Disruption of cellular energy production by xenobiotics

Mechanistic Toxicology (M.Sc. /Pharmacology and toxicology)

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Overview

- Mitochondria are key to the maintenance of many cellular functions and are the powerplants of cellular energy production.
- In addition, mitochondria mediate physiological processes and are involved in signal transduction and regulation of cell proliferation, differentiation, apoptosis, and other processes.
- Because of their pivotal function, however, mitochondria are susceptible and are crucial targets of many adverse activities of xenobiotics.

Mitochondrial targets and xenobiotic-induced bioenergy crisis

- The production of ATP plays an important role for the biosynthesis of endogenous compounds, transport processes, generation of kinetic energy, and other functions.
- the ATP synthetase-mediated synthesis of ATP from ADP and inorganic phosphate (Pi), is the ultimate step in a complex reaction network, **any process that disturbs or blocks this reaction network will ultimately result in an inhibition of ATP synthesis**, which is the common endpoint of toxicity.

Normal mitochondrial function:

- There are number of interrelated mechanisms have to remain operative in mitochondria to guarantee proper function

1) Uptake of the substrates, pyruvate (from glucose degradation) and fatty acids, that will ultimately be oxidized.
2) In the matrix, both pyruvate and fatty acids are converted to acetyl-CoA. The major function of the citrate cycle is to produce reduced NADH.
3) Electrons produced from NADH oxidation are transported via a (the electron transport chain) to finally reduce molecular oxygen to water.
4) The energy released from NADH oxidation is used to pump protons outwards and expel them across the IMM into the intermembraneous space. Due to this process, an inside-out negative membrane potential ($\Delta\Psi$) is generated.
5) The energy of this proton gradient is used to drive the ATP synthetase, and ATP is assembled from ADP and Pi.

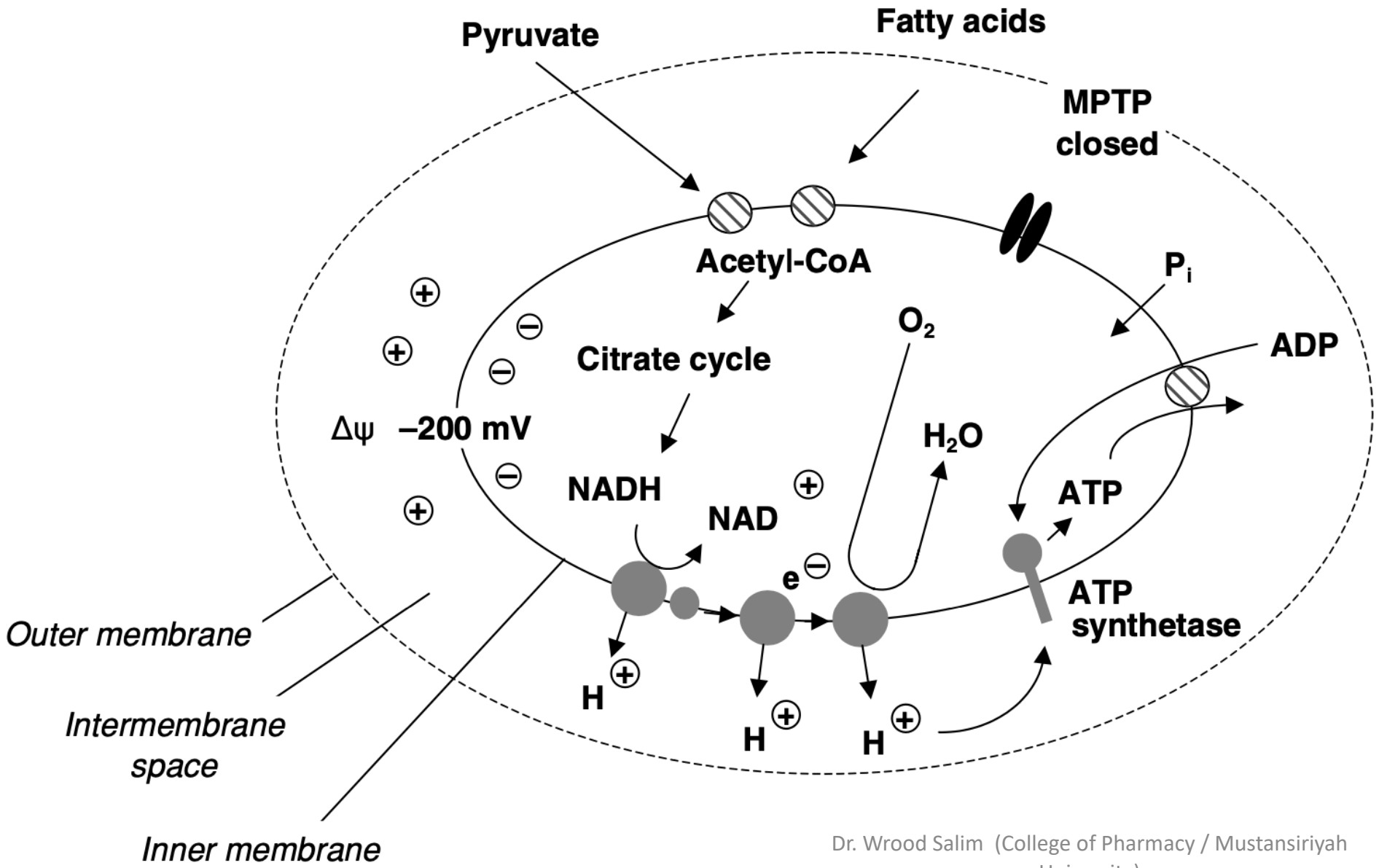

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Figure 15.1. Pivotal steps in normal mitochondrial energy production

Interference with mitochondrial function by xenobiotics:

