38 The production of energy in the electron transfer chain

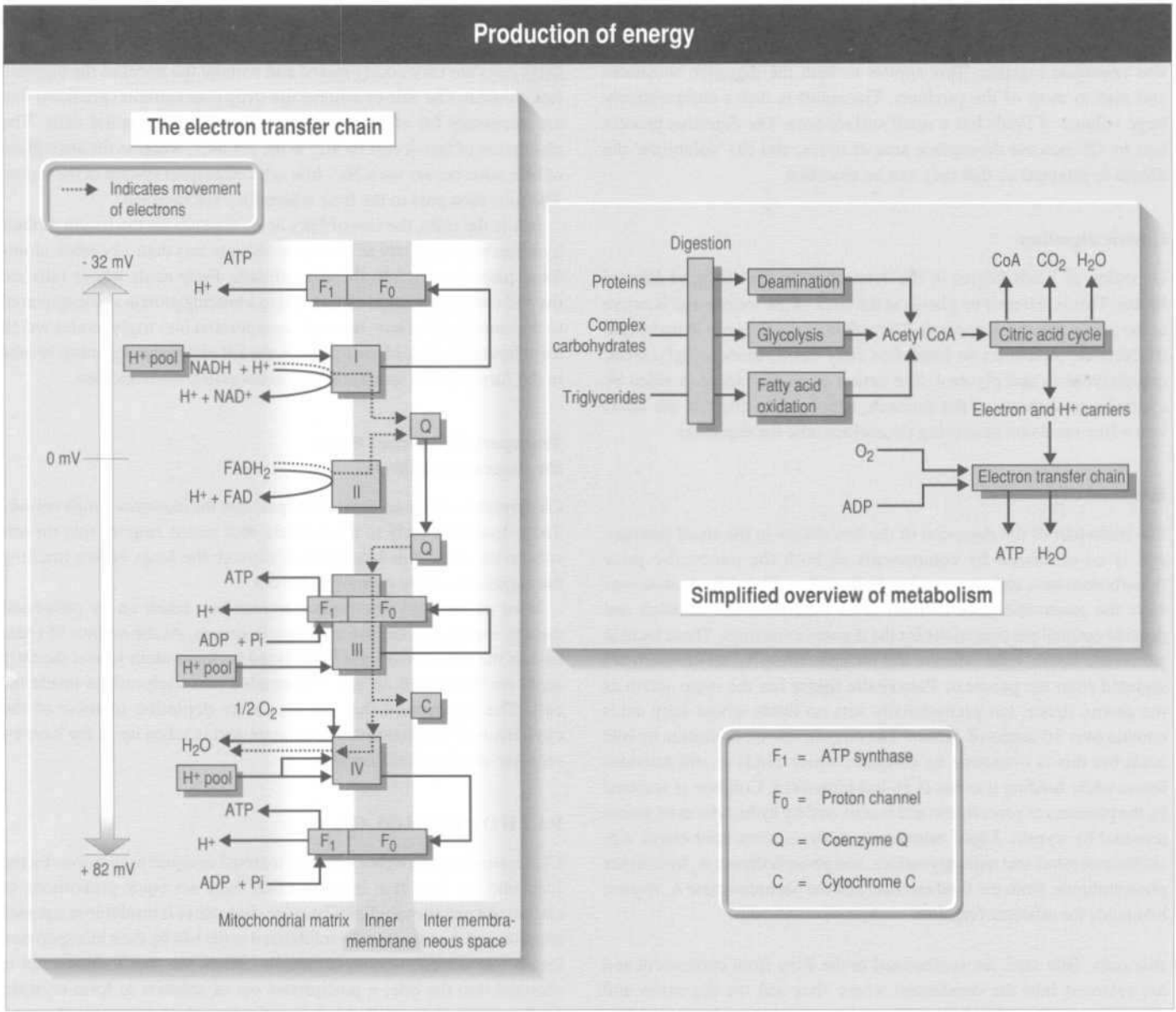

Fig. 38.1

INTRODUCTION

When foodstuffs are digested they are broken down into units that are less complex than their parent compounds. Polysaccharides are broken down into monosaccharides such as glucose, proteins into amino acids and fats into glycerol and fatty acids. Digestion can be thought of as the first stage of energy generation. As this is achieved, the potential energy that is 'contained' in the food molecules is transferred into a form which can be used by the body to fuel its various energy-requiring activities.

COUPLING OF REACTIONS TO THE HYDROLYSIS OF ATP

This principle of coupling reactions is used extensively in biological processes, and one of the main forms of energy currency is **ATP**. The $\Delta G^{\circ\prime}$ for the hydrolysis of ATP is –37 kJ/mol and the products of the reaction are ADP and Pi (see below). Under the conditions that exist intracellularly, the actual change in free energy that accompanies the hydrolysis of ATP is closer to –60 kJ/mol.

$ATP^{4-} + H_2O \rightarrow ADP^{3-} + Pi^{2-} + H^+$

The free energy that is released by the hydrolysis of ATP is used to fuel four types of activities in the body: (i) synthesis of macromolecules; (ii) active transport of ions; (iii) thermogenesis; and (iv) contraction of muscles. Hydrolysis of other phosphorylated nucleotides is also used to provide energy for endergonic reactions, but not to the extent that ATP is used. For example, cytosine triphosphate is hydrolysed to fuel synthesis of phospholipids.

PHOSPHATE TRANSFER POTENTIAL

The ability of a molecule to release free energy with the hydrolytic removal of a phosphate group is known as its **phosphate transfer potential**. Of the phosphorylated molecules involved in metabolism the phosphate transfer potential of ATP occupies a relatively intermediate position. Hydrolysis of the phosphate group of phosphoenolpyruvate has a $\Delta G^{0'}$ of –62 kJ/mol, while that of glucose-1-phosphate is only –14 kJ/mol. It follows, therefore, that hydrolysis of phosphoenolpyruvate can provide enough free energy to phosphorylate ADP to form ATP, while hydrolysis of glucose-1-phosphate cannot. The formation of ATP from ADP is important as there is only a limited quantity of the nucleotide in the body and almost all the cells in the body can phosphorylate ADP.

THE ELECTRON TRANSFER CHAIN

It is the controlled metabolism of digested food molecules that is responsible for the synthesis of ATP. This involves the oxidation of the digested products to CO_2 and H_2O, and involves a large number of reaction-specific enzymes. The oxidative reactions of metabolism all involve the transfer of two molecules of hydrogen (H^+) and two electrons (e^-) from one metabolite to another. The metabolite that loses the two H^+ and the two e^- is then said to be oxidized while the receiving metabolite is said to be reduced and the two make up a redox couple. The principles of redox potential and redox coupling have been discussed in Chapter 30.

In metabolism, when two H^+ and two e^- are removed from a substrate by a dehydrogenase enzyme, they are passed onto a coenzyme. There are three coenzymes that are involved in oxidative processes: **nicotinamide adenine dinucleotide (NAD^+)** and **flavine adenine dinucleotide (FAD^+)** which are involved in the synthesis of ATP, and **nicotinamide adenine dinucleotide phosphate ($NADP^+$)**, which is involved in the synthesis of macromolecules.

$$NAD(P)^+ + 2H^+ + 2e^- \rightarrow NAD(P)H + H^+$$
$$FAD + 2H^+ + 2e^- \rightarrow FADH_2$$

NAD^+ and $NADP^+$ are able to move freely between different dehydrogenase enzymes, but FAD is attached covalently to succinate dehydrogenase. As the coenzymes are reduced, they are effectively absorbing the potential energy which was contained in the food molecules, because the reoxidation of the coenzymes NADH and $FADH_2$ fuels the synthesis of ATP.

The reduced coenzymes transfer their electrons into the electron transfer chain (see Fig. 38.1). This is a series of proteins on the inner mitochondrial membrane organized in order of progressively increasing redox potential. NADH can reduce (and so transfer its electons to) the first complex of the chain, while $FADH_2$ can only reduce the second complex of the chain. The driving force generated by the increasing redox potentials of adjacent components of the chain carries electrons through the chain until, finally, complex IV somehow accumulates four e^- and reduces O_2, which then combines with H^+ to form H_2O. As the electons move down the redox potential gradient, energy is released and at three sites along the chain (complexes I, III and IV) the energy released is sufficient to pump H^+ across the inner mitochondrial membrane from the matrix to the intermembraneous space. The outer surface of the inner membrane subsequently becomes 140 mV more positive than the inner surface of the membrane and the intermembraneous space becomes 1.4 pH units lower than the matrix. As a result, a proton motive force of approximately 200 mV develops across the inner mitochondrial membrane. This membrane is, however, impervious to H^+ and the only way for the H^+ to cross is via a proton channel (F_0) which is coupled to ATP synthase (F_1). The ATP synthase enzyme is activated by the passage of H^+ and catalyses the phosphorylation of ADP to ATP with the passage of three protons through the channel. This method for the generation of ATP from ADP and P_i is known as **oxidative phosphorylation.** Getting ATP out of the mitochondria and ADP into the mitochondria costs one H^+, and so it takes four H^+ to produce one ATP *outside* the mitochondria. $FADH_2$ can only reduce complex II, so its reoxidation yields only two molecules of ATP while that of NADH yields about three.

Complex I is composed of a NADH dehydrogenase with a flavin mononucleotide cofactor and various Fe–S proteins. **Complex II** is composed of various FADH dehydrogenases and Fe–S proteins. **Coenzyme Q** (ubiquinone) shuttles e^- from I and II to III. **Complex III** is composed of cytochromes b and c_1. **Cytochrome c** shuttles e^- from complexes III to IV. **Complex IV** is composed of cytochromes a and a_3.

39 Glycolysis and gluconeogenesis

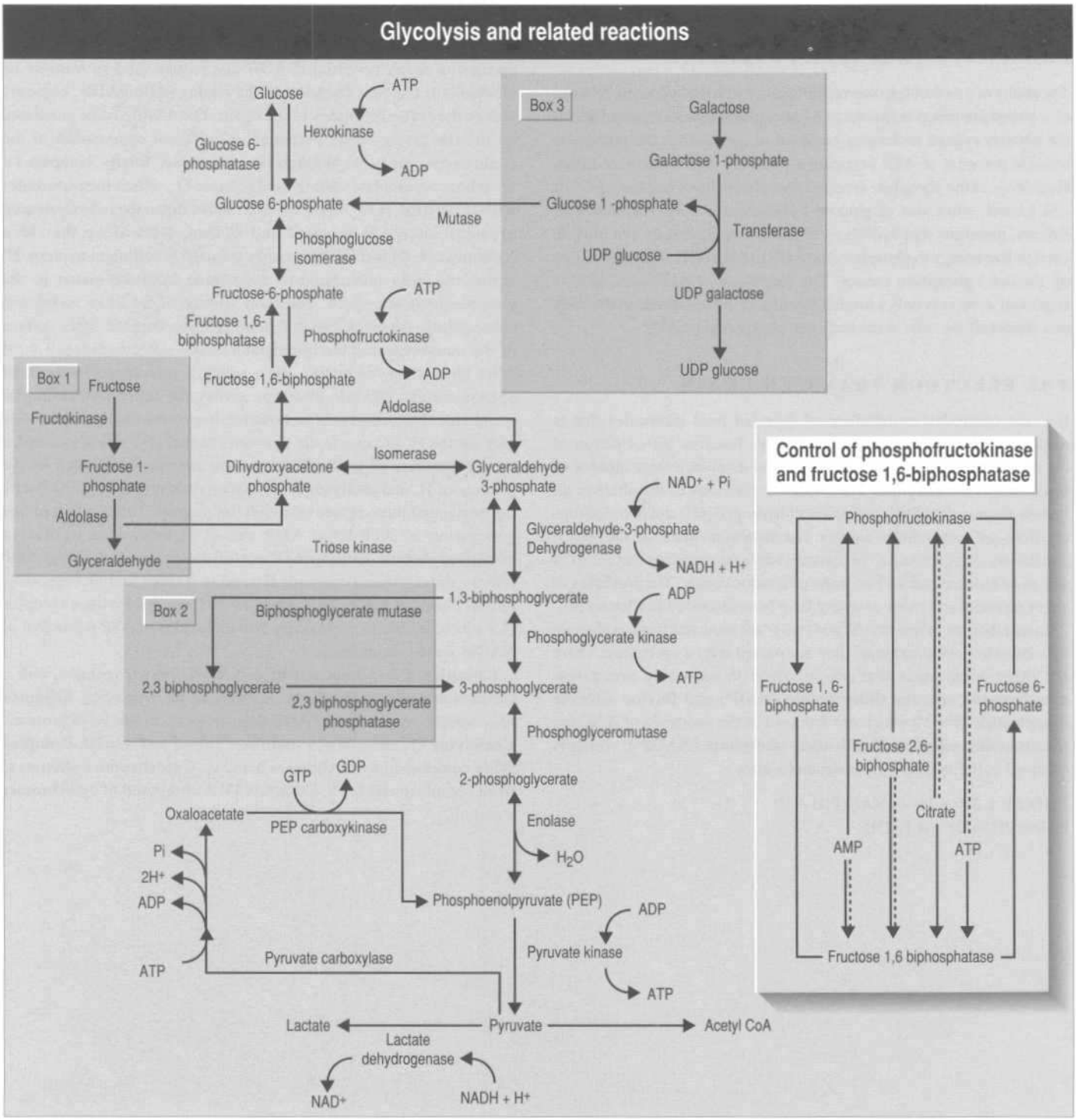

Fig. 39.1

GLYCOLYSIS

Glycolysis, which occurs in the cytosol of the cell, can be divided into two parts.

1 In the first part, the six-carbon monosaccharide is phosphorylated twice and glucopyranose is converted into fructofuranose. The addition of the phosphate groups uses two molecules of ATP. This phosphorylation prevents the sugar from leaving the cell and activates the molecule for later oxidative energy-producing reactions.

2 The initial reaction of the second part of glycolysis splits the six-carbon sugar into two triose phosphate sugars. At equilibrium, 96% of these are in the form of dihydroxyacetone phosphate. This is quickly converted into its isomer, glyceraldehyde-3-phosphate, which is oxidized to 1,3-biphosphoglycerate. As a result, the second half of the pathway is 'repeated' once for each molecule of glucose metabolized.

Substrate level phosphorylation

There are two reactions in glycolysis when ADP is phosphorylated to ATP: (i) the conversion of 1,3-bisphosphoglycerate to 3-phosphoglycerate; and (ii) the conversion of phosphoenolpyruvate to pyruvate.

The two reactions shown above phosphorylate ADP by **substrate level phosphorylation**, which is the formation of ATP by phosphate group transfer from a substrate. The first of these molecules, 1,3-biphosphoglycerate, is formed by the coupling of an endergonic reaction (the phosphorylation of a carboxylate) to an exergonic reaction (the oxidation of an aldehyde — in this case glyceraldehyde-3-phosphate). The second of the 'high-energy' molecules, phosphoenolpyruvate, generates sufficient free energy to phosphorylate ADP by its conversion to an intermediate, enolpyruvate, which can then form pyruvate. The second half of glycolysis will therefore produce four ATP, while the first half metabolizes two ATP.

In summary:

$$\text{glucose} + 2\text{Pi} + 2\text{ADP} + 2\text{NAD}^+ \rightarrow 2\text{pyruvate} + 2\text{H}_2\text{O} + 2\text{ATP} + 2\text{NADH} + 2\text{H}^+$$

There are a couple of points that should be noted about glycolysis.

1 All the reactions of glycolysis discussed above are **reversible** apart from three: those catalysed by hexokinase, by phosphofructokinase and by pyruvate kinase.

2 No O_2 is necessary for the glycolysis to function. This means that in tissues where O_2 is low (e.g. active muscles), poorly supplied (e.g. cornea) or where oxidative metabolism cannot occur (e.g. erythrocytes have no mitochondria), ATP can still be produced from energy sources. In anaerobic respiration, i.e. without O_2, pyruvate is reduced to lactate. This reaction oxidizes NADH, and the coenzyme can then return to the reaction catalysed by glyceraldehyde-3-phosphate dehydrogenase where it is reduced.

Integration of galactose and fructose into glycolysis

Galactose and fructose are also final products of the digestion of carbohydrates and are integrated into glycolysis (see Fig. 39.1, Boxes 1 and 3). Fructose can also be converted to fructose-6-phosphate by hexokinase, but the enzyme's affinity for fructose is much lower than for glucose.

GLUCONEOGENESIS

Under homeostatic conditions, enough carbohydrate is ingested in the diet to supply organs such as the brain, and cells such as the erythrocyte, which require glucose as their primary fuel. Under conditions of deficient dietary intake, glucose can be formed from non-carbohydrate precursors. This pathway is called gluconeogenesis and runs in the opposite direction to glycolysis (i.e. from pyruvate to glucose).

Gluconeogenesis occurs mainly in the liver, but also to a lesser extent in the kidney cortex. Some amino acids can enter this pathway through their conversion to either **oxaloacetate** or **pyruvate,** depending on their initial structure. Of the products of the digestion of triglycerides, only **glycerol** can enter gluconeogenesis through its conversion to dihydroxyacetone phosphate and subsequently to glucose. Lactate is also used in gluconeogenesis through its conversion to pyruvate.

Gluconeogenesis pathway

Although glucose is formed from pyruvate, gluconeogenesis is not simply a reversal of the reactions of glycolysis. Three of the reactions of glycolysis are irreversible and have to be bypassed or catalysed by different enzymes. They are the reactions catalysed by: (i) **hexokinase**; (ii) **phosphofructo-kinase**; and (iii) **pyruvate kinase**.

The overall change in free energy for the gluconeogenic pathway is positive and four ATP and two GTP are metabolized to fuel the pathway.

The reaction catalysed by pyruvate carboxylase occurs in the mitochondiral matrix. The other reactions in the gluconeogenic pathway occur in the cytosol. Oxaloacetate has no carrier through which it can leave the mitochondria and has to be reduced to malate by a NADH-linked dehydrogenase. Malate does have a specific carrier and leaves the mitochondria for the cytosol where it is reoxidized to oxaloacetate which can return to the gluconeogenic pathway. To convert two molecules of pyruvate to one molecule of glucose, four ATP and two GTP are needed.

REGULATORY ENZYMES OF GLYCOLYSIS AND GLUCONEOGENESIS

Glycolysis is one of the central pathways of metabolism as it provides substrates for both energy production and biosyntheses. It is therefore vital that both glycolysis and gluconeogenesis are controlled to prevent wasting of fuel molecules or excessive production of macromolecule precursors.

