegans* through antioxidant treatment



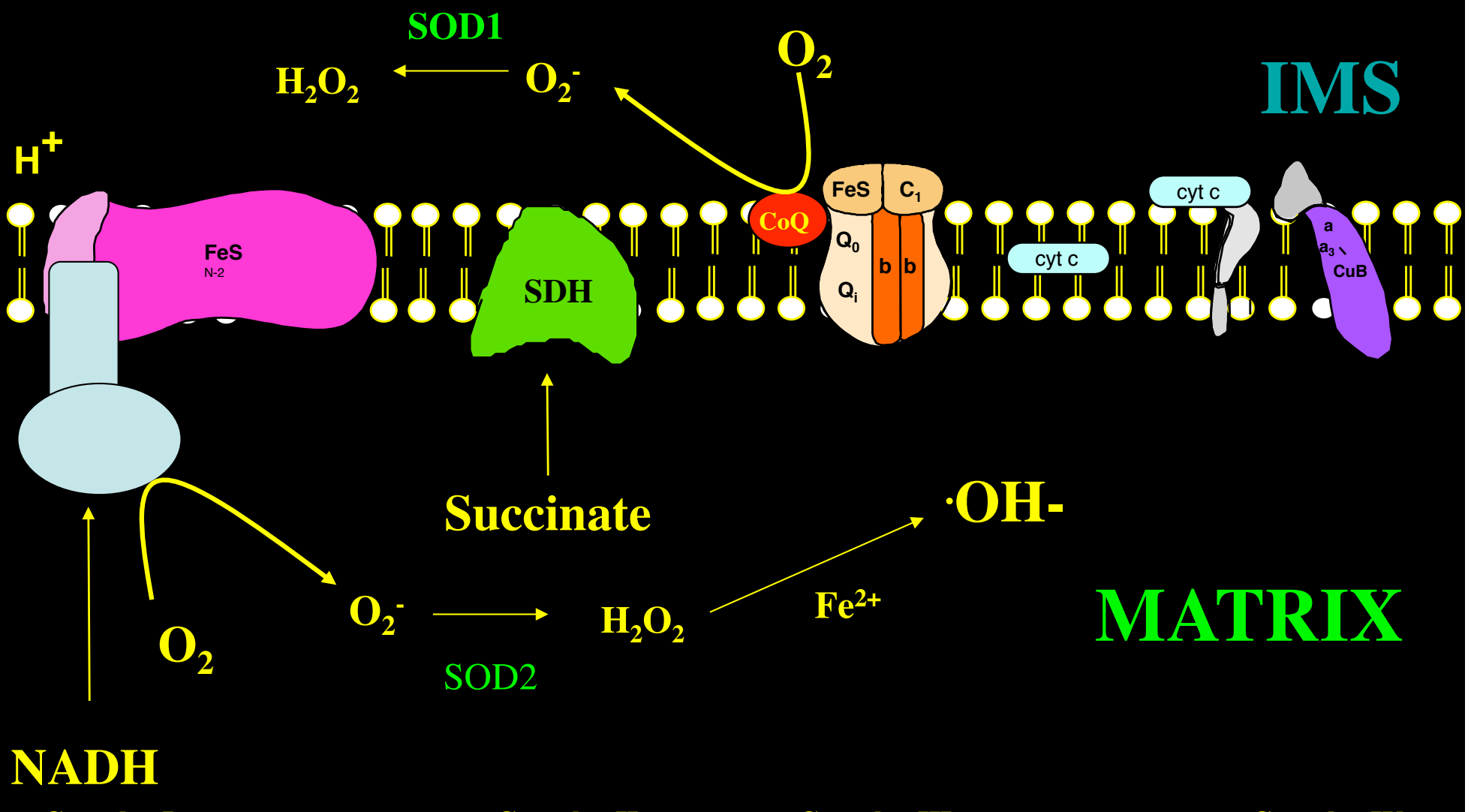
EUK-134 inhibits nitration of tyrosine hydroxylase in DA neurons

1. Control
2. EUK-134
3. MPP^+
4. MPP^+ and EUK-134



K. Pong et al., Brain Res. (2000)

Reactive Oxygen species and the Respiratory Chain



WEAK FORM

Oxygen free radicals generated as a function of metabolic rate cause cumulative oxidative damage, resulting in structural degeneration, functional decline, and age-related diseases.

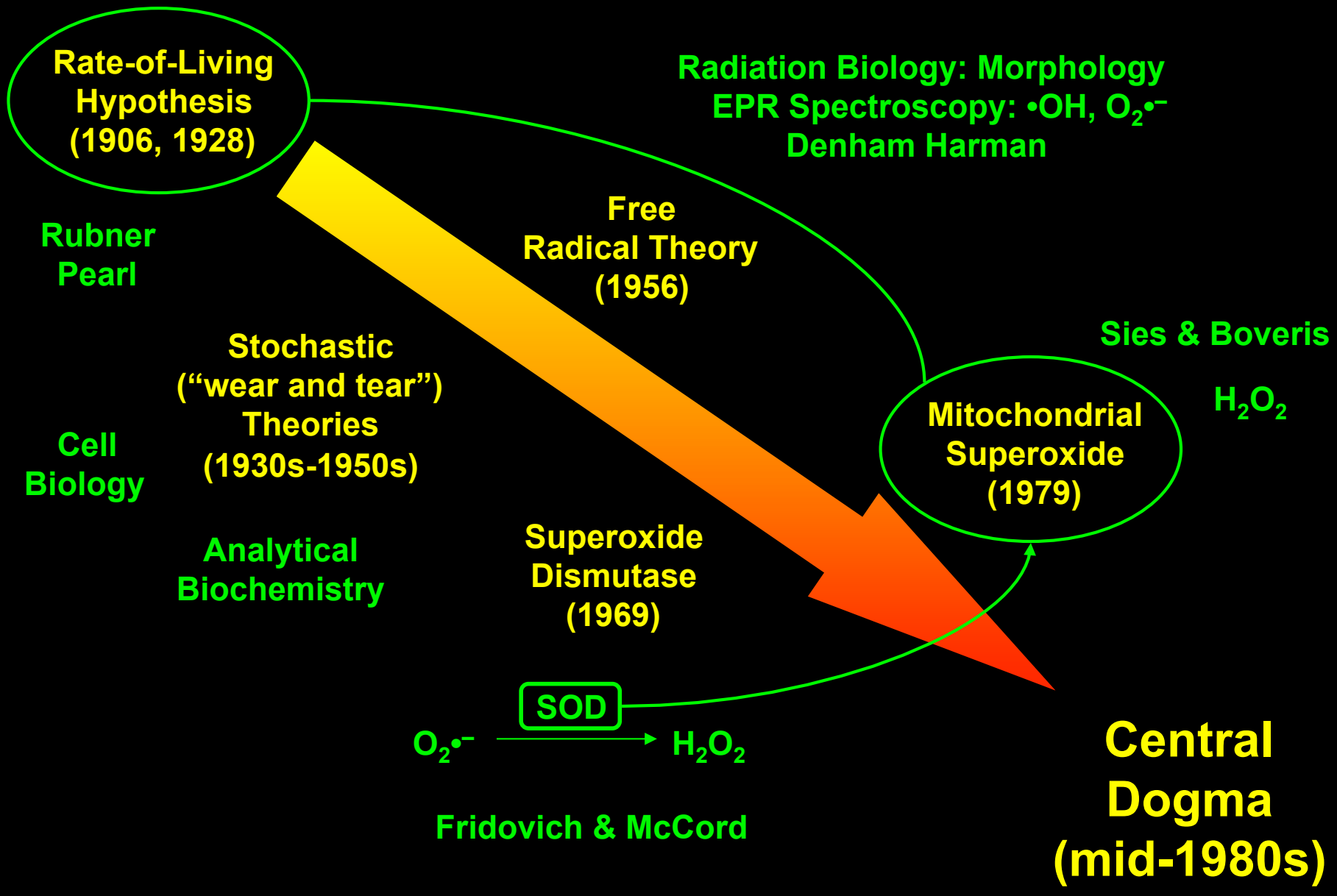
MODERATE FORM

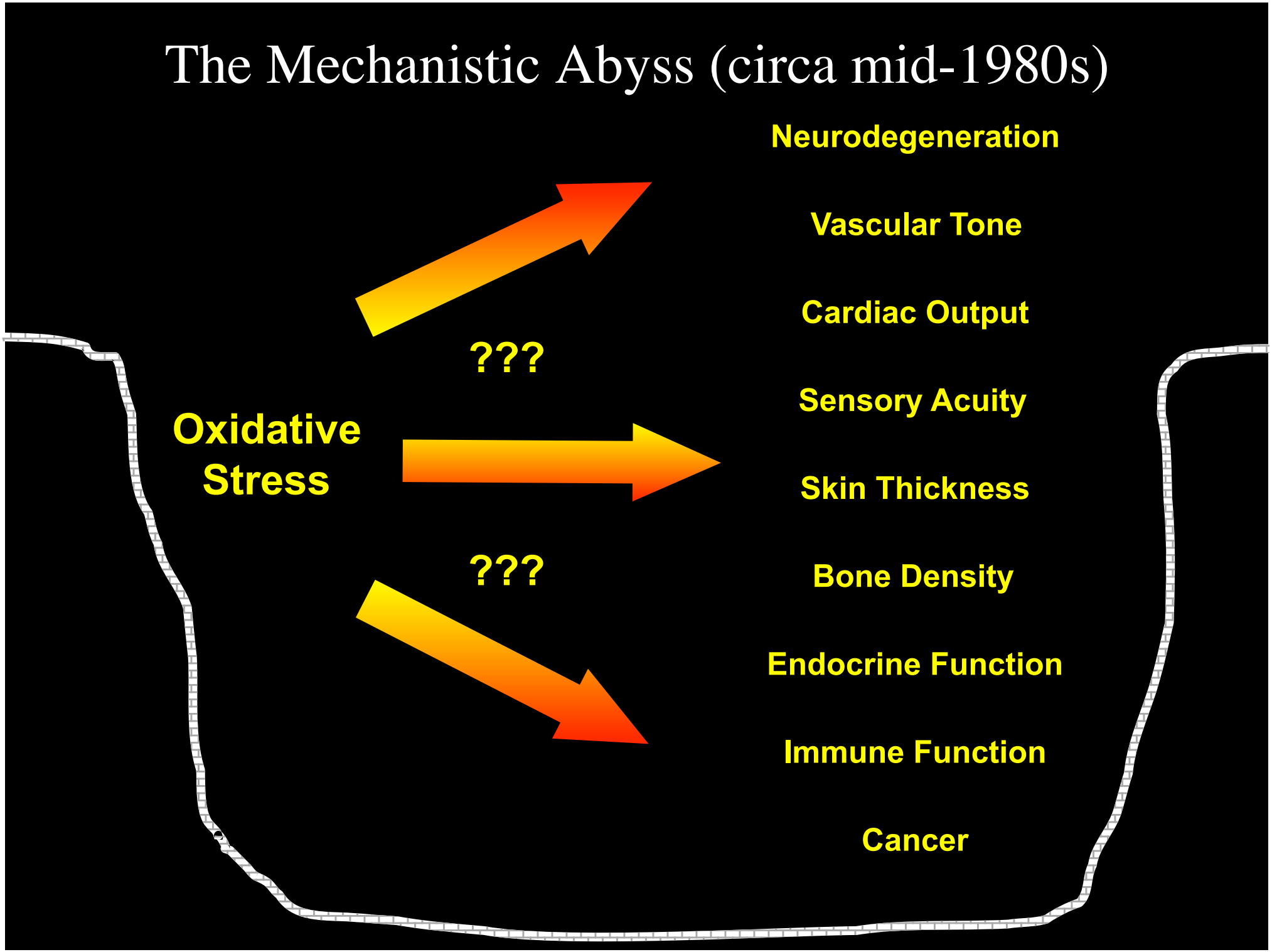
Oxygen free radicals generated as a function of metabolic rate cause cumulative oxidative damage, resulting in structural degeneration, functional decline, and age-related diseases. Oxidative stress is the predominant cause of age-associated degenerative change.

