INTRODUCTION TO Nutrition and Metabolism

SIXTH EDITION

David A. Bender
Shauna M. C. Cunningham

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Preface

The food we eat has a major effect on our physical health and psychological well-being. The Global Burden of Diseases, Injuries and Risk Factors Study has attributed over 10 million deaths globally in 2017 to dietary risk factors. In the United Kingdom, dietary risk factors are second only to alcohol and drug abuse in behavioral risk factors for death. Furthermore, all of the prominent metabolic risk factors – overweight and obesity, high blood pressure, high serum cholesterol levels and high levels of glucose in the blood – are influenced by the quality of our diet.

Therefore, understanding how nutrients are metabolized in the body and how they influence our metabolic status are key to our relationship with food, and for the prevention and management of diet-related disease. Consequently, an understanding of the principles of biochemistry is imperative to our understanding of the scientific basis of what we would call a prudent or healthy diet.

The aim in the following pages is both to explain the conclusions of the many expert committees that have deliberated on the problems of nutritional requirements, diet and health over the years, and the scientific basis on which these experts have reached their conclusions. Much what is now presented as "facts" may well be shown to be incorrect in years to come. This book is intended to provide a foundation of scientific knowledge and understanding on which to interpret and evaluate future advances in nutrition and health sciences.

Nutrition is one of the basic sciences that underlie a proper understanding of health and medical and human sciences and the ways in which human beings and their environment interact. In its turn, the science of nutrition is based on biochemistry and physiology, on one hand, and the social and behavioral sciences, on the other hand. This book contains such biochemistry as is essential to an understanding of the science of nutrition.

In a book of this kind, which is an **Introduction** to Nutrition and Metabolism, it is not appropriate to cite the original scientific literature which provides the (sometimes conflicting) evidence for the statements made; in some of the tables in this book, we have acknowledged our sources of data as a simple courtesy to our follow scientists, and also to guide readers to the original sources of information.

We are grateful to all those students whose perceptive questions have challenged and inspired us, and helped us to formulate and clarify our thoughts on how to approach the relationship between nutrients and metabolism. This new edition has expanded sections on some key emerging areas of research, particularly on the role of the gut microbiome and epigenetics in this relationship.

This book is dedicated to those who will use it as a part of their studies, in the hope that they will be able, in their turn, to advance the frontiers of knowledge, and help their clients, patients and students to understand the basis of the advice they offer.

David A. Bender
Shauna M. C. Cunningham

Authors

David A. Bender was educated at North Ealing Primary School and Greenford County Grammar School in London and then studied biochemistry at the University of Birmingham in England from 1965 to 1968. He joined the Courtauld Institute of Biochemistry at the Middlesex Hospital Medical School as a research assistant in 1968, was appointed as a lecturer in biochemistry in 1970 and received his PhD (on the metabolism of aromatic amino acids) from the University of London in 1971. The Middlesex Hospital Medical School merged with University College London (UCL) in 1987, and he became a member of the Department of Biochemistry and Molecular Biology at UCL. He was appointed as senior lecturer in biochemistry in 1994 and professor of nutritional biochemistry in 2009. He retired from UCL in 2011, with the title of Emeritus Professor. From 1994 until retirement, he was the assistant faculty tutor to the medical students, and from 1998 he was the subdean (Education) and director of studies for the early years of the medical course at UCL.

His research interests have been in the field of amino acid and vitamin nutritional biochemistry, and he was a member of the working group on vitamins that formed part of the expert committee that produced the 1991 report on Dietary Reference Values for Food Energy and Nutrients for the United Kingdom, the European Union expert committee that produced the 1993 report on Nutrient and Energy Intakes for the European Community and the Food Safety Authority of Ireland working party on Safe Micronutrient Levels.

In addition to research publications, he has also written a number of books, including:

- *Nutritional Biochemistry of the Vitamins*, 2nd Edition, 2003, Cambridge University Press.
- *Amino Acid Metabolism*, 3rd Edition, 2012, Wiley-Blackwell.
- *Benders' Dictionary of Nutrition and Food Technology*, 8th Edition, 2006, Woodhead Publishing, Oxford.
- *Dictionary of Food and Nutrition*, 3rd Edition, 2009, Oxford University Press.

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Additional Resources on the Website: Metabolism Online – The Virtual Tutorial

There is a series of virtual tutorials called *Metabolism Online* available on the website. Each exercise is based on a clinical problem or analyzing laboratory data. As each set of information is provided, you are expected to think about it and draw conclusions. You can then proceed to see the answer, and the next set of information to be considered. While you can obviously use this set of exercises working alone, it is most useful if two or three of you gather around the same computer and discuss the problems together – as you would in a real tutorial.

Energy – What Is Metabolism All About?

This set of exercises introduces the main metabolic fuels that are available, and the concepts of metabolic pathways and coenzymes, as well as the idea that the metabolism of metabolic fuels is a process of oxidation to provide metabolically useable energy (in the form of adenosine triphosphate [ATP]) for the various energy-requiring activities of the body. The following exercises are available in this section:

1. A respiratory physiology practical class – and beyond
2. An early morning jog
3. Starving to slim
4. Poisoned by unripe ackee fruit
5. Overheating after overdosing on E – and slimming by taking dinitrophenol
6. Summary – why do we need to eat and how do we survive between meals?

Carbohydrate Digestion and Metabolism

This set of exercises introduces the main pathways of carbohydrate metabolism: glycolysis, gluconeogenesis and the pentose phosphate pathway, as well as the synthesis and utilization of glycogen. The following exercises are available in this section:

1. Sugars, starches and the glycemic index
2. Bloating, flatulence and diarrhea after drinking milk - but not yoghurt or cheese
3. Experiments on glucose metabolism

4. Life-threatening acidosis in an alcoholic - and in a hunger striker given intravenous glucose
5. Breathless after sprinting
6. Weight loss in a patient with advanced cancer
7. How is NADH (reduced nicotinamide adenine dinucleotide) from glycolysis normally reoxidized?
8. An adverse response to antimalarial medication – and a fatal reaction to fava beans
9. Fasting hypoglycemia in an infant – and poor exercise tolerance in two brothers
10. Alanine released from muscle in fasting
11. A hypoglycemic adolescent with an enlarged liver and gout
12. Two diabetic patients in coma
13. Was she murdered by insulin injection?
14. An unusual cause of diabetes – how the pancreas senses a rise in blood glucose

Lipids

The following exercises are available in this section:

1. Fats and oils – are all fats the same?
2. Children with fatty diarrhea
3. Gripping abdominal pain and jaundice
4. Muscle weakness, heart failure and profound hypoglycemia in a young girl
5. Muscle weakness and hypoketotic coma on fasting
6. Do we need to synthesize fatty acids?
7. Is fructose fattening?
8. Not an ounce of fat on her – and extreme emaciation in patient with advanced cancer
9. Two boys with profound fasting hypoglycemia and no ketone bodies
10. Two very hyperketotic children

Amino Acid and Nitrogen Metabolism

This set of exercises introduces the main pathways in the metabolism of amino acids and the estimation of protein and amino acid requirements. The following exercises are available in this section:

1. Why does an adult require so much protein in the diet?
2. Transamination and deamination of amino acids
3. Urea synthesis in the liver, and potentially fatal hyperammonemia in a child
4. An unconscious child with hyperammonemia and ketoacidosis
5. Gout and hyperuricemia – and anticancer drugs

Central Metabolic Pathways and the Integration and Control of Metabolism

This set of exercises introduces pathways that are common to carbohydrate, lipid and amino acid metabolism – the central metabolic pathways. These pathways can be considered to be both catabolic, leading to ATP formation, and anabolic, leading to

synthesis and interconversion of metabolic substrates. The following exercises are available in this section:

1. Experiments with isolated liver cells – the citric acid cycle and warming up post-operative patients
2. Selection of fuels for muscle contraction
3. Hyperammonemic coma due to liver failure

Miscellaneous Topics

This is a selection of problem-solving exercises on various aspects of metabolism other than the central pathways of energy-yielding metabolism, and carbohydrate, lipid and amino acid metabolism. The following exercises are available in this section:

1. Vitamin C and collagen synthesis
2. A problem of bleeding cows and chickens, rat poison and patients with thrombosis – the role of vitamin K in blood clotting
3. Primary hyperoxaluria and kidney stones

chapter one

Why Eat?

An adult eats about a tonne (a metric ton = 1,000 kg) of food a year. This book attempts to answer the question "why?" by exploring the need for food and the uses to which that food is put in the body. Some discussion of chemistry and biochemistry is obviously essential in order to understand the function of food in the body, and why there is a continual need for food throughout life. Therefore, in the following chapters various aspects of biochemistry and metabolism will be discussed. This should provide not only the basis of our present understanding, knowledge and concepts in nutrition, but also, more importantly, a basis on which to interpret future research findings and evaluate new ideas and hypotheses as they are formulated.

We eat because we are hungry. Why have we evolved complex physiological and psychological mechanisms to control not only hunger and satiety, but also our appetite for different types of food? Why do meals form such an important part of our life?

Objectives

After reading this chapter, you should be able to:

- describe the need for water and fluid balance
- describe the need for metabolic fuels and outline the relationship between food intake, energy expenditure, and body weight
- describe in outline the importance of an appropriate intake of dietary fat
- describe the mechanisms involved in short-term and long-term control of food intake
- describe the mechanisms involved in the sense of taste
- explain the various factors that influence peoples' choices of foods
- describe the key features of eating disorders.

1.1 The Need for Water

The body's first need is for water. The human body contains about 60% water – a total of 42 L in a 70-kg person. Water is excreted in the urine as a way of ridding the body of the end products of metabolism, and obviously there is a need for an intake of water to balance the losses from the body. It is possible to survive for several weeks without any food by using body reserves of fat and protein, but without water, death from dehydration occurs within a few days.

Average daily output of urine is often said to be 1.5 L (although the figures in Table 1.1 show that this is an overestimate), and advertisements for bottled water suggest that we should drink at least this much water per day. At first glance it might seem obvious that we would need an intake of the same amount of fluid to replace the loss in urine. However, as shown in Table 1.1, total daily fluid output from the body is about 3 L for an adult man and

Table 1.1 Daily fluid balance

xx	xx	Adult man mL/day	% of total	Adult woman mL/day	% of total
Intake	Fluids	1950	65	1400	67
	Water in food	700	23	450	21
	Metabolic water	350	12	250	12
	Total	3000		2100	
Output	Urine	1400	47	1000	48
	Sweat	650	22	420	20
	Exhaled air	320	11	320	15
	Insensible losses through the skin	530	17	270	13
	Water in feces	100	3	90	4
	Total	3000		2100	

about 2.1 L for a woman; urine accounts for less than half of this. Equally, fluid consumption in beverages accounts for only about two-thirds of total fluid intake.

In addition to the obvious water in beverages, food provides a significant amount of water – around 22% of total intake, and more if you eat the recommended five or more servings of fruit and vegetables per day (Section 6.3). Most fruits and vegetables contain about 60–90% water.

A further source of water is metabolic water; this is the water produced when fats, carbohydrates and proteins are oxidized to yield energy. This accounts for about 12% of total water "intake", and more on a high-fat diet, or when metabolizing fat reserves. The camel is able to survive for a considerable time in desert conditions without drinking because it metabolizes the fat stored in its hump; the water produced from fat oxidation meets its needs.

