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Succinate Dehydrogenase: A Source of Radicals?

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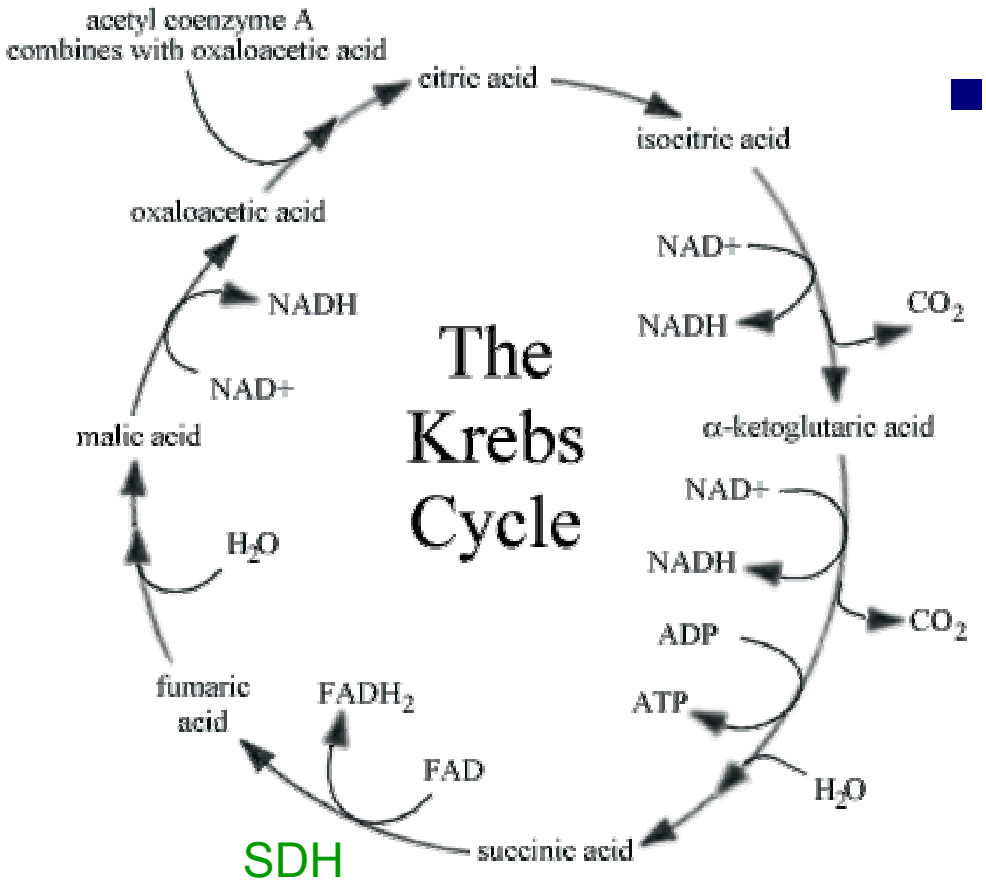
Succinate dehydrogenase (SDH) in the Krebs cycle and electron transport chain

