4

DEATH ON THE FARM

Introduction

The success and profitability of modern agriculture depend on, among other things, tight control of pests so that maximal yields of crops are obtained. To some extent this has been achieved by growing varieties which are naturally resistant to pests. With the advent of the 'biotechnological revolution', we are now moving into the era of 'designer plants' and there will be increased reliance on crops genetically engineered for resistance to specific pests. However, in the meantime, agriculture relies heavily on spraying crops with chemical pesticides.

The ideal pesticide is of course one that is selectively toxic to the pest but harmless to humans and other species. In fact a great deal of effort is put into discovering such chemicals, but rarely is this goal of specificity attained. The difficulty is that eukaryotic species have so much in common that there are very few differences in their metabolism which can be the focal points for differential attack. This problem is not just confined to pest control in agriculture; in treating patients with cancer or with diseases due to eukaryotic parasites, the clinician faces the dilemma of knowing that the drugs used in chemotherapy will often also harm the patient.

Because of this, agents are used in agriculture that are substantially toxic to humans and therefore hazardous to agricultural workers, particularly during crop spraying, unless stringent precautions are taken (Figure 4.1).

Figure 4.1. Agricultural workers spraying crops.

The Problem

Two agricultural workers were employed to spray cereal crops with a preparation containing *o*-dinitrocresol (DNOC) for pest control. On the final day of the spraying programme both men began to feel ill, and one of them died whilst returning to the farm for help. The doctor who examined the corpse shortly afterwards noted that rigor mortis was unusually far advanced. The other man was admitted to hospital. His temperature was 39.3°C and he was sweating profusely with a respiratory rate of 60/min. He was extremely anxious and his pupils were dilated. Despite strenuous attempts to reduce his body temperature, he also died in a coma shortly after admission. Autopsy findings included pulmonary congestion and slight oedema. There was a striking absence of subcutaneous and omental fat. The shaft of the femur contained red marrow throughout, with microscopic findings consistent with anoxaemia. In view of the clinical findings, a sample of the pesticide was tested on a preparation of rat liver mitochondria. Representative recordings obtained with an oxygen electrode are shown in Figure 4.2.

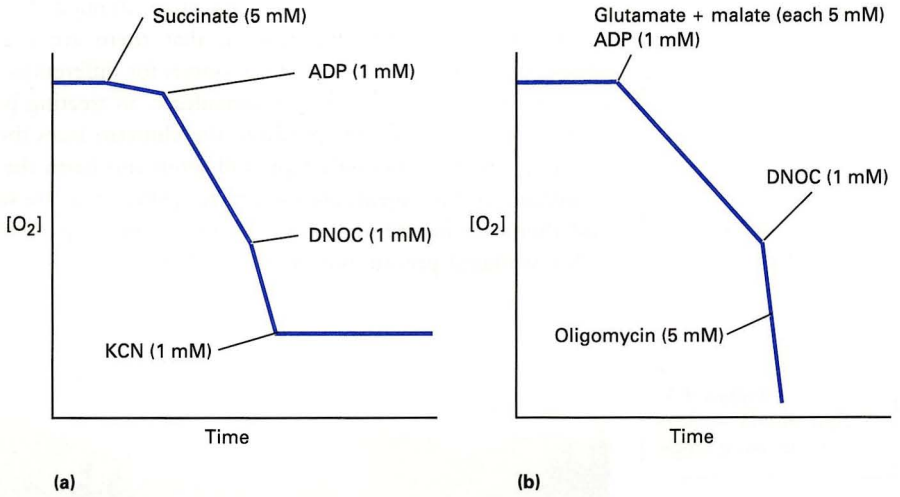


Figure 4.2. Effects of DNOC on rat liver mitochondria. A suspension of freshly-prepared rat liver mitochondria in a suitable buffer was incubated in an oxygen electrode chamber. Various compounds were added to the chamber as indicated (chamber concentrations shown) and changes in the oxygen consumption were recorded.

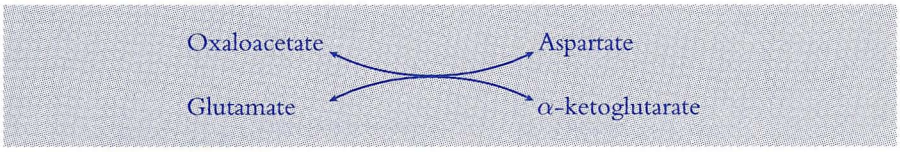
Questions

1. Draw a simple diagram of the mitochondrial electron transport system to indicate the order of the principal redox carriers. Where are the sites of oxidative phosphorylation and the entry points for the substrates used in the oxygen electrode experiments?
2. Why is mitochondrial respiration dependent on ADP (Figure 4.2a)? What is the reason for adding both glutamate and malate (Figure 4.2b)? Why is the rate of respiration in the presence of succinate and ADP (Figure 4.2a) greater than with glutamate, malate and ADP (Figure 4.2b)? Explain the results of adding KCN (Figure 4.2a).
3. Under normal circumstances, what would happen after addition of oligomycin (Figure 4.2b)?
4. What do the data allow you to conclude regarding the metabolic effect of DNOC?
5. Why were the respiratory rate and body temperature elevated?
6. Can you provide explanations for the absence of body fat, the effect on the femur and the rapid onset of rigor mortis?

