Chapter 5 / Biochemistry

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5.1 INTRODUCTION

This chapter gives an account of the major biochemical processes that take place in parasitic protozoa and helminths. The reader may immediately point out that there are other sorts of parasites. Unfortunately, space is limited and even restricting discussion to those groups mentioned severely tests the author's capacity for condensation, for the parasitic protozoa and helminths are not homogeneous groups. The decade since the writing of the first edition of this chapter has seen the flowering of the study of parasite biochemistry, and with it has come the realization of the great *diversity* exhibited within and between taxons.

A single chapter is not much to devote to this diversity; of necessity it must be selective in its treatment. The following pages therefore concentrate on dynamic aspects of biochemistry and on concepts. It is assumed that the reader is familiar with basic mammalian biochemistry; any standard biochemistry text constitutes an adequate reference work. For many of the supporting data on parasites, the reader is particularly referred to the late Theodor Von Brand's heroic compendia *Biochemistry of Parasites* and *Biochemistry and Physiology of Endoparasites*; to Barrett's *Biochemistry of Parasitic Helminths*; to *Biochemical Adaptation in Parasites*, by Bryant & Behm; and to *Biochemical Protozoology*, edited by Coombs & North.

The following abbreviations are used in the text: ATP, ADP, AMP – adenosine tri-, di- and monophosphates; cAMP – cyclic AMP; CoA – coenzyme A; DNA – deoxyribonucleic acid; GTP, GDP – guanosine tri- and diphosphates; LDH – lactate dehydrogenase; MDH – malate dehydrogenase; NAD(P)(H) – nicotinamide adenine dinucleotide (phosphate) (reduced); PEP – phosphoenol pyruvate; PEPCK – phosphoenol pyruvate carboxykinase; PK – pyruvate kinase; RNA – ribonucleic acid; TCA – tricarboxylic acid.

5.2 ENERGY METABOLISM

All parasites need to convert organic molecules into their own substance; to do this, they need to maintain a supply of energy for macromolecular synthesis, growth, mechanical activity, differentiation and reproduction. Parasites characteristically exhibit rapid growth or multiplication, which make great demands on energy generating mechanisms. They also need protection from the immune response of the host, another major energetic cost. 'Energy metabolism', then, refers to those biochemical processes that result in the formation of ATP and other energy-conserving compounds which, in turn, are employed in many energy-dependent reactions. Energy metabolism is particularly important because the establishment of a parasite in a new host depends, in the short term, on its ability to sustain life in a harsh environment. Other crises, such as the evasion of the host's immune response, are subordinated to the immediate crisis of survival.

5.2.1 Environments and life cycles

The environments of parasites are legion. Parasitic protozoa and helminths may be found in all tissues of vertebrates and invertebrates. Each tissue has its own special characteristics. It may be rich in oxygen or carbon dioxide. It may provide the parasite with a well-regulated supply of metabolic precursors, as does the blood vascular system or, as in the intestine, availability of nutrients may depend on the host's diet. It is not

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surprising, therefore, that there is diversity in the pathways of energy metabolism of internal parasites, but there are common features about classes of environments that make a few generalizations possible. One is that, whether a parasite is aerobic or anaerobic, it requires a source of highly reduced organic compounds, an efficient mechanism for energy entrapment and the capacity to maintain its intracellular environments at the right level of oxidation.

Parasites often occupy more than one environment during their life cycles, adding further complications to the study of their biochemistry. Biochemical strategies that enable survival in one environment may not be appropriate to another. The parasite must have the genetic capacity to allow it to recognize and grow in more than one environment. There must be a complex programme for the expression of different genes at different times, leading to the elaboration of different biochemical pathways at each of the life stages. This is often achieved by sensitivity to 'trigger' stimuli. Many parasites embark on the adult stages of their life cycles in response to the high carbon dioxide concentrations, reducing conditions or the high temperatures encountered in their definitive hosts.

5.2.2 Energy stores

The most usual type of macromolecule retained as an energy source is some form of carbohydrate. In trichomonads and *Entamoeba*, glycogen accounts for 10–30% of dry weight, but there is little, if any storage carbohydrate in trypanosomes or malarial parasites. This is probably because those forms that inhabit the vertebrate bloodstream, about which most is known, occupy an environment that contains ample glucose maintained at constant levels by the host's homeostatic mechanisms. In the insect host a very different form of metabolism occurs, which takes advantage of the availability of amino acids. Also of interest is a polyphosphate found in *Crithidia fasciculata*, a kinetoplastid flagellate. It may be an energy store, but may also be important in the regulation of metabolism.

Glycogen storage is a characteristic of helminth parasites, and is often found in large quantities, as much as 10% of dry weight in individuals of some tapeworms. Generally, glycogen is depleted during 'starvation' of helminth parasites in *in vitro* culture, which provides prima facie evidence that it is indeed an energy store. Some helminths also store trehalose, a soluble disaccharide, in their tissues. For example, in the acanthocephalan *Moniliformis dubius* and the larval nematode *Trichinella spiralis*, trehalose accounts for up to 2.5% of tissue solids.

Glucose, too, is universally present in parasites. It is generally not stored, but active transport mechanisms for its uptake from the environment are widespread in protozoa and helminths. In *Entamoeba*, glucose uptake is the rate limiting step in metabolism. There are active transport mechanisms for glucose uptake in cestodes and nematodes, while recent studies have shown that glucose uptake in trematodes may vary depending on the ecological niche occupied by the parasite. Thus, glucose uptake in *Fasciola hepatica* is passive, presumably because there is no shortage of glucose in its predilection site in the mammalian liver. On the other hand, in three different species of the fish fluke *Proterometra*, the possession of an active transport system for glucose uptake seems to be directly related to the availability of glucose in the immediate environment. The species that lives in the more external environment, where little glucose is available, has a well-developed active transport system. In all these parasites, glucose is an intracellular metabolic pool, maintained from the environment or by the breakdown of stored polysaccharide, and is either oxidized for immediate energy yield or converted to storage product.

In adult helminths and protozoa, lipids do not form an energy store. Almost all parasites except some larval helminths lack the enzymes necessary for their oxidation.

5.2.3 Regulation of energy metabolism

A useful aid in understanding metabolic regulation is the concept of the 'adenylate energy charge'. It is a measure of the energy resident in the adenylate system and is given by the following expression:

$$\frac{[ATP] + 1/2[ADP]}{[ATP] + [ADP] + [AMP]}$$

where the square brackets indicate the concentration of the adenylate in question. The value of the adenylate energy charge is about 0.8–0.9 for many mammalian tissues; in parasites it varies between 0.6 and 0.9, the lower values usually being obtained in investigations of anaerobic metabolism. It is less subject to fluctuation than the ATP/ADP ratio but in both cases, high values suggest that the organism is in good health and is probably engaged in synthetic processes. Many anabolic enzymes are activated, while catabolic enzymes are down-regulated, by the allosteric and substrate effects of high concentrations of ATP and low concentrations of ADP. At low adenylate energy charge levels the reverse is true.

Redox state, which can be practically determined as the ratio of the intracellular concentration of NAD(P) to that of NAD(P)H, is another useful indicator. A high concentration of NAD(P) favours catabolic processes, high NAD(P)H favours anabolic ones. Barrett (1991) observed NAD/NADH ratios in *Ascaris* muscle cytoplasm up to 2214 to 1, which indicates that the muscle is maintained at a high level of redox, achieved by coupling NADH oxidation to malic dehydrogenase.

Except in a few instances, metabolic pathways in parasites are regulated the same way as in other organisms. They respond to adenylate energy charge, to the redox state, their enzymes show allosteric activation and, generally, similar enzymes are involved in regulation. The metabolic pathways are subject to feed-forward and feedback control and to product inhibition. Differences lie in the interrelationship of metabolic sequences, and the fact that, in different organisms, different properties of similar enzymes are enhanced or suppressed for the fine-tuning of metabolic pathways that adapt parasites to different environments.

5.3 ENERGY METABOLISM IN PARASITIC PROTOZOA

Protozoan parasites are not a homogeneous group and many of them (for example, the malaria parasites and the trypanosomes) are adapted to more than one host in a single life cycle. As hosts are as different as mammals and insects it is not surprising to discover that metabolism of a given parasite within each of its hosts is quite distinctive and that mechanisms exist for rapidly switching between modes when transmission to another host takes place. There are thus many different types of energy metabolism exhibited by parasitic protozoa, and there is space for little more in this chapter than a few generalizations.

Glucose is the major substrate for energy metabolism in many groups, such as malarial parasites, bloodstream salivarian trypanosomes, trichomonads, *Giardia* and *Entamoeba*. In these organisms, the emphasis is on glycolysis. Others (insect stages of *Trypanosoma cruzi*, *Leishmania* spp. and the salivarian trypanosomes) are capable of at least partial oxidation of fatty and amino acids by a specialized TCA cycle.

