Biochemistry of Parasitic Helminths

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To Sara and Kate

Preface

It is the aim of this book to give a comparative account of the biochemistry of parasitic helminths and to relate the biochemistry of parasites not only to that of mammals but also to that of free-living invertebrates. The emphasis is on regulation and deals with metabolic control both at the cellular and at the developmental level.

A difficulty in parasite biochemistry is that the information is patchy, some aspects are known in great detail and others not at all. In the text, I have tried to indicate those areas of parasite biochemistry that have been neglected, in the past, since, in some ways, what is not known about helminths is almost as interesting as what is known.

The book does not deal with nerve-muscle physiology, nor with osmoregulation, and nor does it discuss, in detail, the mode of action of anthelmintics. However, a table of the possible biochemical modes of action of the more important anthelmintics is given in Appendix 7.1.

For the purpose of this book, I have taken helminths to include acanthocephalans, cestodes, trematodes, nematodes and nematomorphs. Throughout the text, *Ascaris lumbricoides* is used, rather than *A. suum* or var. *suis.* In almost all cases, it is the ascarid from pigs which has been investigated and not the human worm.

The literature has been covered up to December 1978, and the sheer number of references poses a severe problem. The compromise I have adopted is to have a general reading list composed of relevant reviews at the end of each chapter, as well as the specific references. Any reference that can easily be found from the general reading list is not included in the specific list; in particular, T. von Brand's book, *The Biochemistry of Parasites* (2nd edn, 1973), gives comprehensive references to all of the literature prior to 1973. In this way, it should be possible to find the reference to any particular point of interest, yet keep the reference lists to manageable proportions.

It is inevitable that some of the information in this book will rapidly become out of date or prove to be incorrect. What I hope the text will provide is a framework within which the reader can place new developments in context.

In conclusion, I should like to thank those colleagues who have sent me reprints or allowed me to quote from their unpublished work. Also, I am grateful to R. W. Walker, J. M. Paul and, in particular, G. M. Lloyd for reading and commenting on various aspects of the manuscript.

June, 1979 *J.B.*

Contents

1 Introduction to Parasite Biochemistry

Biochemistry consists of both variant and invariant elements. The invariant aspects of biochemistry are features like the use of DNA and RNA and the major metabolic pathways that are common to all living organisms. The variant part of biochemistry covers the ways in which metabolic pathways are modified to suit the environmental needs of the animal and the variations in the details of cofactor requirements and molecular structure. The invariant features of biochemistry emphasise the biochemical similarities between living organisms, and the variant aspects highlight the differences. A comparative approach to the biochemistry of animals or a group of animals, such as will be taken in this book, will tend to emphasise the differences between animals, rather than dwell on the similarities.

Much of the interest in parasite biochemistry comes from the ways in which the metabolic pathways have been modified to suit the highly specialised parasitic mode of life. In addition to this intrinsic interest, parasite biochemistry has great practical importance through chemotherapy and vaccine production. The development of successful vaccines against parasitic helminths necessitates routine *in vitro* culture, and for this an intimate knowledge of parasite biochemistry is required. In chemotherapy, parasite biochemistry can contribute to the development of new drugs and to the elucidation of the mode of action of compounds already discovered. The increased awareness of the possible dangerous side-effects of drugs has led to renewed interest in the mode of action of anthelmintics. For this, the basic biochemistry of parasites must be understood before the specific biochemical lesions caused by drugs can be identified. However, few if any anthelmintics have been reported to have one single mode of action, most of them appearing to disrupt several different cellular activities at once. Anthelmintics, by their very nature, are toxic to parasites, and perhaps it is not surprising that, in the dying parasite, many different metabolic processes become disrupted. So, identifying the primary effect of an anthelmintic (if indeed there is only one) is not a straightforward process. Commercially, the discovery of new anthelmintics involves the screening of large numbers of random compounds, in the hope that a new drug will be found. This empirical approach is, needless to say, very expensive and time-consuming (only 1 in 10 000 compounds screened shows any activity). It has often been felt that, with the increase in knowledge in parasite biochemistry over the last twenty years, a more rational approach to helminth chemotherapy should be possible, the idea being that any differences between the biochemistry of parasites and that of their

mammalian hosts are potential sites for chemotherapy. Once such a difference has been identified, specific inhibitors for the parasite pathway could then be synthesised. Many biochemical differences between parasitic helminths and mammals are now known, but the rational approach to parasite chemotherapy has yet to produce a successful compound. Possibly, in comparison with the empirical method, the rational approach has not had a sufficiently long trial. More promising, perhaps, is the semi-rational approach in which knowledge, gained from the mode of action of empirically discovered drugs, is used as a basis for the synthesis of improved compounds.

