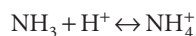


- 34 Smit JJ, Schinkel AH, Oude Elferink RP *et al.* (1993) Homozygous disruption of the murine mdr2 P-glycoprotein gene leads to a complete absence of phospholipid from bile and to liver disease. *Cell* 75 (3), 451–462.
- 35 Deleuze JF, Jacquemin E, Dubuisson C *et al.* (1996) Defect of multidrug-resistance 3 gene expression in a subtype of progressive familial intrahepatic cholestasis. *Hepatology* 23 (4), 904–908.
- 36 De Vree JM, Jacquemin E, Sturm E *et al.* (1998) Mutations in the MDR3 gene cause progressive familial intrahepatic cholestasis. *Proc Natl Acad Sci USA* 95 (1), 282–287.
- 37 Strautnieks SS, Bull LN, Knisely AS *et al.* (1998) A gene encoding a liver-specific ABC transporter is mutated in progressive familial intrahepatic cholestasis. *Nature Genet* 20 (3), 233–238.
- 38 Jansen PL, Strautnieks SS, Jacquemin E *et al.* (1999) Hepatocanalicular bile salt export pump deficiency in patients with progressive familial intrahepatic cholestasis. *Gastroenterology* 117 (6), 1370–1379.
- 39 van Mil SW, van der Woerd WL, van der Brugge G *et al.* (2004) Benign recurrent intrahepatic cholestasis type 2 is caused by mutations in ABCB11. *Gastroenterology* 127 (2), 379–384.
- 40 Bull LN, Carlton VE, Stricker NL *et al.* (1997) Genetic and morphological findings in progressive familial intrahepatic cholestasis [Byler disease (PFIC-1) and Byler syndrome]: evidence for heterogeneity. *Hepatology* 26 (1), 155–164.
- 41 Bull LN, van Eijk MJ, Pawlikowska L *et al.* (1998) A gene encoding a P-type ATPase mutated in two forms of hereditary cholestasis. *Nature Genet* 18 (3), 219–224.
- 42 Paulusma CC, Groen A, Kunne C *et al.* (2006) Atp8b1 deficiency in mice reduces resistance of the canalicular membrane to hydrophilic bile salts and impairs bile salt transport. *Hepatology* 44 (1), 195–204.
- 43 Noe J, Kullak-Ublick GA, Jochum W *et al.* (2005) Impaired expression and function of the bile salt export pump due to three novel ABCB11 mutations in intrahepatic cholestasis. *J Hepatol* 43 (3), 536–543.
- 44 Pauli-Magnus C, Lang T, Meier Y *et al.* (2004) Sequence analysis of bile salt export pump (ABCB11) and multidrug resistance p-glycoprotein 3 (ABCB4, MDR3) in patients with intrahepatic cholestasis of pregnancy. *Pharmacogenetics* 14 (2), 91–102.
- 45 Hayashi H, Takada T, Suzuki H *et al.* (2005) Two common PFIC2 mutations are associated with the impaired membrane trafficking of BSEP/ABCB11. *Hepatology* 41 (4), 916–924.
- 46 Wang L, Soroka CJ, Boyer JL (2002) The role of bile salt export pump mutations in progressive familial intrahepatic cholestasis type II. *J Clin Invest* 110 (7), 965–972.
- 47 Craddock AL, Love MW, Daniel RW *et al.* (1998) Expression and transport properties of the human ileal and renal sodium-dependent bile acid transporter. *Am J Physiol* 274 (1 Pt 1), G157–G169.
- 48 Wong MH, Oelkers P, Dawson PA (1995) Identification of a mutation in the ileal sodium-dependent bile acid transporter gene that abolishes transport activity. *J Biol Chem* 270 (45), 27228–27234.
- 49 Kullak-Ublick GA, Ismail MG, Stieger B *et al.* (2001) Organic anion-transporting polypeptide B (OATP-B) and its functional comparison with three other OATPs of human liver. *Gastroenterology* 120 (2), 525–533.
- 50 Kobayashi D, Nozawa T, Imai K *et al.* (2003) Involvement of human organic anion transporting polypeptide OATP-B (SLC21A9) in pH-dependent transport across intestinal apical membrane. *J Pharmacol Exp Ther* 306 (2), 703–708.
- 51 Dawson PA, Hubbert M, Haywood J *et al.* (2005) The heteromeric organic solute transporter alpha-beta, Ostalpha–Ostbeta, is an ileal basolateral bile acid transporter. *J Biol Chem* 280 (8), 6960–6968.
- 52 Chiang JY (2004) Regulation of bile acid synthesis: pathways, nuclear receptors, and mechanisms. *J Hepatol* 40 (3), 539–551.
- 53 Karpen SJ (2002) Nuclear receptor regulation of hepatic function. *J Hepatol* 36 (6), 832–850.
- 54 Moore JT, Moore LB, Maglich JM *et al.* (2003) Functional and structural comparison of PXR and CAR. *Biochim Biophys Acta* 1619 (3), 235–238.
- 55 Neimark E, Chen F, Li X *et al.* (2004) Bile acid-induced negative feedback regulation of the human ileal bile acid transporter. *Hepatology* 40 (1), 149–156.
- 56 Li H, Chen F, Shang Q *et al.* (2005) FXR-activating ligands inhibit rabbit ASBT expression via FXR-SHP-FTF cascade. *Am J Physiol Gastrointest Liver Physiol* 288 (1), G60–G66.
- 57 Lee H, Zhang Y, Lee FY *et al.* (2006) FXR regulates organic solute transporter alpha and beta in the adrenal gland, kidney and intestine. *J Lipid Res* 47 (1), 1006–1011.
- 58 Denson LA, Sturm E, Echevarria W *et al.* (2001) The orphan nuclear receptor, shp, mediates bile acid-induced inhibition of the rat bile acid transporter, ntcp. *Gastroenterology* 121 (1), 140–147.
- 59 Mukhopadhyay S, Ananthanarayanan M, Stieger B *et al.* (1998) Sodium taurocholate cotransporting polypeptide is a serine, threonine phosphoprotein and is dephosphorylated by cyclic adenosine monophosphate. *Hepatology* 28 (6), 1629–1636.
- 60 Ananthanarayanan M, Balasubramanian N, Makishima M *et al.* (2001) Human bile salt export pump promoter is transactivated by the farnesoid X receptor/bile acid receptor. *J Biol Chem* 276 (31), 28857–28865.
- 61 Plass JR, Mol O, Heegsma J *et al.* (2002) Farnesoid X receptor and bile salts are involved in transcriptional regulation of the gene encoding the human bile salt export pump. *Hepatology* 35 (3), 589–596.
- 62 Handschin C, Meyer UA (2005) Regulatory network of lipid-sensing nuclear receptors: roles for CAR, PXR, LXR, and FXR. *Arch Biochem Biophys* 433 (2), 387–396.
- 63 Guo GL, Lambert G, Negishi M *et al.* (2003) Complementary roles of farnesoid X receptor, pregnane X receptor, and constitutive androstane receptor in protection against bile acid toxicity. *J Biol Chem* 278 (46), 45062–45071.
- 64 Stedman CA, Liddle C, Coulter SA *et al.* (2005) Nuclear receptors constitutive androstane receptor and pregnane X receptor ameliorate cholestatic liver injury. *Proc Natl Acad Sci USA* 102 (6), 2063–2068.

2.3.7 Ammonia, urea production and pH regulation

Dieter Häussinger

Ammonia plays a central role in nitrogen metabolism. It is a major byproduct of protein and nucleic acid catabolism, and its nitrogen can be incorporated into urea, amino acids, nucleic acids and many other nitrogenous compounds. Ammonia is present in body fluids as both NH_3 and NH_4^+ , and these are in equilibrium according to the equation:



The $\text{p}K_a$ of this reaction is 9.25, so that at physiological pH there is a great excess of the ionized form. NH_3 can diffuse freely across membranes via aquaporins [1] and NH_4^+ is carried in liver by an active transport system, the RhB glycoprotein [2].

The blood ammonia concentration is normally below 35 $\mu\text{mol/L}$; this is important as ammonia is neurotoxic at higher concentrations. Excessive cerebral ammonia uptake in hyperammonaemic states leads to astrocytic glutamine accumulation and cerebral oedema, which is important in the pathogenesis of hepatic encephalopathy [3–5].

The liver is the most important site of ammonia metabolism; it removes much of the toxic ammonia presented to it by urea and glutamine synthesis. By doing so, the liver also plays a major role in the metabolic regulation of systemic pH, because hydrogen ions released from NH_4^+ during the synthesis of urea neutralize the excess bicarbonate produced by the breakdown of amino acids (see below).

Urea is electroneutral and is transported across biological membranes by facilitated diffusion. A phloretin-sensitive urea transporter is also present in liver [6], and an aquaglyceroporin, AQP9 [7], and UT-B1 [8] have also been identified as urea transporters in liver. Although urea is not further metabolized by mammalian enzymes, it interferes with the activity of K^+ channels in the plasma membrane [9] and can cause liver cell shrinkage at concentrations found in uraemia. Urea is excreted by the kidney, and is normally present in plasma and body fluids at a concentration of 3.0–6.5 mmol/L.

Sources of ammonia

Whereas urea production takes place largely within the liver, much of the ammonia used in urea synthesis is derived, directly or indirectly, from extrahepatic tissues. Ammonia is released from the intestine and the kidneys, whereas liver, resting muscle, and brain remove ammonia from the blood [10]; 25% of the nitrogen utilized in urea synthesis reaches the liver via the portal vein, from ammonia formed in the small intestine and colon (see below) [10–13]. Most of the nitrogen transported to the liver for incorporation into urea is carried not as ammonia but as amino acids, such as alanine or glutamine (see also Chapter 2.3.3).

