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NADPH Oxidase in Neutrophils

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Introduction

- In 1933 Baldridge and Gerrard observed that neutrophils consume more oxygen when the phagocytized a bacterium
- This phenomenon became known as the "Respiratory Burst" and is catalyzed by NADPH oxidase
- **NADPH oxidase transfers electrons from the cytosol to form superoxide in the phagocytic vacoule**

Baldridge CW, Gerald RW. (1933) *Am J Physiol*. **103:** 235-236.
Cross AR, Segal AW. (2004) *Biochim Biophys Acta*. **1657:** 1-22.
Wientjes FB, Segal AW. (1995) *Cell Bio.* **6:** 357-365.

Respiratory Burst

- Defense mechanism to fight infection
- Neutrophils phagocytize bacteria causing the activation of NADPH oxidase
- Increase in oxygen consumption and release $O_2^{\bullet-}$
- Degranularization into the phagocytic vacuole

Koshkin V *et al*. (1997) *Biochim Biophys Acta.* **1319:** 139-146.
Wientjes FB, Segal AW. (1995) *Cell Bio.* **6:** 357-365.

Respiratory Burst

- The increase in ROS may be involved in killing the bacteria, but alone is not sufficient
  - Studies have shown that phagocytic vesicle without granules could not kill the bacterium

- One hypothesis is that the production of $O_2^{\bullet-}$ optimizes the pH of the vacuole for the enzyme in the granules to effectively work

Wientjes FB, Segal AW. (1995) *Cell Bio.* **6:** 357-365.