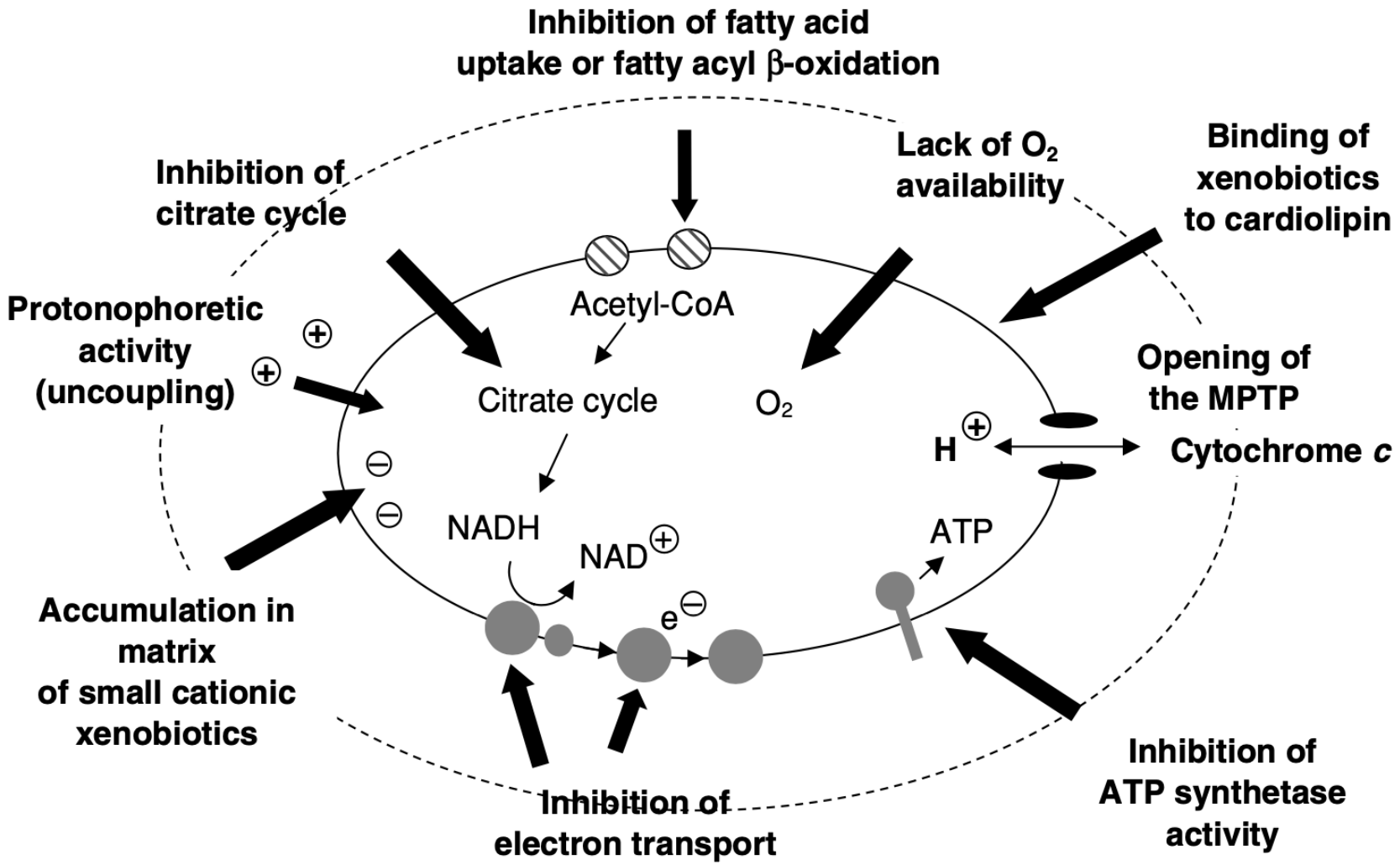

Figure 15.2. Possible sites of xenobiotic interference with mitochondrial function, resulting in mitochondrial toxicity.

Interference with mitochondrial function by xenobiotics:

Chemicals can interfere at any of these steps that participate in mitochondrial energy production.