1.1. THE PARASITE'S ENVIRONMENT

Animal environments can be divided into aquatic, terrestrial and parasitic. The environment of a parasite is, of course, the body of another animal, and this seems to be a relatively difficult environment to invade. However, those organisms which have done so have often been remarkably successful, both in terms of numbers of species and numbers of individuals.

An outstanding feature of many parasites is their complex life-cycles, which can involve up to four different hosts, interspersed with free-living phases. A parasite may be faced with a succession of different environments, ranging from the free-living, to the tissues of an invertebrate, to the tissues of an ectothermal vertebrate, to the tissues of an endothermal vertebrate. Even within the vertebrate host, helminths often undergo extensive migrations, passing, say, from the gut, to the bloodstream, to the lungs and back again to the gut. The change from one phase of the life-cycle to the next in parasites is often extremely rapid and there is little time for acclimatisation. The different environments occupied by a parasite during its life-cycle necessitate different biochemical (and structural) adaptations. So, the life-cycle of a parasitic helminth involves a regular sequence of metabolic switches; the extent of the biochemical changes occurring at each phase depends on the degree of difference between the two environments involved. The actual metabolic switches that take place during the life-cycle of parasitic helminths, and the control of these switches by environmental and genetic factors, will be dealt with in Section 5.2.

Parasites can invade almost every part of the vertebrate and invertebrate body, although some organs are more commonly parasitised than others. In vertebrates, the most favoured habitats are the alimentary system, the bloodstream and the respiratory system, in that order. The number of parasitic habitats in invertebrates is much fewer, and only the alimentary canal, its associated structures and the haemocoel are invaded to any great extent.

A detailed account of all of the different possible parasitic sites in vertebrates and invertebrates would involve a description of virtually every organ system and is obviously outside the scope of this book. Instead, we shall look briefly at the major physico-chemical factors that are especially important in influencing parasite metabolism and development in the different sites.

1.1.1. Oxygen Tension

The oxygen tension in the parasite's environment is important in relation to the possibility of aerobic or anaerobic metabolism. Oxygen tension in different parasite habitats can vary widely (*Table 1.1*). In general, reasonable amounts of oxygen are present in the tissues and body fluids of vertebrates, but in the alimentary canal and excretory system the oxygen tensions are low and rather variable. In the vertebrate gut, the partial pressure of oxygen is much higher at the mucosal surface than in the centre of the lumen, where it can fall to zero. In addition, intestinal pO_2 varies with the feeding cycle, being higher during fasting than after a meal. Gut micro-organisms may create localised anaerobic conditions, and obligate anaerobes like *Trichomonad* protozoa are able to survive in what would appear to be aerobic sites, such as the mouth, because of these local regions. Gut parasites, like hookworms, which feed on blood, may be able to get some oxygen from their blood meal.

Table 1.1 Oxygen tensions in vertebrate tissues

Habitat	Species	O_2 tension (mmHg)
Swim bladder	Fish	>760
Skin	Mammals	50–100
Subcutaneous tissues	Mammals	20–43
Arterial blood	Mammals, birds and fish	70–100
Venous blood	Mammals and birds	40–66
	Fish	15–20
Peritoneal cavity	Mammals	28–40
Pleural cavity	Mammals	12–39
Urine	Mammals	14–60
Bile	Mammals	0–30
Stomach	Mammals (gases)	0–70*
Small intestine	Mammals (gases)	0–65*
	Mammals and birds (contents)	0.5–30†
Large intestine	Mammals (gases)	0–5

*High values due to swallowed air. †Highest values near mucosa.

The oxygen tension in invertebrates has not been measured and it is usually assumed that the tissues of small invertebrates are reasonably aerobic. Some insects only open their spiracles periodically and in these the pO_2 can drop to 20 mmHg when the spiracles are closed. The presence of obligate anaerobes in the intestines of several invertebrate species suggests that there are at least localised anaerobic regions in the gut. The oxygen tension in free-living habitats can also be very variable, anaerobic conditions frequently being found in stagnant water, mud and fermenting faeces.