Commentary

Foods are oxidised to release their stored chemical energy. Oxidation involves transfer of electrons from food substrates to O_2 which is reduced to H_2O. Electrons are not passed directly to O_2 but via a chain of reduction–oxidation (redox) carriers (the electron transport chain) located in the mitochondrial inner membrane (Figure 4.3). The chain consists of four multi-component complexes (I, II, III and IV) linked by mobile redox carriers (CoQ and cytochrome *c*). Each complex uses the energy released in the electron transfer to transport H^+ across the 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.

Metabolic coupling of the electron transport chain and oxidative phosphorylation ensures that substrates are only metabolised when there is a demand for ATP. The fact that O_2 consumption only occurs at a significant rate when both a substrate and ADP are present (Figure 4.2) indicates that the mitochondria are tightly coupled. Malate enters the mitochondrion and is oxidised to oxaloacetate by malate dehydrogenase with concomitant reduction of mitochondrial NAD^+ to NADH which feeds into the electron transport chain. For continued oxidation of malate, oxaloacetate must exit the mitochondrion. However, oxaloacetate itself is only poorly transported so it is transaminated to aspartate, which is readily transported. The transaminase involved, glutamic-oxaloacetic transaminase (also called aspartate transaminase), utilises glutamate and α-ketoglutarate as the other amino acid/keto acid pair:



In effect, the oxygen electrode experiment uses part of the mitochondrial aspartate–malate shuttle, a device for allowing NADH produced in the cytosol by glycolysis to be transported indirectly into the mitochondrion for oxidation back to NAD^+.

By reacting covalently with the Fe^{3+} in cytochrome oxidase, the respiratory poison KCN inhibits the terminal step in the electron transport chain, so respiration ceases.

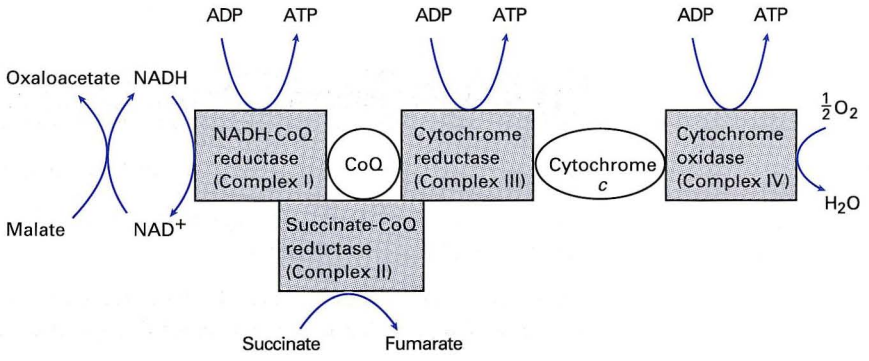


Figure 4.3. Diagrammatic representation of the electron transport chain.

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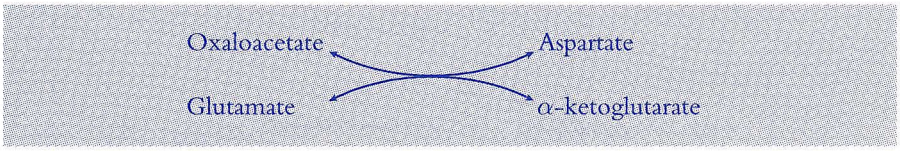

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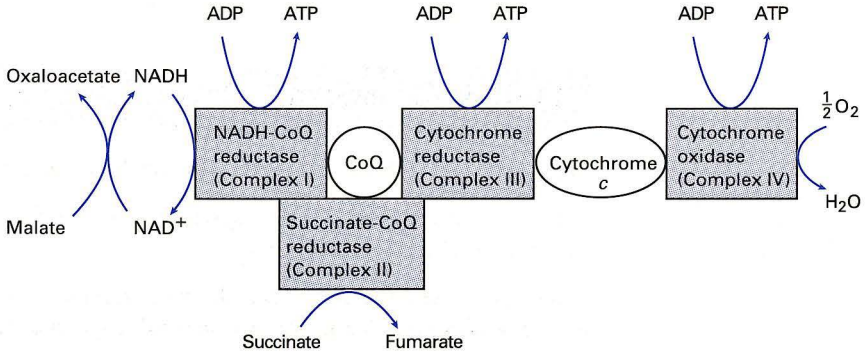

Figure 4.3. Diagrammatic representation of the electron transport chain.