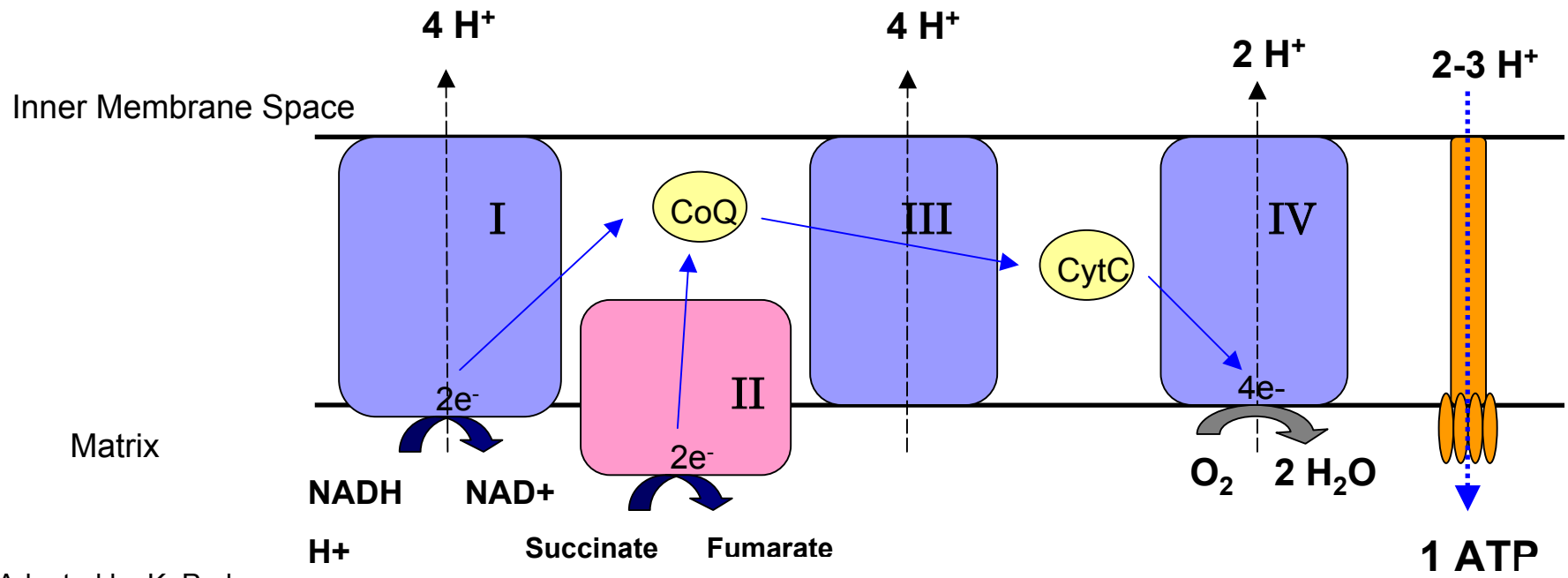


- Krebs Cycle
  - Key metabolic pathway
  - SDH catalyzes the oxidation of succinate to fumarate
    - Donates 2 electrons to the electron acceptor flavin adenine dinucleotide (FAD)