The main step that controls glycolysis and gluconeogenesis is shown in Fig. 39.1. ATP acts at regulatory sites via an allosteric mechanism, thereby preventing excessive energy production when the ATP/AMP ratio is high. Citrate enchances the action of ATP, thus preventing the over-formation of carbon skeletons for biosyntheses, whose levels are reflected by citrate levels. Fructose-2,6-bisphosphate acts to stimulate glycolysis when levels of fructose-6-phosphate rise.

There are two other enzymes that play a smaller part in the control of glycolysis: hexokinase and pyruvate kinase. Hexokinase is inhibited by glucose-6-phosphate, but when glucose levels rise over a critical level in the liver, the same reaction can be catalysed by glucokinase, which has a higher K_m for glucose than hexokinase. The product of the reaction catalysed by pyruvate kinase is pyruvate, which can be used to build molecules or produce energy by its conversion to acetyl CoA. Pyruvate kinase exists in three forms: L (mostly in the liver), M (in muscle) and A (in other tissues). The L form is allosterically inhibited by ATP, thus slowing the production of energy when levels of ATP are high.

40 The citric acid cycle and mitochondrial carriers

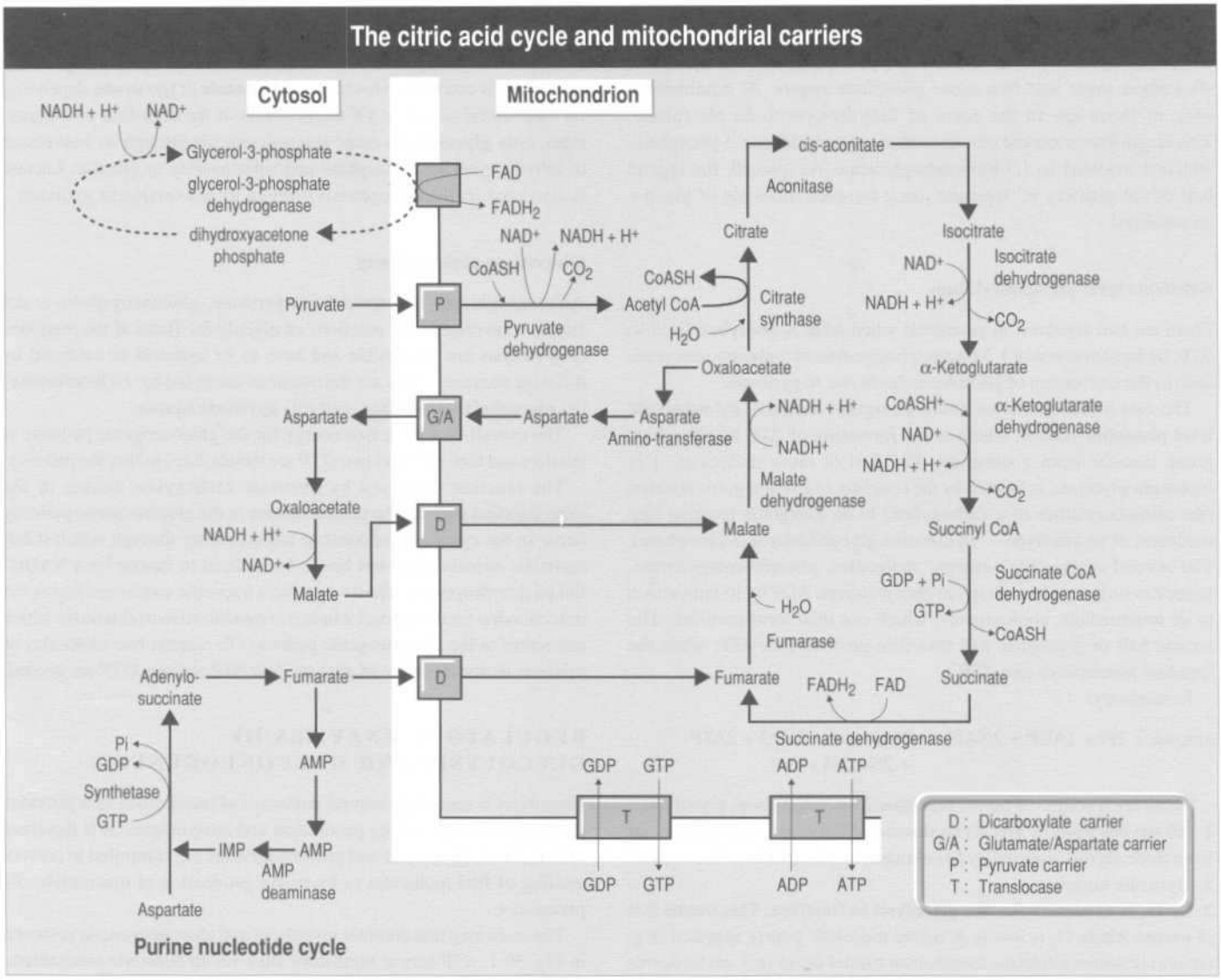

Fig. 40.1

INTRODUCTION

The citric acid cycle is a series of reactions in the matrix of the mitochondria that completes the oxidation of carbohydrates, fatty acids and amino acid skeletons, and in so doing reduces electron carriers so that they can fuel the redox chain. Pyruvate in the cytosol is shuttled into the matrix of the mitochondria where it is converted into acetyl CoA. Acetyl CoA then enters the citric acid cycle by condensing with oxaloacetate to form citrate. The subsequent reactions of the cycle are a series of modifications to the carbon skeleton, which results in the regeneration of oxaloacetate. The overall reaction achieved by one round of the citric acid cycle is:

$$3NAD^+ + FAD + \text{acetyl CoA} + GDP + 2H_2O + Pi \rightarrow 3NADH + 2CO_2 + FADH_2 + CoA + GTP + 2H^+$$

Products and stoichiometry

There are a number of important points that need to be noted about the products and stoichiometry of the citric acid cycle.

1 Within one cycle there are four dehydrogenation reactions, which result in the reduction of three NAD^+ and one FAD. These hydrogen carriers exist in only limited quantities in the mitochondria and need to be reoxidized in the eletron transport chain for the citric acid cycle to continue. Consequently, the **citric acid cycle can only operate under aerobic conditions.**

2 Two carbon atoms in the form of acetyl CoA enter the cycle and two different carbon atoms leave the cycle in the form of CO_2. The decarboxylation reactions are those which are catalysed by α-ketoglutarate dehydrogenase and by isocitrate dehydrogenase.

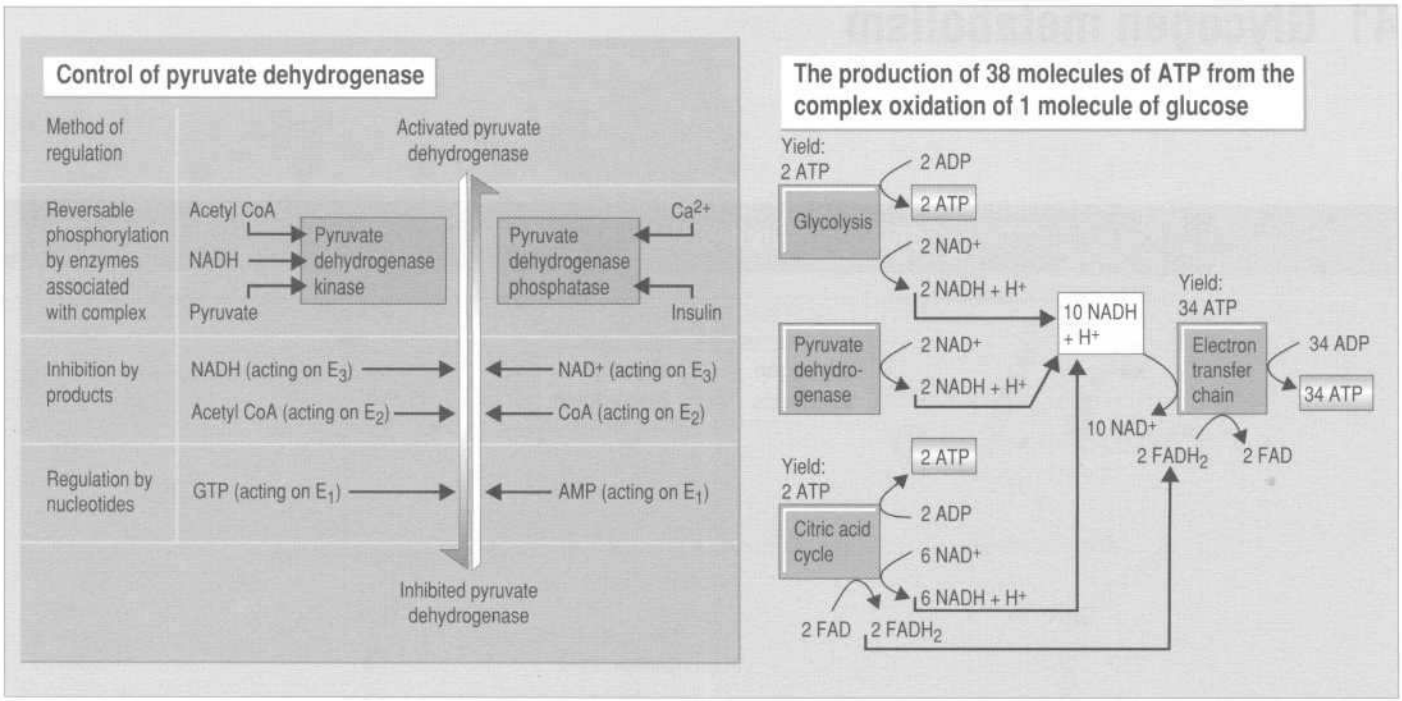

Fig. 40.2

3 The two reactions catalysed by citrate synthase and by fumarase each consume one molecule of H_2O.

4 A molecule of GTP is formed from a molecule of GDP and Pi in the reaction catalysed by succinyl CoA synthetase.

ENZYMES OF THE CITRIC ACID CYCLE AND THEIR CONTROL

The citric acid cycle is vital in the process of **energy generation**, but is also intricately involved in other fundamental metabolic processes such as **gluconeogenesis**, **amino acid metabolism** and **lipogenesis**. It is therefore important that the cycle be precisely controlled so as not to provide an excess of biosynthetic precursors nor to utilize more fuel than is absolutely necessary. There are four main enzymes by which the cycle is controlled: (i) the pyruvate dehydrogenase complex; (ii) citrate synthase; (iii) isocitrate dehydrogenase; and (iv) α-ketoglutarate dehydrogenase.

1 The irreversible conversion of pyruvate to acetyl CoA by the pyruvate dehydrogenase complex is not part of the citric acid cycle but is essential for its efficient functioning. The complex is made up of three enzymes:

(a) E_1 — pyruvate dehydrogenase oxidatively decarboxylates pyruvate;

(b) E_2 — dihydrolipoyl transacetylase transfers the acetyl residue to acetyl CoA; and

(c) E_3 — dihydrolipoyl dehydrogenase oxidatively regenerates a lipoamide.

2 Citrate synthase controls the entry of acetyl CoA into the cycle, and is inhibited allosterically by ATP.

3 Isocitrate dehydrogenase catalyses the conversion of isocitrate to α-ketoglutarate. The enzyme is allosterically stimulated by ADP and is competitively inhibited by NADH which can displace NAD^+.

4 α-Ketoglutarate dehydrogenase catalyses the conversion of α-ketoglutarate to succinyl CoA. It exists as a three-enzyme complex similar to pyruvate dehydrogenase, and shares many of its properties, i.e. its activity is regulated by its products and also by levels of phosphorylated nucleotides in the cell.

THE GLYCEROL-3-PHOSPHATE SHUNT AND THE MALATE/ASPARTATE SHUNT

The glycolytic reaction that oxidizes glyceraldehyde-3-phosphate to 1,3-biphosphoglycerate results in the reduction of NAD^+ to NADH. Quantities of this hydrogen carrier are limited in the cytosol and therefore NADH needs to be reoxidized for glycolysis to be maintained.

The oxidation of NADH in the electron chain occurs in the mitochondria, but the carrier cannot pass across the inner mitochondrial membrane. Thus, two systems have developed so that the cytosolic NADH can be reoxidized in the mitochondria.

1 In the **glycerol-3-phosphate shunt**, a cytosolic dehydrogenase oxidizes NADH as dihydroxyacetone phosphate is reduced to glycerol-3-phosphate. A mitochondrial dehydrogenase operates in the reverse direction, but reduces FAD instead of NAD^+.

2 In the **malate/aspartate shunt** NADH is oxidized by the conversion of oxaloacetate to malate. Malate crosses the inner mitochondrial membrane where it is oxidized to oxaloacetate. Oxaloacetate is then transaminated to form aspartate, which moves back into the cytosol.

PURINE NUCLEOTIDE CYCLE

The purine nucleotide cycle is used to supply fumarate to the citric acid cycle when the availability of acetyl CoA exceeds the availability of oxaloacetate. This imbalance commonly occurs in skeletal muscle during exercise, and the nucleotide cycle is essential for maintaining the efficient functioning of the citric acid cycle.

41 Glycogen metabolism

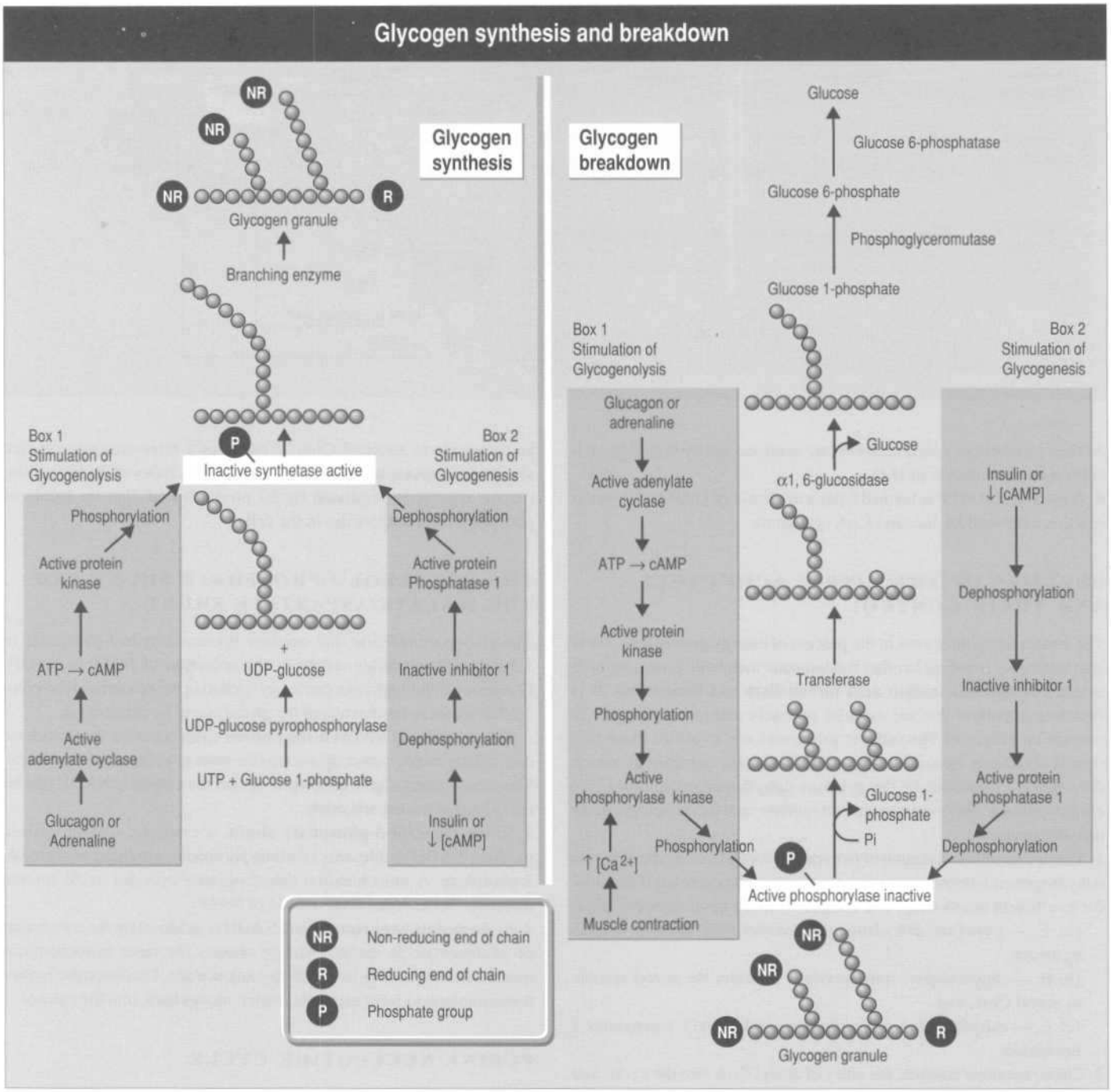

Fig. 41.1

INTRODUCTION

Glycogen is the form in which glucose is stored. It is composed of many glucose residues joined by α-1,4-glycosidic bonds (see Chapter 36) with α-1,6-linked branch points. Glycogen exists in the cytosol of the cell as granules. These granules range from 10 to 40 nm in size and contain the enzymes that are involved in glycogen metabolism. The main sources of glycogen in the body are the liver (100 g after a meal) and skeletal muscle (up to 300 g), although there are also substantial amounts in cells of the intestines, kidneys and brain.

GLYCOGENOLYSIS

1 Phosphorylase catalyses the removal of a glucose residue by the phosphorylysis of an α-1,4-glycosidic bond. Phosphorylase requires the presence of the coenzyme pyridoxal phosphate, which acts as an acid–base catalyst, to work.
2 Phosphorylase can only break α-1,4-glycosidic bonds up to four residues away from a branch point. The three residues leading to the branch are then transferred to another part of the glycogen molecule by transferase.
3 The α-1,6-glycosidic bond forming the branch point is cleaved by α-1,6-glucosidase.
4 Phosphoglucomutase converts glucose-1-phosphate into glucose-6-phosphate, via a glucose-1,6-biphosphate intermediate, which can then enter glycolysis. In the liver, intestines and kidney, glucose-6-phosphatase can remove a phosphate group from glucose-6-phosphate to form glucose, which can then pass out of the cell into the bloodstream. In brain and skeletal muscle tissue, glucose-6-phosphatase is not present and glucose-6-phosphate cannot pass out of the cells.