STRONG FORM

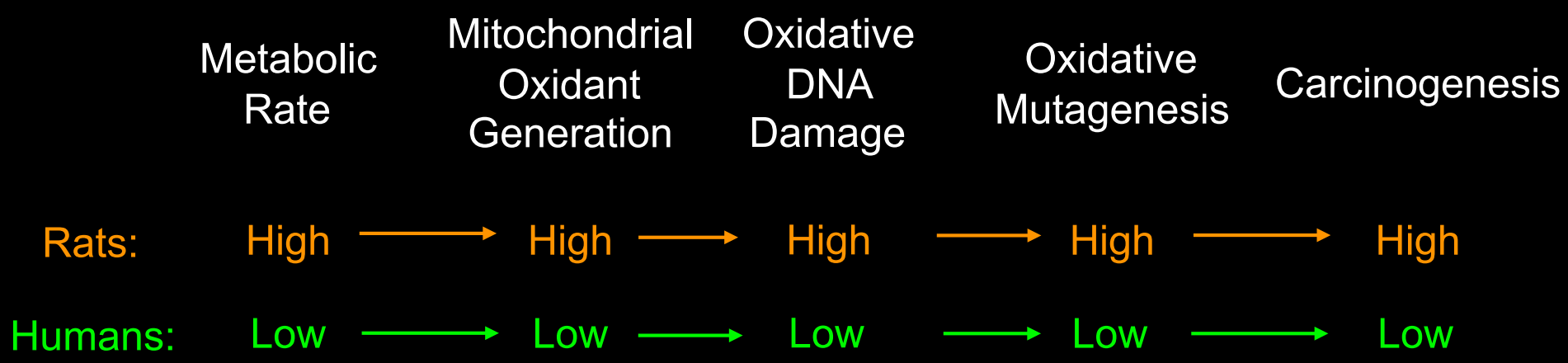
Oxygen free radicals generated as a function of metabolic rate cause cumulative oxidative damage, resulting in structural degeneration, functional decline, and age-related diseases. Oxidative stress is the predominant cause of age-associated degenerative change, and thus the determinant of MLSP.

Brief History

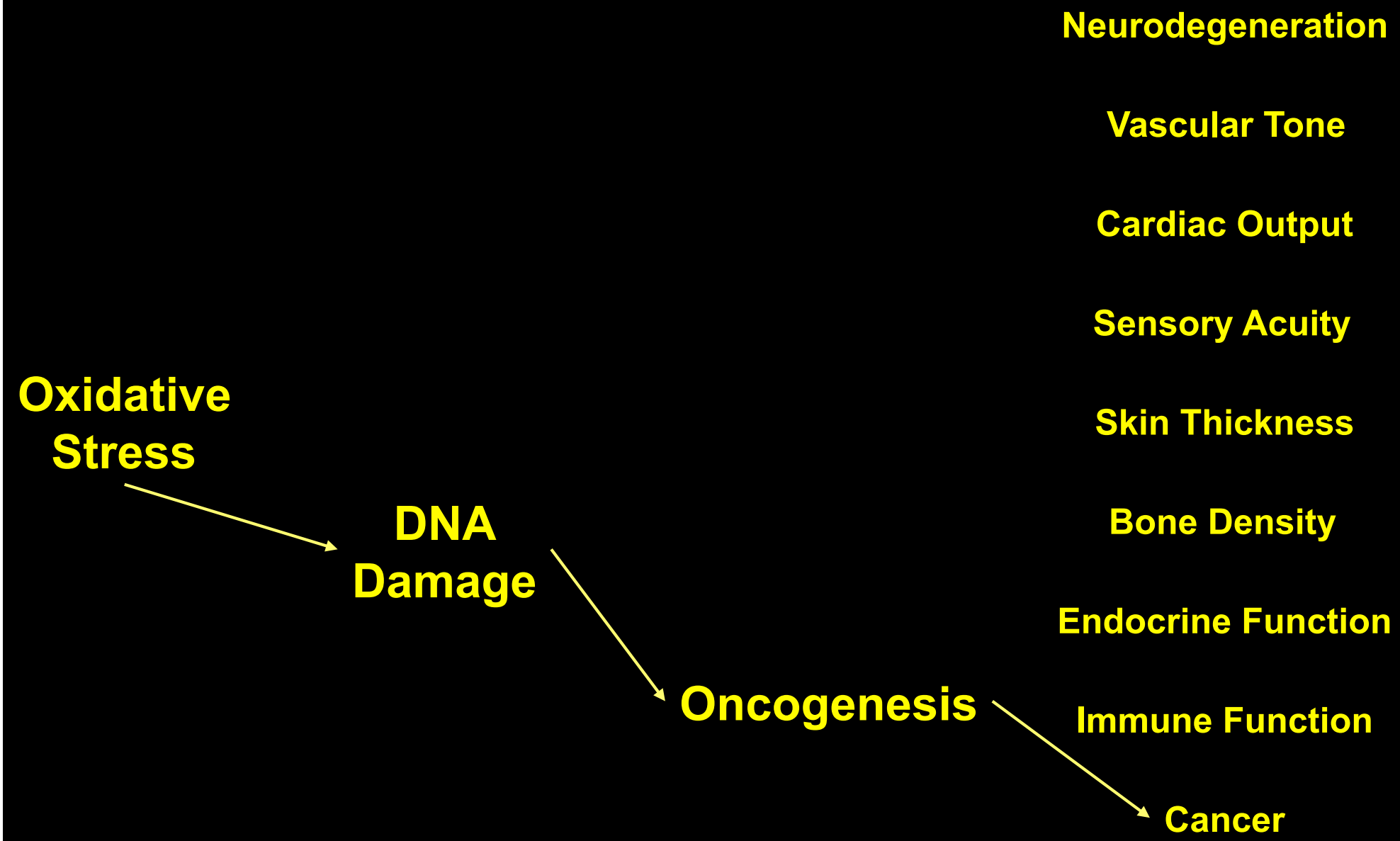



The Mechanistic Abyss (circa mid-1980s)
Neurodegeneration
Vascular Tone
Cardiac Output
???
Sensory Acuity
Oxidative Stress
Skin Thickness
???
Bone Density
Endocrine Function
Immune Function
Cancer