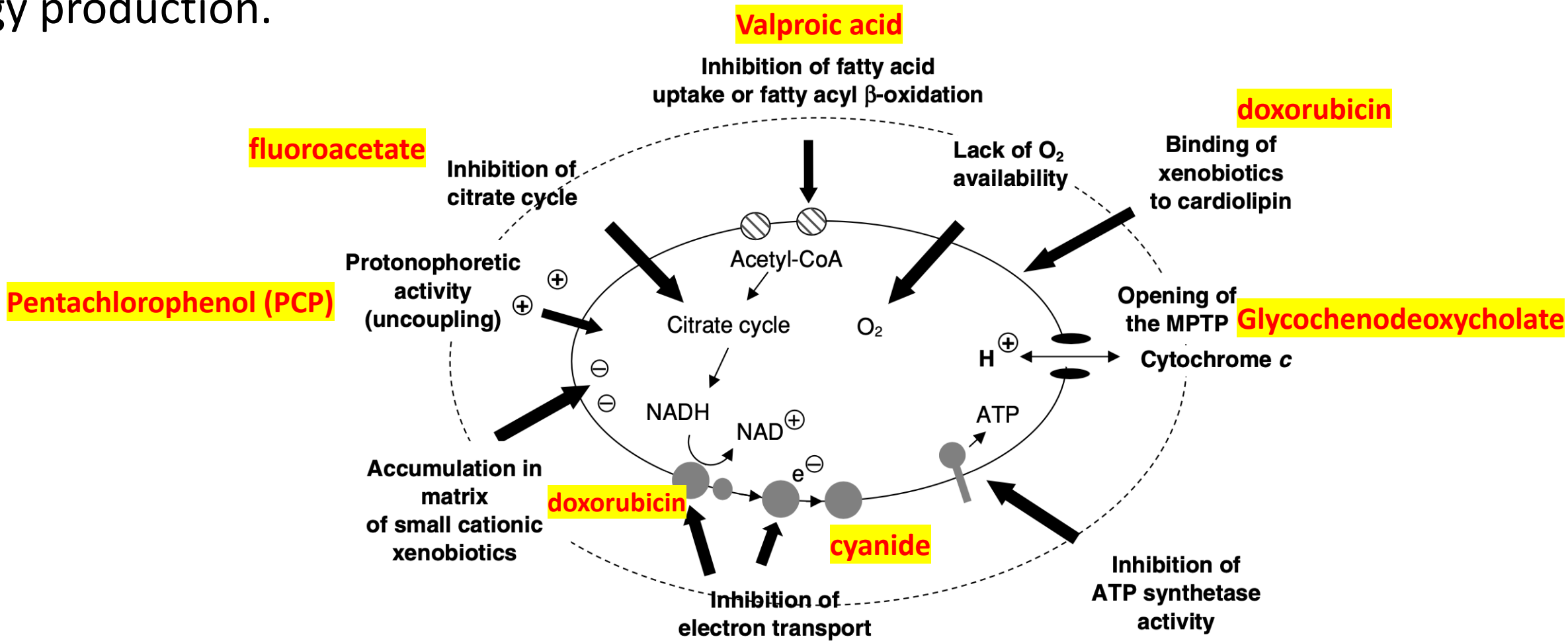

Figure 15.2. Possible sites of xenobiotic interference with mitochondrial function, resulting in mitochondrial toxicity.

A. Protonophoretic and uncoupling activity of xenobiotics

Pentachlorophenol (PCP) is a widely used fungicide and wood impregnating agent.

- **Molecular mechanisms of PCP toxicity:** PCP can easily penetrate OMM and reach the intermembrane space, where it is protonated. The uncharged lipophilic molecule can cross the IMM where it is dissociated. The PCP anion is then expelled back to the intermembrane space, and the cycle is repeated.
- Thus, PCP shuttles back the H+ into the matrix, preventing the H+ from accumulating in the inter membrane space and from driving the ATP synthetase. Thus, PCP is a 'protonophore' (it transports protons). The result is uncoupling of the oxidative phosphorylation

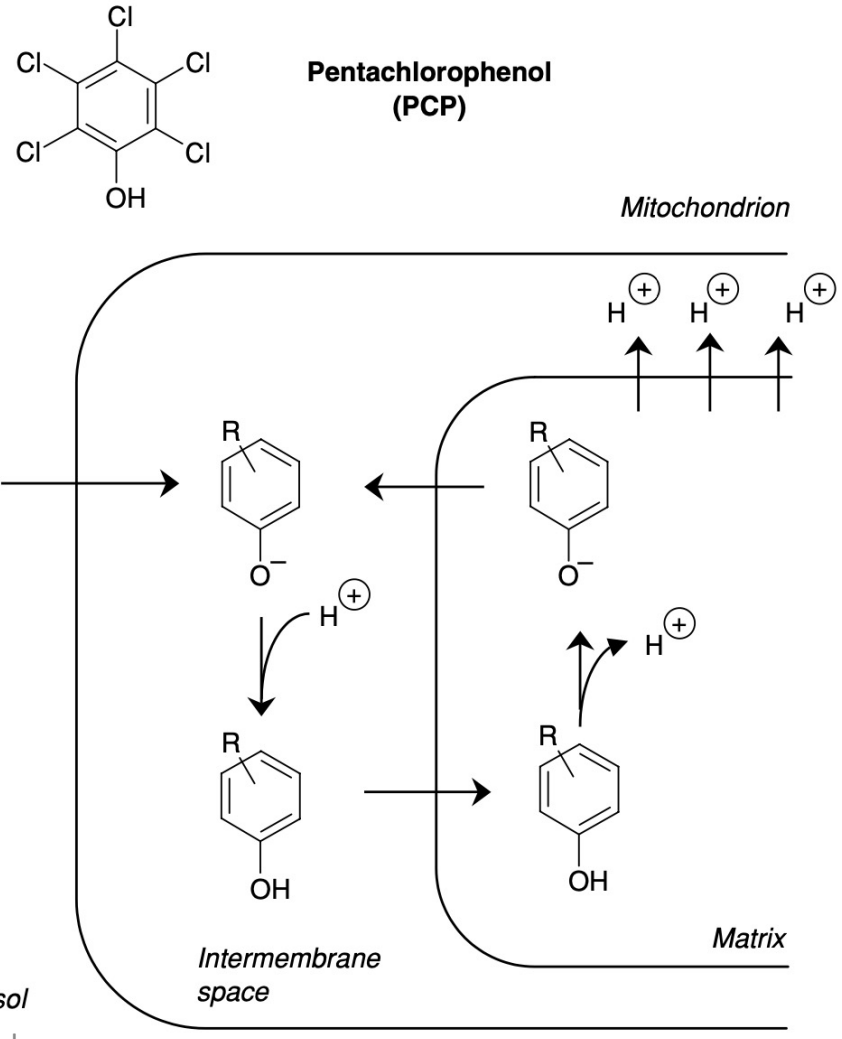

Figure 15.3. Pentachlorophenol is an uncoupler because it transports protons back into the matrix, thus dissipating the mitochondrial membrane potential.

B. Inhibition of NADH production

NADH is the major reducing equivalent in the mitochondrion and is used to:

a) provide electrons for maintaining the proton pumps

b) reducing oxygen to water

c) finally to synthesize ATP from ADP and Pi.

➢ Hence, inhibition of the production of NADH by xenobiotics must invariably lead to impaired energy production and toxicity.

This disruption can occur at several distinct sites including:

1. the β-oxidation pathway, a major energy (NADH)-generating process
2. the citrate cycle.

1. Inhibition of mitochondrial fatty acyl β-oxidation

- Fatty acids are progressively shortened into acetyl-CoA products by a process called β-oxidation.
- Because the enzymes involved in the β-oxidation process are located in the mitochondrial matrix, fatty acids must first cross the inner (impermeable) mitochondrial membrane. **long-chain fatty acids** (i.e. C14–C18) require a specific transport system. In a first step, they are activated by coenzyme A to the CoA thioester, which allows for a subsequent coupling to carnitine, a shuttle molecule that transports the long- chain fatty acids across the membrane and reactivates them by binding again to CoA. In the matrix, the fatty acyl-CoA is then enzymatically shortened into acetyl-CoA, the substrate for the citrate cycle. During this oxidative process, NADH is generated.
- Xenobiotics can interfere with any of these enzymatic steps of the β-oxidation process. For example, if **xenobiotics interfere with the availability of the cofactor, coenzyme A**, then fatty acyl activation and transmembrane transport are severely impaired. An example is the drug valproic acid.

