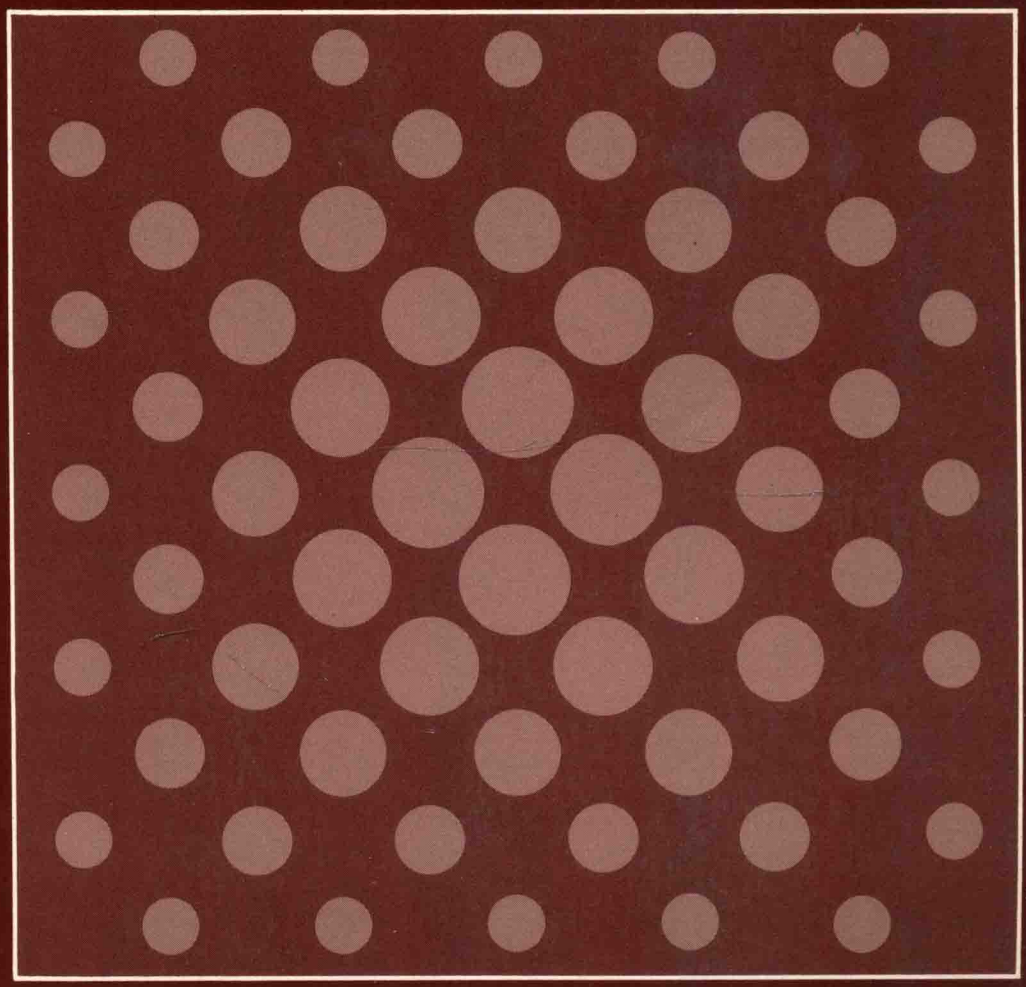
BIOTOL

BIOTECHNOLOGY BY OPEN LEARNING

Biosynthesis and the Integration of Cell Metabolism



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Biosynthesis and the Integration of Cell Metabolism

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The Biotol Project

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Project Manager Dr J.W. James

nor to include all *groups* of biochemicals. Such an approach would be a daunting one. Instead, emphasis is placed on developing a knowledge of the pattern of biosynthesis and on exploring how biosynthesis is interconnected both physically and chemically with catabolism. Strategies for investigating the biochemical problems posed by biosynthesis are developed within the text.