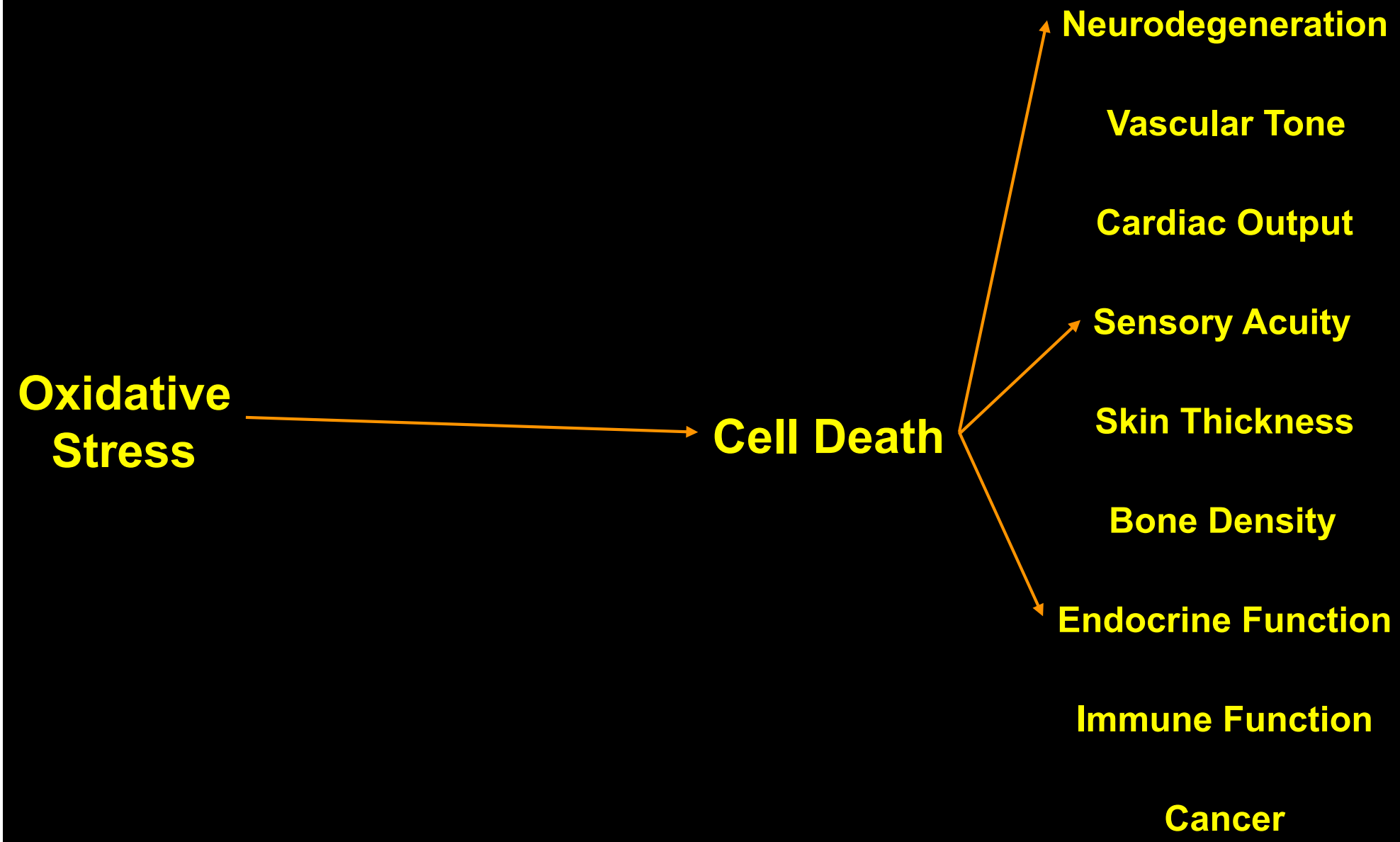
A Simplifying Hypothesis



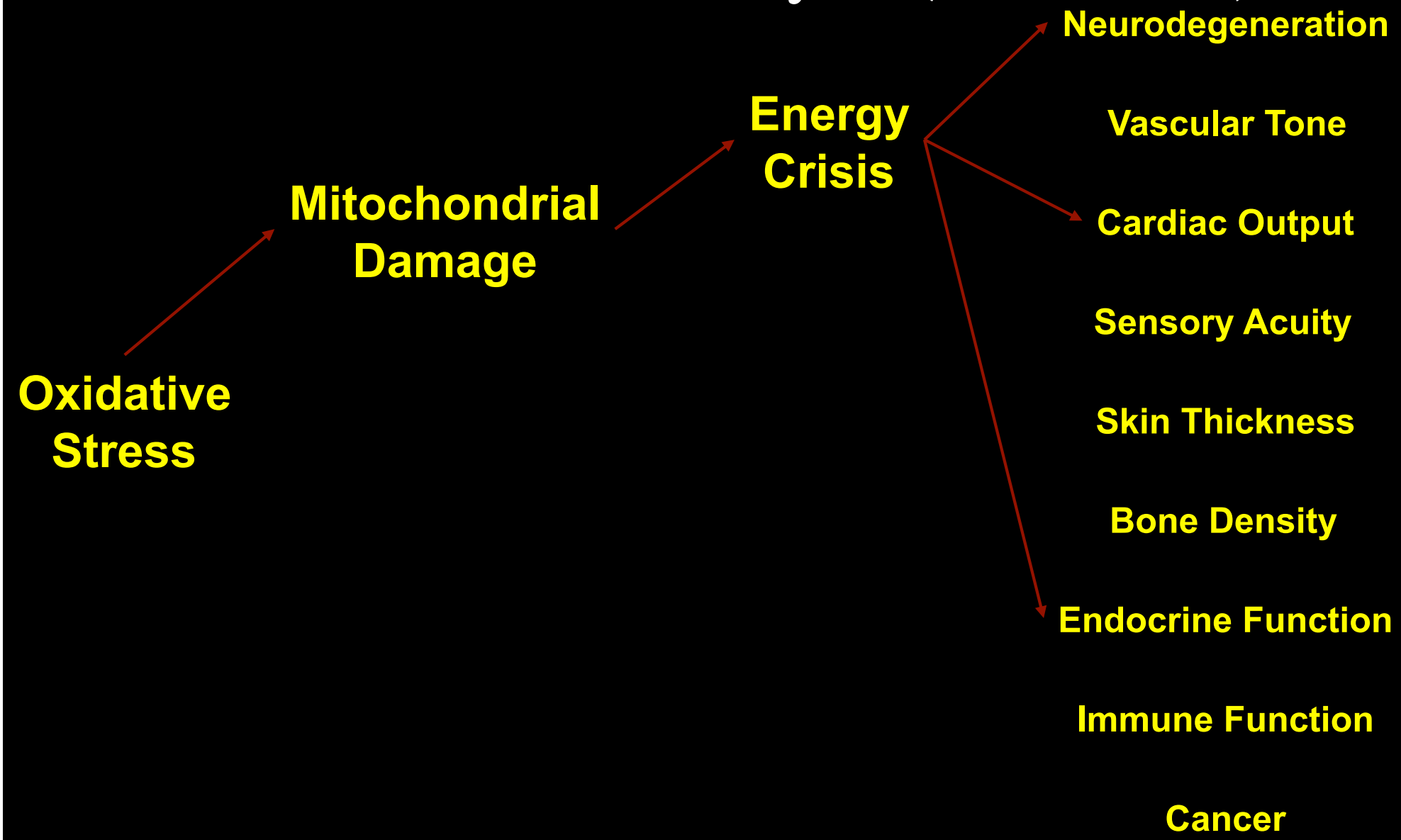
The Mechanistic Canyon (circa 1995)