Mechanisms of valproate toxicity:

At least two mechanisms have been implicated in VPA mitochondrial toxicity:

1. decrease the cellular levels of **free CoA and carnitine.**
2. direct inhibition of the **enzymes** involved in the β-oxidation process.

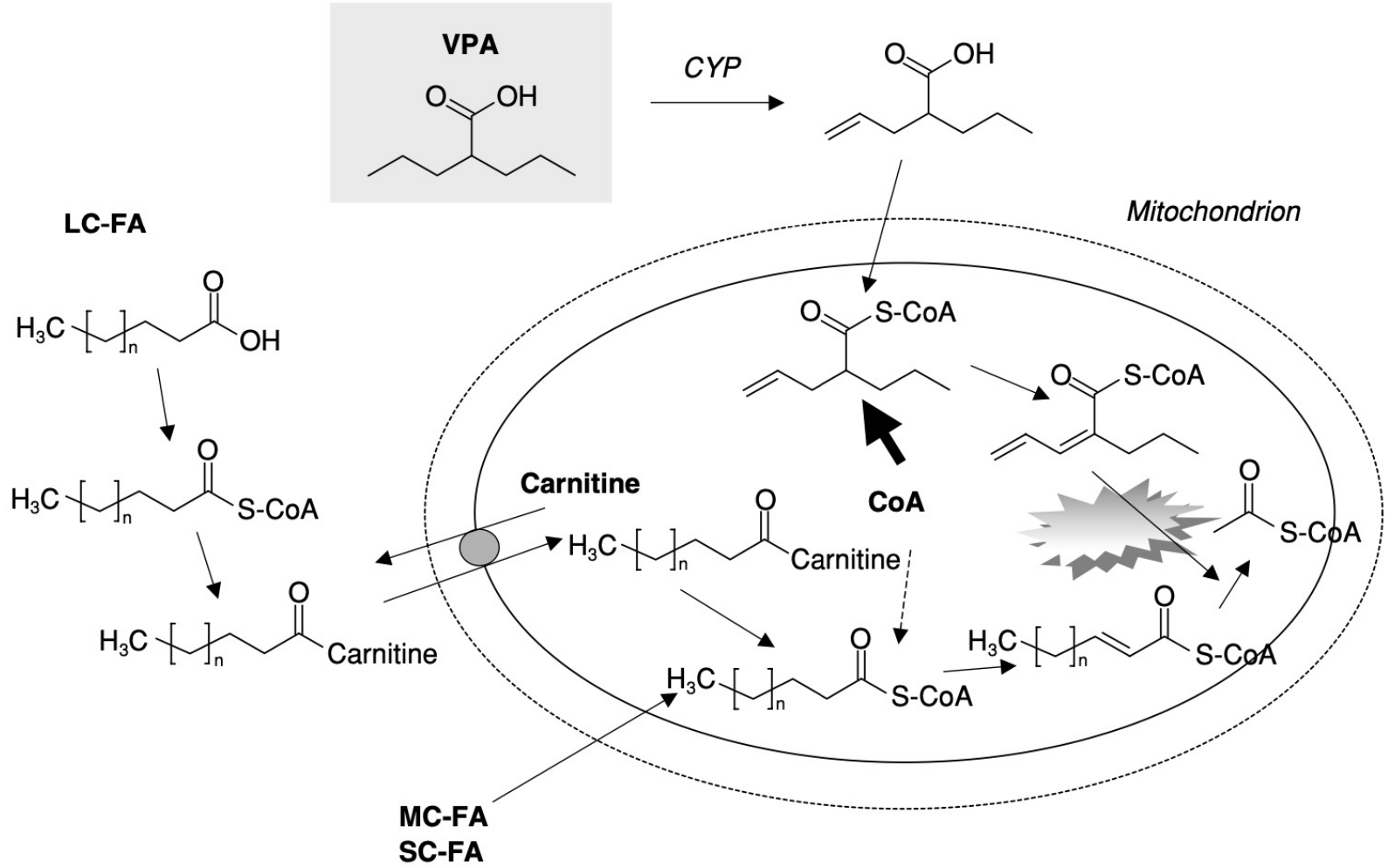

Figure 15.4. Mitochondrial fatty acid β-oxidation and mechanisms of valproic acid (VPA)-induced inhibition of β-oxidation. VPA sequesters intramitochondrial CoA (thick arrow) and therefore prevents fatty acid activation. In addition, its oxidative metabolite, Δ^4-VPA, undergoes the first step of β-oxidation to Δ^2-VPA. The latter is an electrophilic metabolite that directly reacts with and inhibits enzymes involved in β-oxidation. LC-FA, long-chain fatty acid; MC-FA, medium-chain fatty acid; SC-FA, short-chain fatty acid.

Side-effect of the inhibition of β-oxidation by VPA

- Because mitochondrial β-oxidation of fatty acids is the primary source of energy in some organs (liver, heart) in the fasting state, sustained inhibition of β- oxidation can lead to a **severe energy crisis.**
- Another side-effect of the inhibition of β-oxidation is accumulation of fatty acids. This can take extreme forms and lead to the development of microvesicular steatosis.

2. Xenobiotics as pseudosubstrates for the citrate cycle

- Xenobiotics which interrupt the citrate cycle cause a block in the production of NADH.
- Such an interruption of the cycle can occur when a xenobiotic mimics a natural substrate that is part of the citrate cycle. One example is fluoroacetate, which mimics acetate but cannot be fully metabolized like acetate.

Mechanisms of fluoroacetate toxicity:

- Fluoroacetate **is an analog of acetate.**
- Fluoroacetate mimics the first few steps of acetate metabolism, i.e. it couples to CoA to form fluoroacetyl-CoA. Condensation with oxaloacetate yields fluorocitrate. However, the following isomerization step is not possible, because fluorocitrate inhibits aconitase (catalyze the isomerization of citrate to isocitrate). The strongly electronegative fluorine atom interacts with the iron center of aconitase, making an isomerization impossible.
- The entire citrate cycle comes to a stop, and NADH production is inhibited.
- This process is called a '**lethal synthesis**', because the organism synthesizes substrate analogs which in a later step block the metabolism and irreversibly damage the organism.