Not surprisingly, parasitic protozoa show many adaptations which are unique to this diverse assemblage. They include metabolic pathways with a high carbon flux and a high energy output, and specialized organelles for maintaining the appropriate enzymes in close proximity to one another. For example, in the bloodstream forms of the African trypanosomes the process of 'aerobic glycolysis' involves the interaction of two organelles and the cytosol. The enzymes of glycolysis, to the level of phosphoglycerate kinase, are contained in the glycosome, and are present in high concentrations; the cytosol contains phosphoglycerate mutase, and the mitochondrion, a simple tube without TCA cycle activity at this stage, contains the membrane-bound glycerophosphate oxidase complex. Glucose enters the glycosome, is rapidly metabolized to 1,3-diphosphoglycerate and glycerol-3-phosphate. The former leaves the glycosome and enters the cytosol, where it is converted to pyruvate (there is no LDH). Meanwhile, glycerol-3-phosphate leaves the glycosome for the mitochondrion where it is oxidized to dihydroxyacetone phosphate. This then re-enters the glycosome, while its electrons are transferred by the oxidase to oxygen. It is not clear whether this electron transfer results in the synthesis of ATP, but an important outcome is that it ensures the continual reoxidation of NADH. The net effect is to maintain

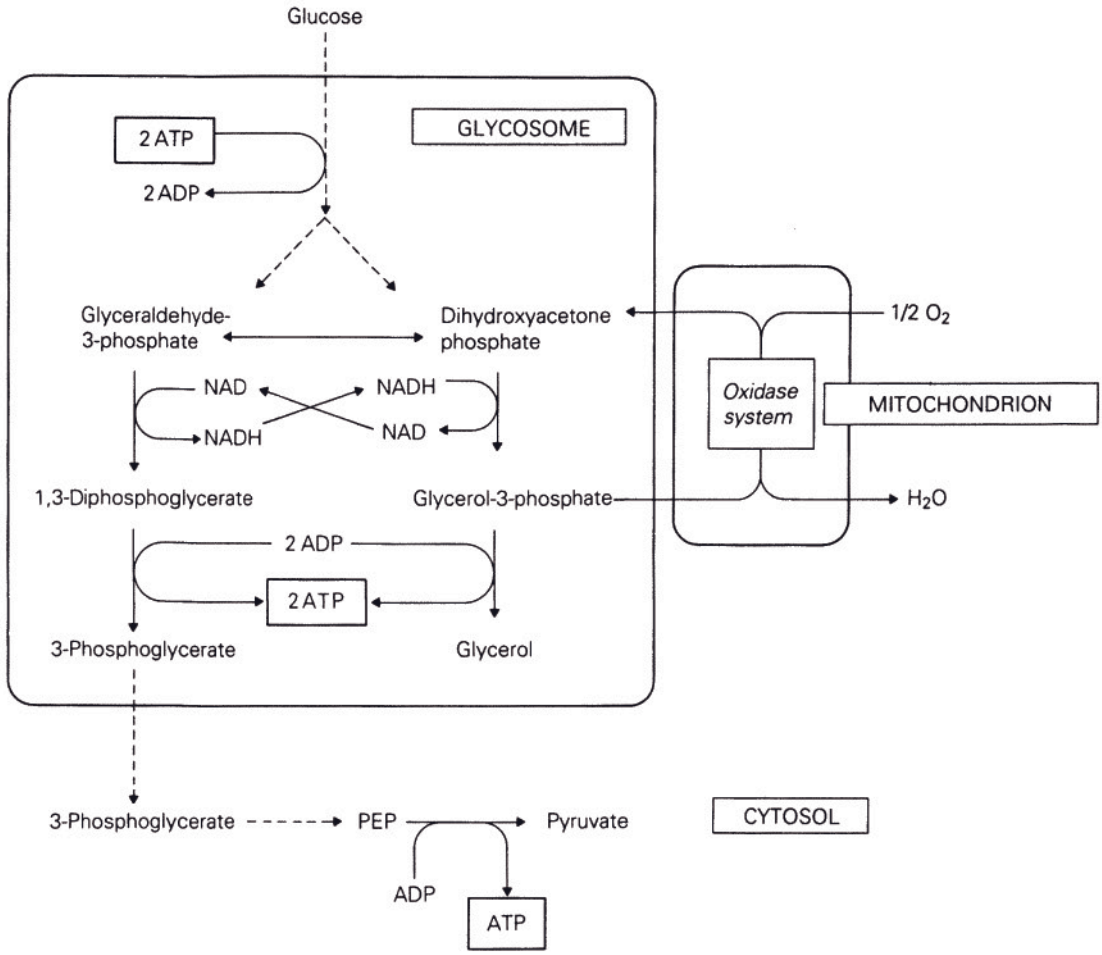

Fig. 5.1 Aerobic glycolysis in long-slender bloodstream forms of trypanosomes. Abbreviated metabolic scheme which shows the cooperation of enzymes from three cellular compartments within the cell. Dotted arrows indicate that several reactions have been omitted for simplification.

rapid glucose oxidation and ATP production in the glycosome and cytosol compartments (Fig. 5.1). The potential yield of ATP from this pathway is only 2 moles per mole glucose utilized, but, as glucose is plentiful, the lack of efficiency of energy conservation is amply compensated for by the high throughput of glucose carbon.

Glycosomes possess only a single membrane, and are unique to the kinetoplastids. Their origin is obscure but they are probably related to peroxisomes. They can sustain a very high rate of glycolysis because of their high concentrations of glycolytic enzymes and their impermeability.

The mitochondrion of trypanosomes is remarkable in that it changes its form and function at each stage of the life cycle. In the 'long-slender' bloodstream forms, there is little else but the glycerate phosphate oxidase complex. In later bloodstream forms – 'intermediate' and 'short-stumpy' – the mitochondrion may be branched and contain cristae, and possess the additional functions of acetate or succinate production from pyruvate. Fumarate reductase is present, and so are the enzymes of the TCA cycle although it does not function as such. The glycosome changes, too, and acquires PEPCK and MDH

activities. The latter assumes the role of reoxidizing NADH as glycolytic activity diminishes.

These changes foreshadow the requirements of survival in the insect host. After ingestion, the parasite transforms to a procyclic trypomastigote and migrates to the salivary glands. It develops a highly branched mitochondrion, with large numbers of cristae, and a fully functional TCA cycle, and is able, at least partially, to oxidize proline and other amino acids from the insect's haemolymph.

Of particular interest among the metabolic adaptations of the parasitic protozoa are the hydrogenosomal pathways of the trichomonads. These organisms are classed as aerotolerant anaerobes and respire stored glycogen. Instead of mitochondria, they possess a unique subcellular inclusion, the hydrogenosome. The hydrogenosome has a double membrane, but lacks cytochromes and DNA, and its activity results in the production of molecular hydrogen. It is tempting to speculate that it owes its origin to an ancient symbiosis between a flagellate and a clostridial-like organism.

Although the details may vary between species of trichomonads, Fig. 5.2 illustrates the principles. Glycogen is metabolized by an augmented glycolytic pathway to PEP on the one hand and to glycerol on the other. PEP is further converted to pyruvate and malate, both of which may enter the hydrogenosome. Within the organelle, a series of redox reactions lead to the production of molecular hydrogen and the synthesis of ATP. If oxygen is present, it acts as the terminal electron acceptor and hydrogen is not produced. Oxygen reduction mostly occurs in the cytosol via a soluble NADH oxidase, and while there is no apparent energy gain it is thought that the process is important in oxygen scavenging, protecting the anaerobic enzyme battery within the hydrogenosome from damage by oxygen derived free radicals.

Like the trichomonads, neither *Entamoeba* nor *Giardia* possesses mitochondria, but neither do they possess any other organelle for energy metabolism. Many aspects of their metabolism appear to be primitive; *Entamoeba*, for example does not have a nucleolus, normal ribosomes,

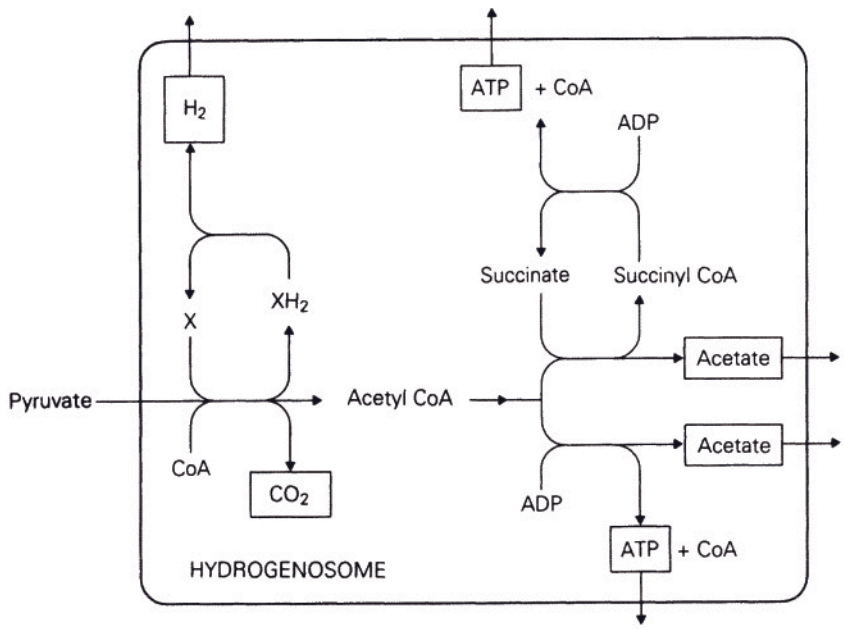

Fig. 5.2 The role of the hydrogenosome in trichomonads. Reactions that are written inside the box take place in the hydrogenosome. X is an unknown carrier that interacts with an unusual hydrogenase to produce molecular hydrogen.

