SFRBM 2010 and SFRR International XV:
Sunrise Free Radical School

From NO to Nitrite and Nitrate….and Back Again:
Role in Mammalian Biology

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Center for Free Radical Biology
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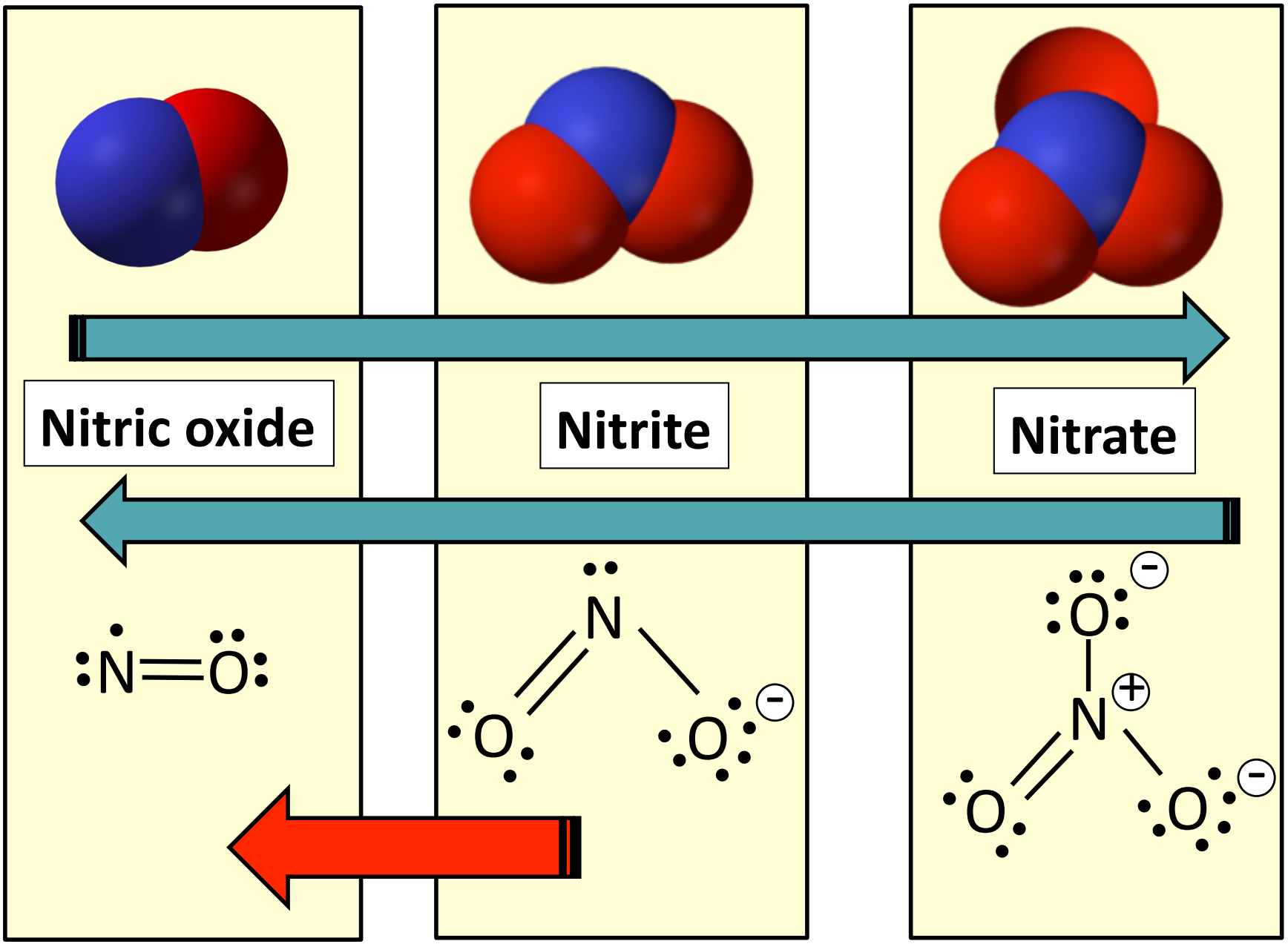
From NO to Nitrite and Nitrate….and Back Again:
Role in Mammalian Biology



Goals:

* Introduce redox reactions that modulate nitrite interconversion between different NO-species

* Put redox reactions of nitrite / nitrate in the context of:

    -physiologic / therapeutic mediator of mammalian NO-signaling at low pO_2

    - toxicity

Historical – Current perspectives on nitrite / nitrate biology

Nitrite and Nitrate….*The classical View to free radical biologists*

$$NO \longrightarrow NO_2^- \longrightarrow NO_3^-$$

i) NO_2^- / NO_3^- 'stable' oxidation products of aerobic NO metabolism

ii) NO_2^- / NO_3^- formation represent NO-detoxification pathway

iii) NO_2^- / NO_3^- formation used as experimental markers for NO-formation

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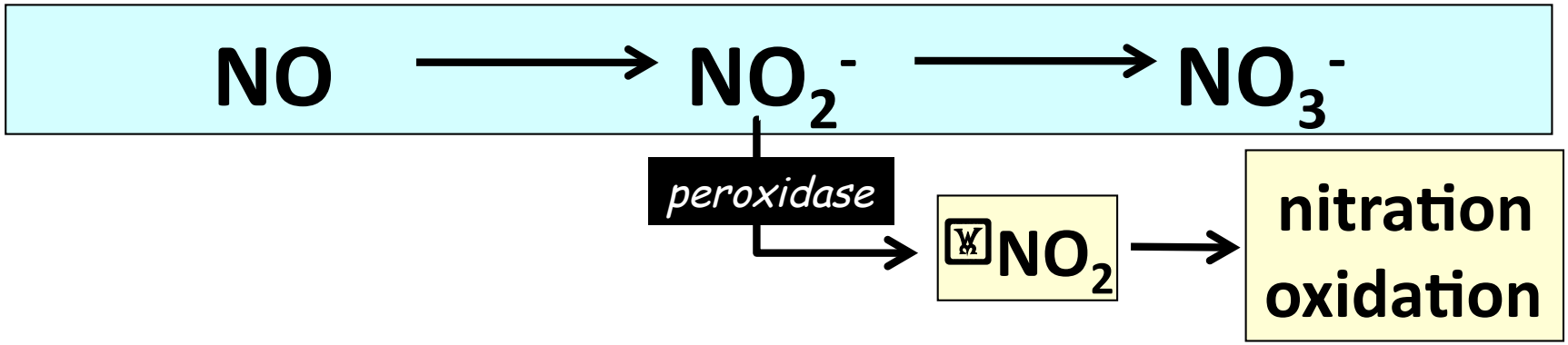

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iv) Pro-inflammatory potential for nitrite via $^{\bullet}NO_2$

Nitrite and Nitrate- inert end products of NO-metabolism

NO-Autoxidation:

a) $2NO + O_2 \longrightarrow 2NO_2$

b) $NO_2 + NO \longrightarrow N_2O_3 \xrightarrow{H_2O} \mathbf{2NO_2^-} + 2H^+$

c) $2NO_2 \longrightarrow N_2O_4 \xrightarrow{H_2O} \mathbf{NO_2^-} + \mathbf{NO_3^-} + 2H^+$

NO-oxidation:

a) $NO + Hb\text{-}Fe^{2+}O_2 \longrightarrow Hb\text{-}Fe^{3+} + \mathbf{NO_3^-}$

b) $NO + Cp\text{-}Cu^{2+} \longrightarrow Cp\text{-}Cu^{+} + \mathbf{NO_2^-}$

Diet
(green leafy vegetables, beets, cured meats

$\mathbf{NO_2^-}$ $\mathbf{NO_3^-}$

NO_2^- oxidation:

a) $NO_2^- + Hb\text{-}Fe^{2+}O_2 \longrightarrow Hb\text{-}Fe^{3+} + H_2O_2 + \mathbf{NO_3^-}$

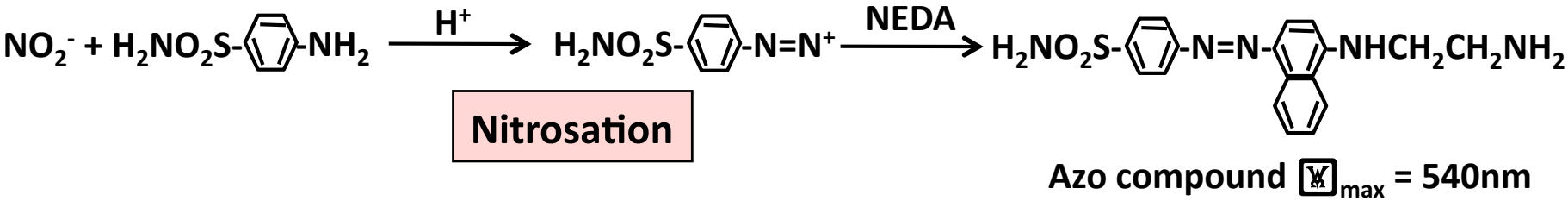
Wink et al (1994) Chem Res Tox 7(4):519
Liu et al (1998) PNAS 95(5): 2175-9
Keszler et al (2008) JBC; 283:9615
Olson et al (2004) FRBM 36(6):685
Cooper (1999) BBA 1411; 290
Shiva et al (2006) Nat Chem Biol (9):486
Carlstron et al (2010) PNAS In Press
Hord et al (2009) Am J Clin Nutr 90; 1-10

Nitrite / Nitrate- Biomarker for endogenous NO-production ….*The classical View*

General Concept:

Nitrite and Nitrate- biological inert - dead end re: mammalian NO metabolism

-1878: Griess showed low nitrite in saliva but high nitrite in urine of volunteer who had a fever- first indication of increased NOx during infection



- 1914: Cruickshank and Moyes showed urinary nitrite derived from bacterial reduction of nitrate…. UTI dipstick test

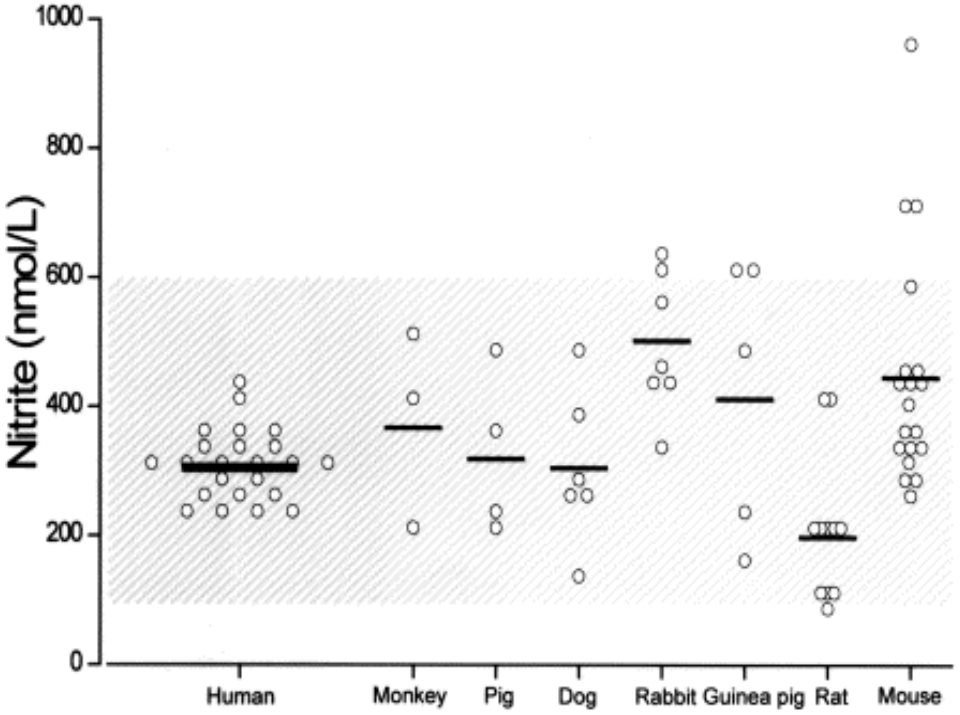
-1916: Mitchell et al. The origin of nitrates in urine JBC 24, 461