Mechanisms of fluoroacetate toxicity:

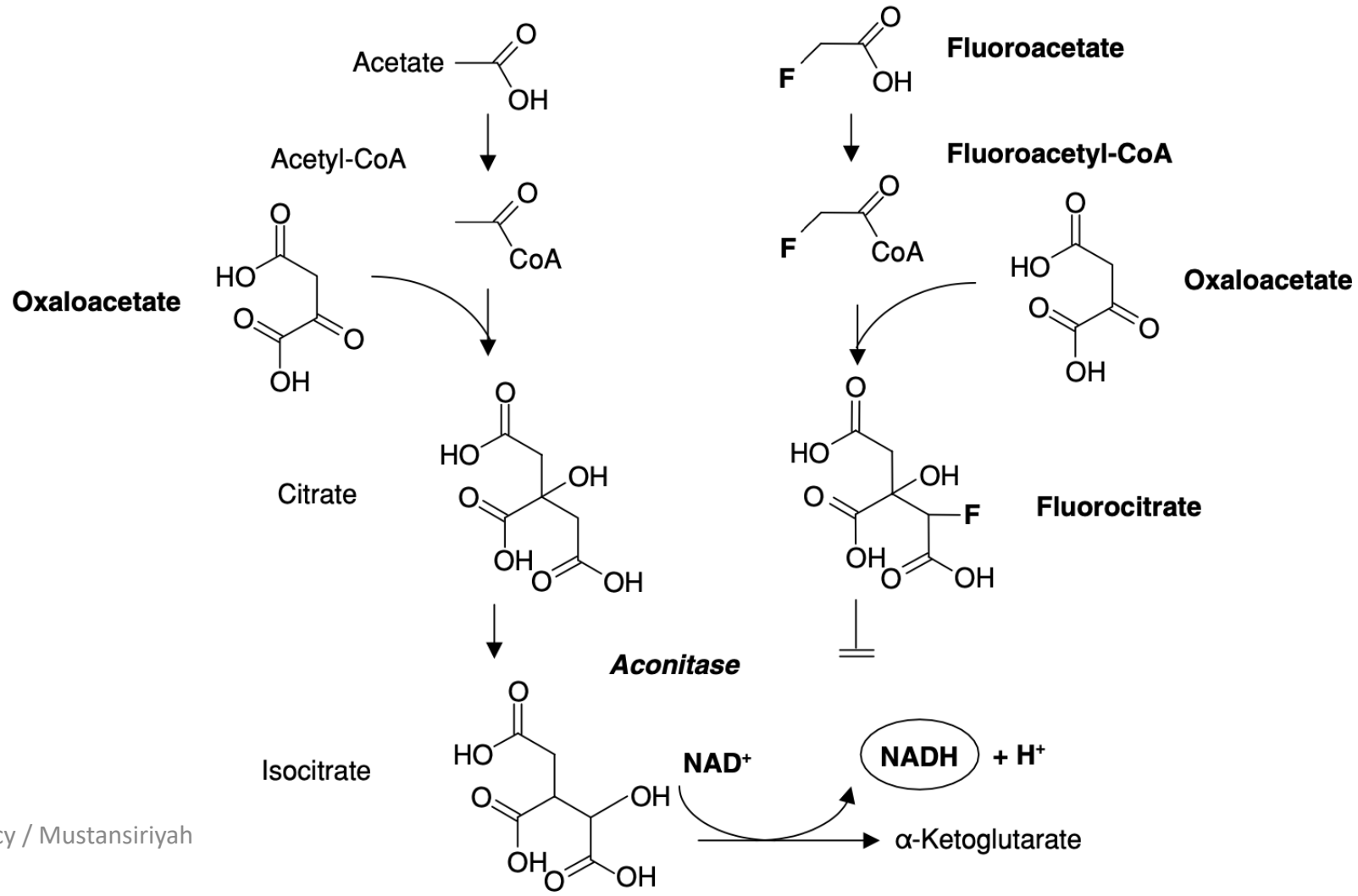

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Figure 15.5. Fluoroacetate is an analog of acetate and is gated into the citrate cycle and processed to fluorocitrate. Fluorocitrate, however, inhibits the enzyme aconitase and, hence, blocks the entire citrate cycle, resulting in a an inhibition of NADH production.