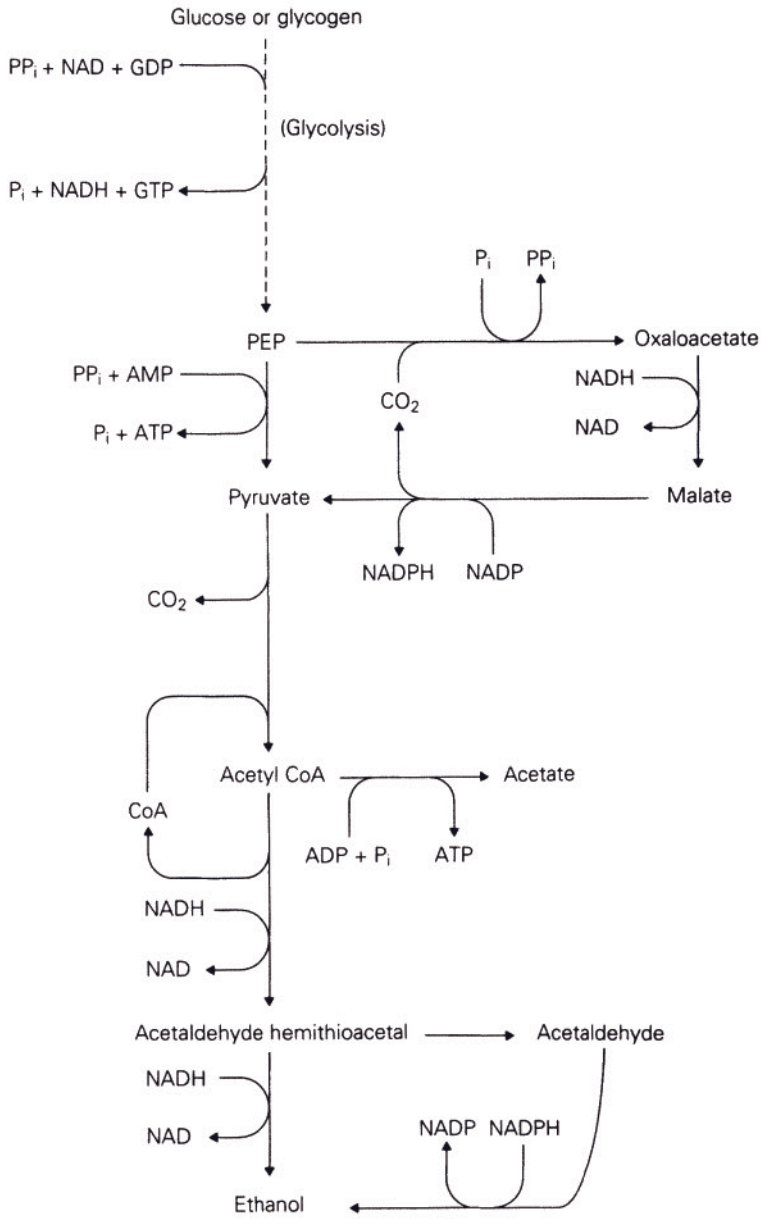

Fig. 5.3 Pathways of anaerobic glucose metabolism in *Entamoeba*. Those in *Giardia* are very similar but less completely known. P_i, PP_i – inorganic phosphate and pyrophosphate.

microtubules, true Golgi apparatus or an extensive endoplasmic reticulum. Both organisms have proved difficult to study because of the presence of ingested bacteria, whose enzymes may contribute to metabolism.

Entamoeba is an aerotolerant anaerobe, consuming oxygen either through NAD(P)H oxidases, or a series of reactions centred on the pyruvate synthase complex. Glucose is the principal respiratory substrate and its transport into the cell appears to be the rate limiting step in metabolism. Glucose may be converted to glycogen and stored, or it may be catabolized to ethanol and carbon dioxide anaerobically, while aerobically, acetate

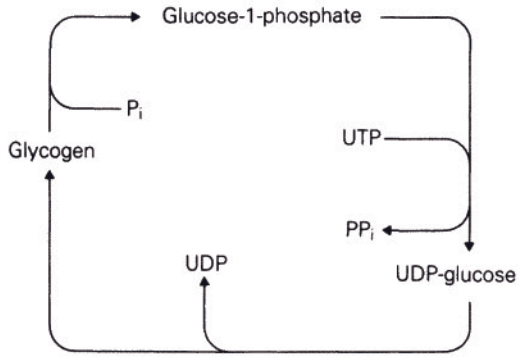

Fig. 5.4 The glycogen cycle generates pyrophosphate (PP_i) in *Entamoeba*, and may be important in metabolic regulation. UDP, UTP – uridine di- and triphosphates.

is produced as well (Fig. 5.3). A glycogen cycle (Fig. 5.4) may contribute pyrophosphate which is important in glucose catabolism. It has been suggested that the pathway of glucose breakdown, with its reliance on pyrophosphate and thiol compounds for energy conservation, may be an ancient evolutionary relict, conserved because of the specialization of *Entamoeba* to parasitic mode of life.

Giardia, also an aerotolerant anaerobe, does not possess the unique enzymes found in *Entamoeba* although its respiratory end-products are similar, perhaps due to its occupation of similar habitats. There is no TCA cycle activity. It consumes oxygen, perhaps, like *Entamoeba*, in order to detoxify it, and grows best *in vitro* when oxygen concentrations are low. The pathway for glucose catabolism has not been fully elucidated, but is probably as shown in Fig. 5.3.

An important characteristic of both *Entamoeba* and *Giardia* is their ability to couple ATP formation directly to the cleavage of the thioester bond:

acetyl CoA + ADP + Pi → acetate + CoA + ATP.

5.3.1 Electron transport in parasitic protozoa

The 'anaerobic' protozoan groups, discussed above, do not possess mitochondria and do not appear to have haem proteins such as cytochromes or catalase. Electron transport is quite different from that in other parasites, and includes low redox potential iron–sulphur compounds, with the properties of ferridoxins, active in both anaerobic and aerobic electron transport. A tentative scheme for aerobic electron transport in *Entamoeba* is shown in Fig. 5.5; it is unlikely that its activity results in energy conservation.

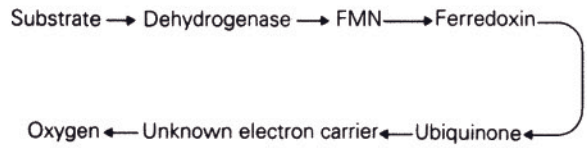

Fig. 5.5 Aerobic electron transport in *Entamoeba*. FMN – flavin mononucleotide.

The other major groups of protozoans, the trypanosomes, leishmaniae and malarial parasites, all possess mitochondria containing cytochromes. They respire aerobically but there is much variation in electron transport between groups and between different stages in their life cycles. Many of them appear to have branched respiratory chains, like the helminths. For example, a branched electron transport chain with two terminal oxidases, has been proposed for *Plasmodium* species. Its role in respiration is unclear, since glucose metabolism in these organisms yields lactate as the only end product. It appears likely that oxygen utilization is associated instead with pyridine metabolism, via dihydroorotate dehydrogenase activity.

5.4 ENERGY METABOLISM IN PARASITIC HELMINTHS

All helminths utilize glucose as a respiratory substrate – indeed, tapeworms incubated *in vitro* are able to absorb almost all the glucose provided in incubation media. They have very active transport mechanisms which bind glucose at very low concentrations. Once glucose is absorbed, it is either converted into glycogen, to act as an energy store, or is metabolized directly via the glycolytic sequence of reactions as far as PEP. There are then a number of different options for its further metabolism. Each of the options may be found within a single taxonomic group of helminths –