Respiratory Burst

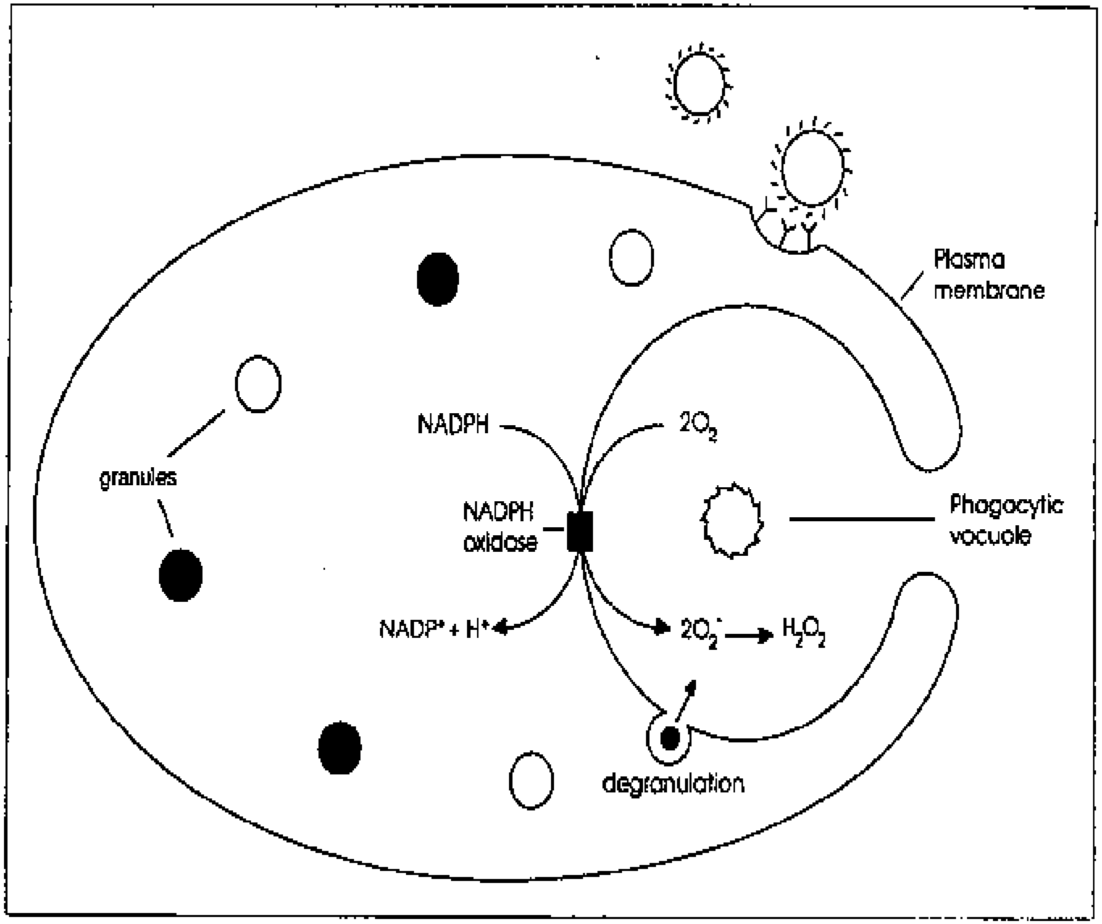


Diagram of the respiratory burst in neutrophils. The cell phagocytizes a bacterium. NADPH is oxidized by NADPH oxidase to $NADP^+$, reducing O_2 to $O_2^{\bullet -}$ in the phagocytic vacuole. This activates degranulation into the vacuole.

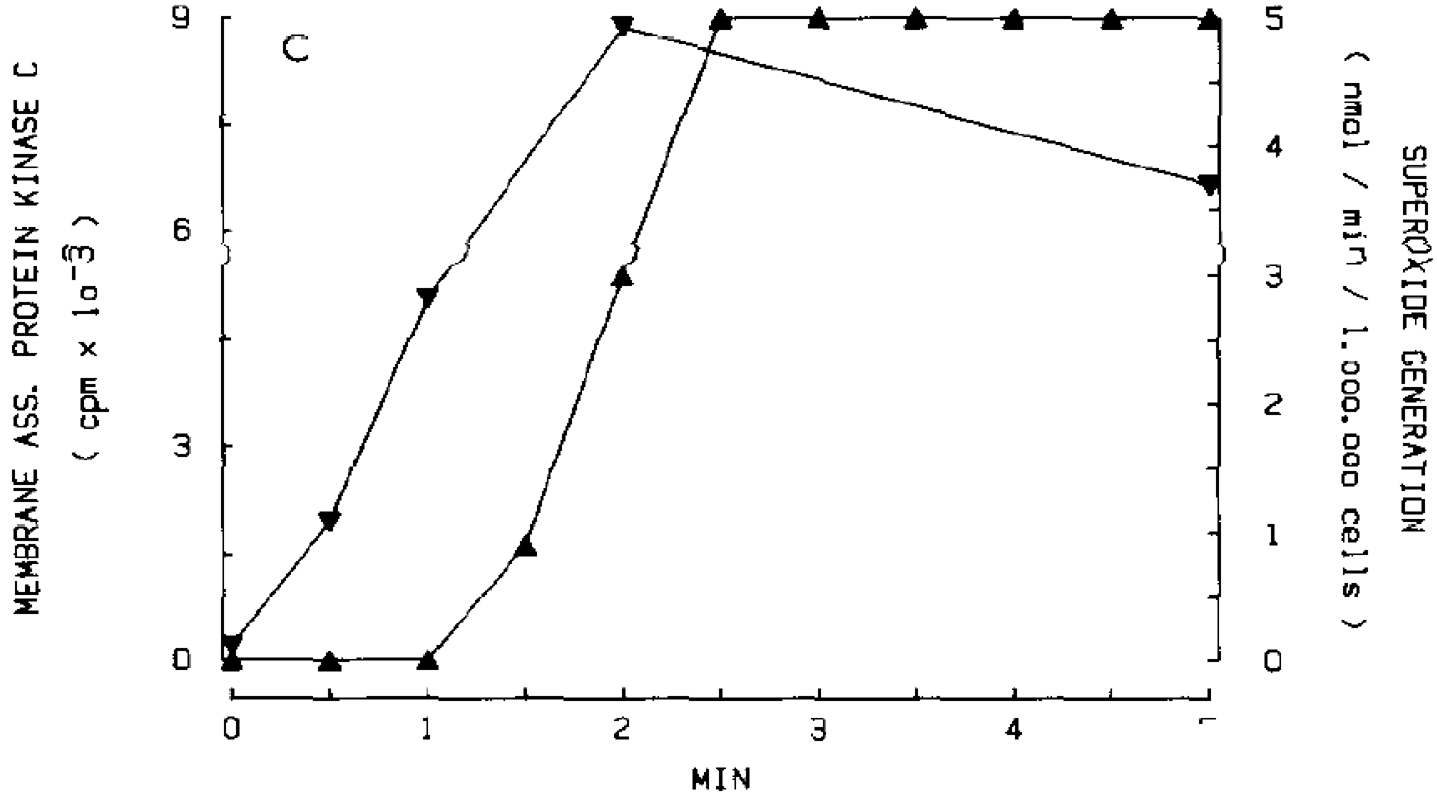
Wientjes, Segal. (1995) *Cell Bio.* **6:** 357-365.