GLYCOGEN SYNTHESIS

The synthesis of glycogen (glycogenesis) follows a different pathway from that of glycogenolysis.
1 To be added onto a chain in a glycogen molecule, the glucose residue must be in the 'activated' form of uridine diphosphate (UDP)–glucose.

glucose-1-phosphate + UTP → UDP–glucose + pyrophosphate

This is catalysed by UDP–glucose pyrophosphorylase. The reaction is driven towards the formation of UDP–glucose by the hydrolysis of pyrophosphate to orthophosphate by the enzyme pyrophosphatase.
2 Glycogen synthase catalyses the addition of glucose from UDP–glucose to the non-reducing end of a chain. The α-1,4-glycosidic bond is made with the hydroxyl group on C_4 and UDP is released. Glycogen synthase can only add UDP–glucose to a chain of five or more residues. Chains smaller than five residues are attached to proteins and are called primers.
3 When a chain contains more than 11 residues, a branching enzyme transfers a block of seven to an interior site within the glycogen molecule, to form a new branch. The branch point must be at least four residues away from any pre-existing branch points.

CONTROL OF GLYCOGEN METABOLISM

The synthesis and breakdown of glycogen is one of the major controlling factors that determine the availability of glucose for energy production in the body. When there is an excess of carbohydrates in the body, for example after a meal, it is important that glucose can be stored for later use. In periods such as these, **insulin** is secreted by the endocrine glands of the pancreas and causes glycogenesis.

On the other hand, if a supply of glucose is needed which is not readily available from the diet, glycogen can be broken down to give **glucose-6-phosphate**, which can be integrated directly into glycolysis or glucose, which can pass into the circulation and be taken up by organs that need it. When concentrations of glucose are low in the blood, **glucagon** is secreted from the endocrine cells of the pancreas, and in periods of stress or excitement **adrenaline** is secreted from the adrenal glands. Both glucagon and adrenaline stimulate glycogenolysis (Note: there are no glucagon receptors in muscle tissue.)

Both glucagon and adrenaline work via a **cascade mechanism** whereby the initial signal by the hormone is amplified manyfold while being relayed to the enzymes of glycogen metabolism. The hormonal signals are dually effective because reversible phosphorylation is used to control the activity of the enzymes. So, for example, when glucose is needed, the enzymes catalysing glycogenolysis are phosphorylated and activated while the enzymes catalysing glycogenesis are phosphorylated and inactivated.

Control of enzyme activity by phosphorylation

The enzyme phosphorylase in skeletal muscle can exist in two forms depending on the phosphorylation of a specific serine residue: **phosphorylase a** and **phosphorylase b**. Phosphorylase a is phosphorylated and is usually always active while phosphorylase b is dephosphorylated and is usually inactive. The two forms are interconvertible by the actions of phosphorylase kinase and phosphatase 1, which also control the activity of glycogen synthase. In turn, the activity of phosphorylase kinase and phosphatase 1 are also controlled by reversible phosphorylation. The activity of phosphatase 1 is modulated by a protein called inhibitor 1. When inhibitor 1 is phosphorylated (e.g. by the action of cAMP-activated protein kinase) it inhibits the activity of phosphatase 1. However, when levels of cAMP fall, or when insulin acts on the cell, inhibitor 1 becomes dephosphorylated and the phosphatase 1 activity resumes. When glucose levels are high, the activity of phosphorylase falls before the activity of synthase rises. This is explained below.
1 Glucose enters the cell and binds to the phosphorylase component of a phosphorylase a: phosphatase 1 complex.
2 Phosphatase 1 dephosphorylates phosphorylase a to yield inactive phosphorylase b.
3 Phosphorylase b dissociates from phosphatase 1.
4 Phosphatase 1 then dephosphorylates glycogen synthase b to convert it into the active glycogen synthase a form.

PATHOPHYSIOLOGY

Von Gierke's disease is a deficiency of the glucose-6-phosphatase enzyme in liver and kidney tissues. The resulting increased levels of glycogen in the affected organs causes enlargement of the organs and hypoglycaemia. **Cori's disease** is a deficiency of the debranching enzyme in liver and muscle tissues. Increased levels of glycogen with short branches result and cause similar symptoms to type 1 disease. **McArdle's disease** is a deficiency in muscle phosphorylase. Only moderately increased levels of glycogen result in the muscle tissue, but severe cramps develop with exercise due to the lack of glucose-6-phosphate available for oxidation.

42 Lipid metabolism I

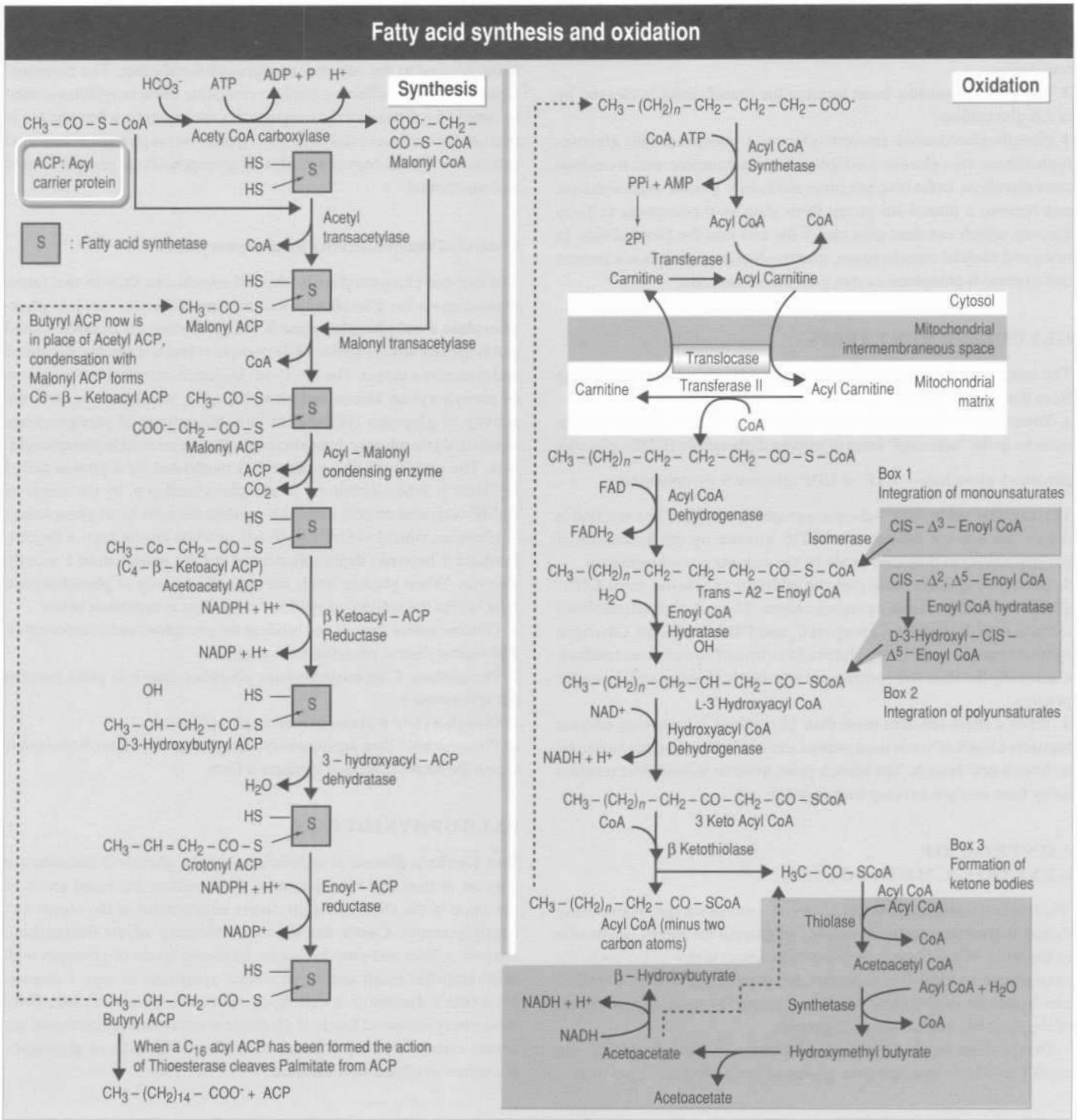

Fig. 42.1

BREAKDOWN OF LIPIDS

Formation of fatty acids and glycerol from triglycerides

The breakdown of lipids begins with the hydrolysis of a cytosolic triglyceride into one molecule of glycerol and three fatty acids. This reaction is catalysed by the enzyme **hormone-sensitive lipase**, which is activated by **glucagon**, **adrenaline**, **noradrenaline** and **adrenocorticotrophin (ACTH)**. These hormones and neurotransmitters act via a cAMP cascade mechanism which leads to the phosphorylation of the enzyme. **Insulin** action results in the dephosphorylation and inactivation of the enzyme.

Glycerol can enter glycolysis by phosphorylation to glycerol-3-phosphate. This is then oxidized to form dihydroxyacetone phosphate.

Fatty acid oxidation

The fatty acids are 'activated' by the formation of a thioester bond between the carboxyl group of the acid to the sulphydryl group of CoA. The reaction is catalysed by acyl CoA synthetase and is driven forward by the hydrolysis of pyrophosphate. The subsequent shuttling of the fatty acid into the mitochondrial matrix and its breakdown (called ß-oxidation) are shown in Fig. 42.1. Carnitine deficiency or a defect in the translocase enzyme impairs energy production from fat oxidation and results in muscle cramps developing with exercise. The overall reaction for the oxidation of a typical fatty acid is shown below:

$$\text{palmitoyl CoA} + 7\text{NAD}^+ + 7\text{FAD} + 7\text{H}_2\text{O} + 7\text{CoA} \rightarrow 8\text{acetyl CoA} + 7\text{NADH} + 7\text{H}^+ + 7\text{FADH}_2$$

The complete oxidation of this fatty acid will thus yield 129 molecules of ATP.

Many fatty acids occur that contain one or more double bonds in the hydrocarbon tail and are said to be **unsaturated**. The enzymes of the ß-oxidation chain are stereospecific and the unsaturated fatty acids have to be modified before they can be fully oxidized. The modification of an acid with only one double bond is shown in Fig. 42.1, Box 1, while those of polyunsaturates are shown in Fig. 42.1, Box 2.

The rate of breakdown is determined by the availability of substrates, the inhibition of carnitine transferase 1 by malonyl CoA and the inhibition of hydroxyacyl CoA dehydrogenase by NADH.

Ketone body formation

When the breakdown of fats exceeds the breakdown of carbohydrates, the supply of acetyl CoA from β-oxidation exceeds the rate that oxaloacetate can be formed. The acetyl CoA then follows a different pathway; that of ketone body formation (Fig. 42.1, Box 3). The ketone bodies, acetoacetate and hydroxybutyrate, diffuse out of the cell and are common fuel molecules in the body, especially in cardiac muscle and the renal cortex where acetyl CoA can be regenerated. The regeneration of acetyl CoA from acetoacetate is catalysed by two enzymes: (i) CoA transferase adds a CoA group; and (ii) thiolase cleaves acetoacetyl CoA, incorporating another CoA, to form two molecules of acetyl CoA.

THE SYNTHESIS OF FATTY ACIDS

There are three major differences between fatty acid synthesis and breakdown:

1 synthesis occurs in the cytosol whereas breakdown occurs in the mitochondria;
2 the enzymes catalysing the breakdown are separate whereas those catalysing the synthesis are joined to form a single polypeptide chain called fatty acid synthase;
3 the reductant used in the synthesis is NADPH whereas the electron donors in the breakdown are FAD and NAD^+.

The principal reactions of fatty acid synthesis are shown in Fig. 42.1 Essentially, the pathway consists of the repetitive addition of a two-carbon unit to the forming fatty acid by the condensation of a three-carbon unit (malonyl CoA) to the end of the fatty acid tail and the release of CO_2.

The committed step of the pathway is the irreversible conversion of acetyl CoA to malonyl CoA. The enzyme catalysing this reaction, acetyl CoA carboxylase, has a prosthetic biotin group attached, which is initially carboxylated by a biotin carboxylase subunit of the enzyme. A transcarboxylase subunit of the enzyme then transfers the CO_2 group to the acetyl CoA.

After the formation of malonyl CoA, the next step in the synthesis pathway is the activation of the substrates by their attachment to the phosphopantetheine group of acyl carrier protein (ACP). The subsequent steps are shown in Fig. 42.1 and the overall reaction for the synthesis of palmitate is as follows:

$$8\text{acetyl CoA} + 14\text{NADPH} + 7\text{ATP} \rightarrow \text{palmitate} + 8\text{CoA} + 6\text{H}_2\text{O} + 14\text{NADP}^+ + 7\text{ADP} + \text{Pi}$$

Fatty acid synthase

This enzyme is dimeric with each subunit composed of three domains.
1 Domain 1: acetyl CoA transferase, malonyl CoA transferase, acylmalonyl condensing enzyme.
2 Domain 2: ACP, β-ketoacyl reductase, hydroxyacyl dehydratase, enoyl reductase.
3 Domain 3: thioesterase.

The dimer is arranged so that the domain 1 of one of the enzymes is facing domains 2 and 3 of the other enzyme. Therefore, by rotating on the phosphopantetheinyl group, the activating reactions can occur on one of the enzymes and the rest of the reactions in the cycle can occur on the opposite enzyme.

The main site of control in fatty acid synthesis is the enzyme acetyl CoA carboxylase. The enzyme's activity is inhibited by high levels of palmitoyl CoA and the actions of glucagon, and is stimulated by high levels of citrate and the actions of insulin.

The assembly of triglycerides: the α-glycerol phosphate pathway

Triglycerides are formed by the esterification of three fatty acyl CoA molecules with one molecule of α-glycerol phosphate.
1 α-Glycerol phosphate can be formed by the phosphorylation of glycerol or by the dehydrogenation of dihydroxy acetone phosphate by NADH.
2 α-Glycerol phosphate combines with two molecules of fatty acyl CoA to form **phosphatidate** and two molecules of CoA.
3 The phosphate group is removed to form a **diglyceride**.
4 The diglyceride combines with a third molecule of fatty acyl CoA to form a **triglyceride** and a molecule of CoA.

43 Lipid metabolism II

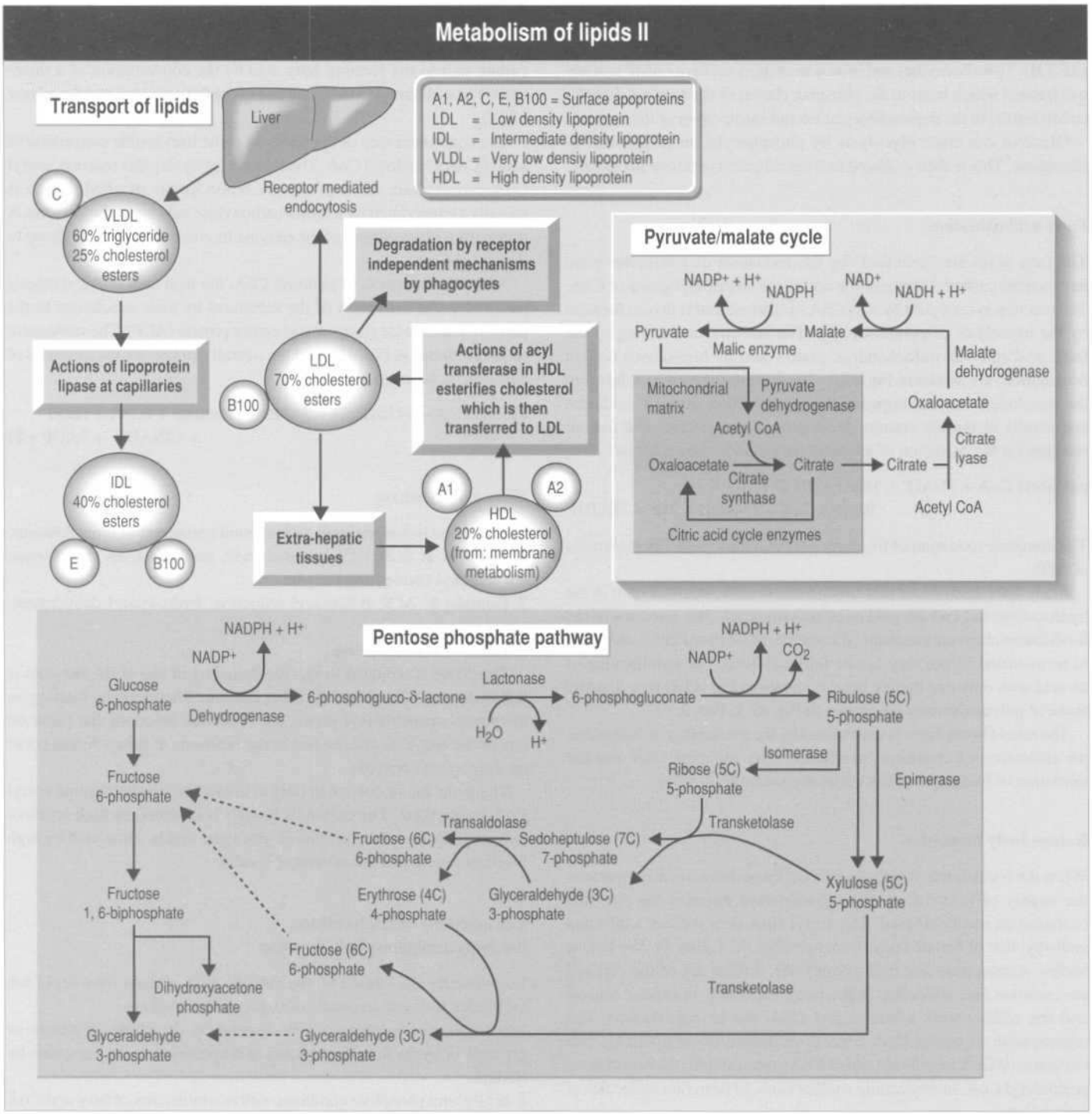

Fig. 43.1

In this chapter, two principles are discussed: (i) the formation of reducing power for biosyntheses in the form of NADPH; and (ii) the carriage of lipids around the body.

PRODUCTION OF NADPH FROM NADP+

NADPH differs from NADH only in that there is a phosphate group

attached to C_2, but their uses in metabolism are clearly differentiated. Both molecules can provide reducing power to fuel reactions by their oxidation to $NADP^+$ and NAD^+, but the reducing power from the oxidation of NADPH is used to fuel biosyntheses while that from NADH is used to fuel the reactions involved in the generation of ATP.

