Metabolism

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The Biochemistry Across the School Curriculum Group (BASC) was set up by the Biochemical Society in 1985. Its membership includes education professionals as well as Society members with an interest in school science education. Its first task has been to produce this series of booklets designed to help teachers of 16+ level biology and related courses to teach the sections of the 16+ level syllabus which have a high biochemical content.

Other topics covered by this series include: Essential Chemistry for Biochemistry, The Structure and Function of Nucleic Acids, Enzymes and their Role in Biotechnology, Immunology, Photosynthesis, and Recombinant DNA Technology.

More information on the work of BASC and these booklets is available from The Education Officer at the Biochemical Society, 59 Portland Place, London W1N 3AJ.

Comments on the contents of this booklet will be welcomed by the series editor, Dr. Colin Wynn, School of Biological Sciences, University of Manchester, Oxford Road, Manchester M13 9PY.

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Preface

An introduction to the study of metabolism is now an important component of most syllabuses published by the GCE Boards for Biology at Advanced Level. The various sections that require an understanding of this subject include Cell Structure and Biochemistry, Nutrition, Respiration, Energetics, Transport and Exchange as well as Metabolism itself. Teachers are encouraged to promote an understanding of the biochemical processes associated with these topics, but Examination Boards emphasise that high school students need to appreciate the principles rather than remember details of metabolic complexity or chemical formulae.

The primary aim of this booklet is to provide teachers with a reasonably detailed but fundamental background to metabolism, that reflects both the current state of knowledge and also emphasises the way in which various processes inter-relate in cells and tissues to provide a molecular explanation for physiological events. Aspects contained within certain chapters may go beyond the immediate needs of the A-level syllabus. Thus, a brief account of ketone bodies, gluconeogenesis and the glyoxylate cycle have been included in order to present the subject in a more balanced way and to prevent misconceptions from arising (e.g. that glucose can be made from fat or that it is the sole respiratory fuel of animals). While some areas may be of particular use in preparing for the Biology S-level paper it is hoped that the text as a whole will provide useful resource material to assist with the teaching of metabolism in the sixth-form.
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1. Basic Concepts in Metabolism

(i) Metabolism deals with molecular interconversions

‘Metabolism’ is the word used to describe the chemical transformations that all cells and tissues perform as an essential part of life, involving the synthesis and degradation of molecules. It is often divided into two distinct phases. Catabolism is the degradative phase of metabolism in which complex nutrient molecules such as carbohydrates, fats and proteins are degraded into simpler molecules such as CO₂, ammonia and water. This supplies the energy needed for all cellular activities as well as providing the starting materials or building blocks for biosynthesis. Anabolism is the biosynthetic phase of metabolism and is the building up of the complex molecules of life such as nucleic acids, proteins, polysaccharides and lipids. The chemical interconversions associated with catabolism and anabolism proceed rapidly at a constant temperature catalysed by specific enzymes. Their actions are integrated and regulated independently.

Two aspects of metabolism are particularly impressive. Firstly there is considerable diversity of chemical activity, with many hundreds of highly specific individual reactions taking place simultaneously within the cell. Secondly the reactions proceed at very high rates, despite the relatively low temperatures that characterise most living systems. Most of the chemical interconversions (e.g. glucose to pyruvate or lactate) do not take place as a single reaction within the cell. Instead there have evolved mechanisms requiring progressive and subtle changes in the structure of a molecule through a series of discrete reactions, each catalysed by a highly specific intracellular enzyme.

\[
\begin{align*}
\text{Overall chemical changes} & \quad A \rightarrow^{\text{ enzyme } b} C \rightarrow^{\text{ enzyme } d} E \rightarrow^{\text{ enzyme } f} X \rightarrow^{\text{ enzyme } y} Z \\
\text{Mechanism occurring within a cell} & \quad A \rightarrow^{\text{ enzyme } b} C \rightarrow^{\text{ enzyme } d} E \rightarrow^{\text{ enzyme } f} X \rightarrow^{\text{ enzyme } y} Z
\end{align*}
\]

Such chains where the product of one reaction is the substrate for the next is known as a metabolic pathway. The individual molecules that undergo transformations as they work their way through these pathways are referred to as metabolites. Enzymes are only capable of catalysing small, but highly-specific
changes to a molecule. This also allows for a controlled release of the energy associated with a chemical change in manageable amounts or 'packets' that the cell can deal with efficiently. Having multiple steps during the course of a chemical interconversion also provides great versatility and flexibility. The metabolites produced can interconnect with other metabolic routes so that a web of chemical interactions becomes possible. With suitable control mechanisms this allows molecules to follow different pathways according to the needs of the cell or the requirements of changing physiological states.

Living organisms are able to utilise a comprehensive range of nutrients and stored materials including sugars, amino acids and fats to generate the energy and biosynthetic precursors that they need. This has not required a massive increase in their metabolic complexity. The processes of energy release during the catabolism of these molecules, and the means by which the end products are subsequently re-assembled into the macromolecular components of the cell, involve relatively few additional steps many of which are common to all organisms. This is because catabolic routes converge and lead into the central pathways of metabolism, from which arise and diverge various anabolic routes.

These central pathways catalyse the reorganisation of carbon–carbon linkages allowing catabolic products to be converted into anabolic precursors. They also provide terminal pathways of energy release for all organisms capable of oxidising their food materials to carbon dioxide and water. Under many circumstances, the central pathways illustrated in Figure 1 are served by the tricarboxylic acid cycle.

![Diagram of metabolic pathways](image)

**Nutrients**

- Sugars
- Amino acids
- Fats

**Catabolic Routes**

**Biosynthetic Routes**

- Proteins
- Polysaccharides
- Lipids
- Nucleic Acids

**Central Pathways**

**Oxidations**

- Energy Reducing Power
- $\text{CO}_2 + \text{H}_2\text{O}$

**Cell Components**

Fig. 1 The main types of metabolic route in cells
(see later) although under special conditions of nutrition, microorganisms can use other cyclic series of reactions.

(ii) Energy exchange occurs between catabolism and anabolism
The oxidation of carbohydrate, protein and fat during catabolism liberates large amounts of energy. However, direct oxidation of these molecules with oxygen would release the energy as heat which could not be used by cells. Therefore the principal method of energy re-distribution involves the breakdown of large molecules using many chemical steps. Some of these steps involve inorganic phosphate ($\text{P}_1$) and the phosphorylation of ADP to ATP. This traps a considerable part of the energy in a form that can be released in a controlled fashion to drive energy-requiring processes such as growth, biosynthesis, active transport and mechanical work. ATP therefore serves as a common medium of exchange between catabolic and anabolic processes.

The metabolic degradation of glucose is an example of a catabolic process that generates ATP, but its end product depends on the availability of molecular oxygen. Tissues and cells that can use oxygen to oxidise glucose are termed aerobes and the overall process is known as respiration. In the absence of oxygen, some cells are able to convert glucose into lactate or ethanol. This way of carrying out metabolism is called anaerobic and the breakdown of glucose without recourse to oxygen is known as fermentation. The oxidation of glucose to pyruvate is termed glycolysis and is a metabolic component of both fermentation and respiration (Figure 2). Glycolysis involves many individual

![Glycolysis Diagram](image)

Fig. 2 A basic outline of glucose catabolism

- 3 -
steps, each catalysed by a specific enzyme and it is only the subsequent metabolism of pyruvate which is influenced by the presence or absence of oxygen.

The daily production of lactate in an adult human has been estimated to be about 140g. In the absence of oxygen, cells can form lactate although the rate varies from tissue to tissue. In a healthy individual doing severe exercise, over 30g of lactate can be produced within minutes because skeletal muscle receives an insufficient oxygen supply to carry out the complete oxidation of glucose. Over a twenty-four hour period the skin and blood each produce about 30g of lactate.

Certain bacteria can ferment glucose to lactate. These organisms are used extensively in the production of yoghurt when lactose, the sugar in milk, is fermented by lactic acid bacteria to lactate, giving yoghurt its characteristic flavour. A number of other fermented dairy products involve using lactic acid bacteria e.g. the production of sour cream and buttermilk.

Yeast are facultative anaerobes, capable of switching their metabolic machinery from an aerobic respiration mode to an anaerobic fermentative one. Pasteur discovered that the rate of glucose fermentation to ethanol by yeast cells is slowed down by the introduction of oxygen into the culture. Under anaerobic conditions the rate of glucose consumption by cells is 6 to 8 times faster than under aerobic conditions. The decreased rate of utilisation observed in the presence of oxygen is known as the Pasteur Effect, and can be explained by comparing the energy production (i.e. number of molecules of ATP produced) of fermentation relative to that of respiration. Anaerobic glycolysis does not fully exploit the energy available in glucose for producing ATP, and the fermentation products, ethanol and lactate, represent a waste of chemical energy by that particular organism since potentially they could be further oxidised into CO₂ and H₂O. Aerobic respiration fully oxidises glucose giving the maximum yield of ATP per molecule of glucose consumed. Under these conditions the cell will utilise glucose at a lower rate than that required for fermentation to meet its energy requirements. The ATP produced as a result of glucose metabolism is used in anabolic processes, providing the required chemical energy and producing ADP. For example in the liver, an important energy requiring process is urea biosynthesis (Figure 3) which provides a means of disposing of excess ammonia that is toxic to the central nervous system. The body actually has to use energy in converting ammonia and carbon dioxide into a neutral, very soluble and non-toxic molecule, urea, which is transported subsequently in the blood to the kidneys and excreted in the urine. An adult human synthesises 30g of urea per
day and the complete oxidation of approximately 10g of glucose would be required to provide the ATP necessary for this.