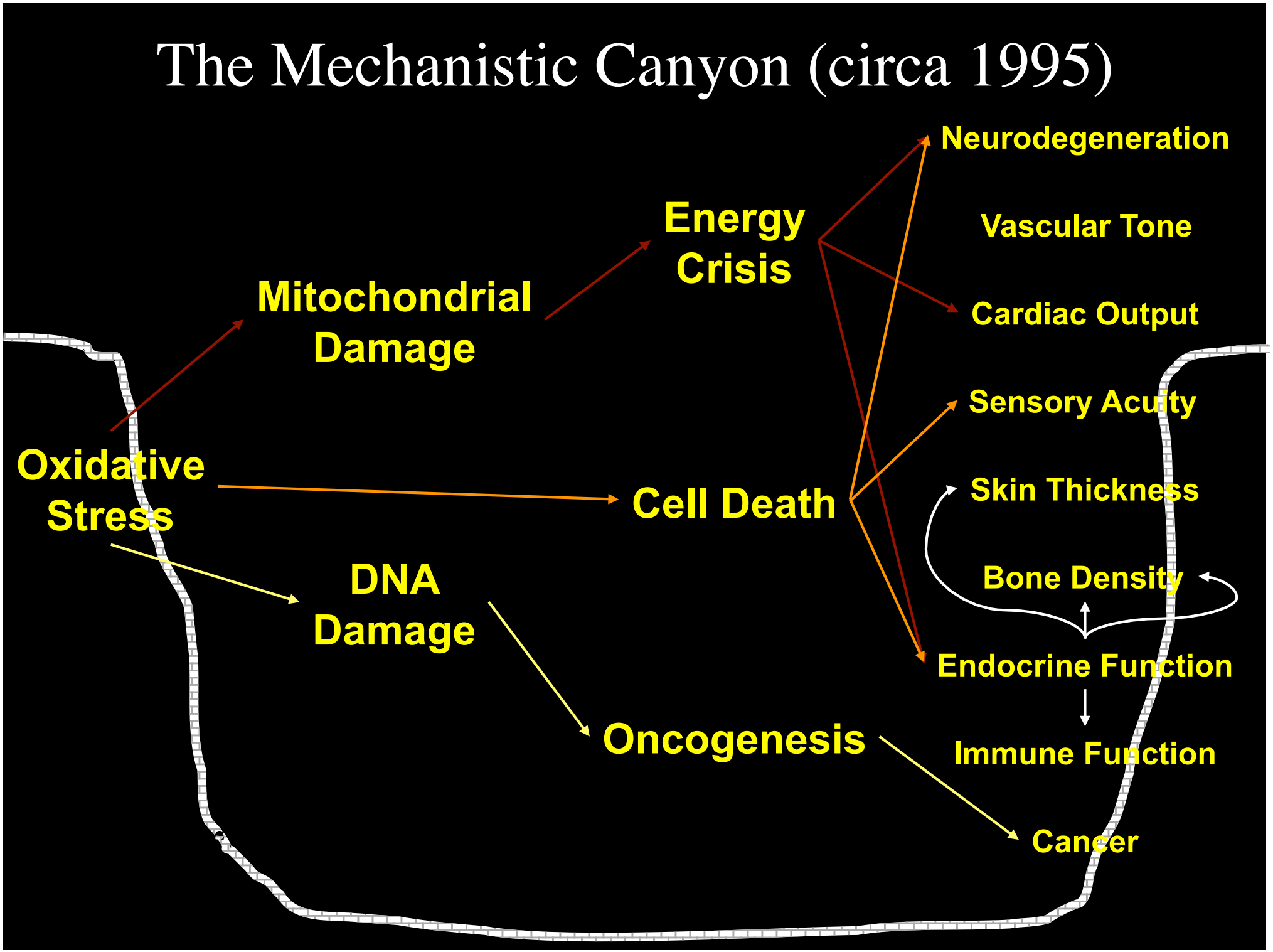


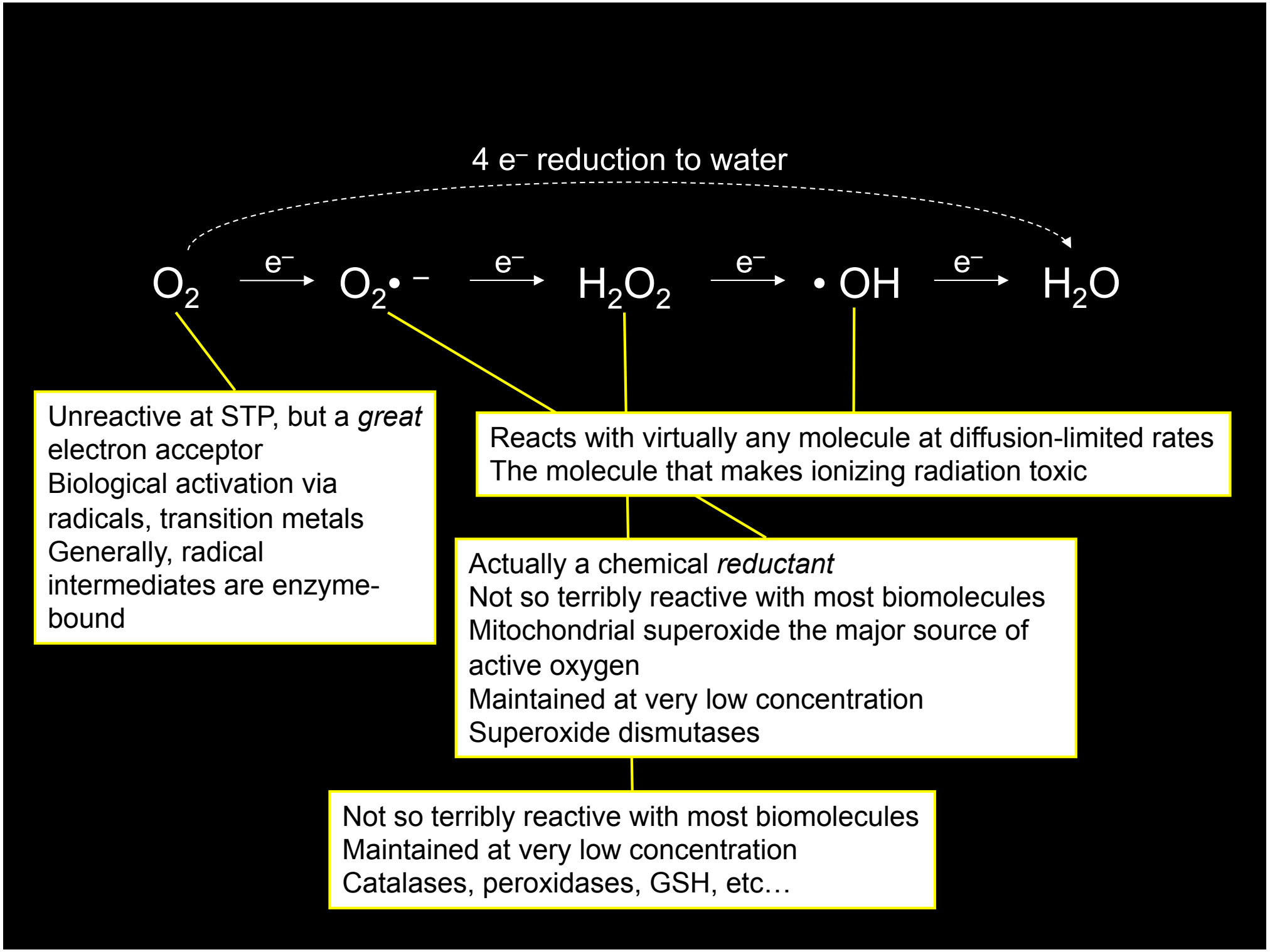
The Mechanistic Canyon (circa 1995)

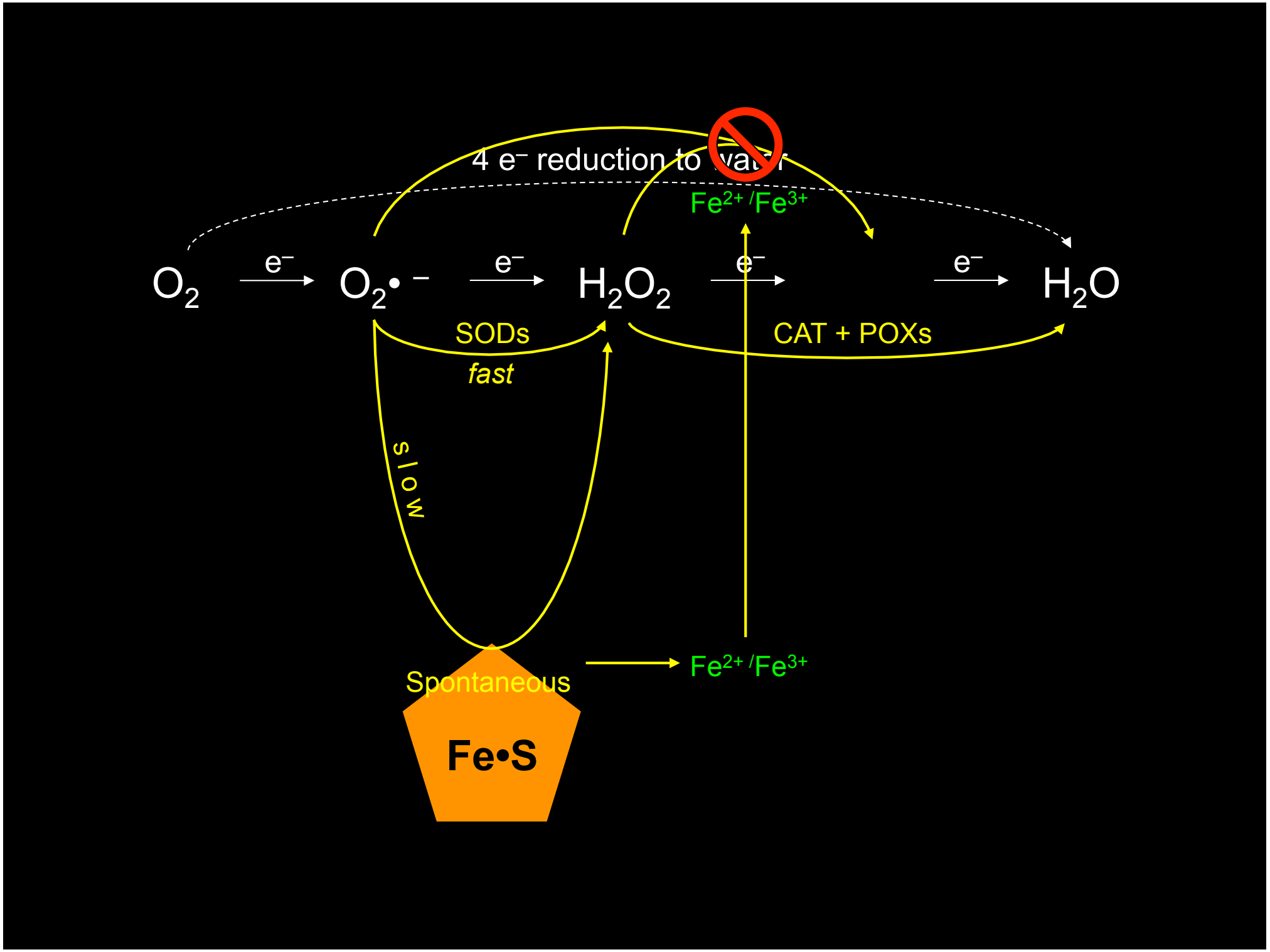


The Mechanistic Canyon (circa 1995)



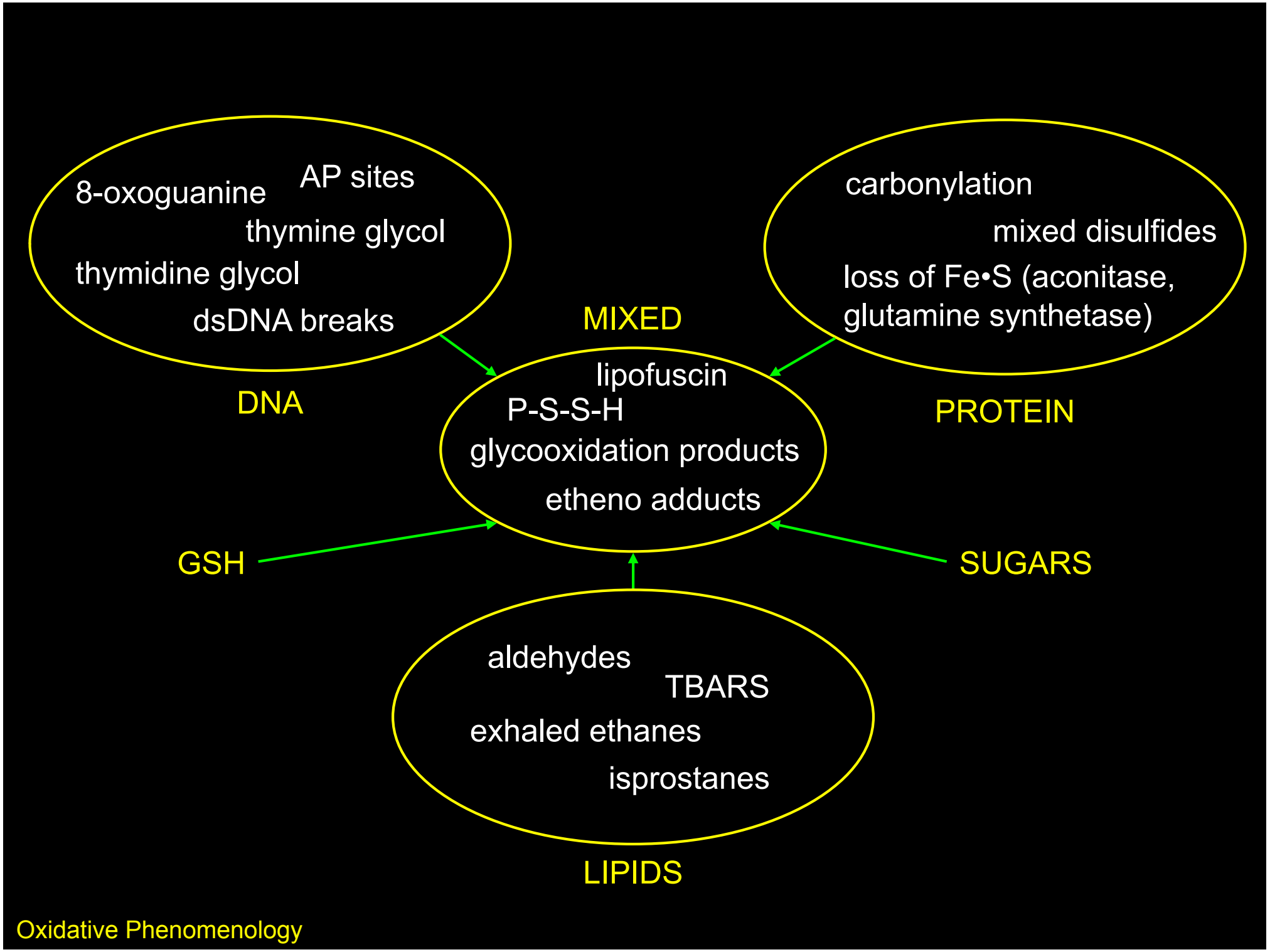

The Mechanistic Canyon (circa 1995)
Neurodegeneration
Energy Crisis
Vascular Tone
Mitochondrial Damage
Cardiac Output
Sensory Acuity
Oxidative Stress
Cell Death
Skin Thickness
Bone Density
DNA Damage
Endocrine Function
Oncogenesis
Immune Function
Cancer