There are two different metabolic pathways that reduce $NADP^+$ to NADPH: (i) the pyruvate malate cycle; and (ii) the pentose phosphate pathway. Approximately 40% of the NADPH needed for fatty acid synthesis is produced by the pyruvate malate cycle (see Fig. 43.1). The remainder is produced by the pentose phosphate pathway.

The pentose phosphate pathway

The pentose phosphate pathway is made up of two component pathways: (i) an oxidative pathway; and (ii) a non-oxidative pathway. These both occur in the cytosol. The oxidative pathway is responsible for the formation of NADPH and involves the irreversible conversion of **glucose-6-phosphate**, through a series of steps, to **ribulose-5-phosphate**. Ribulose-5-phosphate is a precursor for a number of important molecules in the body, which include DNA, RNA, ATP and CoA. If synthesis of these molecules is required in the cell, then ribulose-5-phosphate will be utilized for that purpose. If ribulose-5-phosphate is not required for biosyntheses, the **non-oxidative pathway** is used.

The non-oxidative pathway is a series of reversible carbon skeleton interconversions which result in the formation of two molecules of fructose-6-phosphate and one molecule of glyceraldehyde-3-phosphate from three molecules of ribulose-5-phosphate. The starting substrate of the oxidative pathway and the products of the non-oxidative pathway are all metabolites of the glycolytic pathway, and so the pentose phosphate pathway can act as a shunt off from glycolysis.

The rate-limiting step for the oxidative pathway is the conversion of glucose-6-phosphate to 6-phosphoglucolactone. This reaction is catalysed by the enzyme glucose-6-phosphate dehydrogenase, whose activity is regulated by levels of $NADP^+$. The controlling factors in the non-oxidative pathway are the availabilities of the substrates.

Pathophysiology of the pentose phosphate pathway. A deficiency of the enzyme glucose-6-phosphate dehydrogenase in red blood cells is found in 11% of black Americans. This deficiency leads to decreased levels of NADPH in the red blood cells. NADPH is needed in these cells for the reduction of oxidized glutathione. Glutathione maintains the reduced state of the cell and is also involved in the metabolism of toxins. The deficiency therefore predisposes the cell to damage which might be prevented with sufficient levels of reduced glutathione.

Wernicke–Korsakoff syndrome. The enzyme ketolase, of the non-oxidative pathway, tightly binds a thiamine pyrophosphate prosthetic group. In the Wernicke–Korsakoff syndrome, the prosthetic group is held less tightly than normal and a deficiency of thiamine in the diet (e.g. in alcoholics or malnourishment) precipitates characteristic symptoms which include disorientation and decreased mental function.

CARRIAGE OF LIPIDS AROUND THE BODY

Lipids are transported round the body in structures known as **lipoproteins**. These lipoproteins consist of a **shell** of polar lipids (phospholipids and free cholesterol) and apoproteins and a **core** of non-polar lipids (triglycerides and cholesterol esters). Carriage of the lipids in this way facilitates their transport in an aqueous medium and enables a very specific targeting system for metabolites to occur. Dietary lipids that have been digested and absorbed follow an exogenous pathway involving chylomicrons and chylomicron remnants.

Lipids that have been formed within the body, for example biosynthesis in the liver or membrane turnover, follow an endogenous pathway. This pathway involves four different types of lipoprotein which can be differentiated by density and surface protein markers: (i) very low-density lipoprotein (VLDL); (ii) intermediate-density lipoprotein (IDL); (iii) low-density lipoprotein (LDL); (iv) high-density lipoprotein (HDL).

The passage and interconversions of the lipoproteins are shown in Fig. 43.1. As triglycerides are lost from the core of the lipoproteins, VLDL become IDL, which lose more triglyceride to become LDL. Nascent HDL is released in the liver and acquires further lipid from the mebranes of peripheral cells; its purpose is to transfer cholesterol esters to other lipoproteins for subsequent hepatic metabolism.

Pathophysiology of lipid transport

Familial hypercholesterolaemia (type IIa) is caused by a deficiency of the LDL receptor as a result of a number of types of mutation (e.g. absent receptor; defective ligand binding by receptor; disruption of transport to cell membrane of receptor). Increased blood levels of LDL and cholesterol result and these may be deposited in the walls of arteries leading to premature atheroma.

Familial hyperlipoproteinaemia (type III) is caused by an abnormal apoprotein E and causes increased blood levels of remnant particles (IDL) with raised triglycerides and cholesterol levels.

Familial hypertriglyceridaemia (type IV) has an unknown cause, but results in increased blood levels of VLDL with high triglycerides.

44 Nucleotide synthesis

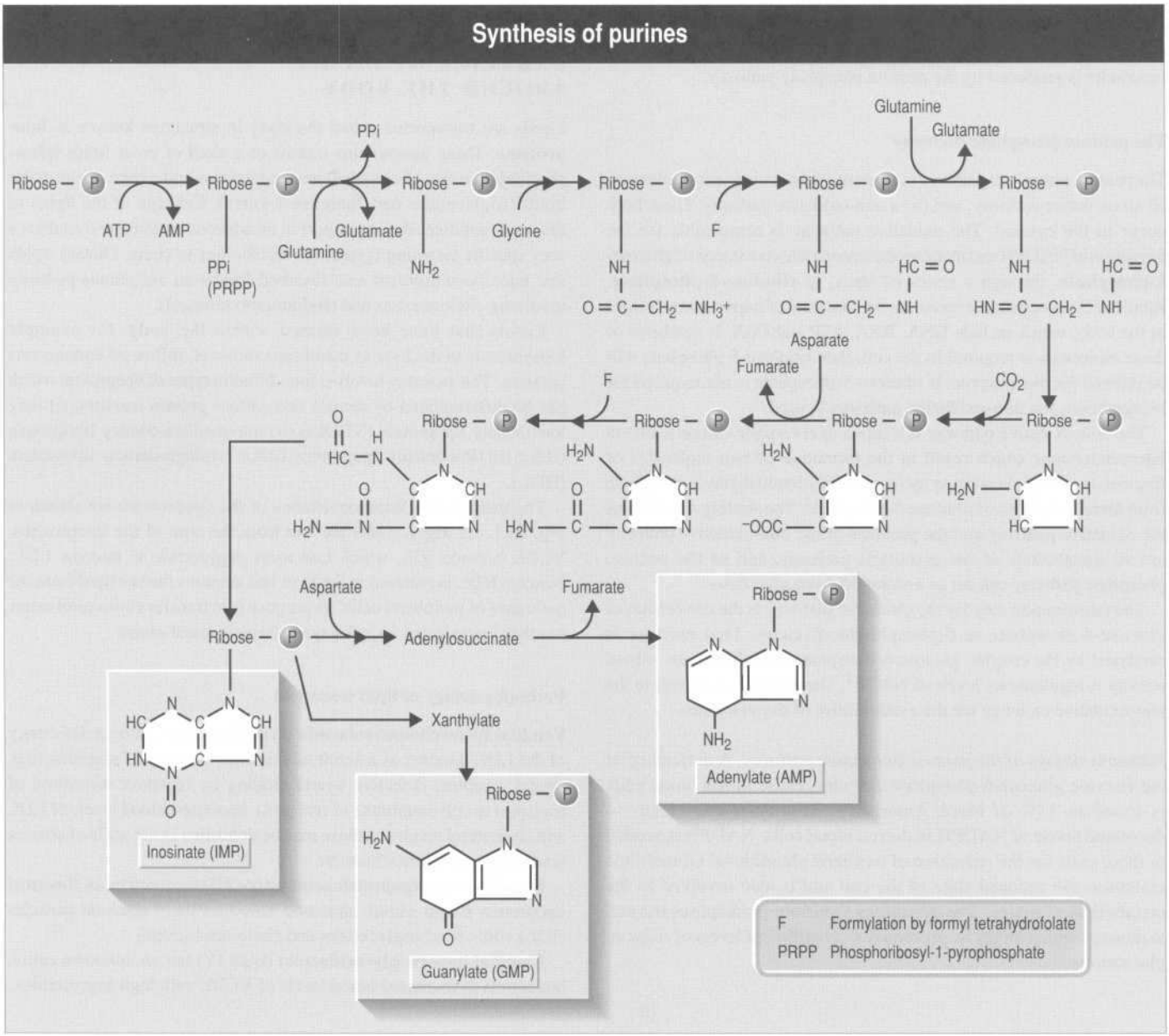

Fig. 44.1

Nucleotides are involved in most of the integral biochemical processes of the cell. To name only a few of their functions, they are involved in energy storage and release (ATP), communication of extracellular signals intracellulary (cAMP) and genetic information processing (DNA and RNA). The structure of nucleotides is described in Chapter 6.

ASSEMBLY OF PURINES

The purine ring is assembled from five precursors: glycine, glutamine, aspartate, CO_2 and formyltetrahydrofolate. The first step in the synthesis is the formation of **5-phosphoribosyl-1-pyrophosphate (PRPP)** from **ribose-5-phosphate** and is catalysed by the enzyme **ribose phosphate pyrophosphokinase.**

The second step is the committed step in the synthesis and is the irreversible formation of **5-phosphoribosylamine** from PRPP. This

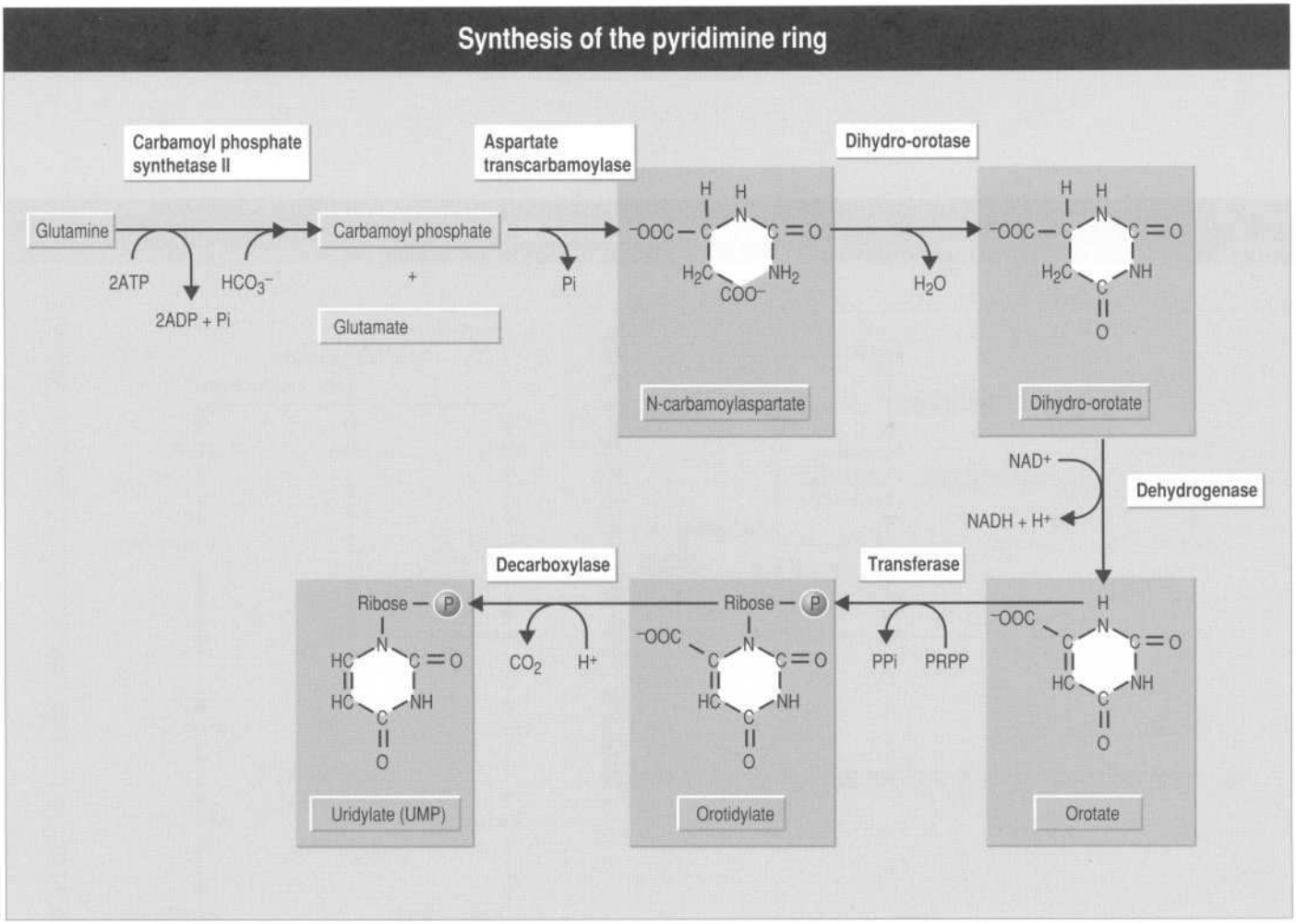

Fig. 44.2

reaction is catalysed by **amidophosphoribosyl transferase** and is driven forward by pyrophosphate hydrolysis.

Control of purine ring synthesis

The synthesis of the purine ring is controlled mainly at four sites:

1 ribose phosphate pyrophosphokinase is inhibited by AMP, inosine monophosphate (IMP) and GMP;

2 amidophosphoribosyl transferase is inhibited by AMP, IMP and GMP;

3 the enzyme converting IMP to adenylosuccinate is inhibited by AMP; and

4 the enzyme converting IMP to xanthylate is inhibited by IMP.

Salvage reactions

A common feature of purine nucleotide synthesis is **salvage reactions.** The breakdown of nucleotides forms free purine bases, and these can be salvaged by their direct addition to PRPP. The enzymes catalysing these reactions are adenine phosphoribosyl transferase (which forms AMP) and hypoxanthine–guanine phosphoribosyl transferase (HGPRTase) (which forms IMP and GMP).

ASSEMBLY OF PYRIMIDINES

The synthesis of pyrimidines differs from the synthesis of purines in that the pyrimidine ring is synthesized first and is then attached to ribose phosphate. The three enzymes that catalyse the conversion of glutamine to dihydroorotate (synthetase, transcarbamoylase and dihydroorotase) are domains on one protein. The two enzymes that catalyse the conversion of orotate to uridine monophosphate (UMP) (transferase and decarboxylase) are domains on another protein.

The committed step in this synthetic pathway is the formation of carbamoylaspartate from aspartate and carbamoyl phosphate.

> *Note*: the term 'committed step' means the first irreversible step of the pathway, and is often the step at which the pathway is controlled.

The enzyme that catalyses this reaction, aspartate transcarbamoylase, is inhibited by cytidine triphosphate (CTP). CTP is formed from UTP and is one of the end products of pyrimidine nucleotide synthesis.

45 Breakdown of nucleotides

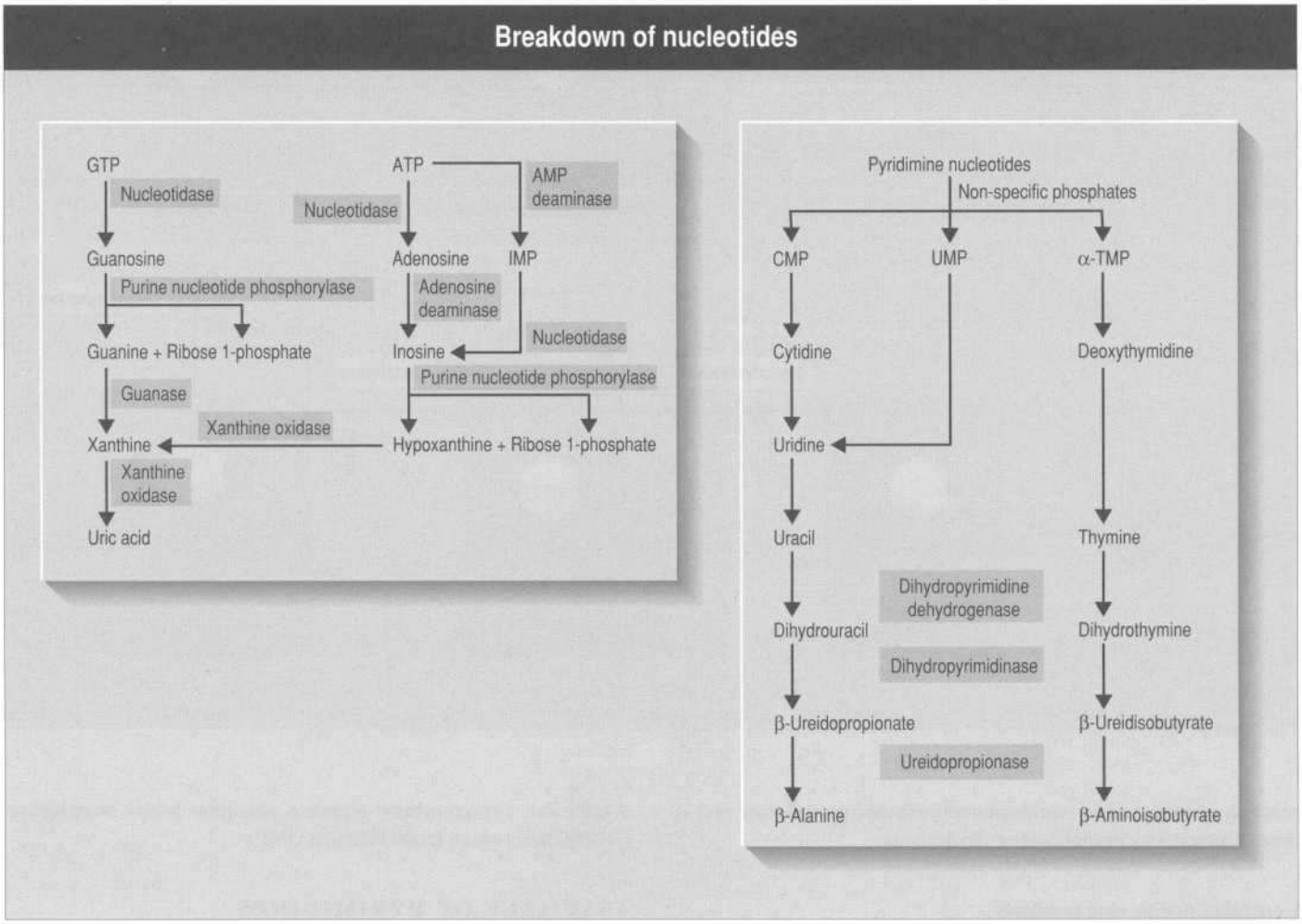

Fig. 45.1

The end product for the degradation of purines is uric acid which is excreted in the urine. The end products of degradation of pyrimidines are amino acids. The enzyme deoxyuridine triphosphatase (dUTPase) catalyses the reaction that converts dUTP to dUMP. It is important that deoxyuridine diphosphate (dUDP) is not present in the cell in high concentrations otherwise it would become incorporated into DNA. The three enzymes that catalyse the final reactions of pyrimidine breakdown (dihydropyrimidine dehydrogenase, dihydropyrimidinase and ureidopropionase) can metabolize uracil or thymine and their proceeding intermediates equally.