CATABOLISM

\[
glucose \rightarrow 2 \text{ pyruvate} \rightarrow 6 \text{ CO}_2 + 6 \text{ H}_2\text{O}
\]

Energy Conservation

\[
\text{ADP} + P_i \rightarrow \text{ATP}
\]

Energy Utilisation

\[
\text{urea} + \text{H}_2\text{O} \rightarrow 2 \text{ NH}_3 + \text{CO}_2
\]

BIOSYNTHESIS

Fig. 3 The coupling of biosynthetic with catabolic pathways

(iii) **ATP and NAD\(^+\)** are involved in energy conservation

ATP is a nucleotide (see *BASC Booklet No. 2*) occurring in all cell types at concentrations between 1.0–5.0 x 10\(^{-3}\) M. It exists as a highly charged molecule, the linear triphosphate structure bearing four negative charges.

\[
\text{NH}_2
\]

\[
\text{Adenosine triphosphate, ATP}
\]

Another molecule associated with energy conservation is NAD\(^+\) (nicotinamide adenine nucleotide). Reducing power, effectively electrons, is released during catabolism and NAD\(^+\) acts as a universal electron carrier in cells. The component of NAD\(^+\) that accepts electrons and becomes reduced resulting in the generation of NADH + H\(^+\) is **nicotinamide**. Nicotinamide is a B group vitamin and is an
essential component in the diet of man and higher animals. A dietary deficiency of nicotinamide results in the disease pellagra, and affected individuals show nervous disorders as a consequence of impaired energy provision in the brain. Cells also contain another electron carrier NADP⁺, that has a very similar structure to NAD⁺ but contains an additional phosphate group. These two molecules have very different biochemical functions. NAD⁺ is concerned with carrying electrons, generated during catabolism, to oxygen during respiration, while NADP⁺ supplies electrons for biosynthetic reductions in anabolic processes.

ATP synthesis is achieved by phosphorylation of ADP:

\[
\text{ADP}^{3-} + \text{H}_2\text{PO}_4^- \rightleftharpoons \text{ATP}^{4-} + \text{H}_2\text{O}
\]

If this reaction is allowed to come to equilibrium, the concentration of ATP is exceedingly small, i.e. the reaction at equilibrium favours hydrolysis. In order to drive the synthesis of ATP an input of energy is required that can do work under conditions of constant temperature.

When chemical reactions occur and reactants and products come to equilibrium, free energy changes (ΔG) take place. Reactions that proceed to equilibrium releasing free energy are known as exergonic ones and the value of ΔG is given a negative sign. Endergonic reactions, by contrast, cannot proceed unless ‘supplied with’ sufficient free energy and now the value of ΔG is given a positive sign. The precise magnitude of the free energy change obtained in a chemical reaction varies, and depends on the initial concentrations of the reactants and products and the equilibrium constant. In order to compare the energetics of different chemical reactions, they are considered under standard conditions. The standard free energy change (ΔG°) considers the reaction taking place at 25°C and 1 atmosphere pressure when all the components are at 1 M concentration. While this concentration is non-physiological it does allow characteristic ΔG° values to be assigned to every chemical reaction. The ΔG° values for certain cellular reactions are given below:

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Nature</th>
<th>ΔG° (\text{kJ mole}^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>glucose + 6O₂ → 6CO₂ + 6H₂O</td>
<td>respiration</td>
<td>−2860</td>
</tr>
<tr>
<td>glucose → 2 lactate</td>
<td>fermentation</td>
<td>−200</td>
</tr>
<tr>
<td>NADH + H⁺ + (\frac{1}{2})O₂ → NAD⁺ + H₂O</td>
<td>electron transport</td>
<td>−220</td>
</tr>
<tr>
<td>ADP + P₁ → ATP + H₂O</td>
<td>ATP synthesis</td>
<td>+30</td>
</tr>
</tbody>
</table>
Fermentation only releases a very small fraction \( \left( \frac{200}{2860} \times 100 \right) = 7\% \) of the potential energy available in glucose.

Exergonic processes can be linked or coupled to a variety of endergonic processes, and in this way free energy can be transferred between reactions. In any sequence of consecutive reactions \( \Delta G^* \) values are additive, and the overall standard free energy change for a process can be obtained from the algebraic sum of the \( \Delta G^* \) values for the individual steps. Thus under standard conditions the amount of energy required to synthesise two moles of ATP from 2 ADP and 2 Pi is \( 30 + 30 = 60 \) kilojoules.

The breakdown of glucose to lactate in theory provides sufficient energy for the phosphorylation of at least 6 molecules of ADP. However, the precise chemical mechanisms involved in fermentation limit the net production of ATP to only two molecules.

**Exergonic:**

\[
glucose \rightarrow 2 \text{ lactate} \quad \Delta G^* = -200 \text{ kJ mole}^{-1}
\]

**Endergonic:**

\[
2 \text{ ADP} + 2 \text{ Pi} \rightarrow 2 \text{ ATP} + 2 \text{ H}_2\text{O} \quad \Delta G^* = + \ 60 \text{ kJ mole}^{-1}
\]

The overall process of fermentation even after allowing for the coupled synthesis of ATP still results in a large decrease in free energy, \((-200 + 60) = -140 \) kJ per mole. The efficiency of energy conservation for this process is about \( \left( \frac{60}{200} \times 100 \right) = 30\% \). During respiration 38 molecules of ATP are synthesised when glucose is completely oxidised, suggesting that the free energy conserved as ATP is \( \left( \frac{38 \times 30}{2860} \times 100 \right) = 40\% \). Such calculations have limited value as the free energy changes used are based on standard 1 M concentrations of metabolites rather than actual intracellular values.

It is also important to recognise that when glucose fermentation is used to make ATP, cells do not carry out separately the two reactions written above. An essential requirement of coupled reactions is that a common intermediate exists to link them chemically, so that energy transfer can take place. During fermentation, key exergonic reactions are involved in producing specific phosphorylated intermediates in which energy is conserved. These intermediates participate subsequently in reactions concerned with transferring a phosphate group to ADP, generating ATP.
In these examples, any energy not conserved in ATP appears as heat and can contribute to the maintenance of body temperature. **Brown adipose tissue** which is found in hibernating and new born animals has the unique ability to dissipate a major proportion of the energy of respiration as heat rather than produce ATP. This ‘metabolically-inefficient’ tissue is an important *thermogenic* organ allowing very young animals (e.g. human babies and newly-born rabbits) to produce heat without having to shiver, and hibernating animals to warm up rapidly despite a low ambient temperature. In some adults, brown adipose tissue may ‘waste’ food energy as heat thus minimising the obesity that could result from excessive food consumption.

(iv) **The metabolic classification of organisms**

So far only the metabolic profile of cells that obtain carbon compounds from their environment in a relatively complex and reduced form and then oxidise them to obtain energy, has been considered. Such organisms are referred to as **heterotrophs** and they represent a large group which includes most microorganisms and the cells of animals. They may be classified more specifically as **chemoorganotrophs** to emphasise that their metabolic routes use chemical oxidation reactions to generate energy from reduced organic substrates.

However, many types of cells can utilise carbon dioxide as a sole source of carbon for the synthesis of all their cellular components. These are referred to as **autotrophs** and have alternative mechanisms for generating metabolic energy because although carbon dioxide is their carbon source, it cannot be further oxidised. Carbon dioxide represents the ultimate biochemical oxidation state of carbon, and no organism can grow on carbon dioxide as a combined carbon and energy source. Energy must therefore be obtained from elsewhere. A small group of microorganisms can obtain their energy by oxidising inorganic material present in their environment, such as ammonia, nitrite and sulphur. These cells are classified as **chemolithotrophs** (the term litho identifying the inorganic nature of the donor) and while they include relatively few species, they none the less play an extremely important role in processing inorganic material in the biosphere.

Thus **Nitrosomonas** and **Nitrobacter** that form the nitrifying bacteria of the **nitrogen cycle**, carry out the oxidation of ammonia to nitrite, and nitrite to nitrate, respectively.
This oxidation of inorganic nitrogen compounds is carried out solely to meet the energetic needs of the bacteria involved, although it turns out to be very important for the nitrogen cycle and the biosphere generally.

The other major group of cells directly utilising carbon dioxide are **phototrophs** which use light as an energy source. In order to fix the carbon dioxide, reducing power may be obtained from inorganic donors. Such organisms are classified as **photolithotrophs**. In the case of both multicellular and unicellular green plants, electrons are obtained from water and by the transduction of light energy in chloroplasts, raised to a high reducing potential. The use of water as a source of electrons results in the liberation of oxygen during **photosynthesis**.

Oxygen evolution also occurs in photosynthetic blue-green bacteria but other photosynthetic bacteria never evolve oxygen, and indeed their particular photosynthetic mode of life is strictly anaerobic. While they carry out a similar photosynthetic process to that of green plants, the reducing power is not derived from water. Instead inorganic donors such as hydrogen sulphide or organic donors such as succinate (a four-carbon compound) are involved. For example, green sulphur bacteria use light energy to obtain reducing power from hydrogen sulphide and use it to fix carbon dioxide using a photosynthetic mechanism that is less complex but similar to that operating in the chloroplasts of plant cells.
2. The Metabolic Profile of Animal Cells

(i) Animal cells have a regulated fuel supply

Animals are chemoorganotrophs, requiring a dietary intake of carbohydrate, fat and protein. To a large extent these are interchangeable as energy sources for omnivorous organisms, whose food sources can vary considerably. An adult in a fed state, receiving an average UK diet will obtain approximately 47% of the energy requirement from carbohydrate, 38% from fat and 15% from protein. On fasting, the energy demands of the body are met from the catabolism of storage molecules, such as glycogen, fat and ultimately expendable protein. Glycogen is particularly abundant in the liver where it acts as a limited reserve of glucose. During the first 24 hours of starvation, glycogen is broken down to glucose (glycogenolysis) depleting the liver concentration to 10% of its normal value. Subsequently carbohydrate makes a smaller contribution to energy provision and this is balanced by a proportionate increase in the energy obtained from fat.