From http://library.thinkquest.org/ 27819/ch4_6.shtml (Accessed 3.17.05)

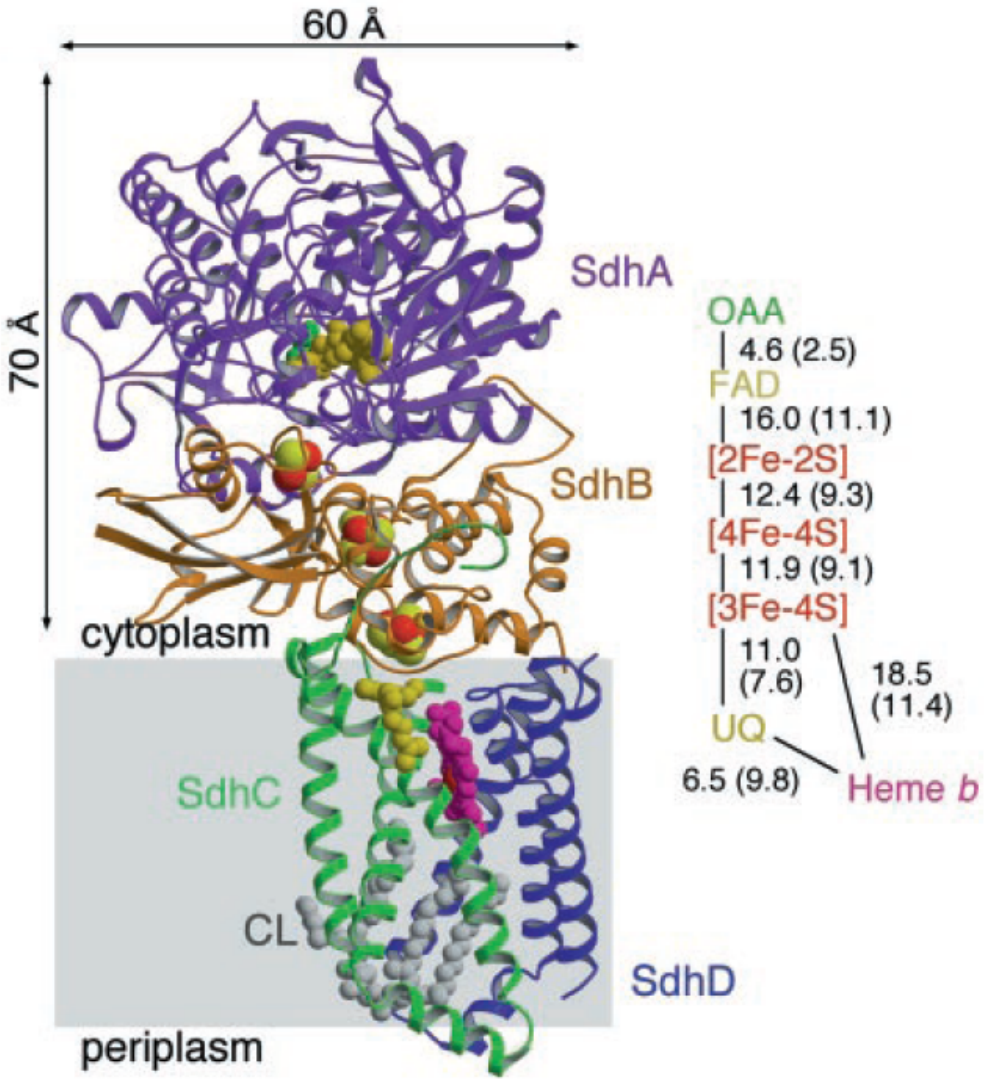
Succinate dehydrogenase (SDH) in the Krebs cycle and electron transport chain