The integration and regulation of catabolic and biosynthetic processes are essential to cells if they are to maintain a balanced metabolism in which energy generation and precursor production are harmonised. This aspect of metabolism is a recurrent theme throughout but it especially is the focus of the final two chapters. The text is, therefore, much more than a collection of metabolic pathways. Although important, the pathways themselves are merely the foundations upon which an understanding of intermediary metabolism can be developed.

Intermediary metabolism underpins so many aspects of applied biology and biotechnology that its importance cannot be overstressed. We urge readers to make full use of the in-text activities (Π) and self-assessed questions (SAQs) to maximise the benefit to be gained from the text.

One final point we wish to make is that, as you will learn from the text, biosynthesis shows remarkable similarities between species including animals, plants and micro-organisms. Nevertheless many differences do exist and the authors have taken opportunities to illustrate these differences especially in the biotechnologically-important groups - micro-organisms and plants. This text has, therefore, a different flavour from many biochemistry texts which carry a human/medical orientation. This, together with its reader-orientated activities makes this and its partner BIOTOL texts, a learning package of distinction.

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Dr C K Leach

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Introduction

Introduction

1.1 Three Biotol texts on cells' metabolism

major areas of metabolism

Metabolism includes a wide range of processes which can be divided into three major groups. These are:

- the generation of a usable form of energy and reducing power;
- the generation of simple molecules to act as precursors of cell constituents;
- the synthesis of new cell constituents (biosynthesis).

The first two of these groups of processes may be regarded as 'fuelling' reactions since they are the processes which provide the fuel for new cell synthesis. The final group is concerned with the use of the products of the first two to make new cell constituents.

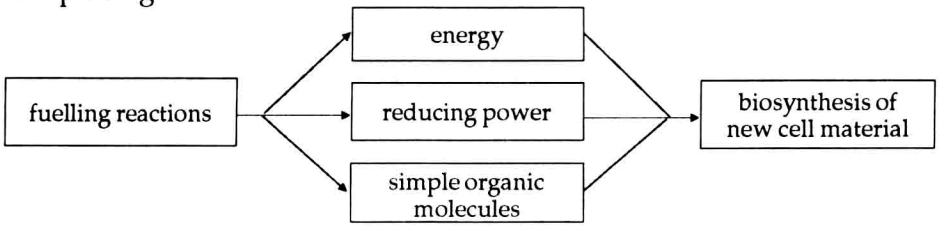
To cover the necessary breadth of chemical and biological knowledge to understand metabolism, three Biotol texts have been produced. The first two texts, ('Principles of Cell Energetics' and 'Energy Sources for Cells') deal with the underlying thermodynamics and enzymology and examine the processes which lead to the production of cellular energy (ATP), reducing power (NADH/NADPH) and simple organic molecules. In other words, these two texts deal with the cellular fuelling reactions. In this, the third text, the biosynthetic processes of cells are examined. It has been written on the assumption that the reader has knowledge of the fuelling reactions including the mechanisms for generating ATP, reducing power and simple organic metabolites by heterotrophic and autotrophic systems.

The purpose of this introductory section is to explain, in outline, the layout of this text and its relationship to the other two Biotol texts committed to cell metabolism. It will also enable readers to check whether they have the requisite knowledge to benefit from this text.

1.2 The fuelling reactions are diverse

1.2.1 The generation of cellular energy and reducing power

Central to our understanding of metabolism is the knowledge that the three main products of the fuelling reactions (energy, reducing power, simple organic molecules), are the starting materials for biosynthetic reactions. We can represent this relationship by a simple diagram:



diverse fuelling reactions

The symmetry of this diagram is, however, misleading. Although the biosynthetic processes are similar but not identical in all systems, there is a much greater diversity in the fuelling reactions. Let us see if we can work out the reasons for this.