Within the liver, glutamate and glutamine are major sources of ammonia. Glutamate is released directly from protein, but more importantly it is formed from other amino acids (except lysine and threonine) released from protein breakdown in aminotransferase reactions. Glutamate is directly formed by deamidation of glutamine, and from proline and histidine. Ammonia is released from glutamate by its oxidative deamination by glutamate dehydrogenase, a mitochondrial enzyme. Glutamate dehydrogenase is present in most tissues, but its activity is highest in the liver.

Amino acids can also be transaminated with glyoxalate to form glycine, which is deaminated by glycine oxidase to yield ammonia; this pathway is thought to be quantitatively important in mammalian ammonia production. Ammonia is generated by the deamidation of glutamine and asparagine and in the histidine lyase reaction. Ammonia is also generated from serine, threonine, cysteine, cystathione and homoserine, in pyridoxal phosphate-dependent deamination reactions, which occur mainly in the liver. Ammonia is also produced by amine oxidases, but the amounts involved are small. In addition, ammonia can be generated from the metabolism of purines and pyrimidines. Most of the ammonia produced from purine nucleotides is derived from adenosine monophosphate (AMP), in a reaction catalysed by adenylate deaminase. This pathway becomes par-

ticularly important during exercise when ammonia formation and release from muscle is increased [14].

Ammonia from the intestine

The intestine is a major site of ammonia production. Some 15–30% of the urea synthesized by the liver is degraded by bacterial ureases in the gut, with the liberation of ammonia and carbon dioxide [3,15]. Urea is hydrolysed in the mucosa or the juxtamucosal area of the colon, and to a lesser extent in the small intestine [16]. The ammonia generated in these reactions is completely absorbed and returns to the liver to be converted back to urea. The oral administration of antibiotics, such as neomycin, reduces the bacterial degradation of urea in the intestine [17].

A second source of ammonia from the gut, quantitatively of equal importance, is the intestinal mucosa itself. The small intestine produces a significant quantity of ammonia, which comes primarily from the metabolism of glutamine removed from arterial blood. The fractional glutamine extraction in the human jejunum and ileum is 24% and 9% respectively [18]. The small intestine also produces ammonia from the intraluminal amino acids, alanine, leucine and glutamine, but not from threonine, serine and glycine [19]. Most of the ammonia produced in the colon comes from bacterial degradation of urea and other nitrogenous substances; nonbacterial production accounts for only 10% of the ammonia produced in the colon; in the dog, ammonia produced by the small intestine is approximately equal to that produced in the uncleansed colon [18]. The ammonia from the intestine enters the portal circulation and the ammonia concentration in the portal vein is up to 10-fold greater than elsewhere in the circulation.

Ammonia detoxication by the liver

There are two major pathways for ammonia detoxication by the liver: urea and glutamine synthesis [10–13,20,21]. Both pathways are embedded into a sophisticated structural and functional organization in the liver acinus [10–13,20,21].

Urea production

Approximately 90% of surplus nitrogen in humans enters the urea cycle for irreversible conversion to urea, which is excreted by the kidneys. Approximately 30 g of urea is excreted daily in healthy adults. Using tracer techniques, it has been observed that calculated urea production exceeds urinary urea excretion by about 20–30% [22]. This difference is attributed to extrarenal losses, largely accounted for by the intestinal hydrolysis of urea.

The urea cycle and its enzymes

The urea cycle comprises five enzymes (Fig. 1): carbamoyl-phosphate synthetase I (CPS I), ornithine transcarbamylase (OTC), argininosuccinate synthetase (ASS), argininosuccinate

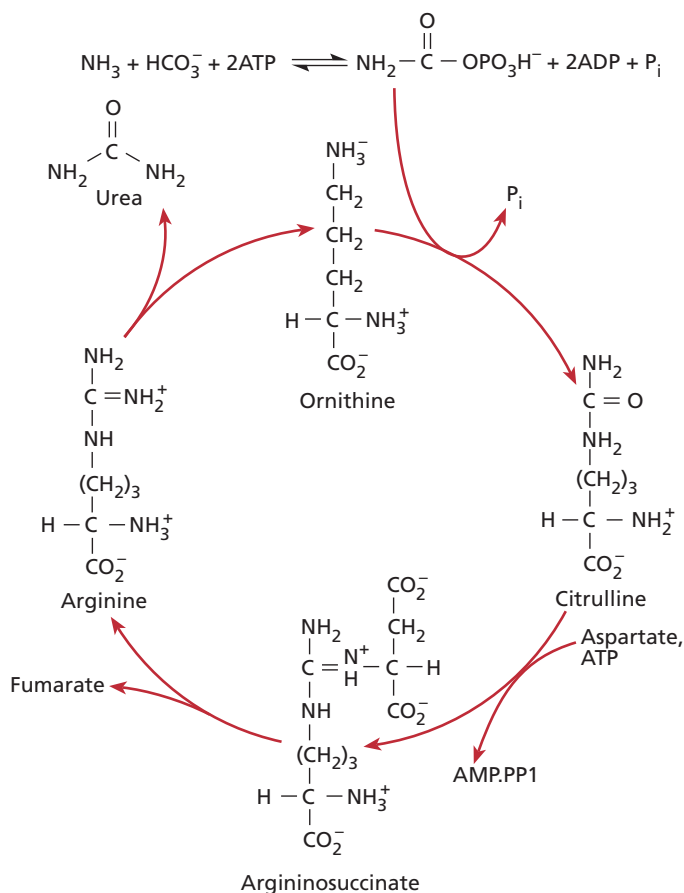


Fig. 1 The urea cycle.

lyase (ASL) and arginase. For efficient functioning of the pathway *in vivo*, however, further proteins are required, such as liver glutaminase [23], mitochondrial carbonic anhydrase V [24], *N*-acetylglutamate synthetase [25], the mitochondrial ornithine/citrulline antiporters ORNT1 and ORNT2 [26,27] and citrin, the mitochondrial aspartate/glutamate antiporter [28].

The liver is quantitatively the major organ involved in urea synthesis and it is doubtful whether other cell types, such as enterocytes, can produce significant amounts of urea [29]. However, at least some urea cycle enzymes are found in extrahepatic tissues, where they are involved in providing arginine for nitric oxide (NO) synthesis (the so-called citrulline-NO cycle) [30].

The initial reaction of the urea cycle is the formation of carbamoyl phosphate from ammonia and bicarbonate (not CO_2), a reaction catalysed by CPS I, which requires *N*-acetylglutamate as an allosteric cofactor. Condensation of carbamoyl phosphate with ornithine yields citrulline (by OTC); this in turn condenses with aspartate to give argininosuccinate (by ASS), a reaction that requires the cleavage of two further high-energy phosphate bonds. Argininosuccinate is hydrolysed to fumarate and arginine (by argininosuccinase). Arginine is cleaved by arginase to give urea and ornithine. OTC, like CPS I, is also a

major mitochondrial protein [31]; the remaining enzymes are in the cytoplasm of hepatocytes. This necessitates the entry of ornithine into mitochondria and the exit of citrulline, which is brought about by the ornithine/citrulline transporters ORNT1 and 2.

This series of reactions, returning to ornithine, is known as the 'urea cycle'. It takes part not only in the removal of potentially toxic ammonia, but also in the irreversible removal of bicarbonate [32,33]. Although the arginase reaction is the major fate of arginine in liver, arginine can also be used for NO synthesis or undergo decarboxylation to form agmatine. Agmatinase converts arginine to putrescine and urea; however, the physiological role of this pathway is unclear [34].

Regulation of urea synthesis

Short-term regulation of urea synthesis occurs at the levels of substrate provision and enzyme activities, whereas long-term control is transcriptionally effected by changes in enzyme concentrations. Substrate provision for the urea cycle depends on amino acid delivery, the activity of amino acid transport systems, and amino acid metabolizing enzymes, and these factors are therefore important determinants of urea synthesis *in vivo*. The hepatic ureagenic response in humans is acutely sensitive to the plasma amino-nitrogen concentration, and urea production from an exogenous amino acid load is directly related to the plasma amino-nitrogen concentration, up to at least a concentration of 15 mmol/L [35]. Urea synthesis cannot therefore be saturated at realistic substrate concentrations.

In vitro, ASS seems to be the rate-limiting enzyme of the urea cycle at saturating ammonia concentrations. At physiologically low ammonia concentrations, however, carbamoyl phosphate synthesis is the rate-controlling step in urea synthesis [36].

Short-term regulation of urea synthesis primarily takes place at the level of ammonia and bicarbonate provision for mitochondrial CPS I, which presumably is the rate-controlling enzyme for the cycle at physiological substrate concentrations [11,12,21]. The K_m (NH_4^+) of CPS I is 1–2 mmol/L, that is, far above the portal NH_4^+ concentration of 0.2–0.3 mmol/L. Thus, the urea cycle has a low affinity for ammonia. This low affinity is in part compensated for by mitochondrial glutaminase, which is activated by its own product, ammonia, in the physiological concentration range [37]. This unique feature of liver glutaminase makes this enzyme a mitochondrial amplification system for ammonia, and glutaminase activity becomes an important determinant of urea cycle flux [12,13,21,23]. All factors known to increase urea synthesis, such as glucagon, ammonia, increased amino acid loads or alkalosis, activate glutaminase and accordingly, via amplification, ammonia provision for CPS I. Urea synthesis is also controlled at the level of mitochondrial bicarbonate supply, which is determined by pH, the CO_2 concentration and the activity of mitochondrial carbonic anhydrase V. This enzyme is required for the fast conversion of CO_2 into HCO_3^- , which is the substrate for CPS I. Its inhibition by acetazolamide blocks urea synthesis [24,38].