PATHOPHYSIOLOGY

Deficiency of adenosine deaminase

The deficiency of this enzyme results in immune impairment. It is thought that this is a result of substrate accumulation which subsequently inhibits other enzymes involved in nucleotide synthesis. Similar to this is a deficiency of purine nucleotide phosphorylase which also results in immune impairment.

Gout

Gout results from deposition of uric acid crystals in joints (causing severe pain and inflammation) as a result of increased levels of uric acid in the blood. It is often due to a metabolic abnormality which has resulted in

an increased production of purine nucleotides. The uric acid may precipitate to form sodium urate crystals which are deposited in joints and kidneys and cause pain and renal impairment.

The biochemical basis for the majority of cases of primary gout is unknown, but the condition is associated with increased uric acid production and/or reduced renal excretion. In rare instances, primary gout may result from specific enzyme defects.

1 A PRPP synthase enzyme which is insensitive to feedback inhibition by GDP or ADP.

2 A partial deficiency of HGPRTase. The decreased rate of the salvage reaction causes increased levels of PRPP which will result in an increase in the activity of PRPP aminotransferase.

The symptoms of gout can be relieved by the drug allopurinol. This is metabolized by xanthine oxidase to alloxanthine which does not dissociate from the active site of the enzyme.

46 Catabolism of amino acids

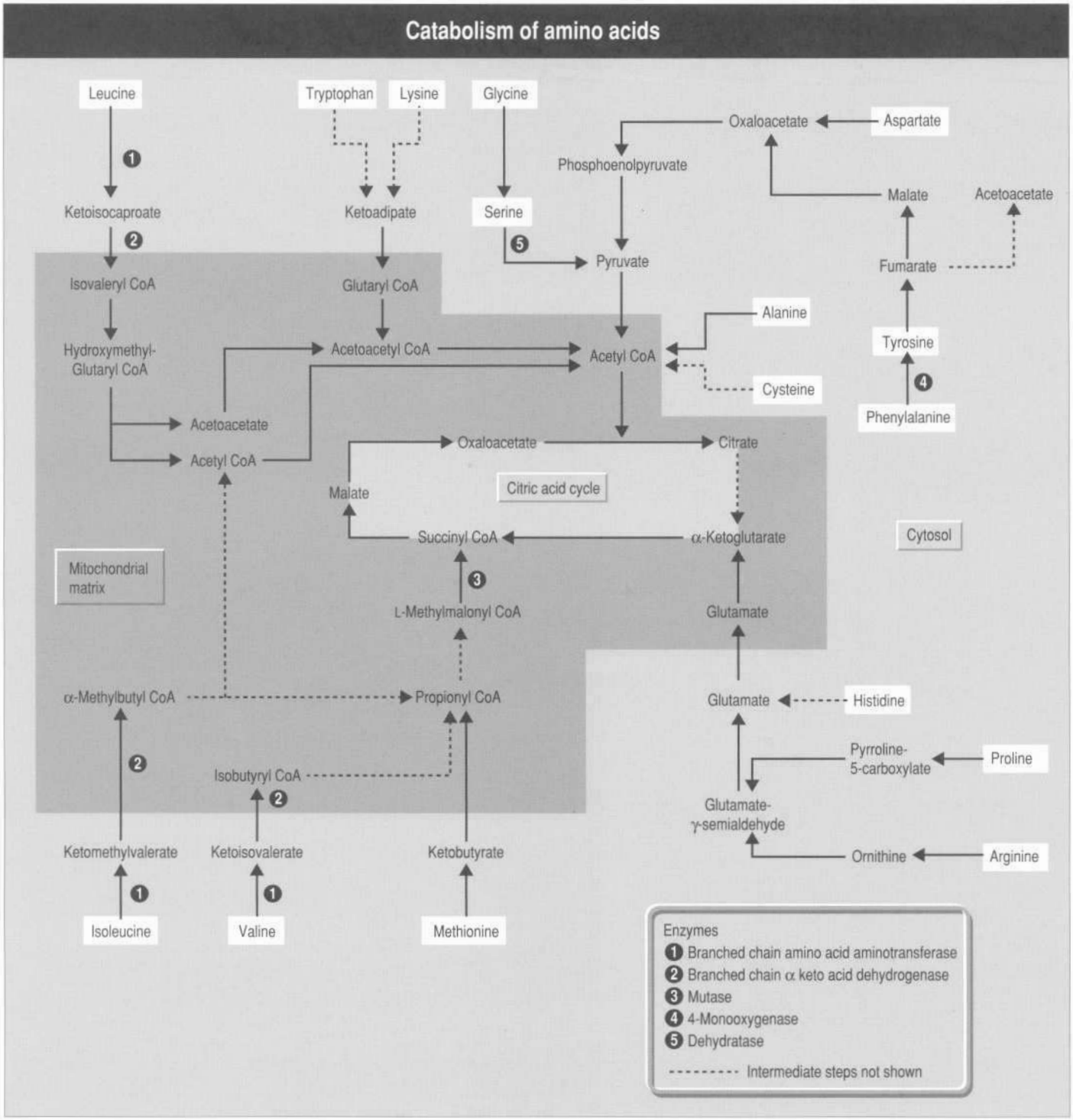

Fig. 46.1

INTRODUCTION

Amino acids in the body that are not used in protein synthesis are not stored or excreted. Instead, they are metabolized to intermediates which can be fully oxidized to provide energy or are converted to glucose, fatty acids or ketone bodies. This occurs mainly in the liver.

The pathway for the breakdown of most amino acids can be divided into two distinct phases: (i) the removal of the amino group and its conversion into urea (the Krebs–Henseleit urea cycle); and (ii) the conversion of the remaining carbon skeleton into pyruvate, one of the metabolites of the citric acid cycle, acetyl CoA or acetoacetyl CoA.

REMOVAL OF AMINO GROUP AND FORMATION OF UREA

Deamination of amino acids

The removal of the α-amino group from most amino acids is achieved by a **transamination reaction**. This reaction is catalysed by specific aminotransferases and involves transferring the amino group onto α-ketoglutarate to form **glutamate**. The loss of the amino group converts the amino acid into an α-keto-acid. The transamination catalysed by alanine aminotransferase is shown below:

alanine + α-ketoglutarate → pyruvate + glutamate

The aminotransferases are dependent on a pyridoxal phosphate prosthetic group which is derived from vitamin B_6 and can accept the amino group and transfer it to α-ketoglutarate. The amino acids serine and threonine are exceptions to the rule in that their amino groups can be converted directly to NH_4^+ by **dehydratases**.

Starvation: role of muscle aminotransferases

The importance of the aminotransferases can be seen when the body is subjected to a period of starvation. After the glycogen reserves have been depleted it is vital to maintain an adequate concentration of glucose in the blood. The body is not able to convert fatty acids to glucose, so gluconeogenesis must be fuelled by amino acids. The sequence of metabolic events is outlined below.

1 Amino acids of the muscle tissue, especially the branched chain amino acids (valine, leucine and isoleucine), are deaminated.

2 The carbon skeletons of the deaminated amino acids are metabolized by the enzymes of the citric acid cycle, phosphoenolpyruvate carboxykinase and pyruvate kinase to form pyruvate.

3 In a reaction catalysed by alanine aminotransferase, pyruvate can be converted to alanine.

4 Alanine is then released into the blood where it passes to the liver. There it is converted back to pyruvate which can then be metabolized to glucose.

The Krebs–Henseleit urea cycle

The glutamate formed by the transamination reactions can either be converted to glutamine or to α-ketoglutarate. The conversion to glutamine is fuelled by the hydrolysis of a high-energy bond of ATP and is catalysed by glutamine synthetase. Glutamine can then be used as a fuel source in the intestines or as a regulator of acid–base balance in the kidney. The conversion of glutamate to α-ketoglutarate is catalysed by glutamate dehydrogenase, and the NH_4^+ which is formed enters the urea cycle. The formation of oxaloacetate from fumarate provides the urea cycle with a link to the citric acid cycle as oxaloacetate can condense with acetyl CoA to form citrate.

The rate at which the urea cycle functions is controlled by acetyl glutamate which stimulates carbamoyl phosphate synthetase. Acetyl glutamate is synthesized from glutamate in a reaction catalysed by a synthetase enzyme. The activity of this enzyme is increased by amino acids, particularly arginine.

INTEGRATION OF THE KETO-ACID SKELETONS INTO METABOLISM

All of the amino acids found in the body are broken down into one or more of seven intermediates in metabolism. They are acetyl CoA, acetoacetyl CoA, pyruvate, α-ketoglutarate, succinyl CoA, fumarate and oxaloacetate.

The amino acids that are degraded purely to either acetyl CoA or acetoacetyl CoA are termed **ketogenic** since their breakdown is directed towards the formation of ketone bodies. Amino acids whose breakdown products can be directed towards the formation of glucose are termed **glucogenic**. The only purely ketogenic amino acids are leucine and lysine, although isoleucine, tryptophan, phenylalanine and tyrosine are ketogenic and glucogenic.

PATHOPHYSIOLOGY

Deficiencies of the urea cycle

Any deficiency of the urea cycle is detrimental to the body, since high concentrations of NH_4^+ are toxic and the urea cycle is the only metabolic pathway which can convert NH_4^+ to a substance that can be excreted. A total deficiency of one of the enzymes of the cycle results in death shortly after birth. Partial deficiencies result in mental retardation and frequent vomiting.

Maple syrup disease

In maple syrup disease, there is a deficiency of the branched chain **α-keto-acid dehydrogenase enzyme**. This leads to an increase in the levels of isoleucine, leucine, valine and their α-keto-acid derivatives in both the blood and urine. The accumulation of these compounds in the urine causes the urine to smell similar to maple syrup, hence the name. If undetected, maple syrup disease leads to physical and mental retardation. This disease can be detected by adding 2,4-dinitrophenylhydrazine to a urine sample. This compound will combine with α-ketoisocaproate, a derivative of leucine, forming 2,4-dinitrophenylhydrazine. Management of maple syrup disease involves a specially formulated diet which is low in leucine, valine and isoleucine.

Phenylketonuria

Phenylketonuria is caused by a deficiency of the **phenylalanine mono-oxygenase enzyme,** or less commonly of its tetrahydrobiopterin cofactor. The disease has an incidence of around 1 in 25 000 newborn babies and shows an autosomal recessive inheritance pattern. In the healthy state, 75% of phenylalanine is converted into tyrosine which can then be metabolized, and the remainder of the phenylalanine is incorporated

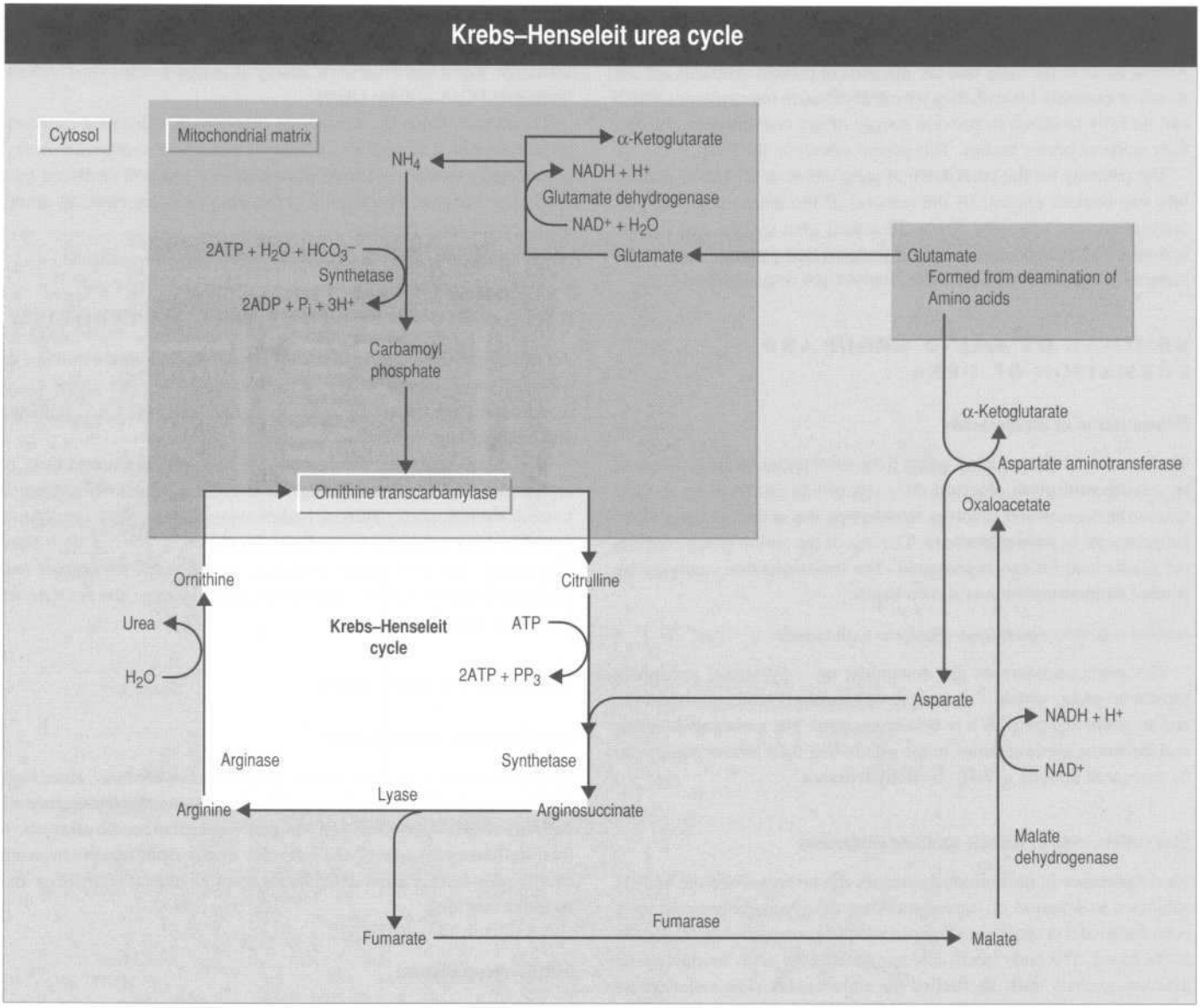

Fig. 46.2

into proteins. In phenylketonurics, the conversion of phenylalanine into tyrosine is blocked and levels of the amino acid rise drastically in the blood and the urine. If undetected and untreated in the early stages, severe complications follow which include abnormal myelination of nerves, hyperreflexia, a low brain weight and mental retardation. These complications are in evidence in untreated 1-year-old phenylketonurics who would have seemed normal and healthy at birth. Untreated phenylketonuria is also life-shortening and will usually be fatal before the patient reaches the age of 30 years.

The phenylketonuria gene has been cloned and screening programmes have ensured that most phenylketonurics are now treated. The treatment for phenylketonuria is a diet which is low in phenlyalanine, but which still contains a sufficient amount of the amino acid for normal growth. Because of the severe nature of the effects of a raised level of phenylalanine, this dietary regime is started soon after birth.

Pernicious anaemia

In pernicious anaemia, there is a deficiency of **intrinsic factor**. Intrinsic factor is responsible for the uptake of vitamin B_{12}, also called cobalamin, from the ileum. Cobalamin is a coenzyme associated with two integral enzymes of amino acid degradation, methylmalonyl CoA mutase and cystathione synthase. The mutase enzyme converts methylmalonyl CoA to succinyl CoA and the synthase enzyme converts homocysteine (a metabolite of methionine) to cystathione. The deficiency of the coenzyme results in increased levels of the enzyme substrates methylmanolyl CoA and homocysteine. Increased levels of methylmalonyl CoA cause acidosis, and increased levels of homocysteine cause homocysteinuria.

There are also a number of defects in the metabolism of methylmalonyl CoA which result in acidosis and increased levels of

methylmalonate in the urine. In approximately 50% of these patients, there is a defect in the conversion of a derivative of colbalamin to colbalamin itself. These patients respond favourably to treatment with vitamin B_{12}. Other patients have a defective enzyme involved in methylmalonyl CoA metabolism, for example methylmalonyl CoA mutase, and will not therefore respond to vitamin B_{12}.

Vitamin B_{12} is also essential in the synthesis of purines and pyrimidines and a lack of cobalamin is damaging to the haematopoietic system due to the rapid turnover of red blood cells.