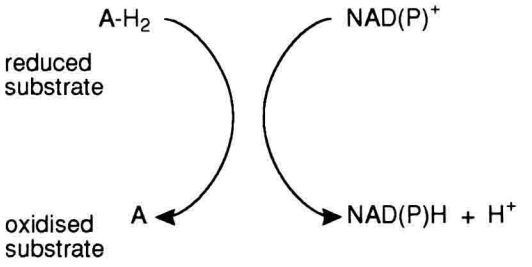
Π Make a list of the sources of energy which may be used by cells.

In your list you probably had a wide range of organic substrates such as carbohydrates, proteins and fats. You may also have included some aromatic and aliphatic hydrocarbons. Careful thought may have enabled you to include light as a source of energy. With such a diversity in energy sources, it is not surprising cellular energy-generating mechanisms are also diverse.

Π Write down a list of agents or systems that can produce reducing power in the form of NADH or NADPH.

heterotrophic systems

This one is not quite so straightforward. Heterotrophic systems (those which use organic nutrients) use the oxidation of organic substrates to produce reducing power mainly, but not exclusively, in the form of NADH or NADPH. Typically we can draw such reactions as:



where A represents an organic molecule.

photo-autotrophs & chemo-autotrophs

In photoautotrophs reducing power is generated by the energy of light in activating electrons to a lower redox potential. In chemoautotrophs, reduced inorganic substrates such as NH_4^+ and H_2S are used to produce reducing power.

Π Write down as many processes as you can by which ATP can be produced from ADP + Pi.

Three types of phosphorylation

We anticipate that you would include in your list oxidative phosphorylation, photophosphorylation and substrate level phosphorylation. The mechanism(s) used by cells for the production of ATP is, of course, related to the energy source that is being used. ATP synthesis and the generation of reducing power are generally linked. Thus in photoautotrophs, reducing power is generated by the energy of light, harvested by chlorophyll, activating electrons to a lower redox potential (ie they become more reducing). ATP is generated by passing some of these strongly reducing (activated) electrons down an electron transport chain. This results in protons (H^+) being pumped across membranes the subsequent pH gradient established across the membrane being used to drive ATP synthesis in a process known as photophosphorylation.

pumping of protons

reduced inorganic substrates

In chemoautotrophs, reduced inorganic substrates (such as H_2S), are used to produce reducing power and to provide electrons for passage down an electron transport chain accompanied by the production of ATP.

reduced organic substrates

Heterotrophs use reduced organic substrates to produce reducing power mainly in the form of NAD(P)H, whilst ATP is generated by substrate level phosphorylation (anaerobes) or by substrate level phosphorylation and oxidative phosphorylation (aerobes). Substrate level phosphorylation is, of course, phosphorylation which takes place when a substrate is converted to a product - for example when phosphoenol pyruvate is converted to pyruvate by the enzyme pyruvate kinase. We remind you that oxidative phosphorylation is the process in which ATP is generated when reduced substrates (eg NADH and $FADH_2$) are oxidised via an electron transport chain. Some anaerobic heterotrophs can, however, produce ATP by using an electron transport mechanism in which an inorganic substrate (eg NO_3^- or $SO_4^=$) acts as an oxidant in place of O_2.

There are many different varieties of organic substrates that may be used by heterotrophs. These include biological products (eg carbohydrates, proteins, lipids, nucleic acids) and aromatic and aliphatic hydrocarbons and their derivatives. Almost all organic molecules, with the possible exception of some man-made polymers such as plastics and halogenated derivatives, can be catabolised (broken down and oxidised) to yield ATP and reducing power.

With these points in mind, it is not surprising that there is a very extensive range of metabolic routes and processes involved in the generation of reducing power and cellular energy.