Short-term increases in ureagenesis are also thought to be mediated via *N*-acetylglutamate, a cofactor that stimulates CPS I activity [39], although its role as a short-term regulator of overall flux through the urea cycle has been disputed [40]. An intraperitoneal injection of a complete amino acid mixture causes rapid activation of ureagenesis. *N*-Acetylglutamate increases fivefold, causing a fivefold increase in the activity of CPS I [41]. Because the half-life of *N*-acetylglutamate in the liver is about 20 min, there is also a rapid response to a reduced protein intake. The concentration of ornithine may also play a role in the regulation of CPS I activity, and of flux through the urea cycle [42].

The activities of CPS I, ASS, and ASL are subject to allosteric regulation by MgATP and free Mg²⁺. There is no feedback inhibition of the urea cycle by urea within the physiological concentration range. Only at very high urea concentrations is there some inhibition of ASL [43].

In the longer term, there are changes in urea-cycle enzyme expression following alterations in dietary protein [43–46]. Glucagon, insulin and glucocorticoids are major mediators of these responses [47]. When protein intake is increased, there is a proportional increase in the total hepatic content of all five urea-cycle enzymes, resulting in increased urea production. Long-term regulation of urea-cycle enzymes [20] primarily occurs at the level of transcription [48,49], and several proteins associated with the urea cycle such as ORNT1 [26] or glutaminase [50] are regulated in parallel. mRNA levels for CPS I and ASS also increase immediately following partial hepatectomy [51]. Glucagon, which increases during starvation, and glucocorticoids, which increase protein breakdown, are both associated with increased enzyme levels [52] and urea production. Glucagon also regulates urea synthesis by rapid mechanisms, by activation of mitochondrial glutaminase [53], amino acid transport [54], *N*-acetylglutamate synthesis [55], and stimulation of hepatic proteolysis [56]. Insulin probably has no direct effect on urea synthesis, but is a powerful modulator of other known hormonal regulators such as glucagon and a potent inhibitor of liver proteolysis [57], and is therefore still a major regulator of urea synthesis *in vivo*. Hypothyroidism is associated with increased activity of the urea-cycle enzymes in rat liver. There is no change in urea-cycle activity with hyperthyroidism [58], although urea synthesis from infused amino acids increases [59]. Growth hormone lowers urea production, possibly by reducing the supply of nitrogen to the liver [60,61]. Pregnancy and ageing are also associated with reduced urea synthesis [62,63]. Caloric restriction upregulates CPS I and glutaminase [64], whereas reduction in protein intake decreases urea synthesis [65].

Endotoxaemia [66] and interleukin-1 increase urea synthesis from amino acids *in vivo* [67]. In livers from endotoxaemic rats, arginase and nitric oxide synthetase can compete for arginine [68,69]. A short circuit of the urea cycle occurs under these conditions, because arginine is converted directly to citrulline and NO by inducible nitric oxide synthase; however, compared with total urea-cycle flux, this pathway of arginine metabolism is

small in the hepatocyte. Flux through this citrulline–NO cycle in nonhepatic tissues is controlled by the expression of arginase and ASS and, like inducible nitric oxide synthase, is primarily regulated by cytokines [30].

Arginine protects against ammonia intoxication by stimulating *N*-acetylglutamate synthetase [70]. Carnitine reduces plasma ammonia and protects against acute ammonia intoxication by increasing urea synthesis [71]. Juvenile visceral steatosis mice with a defect in carnitine transport exhibit decreased expression of urea-cycle enzymes [72]. Here, probably, the accumulation of long-chain fatty acids results in an activated protein (AP)-1-dependent inhibition of the glucocorticoid activation of transcription [73]. In addition, CPS I is irreversibly inhibited by fatty acylation at physiological concentrations of palmitoyl-CoA [74]. Such phenomena provide a link between fatty acid metabolism and urea synthesis.

Drugs may influence urea synthesis. Sodium valproate increases plasma ammonia concentrations and reduces urea synthesis by 30% [75] due to a decrease of CPS I activity, but renal ammoniogenesis is also affected [76]. There is a dose-dependent inhibition of urea synthesis by acetylsalicylic acid.

Studies using isolated perfused liver preparations have shown that the pH of the perfusate influences the rate of urea production [24,77,78]. Such observations are consistent with the hypothesis that the liver plays a central role in the metabolic regulation of systemic pH (see later). Acetazolamide and loop diuretics containing a sulfonamide moiety inhibit the activity of mitochondrial carbonic anhydrase V and act as inhibitors of urea synthesis by impairing bicarbonate supply for carbamoyl phosphate synthesis [79].

Ammonia detoxication by glutamine synthesis

The other major pathway for ammonia fixation in liver is glutamine synthesis. Liver glutamine synthetase is a cytosolic enzyme and its localization in liver is restricted to a small hepatocyte subpopulation at the perivenous end of the liver acinus [23,80]. These cells are free of urea-cycle enzymes [12,21]. Thus, following the direction of sinusoidal blood flow, the two ammonia detoxication systems, urea and glutamine synthesis, are anatomically switched behind each other and present the sequence of a low- and high-affinity system for ammonia detoxication respectively [12,21]. The cells that contain glutamine synthetase have been termed perivenous scavenger cells [81], because they eliminate with high affinity the ammonia that was not used by the upstream urea-synthesizing compartment. They are of crucial importance for the maintenance of nontoxic ammonia levels in the hepatic vein: selective damage of perivenous scavenger cells does not impair upstream urea synthesis, but leads to hyperammonaemia due to a failure of scavenger function [82]. Both *in vivo* and *in vitro*, some 7 to 25% of the ammonia delivered via the portal vein escapes periportal urea synthesis and is used for glutamine synthesis [23,83]. In line

with their specialization to scavenge ammonium by glutamine synthesis, the perivenous scavenger cells strongly express the NH_4^+ transporter RhB glycoprotein [2], ornithine aminotransferase [84] and the glutamate transporter GLT1 [85], and exhibit high-affinity uptake systems for α -ketoglutarate, malate and other dicarboxylates [86,87], which serve as carbon precursors for glutamine synthesis. The mechanisms underlying the strict zonal expression of glutamine synthetase are not settled [88], but may involve an intronic silencer element of the gene [89] and the Wnt/ β -catenin pathway [85,90].

Small amounts of glutamine synthetase are also expressed by Kupffer cells [91]. Glutamine synthetase is strongly induced in activated hepatic stellate cells, which may significantly contribute to ammonia detoxication in fibrotic liver injury [92].

Short-term regulation of glutamine synthesis occurs at the level of substrate supply [87] and covalent modifications of the enzyme [93]. Because of the exclusive downstream localization of glutamine synthetase in the liver acinus, flux through the urea cycle in the periportal compartment will determine the amount of ammonia reaching the perivenous scavenger cells. Accordingly, all factors that regulate urea synthesis will indirectly exert control on glutamine synthesis. Also, the availability of carbon skeletons can control the rate of glutamine synthesis.

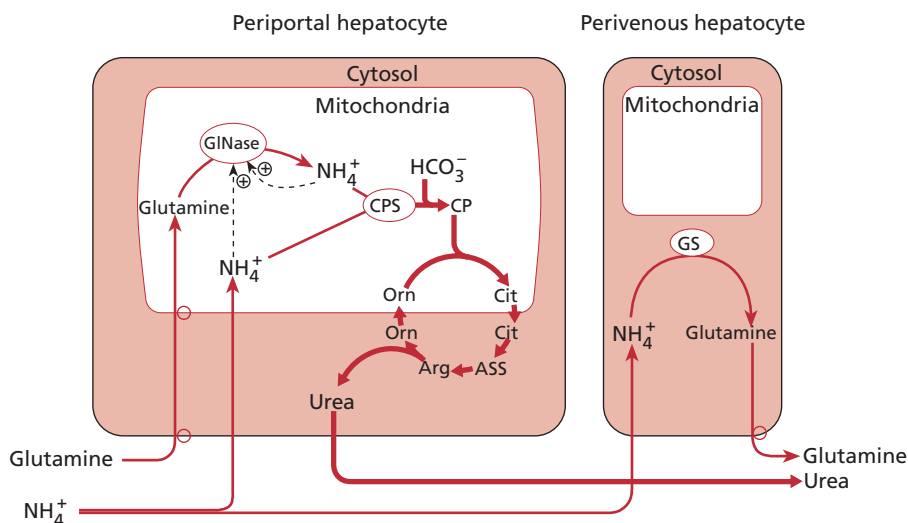
In vivo, glutamine synthetase activity is downregulated by

hypophysectomy [94], endotoxaemia [93] and following porto-caval anastomosis [95,96]. Glutamine and cell swelling decrease, whereas insulin and glucocorticoids increase, the levels of glutamine synthetase expression [97–99]. The age-related increase in oxidized protein in the liver and brain is accompanied by a loss of glutamine synthetase activity [100] and acetaminophen inhibits its catalytic activity [101].