Urine accounts for less than half of the total fluid output from the body. As shown in Table 1.1, the remainder is made up of sweat, water in exhaled air, the so-called insensible losses through the skin (this is distinct from the loss in sweat produced by sweat glands), and a relatively small amount in feces. The latter will also increase on a diet rich in fruit and vegetables because of their dietary fiber content – part of the beneficial effect of a high-fiber diet (Section 6.3.3.2) is that the fiber retains water in the intestinal tract, thereby softening the feces.

Sweat losses obviously depend on the temperature and physical activity, and we do indeed need to drink more in a hot environment or after a strenuous exercise. Losses in exhaled air, feces and other insensible losses are relatively constant; urine output varies widely depending on how much fluid has been consumed. Although average urine volume is between 1 and 1.4 L per day, this reflects the average fluid intake. The output of urine required to ensure adequate excretion of waste material and maintain fluid balance without becoming dehydrated is no more than about 500 mL. Put simply, the more you drink, the more urine you will produce.

A final problem is whether water is the most appropriate liquid to drink to balance large losses in sweat after vigorous exercise or in a hot climate. The answer is probably not– sweating involves loss of mineral salts as well as water, and these losses have to be made good. Various sports drinks contain balanced mixtures of mineral salts in the same proportions as they are lost in sweat, together, usually, with glucose or another carbohydrate as a source of metabolic fuel. Milk and fruit juices also provide mineral salts.

1.2 The Need for Energy

There is an obvious need for energy to perform physical work. Work has to be done to lift a load against the force of gravity, and there must be a source of energy to perform that work. The energy used in various activities can be measured (Section 5.1.3.2), as can the metabolic energy yield of the foods that provide the fuel for that work (Section 1.3). This means that it is possible to calculate a balance between the intake of energy, as metabolic fuels, and the body's energy expenditure. Obviously, energy intake has to be appropriate for the level of energy expenditure; as discussed in Chapters 7 and 8, neither excess intake nor a deficiency is desirable.

Figure 1.1 shows the relationship between food intake, physical work and changes to body reserves of metabolic fuels, as shown by changes in body weight. This was a study in Germany at the end of World War II, when there was a great deal of rubble from bomb-damaged buildings to be cleared, and a large number of people to be fed and found employment. Increasing food intake resulted in an increase in work output – initially with an increase in body weight, indicating that the food supply was greater than was required to meet the (increased) work output. When a financial reward was offered as well, the work output increased to such an extent that people now drew on their (sparse) reserves, resulting in a loss of body weight.

Quite apart from obvious work output, the body has a considerable requirement for energy, even at rest. Only about one-third of the average person's energy expenditure is for voluntary work (Section 5.1.3). Two-thirds of energy expenditure is required for maintenance of the body's functions, homeostasis of the internal environment and metabolic integrity. This energy requirement at rest, the basal metabolic rate (BMR, Section 5.1.3.1) can be measured by the output of heat from the body, or the consumption of oxygen, when the subject is completely at rest. Figure 1.2 shows the proportion of this resting energy expenditure that is accounted for by different organs of the body.

Part of this basal energy requirement is obvious. The heart beats to circulate the blood; breathing continues; and there is considerable electrical activity in nerves and muscles,

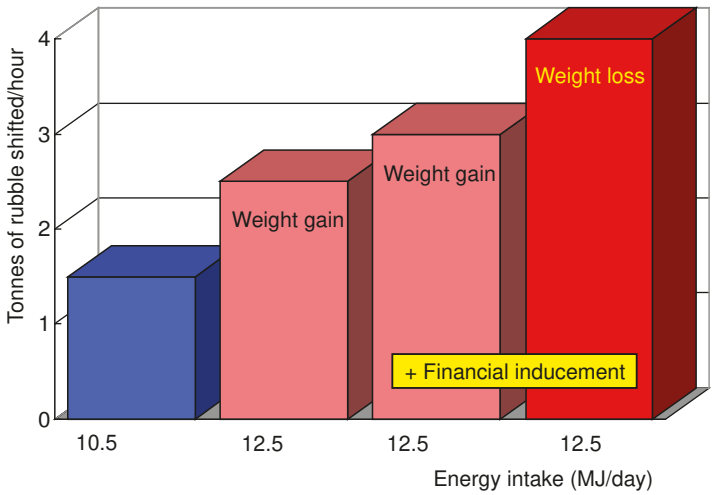

Figure 1.1 The relationship between food intake, work output and body weight. (From Widdowson, E.M., MRC Special Report Series no. 275, London, HMSO, 1951.)

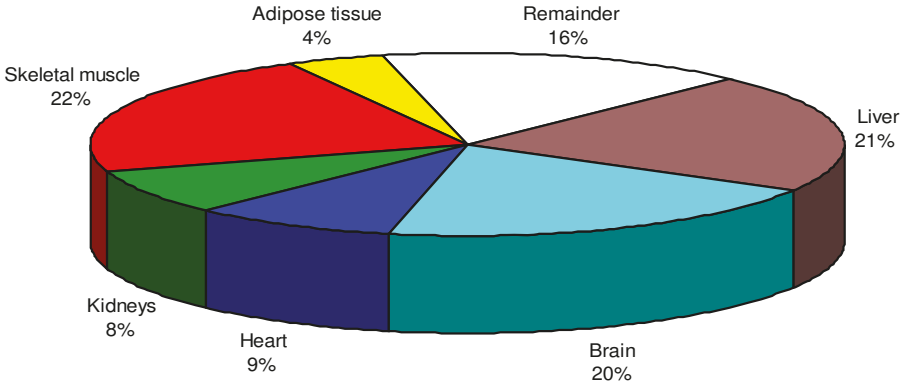

Figure 1.2 Percentage of resting energy expenditure by different organs of the body.

whether they are working or not. The brain and nervous system comprise only about 2% of the total body weight but consume some 20% of resting energy expenditure because of the active transport of ions across nerve membranes to maintain electrical activity (Section 3.2.2). This requires a metabolic energy source. Less obviously, there is also a requirement for energy for the wide variety of biochemical reactions occurring all the time in the body: laying down reserves of fat and carbohydrate (Sections 5.6.1 and 5.6.3); turnover of tissue proteins (Section 9.2.3.3); transport of substrates into, and products out of, cells (Section 3.2.2); and the synthesis and secretion of hormones and neurotransmitters.

1.2.1 Units of Energy

Energy expenditure is measured by the output of heat from the body (Section 5.1). The unit of heat used in the early studies was the calorie – the amount of heat required to raise the temperature of 1 g of water by 1 degree Celsius (°C). In biological systems, the kilocalorie, kcal (sometimes written as Calorie with a capital C), is used. One kcal is 1,000 calories (10^3 cal), and hence the amount of heat required to raise the temperature of 1 kg of water by 1 °C.

Correctly, the Joule is used as the unit of energy. The Joule is an SI unit named after James Prescott Joule (1818–1889), who first showed the equivalence of heat, mechanical work and other forms of energy. In biological systems, the kiloJoule (kJ = 10^3 J) and megaJoule (MJ = 10^6J) are used.

To convert between calories and Joules:

1 kcal = 4.186 kJ (normally rounded off to 4.2 kJ)
1 kJ = 0.239 kcal (normally rounded off to 0.24 kcal)

The average total daily energy expenditure of adults is between 7.5 and 10 MJ (1,800–2,400 kcal) for women and between 8 and 12 MJ (1,900–2,900 kcal) for men.

1.3 Metabolic Fuels

The dietary sources of metabolic energy (the metabolic fuels) are carbohydrates, fats, protein and alcohol. The metabolism of these fuels results in the production of carbon dioxide and water (and also urea in the case of proteins, Section 9.3.1.4). They can be converted to

Table 1.2 The energy yield of metabolic fuels

	kcal/g	kJ/g
Carbohydrate	4	17
Protein	4	16
Fat	9	37
Alcohol	7	29

1 kcal = 4.186 kJ or 1 kJ = 0.239 kcal.

the same end products chemically by burning in oxygen. Although the process of metabolism in the body is more complex, it is a fundamental law of chemistry that if the starting material and end products are the same, the energy yield is the same, regardless of the route taken. Therefore, the energy yield of foodstuffs can be determined by measuring the heat produced when they are burnt in oxygen using a bomb calorimeter. The sample for analysis is placed in a crucible inside a steel bomb, surrounded by water in an insulated container, and the heat produced by combustion is measured by the increased temperature of the water. The physiological energy yields of the metabolic fuels in the body, making allowance for the extent to which they are digested and absorbed, are shown in Table 1.2.

1.3.1 The Need for Carbohydrate and Fat

Although there is a requirement for energy sources in the diet, it does not matter unduly how that requirement is met. There is no requirement for a dietary source of carbohydrate; the body can synthesize carbohydrates from amino acids derived from proteins (the pathways of gluconeogenesis, Section 9.3.2). However, there is an average requirement of about 100 g of carbohydrate per day to maintain a normal blood concentration of glucose to provide for brain and red blood cell metabolism without the need for gluconeogenesis from amino acids. This is less than 2% of total energy intake; as discussed in Section 6.3.3, a desirable level of carbohydrate intake is 50–55% of energy. An intake of 50 g of carbohydrate per day is adequate to prevent the development of ketosis (Section 5.5.3), while very low carbohydrate diets for weight reduction (Section 7.3.4.4) that provide less than 20 g of carbohydrate per day are associated with significant ketosis.

Similarly, there is no requirement for a dietary source of fat apart from the essential fatty acids (Sections 4.3.1.1 and 5.6.1.1), and there is certainly no requirement for a dietary source of alcohol. Diets that provide more than about 35–40% of energy from fat are associated with increased risk of heart disease and some cancers (Section 6.3.2), and there is some evidence that diets that provide more than about 20% of energy from protein are also associated with chronic diseases, including osteoporosis and kidney damage. Therefore, the general consensus is that diets should provide about 55% of energy from carbohydrates, 30% from fat and 15% from protein (Section 6.3).

Although there is no requirement for fat in the diet, fats are nutritionally important, and there is a specific mechanism for detecting the taste of fats in foods (Section 1.4.3.1).

- It is difficult to eat enough of a very low-fat diet to meet energy requirements. As shown in Table 1.2, the energy yield per gram of fat is more than twice that of carbohydrate or protein. The problem in many less developed countries, where undernutrition is a problem (Chapter 8), is that average diets provide only 10–15% of energy from fat, and it is difficult to consume a sufficient bulk of food to meet

energy requirements. By contrast, the problem in Western countries is an undesirably high intake of fat, contributing to the development of obesity (Chapter 7) and chronic diseases (Section 6.3.2).

- Four of the vitamins, A, D, E and K (Chapter 11), are fat-soluble and are found in fatty and oily foods. They are absorbed dissolved in fat; therefore, with a very low-fat diet the absorption of these vitamins may be inadequate to meet requirements even if the diet provides an adequate amount.
- There is a requirement for small amounts of two essential fatty acids (Sections 4.3.1.1 and 5.6.1.1) that cannot be synthesized in the body, but must be provided in the diet.
- In many foods a great deal of the flavor (and hence the pleasure of eating) is carried in the fat.
- Fat lubricates food and makes it easier to chew and swallow.

1.3.2 The Need for Protein

Unlike fats and carbohydrates, there is a requirement for protein in the diet. In a growing child this need is obvious. As the child grows, and the size of its body increases, so there is an increase in the total amount of protein in the body.

Adults also require protein in the diet (Section 9.1.2). There is a continual loss of protein from the body, for example, in hair, shed skin cells, enzymes and other proteins secreted into the gut and not completely digested. More importantly, there is turnover of tissue proteins, which are continually being broken down and replaced. In the fasting state between meals, amino acids arising from tissue protein breakdown are utilized as a source of metabolic fuels rather than being used for replacement protein synthesis (Chapter 9). Although there is no change in the total amount of protein in the body, an adult with an inadequate intake of protein will be unable to replace losses and therefore will lose tissue protein.