1.2.2 Generation of metabolic precursors

carbon cycle

Before we turn our attention to biosynthesis, let us examine the generation of the other products of the fuelling reactions, namely the production of simple organic molecules. In heterotrophs these products arise mainly through the breakdown of the organic substrates being used as an energy source.

breakdown of organic substrates

Π How are these simple organic molecules produced in chemoautotrophs and photoautotrophs?

carbon dioxide fixation

The simple answer is from carbon dioxide (CO_2) by a process known as carbon dioxide fixation. The most common, but not ubiquitous, of the metabolic pathways is known as the Calvin (or Calvin-Benson) cycle.

The material discussed so far is summarised in Figure 1.1. The two Biotol texts, 'Principles of Cell Energetics' and 'Energy Sources for Cells', examine in depth the processes represented by the arrows in Figure 1.1. If in reading this section you have realised that you are not sufficiently familiar with the fuelling reactions of heterotrophs and autotrophs, we would suggest that you take the opportunity to read the Biotol texts mentioned above before proceeding.

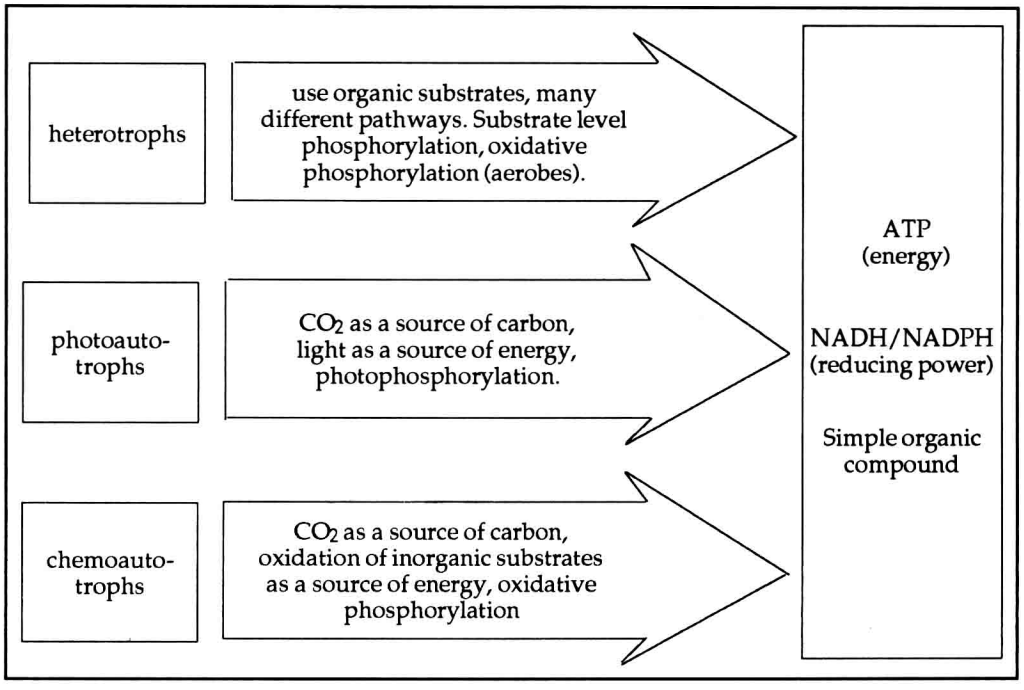


Figure 1.1 The fuelling reactions of cell metabolism.

1.3 Biosynthetic processes are similar in all systems

We begin this section by asking you to respond to a question:

Π Write down the names of the nucleotide bases that are found in nucleic acids. Are different nucleotides used by plants, animals and micro-organisms?

nucleic acids

We would anticipate that you would have written down adenine, guanine, cytidine, thymine and probably uracil. The first four are found in DNA from all sources. There are, of course, differences in the quantities of each nucleotide and in their sequence along the DNA strands. Nevertheless, the point we are attempting to make is that all organisms will need to be able to synthesise the same four bases to make DNA. They will also need to make uracil in order to make RNA.

amino acids

We can apply the same argument to the majority of proteins. We know that about 20 different amino acids are found in proteins irrespective of the organisms from which the proteins are derived. These amino acids are, of course, arranged in different orders and in different ratios within these proteins. Nevertheless all organisms will need to produce the same 20 or so amino acids. It is not surprising, therefore, to find that the biosynthetic pathways are similar (but not necessarily identical) in most organisms.