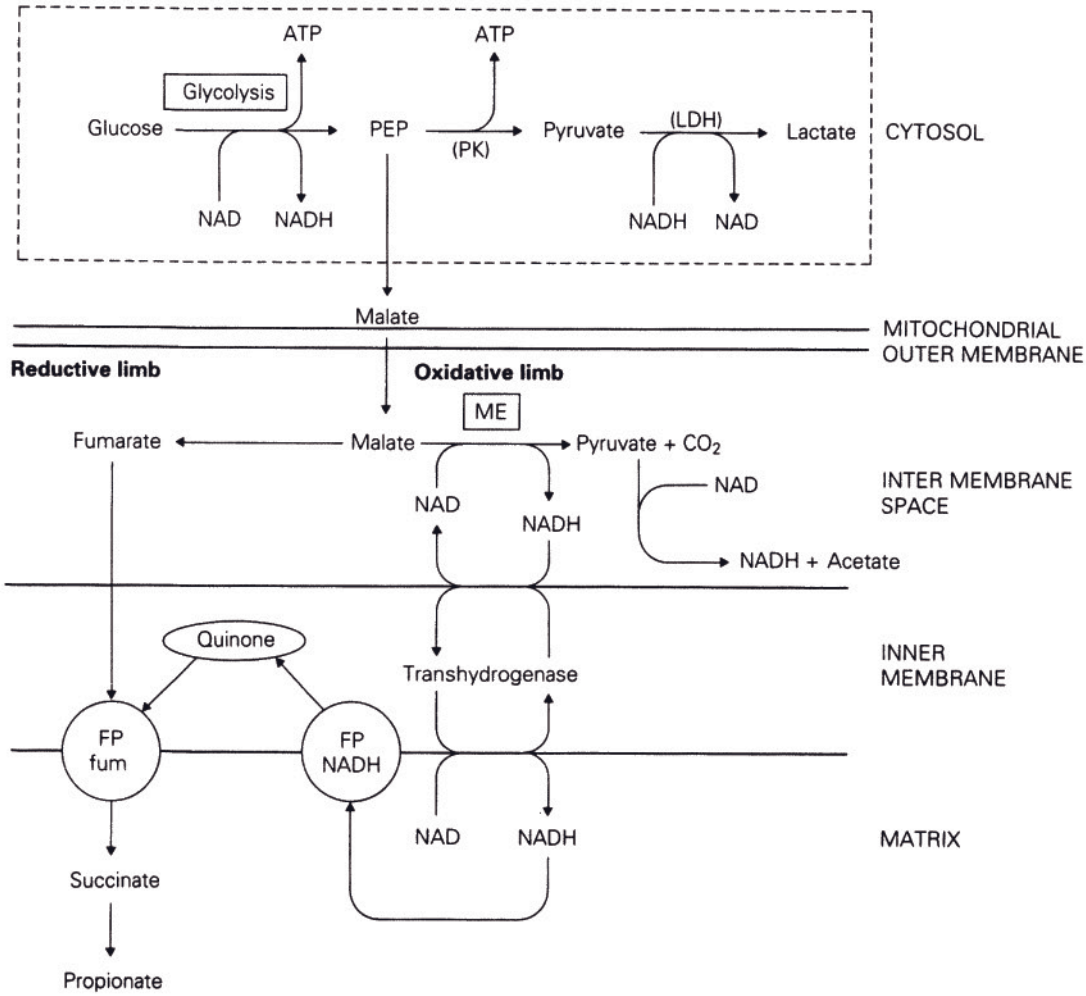

Fig. 5.6 Homolactate fermentation (in the dashed box) and the malate dismutation in parasitic helminths. In the malate dismutation, electrons are transferred from NADH (generated by malic enzyme activity and the pyruvate dehydrogenase system) across the membrane by the transhydrogenase. They are then passed to NADH dehydrogenase (FP NADH), quinone and fumarate reductase (FP fum). In this scheme there is no role for cytochromes. Another view suggests that cytochrome mediated electron transport intervenes at the level of the quinone. FP – flavoprotein; ME – malic enzyme.

there are no predictions that can be made about the type that will be encountered in a particular group.

5.4.1 Homolactate fermentation

True homolactate fermentation (Fig. 5.6) probably does not occur in helminths. In homolactate fermentation glucose is converted exclusively into lactic acid by glycolysis. The end-product, lactic acid, is excreted and energy generation is thus wholly independent of oxygen. ATP is synthesized at the phosphoglycerate kinase and pyruvate kinase steps, and NADH is reoxidized by lactate dehydrogenase, so that the pathway remains in redox balance. There is, however,

evidence that other energy yielding processes also occur in the so-called helminth homolactate fermenters. For example, schistosomes display low levels of TCA cycle activity that contribute significantly to energy metabolism. This is because the aerobic oxidation of glucose generates 18 times more ATP per mole of glucose than homolactate fermentation.

The same is true for the filarial worm, *Litomosoides carinii*. Overall, studies with ^{14}C-labelled substrates suggest that 2% of utilized carbohydrate may, in normal respiration *in vitro*, undergo complete oxidation to carbon dioxide and water. A simple calculation shows that 98 moles of glucose converted to lactate yields 196 moles of ATP, while 2 moles of glucose converted to carbon dioxide and water by the TCA cycle yields 72. From this, it is clear that at least 27% of energy generation in *L. carinii* could be aerobic.

5.4.2 Malate dismutation

The malate dismutation (Fig. 5.6) has been most extensively studied in *Ascaris* spp., *Hymenolepis diminuta* and *Fasciola hepatica*. Glucose is oxidized to the level of PEP. A carbon dioxide fixation step then occurs, catalysed by PEPCK, leading to the formation of oxaloacetate which is subsequently reduced to malate. This can be contrasted with what occurs in mammals. In mammals, PEP is converted to pyruvate, which in turn is converted to acetyl CoA by the pyruvate dehydrogenase system, and the subsequent metabolism of acetyl CoA is by the TCA cycle in mitochondria. The operation of this aerobic system permits the synthesis of large amounts of ATP and the ultimate reoxidation of reduced cofactors by oxygen. The role of PEPCK in mammals is the reverse of that encountered in helminths, the decarboxylation of oxaloacetate during gluconeogenesis. It serves as a bypass of the irreversible pyruvate dehydrogenase step.

Few, if any, parasites possess a TCA cycle as active as that of mammals. Generally, where the cycle is complete, it operates at very low activities. Important enzymes of the cycle are often missing or present in very small amounts. There are no rules as to which ones will be absent. Citrate synthase, aconitate hydratase and isocitrate dehydrogenase are often not detectable in trematodes and cestodes. But there are three enzymes of the TCA cycle which are, almost invariably, present in the mitochondria of parasitic helminths. They are MDH, fumarate hydratase and fumarate reductase. They catalyse the following reductive reactions, a reversed sequence of part of the TCA cycle:

$$\text{oxaloacetate} \xrightarrow[MDH]{} \text{malate} \xrightarrow[\substack{fumarate\\hydratase}]{} \text{fumarate} \xrightarrow[\substack{fumarate\\reductase}]{} \text{succinate.}$$

In the mitochondria, there are also two important oxidative enzymes, malic enzyme (ME) and the pyruvate dehydrogenase complex, that catalyse the following reactions.

$$PEP + CO_2 + GDP \xrightarrow[PEPCK]{} \text{oxaloacetate} + GTP,$$

$$\text{malate} + NAD(P) \xrightarrow[ME]{} \text{pyruvate} + CO_2 + NAD(P)H.$$

Malate then enters the mitochondrion, where it undergoes a dismutation. In a dismutation reaction, one molecule of a given compound is oxidized, while a second is reduced. The oxidation step is coupled to the reductive step and provides the reducing power to drive the reaction. In the malate dismutation, there is an additional need to maintain redox balance which demands the oxidation of one molecule of malate while two molecules are reduced. This is because in the oxidative arm of the pathway there are two reactions that each generate one reducing equivalent per molecule of malate oxidized, while the reductive arm has only one reaction that utilizes reducing equivalents. The products of the dismutation, usually acetate and succinate or propionate, are then excreted as the free acids (Fig. 5.6). The net result is the production of more ATP than is obtained fom homolactate fermentation on its own (Table 5.1).

The malate dismutation involves two subcellular compartments – the cytosol and the mitochondrion – which must both remain in redox balance. Lactate and malate, when produced in the cytosol, are equivalent in metabolic terms. The dehydrogenases that produce them have a similar role in regenerating NAD. Further, the

Table 5.1 ATP yield of different types of fermentation

End product	mol ATP formed per mol glucose
$CO_2 + H_2O$	36
Lactic acid	2
Ethanol	2
Alanine	2
Acetate	2
Acetate + succinate	3.7
Acetate + propionate	5.4
Acetate + propionate and other fatty acids	5

series of reactions that produce either lactate or malate are equivalent energetically, because PK and PEPCK each bring about the synthesis of ATP. PEPCK does it at one remove because its preferred cofactor is GDP not ADP, and it generates GTP. An additional reaction is thus necessary for conversion to ATP:

PK reaction:

$$PEP + ADP \rightarrow pyruvate + ATP.$$

PEPCK reaction:

$$PEP + GDP + P_i + CO_2 \rightarrow oxaloacetate + GTP.$$

PK and PEPCK stand at an important branchpoint of metabolism in helminths. The flow of carbon through PEPCK must be regulated so that the redox balance of each cellular compartment is maintained. The nature of the regulation is dynamic, as it is observed that under different environmental conditions, the proportions of the end-products change. PK and PEPCK are influenced allosterically by purine nucleotides and by metabolic intermediates. The concentrations of these change depending on the availability of terminal electron acceptors, bringing about adjustments to carbon flow through the different metabolic branches.