-1980s: Tannenbaum et al: metabolic studies confirming biological NO production

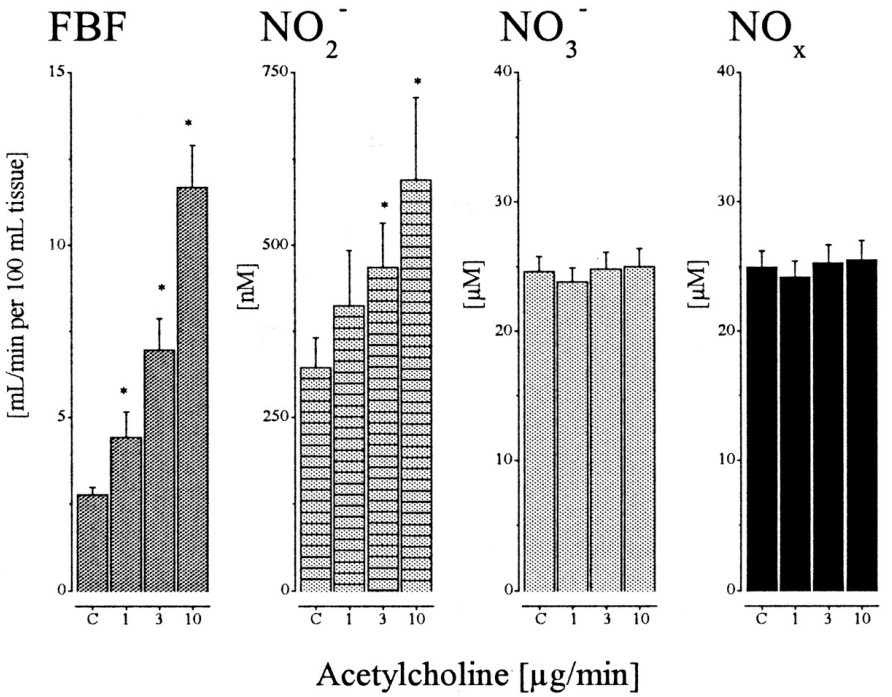
-2001 : Kelm and Feelisch et al nitrite (not nitrate) marker for eNOS activity in vivo

Nitrite – Biomarker for eNOS activity in mammals

eNOS → NO → Nitrite



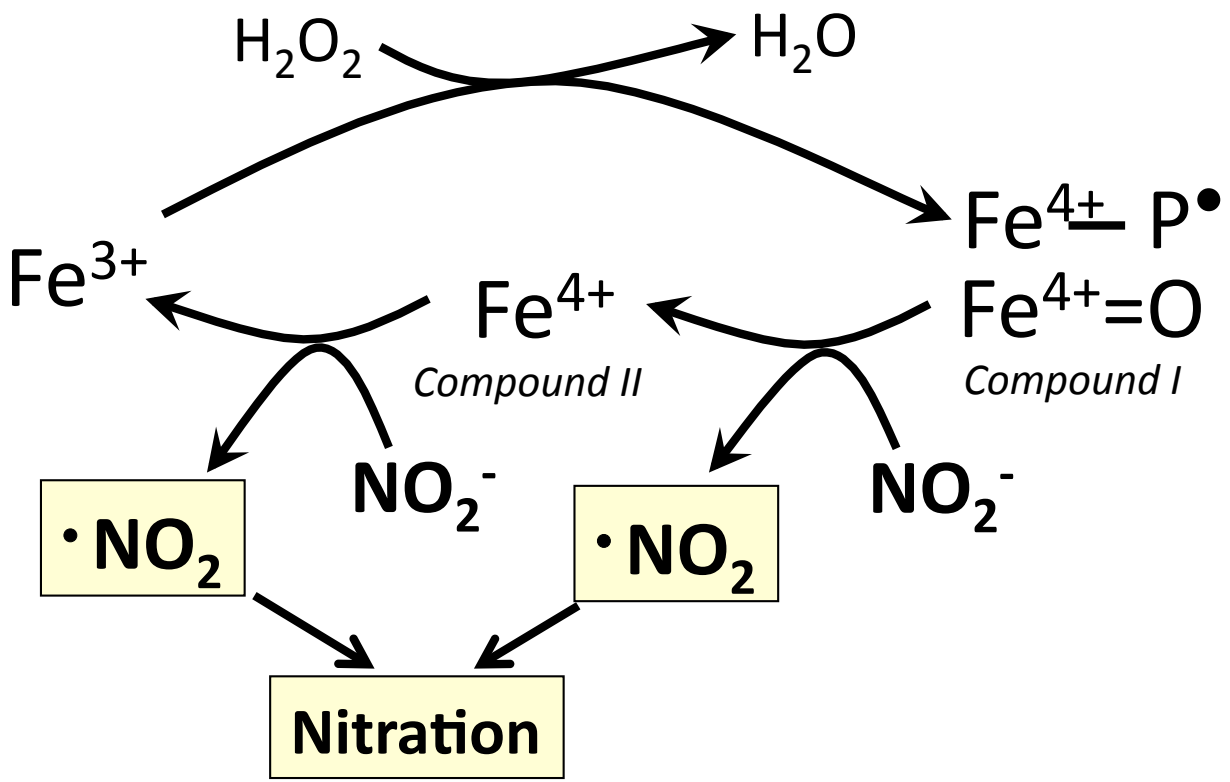
Kleinbongard et al 2004 FRBM



Lauer, Thomas et al. (2001) Proc. Natl. Acad. Sci. USA 98, 12814-12819

Nitrite oxidation and formation of nitrogen dioxide radical: *a biological mechanism for nitration*

Peroxidase-dependent nitrite oxidation:



Peroxidase:

-myeloperoxidase
-eosinophil peroxidase
-horse radish peroxidase
-pseudoperoxidases

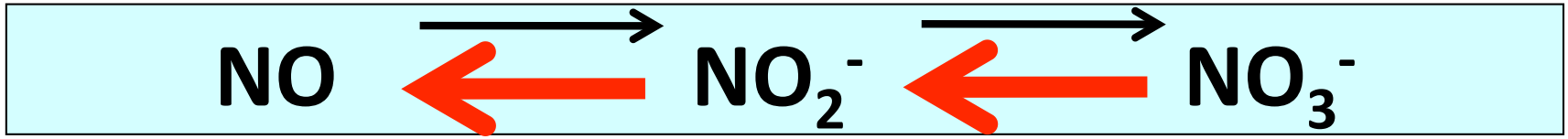
CV disease
Lung disease
Etc…

Van der Vliet (1997) JBC 272, 7617
Eiserich et al (1998) Nature 391: 393
Castro et al (2004) ABB; 421:99
Podrez et al (1999) JCI 103; 1547
Wu et al (1999) JBC 274; 25933
Hazen et al (1999) Circ Res 85:950
Brennan et al (2002) JBC 277: 17415
Sampson (1998) ABB 356; 207

From NO to Nitrite and Nitrate….and Back Again:

Redox perspectives for an evolving view

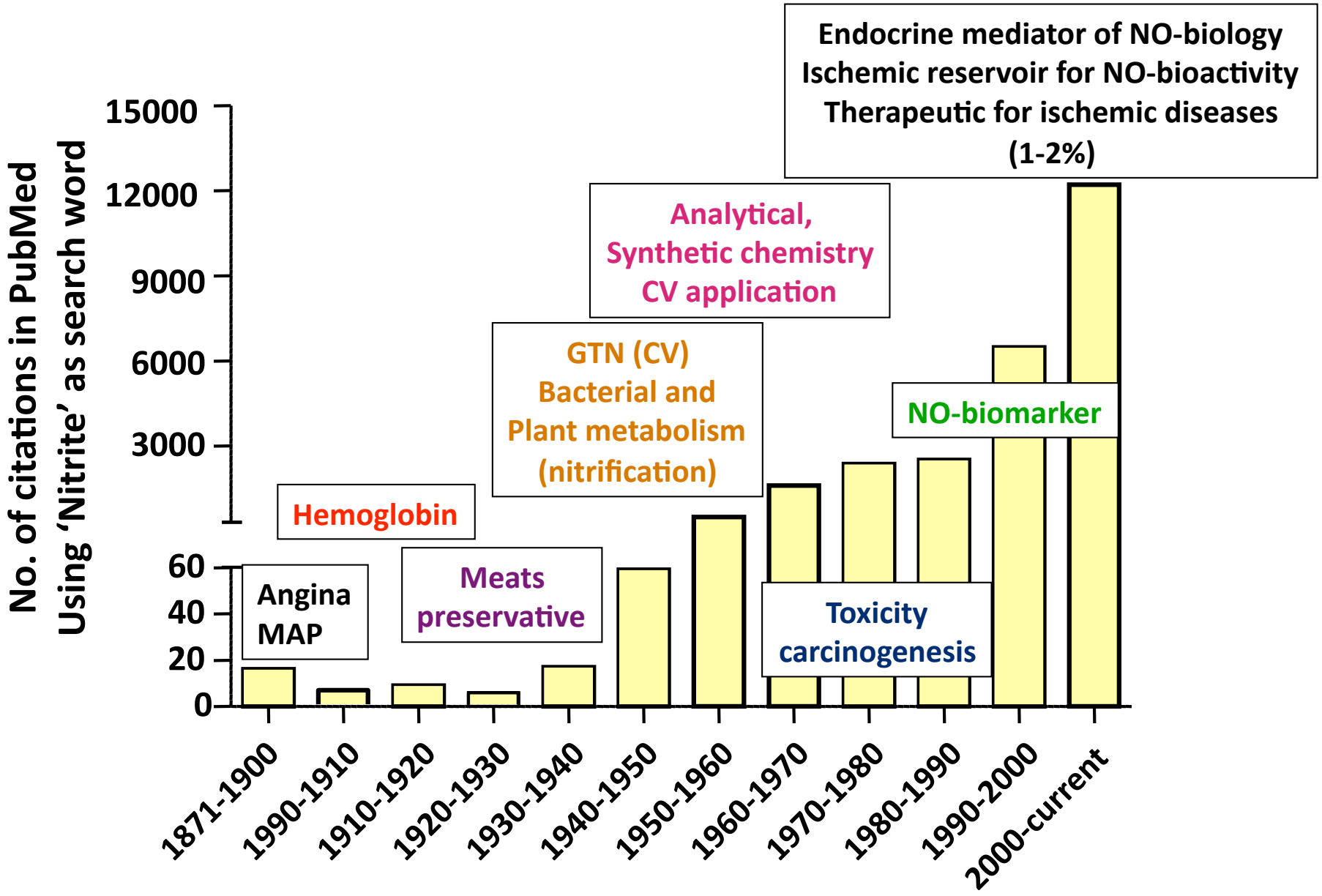
Concept:
Nitrite / Nitrate as a physiologic source of bioactive NO



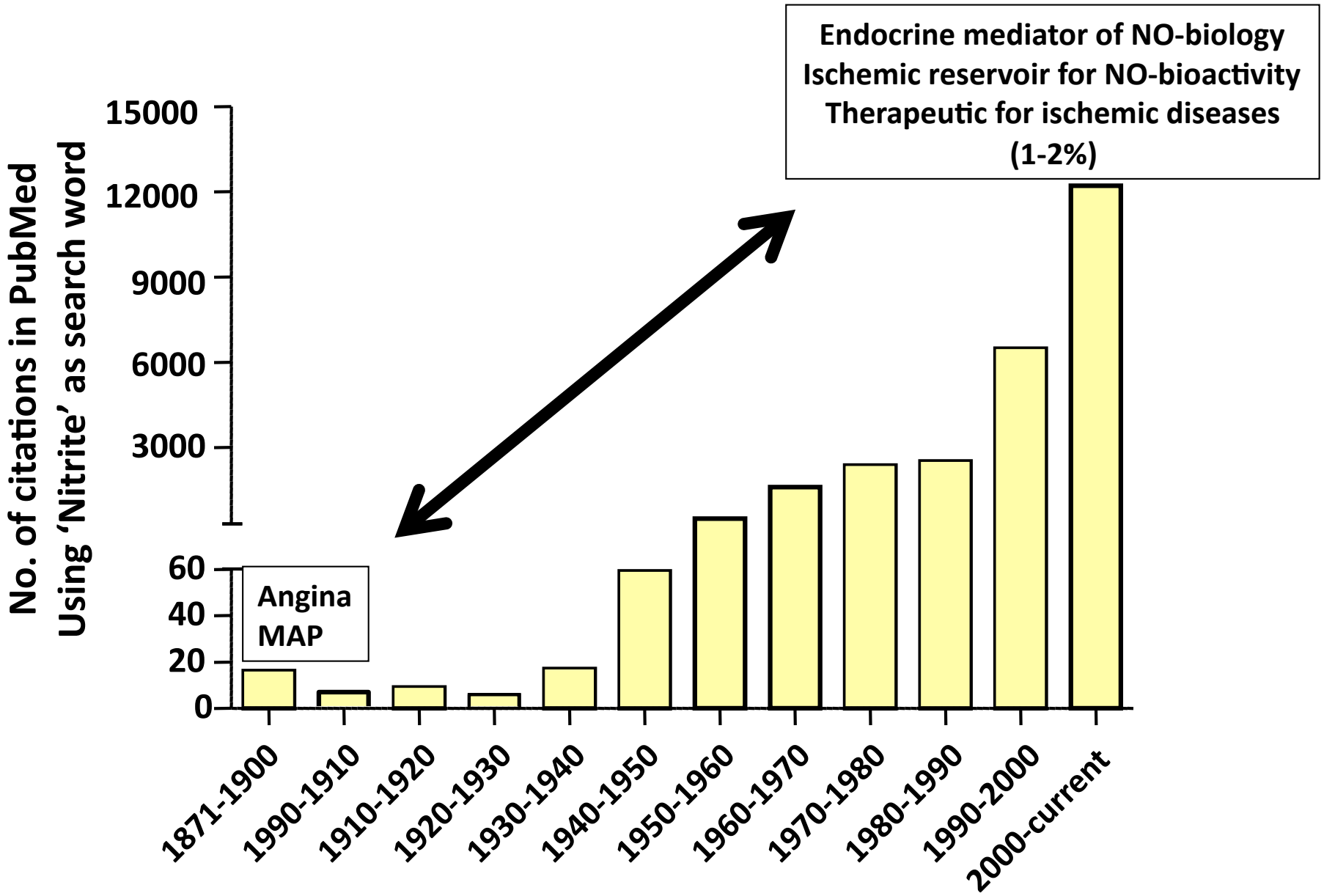
i) NO_2^- / NO_3^- 'biological' storage depots for NO formation during hypoxia at physiologic (non stomach acid) pH

ii) NO_2^- / NO_3^- 'therapeutics' for repleting NO bioactivity in ischemic tissues

Sep 2010- Literature (PubMed) search ...'Nitrite' – 25759 citations



Question: Why has it taken a ¥ 100yr for the potential for nitrite as a biological / therapeutic source of NO to be realized?