The energy provision for the body can be adequately classified under broad headings of carbohydrate, fat and protein. Individual cells and tissues encounter a much more controlled and restricted supply of organic molecules as energy sources. These molecules are referred to as respiratory fuels and arise either as a result of the digestion and absorption of food, or alternatively as a result of the mobilisation of storage molecules. In all circumstances, the liver is the major organ responsible for processing and distributing these nutrients to other tissues of the body.

The major fuels directly used by animal tissues are listed below:

- Glucose – derived predominantly from carbohydrate (dietary or stored)
- Fatty acids – derived from fat metabolism
- Ketone bodies – derived from fatty acid metabolism
- Amino acids – derived from protein (dietary unless starving)
- Lactate – derived from carbohydrate metabolism

Other minor sources of energy include fructose and galactose, which result from the hydrolysis of sucrose and lactose respectively, fruit acids such as citrate, alcohol, glycerol and ribose. For infants feeding on milk (the lactose content of human milk is 7%) galactose is an important nutrient, derived from the intestinal hydrolysis of lactose.
An important source of energy for **herbivores** comes from the breakdown of plant material. Vertebrates cannot manufacture the enzyme cellulase, but ruminants such as sheep have bacteria that hydrolyse cellulose to glucose and then ferment it to short-chain fatty acids, such as acetate, propionate and butyrate. These molecules are readily absorbed from the rumen and constitute a major energy source. In non-ruminants acetate is not a significant food constituent.

**(ii) Metabolic fuels are selectively metabolised**

The tissues of the body show differing abilities to utilise these various metabolic fuels. Glucose is an essential fuel for some tissues, but not all cells are absolutely dependent upon it, and this is summarised in the table below:

<table>
<thead>
<tr>
<th>Tissues depending on glucose for fuel</th>
<th>Tissues using fatty acids as fuel</th>
<th>Tissues using ketone bodies as fuel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red blood cells</td>
<td>Liver</td>
<td>Cardiac muscle</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>Kidney cortex</td>
<td>Kidney cortex</td>
</tr>
<tr>
<td>Brain</td>
<td>Cardiac muscle</td>
<td>Brain (during starvation)</td>
</tr>
<tr>
<td>Skeletal muscle (in severe exercise)</td>
<td>Skeletal muscle</td>
<td>Skeletal muscle</td>
</tr>
</tbody>
</table>

Mature mammalian **red blood cells** are dependent critically on glucose but can only catabolise it to lactate because they lack mitochondria and are unable to carry out respiration. Glucose is the principal fuel of the **brain**, and human brain requires between 100 and 145 g of glucose per day. When the body is at rest, the brain consumes about two-thirds of the total circulating glucose, the remaining one-third going mainly to red blood cells and skeletal muscles. The oxidation of glucose in **muscle** is increased by exercise, accounting for approximately 50% of the total oxygen consumption during moderate exercise. The liver metabolises glucose, but not as a major energy source: it obtains most of its energy from the oxidation of fatty acids and amino acids.

Fat, stored in **adipose tissue**, is the major fuel reserve in man and most terrestrial animals. It is made available to tissues in two readily-oxidisable forms, **free fatty acids** and **ketone bodies**. The liver takes up fatty acids and oxidises them, extracting some energy and producing two-carbon units from which are synthesised ketone bodies (four-carbon units). The ketone bodies are then
released by the liver into the bloodstream providing a fuel derived from fat but one that is much more soluble in water. Their concentration can be raised to sufficiently high levels in the blood so that tissues that do not normally utilise fatty acid will start to utilise ketone bodies, and indeed the brain can adapt to this. Tissues such as heart muscle and kidney cortex even use ketone bodies in preference to glucose.

(iii) Caloric homeostasis

The amount of the individual fuels present in the blood is not constant, but varies according to the dietary and hormonal state of the animal. In starvation the glucose level may be relatively low, but the concentration of free fatty acids rises and this is accompanied by a parallel increase in the ketone body concentration. This elevation in blood ketone body levels is referred to as ketosis and it is part of a physiological mechanism to ensure that a relatively constant energy supply is continually available to the tissues of the body. Caloric homeostasis is the term used to describe the joint and complementary roles that fatty acids, ketone bodies and glucose play in supplying respiratory fuel, and is illustrated in Table II.

<table>
<thead>
<tr>
<th>Dietary state</th>
<th>Glucose $10^{-3}M$</th>
<th>Ketone bodies $10^{-3}M$</th>
<th>Fatty acids $10^{-3}M$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fed</td>
<td>5.5</td>
<td>0.01</td>
<td>0.30</td>
</tr>
<tr>
<td>Starvation (40 hours)</td>
<td>3.6</td>
<td>2.9</td>
<td>1.15</td>
</tr>
<tr>
<td>Starvation (7 days)</td>
<td>3.5</td>
<td>4.5</td>
<td>1.19</td>
</tr>
</tbody>
</table>

The concentration of these metabolic fuels in the blood reflects not only the rate at which they are made available to the circulation, but also the rate at which they are removed. During starvation, fatty acids are the main source of ketone bodies. Fat hydrolysis within adipose tissue (lipolysis) releases free fatty acids into the plasma. The rate at which the liver then removes and oxidises fatty acids by a metabolic process known as β-oxidation is, within limits, proportional to their blood concentration. The β-oxidation of fatty acid provides metabolic energy for the liver and yields a two-carbon product called acetyl coenzyme A (acetyl CoA). The extent to which acetyl CoA is converted into ketone bodies (ketogenesis) depends on the magnitude of hepatic β-oxidation. However, an increased output of ketone bodies by the liver may not immediately be reflected
by a large change in their blood concentration. This is because the rate at which they are utilised by many mammalian tissues is also determined by their availability in the circulation. Thus enhanced hepatic ketone body production can be largely balanced by an increased utilisation by tissues such as brain and muscle, and the consequent rise in plasma ketone body levels may be relatively small. Because both the synthesis and utilisation of ketone bodies is enhanced in starvation, their **turnover** within the body is said to be increased. This is outlined in Figure 4.

**Fig. 4** The metabolic interactions occurring within and between tissues during starvation to ensure caloric homeostasis
In the fasting state, mobilisation of fatty acid and ketone bodies occurs because the supply of glucose is inadequate as a sole source of energy. The liver carries out the synthesis of glucose from precursors which are non-carbohydrate in origin by a process called gluconeogenesis to ensure a supply for those tissues that can only use glucose as a fuel. During the early period of starvation, the body of an adult human will synthesise approximately 160 g of glucose per day. While gluconeogenesis is for the most part confined to the liver, in humans during prolonged starvation, the kidneys are able to make a substantial contribution to glucose synthesis as well. Starvation also causes breakdown of body protein into amino acids. Muscle tissue, because of its large quantity, is the most important site of protein degradation (proteolysis) and muscles release the amino acid L-alanine as a substrate for gluconeogenesis. However a continued breakdown of protein as starvation continues, would jeopardise the survival of an animal, and adaptive mechanisms have evolved to cope with this situation. There is consequently a decreased utilisation and requirement of glucose by the brain, which adapts by oxidising circulating ketone bodies to satisfy its energy requirements. Thus obese human adults who have fasted 5–6 weeks produce only 24 g of glucose per day as a result of liver and kidney gluconeogenesis, and essentially all of this is utilised by the brain. The energy deficit is made up from ketone bodies which the brain oxidises in place of glucose. Because of this switch in metabolic behaviour by the brain, there is a reduced requirement for gluconeogenesis, which in turn serves to spare body protein breakdown. Glucose utilisation and production are therefore markedly diminished and nitrogen excretion (see later) is also drastically reduced.

(iv) Metabolite exchange between organs is important

From the mechanisms described above, it emerges that co-operation between organs is an important characteristic of mammalian metabolism. This is known as inter-organ metabolism and each organ is responsible for carrying out a specific range of metabolic transformations, processing molecules to a certain stage. Then organic material can be released into the bloodstream for further processing elsewhere or ultimately excretion through the kidneys. Figure 4 demonstrates that during starvation, the oxidation of fat requires the co-ordinated metabolism of at least four tissues: adipose, liver, muscle and brain.

Under certain circumstances it is recognised that there can be a constant recycling of material between various tissues. Carl Cori identified that when skeletal muscle derives energy for contraction by anaerobic glycolysis, muscle
lactate accumulates. This does not serve as a fuel for respiration: rather it is resynthesised quantitatively to glucose by the liver. This glucose can then serve to replenish muscle glycogen stores so that there is a continual exchange of glucose and lactate carbon between liver and muscle.

The Cori Cycle is outlined below:

![Cori Cycle diagram]

A similar cycle has been established to explain how the nitrogen (amino groups), liberated during muscle protein breakdown, is efficiently transported to the liver for urea biosynthesis. Skeletal muscle contains almost 60% of the total body protein, and serves as an important metabolic reservoir of amino acid precursors for gluconeogenesis. When muscle proteolysis occurs, the amino acids that are liberated transfer their amino groups onto pyruvate to synthesise alanine. Alanine is the predominant amino acid released by muscle tissue. It is an ideal transport molecule because, unlike many metabolites at physiological pH, it has zero net charge (zwitterion) and can easily permeate cell membranes. In this cycle, glucose taken up by muscle is converted to pyruvate which is then converted to alanine using amino groups liberated during proteolysis. The alanine is released by muscle and taken up by the liver where it is reconverted to pyruvate and thence glucose and the nitrogen is eliminated via conversion to urea.

The glucose–alanine cycle is illustrated below.

![Glucose–Alanine Cycle diagram]
3. General Properties of Metabolic Enzymes and Intermediates

(i) Metabolic enzymes

The general properties of enzymes are reviewed in *BASC Booklet No. 3*, but several points about the enzymes participating in metabolic pathways need to be considered here. Metabolic enzymes are normally highly specific in terms of the nature of the reaction they catalyse and the structure of the substrates involved. They are also subject to a variety of controls in the cell. Their rate of synthesis as well as their final concentration is under genetic control and their activity is influenced by small molecules such as the substrates and products of the reactions they catalyse. Further, they may be present in both inactive and active forms, the rate and extent of the interconversion between one form and the other depending on a variety of physiological factors.