4 e⁻ reduction to water
O_2
e⁻
$O_2\bullet^-$
e⁻
H_2O_2
e⁻
$\bullet OH$
e⁻
H_2O
Unreactive at STP, but a *great* electron acceptor
Biological activation via radicals, transition metals
Generally, radical intermediates are enzyme-bound
Reacts with virtually any molecule at diffusion-limited rates
The molecule that makes ionizing radiation toxic
Actually a chemical *reductant*
Not so terribly reactive with most biomolecules
Mitochondrial superoxide the major source of active oxygen
Maintained at very low concentration
Superoxide dismutases
Not so terribly reactive with most biomolecules
Maintained at very low concentration
Catalases, peroxidases, GSH, etc…


4 e⁻ reduction to water
Fe^{2+}/Fe^{3+}
O_2
e⁻
$O_2^{\bullet -}$
e⁻
H_2O_2
e⁻
e⁻
H_2O
SODs
fast
CAT + POXs
slow
Spontaneous
Fe^{2+}/Fe^{3+}
Fe•S

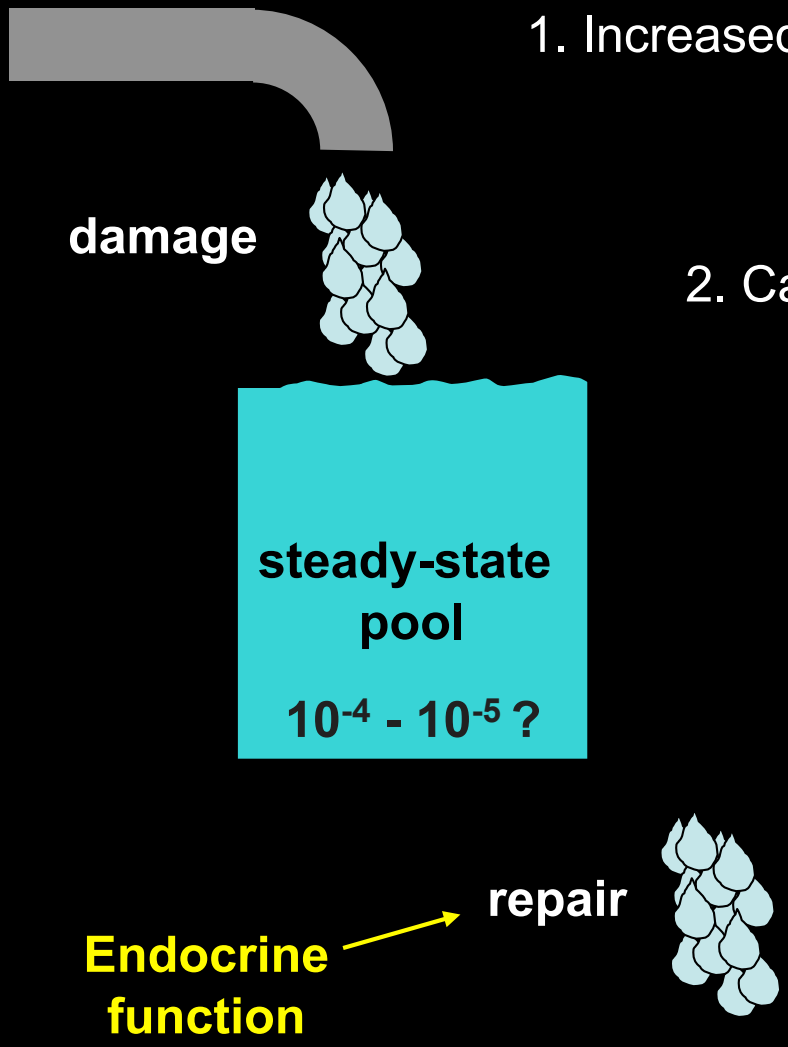
Types of Evidence

1) Oxidative Phenomenology
2) Dietary Restriction
3) Rate-of-Living/Oxygen Tension
4) Dietary Supplementation
5) Pharmacological Intervention
6) Comparative Biology
7) Classical and Population Genetics
8) Transgenic Models
9) Human Degenerative Disease



Oxidative Phenomenology

The Problem With Biomarkers



1. Increased damage or decreased repair?

2. Cause of consequence of aging?

3. False negatives?

4. What does it all mean?

Evidence in favor of the FRTA:

1) "Expected" changes in 100s of studies.
2) Many tissues, many species.
3) Specific repair systems for many end-products characterized.

BUT…

1) Methodological problems with most such work.
2) Negative studies buried?
3) Specific repair systems for many end-products characterized.
4) Absolute and relative magnitude of increases underwhelming.

HOWEVER…

1) Grind-and-find studies *necessary* to establish baselines.
2) Biomarkers most useful in comparative and intervention studies.

Dietary Restriction

Note: dietary restriction does *not* generally decrease metabolic rate or activity in mammals.

Evidence in favor of the FRTA:

1) Generally: decreased age-specific accumulation of biomarkers.
2) Generally: decreased sensitivity to oxidative stress.
3) Sometimes: increased antioxidant activities.

BUT…

1) Almost *all* age-related alterations are slowed by DR.
2) Hence: cause and effect are hopelessly entangled.

HOWEVER…

1) DR is a litmus test, and the FRTA has “passed” it.

Rate-of-Living/Oxygen Tension

Evidence in favor of the FRTA:

1) Models:
   A. Physical restraint of insects.
   B. Thermal manipulation of poikilotherms.
   C. Increased/decreased oxygen tension of invertebrates.

2) Results largely supportive of the FRTA.

   BUT…

3) Applicability of models to other phyla?
4) Decreased life span is not a powerful phenotype.

HOWEVER…

- Negative results would have been robust, so positive results are important.

Dietary Supplementation

Evidence in favor of the FRTA:

1) Some amelioration of age-related degenerative change:
    1) ALCAR/lipoic acid in rat.
    2) Phenolic antioxidants in rodents (blueberries).

2) Data appear to support the weak form of FRTA.

BUT…

1) Most experiments have been negative.
2) Extension of life span: virtually no evidence.

HOWEVER…

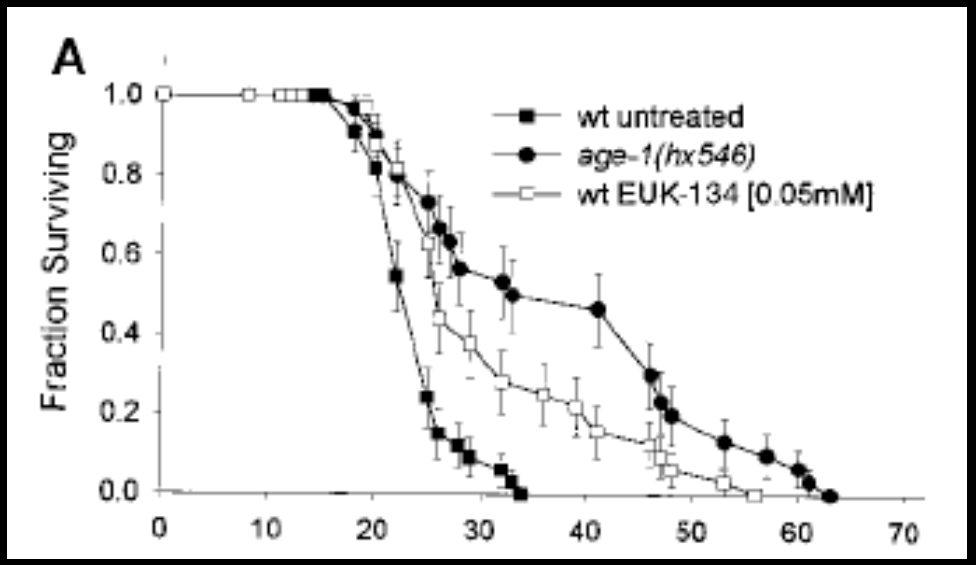
Dietary supplementation is a flawed approach -- physiology restricts degrees of experimental freedom and potency. Falsification is problematic.

Pharmacological Intervention

Evidence in favor of the FRTA:

1) Some amelioration of age-related degenerative change:
    1) PBN in gerbils

2) Extension of life span:
    1) Euk-134 in nematodes
    2) Efficacy in Mammals
    3) Euk-189 in mice?