Stimulation of Neutrophils

- Protein kinase C is needed to activate NADPH oxidase
- Protein kinase C
  - Proposed to directly phosphorylate the enzyme
  - Phorbol 12-mystate 13-acetate (PMA) can stimulate protein kinase C

Christiansen NO. (1988) *FEB.* **239:** 195-198.

Stimulation of Neutrophils



Production of $O_2^{\bullet-}$ after neutrophil stimulus. Cells were stimulated with 300 ng/mL of phorbol 12-myristate 13-acetate (PMA). Superoxide levels were measured by the reduction of cytochrome c at 550 nm.

Christiansen NO. (1988) *FEB.* **239:** 195-198.

Structure of NADPH Oxidase

Components

- Cytochrome *b*-245
  - Heterodimer flavocytochrome
    - α subunit – p22-*phox*
    - Β subunit – gp91-*phox*
  - In the plasma membrane
  - Named cytochrome *b*-245 for its midpoint reduction potential
  - AKA cytochrome *b*-558 for the absorbance of the α subunit
- Cytosolic proteins
  - p47-*phox* and p67-*phox*
  - GTP binding proteins
    - *rac*-1 or *rac*-2
    - Bound to rhoGDI (GDP-Dissociation Inhibitor)

Jones RD *et al.* (2000) *Free Radic Biol Med.* **29:** 416-424.
Koshkin V *et al.* (1997) *Biochim Biophys Acta.* **1319:** 139-146.
Wientjes FB, Segal AW. (1995) *Cell Bio.* **6:** 357-365.