The malate dismutation is, of course, an oversimplification. Other substrates, such as pyruvate may also enter and be oxidized within the mitochondrion. There may be some TCA cycle activity and respiratory patterns may change during development. For example, the juvenile liver fluke, *Fasciola hepatica*, is an almost totally aerobic organism with tricarboxylic acid cycle activity. Three weeks later it has lost much of this and its energy metabolism depends on aerobic acetate formation. By about 2 months a proper malate dismutation has developed.

End-products of metabolism in parasitic helminths include ethanol, lactate, acetate, succinate and volatile fatty acids. No one helminth excretes all of these end-products, and it is difficult, given the present state of knowledge, to know why a particular set of end-products has been adopted by a particular organism. The best answer is that it is an accident of evolutionary history. The formation of such highly reduced end-products can be explained in terms either of 'energetic advantage' or of 'redox advantage'. The first implies that the specialized pathway to a particular end-product leads to energy conservation as, for example, ATP or GTP. The second means that reduced cofactors such as NAD(P)H, generated in other parts of the pathway, are reoxidized during the formation of the end-products. Recycling of cofactors is thus made possible, and the various subcellular compartments are maintained at the appropriate redox level to permit the continued oxidation of glycogen, even under anaerobic conditions.

In *Ascaris* and a number of other nematode genera the malate dismutation is extended by a number of redox and condensation reactions. The main end-products of glucose catabolism are 2-methylvalerate and 2-methylbutyrate, with small amounts of propionate, acetate and other volatile fatty acids (Komuniecki *et al.*, 1981). The pathways for their production involve two reductive steps, in which NADH is consumed, an acyl CoA intermediate is formed, and the regeneration of CoA occurs at the end of the reaction sequences. They are thus remarkably similar to a reversed beta-oxidation pathway for fatty acid breakdown, absent from adult helminths. Helminths have only a very limited capacity for metabolizing fats, although it does occur in some larval nematodes.

Energy metabolism is variable; it varies between and within species and even within individuals. Variability in a system such as that of energy generation, that one feels intuitively ought to be conservative, is intriguing and deserves a little more discussion. Barrett (1981)

remarks that end-products selected by helminths represent a compromise between substrate conservation and rate of working. In other words, a sufficiently high energetic yield can be obtained by incompletely oxidizing large amounts of respiratory substrate. Further, organisms that carry out mixed fermentations in various subcellular compartments have more opportunity for maintaining appropriate redox levels in those compartments, by altering carbon flow. This presumably provides flexibility in adapting to changing environmental circumstances.

The size of a parasite may be important in determining the type of energy metabolism and there is a good inverse correlation between the thickness of the body and the aerobic capacity of nematodes. As worms increase in size, poor diffusion of oxygen into the deeper tissues of large parasites may preclude complete dependence on oxygen as an electron acceptor. Fairbairn (1970) observed that, as the dissociation constants for lactic and succinic acids were high ($K_a = 13.87 \times 10^{-5}$ and 6.63×10^{-5}, respectively), they would be less likely to dissociate at physiological pH and would therefore pass more easily through a lipid membrane than the dissociated ionic species (protons and anions of acids). In other words, Fairbairn considered that there might be an energetic advantage in excreting certain acids. Yet another suggestion was made by Bowlus and Somero (1979). They pointed out that the properties of succinic acid were compatible with the enzyme systems that produced it and that succinate actually stabilizes protein structure. It is difficult to know how to choose between these options. A sensible conclusion is that they all have a part of the truth.

5.4.3 Electron transport in helminths

The mechanism of electron transport in mammals involves the reoxidation of reduced cofactors by a system of enzymes and electron carriers. The latter include flavoprotein dehydrogenases, ubiquinone, cytochromes b, c and a and finally cytochrome oxidase which transfers the electrons to oxygen, with the formation of water. During electron transfer ATP formation occurs at the sites shown in Fig. 5.7.

All parasitic helminths show, *in vitro*, a measurable uptake of oxygen; while some of the oxygen is no doubt used for synthetic reactions, a part of it is used in respiration. It is widely accepted that while many adult helminths possess low activities of classical electron transport systems, their specialized electron transport systems are anaerobic. In addition, they may also be branched and therefore possess several terminal oxidases. In some helminths, for example, there may be three branches: one with cytochrome oxidase, the second with a b-type cytochrome, and a third which generates hydrogen peroxide (Fig. 5.8).

Electrons are transported along the chain of electron carriers either to cytochrome oxidase, to the alternative oxidase or to the fumarate reductase system. The products of respiration are either water, the potentially dangerous hydrogen peroxide (removed either by catalase or

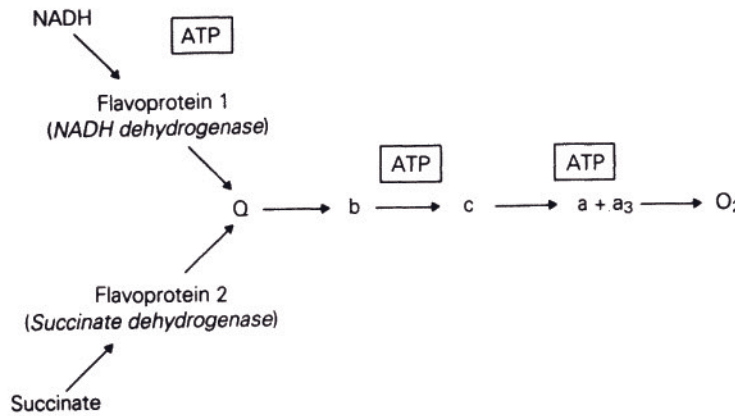

Fig. 5.7 A simplified diagram of the electron transport system in mammals, showing phosphorylation sites. Q is ubiquinone or coenzyme Q.

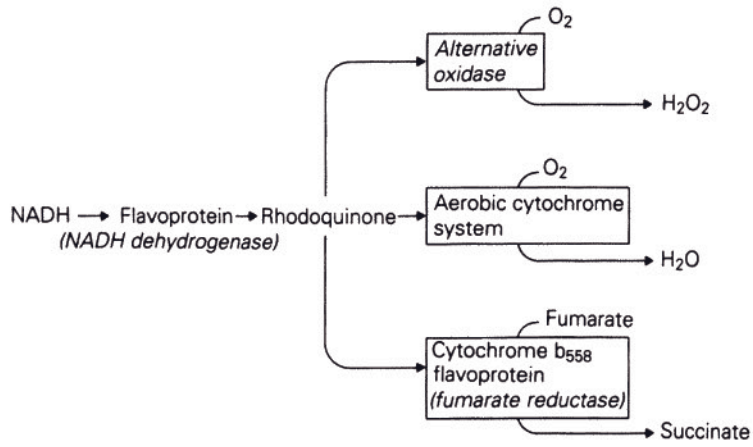

Fig. 5.8 A possible scheme for branched electron transport in parasitic helminths.

peroxidase, both of which are found in helminths at high activities) or succinate and its derivatives. By analogy with the mammalian system, it is presumed that there are three proton translocating sites for ATP synthesis.

Instead of ubiquinone, many helminths possess rhodoquinone, an extremely interesting anaerobic adaptation. The redox potential of rhodoquinone (E_0) is −63 mV, which is considerably lower than ubiquinone (+113 mV) and the fumarate/succinate couple (+33 mV). Electron transport in the direction of fumarate is thus favoured. Helminths also possess an enzyme complex called fumarate reductase, crucial to malate dismutation. Fumarate reductase is distinct from the enzyme that catalyses the reverse reaction in aerobic organisms, succinate dehydrogenase. The enzyme from *Ascaris* has been purified and found to consist of four major and two minor polypeptides, two of which have the same molecular weight as the two subunits of mammalian succinate dehydrogenase. It also contains large amounts of cytochrome b_{558}. A detailed account of helminth fumarate reductase and the evolution

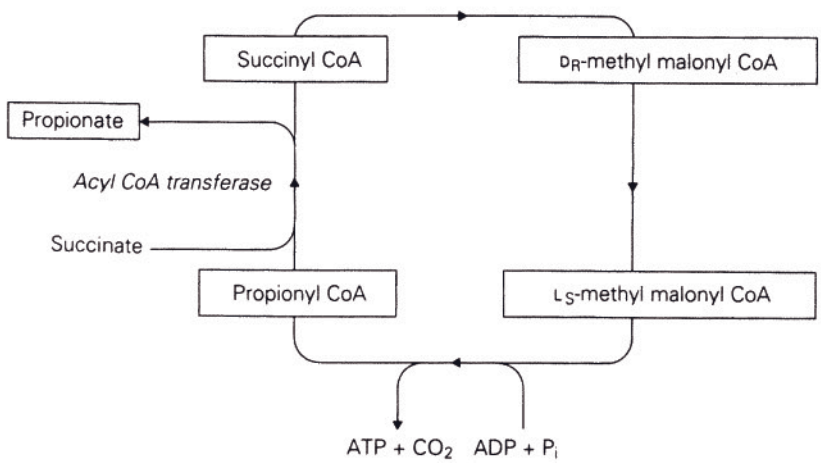

Fig. 5.9 Propionate production by the succinate decarboxylase system in helminth mitochondria. CoA is recycled by the transferase.

of electron transport systems is given by Behm (1991).