During their life-cycles, parasites may have to face wide variations in oxygen availability. Many parasitic habitats have very low oxygen tensions and these are often described as being micro-aerobic. However, no parasitic site can really be described as totally anaerobic and all parasites probably have at least some oxygen available to them, although they may have to cope with extensive periods of anaerobiosis.

1.1.2. Carbon Dioxide Tension and Other Gases

Carbon dioxide levels play an important role in parasitic helminths. In many parasites, carbohydrate breakdown involves carbon dioxide fixation (Section 3.1.2), and pCO_2 plays a major part in the activation of trematode cysts, in the hatching and exsheathment of nematode eggs and larvae and in enhancing the hatching and activation of acanthocephalan eggs and cystacanths. Carbon dioxide is also one of the factors which attracts plant parasitic nematodes to developing roots.

In air, the pCO_2 is extremely low, 0.3 mmHg, but the levels in animal tissues, however, are very much higher (*Table 1.2*). In the blood and tissues of terrestrial vertebrates, the pCO_2 is around 40 mmHg, and in aquatic vertebrates, because of the high solubility of carbon dioxide in water, the pCO_2 is lower, 2–10 mmHg. Few measurements are available from invertebrates, but in insect tissues pCO_2 values of 23 to 48 mmHg have been recorded.

Table 1.2 Carbon dioxide tensions in vertebrate tissues

Habitat	Species	CO_2 tension (mmHg)
Blood	Mammals	34–42
	Birds	21–26
	Reptiles	27–38
	Fish	1.3–10
Small intestine	Non-ruminant mammals and birds	20–600
	Ruminant mammals	500–700

The intestines of mammals have extremely high pCO_2 levels, particularly ruminants where partial pressures of carbon dioxide as high as 700 mmHg have been reported. These excessively high levels of carbon dioxide must pose severe acid/base regulation problems for intestinal helminths (Section 3.2.10). It has also been suggested that the carbon dioxide fixing pathways found in helminths may have arisen in response to the high environmental carbon dioxide levels and are not necessarily adaptations to low oxygen levels (Section 3.7).

Animal tissues are characterised by relatively high carbon dioxide levels, and carbon dioxide may be an important factor in delineating the parasitic environment from the free-living one.

A number of other gases occur in the vertebrate intestine, particularly methane and hydrogen, but it is not known if they play any role in parasite development or metabolism.

1.1.3. Oxidation-Reduction Potential

Low oxidation-reduction (redox) potentials (reducing conditions) are required to initiate hatching or excystment in a number of nematode and trematode life-cycles.

The redox state will also have a bearing on the functioning of helminth cytochrome chains.

Oxidation-reduction potentials are difficult parameters to measure and there is little information available on different environments. For aerobic cells, the oxidation-reduction potential usually lies between −150 and +200 mV. The values for liver and brain are low, about −100 mV, those for muscle and nervous tissue somewhat higher, −50 to +100 mV. In the mammal, the stomach contents tend to be oxidised, around +150 mV, whilst the intestinal contents are reduced, −100 mV. The gut contents of insects are usually positive (up to +200 mV), but reducing conditions do occur, in *Blattela* (−100 mV) and in Mallophaga and clothes moths (−200 mV). In these last two groups, low redox conditions are necessary for the digestion of keratin.

Whether it is really possible to talk about a single redox potential for a particular tissue or environment is, however, questionable. Within tissues or cells there are many different redox couples which are not necessarily in equilibrium, so there is really no single redox value. Some redox couples will interact directly with metal electrodes, and can be measured by redox electrodes. Other systems will react with redox dyes and can be measured in that way. Still other systems do not react with either dyes or electrodes. One very important redox system, the thiol group system, is essentially irreversible and so, although extremely important, cannot be measured. Yet a possible mechanism for the action of reducing agents in the activation of helminth eggs and cysts is the need to keep thiol groups in the reduced form. A single redox value for a tissue or habitat, although it gives a convenient label, does not adequately express the oxidation-reduction states of the different possible redox couples.