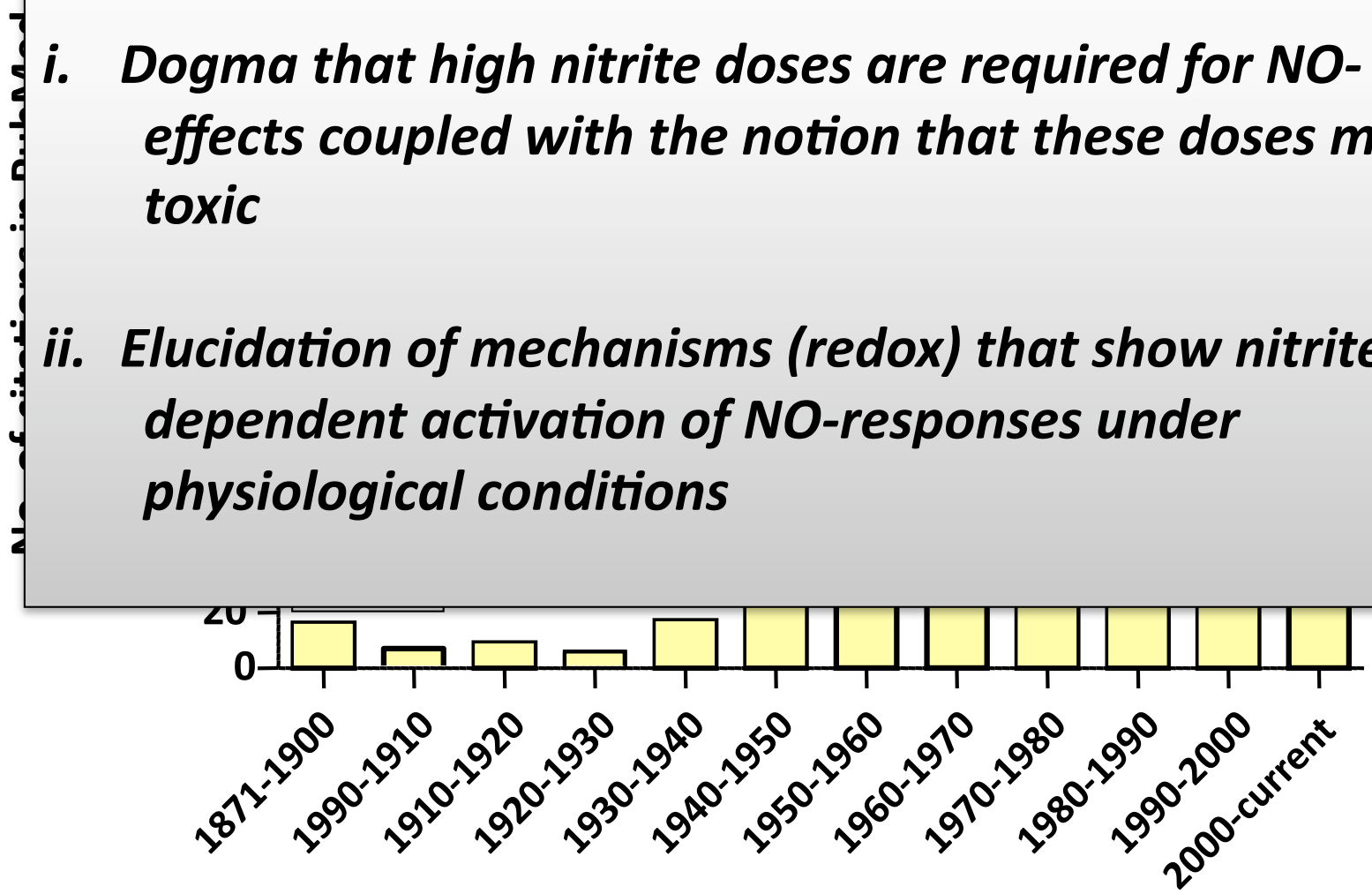


Question: Why has it taken a ¥ 100yr for the potential for nitrite as a biological / therapeutic source of NO to be realized?

Endocrine mediator of NO-biology
Ischemic reservoir for NO-bioactivity

i. Dogma that high nitrite doses are required for NO-effects coupled with the notion that these doses may be toxic

ii. Elucidation of mechanisms (redox) that show nitrite-dependent activation of NO-responses under physiological conditions

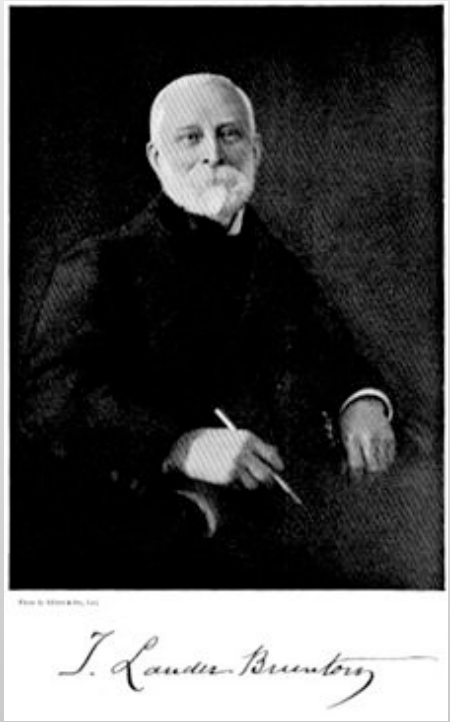


The birth of nitrite biology and therapeutics

Linked to organic nitrite (amyl nitrite) and organic nitrate therapy (GTN)

Angina Pectoris (Chest pain or discomfort due to CHD resulting in ischemia):

1867: Thomas Lauder Brunton, house surgeon at the Edinburgh Royal Infirmary found he could alleviate symptoms by cupping or by venesection… concluding that pain was due to elevated blood pressure.



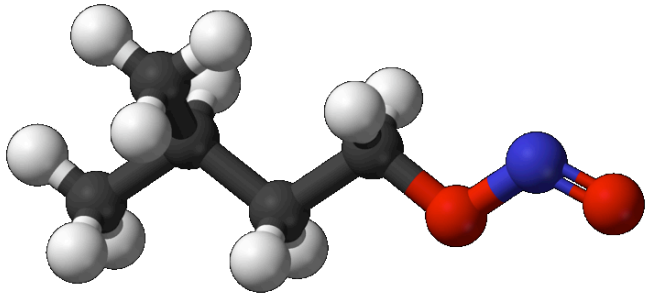
-Venesection not convenient method

-Bruntons colleague Arthur Gamgee had preliminary data showing inhaled amyl nitrite lowered blood pressure

-Brunton impregnated cloth with amyl nitrite and asked patient to inhale…within seconds chest pain was relieved and lasted several minutes (Brunton, Lancet 1867).

The birth of nitrite biology and therapeutics

Amyl Nitrite



Victorian Vasodilator

-**Success of amyl nitrite led to testing of many nitrite / nitrates**

-**Glyceryl trinitrate (GTN, nitroglycerin)- Nobel fame, explosive, tolerance**

-despite concerns, successful, less volatile than amyl nitrite and now standard drug applied transdermally or inhaled- ***although how it works still not clear.***

-Inorganic nitrite therapy- vasodilator action in the *19th century*

- 1880 Reichert and Mitchell - On the physiological action of potassium nitrite
***Amer J Med Sci* 154, 158-80 (Univ of Pennsylvania)**

-tested effects of ingested potassium nitrite on nervous system, brain, spinal cord, respiration, blood pressure

-key findings reproduced by others 1885-1888

-General conclusion:

similar effects to amyl nitrite- but slower onset and effects lasted longer- MECHANISMS NOT KNOWN

1905 National Standard Dispensatory (dictionary for pharmaceutical information)

" potassium nitrite exerts the same influences upon the human organism as do glyceryl trinitrate and amyl nitrite. On account of the slowness of its absorption and elimination its effect is more prolonged. It is given in the dose of 1 to 3 grains"

1-3 grains approx… 500µM for 5L blood (current targets 5-10µM)

-Inorganic nitrite therapy- vasodilator action in the *mid-20th century*

REACTIONS OF STRIPS OF RABBIT AORTA TO EPINEPHRINE, ISOPROPYLARTERENOL, SODIUM NITRITE AND OTHER DRUGS[1]

ROBERT F. FURCHGOTT AND SUCHIN BHADRAKOM[2]

Department of Pharmacology, Washington University School of Medicine, St. Louis, Mo.

Received for publication December 29, 1952

The *in vitro* responses of rings, strips or segments of arteries to various drugs and physical changes have been the subject of numerous investigations since the pioneer work of O. B. Meyer (1905). In most cases the source of arteries has been slaughterhouse animals. However, in the course of studies in this laboratory on the relation between metabolism and contraction of smooth muscles it was found that spirally cut strips of rabbit thoracic aorta were admirably suited for both qualitative and quantitative investigations of the effects of drugs on arterial smooth muscle. The present paper is concerned primarily with the response of such strips to various drugs, and also with their general characteristics as pharmacological test objects.

Nitrite dependent vasodilation of isolated vessels

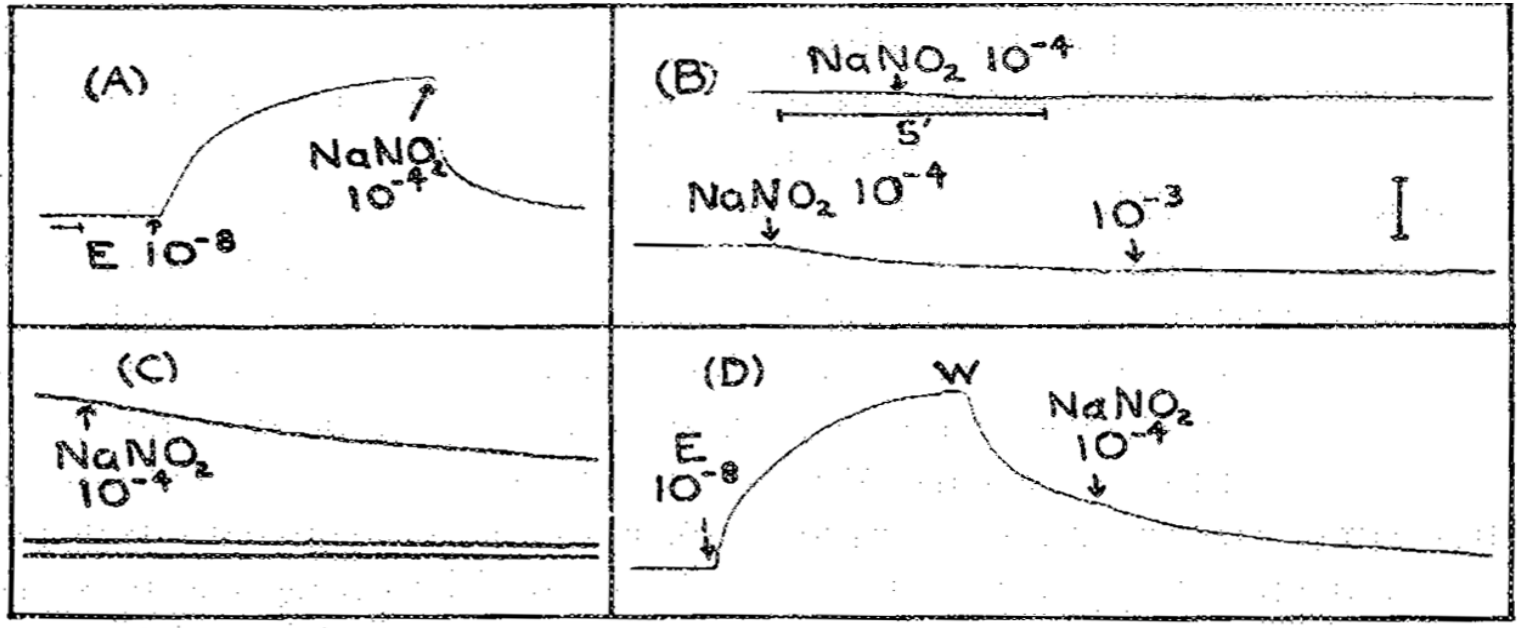


FIG. 6. Responses to sodium nitrite. Record A was obtained with strip approximately 3 cm. in length (under tension of lever), while all other records were obtained with strips approximately 6 cm. in length. Vertical bar in record B is 1 cm. on original record. A. Relaxation by $NaNO_2$ of strip initially brought to a state of moderate tone with epinephrine. B. Effect of $NaNO_2$ on fully stretched strips. Extra elongation after $NaNO_2$ in lower tracing was greatest obtained in present experiments. C. Effect of $NaNO_2$ added to strip undergoing gradual elongation five minutes after attachment to weighted lever. Two lower curves are from same record one hour and two hours later. D. Effect of $NaNO_2$ added at beginning of gradual phase of relaxation following initial rapid phase of relaxation on washout of stimulating dose of epinephrine.