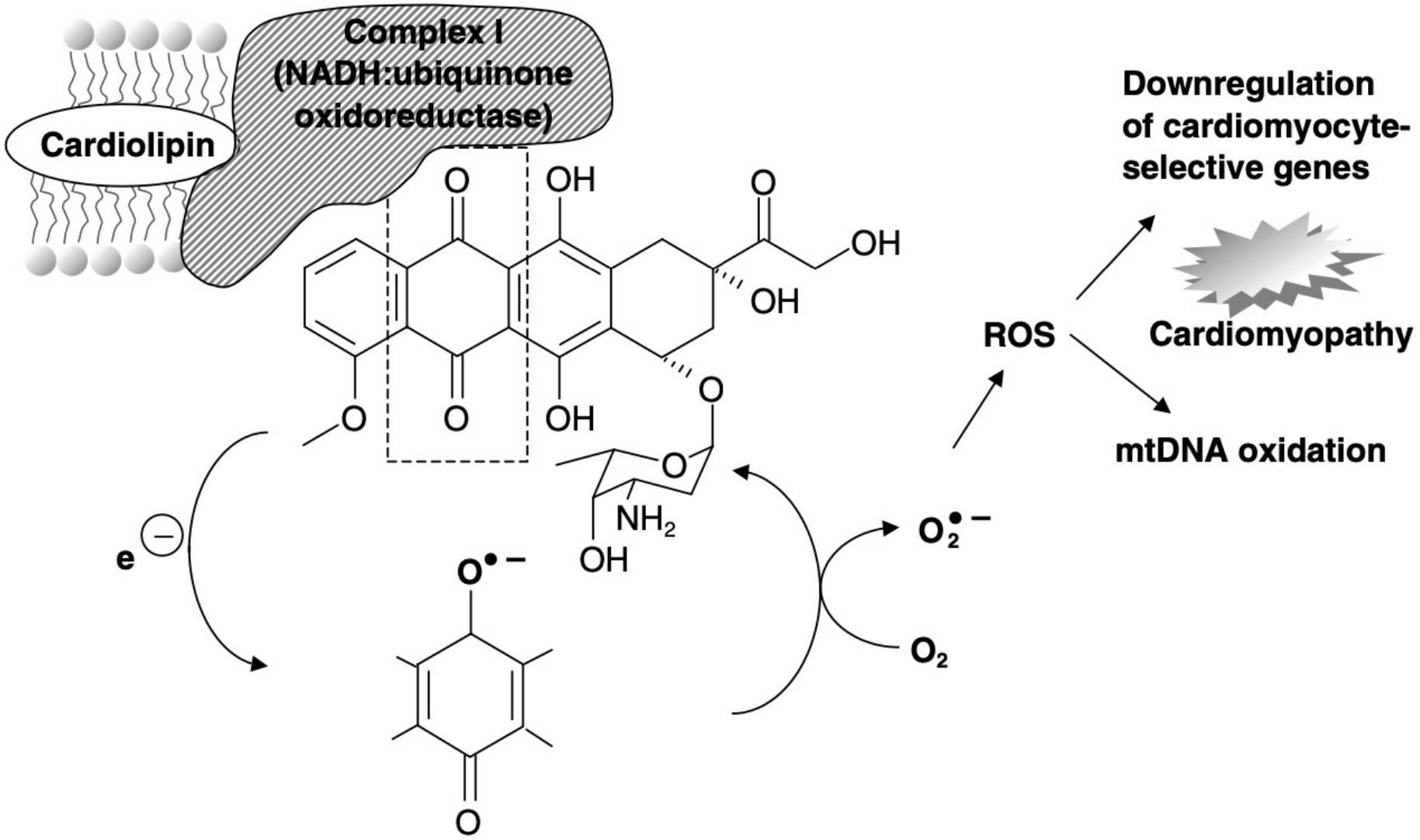
C. Inhibition of the electron transport chain and increased generation of ROS

- Xenobiotics can inhibit the electron flow at several points of the electron transfer cascade. The result will be a decreased proton pump function and, ultimately, inhibition of ATP synthetase.
- There are two types of electron transport chain inhibitors.

1. The first type of **compounds block the electron transport by binding to one of the components of the electron transport chain.** Examples include rotenone and cyanide.
2. The second type are **compounds that actually stimulate electron flow through the initial parts of the transport chain, but at another divert the electron flow** from their normal path by accepting the electrons themselves. Examples are redox cycling agents such as doxorubicin.

Mechanisms of doxorubicin toxicity to mitochondria:

- One of the mechanisms that might explain the mitochondria-selective toxicity is that doxorubicin has a high affinity for cardiolipin at the inner mitochondrial membrane and therefore accumulates at that subcellular location.
- A look at the chemical structure of doxorubicin reveals that it possesses a quinone moiety. Doxorubicin is an excellent electron acceptor from complex I of the respiratory chain, thereby reducing the quinone to its semiquinone radical. This latter is unstable and rapidly autoxidizes to the parent quinone. Due to its high redox potential, it reduces molecular oxygen to the superoxide anion radical and thus undergoes redox cycling . Resulting in production of oxidative stress, include oxidation of mitochondrial glutathione, induction of the MPTP, and cardioselective oxidation of mitochondrial DNA.



production of oxidative stress, include

1. oxidation of mitochondrial glutathione
2. induction of the MPTP
3. cardioselective oxidation of mitochondrial DNA.

Figure 15.6. Redox cycling of the quinone–semiquonone moiety of doxorubicin leads to the generation of oxidative stress which affects the mitochondrial DNA as well as altering cardiomyocyte-specific gene expression.

Why doxorubicin selectively damages the myocardium and exerts a cardiospecific toxicity ?

1. First, the heart simply needs a high and continuous supply of ATP and is thus extremely vulnerable.
2. Second, the heart has a very low antioxidant capacity and is prone to the consequences of oxidative stress. For example, GS-peroxidase and catalase activity in heart muscle are very low.
3. Third, doxorubicin can alter cardiacspecific transcription. For example, **protein kinase C-mediated regulation of intracellular Ca2+** and down-regulate the expression of the heart- and muscle- specific isoform of ADP/ATP translocase .The **ADP/ATP translocase** transports newly synthesized ATP from the inner side of mitochondria into the cytosol, and ADP from the cytosol into mitochondria. This protein is abundant in the inner mitochondrial membrane, and also constitutes part of the MPTP.

Why the effect of doxorubicin is persistent for many weeks after cessation of treatment, and why oxidative stress continues long after discontinuation of the drug ?

doxorubicin directly oxidizes and damages mitochondrial DNA. This effect of doxorubicin could explain why the toxic effects on the heart are irreversible (irreversible cardiomyopathy) in patients, since expression of the mitochondrial genome is crucial to the integrity of the respiratory chain. It is therefore possible that newly synthesized dysfunctional mitochondria may continue to produce high levels of ROS, even after cessation of doxorubicin administration, and thus would provide a continuous source of oxidative damage to the mitochondrial DNA.

Mitochondrial DNA (mtDNA) is highly susceptible to oxidation by ROS. The reasons are manifold.

1. mtDNA in the matrix is in close proximity to the site where relatively large amounts of ROS are generated constantly, even under basal conditions of respiration and in the absence of xenobiotics.
2. Unlike nuclear DNA, mtDNA does not encode RNA and proteins with nonsense sequences, except for a small sequence. Therefore, any mtDNA oxidation is likely to result in a biologically significant effect.
3. mtDNA does not possess any protective histones, and the DNA repair mechanisms for mtDNA are much less effective than those for the nuclear DNA.