The intercellular glutamine cycle and ammonia detoxication [12,13,21]

Whereas glutamine synthetase is localized in perivenous hepatocytes, glutaminase is located in periportal hepatocytes and in the mitochondria together with CPS. Here, glutaminase acts as a pH- and hormone-modulated ammonia amplifier and is an important determinant of urea-cycle flux in view of the physiologically low ammonia concentrations, which are about one order of magnitude lower than the K_m (ammonia) of CPS. Periportal glutaminase and perivenous glutamine synthetase are simultaneously active (the so-called intercellular glutamine cycle) [23]: glutamine utilized for ammonia amplification in periportal hepatocytes is resynthesized in perivenous scavenger cells from the ammonia that escaped upstream urea synthesis (Fig. 2). The regulatory properties of the intercellular glutamine cycle allow the liver to become a net producer or net consumer

Fig. 2 Structural and functional organization of hepatic ammonia and glutamine metabolism. Periportal hepatocytes contain urea-cycle enzymes and glutaminase, whereas glutamine synthetase is located in perivenous scavenger cells. Urea and glutamine synthesis are anatomically switched behind each other and represent functionally the sequence of a periportal high-capacity, but low-affinity, system and a perivenous high-affinity system for ammonia detoxication. Periportal glutaminase acts as a pH-modulated amplifier of the mitochondrial ammonia concentration and is an important determinant of urea-cycle flux. Ammonia that escapes periportal urea synthesis ('low-affinity system') is disposed of by the perivenous scavenger glutamine synthetase ('high-affinity system'). This guarantees the effective detoxication of ammonia, even when urea-cycle flux is inhibited in acidosis. At normal extracellular pH, glutaminase flux equals glutamine synthetase flux (the so-called 'intercellular glutamine cycle'): portal ammonia is completely converted into urea. In acidosis, glutaminase flux and urea synthesis decrease, whereas glutamine synthetase flux increases due to an increased ammonia delivery to perivenous hepatocytes: the liver switches ammonia detoxication from urea to net glutamine synthesis. From ref. 21.



of glutamine depending on the nutritional and hormonal conditions [53,102]. Intercellular glutamine cycling also provides an effective means to shift hepatic ammonia detoxication from urea to net glutamine synthesis in acidosis.

Urea and ammonia metabolism in liver disease

Urea synthesis is reduced in severe liver disease [103] in parallel with glutamine synthesis [104], and this tends to be correlated with deterioration in other markers of hepatocellular function [105,106]. The metabolic zonation in the human hepatic lobules with respect to urea and glutamine metabolism is preserved in fibrotic lesions, but is lost in cirrhotic nodules [107].

In patients with well-compensated cirrhosis, urea production rates are similar to those of healthy controls under basal conditions, but the maximal urea production capacity in response to a protein or amino acid load is significantly reduced [35,108,109]. Reduction in the maximal capacity for urea production in patients with cirrhosis is due to a decreased activity of all five urea-cycle enzymes [110,111]. Near-normal urea production rates in cirrhosis, despite the reduced capacity of the urea cycle, are in part achieved by increased ammonia amplification due to a 4–5-fold increase in glutaminase activity [104,112,113]. Zinc supplementation speeds up the kinetics of nitrogen conversion from amino acids into urea in those with cirrhosis, but not in healthy individuals [114]. This can be explained by the zinc dependence of several urea-cycle enzymes and carbonic anhydrase V, and zinc deficiency, which is frequently found in cirrhosis.

In patients with liver disease, there is a tendency for blood ammonia levels to rise. The main reason for this is presumably the failure of the liver, because of portosystemic shunting and hepatocellular dysfunction, to remove ammonia from the portal venous blood. Liver cirrhosis is characterized by a severe scavenger cell defect [104], which also occurs after portocaval shunting [95,115]. Intestinal ammonia production is only slightly altered in liver cirrhosis [10], but may increase upon intestinal overgrowth with urease-containing bacteria [116,117]. In the presence of severe liver impairment and portosystemic shunting, skeletal muscle becomes an important organ in ammonia homeostasis [118]. In patients with chronic liver failure, muscle takes up more ammonia and releases much more glutamine than in control subjects [119,120]. The extraction of ammonia from the circulation is significantly less in patients with cirrhosis and muscle wasting [121]. Exercise causes a greater release of ammonia from muscle in patients with cirrhosis than in control subjects [122].

In patients with cirrhosis the blood ammonia, which is already high, may occasionally rise further as a result of gastrointestinal bleeding, renal failure, acid–base disturbances, diuretics and infections. Such infections inhibit perivenous glutamine synthetase, due to an inactivating tyrosine nitration of the enzyme [93].

Urea-cycle disorders [123,124]

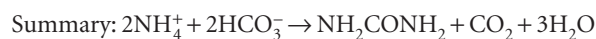
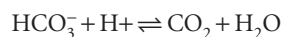
Deficiencies in all five urea-cycle enzymes, *N*-acetylglutamate synthetase [125,126] and glutamine synthetase [127] have been described in humans. A genetic defect of *ORNT1* leads to the hyperornithinaemia–hyperammonaemia–homocitrullinaemia syndrome [26,27], and citrin mutations can cause adult-onset type 2 citrullinaemia [29] and idiopathic neonatal hepatitis [128]. These disorders are rare and their clinical features are discussed in Chapter 22.1.

pH regulation and the liver [12,21,129,130]

The regulation of the acidity of the extracellular fluid is due primarily to the control of plasma CO_2 and HCO_3^- by the lungs, liver and kidneys. Despite its modest buffer capacity at physiological pH, the bicarbonate buffer is of special interest because the concentrations of its constituents, HCO_3^- and CO_2 , can be regulated very effectively. According to the Henderson–Hasselbalch equation:

$$\text{pH} = 6.1 + \log[\text{HCO}_3^-]/[\text{CO}_2]$$

Mechanisms for the maintenance of extracellular pH must keep the $[\text{HCO}_3^-]/[\text{CO}_2]$ ratio constant. Thus, CO_2 and HCO_3^- , which are continuously generated during the oxidation of energy fuels, need to be eliminated from the body at the same velocity as they are generated metabolically. Whereas the complete oxidation of fat and carbohydrates yields CO_2 and water as the only products and the lungs fulfil the role of regulating P_{CO_2} , the hydrolysis of proteins yields bipolar amino acids, whose complete oxidation generates not only CO_2 and water, but also HCO_3^- and NH_4^+ . The latter are derived from the carboxylic and amino/amido moieties of amino acids, respectively, and arise in almost equal amounts. The daily oxidation of 100 g of protein produces about 1 mol of HCO_3^- and 1 mol of NH_4^+ . Thus, protein oxidation creates a strong alkali burden for the body, and the major pathway for removal of HCO_3^- is hepatic urea synthesis, which consumes 2 mol of HCO_3^- per mol of urea produced:



In chemical terms, urea synthesis is an irreversible, energy-driven neutralization of the strong base HCO_3^- by the weak acid NH_4^+ , and the average daily excretion of 30 g of urea is equivalent to the disposal of about 1 mol of HCO_3^- per day. Thus, a major function of hepatic urea synthesis is to effect this neutralization, without which the body would otherwise be confronted by a major load of alkali [32]. *In vitro* studies showed a sensitive control of hepatic urea formation by extracellular pH, which establishes a homeostatic feedback control loop between

bicarbonate-consuming urea synthesis and the actual acid–base status. In this respect, it is important that the structural and functional organization of the pathways of ammonia detoxification in the liver acinus uncouples urea synthesis from the vital need to detoxify ammonia [33]. Whenever the pH and/or the HCO_3^- concentration drop in the extracellular space, the liver responds with a decrease of urea synthesis relative to the rate of protein catabolism and oxidation. Consequently, a fraction of the bicarbonate generated during protein breakdown is retained in the body and can be used to correct the underlying acidosis. The efficiency of such a mechanism is high: a 10% inhibition of ureogenesis relative to protein breakdown in acidosis will retain about 100 mmol HCO_3^- per day. In a well-balanced acid–base situation, however, the rate of bicarbonate removal (urea synthesis) from the organism must match the rate of bicarbonate production (protein catabolism) and the regulation of urea-cycle flux appears to be controlled by the rate of protein breakdown only.

pH control of urea synthesis occurs at the level of substrate provision, but not within the urea cycle itself. The mechanisms involved include pH-dependent changes in the $\text{NH}_3/\text{NH}_4^+$ ratio, the pH dependence of amino acid transport across the hepatocyte membrane, and the regulatory properties of mitochondrial glutaminase and carbonic anhydrase V. These enzymes adjust in a pH-dependent manner the input of the substrates ammonia and HCO_3^- into the CPS reaction, which is, *in vivo*, the rate-controlling step of urea synthesis. Lowering the extracellular pH from 7.4 to 7.3 already inhibits ammonia formation by liver glutaminase by 70% [33], mainly due to an inhibition of glutamine transport across the plasma and mitochondrial membranes [131]. Uptake across the plasma membrane is accomplished by SN1, which couples glutamine/ Na^+ symport to H^+ antiport [132], and these transport characteristics may explain the inhibition of glutamine uptake by periportal cells and simultaneous augmentation of glutamine export from perivenous hepatocytes in acidosis [133]. Also, the uptake of other amino acids into the liver is inhibited in acidosis. This shifts the site of amino acid catabolism from the liver to nonhepatic tissues [134–136]. The other enzyme, mitochondrial carbonic anhydrase V, is also sensitively controlled by pH and plays an important role in providing HCO_3^- inside the mitochondria for CPS [24], because the mitochondrial membrane is impermeable to HCO_3^- but not to CO_2 [137] and the rate of spontaneous (uncatalysed) conversion of CO_2 into HCO_3^- is by far not fast enough to meet the bicarbonate requirements for intramitochondrial biosynthetic pathways [24]. In acidosis, both carbonic anhydrase and glutaminase are inhibited and, consequently, also irreversible bicarbonate consumption and urea synthesis. The regulatory properties of catalysed and uncatalysed intramitochondrial bicarbonate formation also allow discrimination of respiratory from metabolic acidosis, such that urea synthesis is inhibited much more strongly in metabolic than in respiratory acidosis. Even at a normal extracellular pH *in vitro*, the liver can respond to a decrease in buffer capacity of the extracellular

$\text{HCO}_3^-/\text{CO}_2$ system with an inhibition of urea synthesis and a sparing of bicarbonate.