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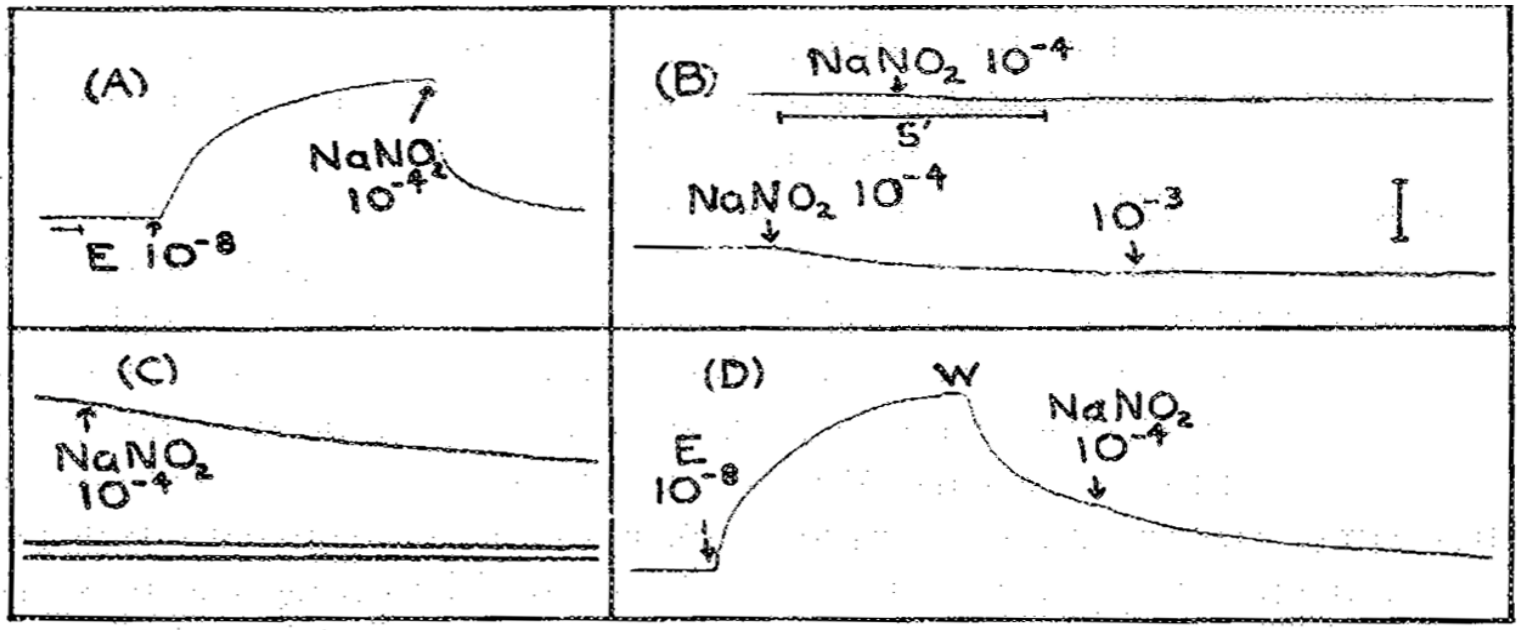

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-High dose
-Normoxia, hyperoxia

BUT- despite promise inorganic nitrite never made it as a front-line therapy for Angina + CV disorders…. *Why not?*

Nitrite- toxicity (1) :

-methemoglobinemia (Haldane et al The actions of poisons of nitrite and other physiologically related substances. J Physiol 1897, 21)

Methemoglobin formation:

a) NO_2^- + Hb-$Fe^{2+}O_2$ ⟶ **Hb-Fe^{3+}** + NO_3^- *(unbalanced)*

Keszler et al JBC 2008

Doses >> 10µM required for significant methemoglobinemia

Nitrite- toxicity (2):

+

→

SFRBM Hospitality Suite

→

High dose Nitrite

→

-Nausea
-Belching
-stomachache
-Diarrhea

+

→

Nitrite- toxicity (2): high dose and nitrate contamination

Nitrite- adverse effects

- **Nausea**
- **Belching**
- **stomachache**
- **Diarrhea**

Blumgarten, 1934

- **WT Law (private practice doctor) used nitrite to treat epilepsy. But he needed high doses (20 grains)- he said gave no adverse effect *(Law 1882, sodium nitrite in the treatment of epilepsy.* Practitioner 28, 420*)***

BUT- when other tried to reproduce patients suffered headaches, dizziness, nausea

REASON: Law's nitrite contaminated with nitrate. This however caused irrevocable damage to inorganic nitrites therapeutic reputation

Physiologic and Therapeutic potential for nitrite as a mediator of NO-signaling: *21st century perspective.*

Over the last 20 years nitrite reduction to NO and modulation of NO-signaling has been demonstrated to occur at low nitrite concentrations:

- ***acidic pH***
- ***Anoxia***
- ***graded hypoxia, neutral pH***

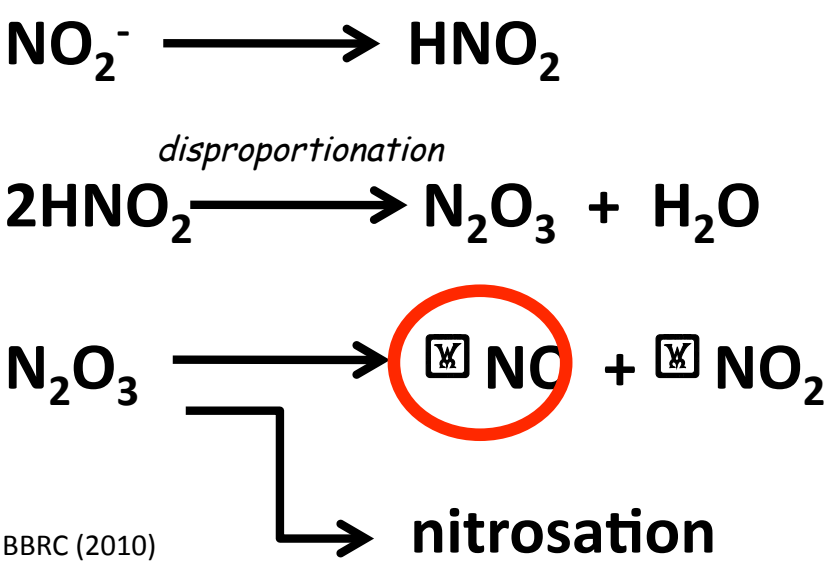
→ **Activate redox mechanisms that reduce nitrite to NO and/or nitrosating species**

Acidified Nitrite dependent NO-formation:

1994: Benjamin et al Stomach NO synthesis *Nature 368, 502*

1994: Lundberg et al Intragastric nitric oxide production in humans: measurements in expelled air. *Gut 35, 1543*

Acidification of nitrite:



pKa = 3.4
At pH 7, 0.025%
and pH 6, 1.7% of nitrite is in HNO_2 form

↓

Compartmentalized to acidic environments

Lundberg et al BBRC (2010)
Lundberg et al Nat Chem Biol (2009)
Lundberg et al Nat Rev Drug Discov (2008)
Lundberg et al Nat Rev Microbiol (2004)
Modin et al Acta Phys Scan (2000)

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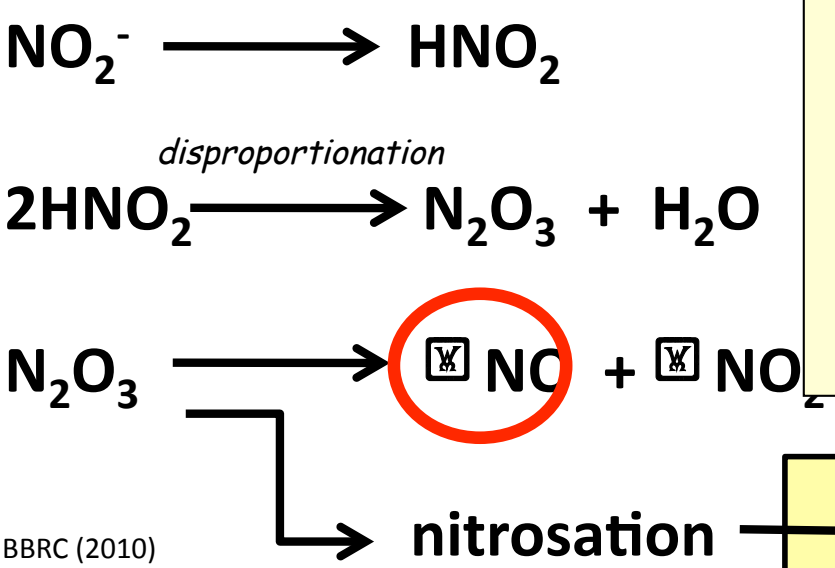

Acidified nitrite:

-Anti-microbial effects in GI tract

-Anti-microbial effects on skin

-Gastric mucosal blood flow and thickness

-*Basis of food preservation*

Basis of carcinogenic effects of nitrite

Lundberg et al BBRC (2010)
Lundberg et al Nat Chem Biol (2009)
Lundberg et al Nat Rev Drug Discov (2008)
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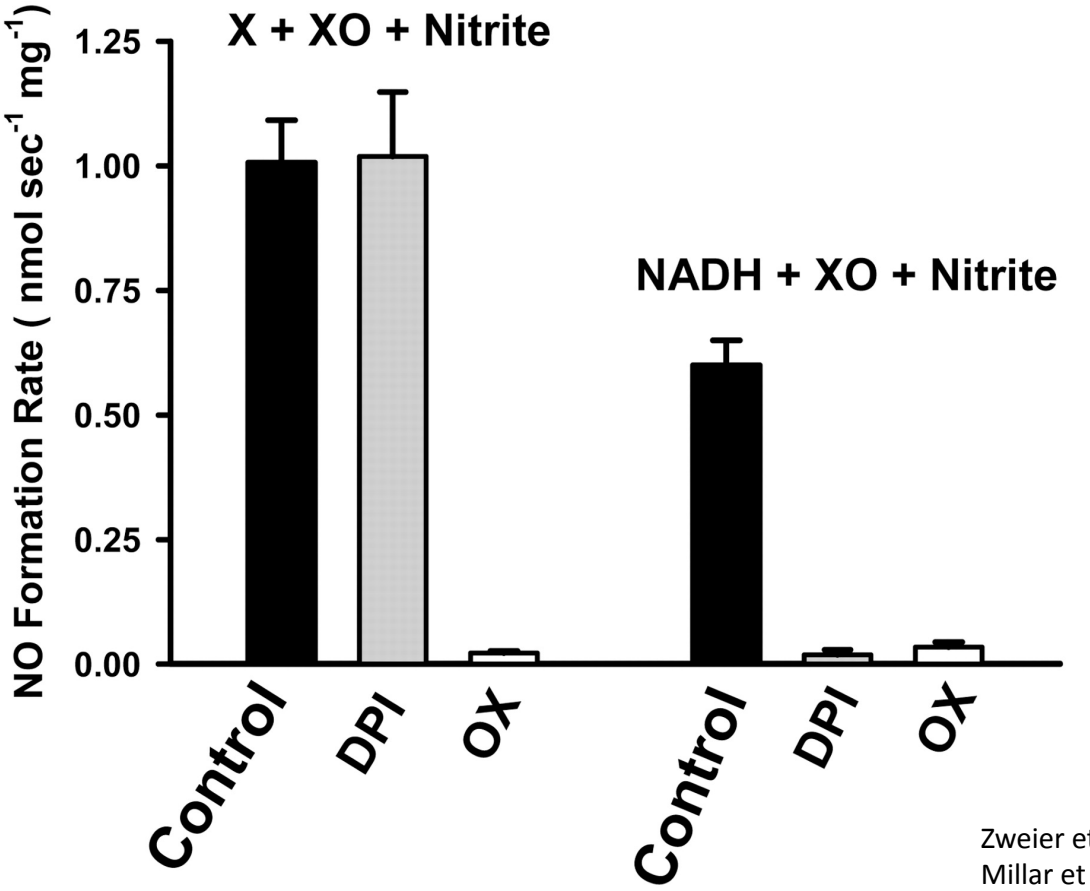
Nitrite-dependent NO-formation during anoxia