Finally, Köhler (1985) points out that acyl CoA transferase reactions and acyl CoA-dependent ATP conservation play an important role in energy metabolism in parasites. Propionate formation within mitochondria is a case in point and occurs in *Fasciola* and *Ascaris* as illustrated in Fig. 5.9. It is another example of the reversal of a cycle found in mammals, this time in ruminants that utilize propionate produced by the rumen microorganisms.

5.5 LIPIDS

Lipids can be divided into three fractions on the basis of their behaviour in experimental separation systems. The fractions are: (1) glycerides, i.e. esters of glycerol and fatty acids – and free fatty acids; (2) phospholipids; and (3) unsaponifiable lipids, which include waxes, sterols, terpenes and related substances. A major part of the structure of membranes comprises many different types of lipids.

While a great deal is known about the distribution of lipids and lipid-like compounds in parasites, their metabolism is a much neglected field of study. All parasites contain lipids in varying amounts and they include a wide variety of molecules, such as the acylglycerols, fatty acids, phosphoglycerides, waxes and sterols. There is no evidence that a particular site within a host requires a particular type of lipid profile, but there is some evidence that, in helminth parasites, lipid content reflects that of the host. The major fatty acids usually contain 16 or 18 carbon atoms, especially the unsaturated oleic and linoleic acids. In parasitic helminths, the range of fatty acids found often depends on host diet and can be changed by changing that diet. They may also reflect the constituent fatty acids of the host. For example, sharks contain C20 and C22 polyunsaturated fatty acids which help to keep them supple at sea-water temperatures, because unsaturated fatty acids have lower melting points than saturated ones. The same fatty acids are found in their tapeworms.

Phylogenetic relationships do not offer any indication of the types and amounts of lipids that a given parasite may possess. Thus the lipid content of five species of *Trypanosoma* ranges from 11 to 20% of dry weight, and for parasitic protozoa as a whole the average is about 14%. Helminth parasites show much greater variation: 1–34%.

The functions of the various lipid components in parasites are understood only by analogy with other organisms, but there is reason to suppose that there are a number of differences. Parasites like other organisms, contain cells with external and internal membranes. The former are especially important since they must protect the parasite from host attack and, at the same time, permit and perhaps control the uptake of nutrients across the membrane. Several unique pathways or enzymes have been identified in some parasitic protozoa. For example, acetate units for lipid synthesis in bloodstream and culture forms of *T. brucei* are preferentially derived from threonine by the action of threonine dehydrogenase and glycine acetyltransferase. This pathway does not occur in the host.

Parasite membranes differ in composition from those of their hosts. Membranes of plasmodial parasites have higher concentrations of unesterified fatty acids, triacylglycerols, 1,2-diacylglycerols, diacyl-phosphatidylethanolamine and phosphatidylinositol and lower concentrations of cholesterol, phosphatidylserine and sphingomyelin than their host's membranes. Parasite membranes thus have different properties from those of their hosts. Presumably, they must also possess quantitatively different synthetic machinery for supplying membrane constituents, or specific uptake processes for obtaining appropriate precursors. Specialized external membranes are present in some parasites. Adult *Schistosoma mansoni* possesses a lipid-rich, double, outer membrane which develops a few days after penetration of the host. It is constantly renewed, and it has a vital function as the interface between the parasite and the host.

In many animals, glycerides and free fatty acids form an energy store which may be drawn upon in times of starvation. While parasites have considerable quantities of glycerides and free fatty acids, they are, usually, not used in the synthesis of ATP. Parasites require an external source of

lipids for their maintenance in culture. For protozoa, it is often sufficient to add whole serum, the essential components of which seem to be cholesterol and fatty acids. Trypanosomes are even capable of absorbing fat droplets. Presumably they are broken down to fatty acids, glycerol and other alcohols. Parasitic helminths also take up lipids and fatty acids, including acetate, from incubation media.

No group of parasitic protozoa or helminths seems capable of synthesizing long-chain fatty acids *de novo*, i.e. from simple precursors. Although many incorporate acetate into long-chain fatty acids, or carbon from glucose or glycerol into the lipid fraction, the process usually involves the comparatively slight modification, such as chain lengthening or the saturation of certain unsaturated bonds in long-chain fatty acids, of an existing molecule.

The apparent neglect of a resource widely exploited in the animal kingdom for energy metabolism needs explaining, but the proximate cause is that parasites often lack all the enzymes necessary for the beta-oxidation of fatty acids and/or an active TCA cycle. The latter is essential for the oxidation of the acetyl CoA that is the end-product of beta-oxidation. An exception to this are the free-living larvae of some parasitic helminths, which are certainly capable of oxidizing stored lipids in the production of energy, e.g. the free-living larvae of *Strongyloides ratti* oxidize palmitic acid by the beta-oxidation pathway.

Finally, there are many compounds in living organisms based on isoprene (2-methyl butadiene):

$$\begin{array}{l} \quad CH_3 \\ \quad | \\ CH_2{=}C{-}CH{=}CH_2. \end{array}$$

They are called terpenes, and are found in oils like camphor and geraniol, in rubber, and in carotenes from plants. They are also found as side chains in various vitamins and cofactors, including the ubiquinones that are important in electron transport and folate metabolism. A ubiquinone has the structure:

$$\begin{array}{l} \quad\quad\quad CH_3 \\ \quad\quad\quad | \\ \text{quinone}{-}(CH_2{-}CH{=}C{-}CH_2)_nH \end{array}$$

where n refers to the number of isoprene units in the side chain. There are many compounds with isoprenoid side chains present in nematodes, including ubiquinone itself, cholesterol, rhodoquinone, farnesol-like compounds and the polypropanol, solanesol. Adult filariae *in vitro* are unable to carry out the synthesis of sterols. Nematodes can synthesize ubiquinone, ecdysteroids, juvenile hormone and farnesol. Some of these compounds play essential roles as hormones in the development of insects. Whether they do so in helminths is not known.

5.6 METABOLISM OF NITROGEN COMPOUNDS

There are about 20 amino acids commonly found in free-living organisms, and a small but variable number of purines and pyrimidines. The same range of molecules are found in parasites. Parasites usually depend on the host for a supply of nitrogenous compounds as the ability to make many of them has either been lost, or less likely, never evolved in these groups.

5.6.1 Amino acid metabolism

All parasites synthesize amino acids. Trypanosomes, for example, are known to make alanine, glycine, serine, aspartic and glutamic acids. The malaria parasite synthesizes alanine, aspartic and glutamic acids and the sulphur-containing amino acids from a range of precursors. Some helminths incorporate ammonia directly into pyruvate and 2-oxoglutarate to form alanine and glutamate, respectively.

Amino acids are readily metabolized by transamination. The two most important reactions, because they interact with pathways of energy metabolism, catalysed by aminotransferases are:

$$\text{2-oxoglutarate} + \text{amino acid} \rightarrow \text{glutamate} + \text{oxoacid},$$
$$\text{pyruvate} + \text{amino acid} \rightarrow \text{alanine} + \text{oxoacid}.$$

They are readily reversible and nearly universally distributed in parasites. Degradation of amino acids is catalysed by L-amino acid oxidases, thus:

$$\text{L-amino acid} + H_2O + O_2 \rightarrow \text{2-oxoacid} + NH_3 + H_2O_2.$$

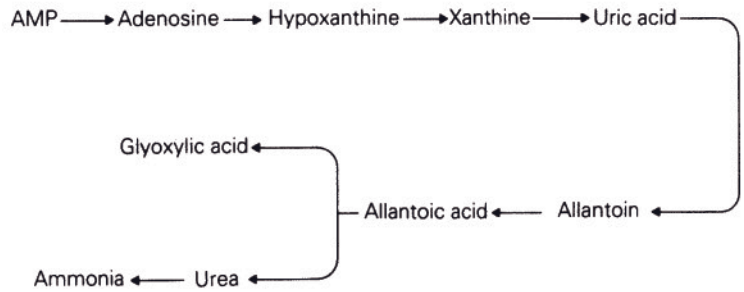

Fig. 5.10 The probable pathway of purine degradation in parasitic nematodes, inferred from the detection of intermediates and end-products after the inclusion of purine compounds in incubation media.

Ammonia is excreted and the oxoacid may be oxidized during respiration.

Another end-product of amino acid and protein metabolism is urea, which in higher animals, is produced by the Krebs–Henseleit (urea) cycle. There is no evidence that any parasite possesses a functional cycle, although they all have some of the enzymes associated with it. Any urea which is detected in parasitic helminths probably derives from purine degradation (Fig. 5.10).