This is necessary to allow cell metabolism to adapt to environmental change, for example to alterations in the dietary intake. Different foods are dealt with by different enzymes. The synthesis of glycogen from glucose, fat from glucose, glucose from amino acids, and urea from amino acids, all occur in the liver, but are processes that can vary greatly in magnitude and in their demand for specific enzymes. The liver cannot accommodate every enzyme in the maximum amounts needed for every situation. It deals with this by synthesising enzymes according to the requirement at any given time, and removing others, so that while its total size varies very little, it can develop a flexible response.

While the spectrum of possible enzyme catalysed reactions is vast, enzymes themselves can be classified into various groups, characterised by common underlying mechanisms. These basic mechanisms are repeated again and again during a metabolic sequence, as various metabolites undergo the necessary structural changes to yield the final product. For example, the conversion of glucose to lactate is catalysed by eleven enzymes but these represent only five of the major types of enzyme catalysed mechanisms. They are:

1. **Kinases** (phosphotransferases) – enzymes that catalyse the transfer of phosphate from ATP to their substrate

2. **Dehydrogenases** – enzymes that catalyse the removal of hydrogens from their substrate and transfer it to either nicotinamide adenine dinucleotide (NAD\(^+\)) or the flavin nucleotides (FMN or FAD)
3. **Isomerases** – enzymes that catalyse isomerisation

4. **Mutases** – enzymes that catalyse the apparent migration of a phosphate group from one hydroxyl group to another within the same molecule

5. **Lyases** – enzymes that catalyse the addition of groups to double bonds

(In lactate fermentation four individual kinases, two dehydrogenases, two lyases, two isomerases and one mutase are involved. There is similar economy of mechanism in the tricarboxylic acid cycle, when the total oxidation of pyruvate to carbon dioxide and water is achieved.)

6. **Synthetases** (ligases) – enzymes catalysing the condensation of two molecules coupled with the cleavage of a pyrophosphate bond of ATP.

(Pyruvate oxidation via the tricarboxylic acid cycle is characterised by nine enzymes, but when basic mechanisms are considered this diminishes to five dehydrogenase steps, three lyase steps and one synthetase step – a total of three fundamental types!)

Once the basic nature of different enzyme mechanisms is understood and the pattern in which they are repeated within a metabolic system is appreciated, the organisation of cell metabolism becomes more easily comprehensible.

**(ii) The nature of metabolic intermediates**

The structures of important cell metabolites is given in Table III, classified according to their carbon content. They are all soluble polar compounds, of which glucose, glycerol, ethanol and ethanal are nonionic. Most intermediates of metabolism are ionic compounds at pH 7, and this prevents them from diffusing out from cells as membranes are normally impermeable to charged molecules. Their anionic nature is due to the presence of carboxylate (COO\(^-\) ) groups. Alanine is an example of a zwitterion, a molecule containing equal numbers of positive and negative groups, so that it has zero net charge. There are certain common structural features between some of these molecules that have important implications for metabolism.

1. Glucose and glycerol contain alcoholic groups (–OH) and a specific one in each compound can be phosphorylated using ATP and a kinase to form a phosphate ester.

\[
\text{R–OH} + \text{ATP} \xrightarrow{\text{kinase}} \text{R–phosphate} + \text{ADP}
\]

where ROH = glucose or glycerol
Table III  The structures of some important cell metabolites

| Six-carbon compounds | \[
\begin{align*}
\text{CH}_2\text{OH} & \quad \text{CH}_2\text{COO}^- \\
\text{H} & \quad \text{HO-C-COO}^- \\
\text{HO} & \quad \text{CH}_2\text{COO}^- \\
\text{H} & \quad \text{citrate}
\end{align*}
| \[
\begin{align*}
\text{CH}_2\text{COO}^- & \quad \text{CH}_2\text{COO}^- \\
\text{CH}_2 & \quad \text{CH}_2\text{COO}^- \\
\text{O}=\text{C}-\text{COO}^- & \quad \text{2-oxoglutarate (α-ketoglutarate)}
\end{align*}
|
| Five-carbon compounds |
| \[
\begin{align*}
\text{CH}_2\text{COO}^- & \quad \text{CH}_2\text{COO}^- \\
\text{H} & \quad \text{C-COO}^- \\
\text{O} & \quad \text{oxaloacetate}
\end{align*}
| \[
\begin{align*}
\text{oxidation} & \quad \text{reduction} \\
\text{H} & \quad \text{malate} \\
\text{OH} & \quad \text{3-hydroxybutyrate}
\end{align*}
|
| Four-carbon compounds |
| \[
\begin{align*}
\text{CH}_3\text{C}-\text{CH}_2\text{COO}^- & \quad \text{CH}_3\text{C}-\text{CH}_2\text{COO}^- \\
\text{OH} & \quad \text{acetoneacetate}
\end{align*}
| \[
\begin{align*}
\text{oxidation} & \quad \text{reduction} \\
\text{H} & \quad \text{lactate} \\
\text{CH}_3\text{C}-\text{COO}^- & \quad \text{pyruvate}
\end{align*}
|
| Three-carbon compounds |
| \[
\begin{align*}
\text{CH}_3\text{C}-\text{COO}^- & \quad \text{CH}_3\text{C}-\text{COO}^- \\
\text{OH} & \quad \text{O} \\
\text{H} & \quad \text{glycerol}
\end{align*}
| \[
\begin{align*}
\text{oxidation} & \quad \text{reduction} \\
\text{H} & \quad \text{alanine} \\
\text{CH}_3\text{C}-\text{COO}^- & \quad \text{HOCH}_2\text{C}-\text{CH}_2\text{OH}
\end{align*}
|
| Two-carbon compounds |
| \[
\begin{align*}
\text{CH}_3\text{CH}_2\text{OH} & \quad \text{CH}_3\text{CHO} \\
\text{oxidation} & \quad \text{reduction} \\
\text{CH}_3\text{COO}^- & \quad \text{acetate}
\end{align*}
| \[
\begin{align*}
\text{oxidation} & \quad \text{reduction} \\
\text{CH}_3\text{CH}_2\text{OH} & \quad \text{ethanol}
\end{align*}
|

Hexokinase catalyses the formation of glucose 6-phosphate from glucose while glycerol kinase catalyses the formation of glycerol 1-phosphate from glycerol.

Phosphorylated intermediates are very common: for example all the metabolites of glycolysis between glucose and pyruvate occur as phosphate esters. The negatively charged phosphate groups make the molecules unable to cross membranes while activating them so they can enter the metabolic network of a cell and function in energy conservation.

- 18 -
2. In an analogous fashion, fatty acids have to be activated before they can be metabolised. The activating agent is coenzyme-A (CoA–SH), a derivative of ADP whose structure incorporates the vitamin pantothenic acid. CoA–SH combines with fatty acids to yield CoA esters that are then processed in a series of enzymic reactions that constitute fat metabolism. All the intermediates of the β-oxidation process for fatty acids, as well as those involved in fatty acid and fat synthesis, occur as coenzyme A derivatives in the cell. Acetate and other fatty acids are activated to their coenzyme A forms through the action of thiokinases (also known as synthetases), as described below.

\[
\text{acetate} + \text{ATP} + \text{coenzyme A} \xrightarrow{\text{thiokinase}} \text{AMP} + \text{acetyl CoA} + \text{pyrophosphate}
\]

3. Ethanol, lactate, 3-hydroxybutyrate and malate all contain single –OH groups that can participate directly in oxidation reactions to generate ethanal, pyruvate, acetoacetate and oxaloacetate respectively. In every case the reaction is catalysed reversibly by a specific dehydrogenase and the reducing equivalents are accepted by NAD⁺.

\[
\begin{align*}
\text{R–CHOH–R'} + \text{NAD}^+ \xrightarrow{\text{dehydrogenase}} & \quad \text{R–C–R'} + \text{NADH} + \text{H}^+ \\
\text{reduced form} & \quad \text{oxidised form}
\end{align*}
\]

The reactions catalysed by these enzymes are reversible, and during normal cell metabolism, the reduced forms of the molecules tend to predominate. For example, the ratio of lactate : pyruvate in the blood is approximately 10 : 1 and results from the reaction catalysed by lactate dehydrogenase present in all body tissues. 3-hydroxybutyrate and acetoacetate (with acetone) are the ketone bodies, and they can be interconverted through the action of 3-hydroxybutyrate dehydrogenase in the liver, before passing into the blood. Malate dehydrogenase functions to regenerate oxaloacetate during the tricarboxylic acid cycle while alcoholic fermentation in yeast requires the production of ethanol from ethanal catalysed by alcohol dehydrogenase.

4. Pyruvate, oxaloacetate and 2-oxoglutarate are known as 2-oxoacids having the following group within their structure:

\[
\begin{align*}
-\text{C}–\text{COO}^- \\
| \\
\text{O}
\end{align*}
\]
This means they can be metabolised in similar ways. All three molecules can accept amino groups from other amino acids during reactions catalysed by aminotransferase enzymes to generate the corresponding amino acids L-alanine, L-aspartate and L-glutamate.

[Diagram]

A phosphorylated form of vitamin B₆, pyridoxal phosphate, is an essential coenzyme in these reactions.