The structural and functional organization of the pathways of nitrogen metabolism in the liver eliminates the threat of hyperammonaemia, which would otherwise result from an acidosis-induced inhibition of urea synthesis: perivenous scavenger cells maintain ammonia homeostasis by glutamine synthesis when upstream urea synthesis is switched off (Fig. 2). Acidosis shifts hepatic ammonia detoxification from bicarbonate-consuming urea synthesis to net glutamine synthesis, which is the result of both increased perivenous glutamine formation and reduced glutamine consumption at periportal glutaminase. Thus, intercellular glutamine cycling in the liver is an elegant means to adjust ammonia flux into either urea or glutamine depending on the acid–base situation. Glutamine formed by the liver and other organs during acidosis is hydrolysed in the kidney and the NH_4^+ is excreted as such into the urine. This process of so-called renal ammoniogenesis has long been known to be stimulated in acidosis and involves the action of kidney glutaminase, which is regulated by pH in an opposite way to the liver enzyme [138].

Thus, the kidneys function as a spillover for surplus NH_4^+ , which cannot be disposed of by urea synthesis [130] (Fig. 3). This renal contribution is critical for the pH-stat function of the liver, because otherwise accumulation of surplus NH_4^+ in the organism, albeit in the form of glutamine, would override the acid–base control of hepatic HCO_3^- elimination by an inadequate NH_4^+ - (or glutamine-)driven stimulation of urea synthesis. With respect to ammonia and HCO_3^- homeostasis, the liver and kidney act as a team and impairment of one organ's function will give rise to acid–base disturbances. It is important to stress that it is the change of urea synthesis relative to protein breakdown that affects the bicarbonate pool in the body, whereas absolute changes of urea synthesis do not allow conclusions on bicarbonate homeostasis unless the rate of protein oxidation is assessed simultaneously. Failure to do so has led in the past to erroneous conclusions.

Clinical consequences [139–141]

Alkalosis is reportedly present in up to 70% of cirrhotic patients, whereas acidosis is found in less than 10%, which is usually a metabolic acidosis [139–141] and follows sepsis, shock and lactate accumulation. In alkalotic cirrhotic patients, the alkalosis was 13–60% metabolic, 20–50% respiratory and 10–30% mixed respiratory/metabolic [139–143]. In fulminant hepatic failure, alkalosis is present in about 75% of patients; in 30% of these cases, the alkalosis is metabolic [140]. Clearly, the acid–base status in patients with liver disease is influenced not only by the severity of liver disease itself, but also by many variables including accompanying morbidities and drugs.

Metabolic alkalosis in cirrhosis is often attributed to potassium deficiency and hyperaldosteronism, particularly as a result of diuretic therapy [144], but it may also be found without these

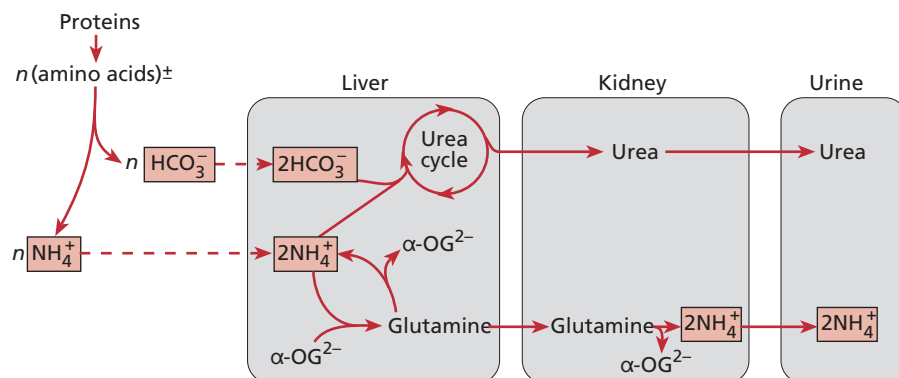


Fig. 3 Coordinated regulation of HCO_3^- and NH_4^+ homeostasis by the liver and kidneys. NH_4^+ and HCO_3^- arise in almost equimolar amounts during protein oxidation. Whereas urea synthesis irreversibly consumes HCO_3^- and NH_4^+ in a 1:1 stoichiometry, HCO_3^- is spared when urea synthesis is switched off in acidosis ('bicarbonate homeostatic response' of the liver). Under these conditions, NH_4^+ homeostasis is maintained by NH_4^+ excretion into urine ('ammonium homeostatic response' of the kidney). A feedback control loop between bicarbonate-consuming urea synthesis and the extracellular pH, $[\text{CO}_2]$ and $[\text{HCO}_3^-]$ adjusts hepatic HCO_3^- consumption to the needs of acid–base homeostasis. In metabolic acidosis, flux through the urea cycle and hepatic glutaminase is decreased, whereas flux through hepatic glutamine synthetase and renal glutaminase is increased. The coordination of these processes results in NH_4^+ disposal from the organism without concomitant HCO_3^- removal in acidosis. From ref. 79.

factors. Metabolic alkalosis in liver cirrhosis may be the result of an impaired urea synthesis [145], as suggested by an inverse relationship between plasma bicarbonate concentration in humans *in vivo* and the capacity for hepatic urea synthesis *in vitro* [104,112,146]. In view of the relationship between urea synthesis and bicarbonate homeostasis, metabolic alkalosis may not only be seen as a consequence of impaired urea synthesis, but also as representing an important driving force for residual urea synthesis in cirrhosis. This may explain why acidotic episodes can provoke hyperammonaemia in cirrhosis. In view of this, careful attention has to be paid to the acid–base status, and metabolic acidosis requires treatment [147].

The pathogenesis of respiratory alkalosis in cirrhotic patients is less clear, but elevated progesterone and oestradiol levels [148], hypoxaemia and hyperammonaemia may play a role [144,149]. It was hypothesized that hyperventilation and respiratory alkalosis represent a compensatory response of the cirrhotic patient in order to counteract the low-grade cerebral oedema that develops in response to hyperammonaemia and other precipitating factors of hepatic encephalopathy [5].

Although the liver plays a key role in lactate metabolism and lactate levels tend to be moderately elevated, overt lactic acidosis is relatively uncommon in liver disease and is usually found only when there are other factors that would increase lactate production or cause further decreases in hepatic uptake of lactate, such as shock, sepsis and bleeding [144]. In fulminant hepatitis, the development of lactic acidosis is associated with a uniformly high mortality; it has been attributed to tissue hypoxia resulting from arteriovenous shunting, as it occurred in patients with an apparently adequate systemic blood pressure, blood flow and arterial oxygen tension [150].

References

- Jahn TP, Moller AL, Zeuthen T *et al.* (2004) Aquaporin homologues in plants and mammals transport ammonia. *FEBS Lett* 574, 31–36.
- Weiner ID, Miller RT, Verlander JW (2003) Localization of the ammonium transporters, Rh B glycoprotein and Rh C glycoprotein, in the mouse liver. *Gastroenterology* 124, 1432–1440.
- Hawkins RA, Jessy J, Mans AM *et al.* (1993) Effect of reducing brain glutamine synthesis on metabolic symptoms of hepatic encephalopathy. *J Neurochem* 60, 1000–1006.
- Häussinger D, Laubenberger J, vom Dahl S *et al.* (1994) Proton magnetic resonance spectroscopy studies on human brain myo-inositol in hypo-osmolarity and hepatic encephalopathy. *Gastroenterology* 107, 1475–1480.
- Häussinger D, Kircheis G, Fischer R *et al.* (2000) Hepatic encephalopathy in chronic liver disease, a clinical manifestation of astrocyte swelling and low-grade cerebral edema? *J Hepatol* 32, 1035–1038.
- vom Dahl S, Häussinger D (1996) Characterization of phloretin-sensitive urea export from the perfused rat liver. *Biol Chem* 377, 25–37.
- Carbrey JM, Gorelick-Feldman DA, Kozono D *et al.* (2003) Aquaglyceroporin AQP9, solute permeation and metabolic control of expression in liver. *Proc Natl Acad Sci USA* 100, 2945–2950.
- Sands JM (2003) Mammalian urea transporters. *Annu Rev Physiol* 65, 543–566.
- Hallbrucker C, vom Dahl S, Ritter M (1994) Effects of urea on K^+ fluxes in perfused rat liver. *Pfluegers Arch Physiol* 428, 552–560.
- Olde Damink S, Deutz N, Dejong C *et al.* (2002) Interorgan ammonia metabolism in liver failure. *Neurochem Int* 41, 177–188.
- Meijer AJ, Lamers WH, Chamuleau RAFM (1990) Nitrogen metabolism and ornithine cycle function. *Physiol Rev* 70, 701–748.
- Häussinger D, Lamers WH, Moorman AFM (1993) Metabolism of amino acids and ammonia. *Enzyme* 46, 72–93.