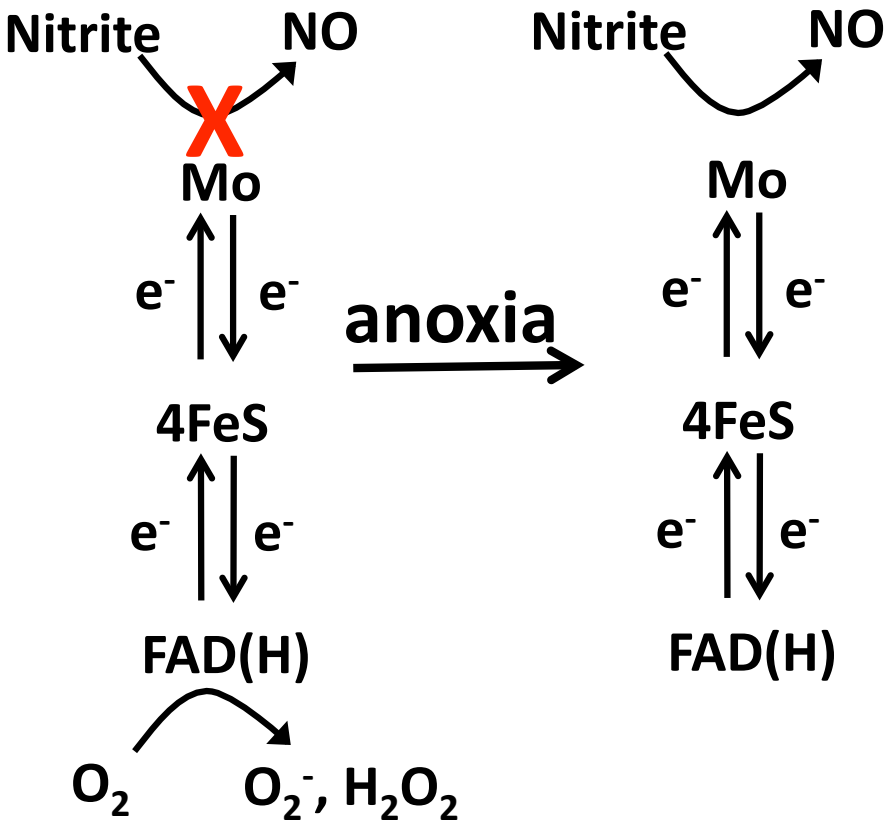
Zweier et al (1995) Enzyme independent formation of nitric oxide in biological tissues. Nat Med 8, 804



Li H et al. J. Biol. Chem. 2001;276:24482-24489

Zweier et al Nitric Oxide (2010)
Millar et al Redox Rep (2002)
Lundberg et al Nat Chem Biol (2009)
Webb et al (2008) Circ Res
Webb et al (2004) PNAS
Calvert et al Nitric oxide (2010)

Anoxic regulation of nitrite reduction to NO
- example of XOR



Molybdenum enzymes (XOR, ALDH)

↓

Anoxic generation of NO from nitrite

↓

Role in physiologic mechanisms for NOS independent NO formation unclear

↓

Current thoughts- protection against ischemia-reperfusion (Tx-related) injury in lungs, heart, kidney….

Adapted from Li H et al. J. Biol. Chem. 2004;279:16939-16946

Physiologic and Therapeutic potential for nitrite as a mediator of NO-signaling: *21st century perspective.*

Over the last 20 years nitrite reduction to NO and modulation of NO-signaling has been demonstrated to occur at low nitrite concentrations:

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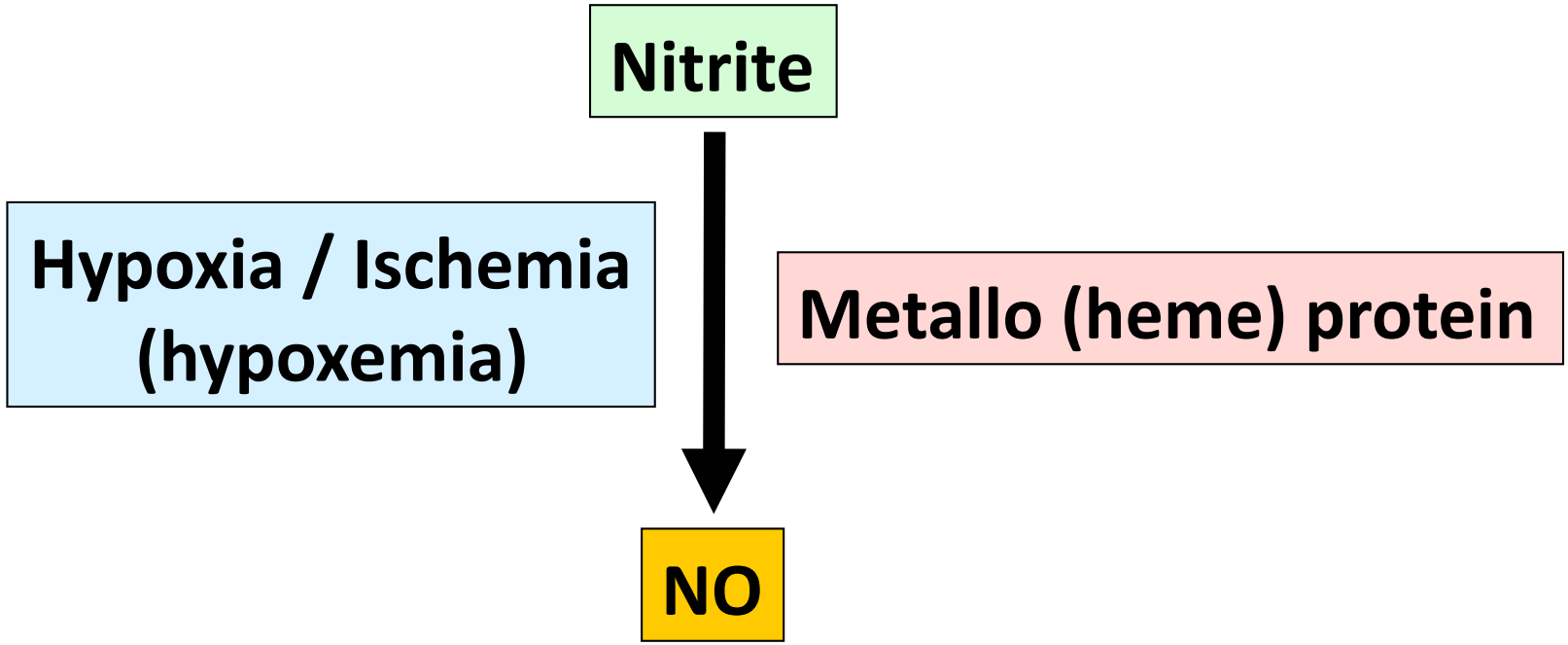
Nitrite-dependent NO formation – 3 component model



-Graded pO_2, NO-formation increases at low pO_2 where NOS dependent NO formation decreases
-Does not require low pH
-Operational in neutral pH range
-Operational at biological (nM) nitrite concentrations

Hypoxemic regulation of nitrite reduction to NO
- example of Hemoglobin

Oxyhemoglobin:

$$4HbO_2 + 4NO_2^- \xrightarrow{4H^+} 4Hb^{3+} + 4NO_3^- + O_2 + 2H_2O$$

Nitrite Oxidation

Deoxyhemoglobin:

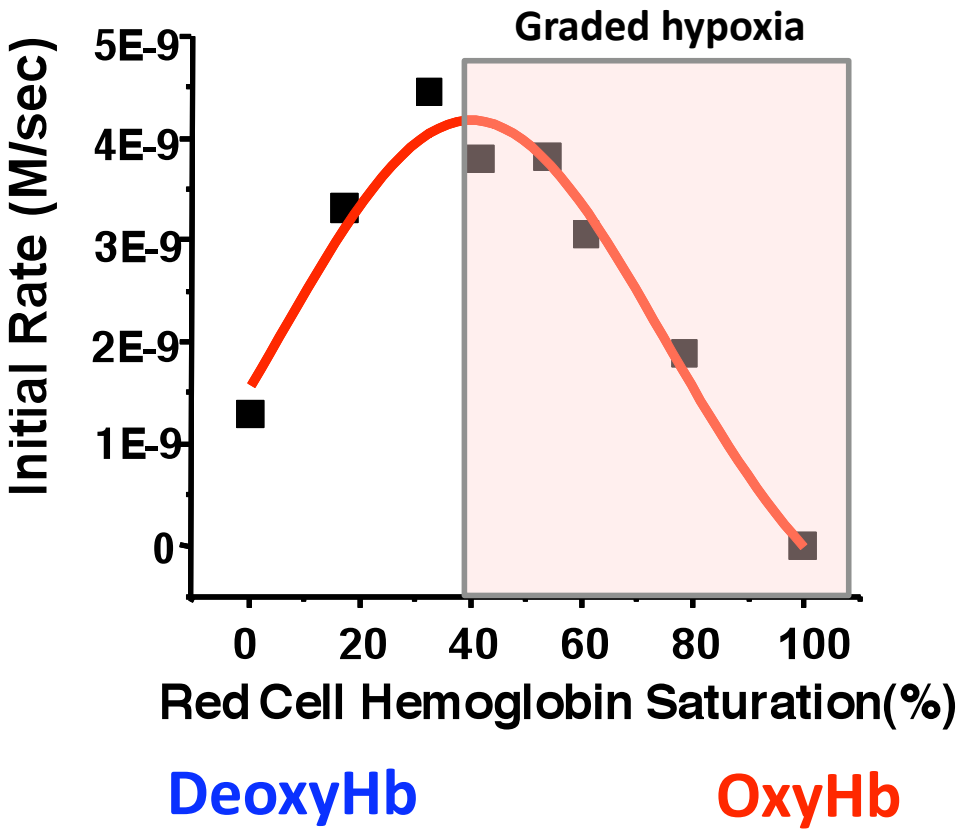
$$Hb^{2+} + NO_2^- + H^+ \longrightarrow Hb^{3+} + NO + OH^-$$

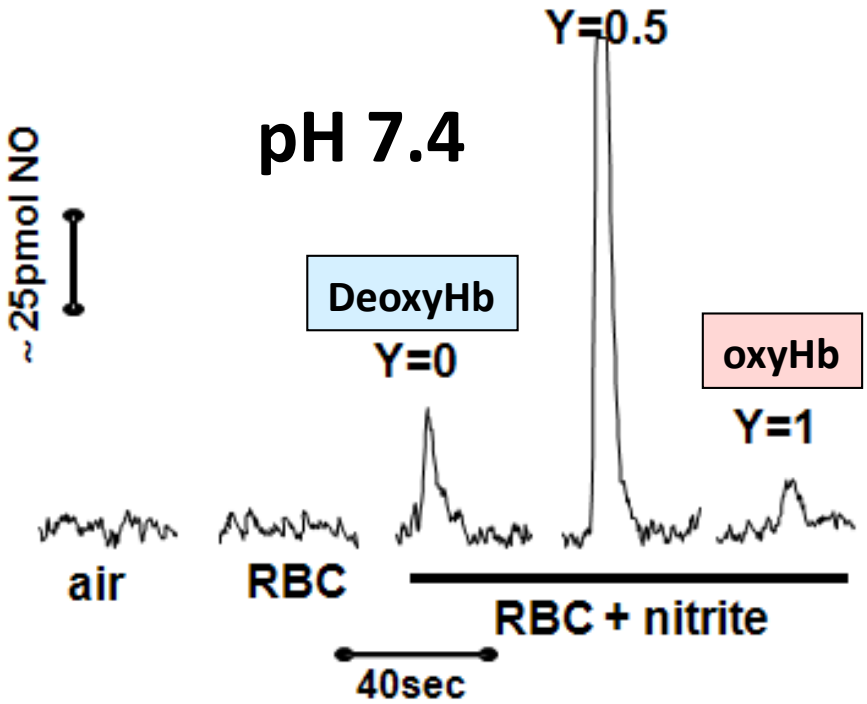
Nitrite Reduction

Kosaka et al BBA (1979)
Brooks (1937) Proc R. Soc Biol Sci
Doyle et al (1981) JBC
Huang et al (2005) JCI
Huang et al (2005) JBC
Keszler et al (2008) JBC
Nagababu et al (2004) JBC
Cosby et al (2004) Nat Med