Pyruvate and 2-oxoglutarate also participate in important irreversible metabolic steps known as oxidative decarboxylation. A specific dehydrogenase is used to catalyse their oxidation and release carbon dioxide. The reaction also involves coenzyme A (CoA) and the product formed is a coenzyme A ester.

\[
\text{R-C-COO}^- + \text{NAD}^+ + \text{CoA} \xrightarrow{\text{dehydrogenase}} \text{R-C-SCoA} + \text{NADH} + \text{H}^+ + \text{CO}_2
\]

Pyruvate is converted into acetyl CoA by pyruvate dehydrogenase. This is the reaction by which carbohydrate molecules are committed via pyruvate to total oxidation in the tricarboxylic acid cycle or to produce precursors for various biosynthetic pathways. 2-oxoglutarate is converted into succinyl CoA by 2-oxoglutarate dehydrogenase, an important enzyme of the tricarboxylic acid cycle.

Both of these enzymes require a coenzyme thiamine pyrophosphate derived from vitamin B₁ (thiamine). A dietary deficiency of this vitamin causes the disease beriberi in man, when certain tissues such as brain show an impaired tricarboxylic acid cycle activity.
4. Glycolysis and Gluconeogenesis

(i) The importance of glycolysis

Glycolysis, the breakdown of glucose to produce pyruvate, is a metabolic process occurring in most living cells. It should be regarded as the first stage in glucose catabolism and the only one that can be completed in the absence of oxygen. Under aerobic conditions, glucose catabolism can proceed by further stages to yield carbon dioxide and water.

Although emphasis is placed on glycolysis as a source of energy the ATP yield is low, only two molecules being produced for each glucose metabolised. This contrasts with the complete oxidation of glucose which yields 38 molecules of ATP. In most animal tissues glycolysis is a minor, yet often vital, source of energy. For mammalian red blood cells it represents their only means of ATP formation. During exercise, when striated muscle receives insufficient oxygen to saturate the tissue, the aerobic generation of ATP is curtailed, but the rate of glycolysis increases to provide energy needed for muscle contraction.

Another function of glycolysis is to supply a cell with necessary biosynthetic precursors. The liver carries out glycolysis to provide precursors for a whole range of molecules it synthesises including fat, cholesterol, bile acids and plasma proteins. In the well-fed animal, once liver glycogen reserves are full, carbohydrate is converted into fat. Glycolysis is then predominantly associated with supplying the initial steps of fat biosynthesis with substrate rather than acting as a source of ATP. For microorganisms such as E. coli or yeast growing on carbohydrate sources, both energy and necessary biosynthetic precursors are obtained from glycolysis.

(ii) The mechanism of glycolysis

Glycolysis occurs in the cytoplasm and may be considered to be divided into three main stages.

Stage 1: Glucose phosphorylation and cleavage

Glucose is converted into glucose 6-phosphate which is isomerised to fructose 6-phosphate and further phosphorylated to give fructose 1,6-bisphosphate. This requires the sequential activity of two kinases, an input of energy in the form of ATP, and an isomerase. The cleavage of the six-carbon sugar phosphate (fructose 1,6-bisphosphate) is catalysed by a lyase (aldolase) to give two, three-carbon
phosphates (glyceraldehyde 3-phosphate and dihydroxyacetone phosphate). Another isomerase allows interconversion between these two isomers so that the number of molecules at every step after this is doubled.

**Stage 2: Substrate-level phosphorylation**

The aldehyde group of glyceraldehyde 3-phosphate is oxidised to a carboxylic acid eventually forming glycerate 3-phosphate. This is achieved through the activity of a dehydrogenase that requires inorganic phosphate as one of its substrates and NADH + H⁺ is produced along with an intermediate that contains a very labile phosphate group. The dehydrogenase is linked in turn to the activity of a kinase so that as oxidation of the aldehyde group proceeds, energy is conserved by phosphorylation of ADP to give ATP, the phosphate coming from the above mentioned intermediate. This sequence has considerable metabolic significance. Apart from being the central energy conserving stage in glycolysis, it can effectively be driven backwards by reducing power and ATP generated during either the light reaction of photosynthesis (see later) or by mitochondrial activity during gluconeogenesis. Both of these processes are concerned with the synthesis of carbohydrate.

**Stage 3: Pyruvate production**

The phosphate group contained within glycerate 3-phosphate is transferred to ADP in a reaction catalysed by a kinase and using the intermediate, phosphoenol pyruvate, generating ATP so that the original investment of energy for activating glucose in Stage 1 is repaid.

The final products of this stage of glucose catabolism are two molecules of pyruvate with a net profit of two molecules each of ATP and NADH + H⁺. This is summarised in Fig. 5.

**(iii) Anaerobic glucose utilisation**

In order for glycolysis to be able to continue, the NADH + H⁺ generated during Stage 2 must be re-oxidised continually to NAD⁺. Aerobically this is accomplished by transferring the reducing power, together with pyruvate, into the mitochondria and completing oxidation using molecular oxygen.

Under anaerobic conditions, NAD⁺ is regenerated, without involving oxygen, using dehydrogenases. In animal tissues, lactate dehydrogenase (LDH) converts pyruvate into lactate which is then transported through the cell membrane into the surrounding fluid. Microorganisms that carry out homolactic fermentation
Fig. 5 The principal stages of glycolysis
yielding lactate as the sole product, also use this enzyme. In yeast, pyruvate is
decarboxylated to ethanal and this is in turn reduced to ethanol using alcohol
dehydrogenase (ADH). This is summarised below:

(iv) Gluconeogenesis
Gluconeogenesis is the synthesis of 'new (neo)' glucose from non-carbohydrate
sources, and in mammals is restricted to the liver and kidney. This cannot be
achieved by simply reversing all the reactions of glycolysis because there are three
steps which are effectively irreversible. These are the reactions catalysed by the
two kinase enzymes in Stage 1 and the terminal kinase reaction in Stage 3 of
glycolysis (see Fig. 5). By contrast, the reaction catalysed by the kinase associated
with substrate level phosphorylation can be driven in reverse. Thus the synthesis
of glucose from pyruvate and lactate uses the glycolytic reactions which are
reversible together with three systems that substitute for the irreversible ones.
Phosphatases, that catalyse the hydrolytic cleavage of phosphate esters are
important here and two specific phosphatases reverse the kinase steps associated
with Stage 1. Gluconeogenesis requires energy and ATP has to be provided by
the mitochondria.
5. The Tricarboxylic Acid Cycle

(i) The discovery of the cycle

The discovery of the tricarboxylic acid cycle was a major advance in biochemistry, providing details of intermediate chemical changes as pyruvate is oxidised to carbon dioxide and water. It subsequently became apparent that this was the predominant central pathway receiving intermediates from most commonly-encountered food materials. The scientist responsible for revealing this unifying pattern was Hans Krebs, who recognised that the discovery of the cycle represented the fulfilment of many years of intensive study by other biochemists as well as himself.

"I must emphasise that although the cycle is named after me, many people contributed to the development of the work. When I entered the field some of the information was already available and I extended this, putting the final touches to it. That the final touches were crucial is true, for they completed a series of reactions by turning them into a cycle".

The work that preceded Krebs’ discovery can be summarised thus.

1. Thunberg identified possible intermediates by examining the ability of organic molecules to be oxidised by a chopped-muscle preparation. Only a few substances reacted with oxygen at rates comparable with that achieved with glucose. These were succinate, fumarate and malate (4-carbon dicarboxylates) and citrate (6-carbon tricarboxylate). As they were oxidised at such high rates, this implied that the muscle possessed an active set of enzymes to metabolise them linked to the main respiratory process.

2. Szent-Györgyi observed that the addition of a small amount of any one of these dicarboxylates, e.g. fumarate, caused an oxygen consumption from muscle tissue far greater than that required just to oxidise it directly to carbon dioxide and water. This experiment suggested that these molecules acted in a catalytic fashion by promoting the oxidation of other substrates already present in the tissue. When the metabolic poison malonate was added, this catalytic effect of fumarate on oxygen consumption was abolished, and instead fumarate was metabolised aerobically to succinate, which accumulated. Malonate has a structure similar to that of succinate, and whilst it is not metabolised by living tissue, it inhibits competitively the activity of the enzyme succinate dehydrogenase which converts succinate into fumarate.
3. It was subsequently shown that citrate was oxidised to succinate via the 5-carbon dicarboxylate 2-oxoglutarate (α-ketoglutarate). In this sequence two dehydrogenases were involved, and in addition to removing hydrogen at each step, a molecule of CO₂ was lost.

Thus a sequence of reactions was established from citrate to oxaloacetate as shown below:

\[
\text{citrate} \rightarrow \text{CO}_2 \quad 2\text{H} \rightarrow \text{2-oxoglutarate} \rightarrow \text{CO}_2 \quad 2\text{H} \rightarrow \text{succinate} \rightarrow 2\text{H}
\]

\[
\text{oxaloacetate} \rightarrow 2\text{H} \quad \text{malate} \rightarrow \text{H}_2\text{O} \rightarrow \text{fumarate}
\]

How this oxidation and interconversion of various organic acids related to glucose metabolism remained obscure. Glycolysis generated molecules of pyruvate each with three carbon atoms, so the reason for citrate with the same number of carbon atoms as the original glucose featuring so prominently was unclear.

It occurred to Krebs that if the end product of citrate oxidation i.e. oxaloacetate, reacted with something derived from glucose which then resynthesised citrate, a cyclic process would result. He had already developed the concept of a metabolic cycle following his work in 1932 on urea biosynthesis (see later). Krebs demonstrated that citrate could be synthesised from oxaloacetate and pyruvate. This provided the missing link with the known enzyme reactions and it was therefore possible to formulate a cyclic scheme and test it in the laboratory. The cycle was experimentally verified by careful measurements using manometric methods. None of the modern techniques of biochemistry such as chromatography or labelling radioactively the individual molecules and tracing
their metabolic fate were then available. Yet all the individual stages that constitute the cycle were shown to occur in muscle tissue, and furthermore they co-operated to achieve pyruvate oxidation under a variety of conditions. An outline of the tricarboxylic acid cycle is given in Figure 6.