1.3.3 The Need for Micronutrients: Minerals and Vitamins

In addition to metabolic fuels and protein, the body has a requirement for a variety of mineral salts. If a metal or inorganic ion has a function in the body, it must be provided by the diet, since the different chemical elements cannot be interconverted. Again, the need is obvious for a growing child; as the body grows, the total amount of minerals in the body will increase. In adults there is a turnover of minerals in the body, and losses must be replaced from the diet (Section 11.16).

There is a requirement for a different group of nutrients in small amounts, vitamins. These are organic compounds that have a variety of functions. They cannot be synthesized in the body and therefore must be provided by the diet. There is metabolic turnover of the vitamins, so there must be replacement of losses (Chapter 11).

A wide variety of other compounds in the diet (especially from fruit and vegetables) are not considered to be nutrients, since they are not dietary essentials, but they may have beneficial effects in reducing the risk of developing various chronic diseases (Section 6.7).

1.4 Hunger and Appetite

Human beings have evolved an elaborate system of complex, overlapping and sometimes apparently redundant, physiological mechanisms to ensure that the body's needs for metabolic fuels and nutrients are met, and to balance energy expenditure with food intake.

The physiological systems for the control of appetite interact with psychological, social, environmental and genetic factors, all of which have to be understood in order to gain a full explanation of eating behavior.

1.4.1 Hunger and Satiety: Short-Term Control of Feeding

Early studies showed that there are hunger centers in the brain that stimulate us to begin eating and satiety centers that signal us to stop eating when hunger has been satisfied. The hunger centers are in the lateral hypothalamus, and the satiety centers are in the ventromedial hypothalamus (see Figure 1.3). In experimental animals, destruction of the hunger centers leads to anorexia – that is, complete loss of appetite – while electrical stimulation leads to feeding even if the animal has eaten enough. Similarly, destruction of the satiety centers leads to more frequent, uncontrolled eating (hyperphagia), and electrical stimulation leads to cessation of feeding, even in a physiologically hungry animal.

Other hypothalamic centers are also involved, and destruction of the paraventricular and arcuate nuclei of the hypothalamus also leads to hyperphagia and obesity. The arcuate nucleus responds to hormones and other signals, and integrates signaling to the hunger and satiety centers. The hunger centers act through neurons that use neuropeptide Y and the agouti-related protein as their transmitters; the satiety centers act through neurons that use two other peptides: pro-opiomelanocortin (POMC) and the so-called cocaine and amphetamine-regulated transcript (CART). To reduce feeding, the satiety centers act primarily by reducing the activity of neurons arising from the hunger centers.

The hunger and satiety centers in the hypothalamus have neuronal connections with the temporal lobe of the amygdala, the nucleus acumbens, the brain stem and higher brain centers, including the cortex. The amydala controls learned food behavior, that is, how you know that something is a food, as opposed to non-food. A young child will put almost anything into its mouth, and gradually learns what is and what is not food. The nucleus

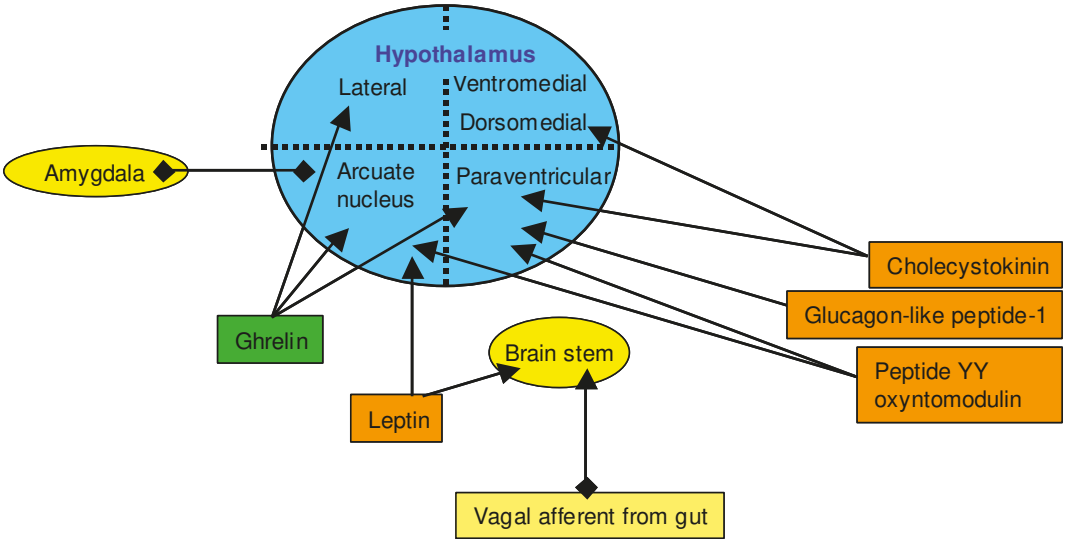

Figure 1.3 The hypothalamic appetite control centers and sites of action of hormones secreted by the gastrointestinal tract.

accumbens is a part of the mesolimbic reward system of the brain, which utilizes dopamine as a transmitter and is concerned with the hedonistic value of food – the pleasure of eating. The brain stem receives direct input via the vagus nerve from the gastrointestinal tract and the liver, signaling the presence of food in the gut and the arrival of nutrients to the liver. Enterochromaffin cells in the intestinal mucosa respond to neurotransmitters produced by intestinal microorganisms (Section 6.3.4) and stimulate vagus nerve transmission; these microbial neurotransmitters can thus affect mood and behavior. The connections to the cortex and other higher brain centers mean that psychological factors (including individual likes and dislikes, Section 1.4.4) can override the physiological control of appetite.

The hypothalamic centers control food intake remarkably precisely. Without conscious effort, most people regulate their food intake to match energy expenditure very closely – they neither waste away from lack of metabolic fuel for physical activity nor lay down excessively large reserves of fat. Even people who have excessive reserves of body fat, and can be considered so overweight or obese as to be putting their health at risk (Section 7.2.2), balance their energy intake and expenditure relatively well considering that the average intake is a tonne of food per year, while very severely obese people weigh about 250–300 kg (compared with average weights between 60 and 100 kg), and it takes many years to achieve such a weight. A gain or loss of 5 kg body weight over 6 months would require only a 1% daily mismatch between food intake and energy expenditure (Section 5.2).

A number of drugs can modify responses to hunger and satiety and can be used to reduce appetite in the treatment of obesity (Section 7.3.4.12), or stimulate it in people with loss of appetite or anorexia. Two appetite suppressant drugs, sibutramine and rimonabant, have been withdrawn because of serious adverse side effects, and two others, phentermine and diethylpropion, are available only on private prescription. There is little evidence of their efficacy in promoting weight loss that is maintained over the long term, although they are effective in the short term. The synthesis and release of neuropeptide Y are dependent on zinc, and the loss of appetite associated with zinc deficiency is a result of impaired secretion of this neurotransmitter.

In addition to direct neuronal input from the gastrointestinal tract and liver, a variety of factors act on the hunger and satiety centers to initiate nerve impulses, including:

- The relative concentrations of the hormones insulin (secreted by the β-islet cells of the pancreas in response to increased blood concentrations of glucose and amino acids, Section 5.3.1) and glucagon (secreted by the α-islet cells of the pancreas in response to a decreased concentration of blood glucose, Section 5.3.2).
- The relative concentrations of glucose, triacylglycerols, non-esterified fatty acids and ketone bodies available as metabolic fuels in the fed and fasting states (Section 5.3). The plasma concentrations of metabolic fuels are mainly regulated by three hormones:
- Insulin stimulates the uptake of glucose into tissues, and its intracellular utilization.
- Glucagon stimulates the synthesis and release of glucose and nonesterified fatty acids into the circulation, and the synthesis of ketone bodies from nonesterified fatty acids.
- Amylin, which is secreted by the pancreas together with insulin, regulates the output of metabolic fuels from tissue reserves into the circulation.
- Hormones secreted by the gastrointestinal tract:
- Ghrelin is secreted mainly by the stomach (Section 1.4.1.2) and stimulates appetite.
- Cholecystokinin, which is secreted by the duodenum and acts mainly to stimulate gall bladder contraction and gastrointestinal tract motility and secretion, suppresses appetite.

- Glucagon-like peptide and oxyntomodulin (both derived from the glucagon gene and secreted by gut endocrine cells) and peptide YY (also secreted by gut endocrine cells, largely in response to the energy yield of a meal) also suppress appetite.
- A peptide derived from the ghrelin gene, obestatin, has anti-ghrelin, appetite-suppressing actions.

1.4.1.1 Nutrient Sensing in the Hypothalamus

There are neurons in the hypothalamus that respond to glucose; some are excited by it, while others are inhibited. In both cases, these neurons contain glucokinase, the high K_m isoenzyme of hexokinase (Section 5.3.1), and in response to a modest increase in the concentration of glucose in the cell there is a considerable increase in the rate of formation of glucose 6-phosphate and its onward metabolism (Section 5.4), leading to an increase in the ratio of ATP : ADP, and the closing of a potassium channel in the cell membrane. POMC neurons, which signal satiety, are excited in response to glucose and increase their rate of firing; neuropeptide Y and agouti-related peptide neurons, which signal hunger, are inhibited in response to glucose and decrease their rate of firing.

1.4.1.2 Ghrelin: The Appetite-Stimulating Hormone

Ghrelin is a small peptide (28 amino acids) that was originally discovered as the hormone that stimulates the secretion of growth hormone; however, it also acts to increase the synthesis of both neuropeptide Y (so increasing hunger – an orexigenic action) and a peptide that antagonizes the appetite-suppressing action of POMC. The action of ghrelin in stimulating neuropeptide Y neurons is antagonized by insulin and leptin. The secretion of ghrelin increases before an expected meal, suggesting that in addition to responding to emptiness of the stomach, there is also central nervous system regulation of secretion.

In addition to its role in stimulating appetite and suppressing satiety signaling, ghrelin is involved in reinforcing the reward system of the nucleus acumbens in response to food, alcohol and narcotics by activating dopaminergic and cholinergic neurons. This means that it has a role in the hedonistic responses to food and motivation to eat, as well as stimulating feeding behavior.

Paradoxically, ghrelin secretion is low in obese people and rises as weight is lost, suggesting that the state of body reserves of adipose tissue, which is signaled by the hormone leptin (Section 1.4.2), may affect ghrelin secretion. This may explain why it is relatively difficult to lose excess weight and maintain a reduced body weight. A short-term weight loss leads to a long-term increase in ghrelin secretion and decrease in peptide YY secretion, so that even after the lost weight has been regained there will be increased hunger signaling and decreased satiety signaling. Ghrelin secretion is paradoxically high in anorexia nervosa (Section 1.4.5.1), suggesting decreased sensitivity of the ghrelin receptor. Some studies have shown that the administration of ghrelin leads to increased food intake in anorexia. The Prader–Willi syndrome involves a voracious appetite and the development of severe obesity; here the problem is indeed excessive secretion of ghrelin.

The main site of ghrelin secretion is the stomach; it is synthesized in, and secreted by, enteroendocrine cells in the gastric fundus – the upper part of the stomach. This is the region that is removed in the surgical procedure of banded gastroplasty for treatment of severe morbid obesity (Section 7.3.4.13), and part of the success of surgery may be due to reduced secretion of ghrelin, as well as the physical effect of reduced stomach capacity. Ghrelin is also secreted by ε-cells of the pancreas and, to some extent, also the small intestine.