Variations are shown particularly by cells that:

- live in extreme environments;
- are components of multicellular systems.

primary metabolism

Cells that live in extreme environments often need to produce specialised structures to protect themselves from environmental damage. In multicellular systems there is also greater opportunity for cellular specialisation bringing with it a greater opportunity for a diversity of biosynthetic products. These include such products as pigments (eg in flowers), hormones and alkaloids. This diversity is mainly in what we might regard as secondary metabolism. Primary metabolism (ie metabolism directly related to cell growth), especially the biosynthesis of the core compounds from which cells are made, remains remarkably similar.

secondary metabolism

1.4 Precursor molecules for biosynthesis

12 precursor molecules

Despite the vast array of molecules needed to make cellular structures, biosynthesis begins with 12 quite simple compounds. The names and structures of these are shown in Figure 1.2.

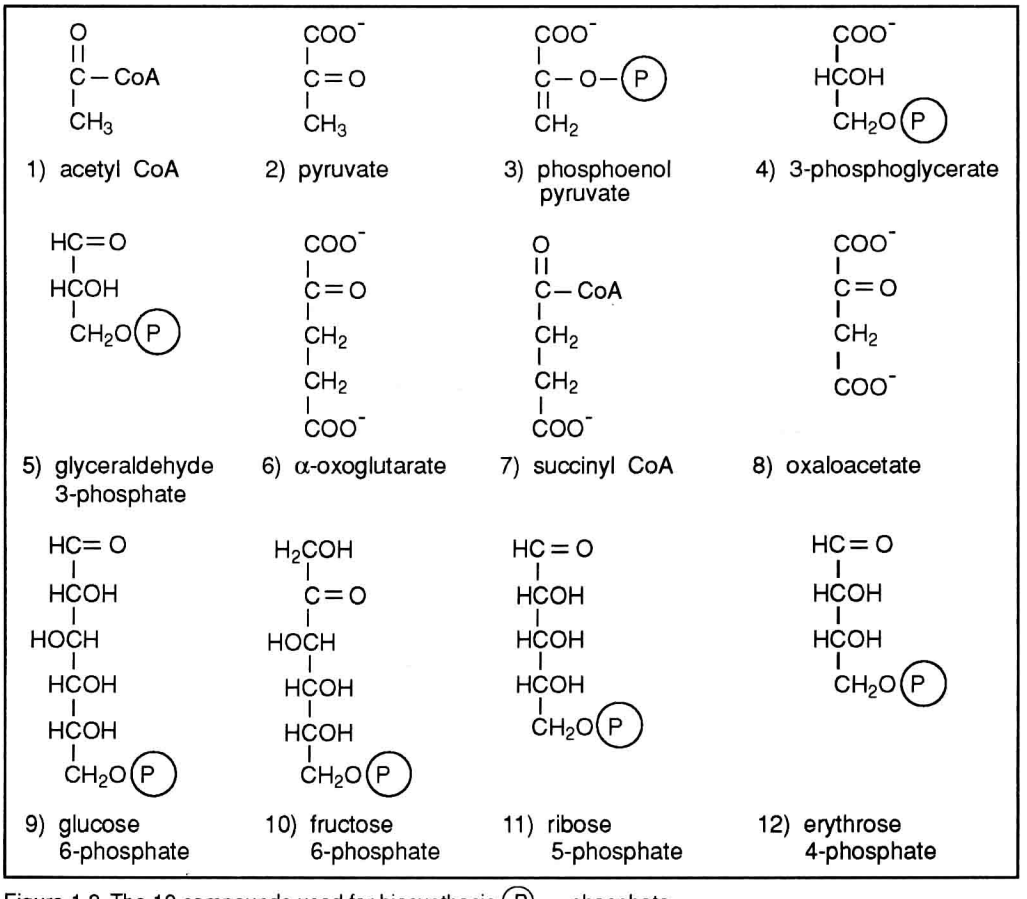


Figure 1.2 The 12 compounds used for biosynthesis. (P) = phosphate.