Fig. 6 The tricarboxylic acid cycle

The involvement of acetyl CoA in directly reacting with oxaloacetate to yield citrate was discovered later. This scheme provided an explanation for the catalytic effect of dicarboxylates observed by Szent-Györgyi, since, as pyruvate is progressively oxidised to three molecules of carbon dioxide, oxaloacetate is regenerated to initiate the cycle again.
(ii) The functions of the cycle

The tricarboxylic acid cycle occurs in aerobic bacteria and the mitochondria of eukaryotic cells. Entry of the two carbon units of acetyl CoA into the cycle is balanced by the loss of two carbons by decarboxylation within the cycle so there is no net formation of oxaloacetate: this acceptor molecule is simply catalytic and is regenerated. In addition to utilising acetyl CoA derived from pyruvate, fatty acids may also enter the cycle after being broken down to acetyl CoA units via the process of β-oxidation. 3-hydroxybutyrate and acetoacetate also generate acetyl CoA when metabolised by various body tissues, so the cycle acts as a common pathway for the degradation of carbohydrate, fatty acids and ketone bodies to carbon dioxide.

During the cycle only one molecule of ATP is effectively made in a reaction catalysed by succinyl CoA synthetase. Much of the energy made available during the degradation of acetyl CoA is conserved by producing the reduced molecules NADH + H⁺ and FADH₂. The complete oxidation of pyruvate generates 4 molecules of NADH + H⁺ and 1 molecule of FADH₂. These reduced molecules are utilised in the further production of ATP by the process of mitochondrial oxidative phosphorylation when the reducing equivalents are oxidised to water by a series of components that comprise the respiratory chain (see later). In addition to providing ATP for biosynthesis, the tricarboxylic acid cycle also functions as the starting point for biosynthetic reactions by producing vital carbon-containing intermediates. Thus oxaloacetate and 2-oxoglutarate can be converted into the amino acids L-aspartate and L-glutamate by aminotransferases. The removal of these 4-carbon and 5-carbon molecules for anabolic processes e.g. protein synthesis, causes a problem because the cycle will be interrupted and the necessary regeneration of the acceptor molecule cannot occur. Ancillary metabolic routes (anaplerotic sequences) therefore exist to provide the necessary role of ‘filling up’ the cycle with intermediates if they are tapped off from it for biosynthetic purposes.

(iii) The synthesis of carbohydrate from fat

In animals carbohydrate is readily converted into fat but the reverse process cannot occur: carbohydrate cannot be synthesised from fat. This follows directly from the nature of the tricarboxylic acid cycle. Fatty acids on oxidation yield acetyl CoA, but the reaction catalysed by pyruvate dehydrogenase is essentially irreversible and acetyl CoA cannot be converted to pyruvate and thence to glucose.
Thus acetyl CoA enters the tricarboxylic acid cycle which effects its complete combustion and the two carbon fragments are lost as CO₂.

Yet many bacteria, fungi, algae and protozoa are capable of growing on acetate as their sole carbon source, obtaining from it both energy and cell components. Seeds rich in oil such as those from castor beans, sunflowers, peanuts and olives, require glucose to be made from fatty acids on germination, until photosynthesis is established. In such instances, this is made possible by an anaplerotic pathway known as the glyoxylate shunt. It is effected by the sequential action of two enzymes not found in animals. They by-pass the CO₂-evolving steps of the cycle (i.e. the sequence between isocitrate and succinate), conserving the two carbons in the molecule glyoxylate which reacts with another molecule of acetyl CoA to give malate. Using succinate, the biosynthesis of glucose and other cell components can be achieved as described in Figure 7.

---

**Fig. 7** The role of the glyoxylate shunt in converting fatty acid into carbohydrate
6. Energy Production by Organelles

(i) Mitochondria and chloroplasts
Eukaryotic organisms have evolved two highly specialised cytoplasmic organelles to convert either light energy, or that derived from chemical nutrients, into physiological energy. These are the chloroplast and the mitochondrion. Although ATP synthesis can occur in metabolic routes such as glycolysis, by far the largest proportion is made by enzyme systems associated with energy-transducing membranes. Such membranes are found within mitochondria and chloroplasts, while in simpler prokaryotic cells lacking these organelles, the plasma membrane fulfils this function.

Mitochondria have an outer and inner membrane, the latter being infolded as cristae in the matrix space. All the enzymes of the tricarboxylic acid cycle are contained within the matrix except for one. Succinate dehydrogenase is bound to the inner membrane which functions as the energy-transducing membrane of this organelle. The shapes of mitochondria vary depending upon the cells from which they originate. Generally the greater the respiratory activity of the tissue the more extensively is the inner membrane folded into cristae.

Chloroplasts are peculiar to green algae and higher plants, and are somewhat larger than most mitochondria. Because chloroplasts conserve energy from sunlight rather than respiratory fuels, it could be expected that there would be considerable differences between these two types of organelle. In fact they show a strikingly similar pattern of organisation, and indeed make ATP in practically the same way. Chloroplasts have an outer and inner membrane that forms an external envelope. The inner membrane surrounds a large central space (stroma) which is analogous to the mitochondrial matrix and contains many enzymes including those catalysing the CO₂ fixation reactions of the Calvin cycle (see later). Within the stroma are a series of membrane vesicles called thylakoids which function as the energy-transducing membranes of the chloroplast. The lumen of each thylakoid interconnects with those of other thylakoids to form an internal compartment called the thylakoid space. Several thylakoids may be stacked together at specific regions to form grana. These structural arrangements are illustrated in Figure 8. Plant cells rely on chloroplasts for the production of energy for biosynthetic purposes during daylight. During darkness they are dependent on mitochondria.
(a) Intact mitochondrion

(b) Intact chloroplast

Fig. 8 Drawings of organelles concerned with energy conservation. The thickest lines indicate energy transducing membranes

(ii) Mitochondrial oxidative phosphorylation

NADH + H⁺ and succinate produced during the tricarboxylic acid cycle are oxidised by an electron transport chain that is linked to ATP synthesis. This process is exclusively associated with the inner mitochondrial membrane. The chain comprises three types of electron carrier, flavoproteins, quinones and cytochromes. These molecules are arranged in a sequence of increasing positivity
so that electrons flow from the most negative component (NADH + H\(^+\)) in a stepwise fashion until they encounter the most positive acceptor, oxygen, which is then reduced to water. As electrons are transferred along the chain, at three sites there is a sufficient decrease in free energy to drive ATP synthesis.

The two flavoprotein enzymes, **NADH dehydrogenase** and **succinate dehydrogenase**, are embedded in the inner membrane. They catalyse the removal of hydrogen in the form of 2H\(^+\) and 2 electrons from NADH + H\(^+\) and succinate respectively. This reducing power is transferred to coenzyme Q (ubiquinone), a lipid-soluble molecule that can diffuse freely in the membrane. The electrons are then passed to a series of five different cytochrome molecules, identified as cytochromes \(b, c_1, c, a\) and \(a_3\). **Cytochromes** are protein molecules containing an iron atom within a haem group that can exist in the Fe\(^{2+}\) (ferrous) and Fe\(^{3+}\) (ferric) forms. Each cytochrome can accept an electron in its ferric form and be reduced to the ferrous state, and then donate the electron to the next cytochrome in the sequence becoming oxidised again. Only one cytochrome, cytochrome \(a_3\) gives up its electron directly to oxygen and this process can be blocked by the poison, **cyanide**.

With the exception of coenzyme Q and cytochrome \(c\), the components of the electron transport chain are organised as four protein complexes in the inner membrane. When two electrons move through them, three of the complexes act as proton pumps. Each extrudes 2H\(^+\) out from the matrix into the intermembrane space. This compartment becomes positively charged while the matrix having lost H\(^+\) ions becomes negatively charged. The mechanism by which this pumping occurs is unclear, but only the complex containing succinate dehydrogenase is unable to perform this task. The result of respiration is therefore not unlike that of a battery with NADH + H\(^+\) oxidation pumping out a total of 6H\(^+\) while succinate oxidation only pumps out 4H\(^+\). This is summarised in Figure 9.

A proton gradient builds up because the inner membrane is impermeable to protons, except at certain sites. One of these sites is the enzyme **ATP synthetase**, a large protein complex with a proton channel embedded in the membrane and a catalytic portion extending into the matrix. It allows 2H\(^+\) to return down the concentration gradient into the matrix, and concomitantly drives the synthesis of ATP from ADP and P\(_i\). (ATP synthetase has an essentially identical structure and function in chloroplasts.) In mitochondria, when NADH + H\(^+\) is oxidised and 6H\(^+\) pumped out, these can return through the ATP synthetase and 3 ATP molecules are made. Succinate oxidation results in only 2 ATP being produced.
The oxidation of pyruvate in the tricarboxylic acid cycle results in 4 NADH + 4 H⁺ and 1 FADH₂ being generated which on further oxidation in the respiratory chain drives the synthesis of 14 ATP. As 1 ATP is generated directly in the cycle, the net ATP production is 15. Glycolysis yields 2 moles of pyruvate, 2 NADH + 2 H⁺ and 2 ATP from 1 molecule of glucose, and the complete aerobic metabolism of this will therefore supply a cell with a total of 30 + 6 + 2 = 38 molecules of ATP.

Fig. 9 The mitochondrial electron transport chain
(iii) Photophosphorylation – the light reaction

ATP can be generated by the thylakoid membrane of chloroplasts in a similar fashion to that outlined for mitochondria. An electron transport chain within the membrane pumps protons into the thylakoid lumen making it acid. ATP synthetase allows protons to return, generating ATP in the stroma. This process, called photophosphorylation, is completely dependent on light to generate the necessary electrons by driving the oxidation of water and liberating oxygen. These electrons apart from powering ATP synthesis can reduce NADP$^+$ to NADPH + H$^+$, and these are the source of energy and reducing power for CO$_2$ fixation in the dark reactions.