Ghrelin undergoes postsynthetic modification by acetylation. Unacetylated ghrelin is inactive, and inhibitors of ghrelin *O*-acetyltransferase are potential drugs for the control of hunger and treatment of obesity (Section 7.3.4.12).

1.4.2 Long-Term Control of Food Intake and Energy Expenditure: The Hormone Leptin

In addition to the immediate control of feeding by sensations of hunger and satiety, there is long-term regulation of food intake and energy expenditure in response to the size of the body's fat reserves. This is largely a function of the peptide hormone leptin, which is secreted by adipose tissue. It was discovered as the normal product of the gene that is defective in the homozygous recessive mutant (*ob/ob*) obese mouse; administration of the peptide to the obese mice caused them to lose weight. Further studies have shown that the administration of leptin to the genetically obese diabetic (*db/db*) mouse had no effect on body weight, and indeed they secreted a normal or greater than normal amount of leptin. The defect in these animals is in the receptor for leptin in the hypothalamus.

The circulating concentration of leptin is determined largely by the mass of adipose tissue in the body, and leptin signals the size of body fat reserves. Low levels of leptin, reflecting adipose tissue reserves that are inadequate to permit a normal pregnancy, not only increase food intake but also lead to cessation of ovulation and menstruation (by decreasing the secretion of gonadotrophin-releasing hormone); a loss of weight to below 45 kg is associated with amenorrhea. In undernourished children, low levels of leptin, reflecting levels of adipose tissue reserves that are inadequate to permit growth, reduce skeletal growth by inhibiting the secretion of growth hormone.

There is reduced food intake in response to leptin, associated with a decrease in the synthesis of neuropeptide Y (the transmitter for neurons from the hunger centers of the hypothalamus) and an increase in the synthesis of POMC (the transmitter for neurons from the satiety centers). However, the resultant weight loss is greater than can be accounted for by reduced food intake alone, and in response to leptin there is a specific loss of adipose tissue, while in response to reduced food intake there is a loss of both adipose and lean tissue. Leptin receptors are found in a variety of tissues, including muscle and adipose tissue itself. In addition to its role in appetite control, leptin acts to increase energy expenditure and promote the loss of adipose tissue by several mechanisms, including:

- Increased expression of mitochondrial uncoupling proteins (Section 3.3.1.5) in adipose tissue and muscle. This results in relatively uncontrolled oxidation of metabolic fuel, unrelated to requirements for physical and chemical work, and increased heat output from the body (thermogenesis).
- Increased activity of lipase in adipose tissue (Section 5.3.2), resulting in the breakdown of triacylglycerol reserves and release of non-esterified fatty acids that may either be oxidized or be re-esterified in the liver and transported back to adipose tissue. This is metabolically inefficient because of the energy cost of synthesizing triacylglycerol from fatty acids (Section 5.6.1.2); such cycling of lipids is one of the factors involved in the weight loss associated with advanced cancer (Section 8.4).
- Decreased expression of acetyl CoA carboxylase in adipose tissue (Section 5.6.1) – this results in both decreased synthesis and increased oxidation of fatty acids as a result of decreased formation of malonyl CoA (Sections 5.6.1 and 10.5.2).
- Increased apoptosis (programmed cell death) in adipose tissue, thereby reducing the number of adipocytes available for storage of fat in the body.

The result of these actions of leptin on adipose tissue and muscle is that there is an increase in metabolic rate, and hence energy expenditure, in addition to the reduction in food intake.

Although most leptin is secreted by adipose tissue, it is also secreted by muscle and the gastric mucosa. After a meal there is an increase in circulating leptin, suggesting that in addition to its role in long-term control of food intake and energy expenditure, it may also be important in short-term responses to food intake. Some of this leptin comes from the gastric mucosa, but in response to food intake, insulin stimulates leptin synthesis in and secretion from adipose tissue. Conversely, leptin not only increases the synthesis and secretion of insulin but also antagonizes its actions so that excessively high levels of leptin, associated with obesity, lead to hyperinsulinemia and insulin resistance – part of the metabolic syndrome associated with obesity (Section 7.2.3).

There is a circadian variation in leptin secretion, with an increase during the night. This is in response to glucocorticoid hormones, which are secreted in increased amount during the night. It is likely that the loss of appetite and weight loss associated with chronic stress, when there is increased secretion of glucocorticoids, is mediated by the effect of these hormones on leptin synthesis and secretion.

When leptin was first discovered, there was great excitement that, as in the obese mouse, human obesity (Chapter 7) might be due to a failure of leptin synthesis or secretion, and that administration of leptin might be a useful treatment for severe obesity. However, most obese people secrete more leptin than lean people (because they have more adipose tissue), and it is likely that the problem is not due to a lack of leptin, but rather to due to a loss of sensitivity of the leptin receptors. Only a very small number of people have been found in whom genetically determined obesity is due to a mutation in the gene for leptin, the leptin receptor or a component of the downstream signaling pathway.

1.4.3 Appetite

In addition to hunger and satiety, which are basic physiological responses, food intake is controlled by appetite, which is related not only to physiological need but also to the pleasure of eating – flavor, texture and a variety of social and psychological factors. The phenomenon of sensory-specific satiety, when there is satiety toward one food, but others may still be tempting, is the result of stimulation of individual neurons that respond to different combinations of taste, flavor, aroma, texture and the sight of food.

1.4.3.1 Taste and Flavor

Taste buds on the tongue can distinguish five basic tastes – salt, savory, sweet, bitter and sour – as well as a less well-understood ability to taste fat. The ability to taste saltiness, sweetness, savoriness and fat allows the detection of nutrients; the ability to taste sourness and bitterness permits avoidance of toxins in foods. There is some evidence that hormones such as leptin (Section 1.4.2) and ghrelin (Section 1.4.1.2) may affect the sensitivity of sweetness taste buds, and possibly also others. In addition, there is sensitivity, not due to taste buds, to chemical irritants such as the tingle of carbon dioxide in carbonated beverages, the cooling effect of menthol and the burning and pungency of peppers and spices.

Altogether there are some 6,000 taste buds on the tongue and soft palate. Each one contains 30–50 sensory cells with microvilli, but not all sensory cells are exposed at the same time; therefore, there is scope to alter the sensitivity to different tastes by changing the number of sensory cells exposed, as well as by modifying the intracellular responses to stimulation of the receptors on the microvilli or transported ions.

Salt (correctly the mineral sodium) is essential for life, and wild animals will travel great distances to a salt lick. Like other animals, human beings have evolved a pleasurable response to salty flavors – this ensures that physiological needs are met. There is evidence that sensitivity to salt changes in response to the state of sodium balance in the body, with an increased number of active salt receptors on the tongue at times of sodium depletion. However, there is no shortage of salt in developed countries; indeed, average intakes of salt are considerably greater than requirements and thus pose a hazard to health (Section 6.3.5).

The sensation of savoriness is distinct from that of saltiness and is sometimes called *umami* (the Japanese term for "savory"). It is largely due to the presence of free amino acids, especially the amino acid glutamate, in foods, and permits the detection of protein-rich foods. Stimulation of the *umami* receptors of the tongue is the basis of flavor enhancers such as monosodium glutamate, an important constituent of traditional oriental condiments that is widely used in manufactured foods.

The other instinctively pleasurable taste is sweetness, which permits the detection of carbohydrates, and hence energy sources. While it is only sugars (Section 4.2.1) and artificial sweeteners (Section 7.3.4.10) that have a sweet taste, human beings (and a few other animals) secrete the enzyme amylase in saliva, which catalyses the hydrolysis of a small amount of starch, the major dietary carbohydrate, to sweet-tasting sugars while the food is being chewed (Section 4.2.2.1).

The tongue is not sensitive to the taste of triacylglycerols, but rather to free fatty acids, and especially polyunsaturated fatty acids (Section 4.3.1.1). This suggests that the lipase secreted by the tongue has a role in permitting the detection of fatty foods as an energy source, in addition to a very minor role in fat digestion (Section 4.3).

Sourness and bitterness are instinctively unpleasant sensations; many of the toxins that occur in foods taste bitter or sour. Learned behavior will overcome the instinctive aversion, but this is a process of learning or acquiring tastes, not an innate or instinctive response.

The receptors for saltiness, sourness and savoriness (*umami*) all act as ion channels, transporting sodium, hydrogen and glutamate ions, respectively, into the cells of the taste buds.

The receptors for sweetness and bitterness act via cell surface receptors linked to intracellular formation of second messengers. There is evidence that both cyclic adenosine monophosphate (cAMP, Section 10.3.2) and inositol trisphosphate (Section 10.3.3) mechanisms are involved, and more than one signal transduction pathway may be involved in the responses to sweetness or bitterness of different compounds. Some compounds may activate more than one subtype of receptor; there are at least 40–80 different cell surface bitterness receptors, all of which are linked to activation of the intracellular protein α-gustducin. Small changes in the structure of compounds can affect whether they taste sweet or bitter; L-tryptophan is intensely bitter, while its stereoisomer D-tryptophan tastes very sweet.

In addition to the sensations of taste provided by the taste buds on the tongue, a great many flavors can be distinguished by the sense of smell. Some flavors and aromas (e.g., fruity flavors, fresh coffee and, at least to a nonvegetarian, the smell of roasting meat) are pleasurable, tempting people to eat and stimulating appetite. Other flavors and aromas are repulsive, warning us not to eat the food. Again this can be seen as a warning of possible danger – the smell of decaying meat or fish tells us that it is not safe to eat.

Like the acquisition of a taste for bitter or sour foods, a taste for foods with what would seem at first to be an unpleasant aroma or flavor can also be acquired. Here things

become more complex; what seems a pleasant smell to one person may be repulsive to another. Some people enjoy the smell of cooked cabbage and Brussels sprouts, while others can hardly bear to be in the same room. The durian fruit is a highly prized delicacy in Southeast Asia, yet to the uninitiated it has the unappetizing aroma of sewage or feces.

1.4.4 Why Do People Eat What They Do?

People have different responses to the same taste or flavor. This may be explained in terms of childhood memories, pleasurable or otherwise. An aversion to the smell of a food may protect someone who has a specific allergy or intolerance (Section 1.4.4.6), although sometimes people have a craving for the foods of which they are intolerant. Most often we simply cannot explain why some people dislike foods that others eat with great relish. A number of factors influence why people choose to eat particular foods (Table 1.3).

1.4.4.1 The Availability and Cost of Food

In developed countries, the simple availability of food is not a significant constraint on choice. There is a wide variety of foods available, and when fruits and vegetables are out of season at home, they are imported; frozen, canned or dried foods are widespread. By contrast, in developing countries, the availability of food may be a major constraint on what people choose. Little food is imported, and what is available will depend on the local soil, climate and season of the year. In normal times the choice of foods may be limited, while in times of drought there may be little or no food available at all, and what little is available will be more expensive than most people can afford. Even in developed countries, the cost of food is important, and for the most disadvantaged members of the community, poverty may impose severe constraints on their choice of foods.

1.4.4.2 Religion, Habit and Tradition

Religious and ethical considerations are important in determining the choice of foods. Observant Jews and Muslims will only eat meat from animals that have cloven hooves and chew the cud. The terms *kosher* in Jewish law and *hallal* in Islamic law both mean "clean"; the meat of other animals, which are scavenging animals, birds of prey and detritus feeding fish, is regarded as unclean (*traife* or *haram*). We now know that many of these forbidden animals carry parasites that can infect human beings, so these ancient prohibitions were based on food hygiene.