Graded Hypoxic regulation of nitrite reduction to NO

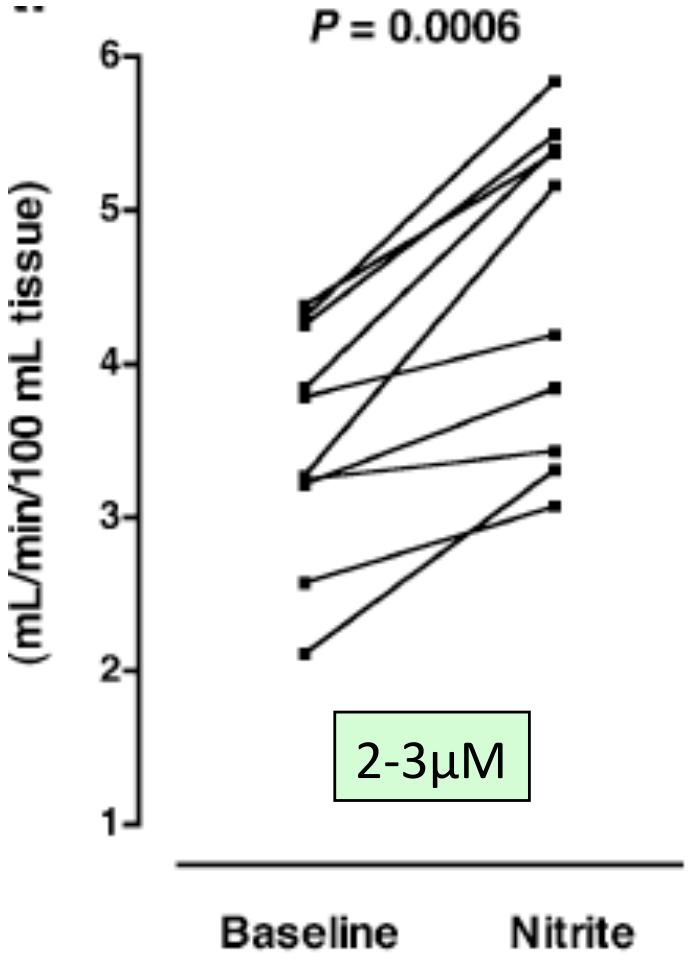
- example of Hemoglobin



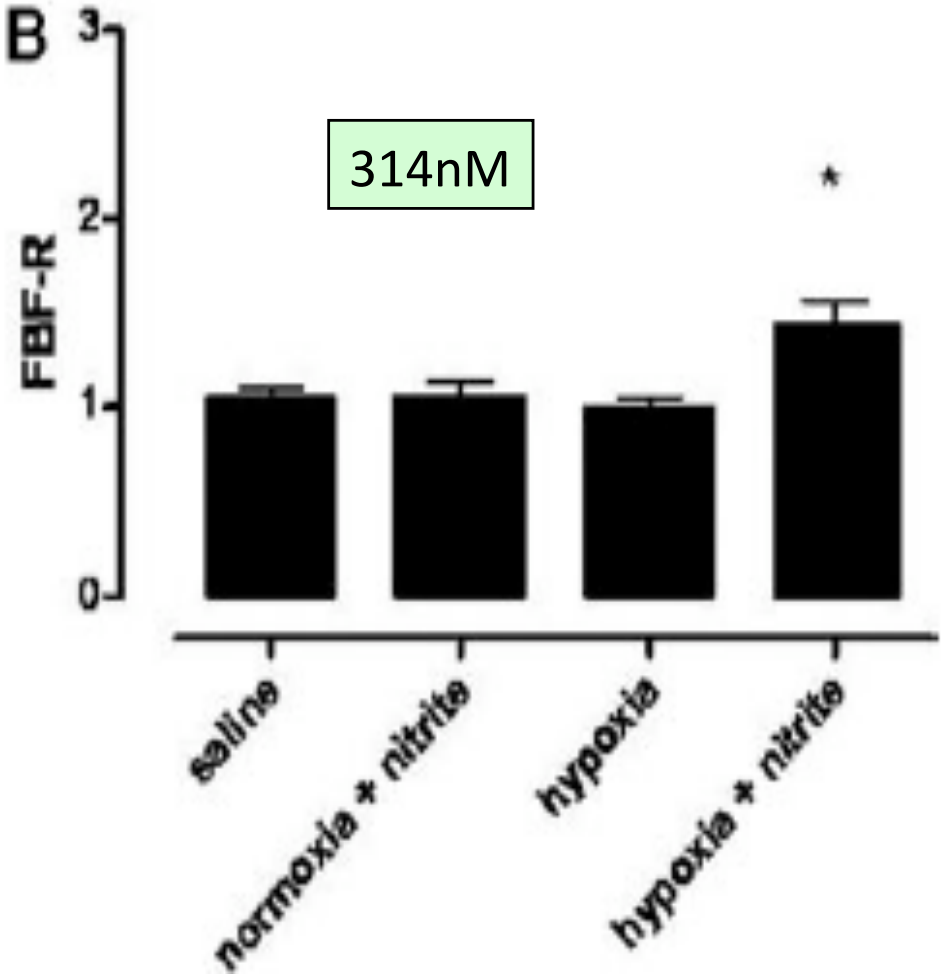


Huang et al (2005) JCI
Huang et al (2005) JBC
Crawford et al (2006) Blood
Cosby et al (2004) Nat Med

Physiologic potential: Human Blood Flow



Cosby et al Nat Med (2003)



Maher et al Circulation (2008)

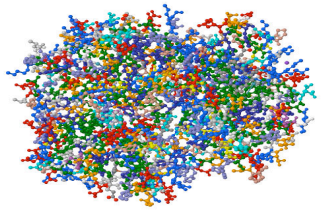
Nitrite-dependent NO formation – 3 component model

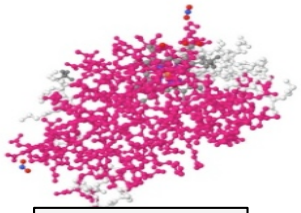
Nitrite

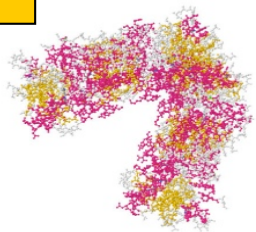
Hypoxia / Ischemia (hypoxemia)

Metallo (heme) protein

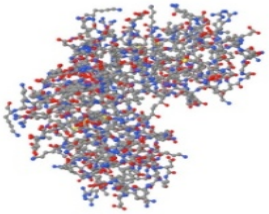
NO

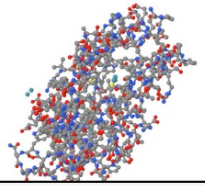

hemoglobin

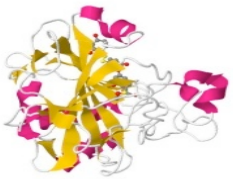

myoglobin


Xanthine oxidoreductase

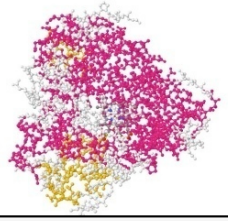

eNOS


Cytochrome c


neuroglobin

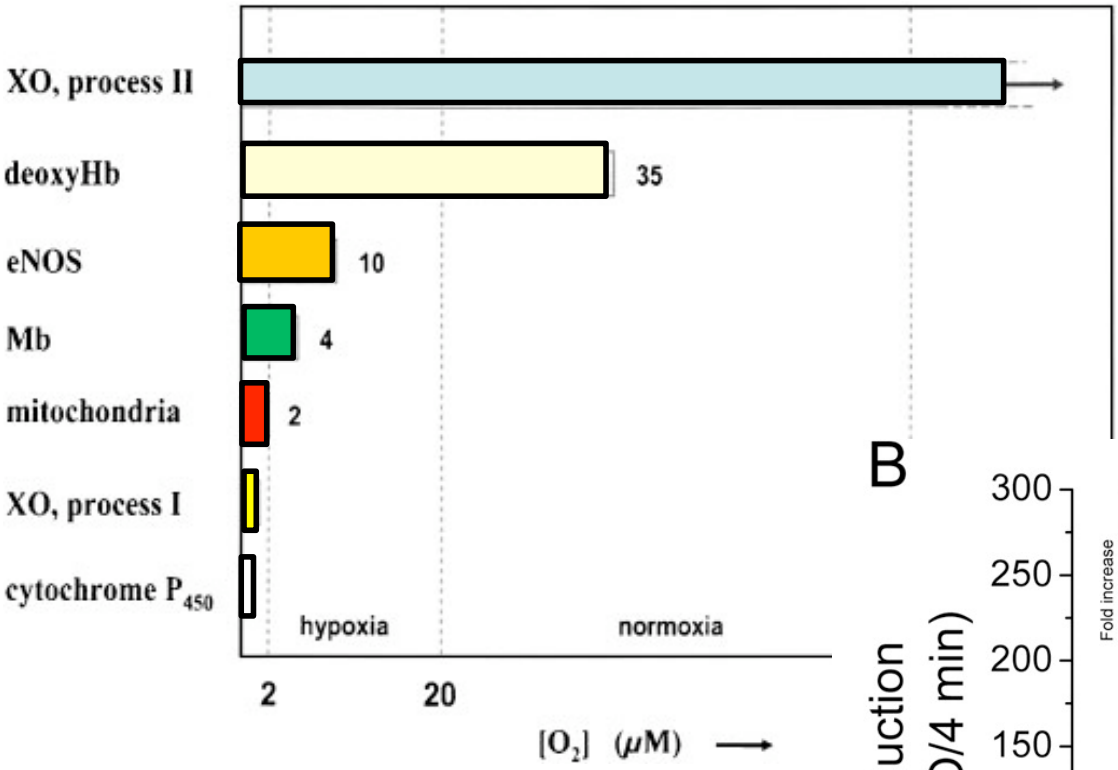

Carbonic anhydrase


Aldehyde oxidase


Cyclooxygenase


Cytochrome c oxidase

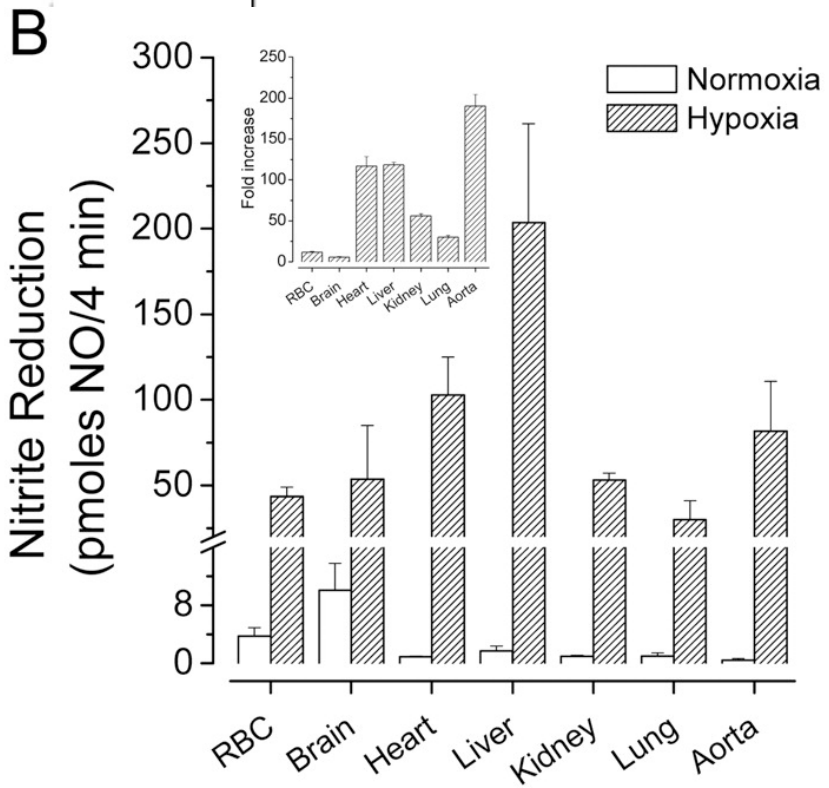
Potential regulatory mechanisms for nitrite–reduction



Van Faasen et al Med Res Rev (2009)

pO$_2$ dependent:

Tissue specific:



Feelisch M et al. J. Biol. Chem. 2008;283:33927-33934

Potential mechanisms of nitrite actions: source of S-nitrosothiols

Nitrite

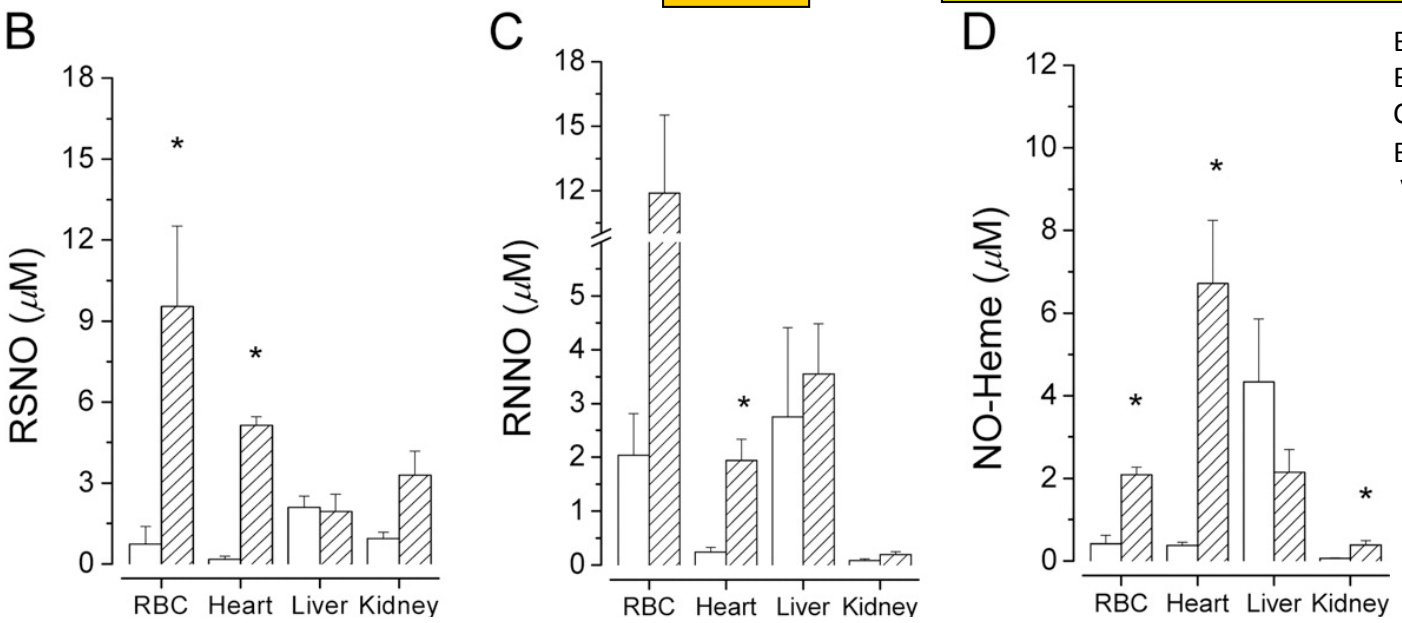
Hypoxia / Ischemia (hypoxemia)