NADH-linked substrates feed electrons into the chain via NADH dehydrogenase (part of Complex I) and so can generate 3 ATP for each oxygen atom reduced (P:O = 3.0). Oxidation of succinate feeds electrons into the chain via $FADH_2$ and succinate dehydrogenase (part of Complex II) thus utilising only two of the phosphorylation sites (P:O = 2.0). So to generate a given quantity of ATP, more succinate must be oxidised than malate and glutamate.

Oligomycin interferes with 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 suggesting that the prior addition of DNOC has uncoupled the mitochondria. Consistent with this is the large increase in mitochondrial O_2 consumption after addition 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 (Figure 4.4).

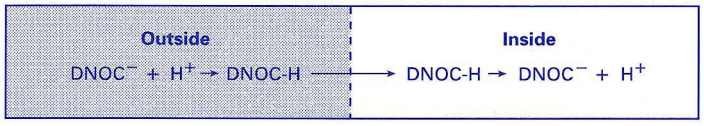

Figure 4.4. Dissipation of the electrochemical gradient by DNOC.

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. The principal fuels used for this uncontrolled respiration are fatty acids from the triglycerides stored in adipose tissue, 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 erythropoiesis in the bone marrow.

The rigor mortis can be explained by considering muscle biochemistry. The power stroke moves muscle actin filaments relative to the 'heads' of the myosin, so shortening the muscle fibre. 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.

Further Questions

1. What other mitochondrial shuttles do you know and what are their roles?
2. What are the 'benefits' of segregating the electron transport chain in the mitochondrial inner membrane?
3. Under some circumstances the electron transport chain may be uncoupled from oxidative phosphorylation. At first sight this appears to 'waste' energy but it may have

a physiological role. In which tissue(s) does this type of uncoupling occur and in what circumstances?

4. So-called 'futile cycles', such as that occurring by the simultaneous operation of phosphofructokinase and fructose bisphosphatase, also appear to 'waste' energy by fruitlessly consuming ATP. What are thought to be the advantages of using ATP in this manner?

5. What would have occurred if rotenone or antimycin had been added along with glutamate and malate, or succinate, in Figure 4.2?

6. Many years ago uncouplers such as dinitrophenol were suggested as drugs to aid slimming. What was the rationale and would there be any dangers?

7. In this problem the victim's tissues became anoxic and the response was increased erythropoiesis. How does the body sense anoxia and how is erythropoiesis stimulated? (See also Problems 6 and 11.)

Connections

- Use this problem to review your understanding of mitochondrial oxidative metabolism to produce energy in the cell. This will include a knowledge of the nature of the electron carriers in the electron transport chain, reduction–oxidation potentials and the mechanism of oxidative phosphorylation. Ensure you understand how an oxygen electrode is used to study respiration, how the order of the electron transport carriers was determined and what is meant by the term P:O ratio. (See also Problem 21.)

- Review the mechanism of muscular contraction – the role of the proteins, actin and myosin, the way in which ATP is used to supply energy for contraction and the importance of calcium. Remember that there are many other types of cellular movement – make a list of these and ensure you understand the biochemical principles involved.

- The cytochromes in the electron transport chain are haem proteins, but so are haemoglobin and myoglobin. Remind yourself of the essential differences in the roles of the haem groups in each of these proteins. Respiratory poisons such as cyanide and carbon monoxide inhibit these proteins. Check that you understand the way in which carbon monoxide from a car exhaust causes death. Studies on haemoglobin have told us a lot about how the structure of a protein determines its functions. Make a list of the more important haemoglobin mutations and what they tell us about this structure–function relationship. (See also Problem 11.)

- Although oxidative phosphorylation is by far the most important source of ATP in the majority of cells, ATP can also be produced under anaerobic conditions, as happens during a sprint. Outline how ATP is produced in muscles working under these conditions and what the problems are in sustaining this type of activity. (See also Problem 1.)

- Carbohydrates and fats are the metabolic fuels of the body and they are eventually broken down into compounds which can undergo complete oxidation to CO_2 in

the mitochondria. However, they are not exactly equivalent fuels and each has its own advantages and disadvantages. Make a list of these.

- Remind yourself of how mitochondria are prepared from tissue homogenates by subcellular fractionation. How would you ascertain the purity and activity of a preparation of mitochondria?
- NADH is a coenzyme whereas the haem group in a cytochrome is a prosthetic group. Remind yourself of the mechanistic difference between these two terms. NADH and $FADH_2$ are derived from vitamins. Use this to revise your knowledge of the roles of all the vitamins required by humans.
- In this problem NADH produced during oxidation of fuels was involved in the generation of ATP. NADPH is utilised for quite different purposes. Remind yourself of what these are and where NADPH is produced in the cell.
- Many pesticides are carcinogenic. Check that you understand how carcinogens act as mutagens, and the relationship between carcinogens and pre-carcinogens. (See also Problem 14.)

Reference

Council of Scientific Affairs report. Cancer risk of pesticides in agricultural workers. *Journal of the American Medical Association* **260**, 959–66.