47 Synthesis of amino acids

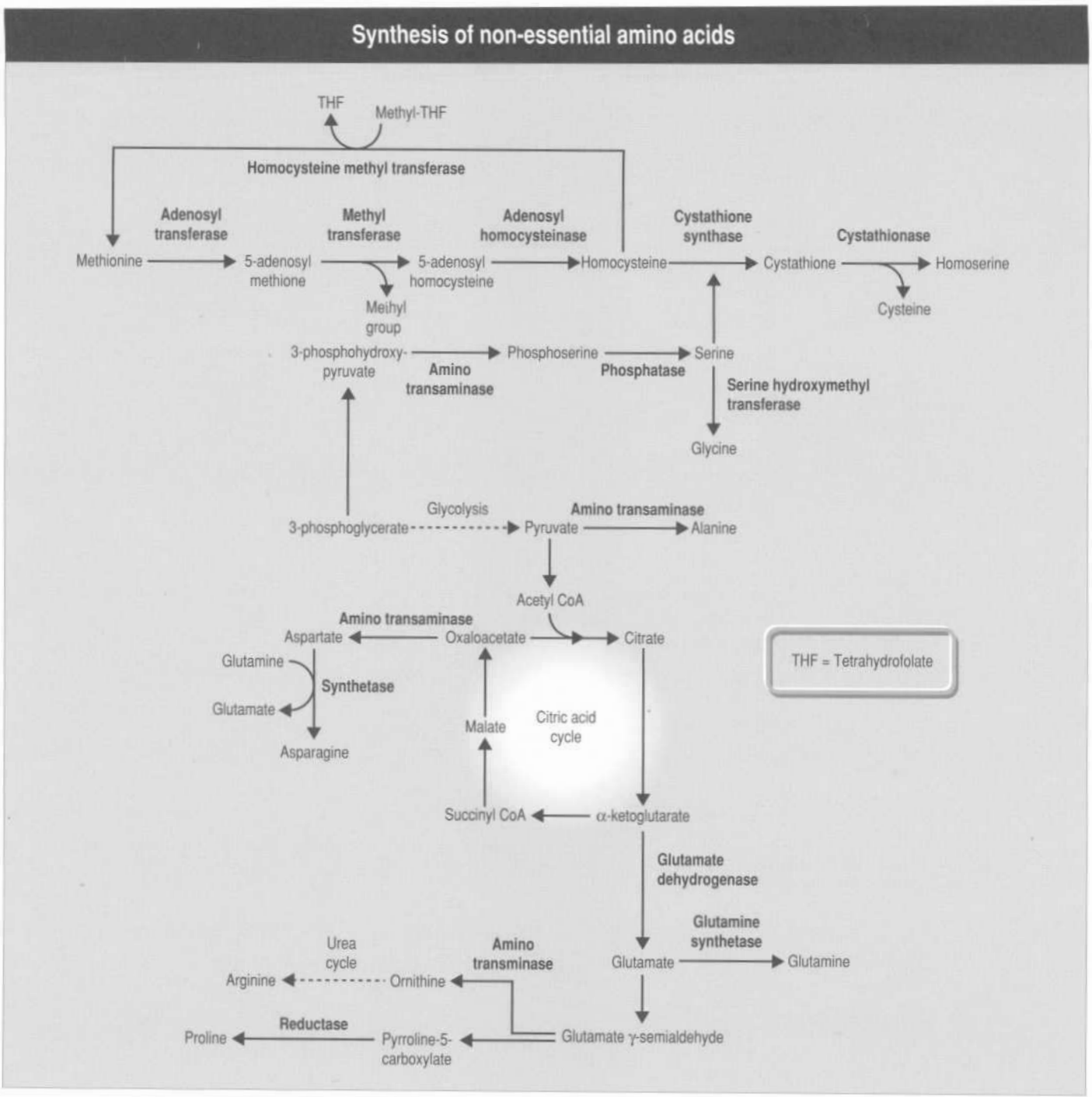

Fig. 47.1

Of the 20 amino acids that exist in the body, nine cannot be synthesized and have to be obtained from the diet. These are called **essential amino acids.** The remaining 11 amino acids can be synthesized in the body and are called non-essential amino acids. This chapter will discuss the synthesis of the non-essential amino acids.

SUPPLY OF THE AMINO GROUP

In the synthesis of most amino acids, the amino group is supplied by **glutamate.** Glutamate is formed in a reaction catalysed by glutamate dehydrogenase:

$NH_4^+ + \alpha$-ketoglutarate + NADPH + $H^+ \rightarrow$ glutamate + $NADP^+ + H_2O$

A further NH_4^+ group can be incorporated by the amination of glutamate to form glutamine. This reaction is catalysed by **glutamine synthase**:

NH_4^+ + glutamate + ATP $\rightarrow$ glutamine + ADP + Pi + H^+

The amino group on glutamate can then be transferred to a keto-acid to form an amino acid by a transamination reaction.

Control of glutamine synthase

Glutamine synthase is a key controller in nitrogen metabolism. Glutamine is involved in the synthesis of a number of important compounds, including the amino acids **tryptophan** and **histidine**, the nucleotide **CTP** and the pyrimidine ring precursor **carbamoyl phosphate**. The final products of glutamine metabolism bind to the enzyme and inhibit its activity. The activity of glutamine synthase is also affected by the reversible covalent attachment of AMP to a tyrosine residue in the enzyme. The addition and removal of the AMP is catalysed by **adenylyl transferase** whose activity in turn is modulated by interchangeable regulator proteins. When levels of ATP or α-ketoglutarate are raised, AMP is removed from glutamine synthase, which increases its activity. When levels of glutamate or glutamine are raised, the AMP is added to the enzyme, which reduces its activity.

Synthesis of the carbon skeletons

The 11 non-essential amino acids that can be synthesized by the body are **alanine**, **arginine**, **asparagine**, **aspartate**, **cysteine**, **glutamine**, **glycine**, **proline**, **serine**, **glutamate and tyrosine**. The formation of all of these amino acids except for tyrosine is shown in Fig. 47.1. **Tyrosine** is formed by the hydroxylation of phenylalanine by phenylalanine hydroxylase:

phenylalanine + O_2 + tetrahydrobiopterin $\rightarrow$ tyrosine + H_2O + dihydrobiopterin

Tetrahydrobiopterin acts as an electron carrier in this reaction and is regenerated by the reduction of dihydrobiopterin, which uses NADPH as a reductant.

Serine, **glycine** and **cysteine** are formed from an intermediate of the glycolytic pathway, 3-phosphoglycerate. The phosphatase enzyme which converts phosphoserine to serine is inhibited by serine, providing a feedback regulation for the pathway.

Glycine is formed from serine by the removal of a one-carbon unit and its attachment to a tetrahydrofolate (THF) carrier. The carbon unit is carried on either the N_5 or N_{10} nitrogen atom of THF as methylene-THF. THF can exist in a number of oxidation states depending on the carbon group carried, for example a methyl-THF donates a methyl group to homocysteine to form methionine.

An example of another carrier of carbon units is **adenosylmethionine**. This carrier has a much higher transfer potential for the release of the carbon unit than has methyl-THF, and is used to methylate a number of important molecules, e.g. noradrenaline. The formation of adenosyl-methionine forms part of a methionine salvage pathway in which methionine is regenerated by the methylation of homocysteine by methyl-THF. The enzyme that catalyses the formation of methionine from homocysteine is a methyltransferase which uses vitamin B_{12} as a coenzyme.

Cleavage of cystathione results in the formation of the amino acids **cysteine** and **homoserine**. Homoserine is then converted into ketobutyrate which is transported into the mitochondria and metabolized to succinyl CoA.

48 Integration of metabolism

Effects of insulin on adipose, liver, and muscle tissue

Increased uptake of nutrients	Glycolysis	Glycogenesis	Amino acid metabolism	Lipid metabolism
Glucose • Muscle • Adipose	Induction of hexokinase and glucokinase	Dephosphorylation and activation of glycogen synthase	Increased protein synthesis	Stimulation of lipoprotein lipase
Amino acids • Muscle	Stimulation of phosphofructokinase	Phosphorylation and activation of glycogen phosphorylation ↓ Stimulation of glycogen synthesis • Liver • Muscle	Decreased protein breakdown • Liver/muscle	Inhibition of hormone-sensitive triglyceride lipase ↓ Breakdown of blood lipids and uptake of products into adipose cells
	Inhibition of gluconeogenesis ↓ Increased rate of glycolysis • Adipose • Muscle • Liver		Excess pyruvate converted to lactate or alanine which can be transported to the liver • Muscle	Activation of pyruvate dehydrogenase and acetyl CoA carboxylase → increased fatty acid synthesis
				Inhibition of carnitine acyl transferase by malonyl CoA → reduced fatty acid breakdown
				Compensatory rise in pentose phosphate pathway • Adipose/liver

Fig. 48.1

Feeding and starvation are associated with changes in the metabolism of the body. Feeding results in a large influx of metabolic substrates into the body and the metabolism has to adapt quickly to store the nutrients as proteins, lipids and glycogen. Starvation is exactly the opposite of feeding and the metabolism of the body must be able to co-ordinate a controlled breakdown of the body's energy storage polymers to provide adequate substrates for energy generation.

FEEDING

Shortly after feeding, insulin is released into the circulation from β-cells in the islets of Langerhans of the pancreas. The overall effect of insulin on metabolism is the stimulation of biosynthetic pathways so that the digested and absorbed substrates such as glucose or amino acids can be converted into a form that can be stored. Some of the effects of insulin on metabolism have been described in earlier chapters and its overall effects are shown in Fig. 48.1.

STARVATION

There are three distinct phases through which the metabolism of the body passes when in a state of starvation: (i) the post absorptive phase; (ii) the gluconeogenic phase; and (iii) the ketotic phase. These three phases and their associated metabolic states are shown in Fig. 48.2.

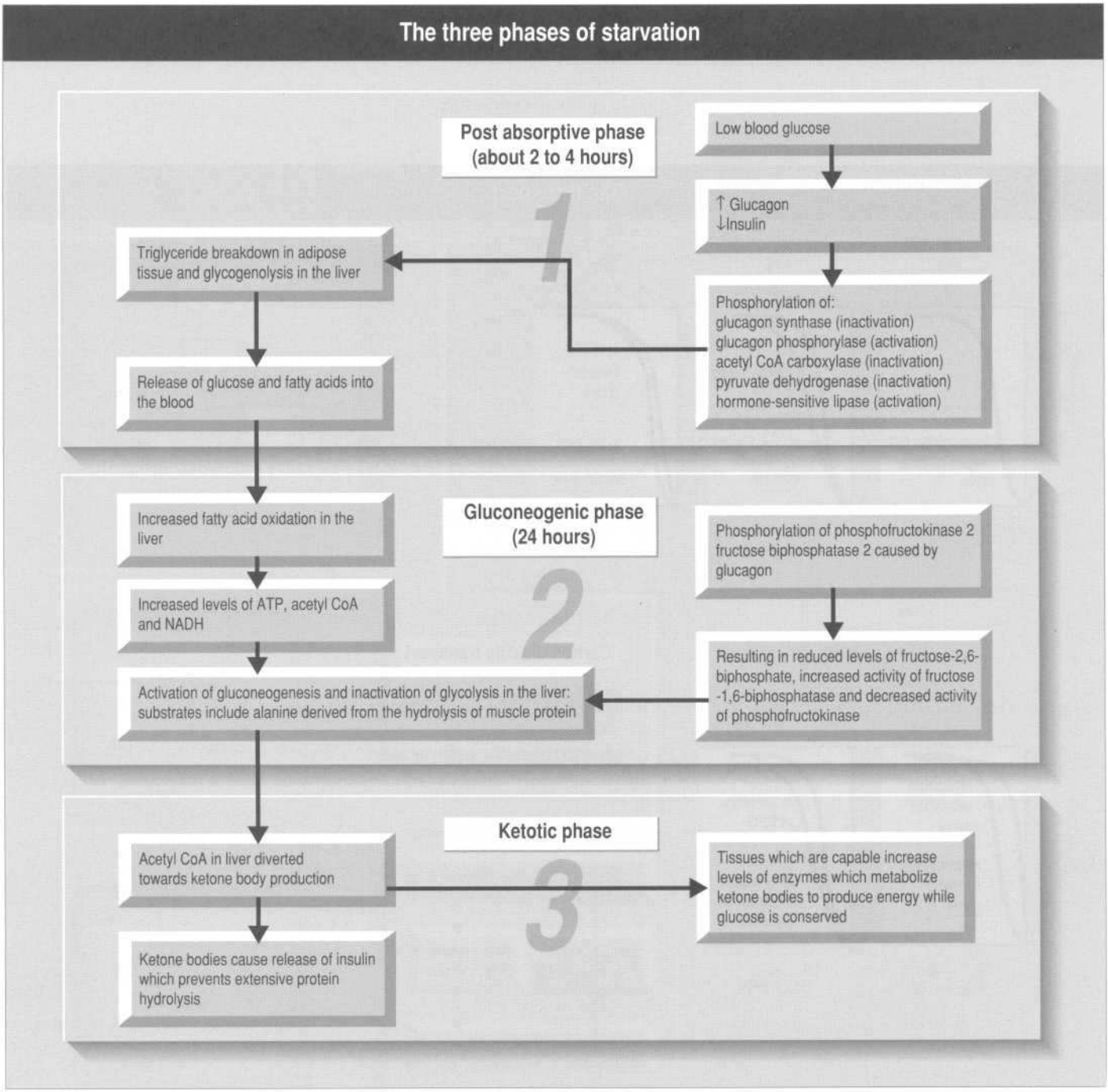

Fig. 48.2

The ketotic phase lasts only as long as the fatty supplies in the body can be broken down to form acetyl CoA for ketone body formation. Oxaloacetate, derived from glucose, is needed for the metabolism of ketone bodies. The glucose is, in turn, derived from the gluconeogenesis of amino acids from protein breakdown. Death eventually occurs from the extensive breakdown of functional proteins.

49 Gas transport

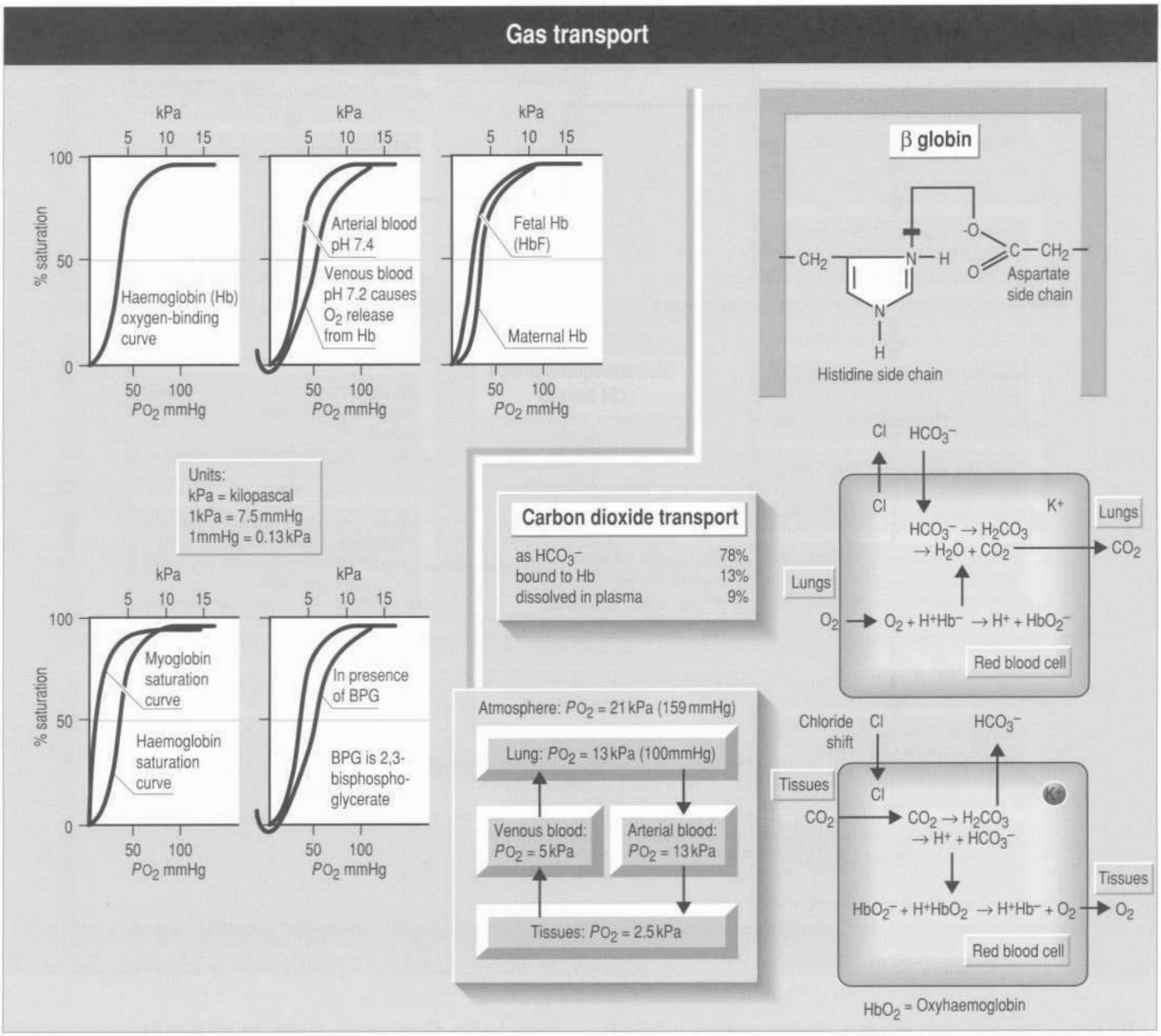

Fig. 49.1

TRANSPORT OF O_2 AND CO_2

In blood, most O_2 is bound to Hb. When O_2 is bound, Hb is called oxyHb. Hb is a red protein present at about 150 g/l. Each gram of Hb binds about 1.36 ml of O_2, i.e. 1 l of blood can carry about 20 ml O_2. The O_2 saturation curve for Hb is sigmoid.

Properties of the curve

The steep part of the curve lies within the range of physiological extrapulmonary P_{O_2} values. Therefore, a small fall in tissue P_{O_2} will result in a large dissociation of O_2 from Hb. The sigmoidal curve is consistent with the occurrence of co-operativity: as O_2 is taken up it

increases the affinity of Hb for more O_2. At a Po_2 of 3.6 kPa, Hb is 50% saturated with O_2 (the P_{50}). A high P_{50} means a low affinity for O_2. The **Hill coefficient** for Hb is 2.8. This indicates positive co-operativity. A small shift of the curve to the right means a sharp loss of O_2 holding, and a small shift to the left implies a sharp affinity increase. Hb binds O_2 weakly at low Po_2, and tightly at high Po_2.

Hb allosterism

The binding of O_2 to Hb causes a **homotrophic** effect, when a ligand changes the affinity of another protomer of a protein for the same ligand (O_2). Hb also shows **heterotrophic** interactions, i.e. the affinity for O_2 is decreased by the binding of other ligands. Three ligands that decrease the affinity of Hb for O_2 are CO_2, H^+ ions and 2,3-bisphosphoglycerate (2,3-BPG), which occurs in red blood cells, and its net formation is a diversion from glycolysis.

CO_2 and H^+ ions

CO_2 and H^+ bind to Hb, decreasing its affinity for O_2. Conversely, when O_2 binds to Hb, it lowers Hb affinity for CO_2 and H^+ ions, and both dissociate more easily from the protein. This interaction between O_2, CO_2 and H^+ with Hb is the **Bohr effect.** In metabolically active tissues, CO_2 and H^+ concentrations are high. These bind to Hb, causing the release of O_2 to the tissues. Hb carrying CO_2 and H^+ is transported to the lung, where O_2 is taken up by free O_2-binding sites on Hb, and CO_2 and H^+ ions are released.