You will recognise many of the compounds as products of the central fuelling process.

Π You may like to see if you can identify which catabolic processes generate the compounds listed in Figure 1.2. If you can identify at least one pathway for each compound, you clearly have a good knowledge of the catabolic fuelling reactions.

Compounds 1-5, 9-10 occur in the Embden Meyerhof pathway. Compounds 6-8 occur in the TCA cycle and compounds 11 and 12 in the pentose phosphate pathway.

All the amino acids, carbohydrates, lipids and nucleic acids needed for cell synthesis are made from the compounds shown in Figure 1.2. We will learn in later chapters that we can group biosynthetic reactions into clusters or families according to the precursor molecules used and the nature of the products made.

Π In addition to these simple precursors and a source of energy and reducing power to drive biosynthesis, other components are needed. Can you list what they are? (Examine the compounds listed in Figure 1.2 and think about the products that will be synthesised - what is missing?)

source of nitrogen and sulphur

The answer we want includes a source of nitrogen which is needed to make amino acids and nucleotides. We would also anticipate the need for a source of sulphur in order to make the sulphur-containing amino acids (cysteine, cystine and methionine) and the sulphonated carbohydrates. There will also be a requirement for the appropriate enzymes to catalyse the biosynthetic reactions. We could also add to this list, elements that are needed to maintain the structure and activity of cell components, such as magnesium, iron, calcium, potassium and the trace elements.

This text is largely concerned therefore with the processes by which the products of the fuelling reactions, together with utilisable nitrogen (usually NH_4^+) and sulphur (usually $SO_4^=$) sources, are used to make the building blocks for cell synthesis. We can represent these processes diagrammatically, as shown in Figure 1.3.

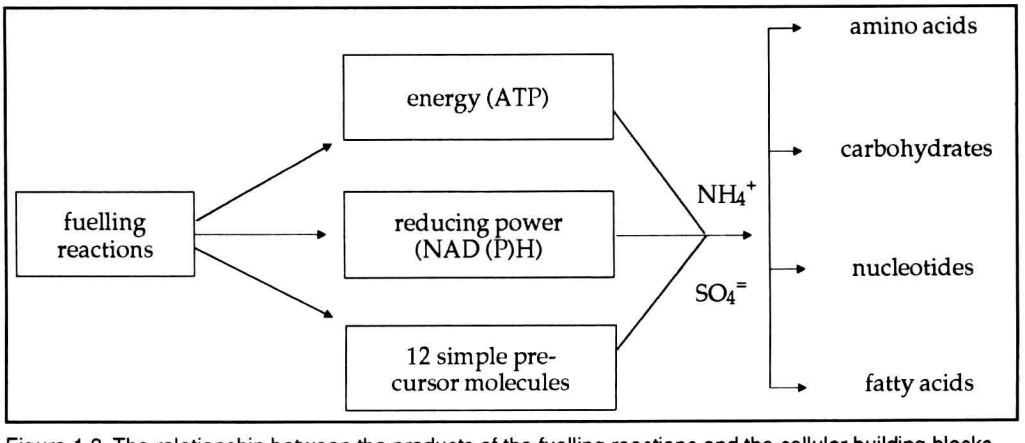


Figure 1.3 The relationship between the products of the fuelling reactions and the cellular building blocks.

You should, therefore, anticipate finding chapters devoted to the pathways and processes leading to each of these major groups of biochemicals. We have, for simplicity, omitted the sequences leading to many of the minor, although important, cell components (eg some cofactors, porphyrins). Three other important aspects of

metabolism are, however, included. These are the uptake of nutrients by cells, the integration of the fuelling and biosynthetic reactions and the regulation of metabolism.