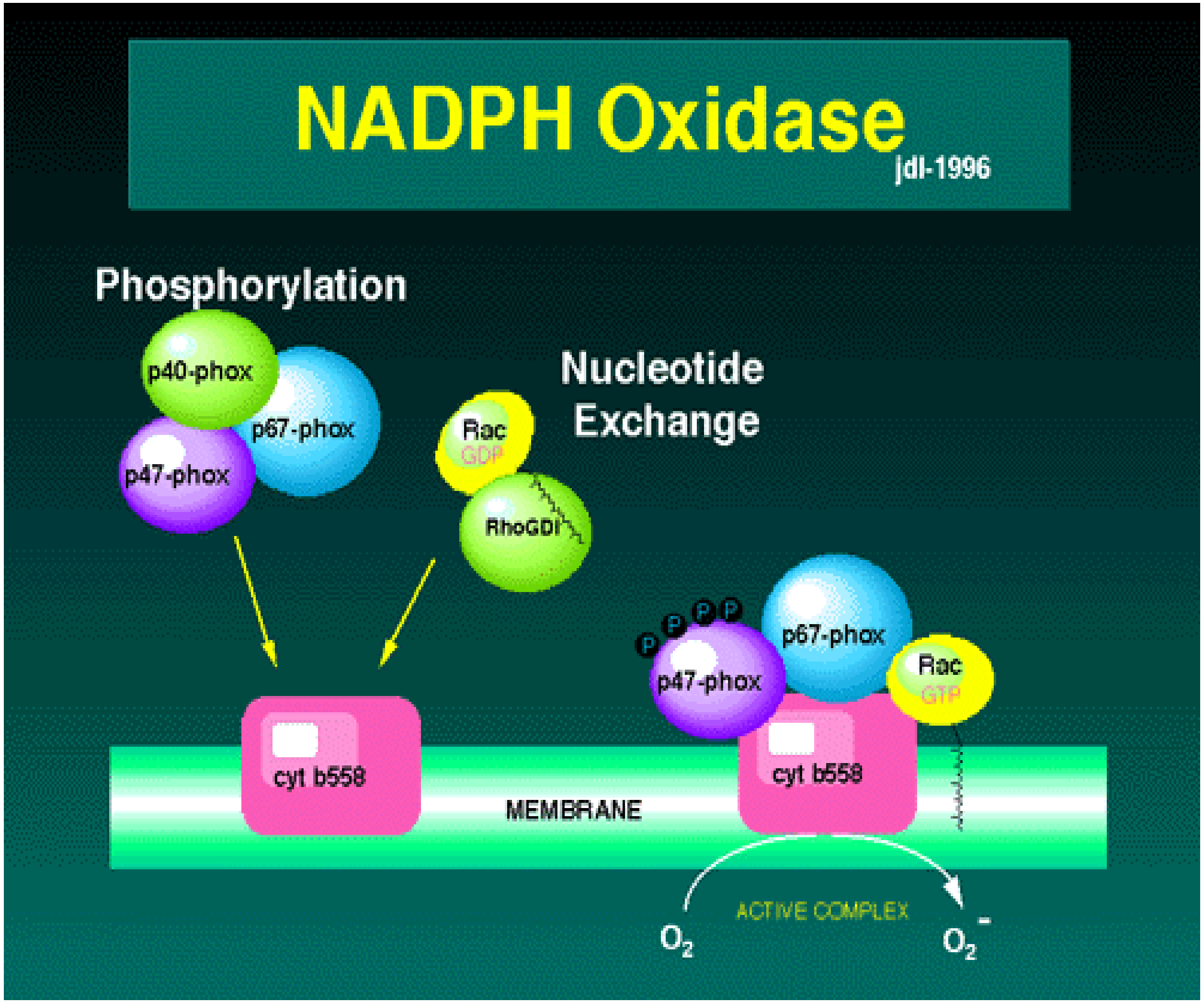
Structure of NADPH Oxidase

Assembly of the Complex

- Cytosolic protein p47-*phox* becomes phosphorylated
- Rac binds GTP and RhoGDI falls off
- The cytosolic proteins assemble on cytochrome *b*-245
- The assembled complex activates the enzyme

Wientjes FB, Segal AW. (1995) *Cell Bio.* **6:** 357-365.

Structure of NADPH Oxidase



Assembly of NADPH on the plasma membrane.

http://www.biochem.emory.edu/labs/dlambe/images/nadphfig.gif. Accessed 18. March 2005.