Table 1.3 Factors that influence the choice of foods

Availability of foods
Cost of foods
Time for preparation and consumption
Disability and infirmity
Personal likes and dislikes
Intolerance or allergy
Eating alone or in company
Marketing pressure and advertising
Religious and ethical taboos
Perceived or real health benefits and risks
Modified diet for control of disease
Illness or medication

Hindus will not eat beef. The reason for this is that the cow is far too valuable, as a source of milk and dung (as manure and fuel), and as a beast of burden, for it to be killed as a source of meat.

Many people refrain from eating meat as a result of humanitarian concern for the animals involved, or because of real or perceived health benefits. Concerns over global warming and the greenhouse gases emitted by farm animals (and especially methane from ruminants) provide a stimulus to many people to become more or less vegetarian, and indeed, with an ever growing global population to be fed, many consider that growing crops to feed animals to provide human food is highly inefficient, and we should consume the primary crops themselves. The term *flexitarian* has been introduced to cover people who aim or wish to reduce their consumption of meat, mainly on the grounds of the environmental impact of livestock farming.

Vegetarians can be divided into various groups according to the strictness of their diet:

- Some avoid red meat but will eat poultry and fish.
- Some specifically avoid beef because of the potential risk of contracting variant Creutzfeld–Jacob disease from BSE-infected animals.
- Pescetarians eat fish but not meat or poultry.
- Ovo-lacto vegetarians will eat eggs and milk but not meat or fish.
- Lacto-vegetarians will eat milk but not eggs.
- Vegans will eat only plant foods and no foods of animal origin.

Foods that are commonly eaten in one area may be little eaten elsewhere, even though they are available, simply because people have not been accustomed to eating them. To a very great extent, eating habits as adults continue the habits learnt as children.

In Britain, haggis and oat cakes travel south from Scotland as specialty items; black pudding is a staple of northern British breakfasts, but is rare in the southeast of England. Until the 1960s yoghurt was almost unknown in Britain, apart from a few health food "cranks" and immigrants from eastern Europe; many British children believe that fish comes as rectangular fish fingers, while children in inland Spain may eat fish and other sea-food three or four times a week. The French mock the British habit of eating lamb with mint sauce – and the average British reaction to such French delicacies as frogs' legs and snails in garlic sauce is one of horror. The British eat their cabbage well boiled; the Germans and Dutch ferment it to produce sauerkraut.

This regional and cultural diversity of foods provides one of the pleasures of travel. As people travel more frequently, and become (perhaps grudgingly) more adventurous in their choice of foods, they create a demand for different foods at home, and there is an increasing variety of foods available in shops and restaurants.

A further factor that has increased the range of foods available has been immigration of people from a variety of different backgrounds, all of whom have, as they have become established, introduced their traditional foods to their new homes. It is difficult to realize that in the 1960s there was only a handful of tandoori restaurants in the whole of Britain, that pizza was something seen only in southern Italy and a few specialist restaurants, or that Balti cooking, Thai food and sushi were unknown until the 1990s. Insects are eaten in some countries and are gradually being introduced to supermarkets and upmarket restaurants as specialty foods.

Some people are naturally adventurous and will try a new food just because they have never eaten it before. Others are more conservative and will try a new food only when they see someone else eating it safely and with enjoyment. Others are yet more conservative in

their food choices; the most conservative eaters "know" that they do not like a new food *because* they have never eaten it before.

1.4.4.3 Organic Foods

Many people choose to eat organically produced foods in preference to those produced by conventional or intensive farming methods. Organic foods are plants grown without the use of (synthetic) pesticides, fungicides or inorganic fertilizers, and prepared without the use of preservatives. Foodstuffs must be grown on land that has not been treated with chemical fertilizers, herbicides or pesticides for at least three years. Organic meat is from animals fed on organically grown crops without the use of growth promoters, with only a limited number of medicines to treat disease, and commonly maintained under traditional, nonintensive or free-range conditions. Within the European Union (EU), foods may be labeled as organic if they contain at least 95% organic ingredients and not more than 0.9% genetically modified ingredients.

People who wish to avoid pesticide, fungicide and other chemical residues in their food, or genetically modified crops, will choose organic produce. Other people will choose organic foods because they believe that they are nutritionally superior to conventional produce, or because they have a better flavor. There is little evidence that organic produce is nutritionally superior to that produced by conventional farming. Although, if organic fruits and vegetables are slower-growing, and possibly lower-yielding varieties, they may have a higher nutrient content. Many of the older, slower-growing and lower-yielding fruits and vegetables have a better flavor than more recently introduced varieties that are grown for their rapid yield of a large crop of uniform size and shape. Flavor does not depend on whether or not they are grown organically, but many organic farmers grow traditional, more flavorful varieties.

The nutrient content of the same variety of a fruit or vegetable may vary widely depending not only on the soil (and any fertilizers used) but also on how much sunlight the plant has received and how frequently it has been watered. The apples from one side of a tree may vary in nutrient content from those on the other side of the same tree. The yield, flavor and nutrient content of the same crop may vary along the field.

While organic produce is indeed free from chemical residues that may be harmful, there is a potential hazard. Animal manure is used in organic farming to a greater extent than in conventional farming, and unless salad vegetables are washed well, there is a potential risk of food poisoning from bacteria in the manure that remains on the produce.

1.4.4.4 Luxury Status of Scarce and Expensive Foods

Foods that are scarce or expensive have a certain appeal of fashion or style; they are (rightly) regarded as luxuries for special occasions rather than everyday meals. Conversely, foods that are widespread and inexpensive have less appeal.

In the 19th century, salmon and oysters were so cheap that the articles of apprentices in London specified that they should not be given salmon more than three times a week, while oysters were eaten by the poor. Through much of the 20th century, salmon was scarce, and a prized luxury food; fish farming has increased the supply of salmon to such an extent that it is again an inexpensive food. Chicken, turkey, guinea fowl and trout, which were expensive luxury foods in the 1950s, are now widely available as a result of changes in farming practice, and they form the basis of inexpensive meals. By contrast, fish such as cod, herring and skate, which were once the basis of cheap meals, are now becoming scarce and expensive as a result of depletion of fish stocks by overexploitation.

1.4.4.5 Social Functions of Food

Human beings are social animals, and meals are important social functions. People eating in a group are likely to eat better, or at least have a wider variety of foods and a more lavish and luxurious meal, than people eating alone. Entertaining guests may be an excuse to eat foods that we know to be nutritionally undesirable, and perhaps to eat to excess. The greater the variety of dishes offered, the more people are likely to eat. As we reach satiety with one food, another, different, flavor is offered to stimulate the appetite. A number of studies have shown that, faced with only one food, people tend to reach satiety sooner than when a variety of foods is on offer. This is the difference between hunger and appetite – even when we are satiated, we can still "find room" to try something different.

Conversely, and more importantly, many lonely single people (and especially the bereaved elderly) have little incentive to prepare meals, and no stimulus to appetite. While poverty may be a factor, apathy (and frequently, in the case of widowed men, ignorance) severely limits the range of foods eaten, possibly leading to undernutrition. When these problems are added to the problems of ill-fitting dentures (which make eating painful), arthritis (which makes handling many foods difficult) and the difficulty of carrying food home from the shops, it is not surprising that we include the elderly among the vulnerable groups of the population who are at risk of undernutrition (Section 8.3.1).

In hospitals and other institutions there is a further problem. People who are unwell may have low physical activity, but they have higher than normal requirements for energy and nutrients, as a part of the process of replacing tissue in convalescence (Section 9.1.2.3), or as a result of fever or the metabolic effects of cancer and other chronic diseases (Section 8.4). At the same time, illness impairs appetite, and a side effect of many drugs is to distort the sense of taste, depress appetite or cause nausea. It is difficult to provide a range of exciting and attractive foods under institutional conditions, yet this is what is needed to tempt the patient's appetite.

1.4.4.6 Food Allergy and Intolerance

Food allergy is an immunological response to proteins or other constituents of a food; after initial exposure to the allergen, antibodies (immunoglobulin E [IgE]) are synthesized. When a person is exposed to the same allergen, there is a massive immune response, which may lead to potentially fatal anaphylactic shock, with the release of histamine and other amines from mast cells, leading to a fall in blood pressure and narrowing of the airways. Most countries require labels on packaged food to contain a list of ingredients, with specified allergens shown in bold type (Table 1.4). In many countries, restaurant menus show a list of allergens with numbers or letters, and when specific allergens are included in a dish, the numbers or letters are shown alongside the description of the dish. Some restaurants that have a more or less fixed menu have a list of allergens in each dish available on their website or through a mobile phone app.

Food intolerance is a physiological problem but does not involve an immune system response to the offending food. The most common intolerance is to the sugar lactose in milk. In most areas of the world, the enzyme lactase, which is required for the digestion of lactose (Section 4.2.2.2), is lost after adolescence so that adults cannot digest lactose, which then provides a substrate for bacterial fermentation in the large intestine. The products of this bacterial fermentation include lactic acid, leading to an increase in osmolality of the intestinal contents, and hence diarrhea, as well as carbon dioxide, leading to bloating and severe abdominal discomfort. It is mainly among people of northern European and

Table 1.4 Food allergens that must be shown on labels

In the European Union:
Celery and celeriac
Cereals containing gluten (wheat, rye, barley and oats)
Eggs
Fish
Lupin
Mollusks
Mustard
Nuts
Peanuts
Sesame
Soya beans
Sulfur dioxide and sulfites at more than 10 mg/kg or 10 mg/L
In the USA:
Crustacean shellfish
Eggs
Fish
Milk
Peanuts
Soya beans
Tree nuts
Wheat
In Canada, as USA plus:
Mustard
Sesame
Sulfites

northern Indian heritage, and the Masai of East Africa, that lactase persists after adolescence, so that they can consume milk with impunity.

1.4.5 Eating Disorders

While obesity is a major public health problem (Section 7.2), one effect of the publicity about obesity is to put pressure on people to reduce their body weight, even if they are within the desirable, healthy weight range. In some cases the pressure for slimness may be a factor in the development of eating disorders, although some evidence suggests that media and peer pressure activate the desire for thinness in people who are already vulnerable because of low self-esteem and dissatisfaction with their body image, but do not cultivate it otherwise. Eating disorders are due to interactions between higher brain centers and the hunger and satiety centers of the hypothalamus; therefore, a variety of psychological factors can override the normal sensations of hunger and satiety. Treatment of eating disorders requires specialist psychiatric help and counselling, as well as nutritional advice and patient support groups.

Those most at risk are adolescent girls; at any time some 25% are dieting to lose weight, whether they need to or not, and 50% think that they are too fat. Eating disorders can occur in older women, and in adolescent boys and men. It is estimated that about 2% of adolescent girls go through at least a short phase of an eating disorder, and another 3% have a

borderline eating disorder. In most cases, the disorder is self-limiting, and normal eating patterns are reestablished as the emotional crises of adolescence resolve. Other people may require specialist counseling and treatment, and in an unfortunate few problems of eating behavior persist throughout the adult life.

It is noteworthy, and perhaps unsurprising, that eating disorders are more common in countries with adequate or superabundant availability of food than in countries where food is scarce. Indeed, in many cultures where food is limited, overweight and obesity are prized as a sign of prosperity and wealth.

1.4.5.1 Anorexia Nervosa

One cause of the problem in adolescent girls is a reaction to the physical changes of puberty. By refusing food, the girl believes that she can delay or prevent these changes. To a considerable extent this is so. Breast development slows down or ceases as energy balance becomes more negative. When body weight falls below about 45 kg, menstruation ceases because of the lower secretion of leptin from the reduced amount of adipose tissue (Section 1.4.2).