Metallo (heme) protein

N_2O_3

NO

Nitroso adducts



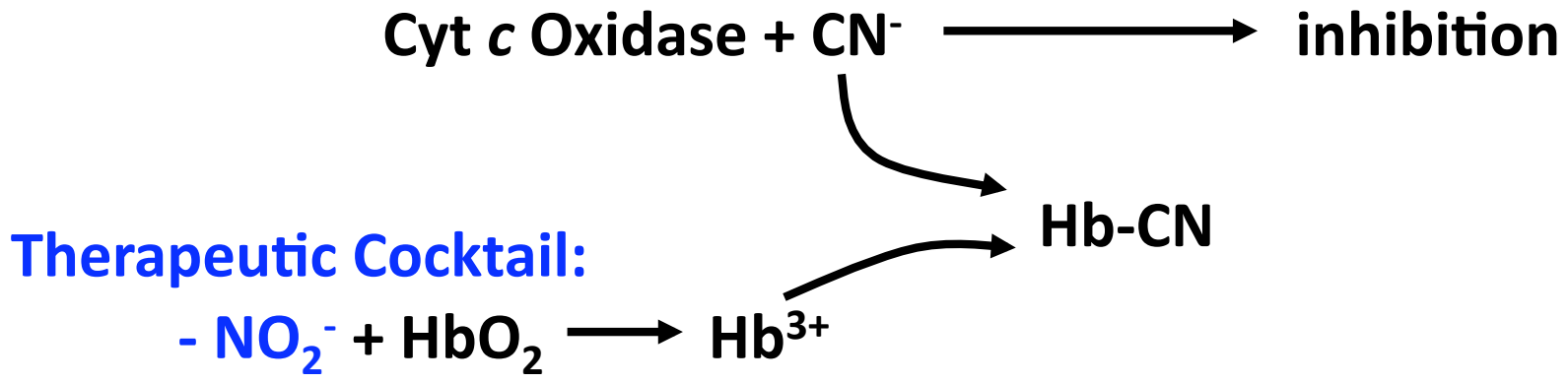
Bryan et al Nat Chem Biol (2005)
Bryan et al PNAS (2004)
Crawford et al Blood (2004)
Basu et al Nat Chem Biol (2007)
Van Faasen et al Med Res Rev (2009)

Feelisch M et al. J. Biol. Chem`. 2008;283:33927-33934

Nitrite Therapeutics:

Currently approved Nitrite based therapy- high dose:

1934: Antidote for cyanide poisoning:



-current practice to use either inhaled amyl nitrite or iv 3%$NaNO_2$- + 50% $Na_2S_2O_3$

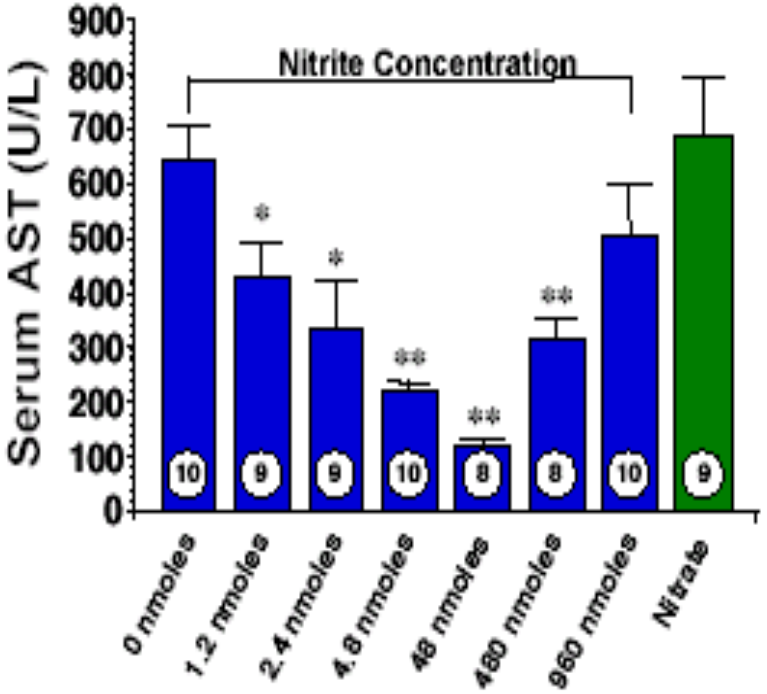
- Similar therapy for H_2S poisoning

high µM- mM nitrite

Future perspectives of nitrite Therapeutics:

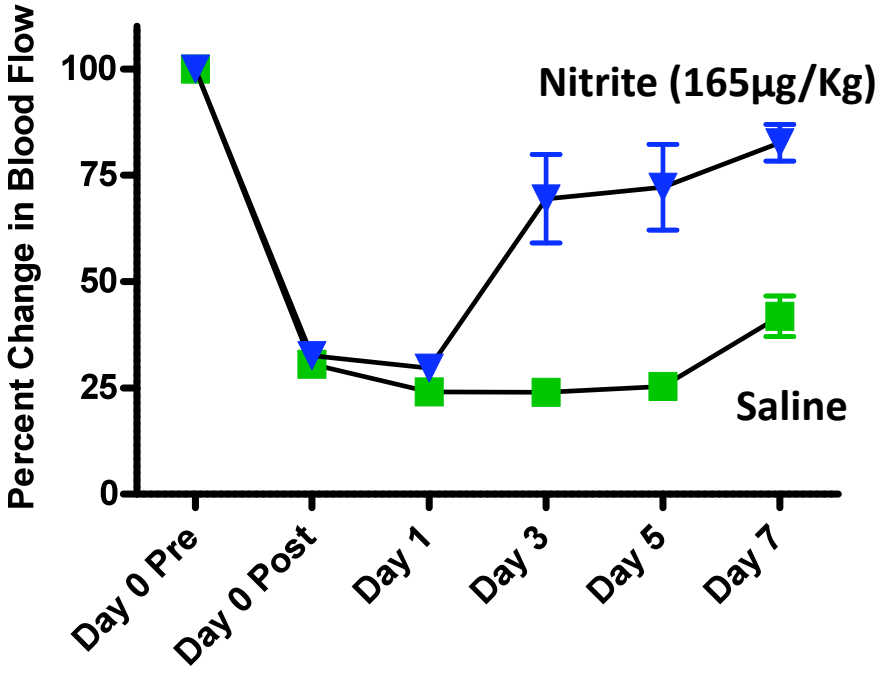
Low dose Nitrite therapy- NO donor to ischemic tissues

Hepatic IR injury



Duranski et al JCI (2005)

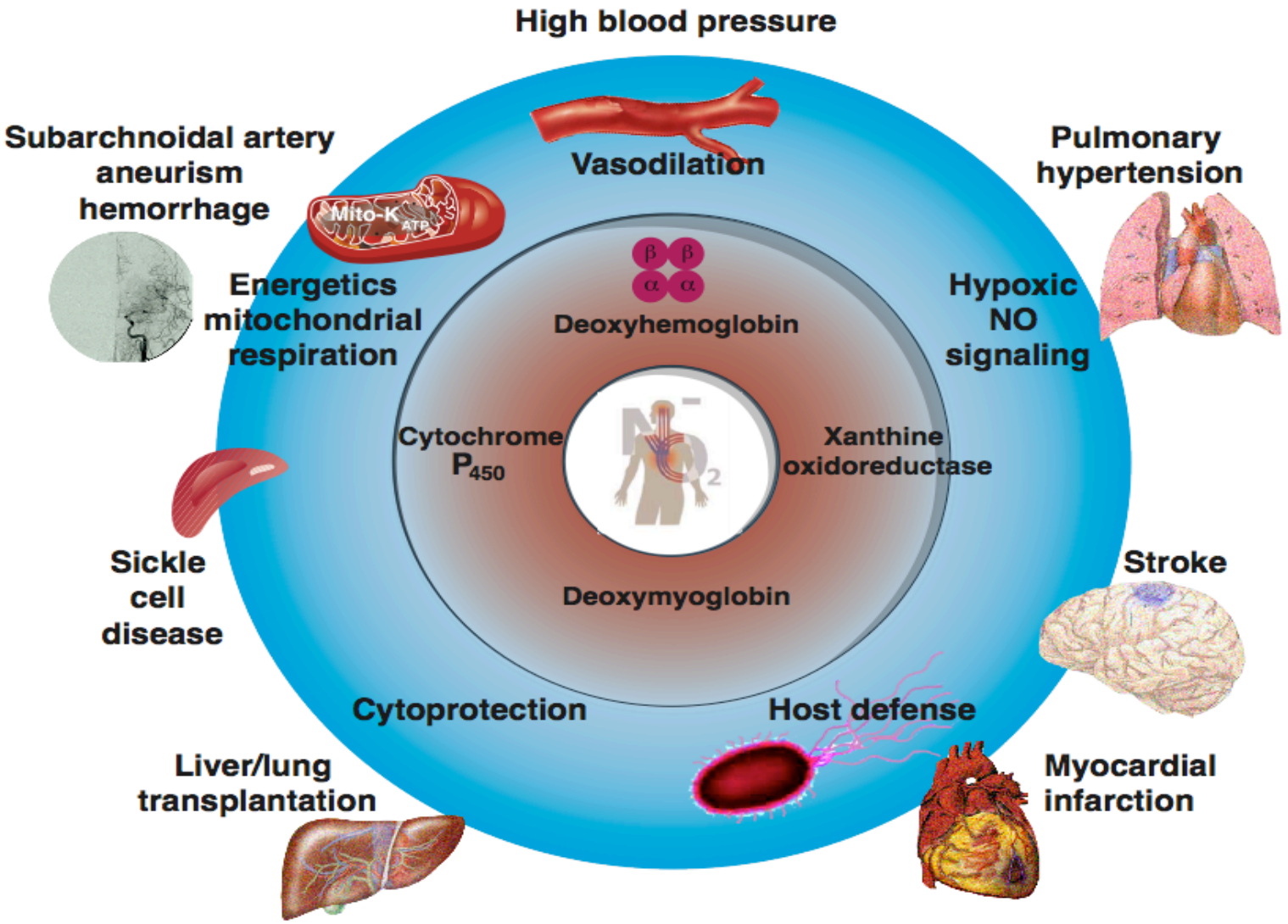
Ischemic Angiogenesis



Kumar et al PNAS (2008)

nM- µM nitrite

Nitrite Bioactivity and therapeutics- *present day*



Gladwin et al Nat Chem Biol (2005)