Mechanism of $O_2^{\bullet -}$ production

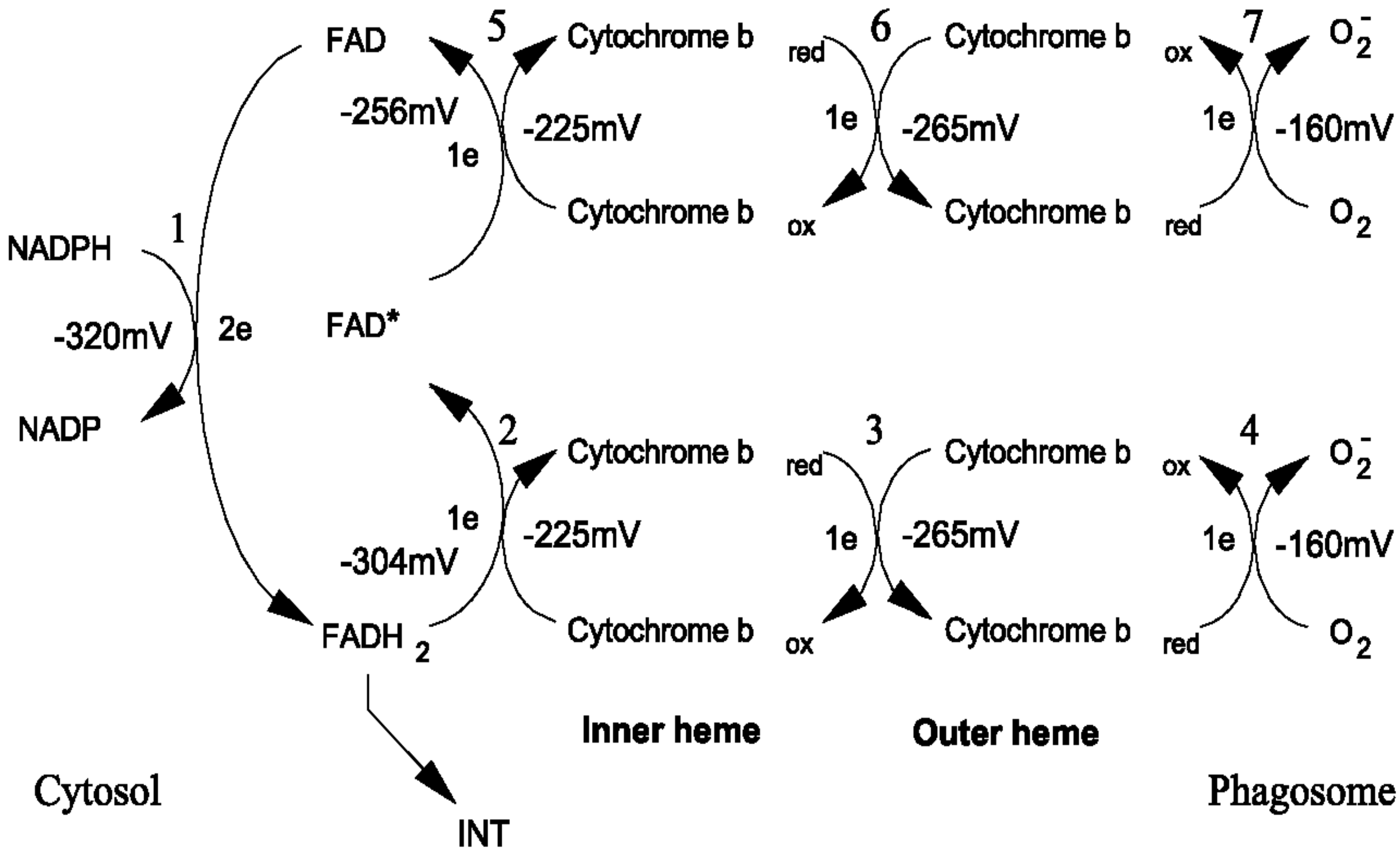
Electron-Transport Pathway

- NADPH donates 2 e^-, 1 at a time, to FAD
  - Max rate = 345 nmol min^{-1} mg protein $^{-1}$
  - NADH also works, but is much less efficient
    - K_m NADPH = 45 μM
    - K_m NADH = 460 μM
- $FADH_2$ and $FAD^{\bullet}$ of cytochrome *b*-245 oxidizes the heme group
- 1-e^- reduction of O_2 to $O_2^{\bullet -}$ by cytochrome *b*- 245
  - The oxidized heme of cytochrome *b*-245 has a $t_{½}$ of 4.7 ms
  - Cytochrome *b*-245 has the lowest reduction potential of the mammalian cytochromes

Cross AR *et al*. (1984) *Biochem J.* **223:** 337-344.
Cross AR *et al.* (1985) *Biochem J.* **226:** 881-884.
Cross AR, Jones OTG. (1991) *Biochim Biophys Acta*. **1057:** 281-298.
Cross AR, Segal AW. (2004) *Biochim Biophys Acta*. **1657:** 1-22.