Transport of CO_2

CO_2 diffuses from the tissues into the blood, and into the red cells, where it is converted into carbonic acid by carbonic anhydrase:

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons HCO_3^- + H^+$$

The reaction is driven to the right because CO_2 is continuously entering the red blood cell. H^+ ions bind to Hb (releasing O_2), and HCO_3^- diffuses down a concentration gradient into the plasma in exchange for Cl^-. Thus, much CO_2 travels to the lungs as bicarbonate. Some CO_2 binds reversibly to unionized amino acid moieties on Hb promoters, forming negatively charged carbamino groups. The carbamino groups form salt bridges with groups on Hb which are positively charged, thereby promoting stability of deoxygenated Hb.

Transport of H^+ ions

H^+ ions generated in the blood must be buffered, to prevent acidosis, and H^+ binds to ionizable groups on Hb globin chains. Thus, Hb acts as a buffer. H^+ ions bind mainly to the imidazole group of C-terminal histidine residues on Hb β-chains. When O_2 dissociates from Hb, the protein polypeptide chains alter shape, bringing imidazole groups of the histidine residues close to $-COO^-$ groups of aspartate residues, and these form a non-covalent electrostatic bond which stabilizes the deoxygenated Hb. When the pH of blood falls, this shifts the O_2 saturation curve to the right, and more O_2 is released, thus increasing the buffering capacity of Hb.

In the lungs, Hb binds O_2 and changes shape. H^+ is released, and oxyHb has a negative charge. This is balanced by the positive charge of K^+ ions in the red blood cell. In other words, oxyHb acts as a stronger acid than Hb. H^+ combines with bicarbonate to form carbonic acid. Thus, the concentration of bicarbonate ions in the red blood cell is reduced, and bicarbonate ions diffuse down a concentration gradient from the plasma into the cell. To maintain electical neutrality in the cell, Cl^- ions diffuse out of the red blood cells into the plasma. This movement of Cl^- ions is termed the **chloride shift**.

In the tissues, O_2 dissociates from Hb and diffuses into the cells. Hb takes up H^+ from carbonic acid, and bicarbonate ions are formed. The negative charges of red cell bicarbonate ions are balanced by K^+ ions. Bicarbonate ions diffuse from the red cell into the plasma, and Cl^- ions diffuse from plasma into the red blood cells in exchange for bicarbonate ions. CO_2 enters the red blood cells and reacts with H_2O to form carbonic acid. Therefore, the Pco_2 within the red blood cell is kept low, and this creates a concentration gradient allowing more CO_2 to diffuse into the red blood cell from the tissues.

Effects of 2,3-BPG

2,3-BPG is present in the red blood cell in concentrations equivalent to those of Hb. 2,3-BPG binds to Hb, and shifts the O_2 saturation curve to the right. When 2,3-BPG is not bound, Hb is saturated with O_2 at the Po_2 of the tissues, and little is given up to them. 2,3-BPG is negatively charged, and binds to positive charges on the β-globin chains of the deoxygenated form of Hb, and slots into a cavity between the two chains. In the lungs, as Hb binds more O_2, the protein shape changes so that the β-globin chains move closer together and compress the sites where 2,3-BPG is bound.

FETAL Hb (HbF)

The fetus derives O_2 from the maternal circulation, and HbF has a higher affinity than does adult Hb (HbA) for O_2. HbF does not have β-chains, but γ-chains, which possess fewer positive charges in the cavity where 2,3-BPG is bound. Therefore, HbF binds 2,3-BPG less tightly than does HbA.

50 Haemoglobin

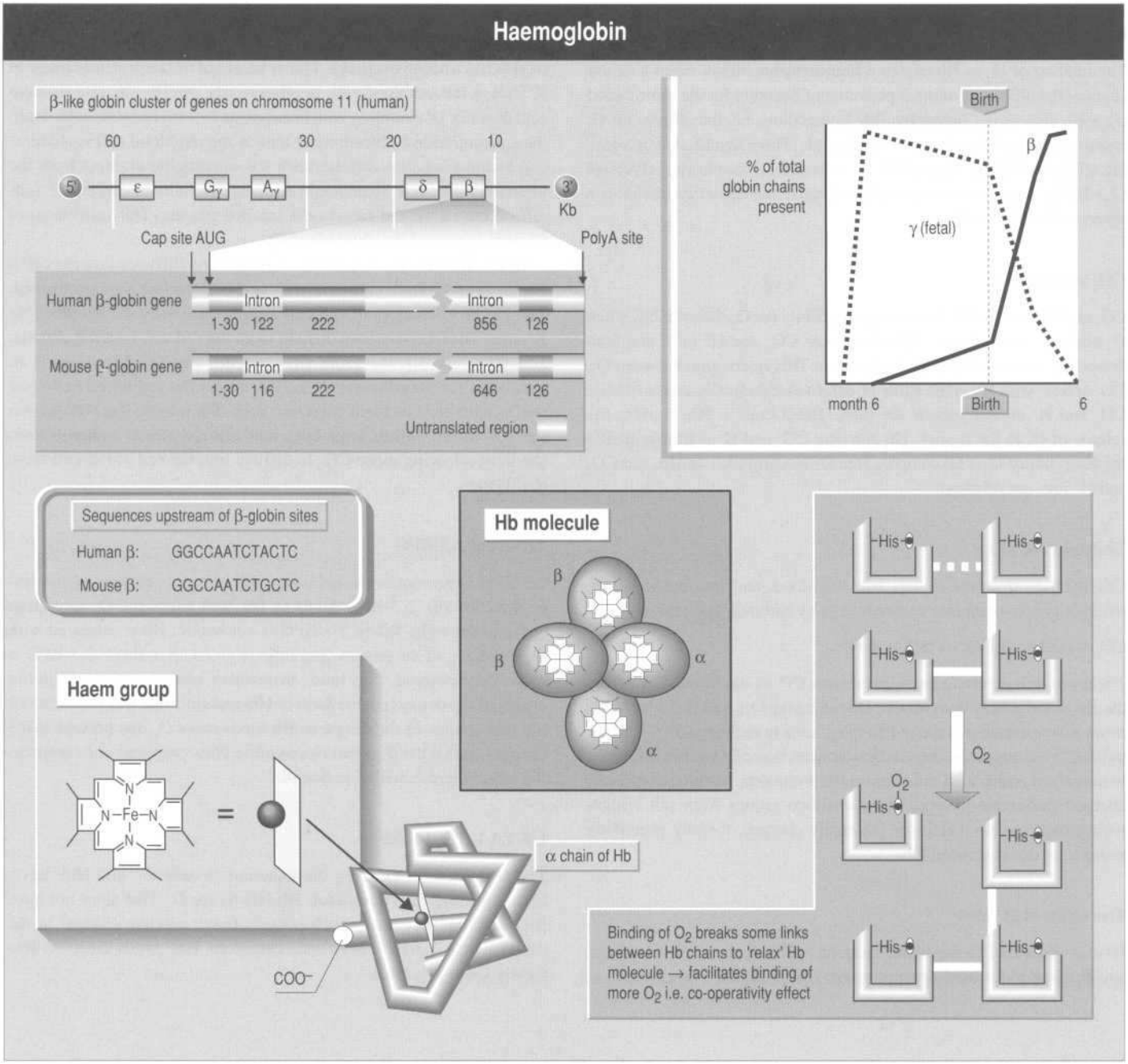

Fig. 50.1

β-GLOBIN FAMILY

Several different forms of β-like globins are formed during embryogenesis and adult life in humans and other vertebrates. In humans, these include β-, δ-, A_γ-, G_γ- and ε-globins. The genes coding for the polypeptide chains occur relatively close together on chromosome 11, and express chains which are very similar in sequence identity. Only the β- and δ-chains continue to be expressed after 6 months of postnatal life in humans.

STRUCTURE OF Hb

Hb consists of **four subunits.** Each is a globin molecule containing a **haem group,** and the four subunits are held together by non-covalent

bonds. **HbA** consists of two α-chains, each 141 amino acids long, and two β-chains, each 146 amino acids long. There is a high degree of uniformity in tertiary structure among the different subunits of Hb, and each chain is arranged in multiple α-helix regions interrupted by turns of the polypeptide chain that force the subunit into a spherical shape.

THE HAEM GROUP

Each subunit of Hb contains a prosthetic haem group. Haem consists of a polyringed **porphyrin** molecule, with an atom of iron (Fe) in the ferrous (Fe(II)) form at its centre. Hb and myoglobin porphyrin is termed protoporphyrin IX. The Fe is bound to the four N atoms of the haem, but is situated outside the plane of the molecule because it is larger than the space between the N atoms. The Fe is also bound to the peptide chain of the subunit through a linkage with one of the histidine (His) residues.

Combination of Hb with O_2

O_2 can combine reversibly with each of the four subunits of Hb. The O_2 binds to the Fe atom on the face of the haem group opposite to that bound to the protein. The first O_2 binds to Fe in an α-subunit. This overcomes a repulsion between a His residue and the porphyrin ring, and allows Fe to move into the plane of the ring. At the same time, several of the ionic bonds linking amino acid residues rupture. This causes a conformational change over the whole Hb molecule. The two ß-subunits move closer to each other, although they do not actually touch. This forces the dissociation of 2,3-BPG. As the β-subunits move closer together, the two α-chains move further apart, and this makes it easier for O_2 molecules to gain access to the haem sites. Thus, as succeeding O_2 molecules bind, so they open or 'relax' the Hb molecule further, and this increases the apparent affinity of the molecule for O_2; the fourth O_2 molecule finds it easiest to bind, i.e. has the highest apparent affinity for Hb.

MYOGLOBIN

Myoglobin is a protein in muscle cells. Like Hb, it binds O_2 but, unlike Hb, myoglobin consists of a single polypeptide chain, 153 amino acids long, with one haem group which binds only one molecule of O_2. Its saturation curve is therefore not sigmoidal.

Functionally, myoglobin provides a binding site for O_2 that can be utilized by the metabolically very active skeletal and cardiac muscle fibres. Together, these two tissues account for about 30% of the O_2 consumption of the human body at rest. The saturation curve for myoglobin, over most of its length, lies to the left of that for Hb. In other words, it has a higher affinity for O_2 than has Hb. Therefore, in the tissues such as muscle, where the Po_2 is about 2.5–3.5 kPa, when Hb is less than 50% saturated with O_2, myoglobin is fully saturated. When the tissues demand more O_2, the Po_2 drops to less than 0.2 kPa, and at this order of magnitude, myoglobin releases most of its bound O_2. Unlike Hb, the binding of O_2 by myoglobin is not influenced by 2,3-BPG concentrations, CO_2 or H^+.

PATHOPHYSIOLOGY OF Hb

Hb is associated with several disease states.

Quantitative determinants

Patients may present with a red blood cell (RBC) count in the normal range, but with a mean cell volume (MCV) outside the normal range (**microcytosis**), abnormal mean cell Hb content (MCH) and abnormal mean cell Hb concentration (MCHC; **hypochromia**). This is **microcytic anaemia**, although in other forms of anaemia the patient may present with a lowered RBC count.

The anaemia may be caused by a dietary shortage of the Fe required for haem synthesis. Individuals at risk include women who are pregnant, menstruating or lactating, and vegetarians, especially vegans.

Microcytic anaemia may result from a failure to utilize Fe. Individuals at risk include those exposed to toxic substances such as lead, which block –SH groups of enzymes that catalyse reactions involved in the synthesis of haem.

Chemicals can cause a quantitative reduction in available Hb. For example, inhalation of carbon monoxide (CO), can be fatal. The CO forms a stable, bright red complex with the haem group and does not dissociate. Another example is the oxidation of the Fe(II) to Fe(III), to form **methaemoglobin**. Methaemoglobin may be formed through hereditary defects in globin synthesis, or through oxidizing compounds. In the Fe(III) states, O_2 is not bound, and, when present together with normal HbA, methaemoglobin shifts the O_2 saturation curve of the HbA to the left, making its bound O_2 less accessible to the tissues.

Qualitative determinants

In **sickle cell anaemia** patients synthesize **HbS**, an aberrant form of HbA, in which a non-polar valine (Val) residue replaces a polar glutamate residue at position six on the β-subunit ($Glu_{6\beta}$). $Glu_{6\beta}$ lies on the outer surface of HbA, and in the deoxy state of Hb, the non-polar Val residue forms hydrophobic bonds with other HbS molecules, which causes polymerization and precipitation of Hb inside the RBC. Consequently, the RBC assumes a characteristic sickle shape, and loses elasticity, which results in blockage of the microcirculation by the cells.

51 Molecular chaperones

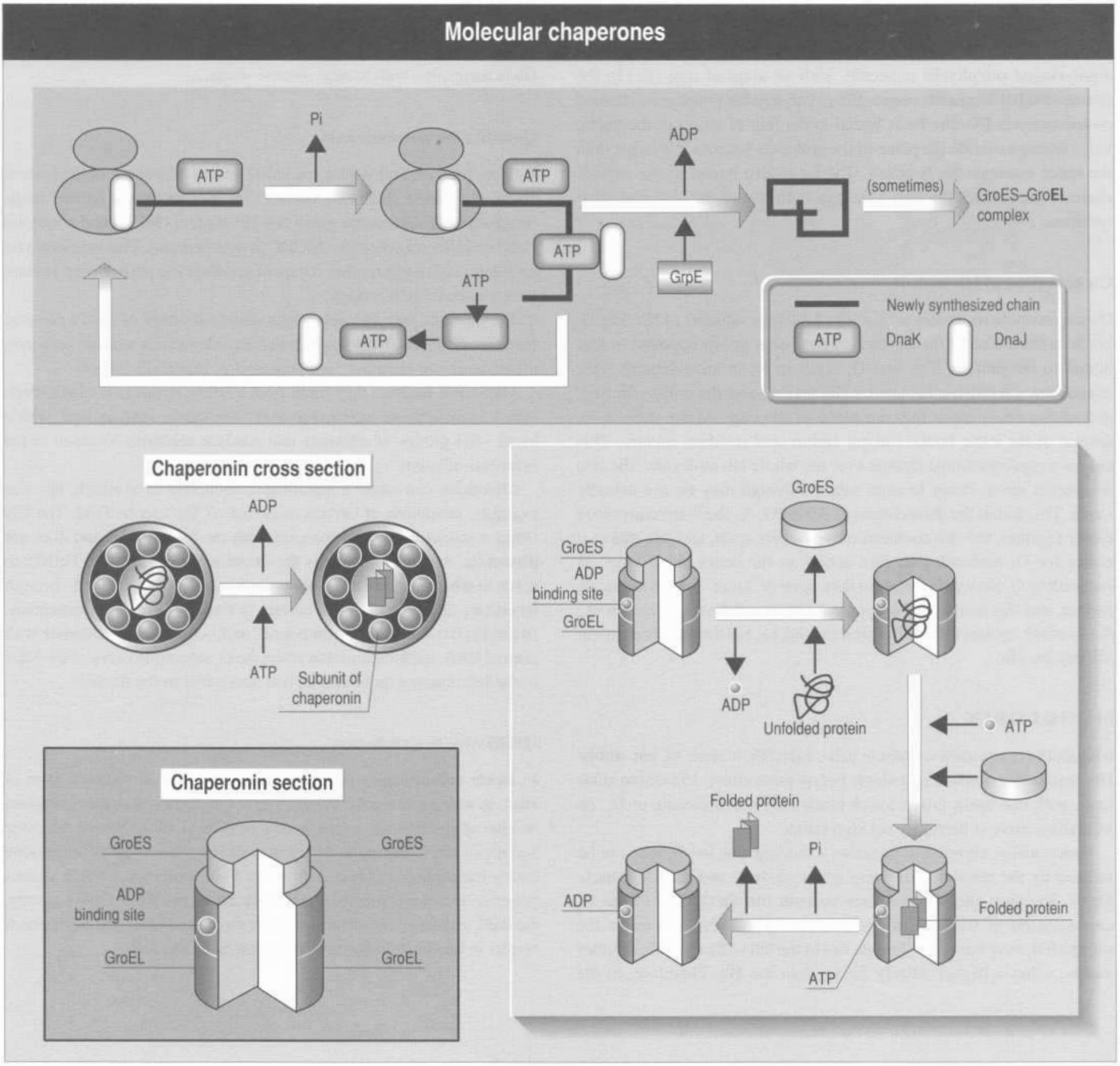

Fig. 51.1

Molecular chaperones regulate the **folding** of many newly synthesized proteins. The chaperones bind newly synthesized proteins, preventing them from aggregating in the cell, and mediate protein folding into their native state. There are several, structurally unrelated families of molecular chaperones. The term 'molecular chaperone' was originally applied to the proteins nucleoplasmin and the chloroplast ribulose biphosphate carboxylase, which promote the assembly of nucleosomes. The molecular chaperones can organize themselves into structures, called **chaperonins**, which contain a central cavity in which an unfolded protein is shielded from the cell.

PRINCIPLES OF CHAPERONE ACTION

Molecular chaperones bind and stabilize proteins in their non-native form, and release them in such a way as to facilitate their folding. They can recognize structural features of proteins in their unfolded form, in particular certain amino acid sequences, but cannot bind folded proteins.

Newly formed proteins extend from the polysomes, amino-terminus leading, as unfolded peptide chains. These cannot start folding until a critical length has been reached: about 100 amino acids. These form a **protein domain**, which facilitates the further folding of the entire protein in a co-operative manner. When the chain is elongated, its hydrophobic residues are repelled by the aqueous environment and tend to bury themselves within the protein. Since so many different proteins are being synthesized simultaneously, hydrophobic residues from different chains could interact to form aggregates, and this has been shown to happen *in vitro* in the absence of molecular chaperones.

Molecular chaperones protect the nascent protein from these interactions by shielding hydrophobic surfaces during peptide elongation, and possibly also during the folding process and during translocation of the protein across membranes, and by binding the completely synthesized, but as yet unfolded protein, and allowing it to fold unhindered by the other cellular processes going on around it.

HSP

HSP60 and HSP70

The principle of molecular chaperones may provide a clue as to why HSP are produced during cellular injury or disease. It is known that the HSP70 proteins can bind proteins **after** they have been incompletely denatured during cellular distress. Therefore, they may provide a means of protecting the cell from undergoing massive protein aggregation.