Arginase, a common enzyme in parasites, splits arginine into ornithine and urea. Kurelec (1975) suggested a unique role for arginine metabolism in the liver fluke, *Fasciola hepatica* (Fig. 5.11). It leads to the formation of proline, which is produced in large quantities by the fluke. The enzyme which catalyses the final step in the production of proline is several times more active than that of the host, and, unlike the mammalian enzyme, is not subject to end-product inhibition. This pathway may be important in the maintenance of redox balance by reoxidizing NADH, or proline may be important in nitrogen excretion. Another suggestion stems from the observation that proline, infused into the peritoneal cavities of rats, causes a bile-duct hyperplasia which is similar to that produced in the early stages of fascioliasis. It is therefore possible

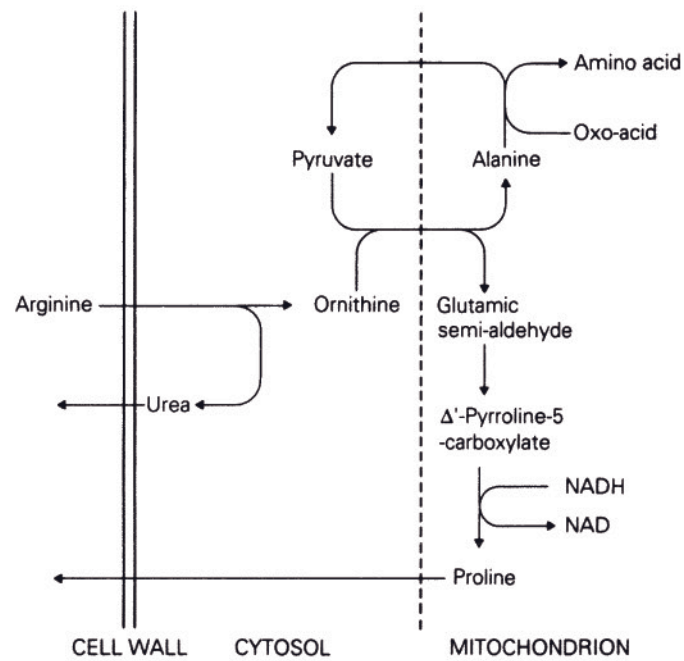

Fig. 5.11 A scheme for arginine catabolism and the production of proline and urea in *Fasciola hepatica*. Arginine is taken up from the environment, converted to ornithine and then, by interaction with a transamination cycle, to glutamic semialdehyde. This is eventually converted into proline in the mitochondrion. Proline and urea, products of the last and first steps of the pathway, are excreted.

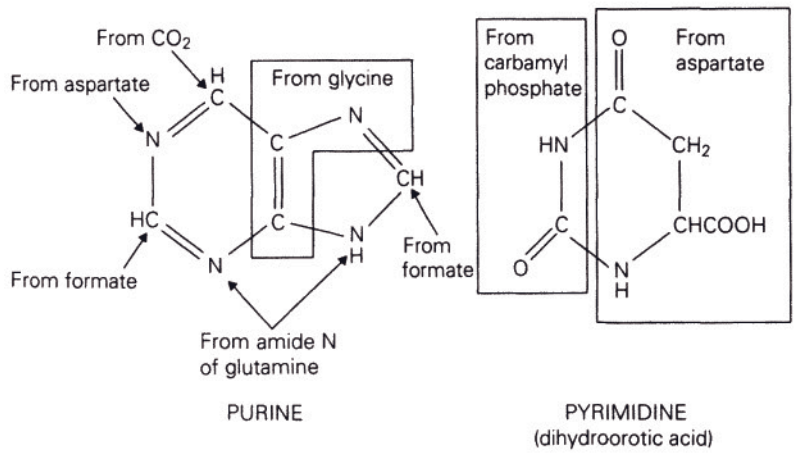

Fig. 5.12 The origins of the atoms that form the purine and pyrimidine rings during *de novo* synthesis.

that proline excretion is a metabolic strategy by which the parasite improves its environment.

5.6.2 Purines, pyrimidines and their salvage

Purines and pyrimidines are bases that possess the heterocyclic ring structures illustrated in Fig. 5.12. Adenine and guanine are purine derivatives; thymine, cytosine and uracil are pyrimidines. A nucleoside is a combination of a heterocyclic base with a sugar, often D-ribose. A nucleotide is the combination of a heterocyclic base with a sugar phosphate. ATP is a nucleotide. Heterocyclic bases are the backbone of the structure of DNA and RNA and provide the basis for the genetic code. Often, incorporated into coenzymes and vitamins, they participate in numerous metabolic reactions. Cyclic nucleotides act as specific signallers within the cell.

It is doubtful whether any protozoan or helminth parasite has the capacity to synthesize the purine ring *de novo*. A possible exception is the kinetoplastid flagellate, *Crithidia oncopelti*; but for the remainder detailed experiments lead to the conclusion that parasites rely on their hosts to provide a source of purines.

Parasites contain the same range of purines and their derivatives that are found in free-living organisms. In the absence of synthetic mechanisms, it is not surprising to find elaborate systems for absorption and interconversion of existing purines. These are the so-called 'salvage' pathways so widely distributed in parasites. Cultivation experiments have demonstrated exactly which purines and purine-containing compounds are essential for growth, especially in the case of the blood dwelling malaria parasites and the schistosomes, as blood itself is a very well-defined medium. The salvage pathways are illustrated in Fig. 5.13. Purines are not, of course, invariably salvaged, as Fig. 5.10 shows.

There are some 'unique' enzymes of purine salvage in parasitic protozoa. For example, *Giardia duodenalis* has a guanine phosphoribosyl transferase that does not recognize hypoxanthine, xanthine or adenine as substrates, in contrast to the analogous enzyme in other organisms. *Leishmania donovani* promastigotes have a xanthine phosphoribosyl transferase, and *Eimeria tenella* possesses a hypoxanthine, guanine, xanthine–phosphoribosyl transferase as a single enzyme, not known in any other organism.

Most parasitic protozoa (except the trichomonads and *G. lamblia*) and all helminths seem to be capable of synthesizing pyrimidines *de novo* from carbamyl phosphate and aspartate (Fig. 5.12). The evidence for the presence of the pyrimidine synthetic pathway is largely circumstantial and, once again, depends heavily on culture experiments with defined incubation media. In parasitic protozoa and in *S. mansoni* the pathways

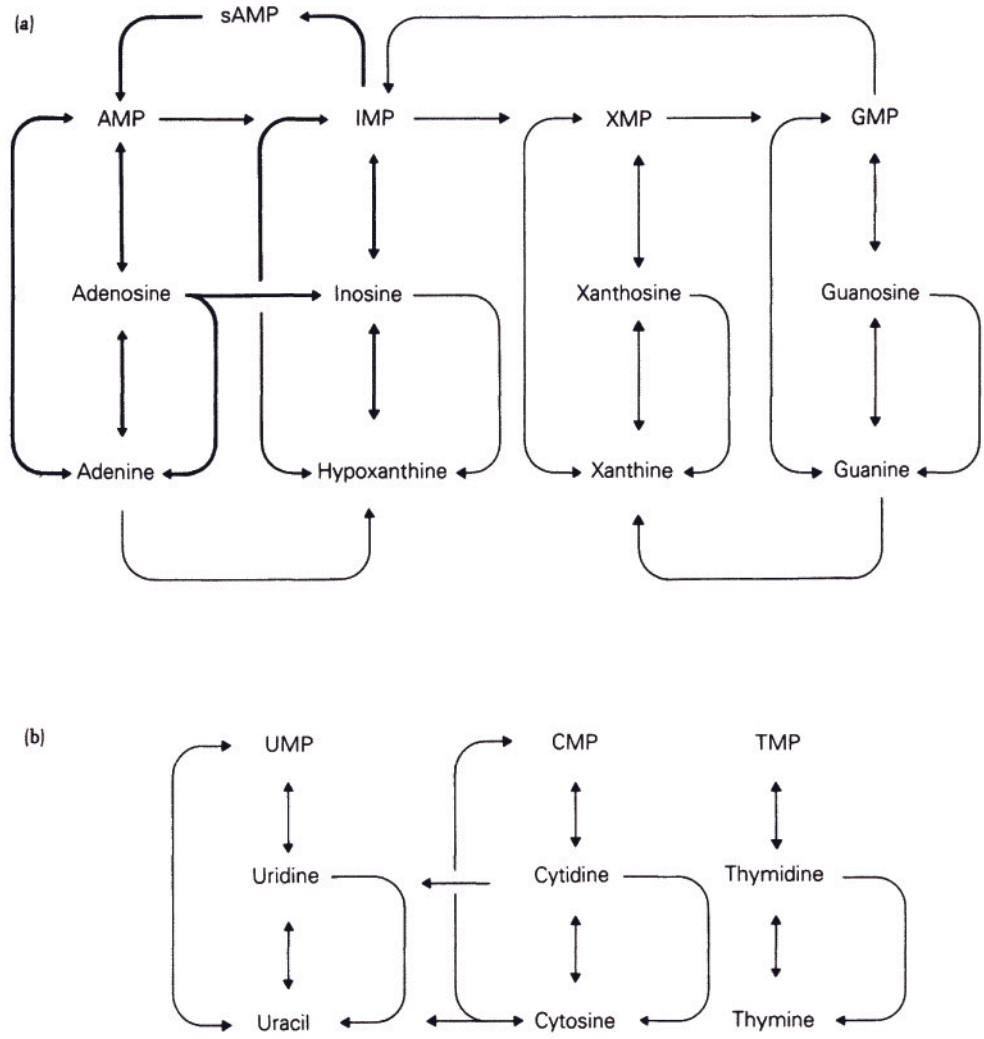

Fig. 5.13 Purine and pyrimidine salvage pathways in parasites. Reactions indicated by bold arrows have been detected in parasitic helminths. Different combinations of all reactions have been detected in parasitic Protozoa but not all are present in a single organism. AMP, GMP, IMP, XMP – adenosine, guanosine, inosine and xanthosine monophosphates; sAMP – succinyl AMP; UMP, CMP, TMP – uridine, cytidine and thymine monophosphates.

are qualitatively similar to those of their hosts.