Mechanism of $O_2^{\bullet -}$ production

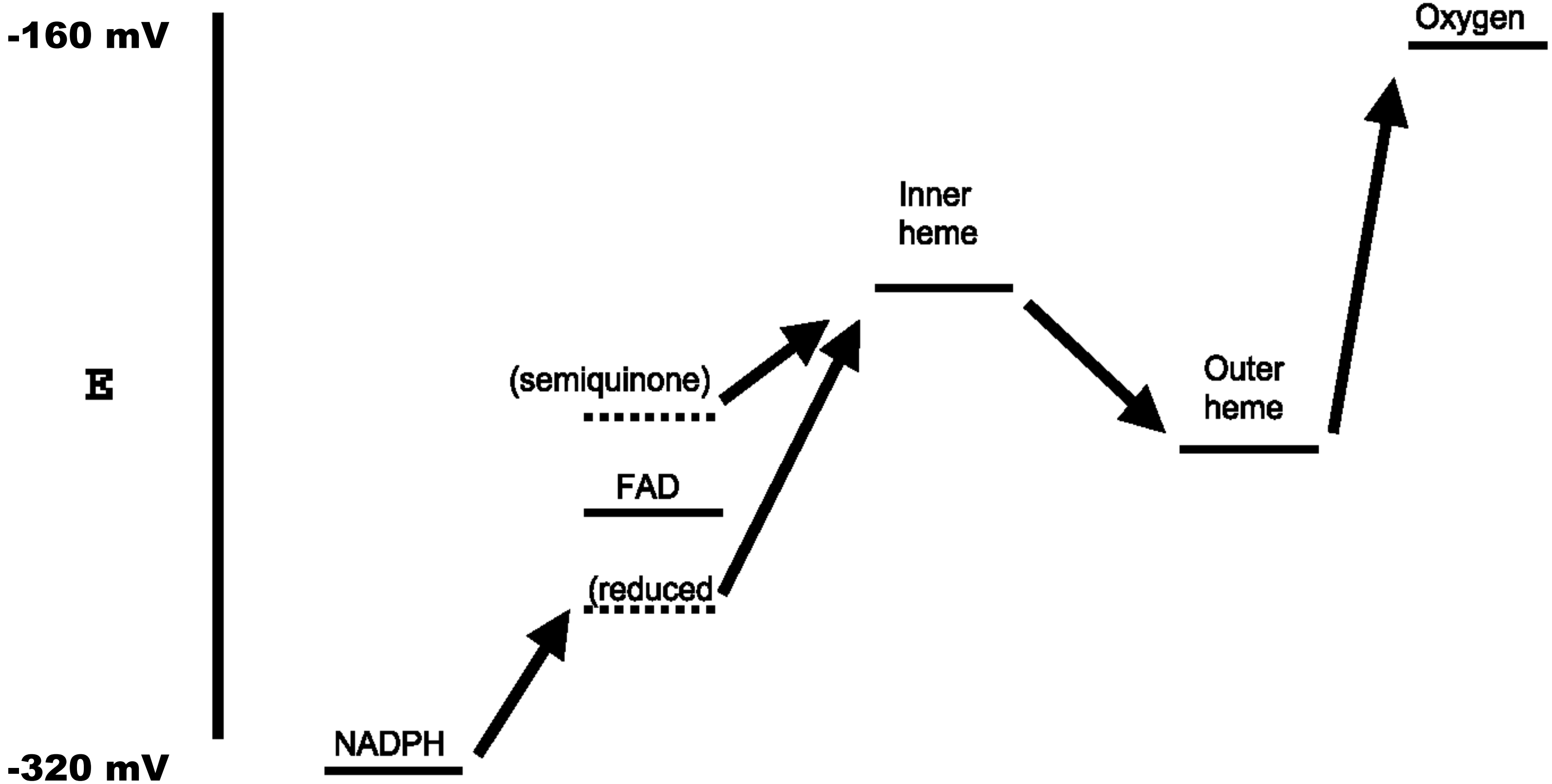
Redox couples and reduction potentials



Cross AR, Segal AW. (2004) *Biochim Biophys Acta*. **1657:** 1-22.

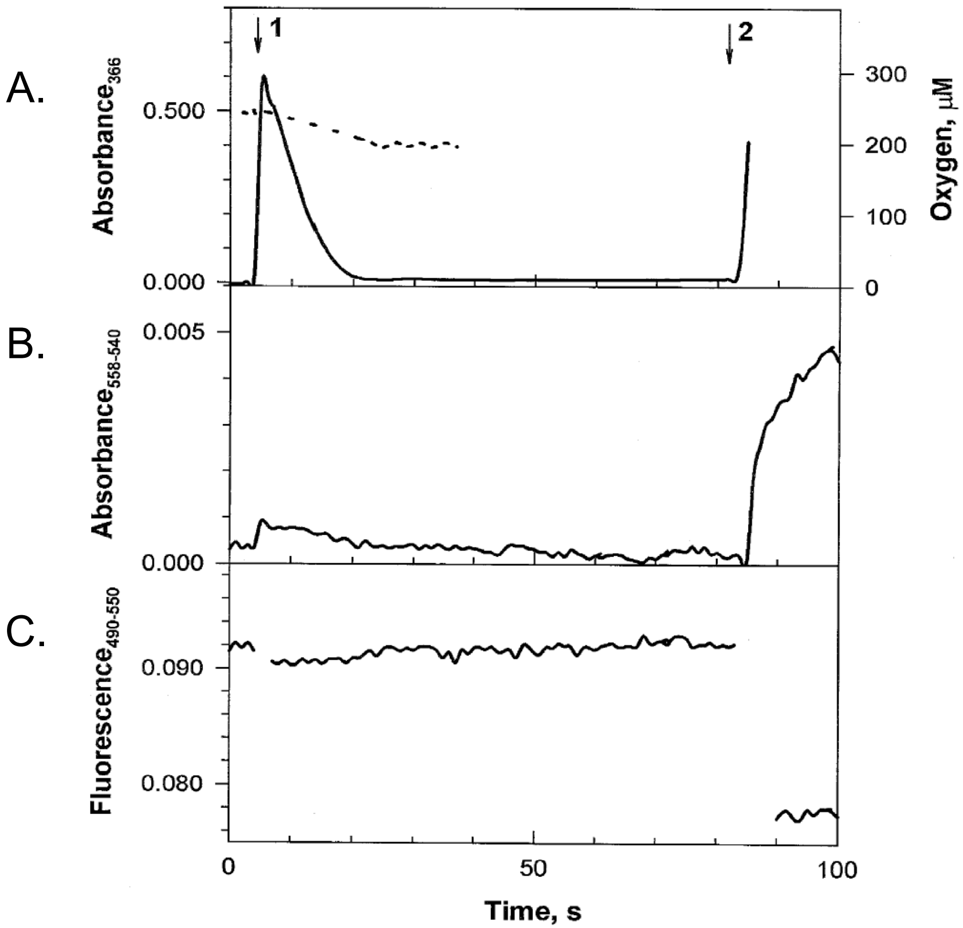
Mechanism of $O_2^{\bullet-}$ production