HSP70 proteins comprise two functional domains: (i) a polypeptide-binding domain at the carboxy terminal; and (ii) a nucleotide-binding site at the amino terminal. There is evidence that ATP is bound and hydrolysed to ADP at the amino terminal of HSP70, and this provides the energy to induce a conformational change in HSP70 that results in the release of the folded polypeptide.

Action of HSP70

The mechanism whereby HSP70 proteins act in concert to regulate protein folding and release in *Escherichia coli* cytosol has been partially elucidated. There are at least five proteins in the cytosol that mediate protein folding, namely **DnaK**, **DnaJ**, **GrpE**, **GroEL** and **GroES**. They regulate the folding of newly synthesized proteins, and may also protect already folded proteins during periods of cellular stress. They have already been shown to inhibit the heat denaturation of a heat-labile protein, luciferase, produced by fireflies. The steps in protein folding are the following.

1 DnaJ and Dnak, with bound ATP attached to it, bind the unfolded polypeptide chain as it is formed by the ribosome, and the bound ATP is hydrolysed to ADP.

2 DnaJ and DnaK bind to each other as the polypeptide starts to fold, and the DnaK–ADP–DnaJ–polypeptide complex is stabilized.

3 The protein GrpE promotes the dissociation of ADP from DnaK, perhaps by binding to the site where ADP is bound. ATP binds to its site on DnaK, and the folded polypeptide is released.

4 In some cases, the released protein is bound by GroEL, which, through an ATP-dependent interaction with GroES, permits protein folding to be completed.

CHAPERONINS

The term 'chaperonins' has been given to the **HSP60** protein family. The term describes a quaternary structure of proteins which assemble to form a compartment in which newly synthesized proteins can fold, protected from other folding proteins, thus eliminating the risk of protein aggregation. Analysis of chaperonins has revealed structures consisting of 14 subunits, stacked in two heptameric rings with a central cavity, which can hold proteins of up to about 90 kDa in size. In *E. coli*, the chaperonins are made up of two HSP60 proteins, GroEL and GroES. The newly synthesized proteins are held in the chaperonin in what has been called a 'molten globule' state. The term describes partially folded proteins whose hydrophobic surfaces have not yet become totally buried in the protein. GroEL and GroES interact via a sequence of ATP binding and hydrolysis, which provide the energy for the binding and release of the folded protein.

Eukaryotic chaperones and chaperonins

In yeast cells, molecular chaperones have been described whose function is to assist in the transfer of proteins across the mitochondrial membrane. Proteins formed in the cytosol must first be unfolded before they can cross the mitochondrial membrane, and refolded once they have done so. This is accomplished through the action of mitochondrial molecular chaperones. There is evidence that proteins in the cytosol are held in an unfolded state by HSP70 proteins, and the energy for this is provided by ATP. The polypeptide chain passes through the lipid bilayer of the mitochondrial membrane, and on the inner surface the polypeptide chain is folded under the direction of HSP70, acting together with HSP60.

A number of other chaperonins have now been described, including one found in eukaryotic cytosols, called **TCP-1**, or **CCT** (also called chaperonin of eukaryotic cytosol), which is particularly abundant in developing embryos, testis and lymphoid tissue. It has been shown that disruption of yeast chaperonins are lethal for the cell.

Abbreviations

A	adenine
ACP	acyl carrier protein
ACTH	adrenocorticotrophin
ADP	adenosine diphosphate
AMP	adenosine monophosphate
ATP	adenosine triphosphate
ATPase	adenosine triphosphatase
bp	base pairs
2,3-BPG	2,3-bisphosphoglycerate
C	cytosine
Ca	calcium
$CaCl_2$	calcium chloride
cAMP	cyclic AMP
CAP	catabolic activator protein
CCK	cholecystokinin
cDNA	complementary DNA
CDP	cytidine triphosphate
cGMP	cyclic guanosine monophosphate
CGRP	calcitonin gene-related peptide
CH_3COOH	acetic acid
CH_3COONa	sodium acetate
Cl_2	chlorine
CO	carbon monoxide
CO_2	carbon dioxide
CoA	coenzyme A
–COOH	carboxyl group
CRS	cytokine receptor superfamily
CTP	cytidine triphosphate
cyt	cytochrome
d	2′-deoxyribo-
Da	dalton
DAG	diacylglycerol
dCTP	deoxycytidine triphosphate
dd	dideoxynucleotide
DFP	di-isopropylphosphofluoridate
DHT	5α-dihydrotestosterone
DNA	deoxyribonucleic acid
DNAase	deoxyribonuclease
dTTP	deoxythymidine triphosphate
dUDP	deoxyuridine diphosphate
dUTPase	deoxyuridine triphosphatase
ECF	extracellular fluid
EF	elongation factor
EGF	epidermal growth factor
emf	electromotive force
ER	endoplasmic reticulum
FAD	flavine adenine dinucleotide
Fe	iron
FGF	fibroblast growth factor
fMET	formyl methionine
GDP	guanosine diphosphate
GH	growth hormone
GMP	guanosine monophosphate
GTP	guanosine triphosphate
GTPase	guanosine triphosphatase
H_2	hydrogen
H_2CO_3	hydrogen carbonate
H_2O	water
H_2O_2	hydrogen peroxide
H_3PO_4	phosphoric acid
Hb	haemoglobin
HbA	adult Hb
HbF	fetal Hb
HDL	high-density lipoprotein
hfr	high frequency of recombination
Hg	mercury
HGPRTase	hypoxanthine–guanine phosphoribosyl transferase
HIV	human immunodeficiency virus
hnRNA	heterogeneous nuclear RNA
HRE	hormone response elements
HSP	heat shock protein
Hyp	hydroxyproline
I	inosinic acid
ICF	intracellular fluid
IDL	intermediate density lipoprotein
IF	initiation factor
IgA	immunoglobulin A
IGF	insulin-like growth factor
Il-1	interleukin 1
IMP	inosine monophosphate
IP_3	inositol trisphosphate
IRS-1	insulin receptor substrate 1
JAK	Janus kinase
K	potassium
K_m	Michaelis constant
LDL	low-density lipoprotein
LTR	long terminal repeats
MAPK	mitogen-activated protein kinase
MCH	mean cell Hb content
MCV	mean cell volume
Mg	magnesium
MGE	mobile genetic element
MCHC	mean corpuscular Hb concentration
MMTV	mouse mammary tumour virus
Mn	manganese
mRNA	messenger RNA
Na	sodium
NADH	nicotinamide adenine dinucleotide
$NADP^+$	nicotinamide adenine dinucleotide phosphate
$–NH_2$	amino group
O_2	oxygen
–OH	hydroxyl group
^{32}P	phosphorus-32
Pa	pascal
PCR	polymerase chain reaction
PDGF	platelet-derived growth factor

PFK	phosphofructokinase
Pi	inorganic phosphate
PIP_2	phosphatidylinositol-4,5-biphosphate
PKC	protein kinase C
PLA_2	phospholipase A_2
PLC	phospholipase C
PLP	pyridoxal phosphate
PRPP	5-phosphoribosyl-1-pyrophosphate
R	gas constant
RBC	red blood cell
RF	release factor
RNA	ribonucleic acid
RNAase	ribonuclease
rRNA	ribosomal RNA
RTF	resistance transfer factor
S	Svedberg unit
scRNA	small cytoplasmic RNA
snRNA	small nuclear RNA
SRP	signal recognition particle
STATS	signal transducers and transcription activators
T	thymine
TGF	transforming growth factor
THF	tetrahydrofolate
TMP	thymidine monophosphate
tRNA	transfer RNA
TPP	thymine pyrophosphate
U	uracil
UDP	uridine diphosphate
UMP	uridine monophosphate
UTP	uridine triphosphate
UV	ultraviolet
VLDL	very low-density lipoprotein
Zn	zinc

Amino acid abbreviations

Ala	alanine
Arg	arginine
Asn	asparagine
Asp	aspartate
Cys	cysteine
Gln	glutamine
Glu	glutamate
Gly	glycine
His	histidine
Ile	isoleucine
Leu	leucine
Lys	lysine
Met	methionine
Phe	phenylalanine
Pro	proline
Ser	serine
Thr	threonine
Trp	tryptophan
Tyr	tyrosine
Val	valine

Glossary

A site ribosomal recognition site where next mRNA codon is exposed to incoming tRNA
activation energy critical energy level for chemical reaction to occur
active transport energy-requiring movement of substances across biomembranes
affinity strength of attraction between two binding sites
agonist ligand that triggers a response (see **ligand**)
allele alternative form of a gene that can occupy a chromosomal genetic locus
allosteric proteins can alter binding site properties in response to ligand occupancy at another site
amphipathic molecule possessing both hydrophilic and hydrophobic properties
anaemic deficient in haemoglobin
anion negatively charged ion
annealing two complementary nucleic acid strands joining
antibody immunoglobulin produced in response to an antigen, which binds it
anticodon set of three consecutive tRNA bases complementary to a mRNA codon
antigen substance, usually foreign to body, that provokes antibody formation
antimetabolite substance blocking a metabolic (usually enzyme) reaction
antiporter membrane protein transporting substance across membrane with simultaneous transport of another substance the opposite way
autocrine hormonal action on cell by substance produced by the same cell
autosomal recessive Mendelian recessive genetic inheritance carried on an autosome
autosome chromosome other than sex chromosome
avian referring to birds
bacteriophage virus that infects bacteria
base proton (H^+) acceptor in solution, e.g. purines, pyrimidines
benign medically, a non-cancerous tumour that does not invade other tissues or destroy any healthy tissue
C terminal free $-COO^-$ group at end of a polypeptide chain
cap-binding protein binds to cap region of mRNA; required for initiation of eukaryotic transcription
carcinogen agent that may cause cancer
cardiotonic heart stimulant
cation positively charged ion
cDNA library collection of DNA strands complementary to source tissue DNA
chi sequence repeated short DNA sequence on bacterial chromosome where RecA-mediated recombination is stimulated
chondroblast cartilage cell producing cartilage matrix
cloning producing identical cells or molecules from single starting cell or molecule
co-operative binding ligand binds to protein and changes affinity for other sites for the same ligand on the protein
codon set of three consecutive bases on DNA or RNA, specifying an amino acid or a signal for the end of translation
coenzyme (cofactor) non-protein required by a protein for bioactivity
cofactor see **coenzyme**
conformation three-dimensional arrangement
constitutive gene a gene continuously expressed without the need for a transcription initiation factor
control element a DNA sequence that influences the expression of nearby genes
cosmid a cloning vector plasmid containing phage λ *cos* sequences
cotransport linked transport of substances across a biomembrane
crista mitochondrial inner membrane infolding
crossover exchange of genes between homologous chromosomes during meiosis
cytoskeleton internal protein skeleton of eukaryotic cell
cytosol soluble cytoplasmic compartment
degenerate one amino acid can be encoded by several different codons
dimer protein formed of two subunits
diploid organism whose cell has two sets of chromosomes
docking protein protein that 'places' another in its binding site
domain structurally defined membrane or chromatin region, or globular region of a protein
electrochemical gradient transmembrane gradient defined by ionic and electrical gradients
endocrine ductless glandular function
enhancer a DNA control site in eukaryotic genes, whose activation by specific proteins increases transcription of the gene
eukaryote organism whose cells have bounded nuclei containing organized chromatin and cytoskeletons
exocytosis secretion of chemicals from eukaryotic cells
exon DNA sequence unit coding for part of a polypeptide, or for rRNA or tRNA
facilitated diffusion membrane transport down a concentration gradient utilizing a carrier system, but no energy
familial traits found in some families; not necessarily inherited
fibril thread-like component of a fibre
fibroblast connective tissue cell
flip-flop transition of a lipid or protein from one membrane surface to the other
G protein guanine nucleotide-binding protein on cytoplasmic surface of cell membrane, forming part of hormone signalling to target cell
genetic recombination meiotic exchange of DNA between homologous chromosomes during gamete formation in sexually reproducing organisms
genome genetic database of a single organism or cell
genomic library collection of DNA chromosomal fragments from one genome
globin protein constituent of haemoglobin
glucocorticoids adrenal steroids influencing carbohydrate metabolism; some are anti-inflammatory
glycosides compounds which when hydrolysed yield a sugar and a non-sugar, e.g. digoxin
glycosylation formation of eukaryotic glycoproteins through addition of oligosaccharide side chain to protein

growing fork locus in DNA replication
haploid cell or organism having one set of chromosomes
helical spiral chain arrangement of protein or nucleic acid molecules forming rod-like helix
hepatocyte liver cell
heterogeneous having dissimilar components
heterotrophic effects allosteric effects due to interactions between different ligands
homodimer protein consisting of two identical subunits
homologous resembling in origin and structure
homotrophic effects allosteric effects due to interactions between identical ligands
hormone response element (HRE) region of DNA that binds hormone–receptor complex
hydrolysis addition of OH^- and H^+ ions of H_2O to a molecule which is consequently split into simpler molecules
hydrophilic water attracting
hydrophobic water repelling
in vitro in test tubes ('in glass')
inducer chemical or physical stimulus to gene expression or enzyme action
intercalate to slip between adjacent bases in DNA
intron non-coding region of DNA that may be transcribed but later spliced out of mRNA
isoelectric pH pH at which protein is uncharged
isomer compound chemically identical to others, but with different spatial arrangement, e.g. stereoisomer
Klenow fragment fragment of DNA polymerase I, containing all the $3' \rightarrow 5'$ exonuclease and polymerase activity
lap-joint intermediate hybrid in genetic recombination
ligand a molecule that binds to another with functional purpose
lipids class of hydrophobic compounds including fats, phospholipids and steroids
lipoprotein compound composed of protein and lipid
lumen internal space of subcellular organelle or of sac-like or tubular organ
malignant tumour that destroys tissue of origin or invades and destroys other tissues
matrix medium or ground substance of tissues
meiosis nuclear division producing haploid daughter cells from diploid parent cell
metastasis spread of cancerous cells to other tissues
micelle spherical ordered arrangement of molecules such as phospholipids in aqueous medium
mitosis nuclear division, when daughter cells have identical chromosomal complement as parent
mobile genetic element DNA sequence able to be inserted on same or other chromosomes and alter gene expression (also called transposable element)
monomer molecule composed of a single unit
mutagenic able to cause a mutation
mutation change in nature or composition of DNA resulting in change in characteristics of gene expression in cell
N terminal amino terminus of polypeptide chain
nascent newly synthesized; not yet active
nonsense codon a termination codon
Okazaki fragments short DNA sequences formed on lagging ($3' \rightarrow 5'$) strand during discontinuous DNA replication
oligomer molecule composed of a few monomer units
oncogene gene carried by cancer cell or virus, that is partly or wholly responsible for tumour formation
operon prokaryotic genetic unit in which several genes are clustered and transcribed into polycistronic mRNA
organelle functional structure within a eukaryotic cell
osteoblast bone-forming cell
P site ribosomal site where the last mRNA codon was read (see also **A site**)
palindrome sequence reading the same both ways, e.g. in DNA: AACAA
paracrine local hormone acting on neighbouring cells
passive transport simple diffusion down a concentration gradient; includes facilitated diffusion
pentose a monosaccharide with formula $(CH_2O)_5$, e.g. ribose
phage see **bacteriophage**
phasmid vector consisting of a combination of a plasmid with phage λ
phenotype biochemical and physical characteristics of an organism
pinocytosis uptake of liquid into the cell
plasmid circular bacterial or yeast DNA replicating independently of chromosomes
polycistronic mRNA codes for more than one polypeptide
polymer macromolecule consisting of several similar or identical subunits
polysaccharide macromolecular carbohydrate polymers, e.g. glycogen
polysome ribosomal aggregate on mRNA during translation
primer short fragment of RNA necessary to initiate DNA polymerase action
primosome prepriming protein assembly necessary for primer synthesis
prohormone hormone precursor
prokaryote unicellular organisms, e.g. bacteria, lacking membrane-bounded nucleus and other organelles such as mitochondria
promoter DNA sequence necessary for initiation of transcription
proofreading property of DNA polymerase to detect base mismatches
prosthetic group non-protein moiety, e.g. haem, forming part of protein active site
protomer inactive enzyme form
proton H^+ (H_3O^+ in some texts)
purine base, commonly adenine or guanine in nucleic acids
pyrimidine base, commonly cytosine, thymine or uracil in nucleic acids
receptor protein that recognizes a ligand, and that constitutes the primary member in the chain of communication between ligand and cell
receptor antagonist ligand that binds receptor, blocks agonist binding and produces no response
receptor superfamily group of intracellular receptors, with structural similarities, which act as transcription activators, e.g. glucocorticoid and retinoic acid receptors
recombination exchange of DNA between homologous chromosomes during meiosis
replication fork Y-shaped point where DNA is unwound and simultaneously replicated
replicon unit of DNA that replicates sequentially and contains an origin of replication
replisome protein assembly on DNA, needed for replication
repressor bacterial protein that binds to the operon to repress transcription
resistance transfer factor (RTF) factor on plasmid that enables transfer of drug resistance to another bacterial cell

second messenger intracellular chemical signal synthesized in response to stimulation of receptor on cell membrane

sedimentation coefficient estimate of size of macromolecule from its rate of sedimentation in a sucrose gradient

semi-conservative replication means of DNA replication

sex pilus means of transmitting F factors from one bacterium to another

Shine–Dalgarno sequence bacterial mRNA 5′-AGGAGG-3′ sequence before initiation codon

signal sequence temporary hydrophobic sequence of amino acids at amino terminal, important for transfer of secretory products across membranes

SOS response sequence of repair responses in *E. coli* in response to damage stimuli

splicing cutting nucleic acid in order to insert sequences, thus creating recombinant nucleic acid

splicosome assembly of ribonucleoprotein that splices RNA

stop signal signal that stops macromolecular elongation

supercoiling twisting of the double helix on itself

symport simultaneous, linked transport of two molecules across a biomembrane, both in the same direction

template pattern from which a molecule is synthesized, e.g. DNA strand is a template for its replication

transcription synthesis of complementary RNA from DNA

transition DNA base substitution causing mutation

translation protein synthesis by ribosomes on mRNA template

translocation removal of part of a chromosome to another, non-homologous chromosome

transposable element see **mobile genetic element**

transposition replication of a DNA sequence on one chromosome in another chromosome

transposon replicated transposed sequence

transversion substitution of a pyrimidine for a purine or vice versa

Uniport membrane transport of a molecule by a membrane protein

van der Waals forces weak form of non-covalent bonding between neutral molecules

vector agent that carries a message, e.g. plasmid, bacteriophage

zwitterion ion with negative and positive charges, e.g. amino acids

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