![Diagram of light reactions and dark reactions]

To ensure that the light reactions occur, chloroplasts possess two unique components in the thylakoid membrane, a light-harvesting system or antenna unit, and a reaction centre. Light between 400–700 nm is photosynthetically active and the antenna unit contains a variety of molecules to absorb this radiation. The green pigment chlorophyll, is an important component. Its structure resembles the haem prosthetic group found in cytochromes, but the central Fe atom is replaced by Mg. The two principal forms of chlorophyll in plants, chlorophyll $a$ and $b$, absorb light particularly at relatively long wavelengths (640–680 nm). Carotenoids, a group of accessory pigments absorb those wavelengths of light not absorbed by chlorophyll. When photons are absorbed by chlorophylls $a$ and $b$, electron displacement within the molecule occurs, exciting them. In an antenna unit, pigment molecules are packed tightly together, and energy is transferred from one neighbour to the next until sufficient energy is trapped to cause a special chlorophyll $a$ molecule called a reaction centre to lose an electron. This energetic electron enters a series of enzyme catalysed reactions responsible for using its
energy to drive the synthesis of ATP and NADPH + H⁺. The reaction centre is left with a positively charged hole of high electron affinity that withdraws an electron from a suitable donor.

The antenna unit plus reaction centre is called a photosystem, and its function in collecting light energy and funnelling this into the release of an energetic electron of considerable reducing power is illustrated below.

In the thylakoid membrane two distinct types of photosystems are found with different functions, each possessing pigment systems with characteristic light-absorbing properties. The transfer of electrons from water to NADP⁺ requires these two photosystems to operate in series, being connected by the electron transport chain. Light absorbed by Photosystem II generates an electron with sufficient energy to reduce plastoquinone (PQ) a lipid-soluble quinone at the commencement of the electron transport chain. The reaction centre of Photosystem II removes electrons from water, oxidising it to oxygen. Photosystem I absorbs light and generates an electron that can reduce NADP⁺ to NADPH + H⁺ via the iron protein ferredoxin (Fd). Its reaction centre subsequently removes an electron from a copper protein called plastocyanin (PC) at the end of the electron transport chain. Plastoquinone and plastocyanin are connected by two cytochromes, b and f, that are characteristic of photosynthetic systems. As electrons move down the chain, protons are pumped out of the stroma as shown in Figure 10 and ATP made subsequently.
The movement of a single electron through the thylakoid membrane requires each photosystem to absorb one photon. In order to liberate O₂ from 2 molecules of water, 4 electrons are transferred to 2 NADP⁺ molecules, and this requires 8 photons of light. This process, generating both ATP and NADPH + H⁺ is called non-cyclic photophosphorylation. If an excited electron returns to the chlorophyll molecule that ejected it via the electron transport chain, only ATP is made. This is called cyclic photophosphorylation and can occur with Photosystem I. It represents a means by which the chloroplast can make the extra ATP needed to fix CO₂ in the dark reactions without producing additional NADPH + H⁺ as well.

(iv) Photosynthetic carbon fixation – the dark reactions

The principal route of CO₂ fixation involves its reaction with a 5-carbon acceptor molecule, ribulose 1,5-bisphosphate (RuBP). The resulting 6-carbon product is unstable and splits into two molecules of glyceraldehyde 3-phosphate (GP) an intermediate also found in glycolysis. This is reduced using NADPH + H⁺ and ATP from the light reactions to a 3-carbon sugar from which glucose is produced and the acceptor molecule regenerated.

\[ C_1 + C_6 \rightarrow [C_6] \rightarrow 2C_3 \]

\[ \frac{1}{6} \text{glucose} \]

This was previously called phosphoglyceric acid (PGA)
The radioactive isotope of carbon, $^{14}\text{C}$, was used to clarify the details of this mechanism. Calvin and his colleagues exposed green algae to $^{14}\text{CO}_2$, then rapidly killed the cells, extracted metabolites and identified them using paper chromatography. Radiolabel was incorporated into a range of molecules including alanine, malate, sucrose, sugar phosphates and GP. However if the cells were exposed to light and $^{14}\text{CO}_2$ for just a few seconds, GP (‘PGA’) became the most heavily labelled molecule, suggesting it is an early photosynthetic intermediate. If the light was suddenly turned off radioactivity in GP increased while that in sugar phosphates fell. This indicates that light supplies the NADPH + H$^+$ to reduce GP to sugar phosphate.

RuBP was subsequently identified as the acceptor reacting with CO$_2$ to yield GP. A sudden fall in the CO$_2$ concentration breaks the reaction sequence so that RuBP accumulates and GP decreases. The enzyme catalysing CO$_2$ fixation is called Ribulose 1,5-bisphosphate carboxylase (Rubisco). It is one of the most abundant proteins in nature. It and the other enzymes of the Calvin cycle are located in the chloroplast stroma and the basic steps of CO$_2$ fixation are shown in Figure 11.

![Diagram of the Calvin cycle](image)

**Fig. 11** The Calvin cycle in outline
GP is converted into glyceraldehyde 3-phosphate using ATP and NADPH + H⁺ in a reaction essentially the reverse of substrate-level phosphorylation in glycolysis. Glyceraldehyde 3-phosphate is the major product of CO₂ fixation, but five-sixths of this must be re-cycled to generate the substrate RuBP. The remaining one-sixth can either be retained in the chloroplast and converted into starch or be transported from the chloroplast into the cytoplasm where sucrose synthesis occurs. Sucrose represents the major transport form of the fixed carbon in most plants. It is transported through the phloem to other parts of the plant where it is utilised for growth and to supply energy to non-green tissues such as roots and seeds.

The Calvin cycle has a strict requirement for 3 ATP and 2 NADPH + H⁺ for every molecule of CO₂ fixed. The rate of CO₂ assimilation depends on a number of factors including light, temperature and CO₂ availability. The cycle is controlled by light so that there is little or no CO₂ fixation in the dark. When light shines, electron transport generates ATP and NADPH + H⁺ and causes the stroma to become alkaline so that the optimum conditions for many of the enzymes result.

(v) ‘C₄’ photosynthesis and photorespiration
The atmospheric content of CO₂ is about 0.03% by volume and this limits the rate of photosynthesis, the optimum level being about 0.1%. Some tropical plants, e.g. sugar cane and maize, close their stomata for considerable time periods to prevent water loss. This could result in the CO₂ level within the leaf falling and impairing photosynthesis. To overcome this and become photosynthetically efficient these plants have evolved a two-step pathway of CO₂ fixation. The initial capture of CO₂ takes place in mesophyll cells adjacent to the leaf surface. Phosphoenol pyruvate, a 3-carbon molecule is carboxylated to give a 4-carbon metabolite oxaloacetate which is reduced to malate. Malate is transferred to specific bundle-sheath leaf cells that contain numerous chloroplasts where it is decarboxylated and the CO₂ is assimilated into the Calvin cycle in the normal way.

This supplementary ‘C₄’ CO₂ assimilation system is then completed when pyruvate returns to the mesophyll cell to initiate the process again. The ‘C₄’ route has been described as an ATP-dependent CO₂ pump maintaining a high local concentration of CO₂ so that Rubisco can fix CO₂ very efficiently.

Rubisco is also responsible for photorespiration a process involving O₂ uptake and CO₂ output that takes place concurrently with photosynthetic carbon
assimilation. This is a non-mitochondrial system and results from Rubisco having two catalytic activities. O$_2$ can replace CO$_2$ in the Rubisco-catalysed reaction so that instead of carboxylation an organic acid phosphoglycolate is formed. This can undergo oxidation leading to loss of CO$_2$. The ability of photorespiration to re-oxidise some of the recently assimilated carbohydrate back to CO$_2$ is wasteful energetically and results in a significant loss of photosynthetic capacity in plants. Current research involving genetic manipulation of Rubisco to increase its carboxylase activity and suppress its oxygenase activity could lead to substantial increases in net photosynthesis for several important crop plants.
7. Detoxification and Urea Biosynthesis

During their lifetimes most organisms stand the chance of being exposed to poisonous chemicals. In order to transform such substances into less harmful products, a group of enzymes have evolved to catalyse the process of **detoxification**. The liver is the most biochemically active organ concerned with detoxification in mammals. It can metabolise or detoxify a large variety of potentially injurious molecules into products less toxic and more easily eliminated from the body. For example it has a battery of enzymes concerned with transforming drugs such as paracetamol and amphetamine into non-toxic excretable metabolites thereby ensuring that their pharmacological action is curtailed and the substances themselves eliminated from the body.

While many poisons present in the environment result from plant and microbial activity, or chemical pollution, toxic molecules may also arise from normal metabolism of cells. **Ammonia** is an important, but potentially toxic, cellular constituent. The liver is responsible for ensuring low blood levels of ammonia, but in disease states such as liver failure, abnormally high levels of ammonia may occur. This affects the brain causing severe neurological disturbances that may be followed by convulsions and coma.

The continual production of ammonia is a consequence of protein metabolism. Many amino acids undergo transamination to give L-glutamate which can be deaminated using a dehydrogenase enzyme to produce ammonia.

\[
L\text{-glutamate} + NAD^+ + H_2O \rightleftharpoons 2\text{-oxoglutarate} + NADH + H^+ + NH_3
\]

Animals that excrete ammonia directly as their major terminal product of nitrogen metabolism are termed **ammonotelic**. This is characteristic of aquatic organisms, since ammonia requires a plentiful supply of water for its efficient excretion. **Fish** use their gills to eliminate 80% of their nitrogenous excretory material as ammonia. However the change from an aquatic existence to a terrestrial environment results in an alteration in the pattern of nitrogen excretion. Because of a restricted availability of water many terrestrial animals synthesise urea as their principal nitrogenous excretory product. Such animals are termed **ureotelic**, and urea is a highly soluble diffusible non-toxic molecule, produced via a metabolic route known as the **ornithine cycle**.
The ornithine cycle was discovered by Krebs and Henseleit in 1932. They observed that slices of mammalian liver can synthesise urea at high rates providing the amino acid L-ornithine is present together with ammonia in the incubation medium. Ornithine is not consumed in this process: rather it acts as a carrier on which a urea molecule is assembled from two molecules of ammonia and one molecule of CO₂. Like oxaloacetate in the tricarboxylic acid cycle, L-ornithine is regenerated once urea is made. An intermediate in this process is the amino acid L-citrulline, formed after the incorporation of one molecule of ammonia and CO₂. A further input of ammonia results in the synthesis of the amino acid L-arginine. The enzyme arginase which is highly active in the liver of some animals (mammals, amphibians and certain reptiles) catalyses the production of urea and the regeneration of L-ornithine so that the cyclic interconversion of these amino acids can continue as shown below.