- Electron transport (oxidative phosphorylation)
  - Uses energy harvested from electron transport to create a proton gradient across the mitochondria's inner membrane. Protons power ATP synthesis.
  - SDH comprises complex II
    - Takes electrons from succinate and eventually donates them to ubiquinone (CoQ)



Adapted by K. Pedersen

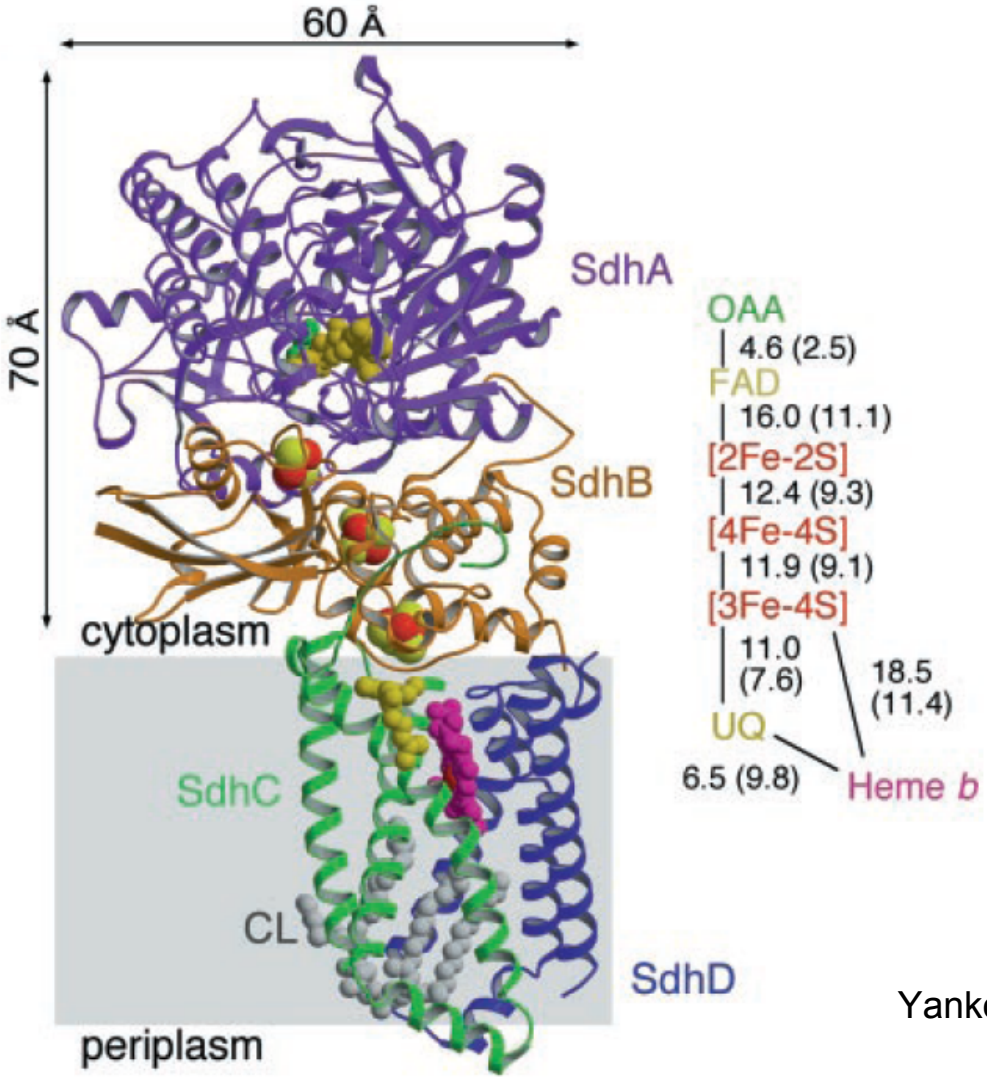
SDH structure



Yankovskaya V *et al.* (2003) *Science* **299**: 701-704

- SDH consists of 4 protein subunits
  - SDHA: hydrophilic, contains FAD
  - SDHB: hydrophilic, contains 3 iron-sulfur centers (only 2 are used in electron transport)
  - SDHC & SDHD: lipophilic, anchor SDH to mitochondrial membrane, coordinate heme b, form CoQ binding pocket with SDHB

SDH structure: Electron motility



- Reactive centers of SDH create a path of 40 A for electrons to travel from FAD to CoQ
- Electrons may travel up to 14 A (Page *et al. Nature* **402**: 47)
  - Distances between components of SDH are within this limit [center to center (edge to edge) distances listed at left]

Yankovskaya V *et al.* (2003) *Science* **299**: 701-704

Electron flow through SDH

- Redox potentials and electron distributions for each complex in SDH indicate that electrons do not travel to each iron sulfur complex (4Fe-4S)
  - Electron residence time is very brief on FAD (because electron distribution based upon equilibrium distribution is small at 0.02)

Table 2. Electron distribution among the redox centers of *E. coli* SQR and QFR.

	Redox potentials (mV)		Electron distribution* (number of electrons)		
	SDH	QFR	SDH	SDH (without heme)	QFR
FAD	−79†	−50	0.02	0.18	1.0
[2Fe-2S]	+10	−35	0.43	0.84	0.65
[4Fe-4S]	−175	−310	0.00	0.00	0
[3Fe-4S]	+65	−67	0.87	0.98	0.34
heme *b*	+35	–	0.68	–	–

*The distribution of two electrons was calculated assuming an equilibrium distribution among independent redox centers of given reduction potentials at 298 K.

Note: QFR=fumarate reductase (catalyzes the reverse reaction of SDH in anaerobic bacteria) and serves as a point of comparison for SDH

Yankovskaya V *et al.* (2003) *Science* **299**: 701-704

Electron flow through SDH

- While electrons are found associated with heme b, it is not likely a part of the regular electron pathway
  - electrons normally flow the shorter distance to the CoQ acceptor
  - Hypothesized to prevent electron leakage off the complex (prevent superoxide formation)