The main feature of anorexia nervosa is a very severe restriction of food intake – with the obvious result of considerable weight loss. The diagnostic criteria of anorexia nervosa include a persistent restriction of energy intake leading to body weight significantly below the minimum required for age, gender and physical health, as well as an intense fear of gaining weight or becoming fat, and persistent behavior that interferes with weight gain. There is disturbance of perception of body weight or shape, undue influence of body weight and shape on self-evaluation, or persistent failure to recognize the seriousness of current low body weight. Dieting becomes the primary focus of life. The anorectic person has a preoccupation with, and often a considerable knowledge of, food and frequently has a variety of stylized compulsive behavior patterns associated with food. As a part of the pathological obsession with thinness, the patient frequently takes a great deal of strenuous exercise, often exercising to exhaustion in solitude.

Surprisingly, many anorectic people are adept at hiding their condition, and it is not unknown for the problem to remain unnoticed, even in a family setting. Food is played with, but little or none is actually eaten; excuses are frequently made to leave the table in the middle of the meal, perhaps on the pretext of going into the kitchen to prepare the next course.

There are two types of anorexia nervosa:

- **Restricting type:** During the current episode of anorexia nervosa, the person has not regularly engaged in binge eating or purging behavior (self-induced vomiting or misuse of laxatives, diuretics or enemas).
- **Binge eating–purging type:** During the current episode of anorexia nervosa, the person has regularly engaged in binge eating or purging behavior (self-induced vomiting or the misuse of laxatives, diuretics or enemas).

Some estimates suggest that the lifetime prevalence of anorexia nervosa is between 1 and 2% for women, and although it is less common in men, perhaps 25% of anorectic people undergoing treatment are male. Twin studies have shown that there is some evidence of genetic factors in anorexia nervosa; there is a 55% concordance between identical (monozygotic) twins compared with only 5% in nonidentical (dizygotic) twins who nonetheless share the same environment and family pressures.

1.4.5.2 Bulimia Nervosa

Bulimia nervosa is characterized by recurrent episodes of binge eating, which means eating within a discrete period of time (e.g. a 2-hour period) an amount of food that is considerably larger than most people would eat in that time period under similar circumstances. The bulimic person senses a lack of control over eating during the binging episode, feeling unable to stop eating or control how much is eaten. There is then recurrent compensatory behavior in order to prevent weight gain, including self-induced vomiting, inappropriate use of laxatives, diuretics and other medication, fasting and excessive exercise.

Both the binge eating and inappropriate compensatory behavior occur on average at least once a week over three or more months, and the disturbance does not occur exclusively during episodes of anorexia nervosa. Indeed, the bulimic patient may have a body weight within the normal range, and patients are generally older than those with anorexia nervosa; typically in late teens and early 20s. Unlike anorexia nervosa, there is no evidence of a genetic factor in bulimia; concordance is the same for identical and nonidentical twins.

1.4.5.3 Binge Eating Disorder

Binge eating disorder involves episodes of binge eating as described above for bulimia nervosa. They are associated with eating much more rapidly than normal; eating until feeling uncomfortably full; eating large amounts when not feeling physically hungry; eating alone because of embarrassment about the amount of food being eaten; and feeling disgusted, depressed or very guilty afterwards. Binge eating occurs on average at least once a week for three or more months, and is not associated with inappropriate compensatory behavior, as occurs in bulimia nervosa.

1.4.5.4 Other Eating Disorders

Pica is persistent eating of nonnutritive substances, including earth or soil, that is not part of a culturally or socially accepted normal practice.

Rumination disorder is repeated regurgitation of food, which may then be re-chewed, re-swallowed or spat out, but not when this is the result of a gastrointestinal condition or medication.

Some people eat an inappropriately limited range of foods as a result of real or perceived food allergy and intolerance (Section 1.4.4.6); such extreme diets lead to significant weight loss and nutrient deficiencies. This is avoidant/restrictive food intake disorder.

Key Points

- The first need of the body is for water. In addition to obvious water in beverages, there is water in most foods, and water formed in the metabolism of metabolic fuels makes a significant contribution to fluid balance.
- There is a relationship between energy intake from food, energy expenditure in physical activity and body weight, and two-thirds of total energy expenditure is required to maintain nerve and muscle tone, circulation and breathing, and metabolic homeostasis.
- The main metabolic fuels are carbohydrate and fat; there is no absolute requirement for either (apart from a small amount of essential fatty acids). It is difficult to achieve an adequate energy intake on a very low fat diet, and fat is essential for the absorption of vitamins A, D, E and K.
- There is a requirement for protein in addition to its role as a metabolic fuel.

- There is a requirement for minerals that have a function in the body and for vitamins.
- Centers in the brain control hunger and satiety in response to circulating concentrations of metabolic fuels and hormones secreted by the gastrointestinal tract and pancreas. Ghrelin stimulates appetite, while the other gastrointestinal hormones signal satiety.
- Long-term control of food intake and energy expenditure is largely by the hormone leptin, which is secreted mainly by adipose tissue; circulating concentrations of leptin reflect the adequacy (or inadequacy) of body fat reserves. Leptin acts on the hypothalamus to regulate food intake, and on muscle and adipose tissue to regulate energy expenditure.
- The sense of taste on the tongue permits the detection of nutrients and avoidance of potential toxins.
- Food choices are complex. In addition to the cost and availability of foods, a variety of religious and ethical beliefs and social and individual factors affect what people choose to eat.
- Higher brain centers and psychological factors can override the physiological signals for hunger and satiety, leading to eating disorders, including anorexia nervosa, bulimia nervosa and binge eating disorder.

chapter two

Enzymes and Metabolic Pathways

All metabolic processes depend on reaction between molecules, with breaking of some covalent bonds and the formation of others, yielding compounds that are different from the starting materials. In order to understand nutrition and metabolism, it is therefore essential to understand how chemical reactions occur, how they are catalyzed by enzymes and how enzyme activity can be regulated and controlled.

Objectives

After reading this chapter, you should be able to:

- explain how covalent bonds are broken and formed; what is meant by thermoneutral, endothermic and exothermic reactions, and how reactions come to equilibrium
- explain how a catalyst increases the rate at which a reaction comes to equilibrium and how enzymes act as catalysts
- explain how an enzyme exhibits specificity for both the substrates bound and the reaction catalyzed
- define a unit of enzyme activity
- explain how pH, temperature and the concentration of enzyme affect the rate of reaction
- describe and explain the dependence of the rate of reaction on the concentration of substrate, define the kinetic parameters K_m and V_{max} and explain how they are determined experimentally
- explain how enzymes may show cooperative binding of substrate and how this affects the substrate dependence of activity
- describe the difference between reversible and irreversible inhibitors of enzymes, their clinical relevance and how they may be distinguished experimentally
- describe the difference between competitive, noncompetitive and uncompetitive reversible inhibitors of enzymes, their clinical relevance and how they may be distinguished experimentally
- explain what is meant by the terms "coenzyme and prosthetic group," "apoenzyme" and "holoenzyme"
- describe the roles of coenzymes in oxidation and reduction reactions
- describe the classification of enzymes on the basis of the reaction catalyzed
- describe and explain what is meant by a metabolic pathway, and what is meant by linear, branched, spiral (looped) and cyclic pathways.

2.1 Chemical Reactions: Breaking and Making Covalent Bonds

Breaking of a covalent bond requires an initial input of energy in some form – normally as heat, but in some cases also light or other radiations. This is the activation energy of the

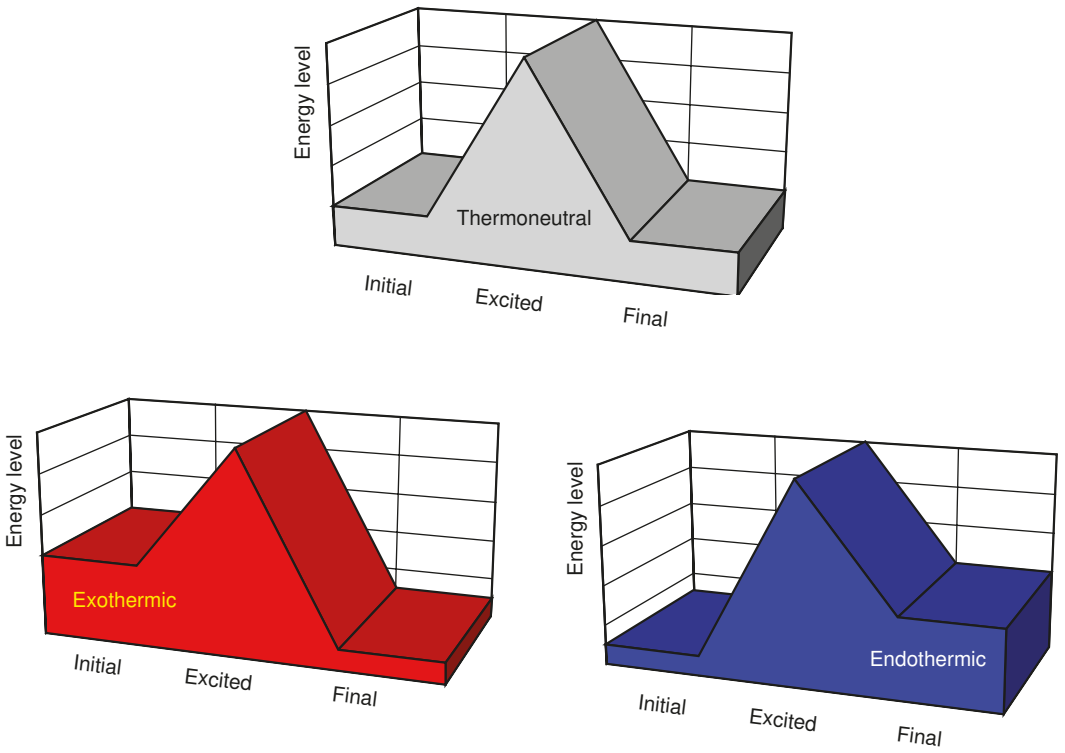

Figure 2.1 Energy changes in chemical reactions: thermoneutral, exothermic and endothermic reactions.

reaction. The process of breaking a bond requires activation of the electrons forming the bond – a temporary movement of electrons from orbitals in which they have a stable configuration to orbitals further from the nucleus. Electrons that have been excited in this way have an unstable configuration, and the covalent bonds they contributed to are weakened and broken. Electrons cannot remain in this excited state for more than a fraction of a second. Sometimes they simply return to their original unexcited state, emitting the same energy as was used to excite them, but usually as a series of small steps, rather than as a single step. Overall, there is no change when this occurs.

More commonly, the excited electrons adopt a different stable configuration by interacting with electrons associated with different atoms and molecules. The result is the formation of new covalent bonds, and hence the formation of new compounds. In this case, there are three possibilities, as shown in Figure 2.1:

- There may be an output of energy equal to the activation energy of the reaction so that the energy level of the products is the same as that of the starting materials. Such a reaction is energetically neutral (thermoneutral).
- There may be an output of energy greater than the activation of the reaction so that the energy level of the products is lower than that of the starting materials. This is an exothermic reaction – it proceeds with the output of heat. An exothermic reaction will proceed spontaneously once the initial activation energy has been provided.

- There may be an output of energy less than the activation energy so that the energy level of the products is higher than that of the starting materials. The solution will take up heat from its surroundings, and will have to be heated for the reaction to proceed. This is an endothermic reaction.

In general, reactions in which relatively large complex molecules are broken down to smaller molecules (catabolic reactions) are exothermic, while reactions that involve the synthesis of larger molecules from smaller ones (anabolic or biosynthetic reactions) are endothermic.