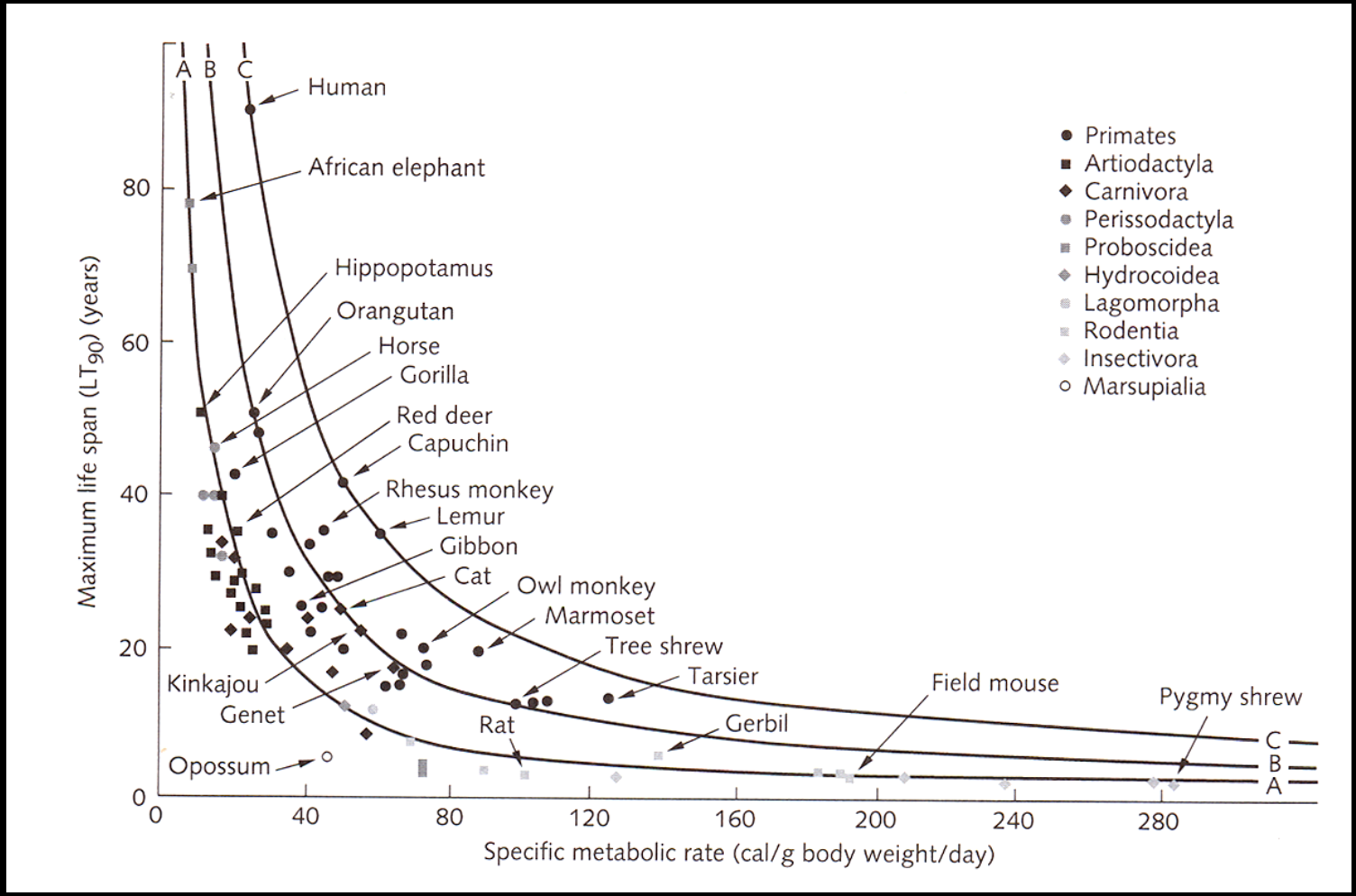
BUT…

1) Many experiments have been negative.

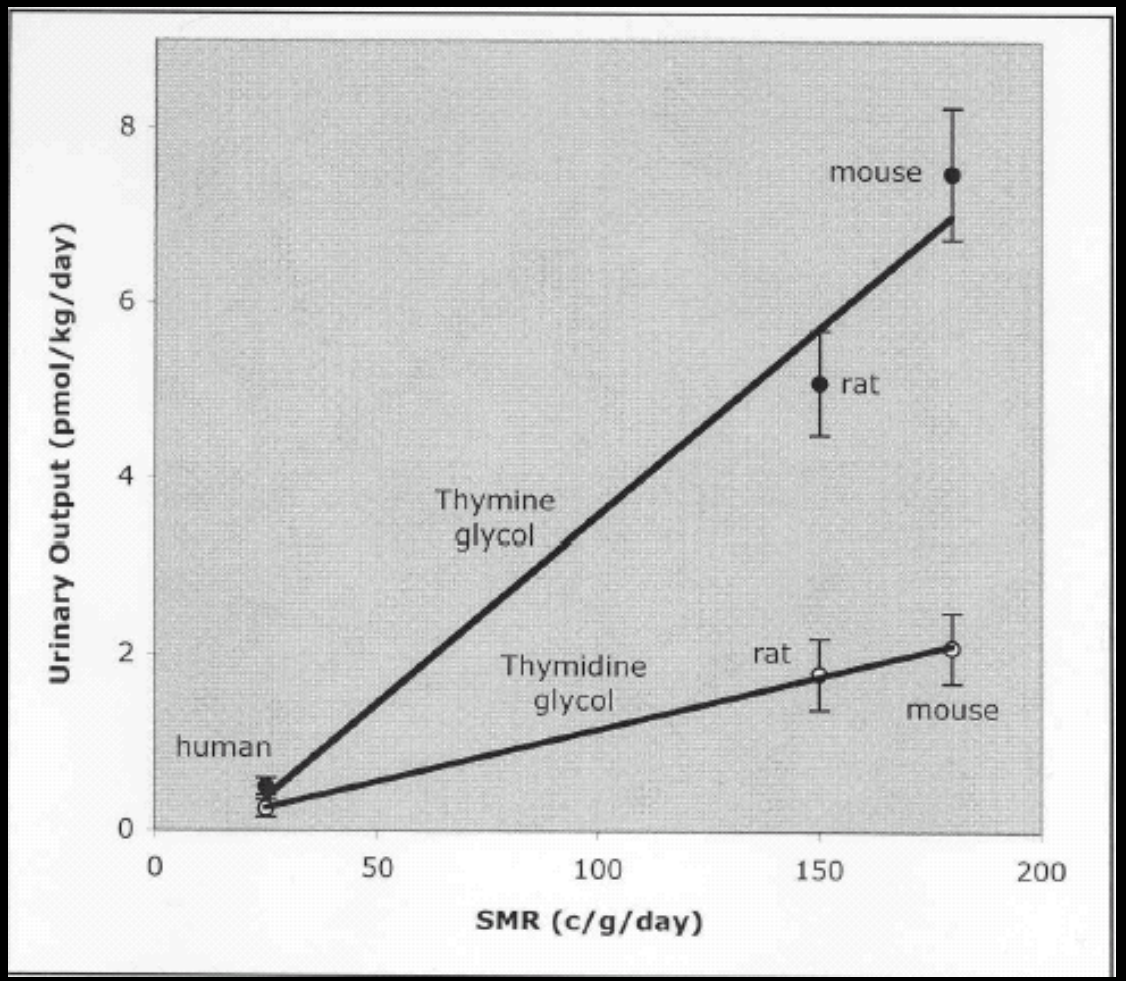
HOWEVER…

Falsification of the FRTA with drugs will be difficult.

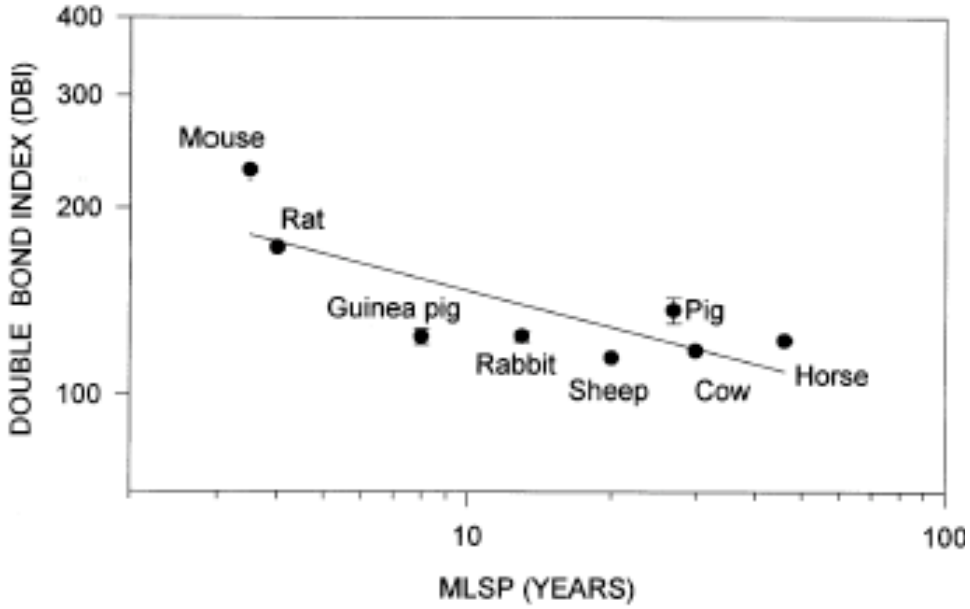
Comparative Biochemistry

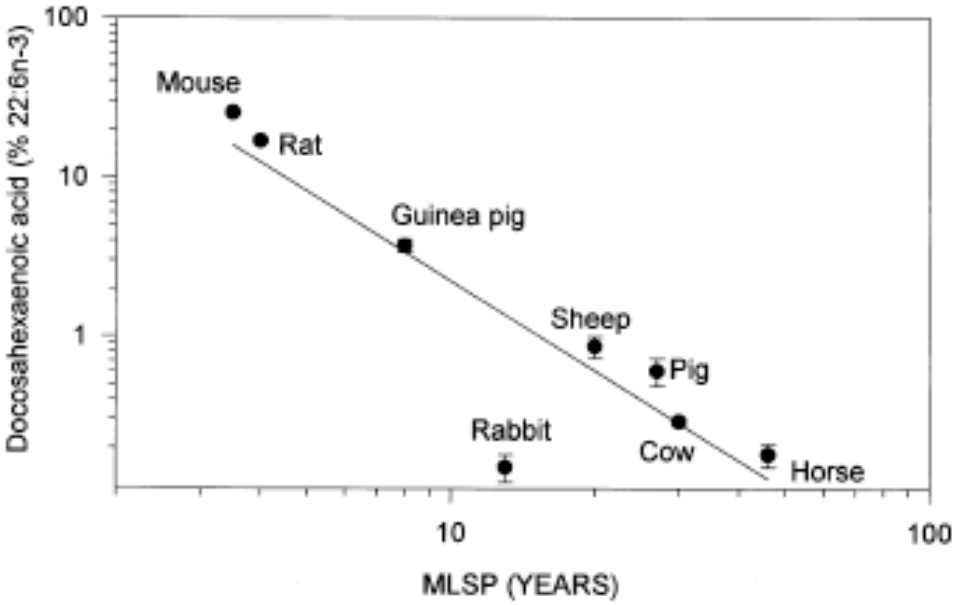


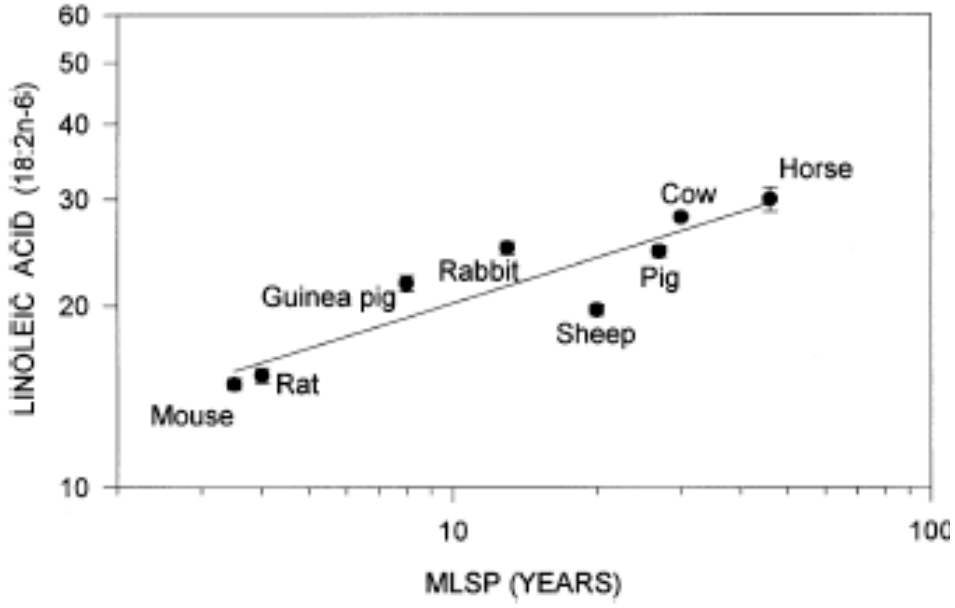
Oxidative DNA damage rates correlate with metabolic rate