- 13 Häussinger D (1998) Hepatic glutamine transport and metabolism. *Adv Enzymol Rec Adv Mol Biol* 72, 43–86.
- 14 Lowenstein JM (1972) Ammonia production in muscle and other tissues, the purine nucleotide cycle. *Physiol Rev* 52, 382–414.
- 15 Jones EA, Smallwood RA, Craigie A *et al.* (1969) The enterohepatic circulation of urea nitrogen. *Clin Sci* 37, 825–836.
- 16 Wolpert E, Phillips SF, Summerskill WH (1971) Transport of urea and ammonia production in the human colon. *Lancet* 2, 1387–1390.
- 17 Wolpert E, Phillips SF, Summerskill WH (1970) Ammonia production in the human colon. Effects of cleansing, neomycin and acetohydroxamic acid. *N Engl J Med* 283, 159–164.
- 18 Weber FL, Veach G (1979) The importance of the small intestine in gut ammonium production in the fasting dog. *Gastroenterology* 77, 235–240.
- 19 Weber FL Jr, Friedman DW, Fresard KM (1988) *Am J Physiol* 254, G264–G268.
- 20 Morris SM (2002) Regulation of enzymes of the urea cycle and arginine metabolism. *Annu Rev Nutr* 22, 87–105.
- 21 Häussinger D (1990) Nitrogen metabolism in liver: structural and functional organization and physiological relevance. *Biochem J* 267, 281–290.
- 22 Walser M, Bodenlos LJ (1959) Urea metabolism in man. *J Clin Invest* 38, 1617–1626.
- 23 Häussinger D (1983) Hepatocyte heterogeneity in glutamine and ammonia metabolism and the role of an intercellular glutamine cycle during ureogenesis in perfused rat liver. *Eur J Biochem* 133, 269–275.
- 24 Häussinger D, Gerok W (1985) Hepatic urea synthesis and pH regulation. Role of CO₂, HCO₃⁻, pH and the activity of carbonic anhydrase. *Eur J Biochem* 152, 381–386.
- 25 Beliveau-Carey G, Cheung CW, Cohen NS *et al.* (1993) Regulation of urea and citrulline synthesis under physiological conditions. *Biochem J* 292, 241–247.
- 26 Camacho JA, Obie C, Biery B *et al.* (1999) Hyperornithinaemia-hyperammonaemia-homocitrullinuria syndrome is caused by mutations in a gene encoding a mitochondrial ornithine transporter. *Nat Genet* 22, 151–158.
- 27 Camacho JA, Riaseco-Camacho N, Andrade D *et al.* (2003) Cloning and characterization of human ORNT2, a second mitochondrial ornithine transporter that can rescue a defective ORNT1 in patients with the hyperornithinemia-hyperammonemia-homocitrullinuria syndrome, a urea cycle disorder. *Mol Genet Metab* 79, 257–271.
- 28 Kobayashi K, Sinasac DS, Iijima M *et al.* (1999) The gene mutated in adult-onset type II citrullinaemia encodes a putative mitochondrial carrier protein. *Nat Genet* 22, 159–163.
- 29 Wu G (1995) Urea synthesis in enterocytes of developing pigs. *Biochem J* 312, 717–723.
- 30 Husson A, Brasse-Lagnel C, Fairand A *et al.* (2003) Argininosuccinate synthetase from the urea cycle to the citrulline-NO cycle. *Eur J Biochem* 270, 1887–1899.
- 31 Clarke S (1976) The polypeptides of rat liver mitochondria, identification of a 36, 000 dalton polypeptide as the subunit of ornithine transcarbamylase. *Biochem Biophys Res Comm* 71, 1118–1124.
- 32 Atkinson DE, Bourke E (1984) The role of ureagenesis in pH homeostasis. *Trends Biochem Sci* 9, 297.
- 33 Häussinger D, Gerok W, Sies H (1984) Hepatic role in pH regulation, role of the intercellular glutamine cycle. *Trends Biochem Sci* 9, 300–302.
- 34 Morris SM Jr (2004) Recent advances in arginine metabolism. *Curr Opin Clin Nutr Metab Care* 7, 45–51.
- 35 Vilstrup H (1980) Synthesis of urea after stimulation with amino acids, relation to liver function. *Gut* 21, 990–995.
- 36 Meijer AJ, Lof C, Ramos IC *et al.* (1985) Control of ureogenesis. *Eur J Biochem* 148, 189–196.
- 37 Häussinger D, Sies H (1979) Hepatic glutamine metabolism under the influence of the portal ammonia concentration in the perfused rat liver. *Eur J Biochem* 101, 179–184.
- 38 Dodgson SJ, Forster RE II (1986) Carbonic anhydrase, inhibition results in decreased urea production by hepatocytes. *J Appl Physiol* 60, 646–652.
- 39 Shigesada K, Aoyagi K, Tatibana M (1978) Role of acetylglutamate in ureotelism. Variations in acetylglutamate level and its possible significance in control of urea synthesis in mammalian liver. *Eur J Biochem* 85, 385–391.
- 40 Lund P, Wiggins D (1984) Is N-acetylglutamate a short-term regulator of urea synthesis? *Biochem J* 218, 991–994.
- 41 Stewart PM, Walser M (1980) Short term regulation of ureagenesis. *J Biol Chem* 255, 5270–5280.
- 42 Lund P, Wiggins D (1986) The ornithine requirement of urea synthesis. Formation of ornithine from glutamine in hepatocytes. *Biochem J* 239, 773–776.
- 43 Menyhart J, Grof J (1977) Urea as a selective inhibitor of argininosuccinate lyase. *Eur J Biochem* 75, 405–409.
- 44 Schimke RT (1962) Differential effects of fasting and protein-free diets on levels of urea cycle enzymes in rat liver. *J Biol Chem* 237, 1921–1924.
- 45 Schimke RT (1963) Studies on factors affecting the levels of urea cycle enzymes in rat liver. *J Biol Chem* 238, 1012–1018.
- 46 Snodgrass PJ, Lin RC (1981) Induction of urea cycle enzymes of rat liver by amino acids. *J Nutr* 111, 586–601.
- 47 Morris SM Jr, Moncman CL, Rand KD *et al.* (1987) Regulation of mRNA levels for five urea cycle enzymes in rat liver by diet, cyclic AMP, and glucocorticoids. *Arch Biochem Biophys* 256, 343–353.
- 48 Morris SM Jr (1992) Regulation of enzymes of urea and arginine synthesis. *Annu Rev Nutr* 12, 81–101.
- 49 Takiguchi M, Mori M (1995) Transcriptional regulation of genes for ornithine cycle enzymes. *Biochem J* 312, 649–659.
- 50 Dhahbi JM, Mote PL, Wingo J *et al.* (1999) Calories and aging alter gene expression for gluconeogenic, glycolytic, and nitrogen-metabolizing enzymes. *Am J Physiol* 277, E352–E360.
- 51 Tygstrup N, Bak S, Krog B *et al.* (1995) Gene expression of urea cycle enzymes following two-thirds partial hepatectomy in the rat. *J Hepatol* 22, 349–355.
- 52 Sigsgaard I, Almdal T, Hansen BA *et al.* (1988) Dexamethasone increases the capacity of urea synthesis time dependently and reduces the body weight of rats. *Liver* 8, 193–197.
- 53 Häussinger D, Gerok W, Sies H (1983) Regulation of flux through glutaminase and glutamine synthetase in isolated perfused rat liver. *Biochim Biophys Acta* 755, 272–278.
- 54 Fehlmann M, Le Cam A, Freychet P (1979) Insulin and glucagon stimulation of amino acid transport in isolated rat hepatocytes. Synthesis of a high affinity component of transport. *J Biol Chem* 254, 10431–10437.
- 55 Staddon JM, Bradford NM, McGivan JD (1984) Effects of glucagon *in vivo* on the N-acetylglutamate, glutamate and glutamine contents of rat liver. *Biochem J* 217, 855–857.