Thermodynamic Scheme of the ElectronTransport Pathway



Cross AR, Segal AW. (2004) *Biochim Biophys Acta*. **1657:** 1-22.

Mechanism of $O_2^{\bullet -}$ production



Reaction time of NADPH oxidase components in aerobic conditions. NADPH was added at arrow 1. The reaction was completed when sodium dithionite was reduced (arrow 2).

A. NADPH (—) and oxygen (- -)

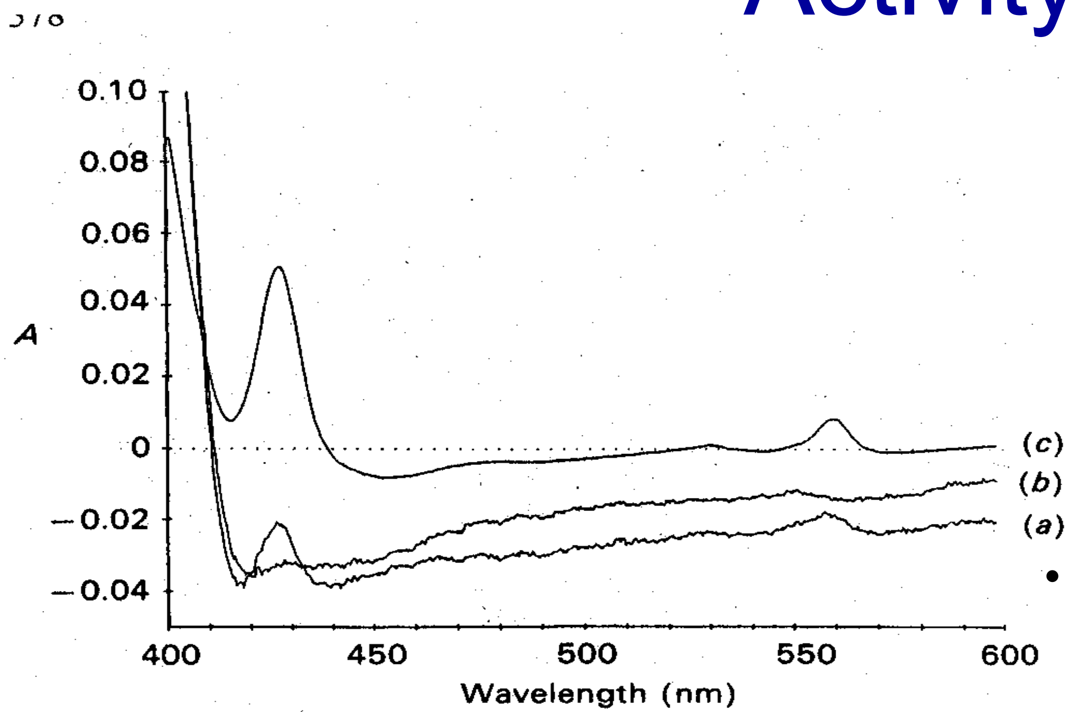
B. Heme.

C. FAD.

Koshkin V *et al*. (1997) *Biochim Biophys Acta.* **1319:** 139-146.

$$V_{NADPH \rightarrow FAD} = V_{FAD \rightarrow heme} = V_{heme \rightarrow oxygen} = k[heme^{2+}] = 1720\ s^{-1}$$

Detection of NADPH Oxidase Activity



Absorption spectra of NADPH oxidase in the presence of 1 mM NADPH.