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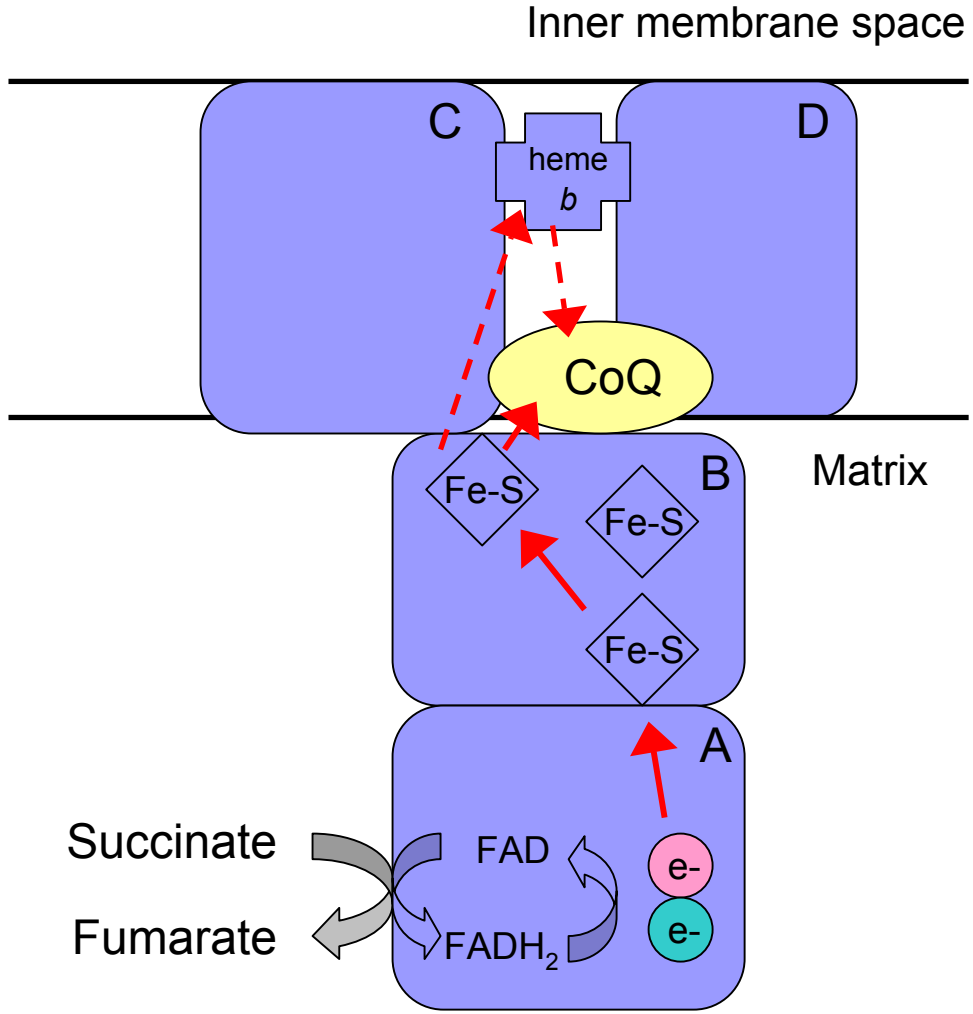
Electron flow through SDH

1. 2 electrons from succinate reduce FAD
2. $FADH_2$ donates one electron at a time to the iron-sulfur complexes in SDHB
3. 1 or 2 electrons reduce CoQ (ubiquinone)
4. CoQ is mobile—can donate electrons to complex III