- Therefore, oxidative injury in the mitochondria leads to accumulation of oxidative base damage.
- In fact, **mtDNA exhibits a more than ten-fold higher basal incidence of oxidized deoxyguanosine than nuclear DNA.**

Mechanisms of cyanide toxicity:

- Cyanide binds to ferric iron-containing heme proteins such as the **cytochrome α3 moiety of the complex IV**, which is the terminal protein complex of the electron transport chain.
- Block of complex IV by cyanide causes a rapid and severe depletion of cellular ATP content that results in cell death due to energy impairment

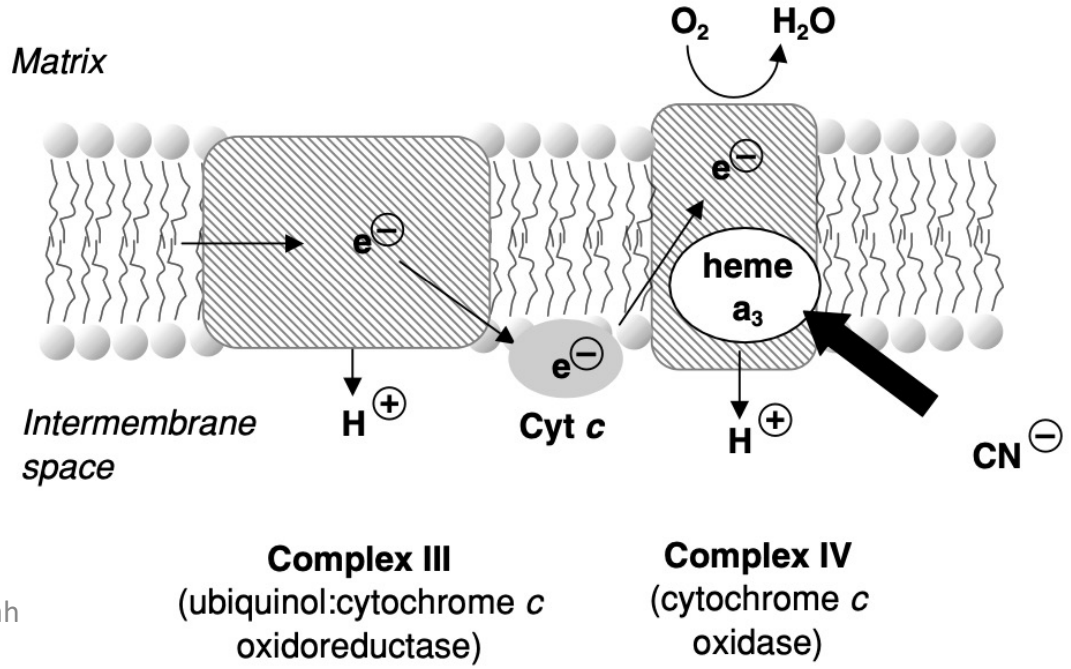


Figure 15.7. Cyanide binds to the heme a_3 subunit and inhibits the terminal cytochrome *c* oxidase (complex IV) of the electron transport chain.

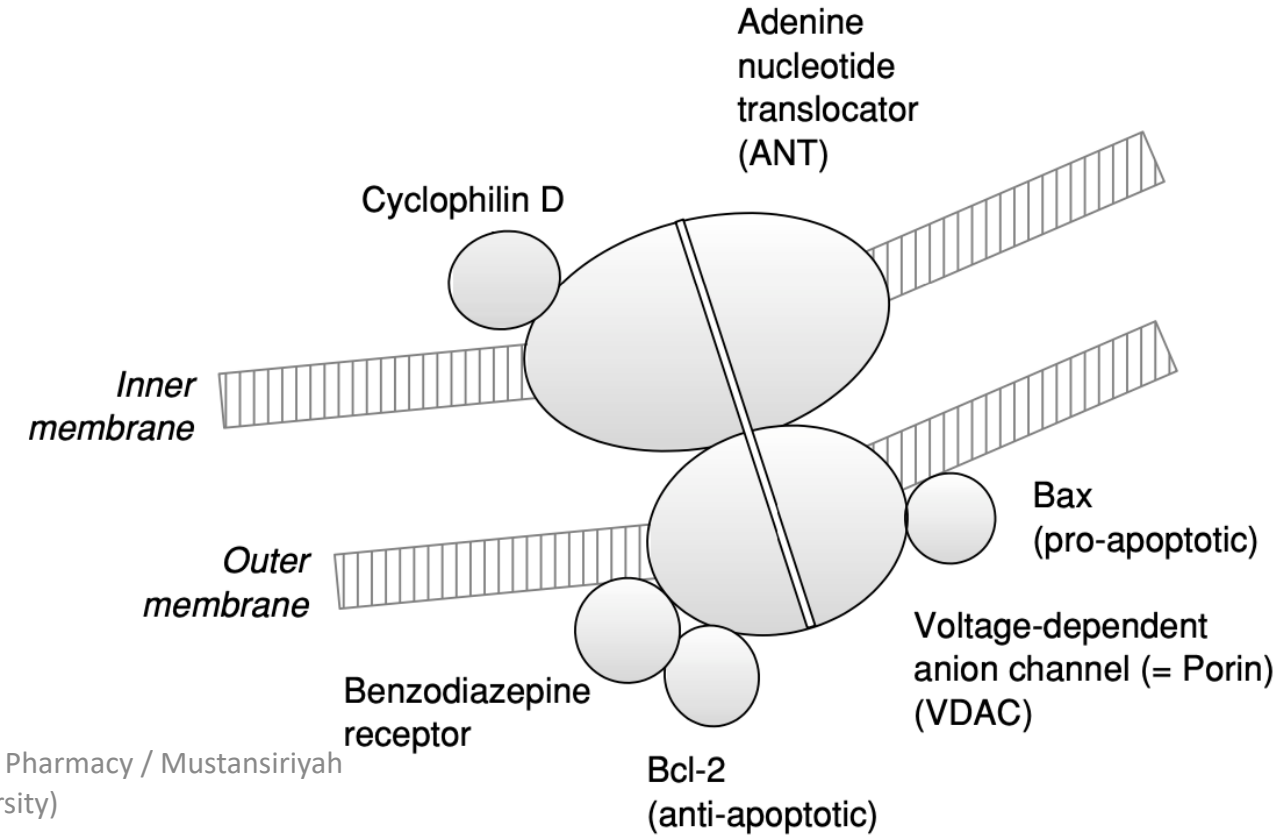
Opening of the mitochondrial membrane permeability transition pore (MPTP)

- the phenomenon of ‘membrane permeability transition’ has been increasingly recognized as an important mechanism of cell death.
- Indeed, many xenobiotics are able to cause an opening MPTP and thus rapidly increase the permeability for solutes of the otherwise largely impermeable inner mitochondrial membrane. This has dramatic consequences.