![Diagram of the ornithine cycle]

Fig. 12 The ornithine cycle

Three molecules of ATP are required during urea biosynthesis. The development of the ornithine cycle can be observed during the metamorphosis of amphibia that adapt to a terrestrial life. Tadpoles (larvae of frogs) excrete ammonia, but with the onset of metamorphosis there is an irreversible change to ureotelism, and the activity of the ornithine cycle enzymes increase as development progresses to the adult frog stage. In contrast earthworms undergo a reversible transition from ammonotelism to ureotelism with alternating periods
of starvation and feeding. Earthworms are normally ammonotelic but become ureotelic when starved, the ornithine cycle detoxifying excess ammonia liberated by increased protein catabolism.

**Mammals** are ureotelic and urea synthesis occurs entirely in the liver. (The kidney also produces ammonia, but this plays a role in the acid–base balance of the body. Ammonia diffuses into the tubular urine and becomes trapped after accepting a proton to form the \( \text{NH}_4^+ \) ion. This cation exchanges for the physiologically-important \( \text{Na}^+ \) present in the urine, and subsequent excretion of ammonium salts ensures that essential cations are conserved while acid is removed.)

While **bony fish** (teleosts) are ammonotelic and contain only low concentrations of urea (0.01–0.03%) in their body tissues, **cartilaginous fish** (elasmobranchs) maintain high levels (2%) of urea in their blood. By conserving urea rather than excreting it, elasmobranchs (particularly marine species) maintain osmotic equilibrium with their surrounding environment, while nitrogen is eliminated as ammonia via the gills. The most extreme adaptation to terrestrial life is seen in **birds, certain reptiles** and **insects**, which have abandoned urea in favour of uric acid as their principal nitrogenous end-product. These animals are termed **uricotelic**, and the pathway concerned with uric acid formation from ammonia is complex. The final stages of this metabolic route involve oxidation of purine bases into uric acid which is insoluble and can be excreted in a semi-solid form ensuring a minimum of water loss.
8. Examination Questions

Most Examination Boards use various types of question in the A-level theory papers in order to assess a student's understanding of the subject. The skills required to produce a competent answer extend beyond the fundamental ability to recall factual information correctly. Considerable emphasis is placed on understanding the principles associated with each topic and appreciating the concepts and ideas behind them. Essay questions encourage students to integrate information from several sources and credit is given for careful organisation and presentation of material. Multiple-choice or short-answer questions can have a variable mark allocation reflecting the need for specific points to be identified or included in the answer. The interpretive questions often provide the greatest challenge, presenting information that has to be analysed and interpreted in a thoughtful and logical way.

Examples of these various types of question are given below, together with a page reference to indicate where the solutions may be found in the booklet.

The examination questions included in this booklet are reproduced by permission of the following Examination Boards.

- The Associated Examining Board
- The University of Cambridge Local Examinations Syndicate (UCLES)
- The University of London School Examinations Board (London)
- Oxford and Cambridge Schools Examination Board
- University of Oxford Delegacy of Local Examinations (Oxford)

The Examination Boards accept no responsibility whatsoever for the accuracy or method of working in any answers given.
(i) **Essay questions**

1. Write an essay on mitochondria.  
   
   [Information provided on pages 30–33]  
   
   (30 marks)  
   
   (London, 1988)

2. Discuss the importance of glycolysis and the tricarboxylic acid cycle (Krebs cycle) in supplying energy for cell metabolism during periods of rest and strenuous activity.  

   [Information provided on pages 21–28]  
   
   (20 marks)  
   
   (London, 1987)

3. (a) Explain the meaning of the term cellular respiration.  

   (3 marks)

(b) Describe the part played by each of the following in cellular respiration:  

(i) Glycogen  

(ii) Fermentation  

(iii) Oxidative phosphorylation  

(12 marks)

(c) What are the roles of electron transport systems in cell metabolism  

(5 marks)

[Information for (a) given on page 3, (b, i) on page 10, (b, ii) on pages 21–24, and (b, iii) and (c) on pages 31–33]  

(London, 1988)

4. Describe how light energy is converted into chemical energy in the light reaction of photosynthesis.  

(9 marks)

[Information provided on pages 34–36]  

(London, 1987)

5. Write an account of the dark reaction in photosynthesis and explain how radioactive tracers have been used to study this pathway.  

(20 marks)

[Information provided on pages 36–38]  

(London, 1988)

6. (a) Give an account of the processes involved in the formation of urea by mammals  

(6 marks)

(b) What other nitrogenous waste products are excreted by vertebrates? How are these related to the mode of life of the animal?  

(8 marks)

[Information provided on pages 40–42]  

(UCLES, 1988)
(ii) **Short-answer questions**

7. The diagram below shows an outline of cellular respiration.

![Cellular Respiration Diagram](image)

a) Name a polysaccharide stored in (i) green plants and (ii) mammals.

..........................................................................................................................(2 marks)

b) (i) Name the process by which the 6-carbon sugar is converted to pyruvic acid.

..........................................................................................................................

(ii) Where in the cell does this process occur?

..........................................................................................................................

(iii) Why is ATP used in this process?

..........................................................................................................................(4 marks)

c) Name the compound formed from pyruvic acid in muscle cells under conditions of oxygen debt.

..........................................................................................................................(1 mark)

d) Name the type of enzyme involved at stage X.

..........................................................................................................................(1 mark)

e) What happens finally to the hydrogen ions released from the tricarboxylic acid cycle?

..........................................................................................................................(2 marks)
f) Make a labelled drawing of a mitochondrion, and on your drawing indicate where ATP synthesis occurs.  

(4 marks)

[Information for part: (a, i) starch (a, ii) glycogen; (b, i) on page 3, (b, ii) and (b, iii) on page 21; (c) on page 22; (d) on page 16; (e) on pages 31–33; and (f) on page 31].

(London, 1987)

8. (a) Why does the breakdown of glucose occur by a series of enzyme catalysed reactions?

..............................................................................................................................(4 marks)

(b) State where, in a cell, the processes involving the breakdown of glucose to carbon dioxide and water occur.

..............................................................................................................................(2 marks)

(c) The energy change for the complete oxidation of glucose is approximately 2800 kJ per mole. The conversion of ADP to ATP requires approximately 29 kJ per mole. If the efficiency of coupling ATP synthesis to respiration is 40%, calculate the number of ATP molecules which may be synthesised from ADP and phosphate by the complete oxidation of one mole of glucose

..............................................................................................................................(2 marks)

[Information for part (a) given on pages 1–2, (b) on pages 21 and 28 and (c) is 38.6 calculated as described on page 7]. (Cambridge, Oxford and Southern Specimen paper)

(iii) Interpretive questions

9. Muscle cells were broken up and separated into fractions. Samples of each fraction were incubated with (i) glucose and (ii) pyruvate. Tests were then made for the production of carbon dioxide and lactate in each sample. The results are given in the table below.

<table>
<thead>
<tr>
<th>Cell Fraction</th>
<th>Incubated with glucose</th>
<th>Incubated with pyruvate</th>
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<tr>
<td></td>
<td>Carbon dioxide</td>
<td>Lactate</td>
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<td>Mitochondria</td>
<td>absent</td>
<td>absent</td>
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<tr>
<td>Cytoplasmic residue</td>
<td>absent</td>
<td>produced</td>
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(a) Explain the significance of the results obtained with each of the fractions.

..............................................................................................................................(4 marks)
(b) Explain why no carbon dioxide is evolved when cyanide is added to the mitochondrial fraction before incubation.

.................................................................(2 marks)

[(a) mitochondria catalyse pyruvate oxidation, not glycolysis which is exclusively cytoplasmic (see pages 21 and 30). Information for (b) is given on page 32].

(London, 1984)

10. The graph shows changes in the relative amounts of ribulose bisphosphate (RuBP) and glycerate 3-phosphate (GP) produced in the Calvin cycle of photosynthesis before and after the light is switched off. All other conditions are constant.

Account for the changes in the relative amounts of GP and RuBP after the light is switched off.

.................................................................(3 marks)

[Information is given on page 37].

(Associated Examining Board, 1987)

NOTE: The possible solutions suggested here may not necessarily constitute the only ones.
9. Source Materials

(i) Textbooks

(ii) Articles


(iii) Practical work


(iv) Teaching aids

The Biochemist’s Songbook. H. Baum (1982) Pergamon Press. A companion cassette tape of songs from the book is also available, from Prof. H. Baum. Biochemistry Section, Kings College London, Campden Hill Road, Kensington, London W8 7AH.


The Urea Cycle, D.A. Bender (1985) A computer simulation of laboratory exercises. BBC Microcomputer version with explanatory notes available from D.A. Bender, Department of Biochemistry, University College, Gower Street, London WC1E 6BT.

Citric Acid Cycle – Magic Wheel. A mobile laminated colour printed teaching aid that displays the names of enzymes, coenzymes and reactions and structures. David Weitzman, Metabaid, P O Box 359, Cardiff CF2 6YD.
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