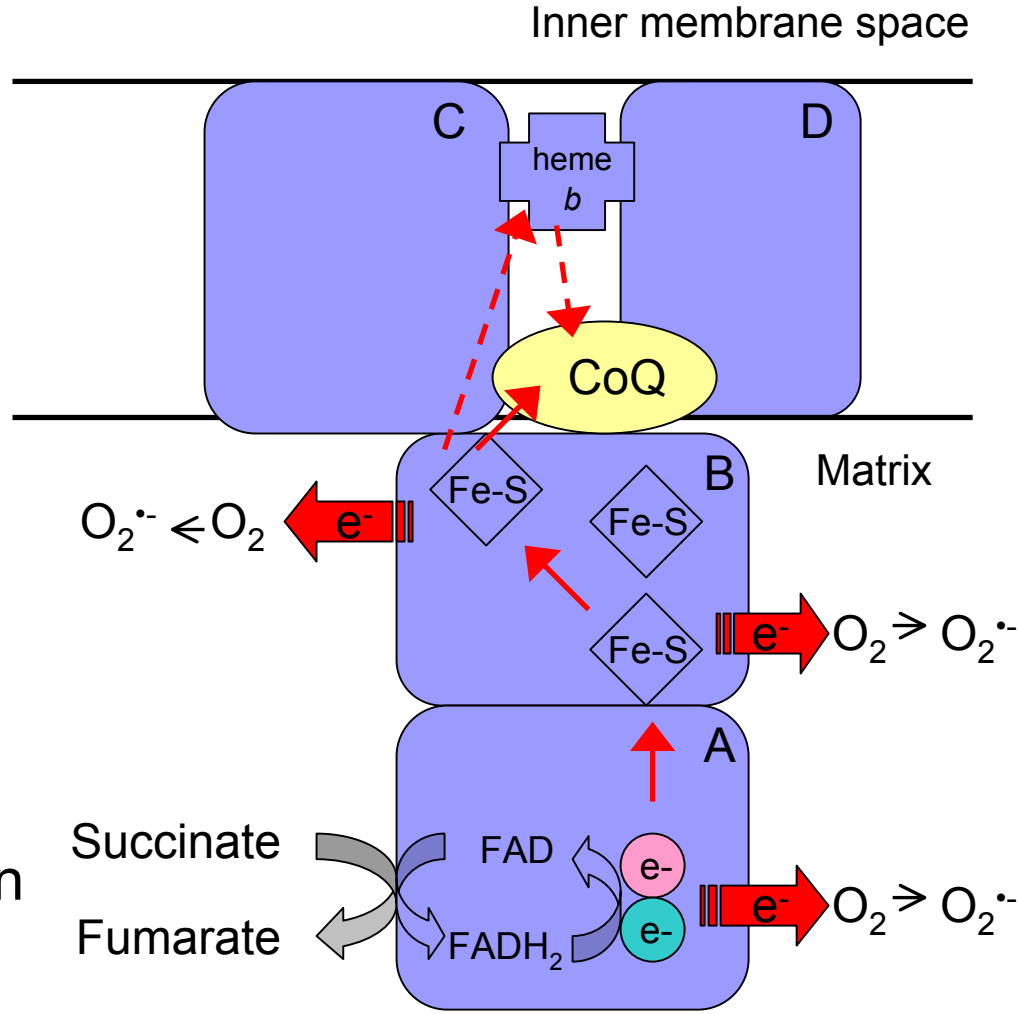
Mitochondria are major sites of ROS formation

- Complexes I and III of the electron transport chain believed to be major contributors to oxidative burden in normal cells
  - Electrons “leak off” the complexes and interact with oxygen in the matrix
    - Forms superoxide and other ROS
- Complex II more recently shown to play larger role in ROS formation
  - Still controversial

McLennan and Degli Esposti (2000) *J Bioenerg Biomembr*. **32**: 153-162.

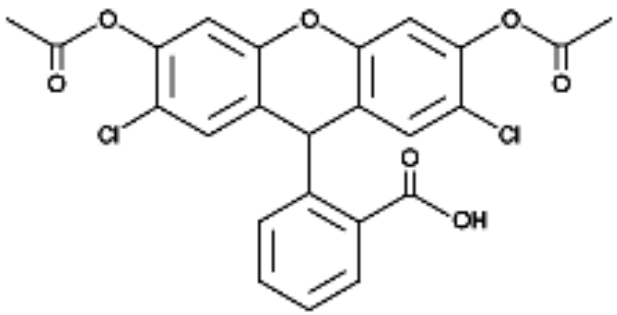
Proposed Model for ROS formation

- Electrons flowing through SDH may interact with oxygen in the matrix
  - Electrons most likely to do so from the flavin (FAD) or iron-sulfur (Fe-S) molecules
  - Effect may be exacerbated by mutations in SDH protein structure