2.1.1 Equilibrium

Some reactions, such as the burning of ethanol (see Figure 2.21) or a hydrocarbon in air to form carbon dioxide and water, are highly exothermic, and the products of the reaction are widely dispersed. Such reactions proceed essentially in one direction only. However, most reactions do not proceed in only one direction. If two compounds A and B can react together to form X and Y, then X and Y can react to form A and B. The reactions can be written as follows:

$$(1) \quad A + B \rightarrow X + Y$$

$$(2) \quad X + Y \rightarrow A + B$$

Starting with only A and B in the solution, at first only reaction (1) will occur, forming X and Y. However, as X and Y accumulate, they will undergo reaction (2), forming A and B. Similarly, starting with X and Y, at first only reaction (2) will occur, forming A and B. As A and B accumulate, they will undergo reaction (1), forming X and Y.

In both cases, the final result will be a solution containing A, B, X and Y. The relative amounts of [A+B] and [X+Y] will be the same regardless of whether the starting compounds (substrates) were A and B or X and Y. At this stage the rate of reaction (1) forming X and Y, and reaction (2) forming A and B, will be equal. This is equilibrium, and the reaction can be written as:

$$A + B \rightleftharpoons X + Y$$

If there is a large difference in energy levels between [A+B] and [X+Y] (i.e. if the reaction is exothermic in one direction, and therefore endothermic in the other), then the position of the equilibrium will reflect this. If reaction (1) is exothermic, then at equilibrium there will be very little A and B remaining, most will have been converted to X and Y. Conversely, if reaction (1) is endothermic, then at equilibrium relatively little of A and B will be converted to X and Y.

At equilibrium, the ratio of [A+B] : [X+Y] is constant for any given reaction. Therefore, if there is a constant addition of substrates, this will disturb the equilibrium and increase the amount of products formed. Similarly, continual removal of the products will increase the rate at which the substrate is utilized.

A metabolic pathway is a sequence of reactions, and *in vivo* very few reactions actually come to equilibrium. The product of one reaction is the substrate for the next; therefore, there is a continual supply of substrate, and removal of products, for each reaction, and there is a constant flux through the pathway – a dynamic steady state rather than equilibrium.

2.1.2 Catalysis

A catalyst increases the rate at which a reaction comes to equilibrium, without itself being consumed in the reaction; therefore, a small amount of catalyst can lead to the reaction of many thousands of molecules of substrate. Although a catalyst increases the rate at which a reaction comes to equilibrium, it does not affect the position of the equilibrium.

Catalysts affect the rate of reaction in three main ways:

- By providing a surface on which the molecules that are to undergo reaction can come together at a higher concentration than would be possible in free solution, thus increasing the probability of them colliding and reacting. This also aligns the reactants in the correct orientation to undergo reaction.
- By providing a micro-environment for the reactants that is different from the solution as a whole.
- By participating in the reaction by withdrawing electrons from, or donating electrons to, covalent bonds. This enhances the breaking of bonds that is an essential prerequisite for chemical reaction and lowers the activation energy of the reaction.

2.2 Enzymes

Enzymes are proteins that catalyze metabolic reactions. There are also a number of enzymes that are not proteins but are catalytic molecules of ribonucleic acid (RNA) (Section 9.2.2); these are sometimes referred to as ribozymes.

Proteins are linear polymers of amino acids (Section 4.4.2). Any protein adopts a characteristic pattern of folding, determined largely by the amino acids in its sequence, and their interactions with each other and the surrounding environment. This folding of the protein chain results in reactive groups from amino acids that may be widely separated in the primary sequence coming together at the surface and creating a site that has a defined shape and an array of chemically reactive groups. This is the active site of the enzyme, which can be divided into two distinct domains: the binding site for the compounds that are to undergo reaction (the substrates) and the catalytic site. Figure 2.2 shows how three amino acids that are widely separated in the primary sequence of the enzyme trypsin come together to form a catalytic triad as a result of folding of the protein chain.

Many enzymes also have a nonprotein component of the catalytic site; this may be a metal ion, an organic compound that contains a metal ion (e.g., heme, Section 3.3.1.2) or an organic compound, which may be derived from a vitamin (Table 2.1 and Chapter 11) or may be a compound that is readily synthesized in the body. This nonprotein part of the active site may be covalently bound, when it is generally referred to as a prosthetic group, or may be tightly, but not covalently, bound, when it is usually referred to as a coenzyme (Section 2.4).

Reactive groups in amino acid side-chains and coenzymes or prosthetic groups at the active site facilitate the making or breaking of specific chemical bonds in the substrate by donating or withdrawing electrons. In this way, the enzyme lowers the activation energy of a chemical reaction (Figure 2.3) and increases the rate at which the reaction attains equilibrium, under much milder conditions than those that are required for a simple chemical catalyst. In order to hydrolyze a protein into its constituent amino acids in the laboratory, it is necessary to use concentrated acid as a catalyst, and heat the sample in a sealed tube at 105°C overnight to provide the activation energy of the hydrolysis. This is the process

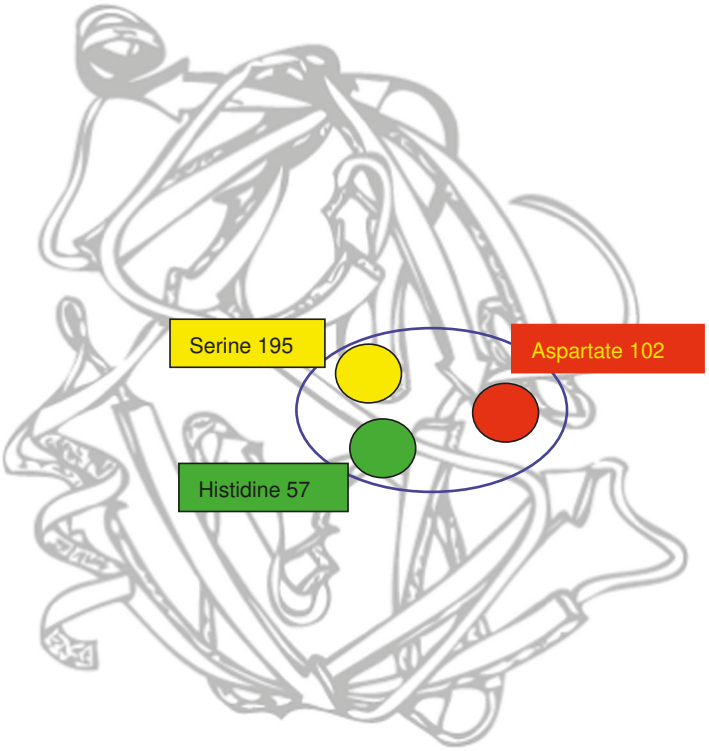

Figure 2.2 The formation of an active site in an enzyme as a result of folding of the protein chain.

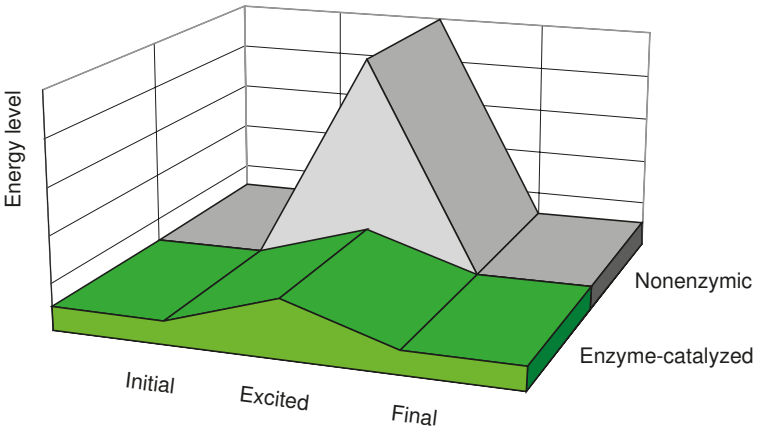

Figure 2.3 The effect of enzyme catalysis on the activation energy of a reaction. The enzyme does not affect the initial or final energy level, but only the activation energy.

of digestion of proteins, which occurs under relatively mild acid or alkaline conditions, at 37°C, and is complete within a few hours of eating a meal (Section 4.4).

2.2.1 Specificity of Enzymes

The binding of substrates to enzymes involves interactions between the substrates and reactive groups of the amino acid side-chains that make up the active site of the enzyme. This means that enzymes show a considerable specificity for the substrates they bind. Normally several different interactions must occur before the substrate can bind in the correct orientation to undergo reaction, and binding of the substrate often causes a change in the conformation of the active site, bringing reactive groups closer to the substrate.

Figure 2.4 shows the active sites of three enzymes that catalyze the same reaction – hydrolysis of a peptide bond in a protein (Section 4.4.3); in all three enzymes, the catalytic site is the same as that shown for trypsin in Figure 2.2. The three enzymes show different specificity for the bond they hydrolyze:

- Trypsin catalyzes cleavage of the esters of basic amino acids.
- Chymotrypsin catalyzes hydrolysis of the esters of aromatic amino acids.
- Elastase catalyzes hydrolysis of the esters of small neutral amino acids.

This difference in specificity for the bond to be hydrolyzed is explained by differences in the substrate binding sites of the three enzymes. In all three, the substrate binds in a groove at the surface in such a way that it brings the bond to be cleaved over the serine residue that initiates the catalysis. The amino acid providing the carboxyl side of the peptide bond to be cleaved sits in a pocket below this groove, and it is the nature of the amino acids that line this pocket that determines the specificity of the enzymes:

- In trypsin there is an acidic group (from aspartate) at the base of the pocket – this will attract a basic amino acid side chain.
- In chymotrypsin the pocket is lined by small neutral amino acids so that a relatively large aromatic group can fit in.
- In elastase there are two bulky amino acid side chains in the pocket so that only a small neutral side chain can fit in.

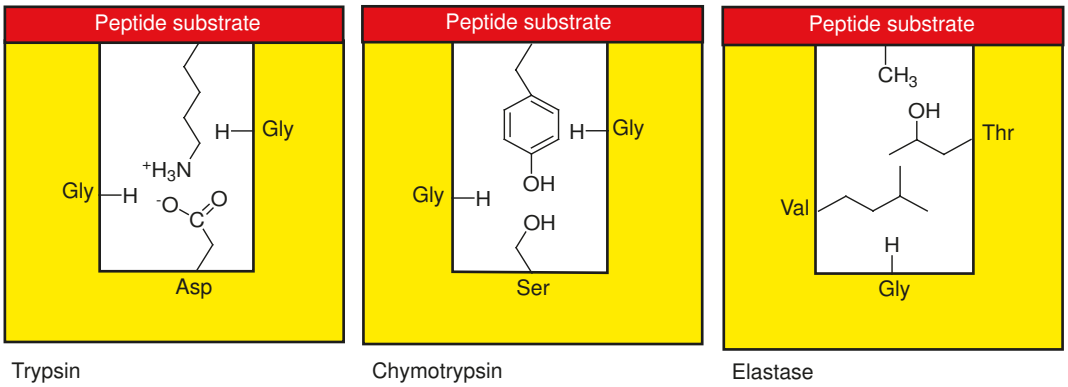

Figure 2.4 Enzyme specificity: the substrate binding sites of trypsin, chymotrypsin and elastase.