A. NADPH oxidase.

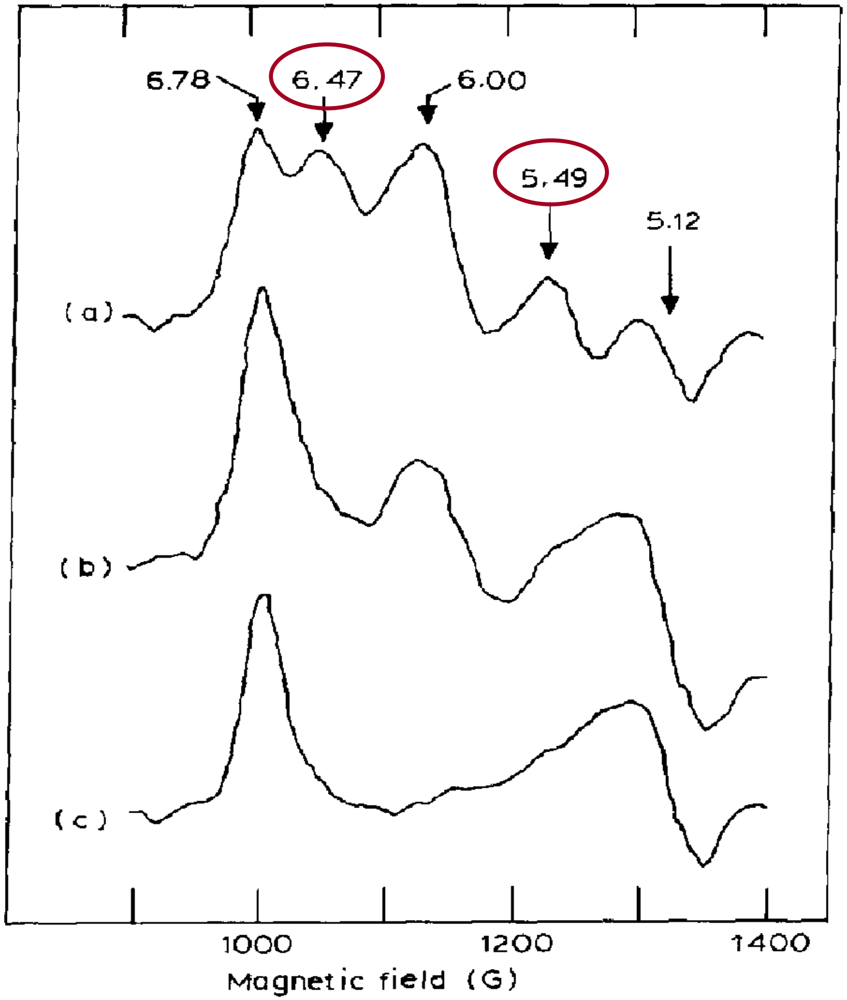
B. NADPH oxidase with imidozole

C. NADPH oxidase reduced with $Na_2S_2O_4$.

- The decrease of the 450 nm peak is due to the reduction of the flavin protein
- The cytochrome peak can be seen at 558 nm.

Cross AR *et al*. (1984) *Biochem J.* **223:** 337-344.

Detection of NADPH Oxidase Activity



EPR spectra from neutrophils.

A. Stimulated neutrophils.
B. Resting cells.
C. Purified myeloperoxidase

g = 6.78 and 5.12 is due to myeloperoxidase

g = 6.00 is due to cytochrome *b*-245

g = 6.47 and 5.49 are only found in stimulated cells, due to cytochrome *b*-245 in the NADPH oxidase complex

Hata-Tanaka A *et al*. (1987) *FEB*. **214:** 279-284.

Chronic Granulomatous Disease (CGD)

- Disorder with deficient NADPH oxidase cytochrome *b*-245
- Patients are very susceptible to infection
- X-linked disorder
  - Most common
  - Loss of gp91-*phox*
- Autosomal recessive disorder
  - Rare
  - Loss of p21-*phox*

Wientjes, Segal. (1995) *Cell Bio.* **6:** 357-365.

Summary

- A functional NADPH oxidase is necessary to fight off bacterial infections
- The enzyme complex catalyzes the one-electron reduction of oxygen to superoxide in the phagocytic vesicle.
- This uses NADPH in the cytosol as an electron donor
- The increase in oxygen consumption leading to a rise superoxide in the phagocytic vesicle is known as the respiratory burst

Cross AR, Segal AW. (2004) *Biochim Biophys Acta*. **1657:** 1-22.
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- Wientjes FB, Segal AW. (1995) NADPH oxidase and the respiratory burst. *Cell Bio.* **6:** 357-365.