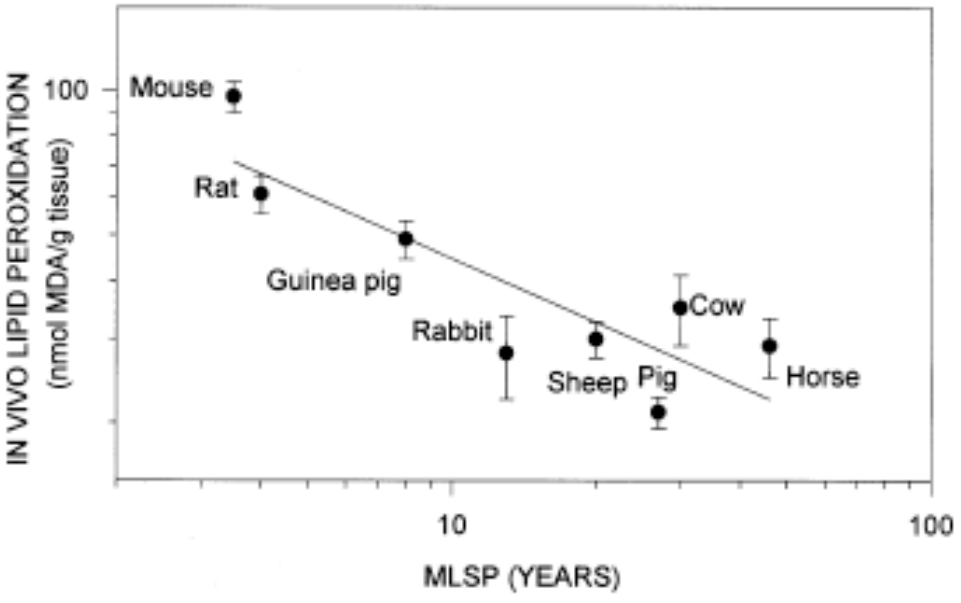


Mitochondrial Lipid Content









Genetics

Evidence in favor of the FRTA:

1) Many long-lived mutants demonstrate increased antioxidant defenses and better tolerance of oxidative stress.
2) Population selection for increased life span sometimes (not always) associated with increased SOD activity in long-lived strains.
3) Short-lived mutants often associated with decreased antioxidant defenses, increased ROS generation.

BUT…

4) Long-lived mutants possess generally better resistance against many stressors.

HOWEVER…

Many stressors may act via oxidative mechanisms.

Transgenic Models

Evidence in favor of the FRTA:

1) Life span extensions with transgenic SOD *Drosophila*.

   BUT…

2) Negative results with various Tg KO and heterozygous organisms.

HOWEVER…

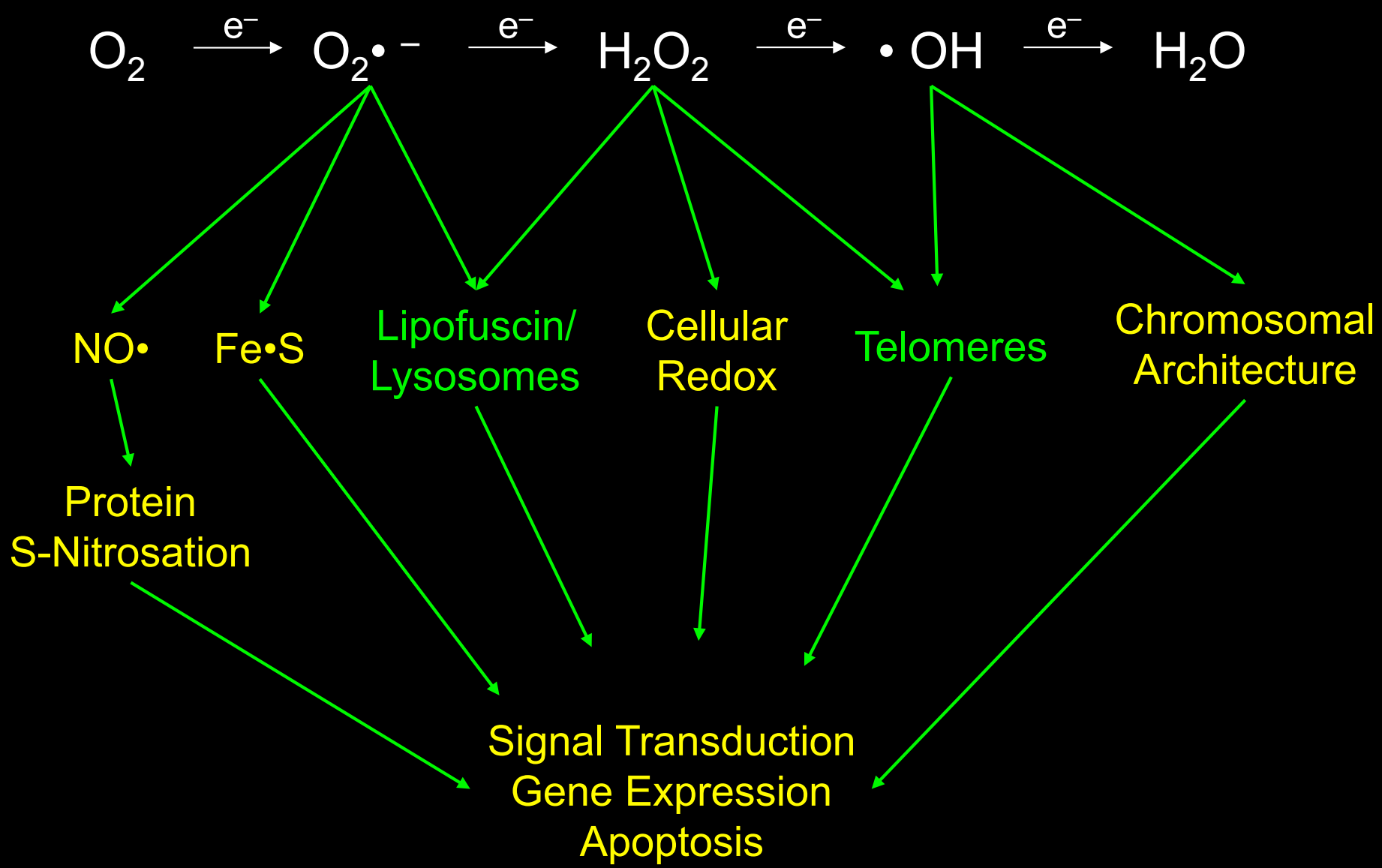
1. Many stressors may act via oxidative mechanisms.
2. Oxidative defenses are both redundant and interconnected -- crude genetic engineering is likely to be often compromised.
3. Overexpression of SOD prohibited *in vitro*.

Value of Evidence

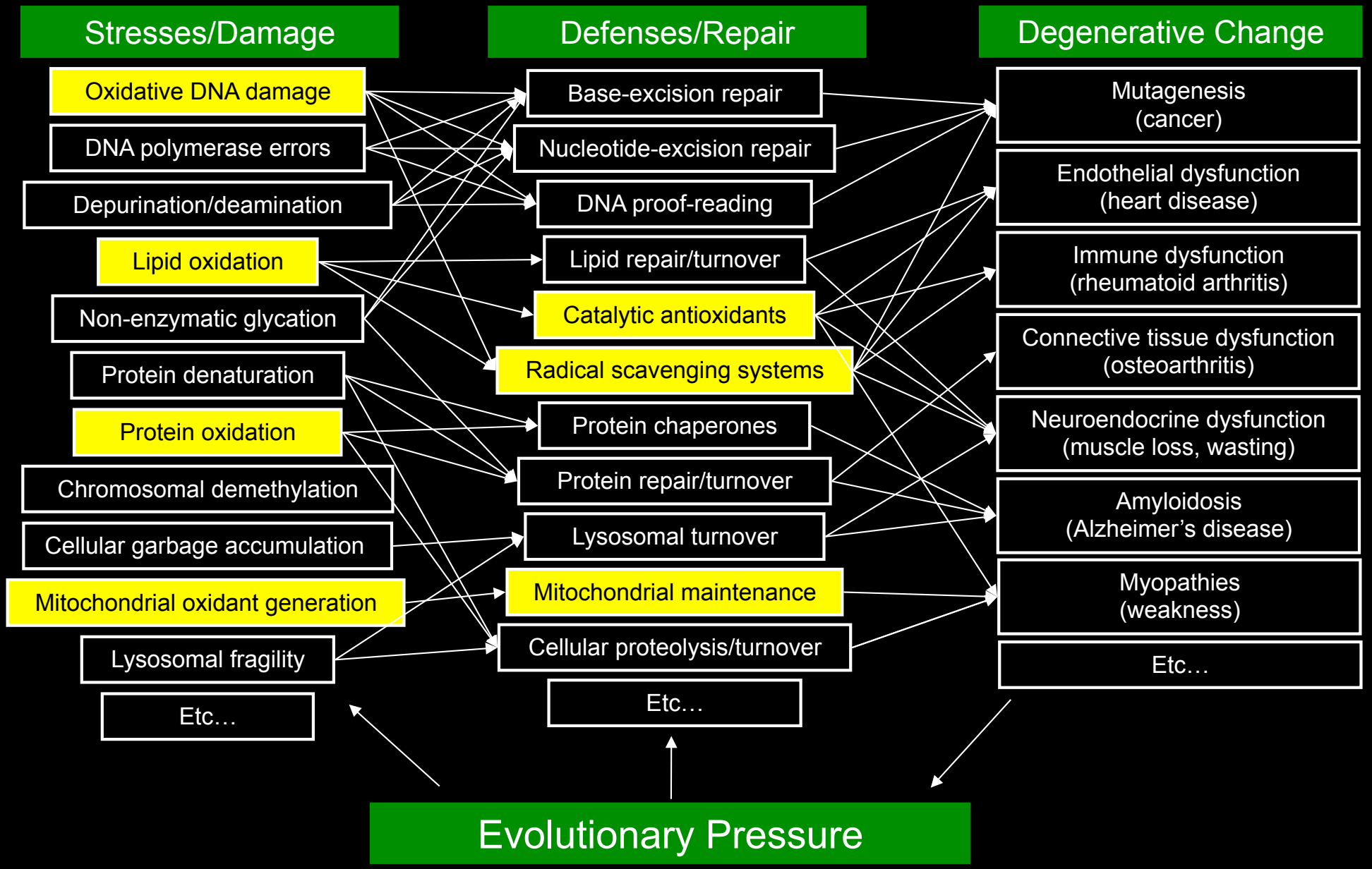
1) Oxidative Phenomenology - LOW
2) Dietary Restriction - MODERATE
3) Rate-of-Living/Oxygen Tension - MODERATE
4) Dietary Supplementation - LOW
5) Pharmacological Intervention - HIGH
6) Comparative Biology - VERY HIGH
7) Genetics - VERY HIGH
8) Transgenic Models - VERY HIGH
9) Human Degenerative Disease - LOW

Functional and Comparative Genomics
VERY HIGH

Amplification Mechanisms?