- 56 vom Dahl S, Hallbrucker C, Lang F *et al.* (1991) Regulation of liver cell volume and proteolysis by glucagon and insulin. *Biochem J* 278, 771–777.
- 57 Hallbrucker C, vom Dahl S, Lang F *et al.* (1991) Inhibition of hepatic proteolysis by insulin. Role of hormone-induced alterations of the cellular K⁺ balance. *Eur J Biochem* 199, 467–474.
- 58 Marti J, Portoles M, Jimenez-Nacher I *et al.* (1988) Effect of thyroid hormones on urea biosynthesis and related processes in rat liver. *Endocrinology* 123, 2167–2174.
- 59 Marchesini G, Fabbri A, Bianchi GP *et al.* (1989) Hepatic conversion of amino-nitrogen to urea in thyroid diseases. II. A study in hyperthyroid patients. *Metabolism* 43, 1023–1029.
- 60 Dahms WT, Owens RP, Kalhan SC *et al.* (1989) Urea synthesis, nitrogen balance, and glucose turnover in growth-hormone-deficient children before and after growth hormone administration. *Metabolism* 38, 197–203.
- 61 Wothers T, Grofte T, Jorgensen JO *et al.* (1994) Effects of growth hormone (GH) administration on functional hepatic nitrogen clearance: studies in normal subjects and GH-deficient patients *J Clin Endocrinol Metab* 78, 1220–1224.
- 62 Kalhan SC, Tserng KY, Gilfillan C *et al.* (1982) Metabolism of urea and glucose in normal and diabetic pregnancy. *Metabolism* 31, 824–833.
- 63 Fabbri A, Marchesini G, Bianchi G *et al.* (1994) Kinetics of hepatic amino-nitrogen conversion in ageing man. *Liver* 14, 288–294.
- 64 Dhahbi JM, Mote PL, Wingo J *et al.* (2001) Caloric restriction alters the feeding response of key metabolic enzyme genes. *Mech Ageing Dev* 122, 1033–1048.
- 65 Picou D, Phillips M (1972) Urea metabolism in malnourished and recovered children receiving a high or low protein diet. *Am J Clin Nutr* 25, 1261–1266.
- 66 Nielsen SS, Grofte T, Tygstrup N *et al.* (2005) Effect of lipopolysaccharide on in vivo and genetic regulation of rat urea synthesis. *Liver Int* 25, 177–183.
- 67 Heindorff H, Almdal T, Vilstrup H (1994) The in vivo effect of interleukin-1 beta on urea synthesis is mediated by glucocorticoids in rats. *Eur J Clin Invest* 24, 388–392.
- 68 Wettstein M, Gerok W, Häussinger D (1994) Endotoxin-induced nitric oxide synthesis in the perfused rat liver: effects of L-arginine and ammonium chloride. *Hepatology* 19, 641–647.
- 69 Stadler J, Barton D, Beil-Moeller H *et al.* (1995) Hepatocyte nitric oxide biosynthesis inhibits glucose output and competes with urea synthesis for L-arginine. *Am J Physiol* 268, G183–G188.
- 70 Kim S, Paik WK, Cohen PP (1972) Ammonia intoxication in rats: protection by N-carbamoyl-L-glutamate plus L-arginine. *Proc Natl Acad Sci USA* 69, 3530–3533.
- 71 Costell M, O'Connor JE, Miguez MP *et al.* (1984) Effects of L-carnitine on urea synthesis following acute ammonia intoxication in mice. *Biochem Biophys Res Commun* 120, 726–733.
- 72 Tomomura M, Imamura Y, Horiuchi M (1992) Abnormal expression of urea cycle enzyme genes in juvenile visceral steatosis (jvs) mice. *Biochim Biophys Acta* 1138, 167–171.
- 73 Tomomura M, Tomomura A, Dewan MA *et al.* (1996) Long-chain fatty acids suppress the induction of urea cycle enzyme genes by glucocorticoid action. *FEBS Lett* 399, 310–312.
- 74 Corvi MM, Soltys CL, Berthiaume LG (2001) Regulation of mitochondrial carbamoyl-phosphate synthetase 1 activity by active site fatty acylation. *J Biol Chem* 276, 45704–45712.
- 75 Hjelm M, Oberholzer V, Seakins J *et al.* (1986) Valproate-induced inhibition of urea synthesis and hyperammonaemia in healthy subjects. *Lancet* ii(8511), 859.
- 76 Marini AM, Zaret BS, Beckner RR (1988) Hepatic and renal contributions to valproic acid-induced hyperammonemia. *Neurology* 38, 365–371.
- 77 Lueck JD, Miller LL (1970) The effect of perfusate pH on glutamine metabolism in the isolated perfused rat liver. *J Biol Chem* 245, 5491–5497.
- 78 Häussinger D, Gerok W, Sies H (1986) The effect of urea synthesis on extracellular pH in isolated perfused rat liver. *Biochem J* 236, 261–265.
- 79 Häussinger D, Kaiser S, Stehle T *et al.* (1986) Liver carbonic anhydrase and urea synthesis. The effect of diuretics. *Biochem Pharmacol* 35, 3317–3322.
- 80 Gebhardt R, Mecke D (1983) Heterogeneous distribution of glutamine synthetase among rat liver parenchymal cells in situ and in primary culture. *EMBO J* 2, 567–570.
- 81 Häussinger D, Stehle T (1988) Hepatocyte heterogeneity in response to icosanoids. The perivenous scavenger cell hypothesis. *Eur J Biochem* 175, 395–403.
- 82 Häussinger D, Gerok W (1984) Hepatocyte heterogeneity in ammonia metabolism: impairment of glutamine synthesis in CCl₄ induced liver cell necrosis with no effect on urea synthesis. *Chem Biol Interact* 48, 191–194.
- 83 Cooper AJ, Nieves E, Coleman AE *et al.* (1987) Short-term metabolic fate of [¹³N]ammonia in rat liver in vivo. *J Biol Chem* 262, 1073–1080.
- 84 Kuo FC, Hwu WL, Valle D *et al.* (1991) Colocalization in pericentral hepatocytes in adult mice and similarity in developmental expression pattern of ornithine aminotransferase and glutamine synthetase mRNA. *Proc Natl Acad Sci USA* 88, 9468–9472.
- 85 Cadoret A, Ovejero C, Terris B *et al.* (2002) New targets of beta-catenin signaling in the liver are involved in the glutamine metabolism. *Oncogene* 21, 8293–8301.
- 86 Stoll B, Hussinger D (1989) Functional hepatocyte heterogeneity. Vascular 2-oxoglutarate is almost exclusively taken up by perivenous, glutamine-synthetase-containing hepatocytes. *Eur J Biochem* 181, 709–716.
- 87 Stoll B, McNelly S, Buscher HP *et al.* (1991) Functional hepatocyte heterogeneity in glutamate, aspartate and alpha-ketoglutarate uptake: a histautoradiographical study. *Hepatology* 13, 247–253.
- 88 Lie-Venema H, Hakvoort TBM, van Hemert FJ *et al.* (1998) Regulation of the spatiotemporal pattern of expression of the glutamine synthetase gene. *Prog Nucleic Acid Res Mol Biol* 61, 243–308.
- 89 Gaunitz F, Deichsel D, Heise K *et al.* (2005) An intronic silencer element is responsible for specific zonal expression of glutamine synthetase in the rat liver. *Hepatology* 41, 1225–1232.
- 90 Ueberham E, Arendt E, Starke M *et al.* (2004) Reduction and expansion of the glutamine synthetase expressing zone in livers from tetracycline controlled TGF-beta1 transgenic mice and multiple starved mice. *J Hepatol* 41, 75–81.
- 91 Bode JG, Peters-Regehr T, Kubitz R *et al.* (2000) Expression of glutamine synthetase in macrophages. *J Histochem Cytochem* 48, 415–422.
- 92 Bode JG, Peters-Regehr T, Gressner AM *et al.* (1998) De novo expression of glutamine synthetase during transformation of hepatic stellate cells into myofibroblast-like cells. *Biochem J* 335, 697–700.
- 93 Görg B, Wettstein M, Metzger S *et al.* (2005) Lipopolysaccharide-induced tyrosine nitration and inactivation of hepatic glutamine synthetase in the rat. *Hepatology* 41, 1065–1073.

- 94 Wong BS, Chenoweth ME, Dunn A (1980) Possible growth hormone control of liver glutamine synthetase activity in rats. *Endocrinology* 106, 268–274.
- 95 Girard G, Butterworth RF (1992) Effect of portacaval anastomosis on glutamine synthetase activities in liver, brain, and skeletal muscle. *Dig Dis Sci* 37, 1121–1126.
- 96 Häussinger D, Lamers WH, Moorman AF (1993) Hepatocyte heterogeneity in the metabolism of amino acids and ammonia. *Enzyme* 46, 72–93.
- 97 Abcouwer SF, Bode BP, Souba WW (1995) Glucocorticoids regulate rat glutamine synthetase expression in a tissue-specific manner. *J Surg Res* 59, 59–65.
- 98 Warskulat U, Newsome W, Noe B *et al.* (1996) Anisoosmotic regulation of hepatic gene expression. *Biol Chem* 377, 57–65.
- 99 Labow BI, Souba WW, Abcouwer SF (2001) Mechanisms governing the expression of the enzymes of glutamine metabolism – glutaminase and glutamine synthetase. *J Nutr* 131, 2467S–2474S.
- 100 Stadtman ER, Starke-Reed PE, Oliver C *et al.* (1992) Protein modification in aging. *EXS* 62, 64–72.
- 101 Bulera SJ, Birge RB, Cohen SD *et al.* (1995) Identification of the mouse liver 44-kDa acetaminophen-binding protein as a subunit of glutamine synthetase. *Toxicol Appl Pharmacol* 134, 313–320.
- 102 Cooper AJ, Nieves E, Rosenspire KC *et al.* (1988) Short-term metabolic fate of ¹³N-labeled glutamate, alanine, and glutamine(amide) in rat liver. *J Biol Chem* 263, 12268–12273.
- 103 Rudman D, DiFulco TJ, Galambos JT *et al.* (1973) Maximal rates of excretion and synthesis of urea in normal and cirrhotic subjects. *J Clin Invest* 52, 2241–2249.
- 104 Kaiser S, Gerok W, Häussinger D (1988) Ammonia and glutamine metabolism in human liver slices: new aspects on the pathogenesis of hyperammonaemia in chronic liver disease. *Eur J Clin Invest* 18, 535–542.
- 105 Vilstrup H, Bucher D, Krog B *et al.* (1982) Elimination of infused amino acids from plasma of control subjects and of patients with cirrhosis of the liver. *Eur J Clin Invest* 12, 197–202.
- 106 Vilstrup H, Iversen J, Tygstrup N (1986) Glucoregulation in acute liver failure. *Eur J Clin Invest* 16, 193–197.
- 107 Racine-Samson L, Scoazec JY, D’Errico A *et al.* (1996) The metabolic organization of the adult human liver: a comparative study of normal, fibrotic, and cirrhotic liver tissue. *Hepatology* 24, 104–113.
- 108 Rypins EB, Henderson JM, Fulenwider JT *et al.* (1980) A tracer method for measuring rate of urea synthesis in normal and cirrhotic subjects. *Gastroenterology* 78, 1419–1424.
- 109 Rafoth RJ, Onstad GR (1975) Urea synthesis after oral protein ingestion in man. *J Clin Invest* 56, 1170–1174.
- 110 Khatra BS, Smith RB III, Millikan WJ (1974) Activities of Krebs-Henseleit enzymes in normal and cirrhotic human liver. *J Lab Clin Med* 84, 708–715.
- 111 Maier KP, Talke H, Gerok W (1979) Activities of urea-cycle enzymes in chronic liver disease. *Klin Wschr* 57, 661–665.
- 112 Häussinger D, Steeb R, Gerok W (1990) Ammonium and bicarbonate homeostasis in chronic liver disease. *Klin Wschr* 68, 175–182.
- 113 Matsuno T, Goto I (1992) Glutaminase and glutamine synthetase activities in human cirrhotic liver and hepatocellular carcinoma. *Cancer Res* 52, 1192–1194.
- 114 Marchesini G, Fabbri A, Bianchi G *et al.* (1996) Zinc supplementation and amino acid-nitrogen metabolism in patients with advanced cirrhosis. *Hepatology* 23, 1084–1092.
- 115 Häussinger D, Lamers WH, Moorman AF (1992) Hepatocyte heterogeneity in the metabolism of amino acids and ammonia. *Enzyme* 46, 72–93.
- 116 Hansen BA, Vilstrup H (1985) Increased intestinal hydrolysis of urea in patients with alcoholic cirrhosis. *Scand J Gastroenterol* 20, 346–350.
- 117 Lai D, Gorbach SL, Levitan R (1972) Intestinal microflora in patients with alcoholic cirrhosis: urea-splitting bacteria and neomycin resistance. *Gastroenterology* 62, 275–279.
- 118 Lockwood AH, McDonald JM, Reiman RE *et al.* (1979) The dynamics of ammonia metabolism in man. Effects of liver disease and hyperammonemia. *J Clin Invest* 63, 449–460.
- 119 Bessman SP, Bessman AN (1955) The cerebral and peripheral uptake of ammonia in liver disease with an hypothesis for the mechanism of hepatic coma. *J Clin Invest* 34, 622–628.
- 120 Fazekas JF, Ticktin HE, Shea JG (1957) Effect of L-glutamic acid on metabolism of patients with hepatic encephalopathy. *Am J Med Sci* 234, 145–149.
- 121 Ganda OP, Ruderman NB (1976) Muscle nitrogen metabolism in chronic hepatic insufficiency. *Metabolism* 25, 427–430.
- 122 Allen SI, Conn HO (1960) Observations on the effect of exercise on blood ammonia concentration in man. *Yale J Biol Med* 33, 133–144.
- 123 Bachmann C (2002) Mechanisms of hyperammonemia. *Clin Chem Lab Med* 40, 653–662.
- 124 Brusilow SW, Horwich AL (2001) Urea cycle enzymes. In: Scriver CR, Beaudet AL, Sly WS *et al.* (eds) *The Metabolic Basis of Inherited Disease*. 8th edn. New York: McGraw Hill, pp. 1909–1963.
- 125 Caldovic L, Morizono H, Panglao MG *et al.* (2003) Null mutations in the N-acetylglutamate synthase gene associated with acute neonatal disease and hyperammonemia. *Hum Genet* 112, 364–368.
- 126 Schmidt E, Nuoffer JM, Haberle J *et al.* (2005) Identification of novel mutations of the human N-acetylglutamate synthase gene and their functional investigation by expression studies. *Biochim Biophys Acta* 1740, 54–59.
- 127 Häberle J, Gorg B, Rutsch F *et al.* (2005) Congenital glutamine deficiency with glutamine synthetase mutations. *N Engl J Med*, 353, 1956–33.
- 128 Saheki T, Kobayashi K, Iijima M *et al.* (2004) Adult-onset type II citrullinemia and idiopathic neonatal hepatitis caused by citrin deficiency: involvement of the aspartate glutamate carrier for urea synthesis and maintenance of the urea cycle. *Mol Genet Metab* 81, S20–S26.
- 129 Häussinger D (1988) *pH Homeostasis*. London: Academic Press.
- 130 Bourke E, Häussinger D (1992) pH homeostasis: the conceptual change. *Contrib Nephrol* 100, 58–88.
- 131 Lenzen C, Soboll S, Sies H *et al.* (1987) pH control of hepatic glutamine degradation. Role of transport. *Eur J Biochem* 16, 483–488.
- 132 Fei YJ, Sugawara M, Nakanishi T *et al.* (2000) Primary structure, genomic organization, and functional and electrogenic characteristics of human system N 1, a Na⁺- and H⁺-coupled glutamine transporter. *J Biol Chem* 275, 23707–23717.
- 133 Bode BP (2001) Recent molecular advances in mammalian glutamine transport. *J Nutr* 131, 2475S–2485S.
- 134 Fafournoux P, Demigne C, Remesy C *et al.* (1983) Bidirectional transport of glutamine across the cell membrane in rat liver. *Biochem J* 216, 401–408.
- 135 Boon L, Blommaert PJ, Meijer AJ *et al.* (1994) Acute acidosis inhibits liver amino acid transport: no primary role for the urea cycle in acid-base balance. *Am J Physiol* 267, F1015–F1020.