Salvage pathways for pyrimidines also occur. They are of different importance in different parasites; malaria parasites make little use of them, whereas the trypanosomes rely equally on salvage and synthesis. Some of the enzymes involved in purine metabolism are peculiar to particular taxons. In kinetoplastids, for example, dihydroorotate oxidase (orotate reductase) is soluble, contains flavin, and appears to donate electrons directly to oxygen, forming hydrogen peroxide, whereas in most organisms it is bound to the mitochondrial membrane and joins the electron transport chain at the level of ubiquinone. Further, thymidylate synthetase in kinetoplastids and plasmodia is bound to dihydrofolate reductase to form a bifunctional enzyme complex. In mammals these two enzymes are separate.

Trypanosoma foetus, *T. vaginalis* and *G. lamblia* do not possess either enzyme; they are unique amongst parasitic protozoa in requiring exogenous thymidine. *Trypanosoma vaginalis* is different from all other protozoa, in that it cannot convert purine and pyrimidine ribonucleotides to the deoxyribonucleotides required for DNA synthesis, because it does not have a ribonucleotide diphosphate reductase. Deoxyribonucleotides must be supplied by a deoxyribonucleotide phosphotransferase acting on salvaged deoxyribonucleosides.

5.6.3 Nucleic acid metabolism

Nucleic acid synthesis is essential for rapidly growing cells and requires nucleosides and nucleotides. Nucleic acid synthesis (especially DNA synthesis) may be cyclic or stage-specific. In protozoa, which undergo rapid multiplication, DNA synthesis may be limited to specific phases of the life/cell cycle. In adult helminths, DNA and RNA synthesis is largely restricted to the reproductive tissues, except in the case of continuously-growing cestodes.

The DNA of parasites is unremarkable. There is no evidence for the presence of any unusual base, neither is there evidence to suggest that the organization of the genome is unusual. The argument that parasites should possess a diminished genome because they depend for many of their requirements on the genetic information of their hosts is not borne out by observation. In fact, parasitic nematodes and flatworms have more complex genomes than their free-living relatives.

With one exception, mitochondrial DNA from parasites is likewise unremarkable. The exception is DNA from the trypanosome kinetoplast. The kinetoplast is an analogue of the mitochondrion. Kinetoplast DNA occurs in relatively large amounts and is organized into maxicircles and minicircles. Maxicircles contain the equivalent of mitochondrial DNA, but the function of minicircle DNA, which is heterogeneous and rapidly evolving, is not understood. Maxicircles and minicircles are not associated with histones and occur in a concatenated mass that must be precisely divided between daughter kinetoplasts at cell division. It is therefore likely that the mechanisms of unravelling and replication are different from those in all other organisms.

5.7 OTHER PROCESSES

Dihydrofolate is essential for growth and reproduction in all organisms because it or its derivatives participate as cofactors in many methylation and other carbon transfer reactions. Intracellular sporozoa synthesize dihydrofolate *de novo* from the precursors GTP, *p*-aminobenzoate and glutamate (Fig. 5.14). Their mammalian hosts do not have this pathway; they recover folate from their diet and reduce it to dihydrofolate directly. Little is known about folate metabolism in helminths. Filarial worms do not appear to synthesize dihydrofolate *de novo*, but obtain 5-methyltetrahydrofolate as their major source of folate from the host.

The secondary messenger, cAMP, is essential in metabolic regulation. It causes the activation of some regulatory enzymes, and increases the phosphorylation state of some membrane proteins, which may be important in the regulation of neuromuscular activity. The intracellular concentration of cAMP is determined by the relative activities of adenylate cyclase and cAMP-phosphodiesterase.

Adenylate cyclase is present in *F. hepatica* in high activities. In this parasite, in *S. mansoni* and in *Ascaris suum*, it is activated by 5-hydroxytryptamine (but not epinephrine as in mammals) and is important in the regulation of carbohydrate metabolism and motility. Among the parasitic protozoa, cAMP inhibits cell division *in vitro* in trypanosomatids. In *Plasmodium falciparum* it may inhibit asexual multiplication

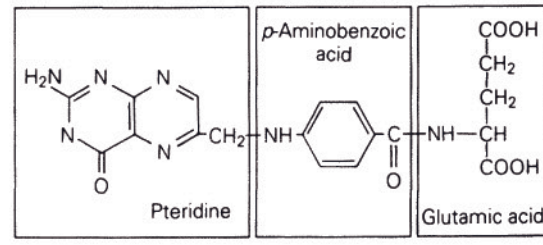

Fig. 5.14 The structure of folic acid.

and gametocyte formation. The major cellular receptors for cAMP in eukaryotes are the regulatory subunits of certain types of protein kinase. The trypanosomes have cAMP receptors, but their properties are different from those in other eukaryotes. Protein kinases, whether cAMP-dependent or independent, are important metabolic regulators in helminths. They have been found in filariae and other nematodes, and in schistosomes. They are probably ubiquitous and presumably have a similar regulatory function in helminth metabolism as in mammals.

Calmodulin is an intracellular receptor of calcium ions. It is widely distributed in the protozoan, animal and plant kingdoms. The calmodulin-calcium ions complex activates many intracellular enzymes and processes. Calmodulin has been isolated from *Hymenolepis diminuta*. Its properties closely resemble those of mammalian calmodulin. Protozoan parasites also contain calmodulin which, in *T. brucei*, *in vitro*, probably controls the calcium induced changes in the activities of adenylate cyclase and endoribonuclease, and the release of variable surface glycoproteins which provide the organism with its elegant immunoevasion mechanism.

Polyamines include putrescine, spermidine and spermine. Their importance in the regulation of many cellular processes in protozoa is only now becoming clear. They bind ionically to nucleic acids and play a role in the cell cycle, in cell division and in differentiation. They may be especially important in parasitic protozoa because of their role of cell multiplication.

5.8 ENVOI

An important phenomenon exhibited by parasites is their capacity for metabolic variation. Both the protozoa and the helminths possess it in marked degree. Its enormous extent and significance has been highlighted in the last decade, as more and more reports of resistance to antiparasite drugs accumulate. Differences between strains (and the development of resistance) arise from the persistence of a particular selection pressure (such as antiparasite drugs) in the ecosystem. An excellent example is the resurgence of human malaria as resistance to chloroquine spreads, due to widespread and indiscriminate use of that otherwise very useful antimalarial.

Metabolic variation is a problem compounded by the procedures of parasitological laboratories themselves. The establishment of a given parasite in culture, whether *in vitro* or by passage through living hosts, is a severe selection step, which permits the 'founder principle' full play. The parasite is removed, by cultivation, from access to the gene pool of its parent population, thus distorting the genetic profile of the cultivar. Further inadvertent selection by research workers, using procedures like cryopreservation that may cause high mortality, or by passage through a single strain of laboratory host, intensifies any differences. Bottlenecking the gene pool in this way leads to atypical frequencies of certain genes in the cultivated population and the emergence of a strain whose metabolic properties may be different from those of cultivars from other laboratories and from wild types. Generalizations based on work done with a single laboratory or field isolate may not be valid, and the alternative, that of working with several strains simultaneously, is expensive and often poses enormous logistical problems. It is a dilemma that has yet to be properly confronted.

None of the biochemical strategies described in this chapter can unequivocally be ascribed to adoption of the parasite habit. There is evidence that many of the same biochemical adaptations are also encountered in free-living lower organisms occupying habitats subject to low or fluctuating oxygen tensions and high carbon dioxide concentrations. If a particular metabolic capacity is absent, one may *suspect* that excision has occurred to effect economies in the energy expenditure of the parasite. For example, the loss of the ability to synthesize purines is a conundrum. Is this indeed an example of parasitic adaptation, the parasite relinquishing a costly suite of enzymes because of the universal availability of a source of purines from a host? Bearing in mind the importance of purines in constructing genetic material, this is giving up a real hostage to fortune. If purines, why not pyrimidines? Or any other major synthetic pathway? Perhaps it was the accidental loss of purine synthetic capacity early in evolutionary history that predisposed to

parasitism? Fairbairn (1970) wrote of helminths 'No unequivocal loss of genetic capacity is known. Either ... the genetic information is present but repressed, or an insufficient study of all stages of a life cycle has been made'. This last *caveat* should also be applied to protozoa. The only safe conclusion is that absence of evidence is not evidence of absence.

I acknowledge with gratitude research workers in the field of parasite biochemistry far too numerous to name here.

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