Model of the membrane permeability transition pore (MPTP) and its components.

The membrane permeability transition pore (MPTP) is a membrane-bound protein complex in mitochondria. This complex forms a pore which under normal conditions is closed. It is composed of a number of proteins including:

1) the voltage-dependent anion channel (VDAC), located in the OMM
2) the ANT (ADP/ATP) exchanger, located in the IMM
3) cyclophilin-D, located in the matrix.
4) This complex is associated with a number of other proteins including Bax and Bcl-2 which play a role in apoptosis.



xenobiotic-induced opening of MPTP

- One important group of compounds that are known to produce oxidative stress and, hence, induce MPTP opening are the toxic hydrophobic bile acids which accumulate in cholestasis.
- **Toxic hydrophobic bile acids** are produced from :

1. metabolism bile acid by the intestinal flora and can accumulate in the liver under conditions of cholestasis.
2. This occurs also by xenobiotics which have the potential to induce cholestasis .For example, the glycine conjugate of chenodeoxycholic acid becomes toxic when it accumulates and reaches high cellular concentrations (increased up to 20-fold in the liver)

Mechanism of Toxic hydrophobic bile acids -induced cell death

1) induction oxidative stress in hepatocytes, by interfering with the electron transport chain.
2) recruitment the pro-apoptotic protein Bax, inducing the MPTP opening.

❖ **Oral administration of ursodeoxycholic acid (UDCA) can protect against cholestasis-induced liver damage HOW??**

➢ The cytoprotective mechanisms indicates that UDCA can inhibit the opening of the MPTP bec. it prevents the recruiting of Bax from the cytosol

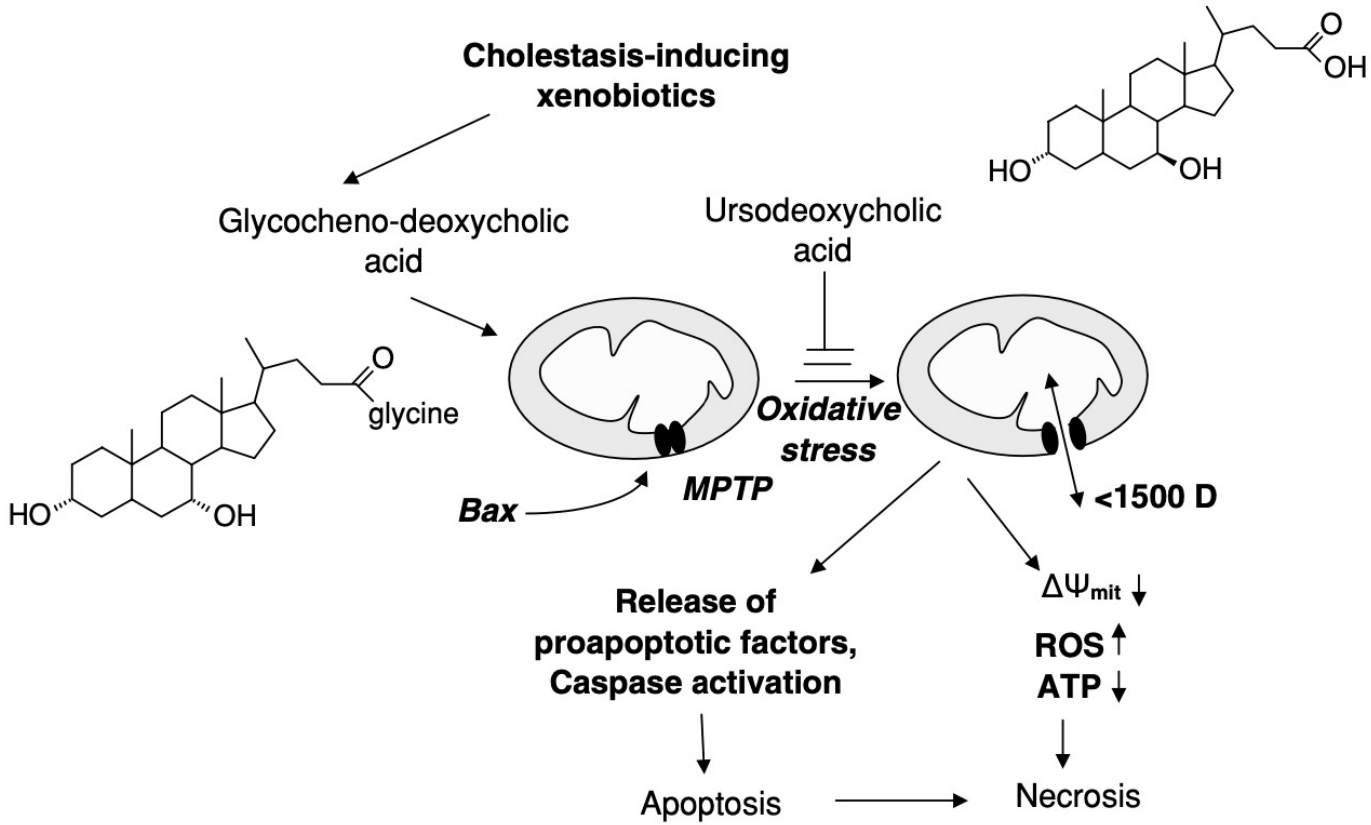


Figure 15.9. Toxic hydrophobic bile acids can induce opening of the MPTP through oxidative stress, leading to both apoptosis and necrosis. The hydrophilic bile acid, ursodeoxycholate, is protective because it inhibits the opening of the pore and blocks cell death.