Methods to detect ROS from SDH

- DCF-DA: dichlorodihydrofluorescein diacetate



- Acetate groups must be cleaved before probe may be oxidized
- DCF-DA probe oxidation emits fluorescence (500-520 nm); used as marker for ROS
  - May be detected with fluorescence microscopy, absorbance spectroscopy, or flow cytometry

From: www.caymanchem.com (Accessed on 4.2.05)

Methods to detect ROS from SDH

- EPR
  - α,α diphenylpicrylhydrazil may be used to aid radical quantification to compare signal intensities (characteristic spectrum at right)
  - Quantitate radicals in conjunction with SDH enzyme activity to determine overall contribution to oxidative burden (see below)

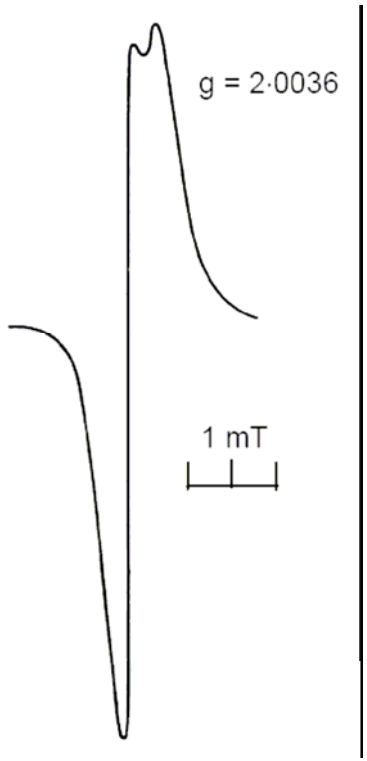


Table 1. Concentration of free radicals and activity of enzymes in myometrial tissues in full-term pregnancy with normal labour and uterine inertia.

Marker	Group of tissues	Normal labour	Uterine inertia
Free radicals*	1	$(4{\cdot}82 \pm 1{\cdot}20) \times 10^{15}$	$(1{\cdot}58 \pm 0{\cdot}20) \times 10^{15}$
	2	$(1{\cdot}20 \pm 0{\cdot}28) \times 10^{15}$	$(0{\cdot}60 \pm 0{\cdot}08) \times 10^{15}$
Succinate dehydrogenase**	1	$0{\cdot}28 \pm 0{\cdot}03$	$0{\cdot}09 \pm 0{\cdot}02$
	2	$0{\cdot}15 \pm 0{\cdot}03$	$0{\cdot}04 \pm 0{\cdot}01$

*Content of free radicals-paramagnetic centers/g of dry tissue. **Activity of enzymes – relative units of optical density per histochemical samples.

Zyrianov *et al.* (2003) *J Biosci.* **28**: 19-21

SDH and ROS

- Carboxin is a complex II inhibitor
- DCF-DA used as marker for ROS
  - Oxidation decreased to control levels when carboxin added
    - Indicates complex II is source of ROS since fluorescence decreases when complex II is inhibited