1.1.4. Hydrogen Ion Concentration

The pH of parasitic habitats can vary widely. In body fluids and tissues, the pH is usually about neutral (pH 6.8-7.4), although minor fluctuations do occur; for example, in active muscle, lactic acid accumulation can cause a drop of 0.1 to 0.3 pH units. In the vertebrate gut, the stomach is extremely acidic (pH 1-3), whilst in the small intestine it is nearly neutral (pH 5-7.8). As the acidic stomach contents pass into the small intestine, there is a transitory drop in pH. In invertebrates, there is usually no acidic gastric phase in digestion. Instead, in insects the mid-gut is usually neutral to alkaline (pH 6-8) and may be as high as pH 10 in Lepidoptera. The hind-gut is usually more acidic due to excretions from the malpighian tubules and there may also be localised acidic regions. Values for intestinal pH in other invertebrates are, for crustacea pH 4.7-6.6, for annelids pH 6-7.8 and for molluscs pH 5.5-7.6.

External pH usually only slowly affects intracellular pH and, in general, the metabolism of invertebrates is relatively unaffected by external changes in pH over a limited range. Nevertheless, the extremely acidic vertebrate stomach is a major obstacle for parasites which must traverse it, and the low pH of the stomach is possibly the main reason why relatively few parasites are found there.

1.1.5. Osmotic Pressure

The osmotic pressure of the environment determines the amount of osmotic work that an organism has to do. Changes in osmotic pressure also trigger hatching in a number of helminth eggs. An example of this is *Schistosoma mansoni*; the eggs of this trematode pass out in the faeces and hatching is inhibited at osmotic pressures above about $\Delta = -0.5\,^{\circ}C$. On dilution of the faeces by water, there is a massive hatching of eggs.

Table 1.3 Osmoconcentration in different parasite environments

Habitat	Δ (°C)	Animal	Body fluid Δ (°C)
Fresh water	−0.01	Molluscs	−0.08 to −0.22
		Crustacea	−0.8
		Fish	−0.5 to −0.55
		Amphibia	−0.45
Brackish water	−0.2 to −0.5	Euryhaline invertebrates	−0.5 to −1.8
Sea water	−1.85	Invertebrates	−1.8 to −1.85
		Elasmobranchs	−1.85 to −1.92
		Teleosts	−0.65 to −0.7
Terrestrial		Annelids	−0.3 to −0.4
		Molluscs	−0.5
		Insects	−0.5 to −1.0
		Reptiles	−0.6 to −0.7
		Mammals	−0.5 to −0.58

The range of osmotic pressures found in different free-living environments and in the tissues of different hosts is summarised in *Table 1.3*. During its life-cycle, a parasite may have to cope with a wide range of osmotic pressures, passing, say, from the tissues of a mammal ($\Delta = -0.58\,^{\circ}C$), to fresh ($\Delta = -0.01\,^{\circ}C$) or sea water ($\Delta = -1.85\,^{\circ}C$), to the tissues of a marine or freshwater invertebrate ($\Delta = -0.08\,^{\circ}C$ to $-1.85\,^{\circ}C$). In general, tissue parasites are isosmotic with their environments, gut parasites slightly hypotonic. In the mammalian gut, the osmolarity of the contents tends towards that of the plasma ($\Delta = -0.58\,^{\circ}C$). However, the volume of the free water in the gut varies with the feeding cycle, there being almost no fluid present in the fasting intestine.

1.1.6. Temperature

Temperature affects the rates of chemical reactions and the stability of biological macromolecules. Parasites of endotherms are subject to dramatic temperature changes during the course of their life-cycles. When an infective stage invades its mammalian or avian host, there is a sudden increase in environmental temperature to 35–43 °C. Conversely, when parasite eggs or larvae are shed from their endo-

thermic host, there is an equally sudden drop in ambient temperature. These extensive temperature changes pose major problems for the functioning of metabolic pathways and for the stability of lipid membranes and cell proteins. The effects of temperature change on the properties of enzymes and on the composition of parasite lipids will be considered in detail in Section 5.2. For parasites of endotherms, a temperature above about 35 °C is usually required before infective stages can be activated.

1.1.7. Bile Salts

Bile salts are steroid derivatives and are involved in the hatching of helminth eggs, in the activation of trematode cysts and in the evagination of cestode scoleces. The role of bile salts in triggering these different events is probably related to their surfactant properties, and in many cases it can be mimicked by detergents.