- 136 Christensen HN, Kilberg M (1995) Hepatic amino acid transport primary to the urea cycle in regulation of biologic neutrality. *Nutr Rev* 53, 74–76.
- 137 Balboni E, Lehninger AL (1986) Entry and exit pathways of CO₂ in rat liver mitochondria respiring in a bicarbonate buffer system. *J Biol Chem* 261, 3563–3570.
- 138 Curthoys NP, Watford M (1995) Regulation of glutaminase activity and glutamine metabolism. *Annu Rev Nutr* 15, 133–159.
- 139 Oster JR, Perez GO (1988) Acid–base homeostasis and pathophysiology in liver disease. In: Epstein M (ed.) *The Kidney in Liver Disease*, 3rd edn. Baltimore: Williams & Wilkins, pp. 119–131.
- 140 Record CO, Iles RA, Cohen RD *et al.* (1975) Acid–base and metabolic disturbances in fulminant hepatic failure. *Gut* 16, 144–149.
- 141 Döller W (1965) *Der Säure-Basenstoffwechsel bei Leberzirrhose*. Hüthig Verlag, Heidelberg.
- 142 Moreau R, Hadengue A, Soupison T *et al.* (1993) Arterial and mixed venous acid–base status in patients with cirrhosis. Influence of liver failure. *Liver* 13, 20–24.
- 143 Bernardi M, Predieri S (2005) Disturbances of acid–base balance in cirrhosis: a neglected issue warranting further insights. *Liver Int* 25, 463–466.
- 144 Oster JR, Perez GO (1986) Acid–base disturbances in liver disease. *J Hepatol* 2, 299–306.
- 145 Guder W, Häussinger D (1987) Renal and hepatic nitrogen metabolism in systemic acid–base regulation. *J Clin Chem Clin Biochem* 25, 457–466.
- 146 Häussinger D (1990) Organization of hepatic nitrogen metabolism and its relation to acid–base homeostasis. *Klin Wschr* 68, 1096–1101.
- 147 Häussinger D, Steeb R, Gerok W (1992) Metabolic alkalosis as driving force for urea synthesis in liver disease: pathogenetic model and therapeutic implications. *Clin Invest* 70, 411–415.
- 148 Lustik SJ, Chhibber AK, Kolano JW *et al.* (1997) The hyperventilation of cirrhosis: progesterone and estradiol effects. *Hepatology* 25, 55–58.
- 149 Wichser J, Kazemi H (1974) Ammonia and ventilation: site and mechanism of action. *Respir Physiol* 20, 393–406.
- 150 Bihari D, Gimson AE, Lindridge J *et al.* (1985) Lactic acidosis in fulminant hepatic failure. Some aspects of pathogenesis and prognosis. *J Hepatol* 1, 405–416.

2.3.8 Protein synthesis and degradation in the liver

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Introduction

The proper physiological function of various organs relies on the expression of biologically active molecules such as antibodies, hormones and neurotransmitters as well as various ligands and receptors, and the signalling and other molecules by which they act. Many of these molecules are proteins, encoded in genes. Some are non-protein molecules (e.g. lipids, sugars and other small molecules such as nitric oxide gas) whose existence requires the action of proteins (e.g. enzymes that are involved in their synthesis). The DNA of the genes encoding these

proteins must be transcribed and processed into mRNAs and exported out of the nucleus into the cytoplasm, as a prerequisite for gene expression. This occurs by complex mechanisms that are outside the scope of this review. Once in the cytoplasm, mRNAs can be either quiescent, as a result of binding proteins that prevent their translation into proteins, or actively engaged in making their encoded polypeptides.

One important function of the liver is the synthesis and secretion of a wide range of proteins into the bloodstream, including blood clotting factors and transporter proteins such as lipoproteins and albumin. Another important function is the synthesis and correct localization of integral membrane proteins of the plasma membrane and other compartments. A common feature of these classes of proteins is that their fates are determined very early during translation of the mRNAs in which they are encoded. The nascent chains of these proteins are targeted to the membrane of the endoplasmic reticulum (ER), a specialized intracellular compartment that initiates their journey by subsequent vesicle fission and fusion through the secretory pathway (Fig. 1). Regulatory steps along the way ultimately determine the amount, function and/or localization of these proteins. Some of these steps, such as the initial event of translocation across (for a secretory protein) or integration into (for a membrane protein) the membrane of the ER, are, in their simplest manifestation, irreversible events. However, under certain conditions, these irreversible steps are overcome by cellular machineries that degrade undesired proteins, and thereby end their action. In this chapter, we describe first the fundamentals of early events in mRNA translation and degradation of newly synthesized secretory and membrane proteins. Subsequently, we discuss regulatory mechanisms of protein synthesis and degradation with emphasis on examples relevant to liver function and pathophysiology.

Principles of posttranscriptional gene expression

Translation of mRNA to protein

Translation of mRNA into protein starts by (i) binding of methionyl-tRNA (tRNA_{Met}) to the small ribosomal subunit (40S), (ii) recruitment of the complex to mRNA at the first AUG codon and (iii) joining of the large ribosomal subunit (60S) to the mRNA–40S complex. The eukaryotic initiation factors (eIF) are involved in all three steps of translation initiation [1]. Shortly before the start of translation, the eIF2 (composed of α , β and γ subunits) forms a complex with GTP and tRNA_{Met} to facilitate binding to the small ribosomal subunit. The binding of this preinitiation complex, known as the 43S complex, to mRNA is assisted both in *cis* and in *trans*. The *cis* element referred to is the ‘cap’, a modified guanine nucleotide at the 5′ end of mRNA, which in many cases is critical for the recruitment of 43S by the eIF4 complex [2]. The eIF4 complex is composed of several proteins including: eIF4E, which physically binds the