1.5 The arrangements of chapters

nutrient uptake

It is perhaps obvious that before any biosynthesis can take place, nutrients have to be absorbed from the cell's environment. We examine the uptake of nutrients in chapter 2. This is followed by the examination of the special status of nitrogen and sulphur uptake and metabolism. Armed with this knowledge and that of the fuelling reactions gained from the Biotol texts, 'Principles of Cell Energetics' and 'Energy Sources for Cells', you will be ready to study the metabolic pathways which lead to the production of the building blocks of cellular materials. Thus, in subsequent chapters the biosynthesis of fatty acids, sugars, amino acids and nucleotides is examined. These monomers provide the building blocks for the cellular macromolecules. In the chapters relating to fatty acid and sugar biosynthesis we also consider the incorporation of these monomers into lipids and polysaccharides. The polymerisation of nucleotides and amino acids to form nucleic acids and proteins is, however, a rather special case. The polymerisation of these is specified by the sequence of nucleotides in the genome and the consideration of the processes of nucleotide polymerisation (gene replication and transcription) and amino acid polymerisation (translation) should more correctly be discussed in the context of molecular genetics. These processes are described briefly in the Biotol text , 'Infrastructure and Activities In Cells' and more fully in the Biotol texts, 'Genome Management in Prokaryotes' and 'Genome Management in Eukaryotes'.

biosynthesis of building blocks

polymerisation

Thus we can represent the subject matter covered by this text within the context of the total metabolic activities of cells by the shaded boxes shown in Figure 1.4.

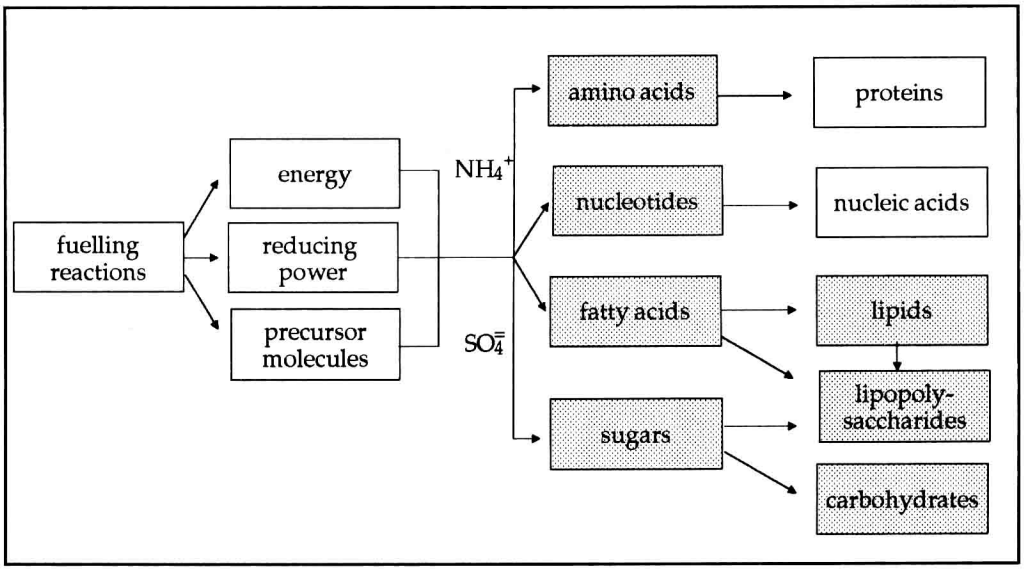


Figure 1.4 The area of metabolism covered by this text (shaded boxes).

The assembly of macromolecules into cellular structures such as the membranes, ribosomes, Golgi apparatus etc, are dealt with in the context of cell biology (Biotol text 'Infrastructure and Activity of Cells').