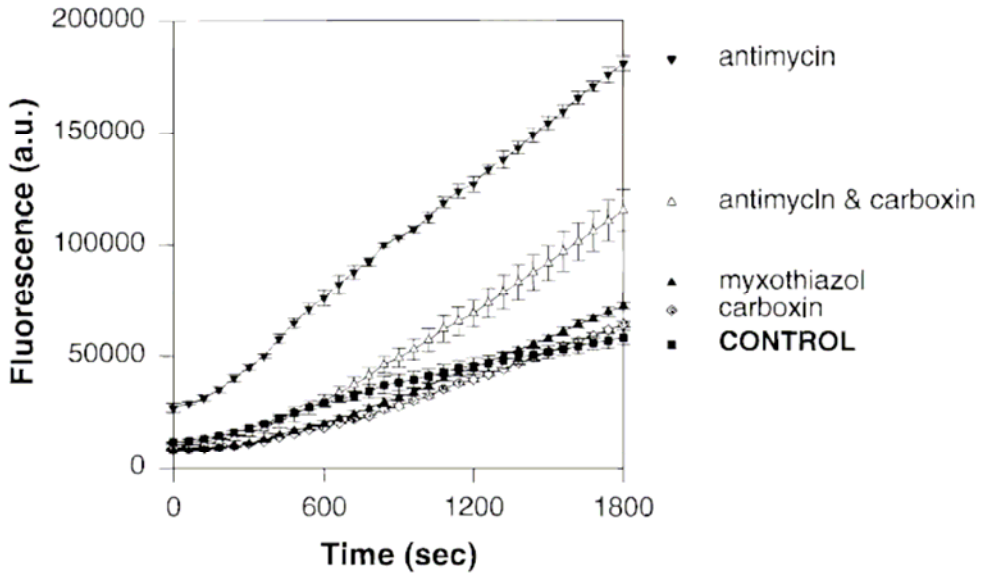
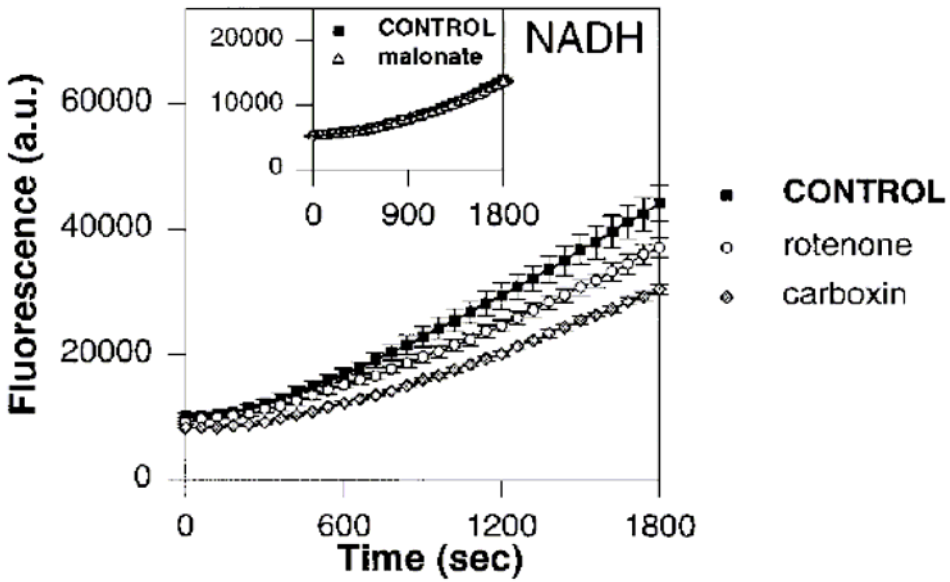


Fig. 3. Inhibition of ROS production with succinate. The experimental conditions were identical to those of Fig. 2B, except that 1 mM malonate was added to the submitochondrial particles to activate complex II. Carboxin was added at 20 μM and induced over 80% inhibition of complex II activity. Succinate concentration was 10 mM.

McLennan and Degli Esposti (2000) *J Bioenerg Biomembr*. **32**: 153-162.

SDH and ROS

- NADH oxidized by complex I, and inhibited by rotenone
  - Use of NADH substrate in a system inhibited with carboxin decreases ROS more greatly
    - Indicates that SDH plays a role in NADH-stimulated ROS production
    - Complex I must contribute less to overall ROS than previously believed



McLennan and Degli Esposti (2000) *J Bioenerg Biomembr*. **32**: 153-162.

Biological implications of ROS formation by SDH

- Increased ROS affect mostly the mitochondria and metabolic processes
- Pathologies linked to SDH dysfunction:
  - Leigh Syndrome
  - Neuroendocrine tumorigenesis
    - Paraganglioma/pheochromocytoma
    - Carcinoids
    - Merkel cell carcinoma
  - Aging/senescence?

Baysal BE *et al.* (2001) *J Mol Med.* **79**: 495-503

Summary

- SDH key metabolic enzyme
  - Serves to move unpaired electron through series of redox interactions with flavo- and metalloproteins to CoQ
- Previously not believed to contribute greatly to mitochondrial oxidative burden
  - Current data appears to show that complex II is a main contributor of ROS in mitochondria