The composition of bile varies substantially within different vertebrate groups and in some cases, at least, these differences may play a part in determining host specificity. In the vertebrate, there is a correlation between diet and the type of bile salts produced, herbivores having mainly dihydroxy and monohydroxymonoketo bile salts, and carnivores having trihydroxy bile salts.

Bile salts are not found in invertebrates. However, surfactant agents, such as sarcosyltaurine, occur in the intestines of crustaceans and possibly other higher invertebrates. Whether these compounds are involved in the activation of helminth stages in the invertebrate intestine is not known.

1.1.8. Other Factors

Environmental factors such as pCO_2, pO_2, temperature, pH and E_h act as developmental triggers in helminth life-cycles. These factors also influence metabolism directly; carbon dioxide and oxygen are substrates for carboxylating and oxidase reactions, respectively, temperature affects a variety of enzyme parameters (Section 5.2.3), as does pH, whilst E_h influences redox-linked reactions. Osmotic pressure and the presence or absence of bile salts also act as major environmental stimuli in different helminths. In addition, there are a number of other physicochemical parameters which change from environment to environment and may act as developmental stimuli or necessitate specific metabolic adaptations.

Hosts, both vertebrate and invertebrate, mount, or attempt to mount, an immune response to invading parasites. The immune response can involve phagocytic and cytotoxic cells, specific antibodies and non-specific agglutinins and lysins. For the parasite, coping with the immune response involves tegumental modifications and in some cases the production of immunosuppressive or immunodisruptive compounds (Section 2.8.2). Hormones are another host product which affects parasites. Some helminths are able to respond to host hormone changes and in this way integrate their reproductive cycles with those of their hosts (Section 2.8). In other situations, parasites need to remain unaffected by host endocrine changes.

Intestinal helminths must protect themselves against digestion by host enzymes (Section 4.1.3), but partial digestion by host enzymes is necessary for hatching in a number of helminth eggs and cysts. For acanthocephalans and cestodes, which produce no digestive enzymes of their own, the activity of host enzymes is essential for providing nutrients of low molecular weight.

Within their hosts, parasites occupy high-nutrient habitats, although there are both quantitative and qualitative differences between sites. For example, intestinal amino acid ratios may be different in different hosts (Section 4.1.8) and, because of fermentation in the rumen, carbohydrate levels in the intestines of ruminants are much lower than those in non-ruminants. The nutritional requirements of helminths are poorly understood (Section 4.9) and the extent to which the availability of a particular nutrient or group of nutrients limits the distribution of helminths is not known. In general, it is probably the overall nutritional requirements that limit a parasite to a particular host or host group, rather than the requirement for a specific metabolite. Examples of specific requirements in parasitic helminths are rare; some shark tapeworms need urea for normal development, and several of the minor orders of tapeworms accumulate large amounts of vitamin B_{12} (Section 2.7.1). Egg hatching in the plant parasitic nematode *Globodera rostochiensis* is greatly stimulated by root exudates from host plants. The active factor in root exudate has been named ecleptic acid, and is an unstable lactone with an empirical formula of $C_{18}H_{24}O_8$.

Two other environmental factors that can affect parasites are the viscosity of the medium, which is important in locomotion and attachment, and light. A number of trematode and cestode eggs require light for hatching. Although action spectra have been constructed for the hatching of *Diphyllobothrium latum* eggs, the nature of the photosensitive pigment is not known.

The presence of a parasite in a particular site within a host may result in changes in the physico-chemical and biotic parameters of that site. A good example of this is the rat tapeworm *Hymenolepis diminuta*. The presence of this parasite causes a rise in intestinal redox potential, pCO_2 and pO_2 and a fall in intestinal pH and water; the number of micro-organisms in the intestine is reduced and the proportions of the different species altered. The physiology of the infected host is thus often different from the physiology of the uninfected host.

1.2. PARASITES AS INVERTEBRATES

Parasitic helminths belong to four different phyla, the Platyhelminthes, the Nematoda, the Nematomorpha and the Acanthocephala. Of these, two, the Nematomorpha and the Acanthocephala are totally parasitic groups. The platyhelminths and nematodes have both parasitic and free-living members and, in these two groups, parasitism has arisen independently on more than one occasion. So taxonomically, parasitic helminths represent a very miscellaneous collection of organisms and one would not necessarily expect their biochemistry to be very similar. On the contrary, it is perhaps remarkable that in many cases the metabolism of the different groups of parasitic helminths is often so alike.