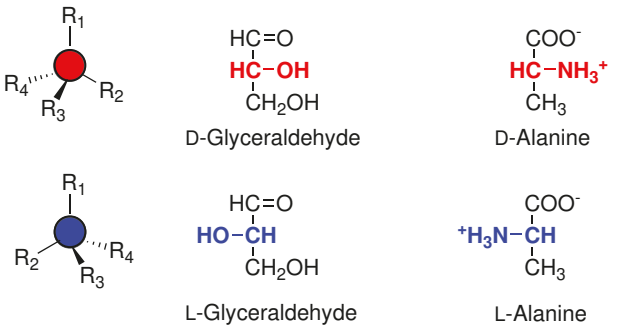

Figure 2.5 DL-Isomerism: the arrangement of substituent groups around an asymmetric carbon atom.

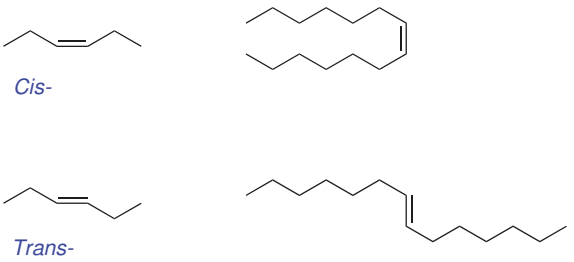

Figure 2.6 *Cis-trans* isomerism: the continuation of the carbon chain on one side or the other of a carbon–carbon double bond.

Chemically, D- and L-isomers of a compound (Figure 2.5) and *cis*- and *trans*-isomers (Figure 2.6) behave identically, and it can often be difficult to distinguish between isomers. However, the isomers have different shapes, and enzymes readily discriminate between them – the shape and conformation of the substrate are critically important for binding to an enzyme. Most of the naturally occurring and physiologically relevant sugars are D-isomers, and most amino acids are L-isomers; the nutritional and health importance of *trans*-isomers of unsaturated fatty acids is discussed in Section 6.3.2.1.

The participation of reactive groups at the active site of the enzyme provides specificity not only for the substrates that will bind, but also for the reaction that will be catalyzed. For example, in a nonenzymic model system, an amino acid may undergo α-decarboxylation to yield an amine, transfer of the α-amino group and replacement with an oxo-group (Section 9.3.1.2), isomerization between the D- and L- isomers, or a variety of reactions involving elimination or replacement of the side chain. In an enzyme-catalyzed reaction, only one of the possible reactions will normally be catalyzed by a given enzyme.

2.2.2 *Stages in an Enzyme-Catalyzed Reaction*

An enzyme-catalyzed reaction can be considered to occur in three distinct steps, each of which is reversible:

- binding of the substrate (S) to the enzyme (Enz) to form the enzyme-substrate complex: Enz + S ⇌ Enz-S

- reaction of the enzyme-substrate complex to form the enzyme-product complex: Enz-S ⇌ Enz-P
- breakdown of the enzyme-product complex, with release of the product (P): Enz-P ⇌ Enz + P

Overall, the process can be written as follows: Enz + S ⇌ Enz-S ⇌ Enz-P ⇌ Enz + P.
There are two models for the binding of a substrate to an enzyme:

1. The lock and key model, in which the reactive groups at the substrate binding site and the catalytic site are perfectly aligned to permit substrate binding and catalysis.
2. The induced fit model, in which binding of the substrate causes a conformational change in the catalytic site, bringing the reactive groups into the correct alignment with the substrate to catalyze the reaction. An extreme form of induced fit is seen in multi-subunit enzymes (allosteric enzymes, Section 2.3.3.3) that show cooperative binding of substrate. Binding substrate at one of the binding sites affects the conformation at the other active sites, enhancing the binding of further molecules of substrate.

2.2.3 *Units of Enzyme Activity*

In relatively rare cases when an enzyme has been purified, it is possible to express the amount of the enzyme in tissues or plasma as the number of moles of enzyme protein present, for example, by raising antibodies against the purified protein for use in an immunoassay. However, what is more important is not how much of the enzyme protein is present in the cell, but rather how much catalytic activity there is – how much substrate can be converted to product in a given time. Therefore, the amount of enzymes is usually expressed in units of activity.

The SI unit of catalysis is katal = 1 mol of substrate converted per second. However, enzyme activity is usually expressed as the number of micromoles (μmol) of substrate converted (or of product formed) per minute. This is the standard unit of enzyme activity, determined under specified optimum conditions for that enzyme, at 30°C. This temperature is a compromise between mammalian biochemists, who would work at body temperature (37°C for human beings), and microbiological biochemists, who would normally work at 20°C.

2.3 *Factors Affecting Enzyme Activity*

Any given enzyme has an innate activity – for many enzymes the catalytic rate constant is of the order of 1000–5000 mol of substrate converted per mol of enzyme per second or higher. However, a number of factors affect the activity of enzymes.

2.3.1 *The Effect of pH*

Both the binding of the substrate to the enzyme and catalysis of the reaction depend on interactions between the substrates and reactive groups in the amino acid side chains that make up the active site. They have to be in the appropriate ionization state for binding and reaction to occur, and this depends on the pH of the medium. Any enzyme will have maximum activity at a specific pH – the optimum pH for that enzyme. If the pH rises above or falls below the optimum, then the activity of the enzyme will decrease. Most enzymes have

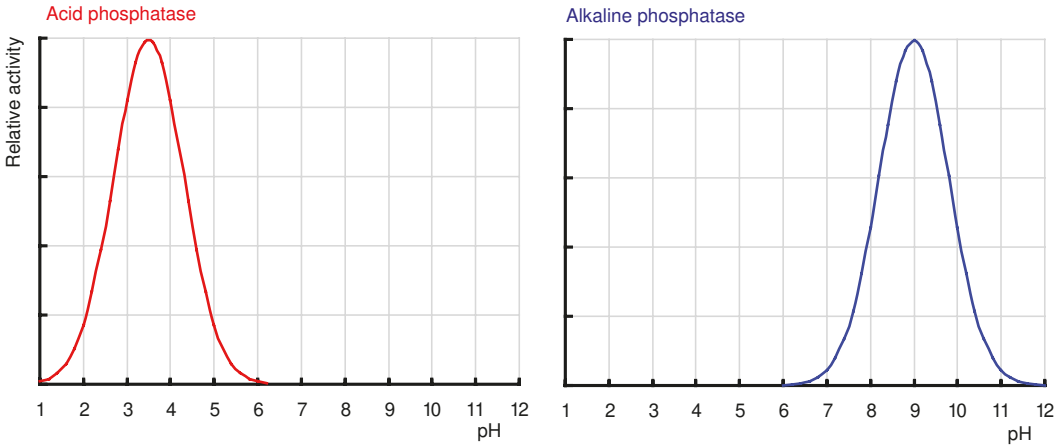

Figure 2.7 The effect of pH on enzyme activity.

little or no activity at a pH of 2–3 units away from their optimum. Although the average pH of cell contents (and plasma) is around 7.4, individual subcellular compartments and organelles may be very acidic or alkaline.

Figure 2.7 shows the activity of two enzymes that are found in plasma and catalyze the same reaction, hydrolysis of a phosphate ester: acid phosphatase (released from the prostate gland) has a pH optimum of about 3.5, while alkaline phosphatase (released from liver and bone) has a pH optimum of about 9.0. Neither has any significant activity at a pH of 7.35–7.45, which is the normal range in plasma. However, alkaline phosphatase is significantly active in the alkaline micro-environment at cell surfaces, and is important, for example, in the hydrolysis of pyridoxal phosphate (the main form of vitamin B_6 in plasma, Section 11.9.1) to free pyridoxal for uptake into tissues.

2.3.2 *The Effect of Temperature*

Chemical reactions proceed faster at higher temperatures for two reasons:

- Molecules move faster at higher temperatures and hence have a greater chance of colliding to undergo reaction.
- At a higher temperature it is easier for electrons to gain activation energy and hence to be excited into unstable orbitals to undergo reaction.

With enzyme-catalyzed reactions, although the rate at which the reaction comes to equilibrium increases with temperature, there is a second effect of temperature; denaturation of the enzyme protein (Section 4.4.2.3), leading to irreversible loss of activity. As the temperature increases, so does the movement of parts of the protein molecule relative to each other, leading to disruption of the hydrogen bonds that maintain the folded structure of the protein. When this happens, the protein chain unfolds, and the active site is lost. As the temperature increases further, the denatured protein becomes insoluble and precipitates out of solution.

Temperature thus has two opposing effects on enzyme activity (Figure 2.8). At relatively low temperatures (up to about 50–55°C), increasing temperature results in an increase in the rate of reaction. However, as the temperature increases further, denaturation

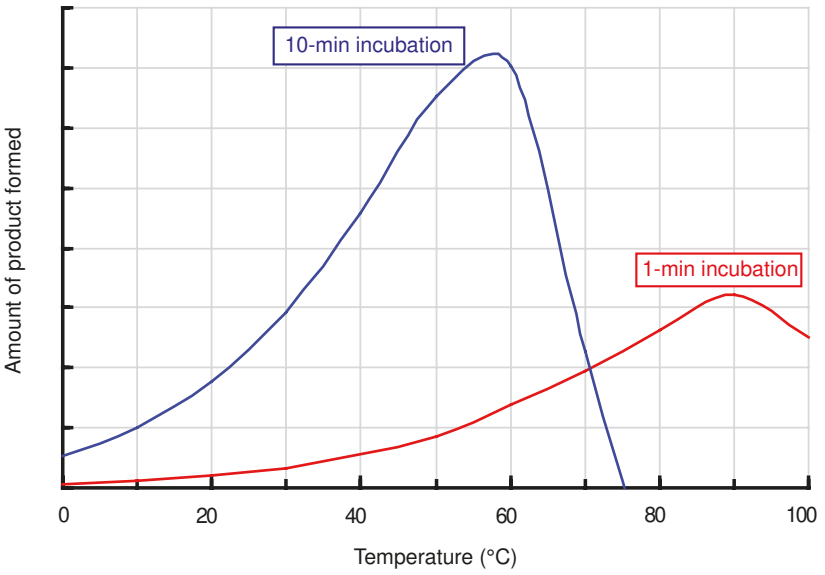

Figure 2.8 The temperature dependence of enzyme activity. In a short incubation (1 minute), the enzyme may have an optimum temperature as high as 90°C, but in longer incubations this falls because of denaturation of the enzyme; therefore in a 10-minute incubation, the optimum temperature is about 55°C.

of the enzyme protein becomes increasingly important, resulting in a rapid fall in activity at higher temperatures. The rate of increase in the rate of reaction with increasing temperature depends on the activation energy of the reaction being catalyzed; the rate of decrease in activity at higher temperatures is a characteristic of the enzyme itself.

The apparent temperature optimum of an enzyme-catalyzed reaction depends on the time for which the enzyme is incubated. During a short incubation (e.g. 1 minute), there is negligible denaturation, and so the apparent optimum temperature is relatively high, while during a longer incubation denaturation is important, and so the apparent optimum temperature is lower.

The effect of temperature is not normally physiologically important, since body temperature is maintained close to 37°C. However, some of the effects of fever (when body temperature may rise to 40°C) or hypothermia may be due to changes in the rates of enzyme-catalyzed reactions. Because different enzymes respond differently to changes in temperature, there may be a loss of the normal integration between different reactions and metabolic pathways.

2.3.3 *The Effect of Substrate Concentration*

In a simple chemical reaction involving a single substrate, the rate at which a product is formed increases linearly as the concentration of the substrate increases. When more substrate is available, more will undergo reaction.

With enzyme-catalyzed reactions, the change in the rate of formation of product with increasing concentration of substrate is not linear, but hyperbolic (Figure 2.9). At relatively low concentrations of substrate (region A in Figure 2.9), the catalytic site of the enzyme will