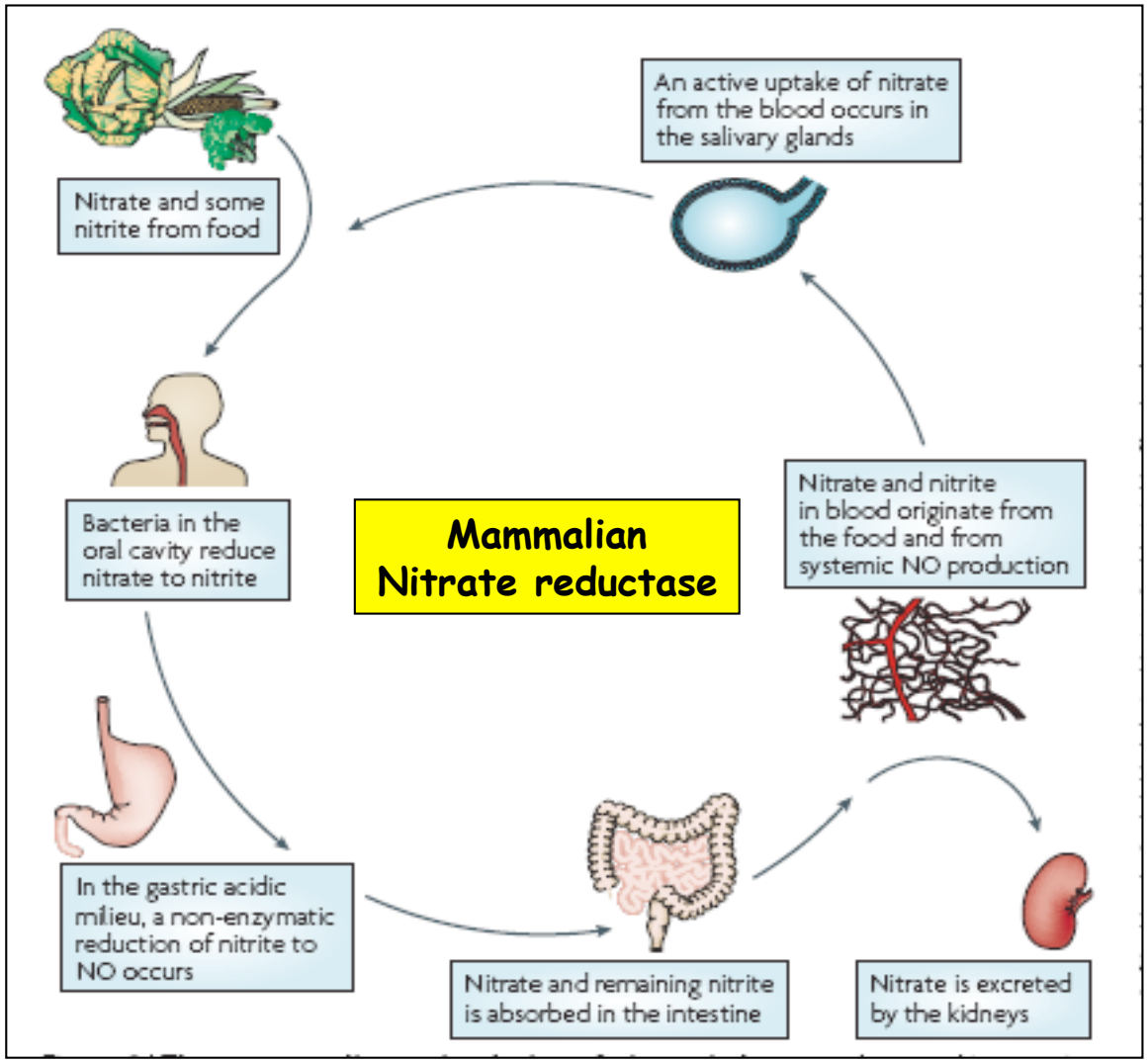
Final thoughts: Emerging importance for nitrate as a mediator

Nitric oxide ⇌ **Nitrite** ⇌ **Nitrate**

H+, hypoxia

hypoxia (XOR)

oral cavity bacterial nitrate reductases



Lundberg et al Nat Rev Drug Disc (2008)
Jansson et al Nat Chem Biol (2008)
Carlstrom et al PNAS (2010)
Larsen et al FRBM (2010)
Kapil et al Hypertension (2010)
Gilchrist et al Cardiovasc Res (2010)
Vanhatalo et al Am J Physiol (2010)
Bailey et al (2009) J Appl Physiol
Li et al JBC (2005)

Hypotensive effects of inorganic dietary nitrate

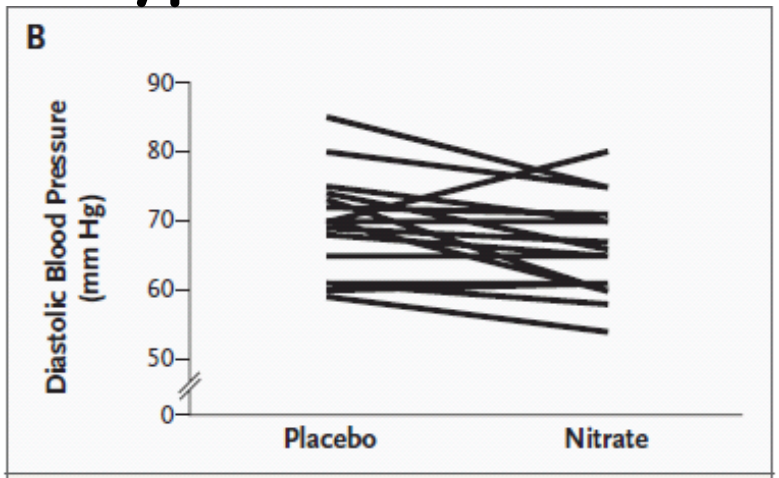
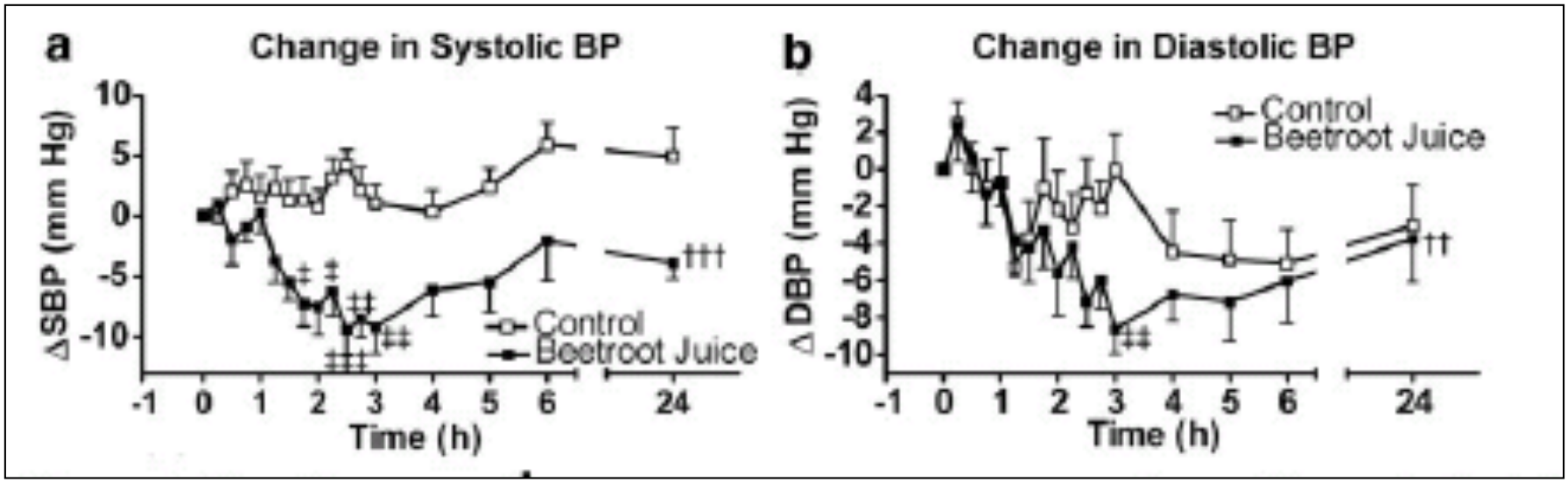


Figure 1. Effects of 3-Day Dietary Supplementation with Inorganic Nitrate or Placebo on Systolic (Panel A) and Diastolic (Panel B) Blood Pressure in 17 Healthy Volunteers.

The diastolic pressure was reduced by 3.7 mm Hg after nitrate supplementation ($P<0.02$), whereas systolic blood pressure was not significantly changed.

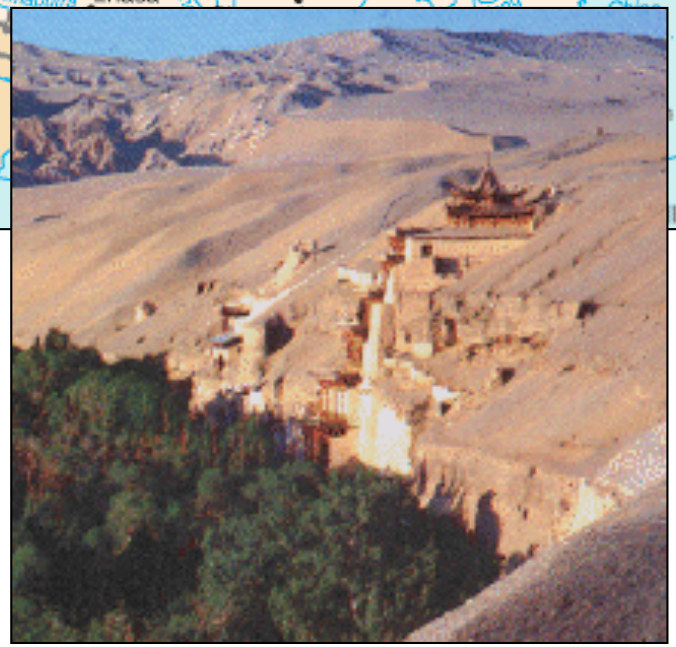
Larsen et al (2006) NEJM

Webb et al (2008) Hypertension



Chinese 8th century medical manuscript found in Buddhist grotto in Dunhuang

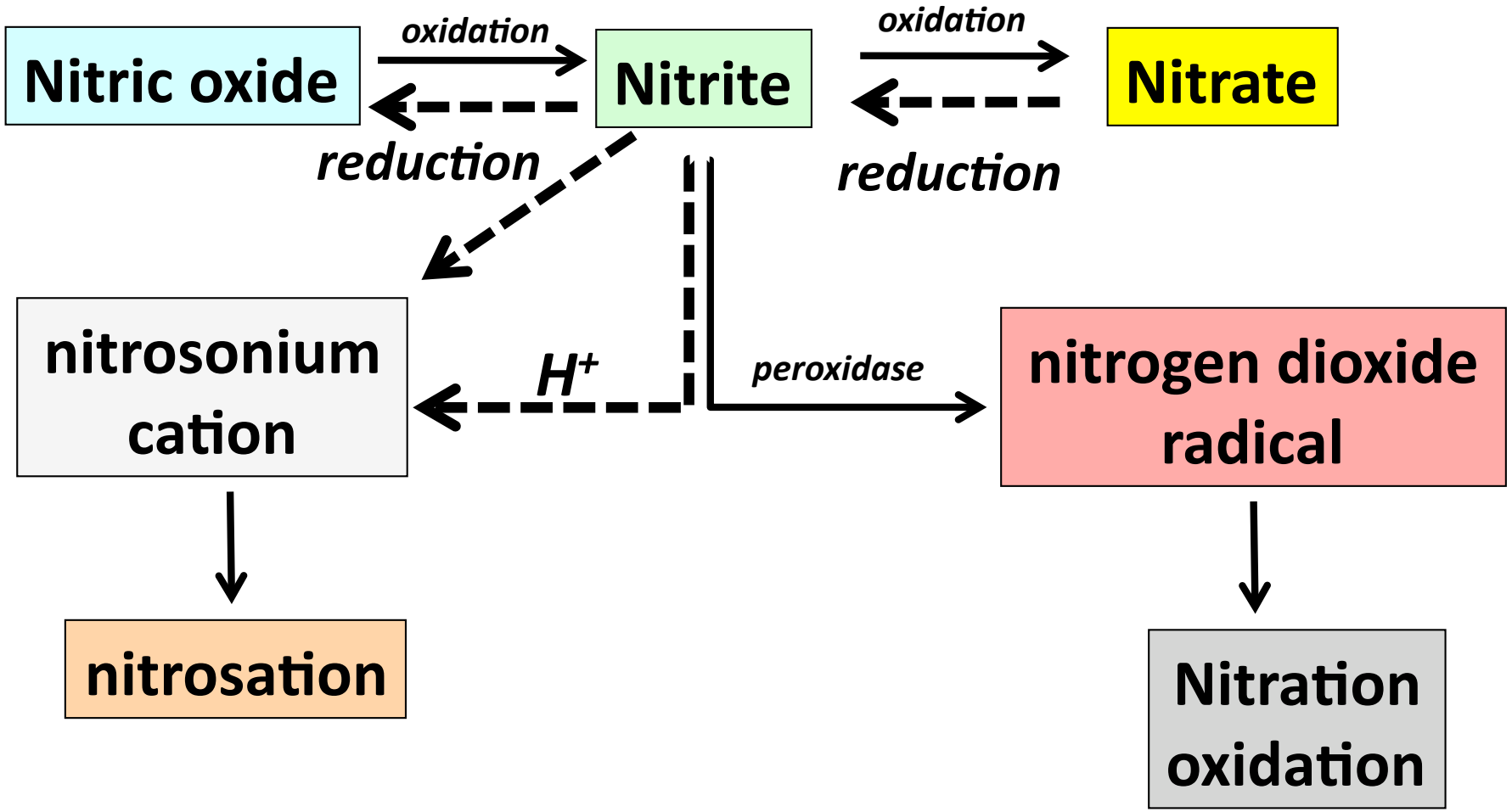
(Butler et al , Medical Chinese Medicine: The Dunhuang Medical Manuscripts, London, Routledge 2004)



着舌以通心氣。治中惡，急心痛，手足逆冷者，傾刻可殺人。
看其人指，爪青者是。
硝石 五錢匕　雄黄 一錢匕
右二味，共爲極細末。啓病者舌，着散一匕於舌下，若有涎出，令病者隨涎咽下，必愈。

"Putting under the tongue to cause heart qi to flow freely for treating symptoms such as struck by evil, acute heart pains and cold in the hands and feet, which can kill a patient in an instant.	Look at patient's fingers and those with greenish-black nails are such cases. Take potassium nitrate (5 measures of a bi spoon) and arsenic sulphide (1 measure of a bi spoon) and combine the two into a fin powder.	lift the patient's tongue and sprinkle one measure of a bi spoon under the tongue. If saliva is produced, have the patient swallow it. This is a certain cure." qi: fluid thought to circulate around the body

Central role for redox reactions in nitrite / nitrate dependent formationof NO-containing metabolites



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