Homeostasis: Is Oxidative Stress Special?



Questions/Answers

- WEAK: Are oxygen free radicals important in aging?

  Yes.

- STRONG: Do oxygen free radicals *determine* MLSP?

  No.

- MODERATE: Are oxygen free radicals *predominant* in aging?

  ???

  - energetics
  - cell division
  - cell arrest
  - cell death
  - chromosomal stability
  - gene expression
  - signal transduction

Questions/Answers

Does CO_2 determine plant growth? No…

Does transcription determine embryogenesis? No…

CO_2 has "nothing to do with" the determination of plant growth.

Transcription has "nothing to do with" embryogenesis.

Mechanisms — not measurement.

The FRTA is no longer theoretical in the "weak" form.

The FRTA is unintelligible in the "strong" form.

The Free Radical "Perspective" on Aging has been productive, and is an object lesson for homeostasis.

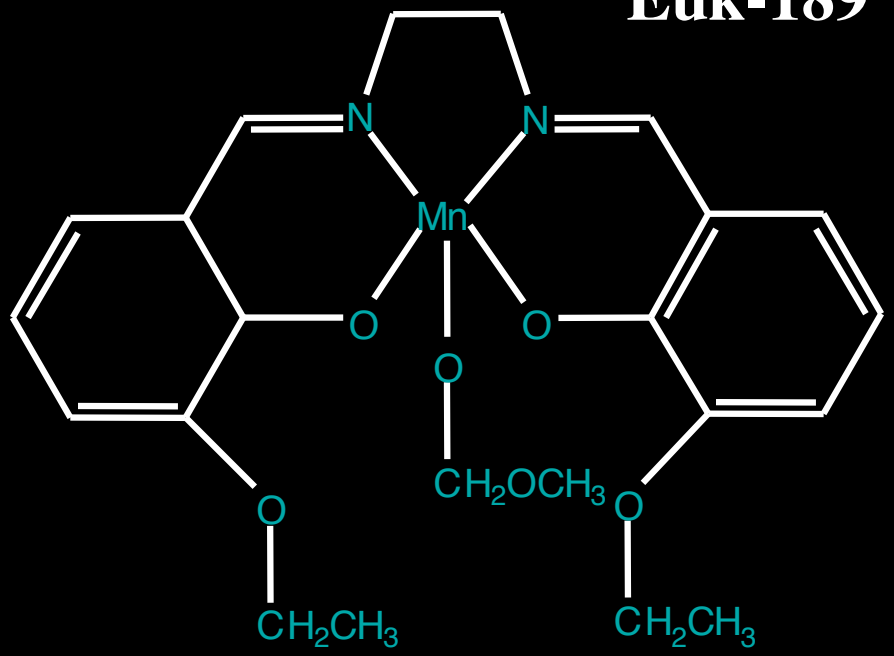
Oxidative stress is ubiquitous, and *may* be the single most significant category of cellular stress.

This is clearly something which can be therapeutically targeted

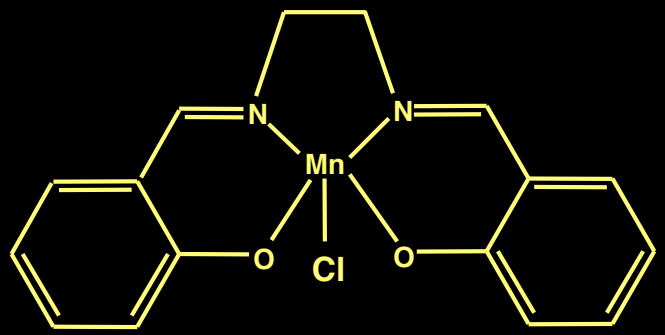
It's not a question of *whether*, but rather of *when*, *how*, and *how much*.

Catalytic antioxidants tested in *Sod2-/-* mice, and in aging paradigms

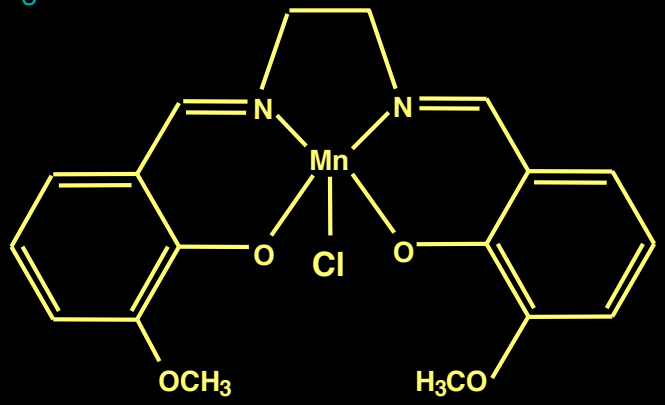
Euk-189

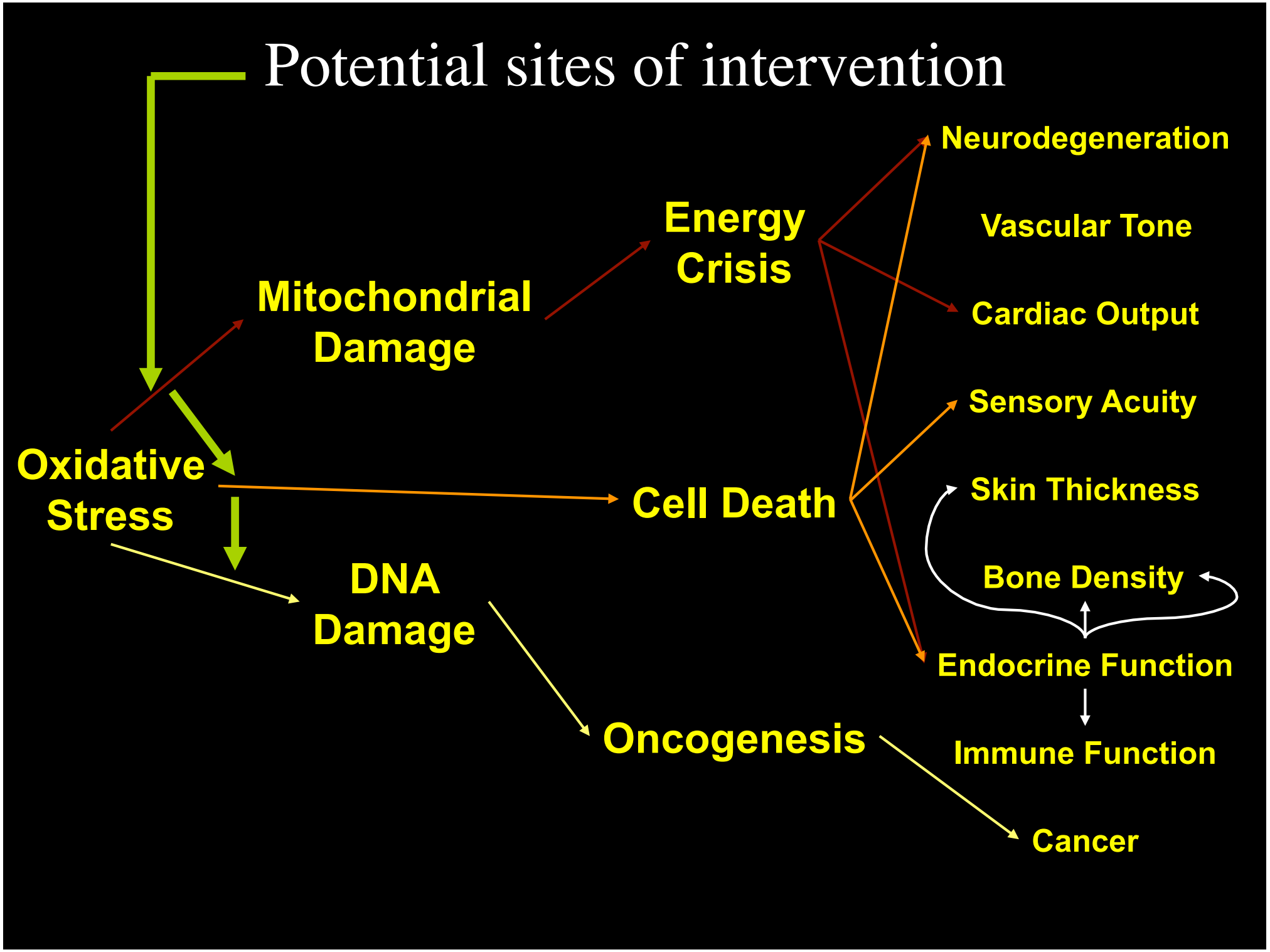


Euk-8



Euk-134




Potential sites of intervention
Neurodegeneration
Energy Crisis
Vascular Tone
Mitochondrial Damage
Cardiac Output
Sensory Acuity
Oxidative Stress
Cell Death
Skin Thickness
Bone Density
DNA Damage
Endocrine Function
Oncogenesis
Immune Function
Cancer