# **MCD Tutorial 3 – Metabolic poisons**

**Take home messages**

**Points to consider**

The main concept to understand in this tutorial is that there is a direct relation between the supply of utilisable energy in the form of ATP and the demand for energy use at any one time. In other words ATP is not made unless ATP is required to drive another process.

The fundamental cellular structures/processes involved are the mitochondrion and the Kreb's cycle and oxidative phosphorylation that they house.

**Question: *Why is mitochondrial respiration dependent on ADP?***

This relates to the physico-chemical coupling of the mitochondrion. In other words, as substrates move along the electron transfer chain each component of the complex uses the energy released in the electron transfer to transport H+ across the inner mitochondrial membrane, so the [H+] is higher on the outer side of the membrane. This electrochemical gradient of H+ represents a temporary store of energy that is used by ATP synthase to phosphorylate ADP to ATP, the H+ flowing back across the membrane in the process.

This “metabolic coupling” of the electron transport chain and oxidate phosphorylation ensures that substrates are only metabolised when there is a demand for ATP. In other words O2 consumption at the end of the chain only occurs in the presence of substrate and ADP (see Figure a. in MCT handbook).

**Question:**  ***Why is the rate of respiration in the presence of succinate and ADP greater than with glutamate plus malate and ADP?***

NADH-linked substrates such as malate feed electrons into the electron transfer chain via NADH dehydrogenase (part of complex1) and so can generate 3 ATP for each oxygen atom reduced to H20. In contrast, oxidation of succinate feeds electrons into the chain via FADH2 and succinate dehydrogenase (part of Complex II) thus utilising only two of the phosphorylation sites. So, to generate a given quantity of ATP, more succinate must be oxidised than malate and glutamate and hence more O2 used.

Note: why is it necessary to add simultaneously glutamate and malate as NADH-linked substrates?

*In vivo* these two substrates are required for the malate/aspartate shuttle: the principal mechanism for the movement of reducing equivalents (e.g. the NADH produced during glycolysis) from the cytoplasm to the mitochondria matrix. The oxaloacetate concentration in mitochondria *in vivo* is exceedingly low (0.1 µM) whereas malate concentration is about 10 mM, due to the unfavourable reaction catalyzed by malate dehydrogenase. In the experiments involving isolated mitochondria the addition of malate alone causes the build-up of oxaloacetate, which would inhibit the malate dehydrogenase and therefore the production of NADH (the electron source for the respiratory electron transport). However, the further addition of glutamate allows the excessive oxaloacetate to be removed from the mitochondrial matrix via the malate/aspartate shuttle (as described in your lectures on **Metabolic Pathways**) and therefore the production of NADH to continue while malate is available. Moreover, an active malate/aspartate shuttle allows for -ketoglutarate to be produced in the mitochondrial matrix making it available for its exchange for malate by the malate/-ketoglutarate antiporter, allowing a constant flow of malate into the mitochondria.

**Question:**  *What happens when KCN is added?*

By reacting covalently with the Fe3+ in cytochrome oxidase, the respiratory poison KCN inhibits the terminal step in the electron transport chain, so respiration ceases.

**Question:**  ***Under normal circumstances, what would happen after addition of oligomycin?***

Oligomycin interferes with and hence reduces the ability of the ATP synthase to utilise the H+ electrochemical gradient. So, in coupled mitochondria, oligomycin would be expected to inhibit respiration. However, in this instance oligomycin is without effect (Fig. b) suggesting that the prior addition of DNOC has uncoupled the mitochondria. In other words the Kreb’s Cycle and oxidative phosphorylation are running maximally but the link to the generation of ATP is broken so the free energy released from substrates is lost as heat. Consistent with this is the large increase in mitochondrial O2 consumption after addition of DNOC.

**Question: *What do the data allow you to conclude regarding the metabolic effect of DNOC?***

Aromatic weak acids such as DNOC and dinitrophenol are thought to pass readily across the mitochondrial inner membrane in their undissociated form thus dissipating the electrochemical gradient.

**Inside**

**DNOC-H DNOC- + H+**

**Outside**

**DNOC- + H+ DNOC-H**

**Question: *Why were the respiratory rate and body temperature elevated?***

DNOC acts as a pesticide by uncoupling mitochondrial respiration in insects so that their electron transport chain runs uncontrollably and unproductively. The same has happened in the two agricultural workers. Large amounts of metabolic fuels are consumed with the released energy being wasted as heat.

**Question: *Can you provide explanations for the post mortem findings (absence of body fat, the effect on the femur, and the rapid onset of rigor mortis)?***

The principal fuels used for this uncontrolled respiration are fatty acids from the triglycerides stored in adipose tissues, thus depleting the body's fat stores. The accompanying excessive oxygen consumption leads to tissue hypoxia, which the body attempts to alleviate by increased pulmonary respiration and by erythropoesis in the bone marrow.

The rigor mortis can be explained by considering muscle biochemistry. The power stroke moves actin filaments relative to the heads of the myosin, so shortening the muscle fibres. This involves ATP hydrolysis to ADP by myosin ATPase. To relax the fibre for the next power stroke, the ADP must be displaced by incoming ATP. Since DNOC poisoning greatly decreases the concentration of ATP, the contractile system is left in rigor.